Gdańsk University of Technology
Faculty of Chemistry
Department of Analytical Chemistry

Ph.D thesis

# THE CHARACTERIZATION OF ENANTIOMERIC COMPOSITION AND IMPURITY PROFILE OF METHAMPHETAMINE AND ITS CHLORO-INTERMEDIATES SYNTHESIZED BY EMDE METHOD

mgr inż. Justyna Małgorzata Płotka

Supervisors: Prof. Marek Biziuk
Ph.D Calum Morrison

Gdańsk 2014

*I would like to express my sincere gratitude to my advisors,*
*Professor* **MAREK BIZIUK** *and Doctor* **CALUM MORRISON**,
*for the continuous support of my Ph.D study and research, for their patience, motivation, enthusiasm, and immense knowledge.*

*I would like to gratefully and sincerely thank Professor* **JACEK NAMIEŚNIK**
*for his scientific advice and knowledge and many insightful discussions and suggestions.*

*I am very grateful to Professor* **VASIL SIMEONOV**
*for cooperation and advice during the project.*

*Thanks to all of my* **FRIENDS** *from Department of Analytical Chemistry*
*for spiritual support and creating a pleasant working atmosphere.*

*I would like to thank my loved ones,*
**MUM, DAD, SISTER, BROTHER, SISTER-IN-LAW, AND MY NEPHEWS**
*who have supported me throughout entire process, both by keeping me harmonious and helping me putting pieces together. I will be grateful forever for your love.*

*I would like to thank my husband* **ŁUKASZ**
*for being with me in good and bad times, for his love and friendship,*
*for support and patience.*

**TABLE OF CONTENTS**

## ABSTRACT

Because the number of people who abuse methylamphetamine (MAMP) is increasing every year, it has become a global threat. Background knowledge about MAMP is significant for many reasons with the most important issues being associated with human health in terms of prediction and protection applied to reduction in drug addiction.

The project aims to develop and optimize the new analytical methodology in order to create a comprehensive characterization of MAMP in terms of impurity and chirality profile, and also enantiomeric characterization of chloro-intermediates of MAMP that are not commercially available, and necessary as reference materials for the further analysis.

The configuration of chloro-intermediates were determined by 1D and 2D NMR analysis in combination with achiral GC-MS analysis. It was shown that chlorination of the ephedrine (EP)/pseudoephedrine (PSEP) compounds occurs via inversion and retention of configuration around the α carbon and mixture of diastereoisomers (chloroephedrine and chloropseudoephedrine) were formed, with the ratio of the resulting compounds dependent on the precursors used.

A GC-MS method using a γ-cyclodextrin chiral stationary phase was developed and optimized for the simultaneous enantiomeric separations of MAMP and its common precursors, EP and PSEP, as well as its chloro-intermediates formed during MAMP synthesis by Emde method, after derivatization with trifluoroacetic anhydride. Cluster analysis and principal components analysis were used as multivariate statistical methods in the interpretation of data obtained from the derivatization process. The new methodology in comparison with other existing procedures is innovative, because it is more economical and ecological (the chiral separation of analytes of interest is performed in a single analysis run in short time, what has not been presented in any literature data). Moreover, the new methodology is easy to apply and rapid. Methylamphetamine samples were successfully analyzed using the proposed method. Several new impurities were detected in this study in MAMP classified as manufactured by Emde. The developed analytical procedure and characterization of CEP analogs synthesized by the Emde method leads to an indication of the method of synthesis as well as which precursors were used for MAMP manufacture indicating that this research would provide valuable information to law enforcement agencies regarding the provenance of MAMP seizures.

## LIST OF ABBREVIATIONS AND ACRONYMS

| 1 | 2 | 3 |
|---|---|---|
| **Abbreviations/ acronyms** | **English term** | **Polish term** |
| **1D** | One dimensional | Jednowymiarowa |
| **2D** | Two dimensional | Dwuwymiarowa |
| **α** | Separation factors | Współczynnik rozdzielenia |
| **Ac.B** | Acetate buffer | Bufor octanu |
| **ADHD** | Attention Deficit Hyperactivity Disorder | Zespół nadpobudliwości psychoruchowej z deficytem uwagi |
| **AE** | Atom Economy | Ekonomia atomów |
| **AE,exp** | Experiment Atom Economy | Ekonomia atomów, wartość doświadczalna |
| **AE'** | Atom Efficiency | Efektywność atomowa |
| **AITC** | 2,3,4-tri-O-acetyl-α-D-arabinopyranosyl isothiocyanate | Izotiocyjanian 2,3,4-tri-O-acetylo-α-D-arabinopiranozylo |
| **AM** | Amphetamine | Amfetamina |
| **ATS** | Amphetamine – type stimulant | Fenetylaminy psychoaktywne |
| **B** | Blood | Krew |
| **CA** | Cluster analysis | Analiza skupień |
| **CAN** | Acetonitrile | Acetonitryl |
| **CB** | Carbethoxyhexafluorobutyryl chloride | Chlorek karboetoksyheksafluorobutyrylu |
| **CD** | Cyclodextrin | Cyklodekstryny |
| **CDFA** | Chlorodifluoroacetic anhydride | Bezwodnik kwasu chlorodifluorooctowego |
| **CE** | Capillary electrophoresis | Elektroforeza kapilarna |
| **CE- β -CD** | Carboxyethyl- β -cyclodextrin | Karboksyetylo-β-cyklodekstryna |
| **CEP** | Chloroephedrine | Chloro efedryna |
| **Chiral-CBH** | Chiral protein phase | Chiralna faza proteinowa |
| **CM- β -CD** | Carboxymethyl- β -cyclodextrin | Karboksymetylo-β-cyklodekstryna |
| **CNS** | Central Nervous System | Centralny układ nerwowy |
| **COSY** | Correlation spectroscopy | Spektroskopia korelacyjna |
| **CPSEP** | chloropseudoephedrine | Chloropseudoefedryna |

**LIST OF ABBREVIATIONS AND ACRONYMS – *CONT.***

| 1 | 2 | 3 |
|---|---|---|
| **CS** | Chiral selector | Selektor chiralny |
| **CSP** | Chiral stationary phase | Chiralna faza stacjonarna |
| **CV** | Coefficient of variation | Współczynnik zmienności |
| **DA** | Discriminant analysis | Analiza dyskryminacyjna |
| **DAD** | Diode-array detector | Detektor z matrycą diodową |
| **DEPT** | Distortionless enhancement by polarization transfer | Bezzakłóceniowe wzmocnienie sygnału (jąder niskoczułych) poprzez transfer polaryzacji |
| **d-MTPAA** | D-α-methoxy-α-(trifluoromethyl)phenyl acetic acid | Kwas d-α-metoksy-α-(trifluorometylo) fenylooctowy |
| **DOM** | 2,5- dimethoxy-4-methylamphetamine | 2,5 - dimetoksy-4-metyloamfetamina |
| **DR** | Derivatization reagent | Odczynnik derywatyzujący |
| **E -factor** | Environmental factor | Czynnik Środowiskowy |
| **EA** | Ethyl acetate | Octan etylu |
| **EI-MS** | Electrospray- Mass spectrometry | Spektrometrii mas z elektrorozpylaniem |
| **El.D.** | Electrochemical Detector | Detektor elektrochemiczny |
| **EMCDDA** | European Monitoring Centre for Drugs and Drug Addition | Europejskie Centrum Monitorowania Narkotyków i Narkomanii |
| **EP** | Ephedrine | Efedryna |
| **f** | Solvent and catalyst environmental impact parameter | Współczynnik oddziaływania środowiskowego rozpuszczalników i katalizatorów |
| **FA** | Factor analysis | Analiza czynnikowa |
| **FID** | Flame-ionization detector | Detektor płomieniowo-jonizacyjny |
| **FLD** | Fluorescence detector | Detektor fluorescencyjny |
| **FLEC** | 9-fluorenylethyl chloroformate | 9-fluorenyloetylenoksykarbonyl |
| **FMOC** | 9-fluorenylmethyl chloroformate | 9-fluorenylometylenoksykarbonyl |
| **GC** | Gas chromatography | Chromatografia gazowa |
| **GITC** | 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate | Izotiocyjanian 2,3,4,6-tetra-O-acetylo-β-D-glukopiranozylu |
| **H** | Hair | Włosy |

**LIST OF ABBREVIATIONS AND ACRONYMS – *CONT.***

| 1 | 2 | 3 |
|---|---|---|
| **HMA** | Hydroxymethamphetamine | Hydroksymetamfetamina |
| **HP- β -CD** | Hydroxypropyl- β -cyclodextrin | Karboksypropylo-β-cyklodekstryna |
| **HSQC** | Heteronuclear single quantum correlation | Heterojądrowa pojedyncza kwantowa korelacja 1H-13C |
| **IR** | Infrared spectroscopy | Spektroskopia w podczerwieni |
| **IRD** | Infrared detector | Detektor podczerwieni |
| **IS** | Internal standards | Wzorzec wewnętrzny |
| **IUPAC** | International Union of Pure and Applied Chemistry | Międzynarodowa Unia Chemii Czystej i Stosowanej |
| **k** | Retention factors | Współczynnik retencji |
| **LC** | Liquid chromatography | Chromatografia cieczowa |
| **LDA** | Linear discrimination analysis | Liniowa analiza dyskryminacji |
| **l-HFBOPCl** | (2S, 4R)-N-heptafluorobutyryl-4-heptafluorobutyloxy-prolyl chloride | Chlorek (2S, 4R)-N-heptafluorobutyrylo-4-heptafluorobutyloksy-prolylu |
| **l-HPC** | (S)-(-)-N-(heptafluorobutyryl)prolyl chloride | Chlorek (S)-(-)-N-(heptafluorobutyrylo)prolylu |
| **LOD** | Limit of detection | Granicawykrywalności |
| **LOQ** | Limit of quantification | Granica oznaczalności |
| **L-Pro** | L-proline | L-prolina |
| **l--TPC** | N-trifluoroacethyl-l-prolyl chloride | Chlorek N-trifluoroacetylo-l-prolylu |
| **m** | Final mass | Masa końcowa |
| **M- β-CD** | Methyl- β -cyclodextrin | Metylo- β-cyklodekstryna |
| **$m_0$** | Initial mass | Masa początkowa |
| **MAMP** | Methylamphetamine | Metamfetamina |
| **MBDB** | Methylenedioxyphenyl-N-methyl-2-butanamine | *N-metylo*-I-fenylo-*2-butanoamina* |
| **MBTFA** | N-methyl-bis(trifluoroacetamide) | N-metylo-bis(trifluoroacetamid) |
| **MDA** | 3,4-methylenedioxyamphetamine | 3,4-metylenodioksy-α-metylofenyloetyloamina |
| **MDEA** | 3,4-methylenedioxy-N-ethylamphetamine | 3,4-metylenodioksy-N-etylo-α-metylofenyloetyloamina |
| **MDMA** | 3,4-methylenedioxymethamphetamine | 3,4-metylenodioksy-N-metylo-α-metylofenyloetyloamina |

## LIST OF ABBREVIATIONS AND ACRONYMS – *CONT.*

| 1 | 2 | 3 |
|---|---|---|
| **MI** | Mass intensity | Intensywność mas |
| **MOH** | Methanol | Metanol |
| **$m_p$** | Melting point | Temperatura topnienia |
| **$m_{p, lit}$** | Literature value of melting point | Literaturowa wartość temperatury topnienia |
| **MPEP** | Methylpseudoephedrine | Metylopseudoefedryna |
| **MS** | Mass spectrometer | Spektrometr mas |
| **MTPA** | α–methoxy-α-(trifluoromethyl)phenylacetyl chloride | Chlorek metoksy-α-(trifluorometylo)fenyloacetylu |
| **NAC** | N-acetyl-L-cysteine | N-acetylo-L-cysteina |
| **NEP** | Norephedrine | Norefedryna |
| **NITC** | Naphthyl isothiocyanate | Izotiocyjanian naftylu |
| **N-MEP** | N-methylephedrine | N-metyloefedryna |
| **NMR** | Nuclear magnetic resonance | Magnetyczny rezonans jądrowy |
| **NOE** | Nuclear Overhauser effect | Jądrowy efekt Overhausera |
| **NOESY** | Nuclear Overhauser effect spectroscopy | Spektroskopia pomiaru jądrowego efektu Overhausera |
| **OPA** | O-phthalaldehyde | Aldehyd o-ftalowy |
| **P** | Pharmaceuticals | Farmaceutyki |
| **P-2-P** | 1-phenyl-2-propanone | 1-fenylo-2-propanon |
| **PCA** | Principal component analysis | Analiza głównych składowych |
| **PCI** | Positive ion chemical ionization | Jonizacja chemiczna jonów dodatnich |
| **PFP** | Pentafluoropropionic acid | Kwas pentafluorooctowy |
| **PFPA** | Pentafluoropropionic anhydride | Bezwodnik kwasu pentafluoropropionowego |
| **Ph.B** | Phosphate buffer | Bufor fosforanowy |
| **PITC** | Phenyl isothiocyanate | Izocyjanian fenylu |
| **PSEP** | Pseudoephedrine | Pseudoefedryna |
| **RME** | Reaction Mass Efficiency | Efektywność masowa reakcji |
| **RRF** | Relative response factors | Czynnik odpowiedzi względnej |
| **$R_s$** | Resolution factors | Rozdzielczość pików |

**LIST OF ABBREVIATIONS AND ACRONYMS – *CONT.***

| 1 | 2 | 3 |
|---|---|---|
| **$R_t$** | Retention time | Czas retencji |
| **SELNOESY** | Selective excitation nuclear Overhauser effect spectroscopy | Spektroskopia pomiaru jądrowego efekt u Overhausera z wykorzystaniem selektywnego wzbudzenia |
| **SFC** | Supercritical fluid chromatography | Chromatografia z płynem w stanie nadkrytycznym |
| **$S_N2$** | bimolecular nucleophilic substitution | Dwucząsteczkowe podstawienie nukleofilowe |
| **$S_Ni$** | internal nucleophilic substitution | Wewnątrzcząsteczkowe podstawienie nukleofilowe |
| **SPE** | Solid phase extraction | Ekstrakcja do fazy stacjonarnej |
| **(S,S)-PDITC** | (1S,2S)-N-[(2-isothiocyanato)-cyclohexyl)] pivalinoyl amide | (1S, 2S)-N-[(2-izotiocyjaniano) cykloheksylo)] amid kwasu piwylowego |
| **T** | Tablet | Tabletka |
| **TFA** | Trifluoroacetic acid | Kwas trifluorooctowy |
| **TFAA** | Trifluoroacetic anhydride | Bezwodnik kwasu trifluorooctowego |
| **TFMPA** | 3-(Trifluoromethyl)phenethylamine | 3-(Trifluorometylo)-fenyloetyloamina |
| **TLC** | Thin layer chromatography | Chromatografia cienkowarstwowa |
| **U** | Urine | Mocz |
| **UNODC** | United Nations Office on Drugs and Crime | Biuro Narodów Zjednoczonych ds. Narkotyków i Przestępczości |
| **UVD** | Ultraviolet-visible variable wavelength detector | Detektor promieniowania UV |

# I.INTRODUCTION

Methylamphetamine (MAMP) is one of the most dangerous psychoactive substances. Within a new report published by EMCDDA the number of people abusing MAMP is increasing every year, thus it has became a global threat. In Poland, where MAMP is illegal and strictly controlled, increased consumption of psychoactive substances is apparent.

Many laboratories are engaged in clandestine manufacture of MAMP. The chemistry is well understood, and widely published, notably on the web, though not easy to achieve. There are various routes of clandestine synthesis of MAMP but the most commonly used are: Leuckart, Emde, Nagai and Birch method. MAMP coming from clandestine laboratories has variable composition because each of these methods may produce different by-products during the synthesis of MAMP. Additionally, the samples synthesized using the same method may show different impurity patterns according to the various conditions, and the impurities may disappear or co-precipitate with MAMP during the crystallization process.

Subsequent analysis of the impurities of MAMP may supply valuable information about the conditions and the chemicals used in the illicit MAMP manufacture provides information on the original source of sample and therefore analysis of the impurities requires techniques which offer a high degree of resolution, specificity and sensitivity.

Methylamphetamine, because of its stereogenic center, has two optically active isomers of which (S)-(+)-enantiomer is more frequently abused due to its stronger CNS stimulatory activity than the (R)-(-)-enantiomer, which does not possess any significant CNS activity or addictive properties. For its high stimulant effect, (S)-(+)-MAMP is predominantly abused and illicitly smuggled on the black market. On the other hand, (R)-(-)-MAMP can be used as a precursor for the manufacturing of l-deprenyl, an effective antiparkinsonian and antidepressant. The enantiomeric composition of a MAMP sample is determined by stereochemical configuration of the precursors. Both, (1R,2S)-(-)-ephedrine and (1S,2S)-(+)-pseudoephedrine and their derivatives have the same C-2 configuration with (S)-(+)-MAMP, so they can be used as starting materials for synthesis. On the contrary, (R)-(-)-MAMP can be synthesized from (1R,2R)-(-)-pseudoephedrine, (1S,2R)-(+)-ephedrine and their derivatives. Because the enantiomeric ratio of MAMP is closely related to the optical activity of precursors and reagents used for the synthesis, this knowledge can provide useful information concerning the origins and synthetic methods used for illicit manufacture. This information is important, since it can be utilized for regulation of the precursors, investigation of the

manufacturing sources, and resultant prevention of abuse. To obtain this information, analytical techniques which offers a high degree of enantio-resolution are required.

Because of above, the knowledge about MAMP, analytical procedures to determine purity of drug as well as impurity profiling and enantiomeric composition, and also the detection and identification in different matrix types is significant for many reasons, including prediction and protection applied to reduction in drug addiction.

Although in recent years, the amount of literature data about analytical methodologies used for the detection and determination of MAMP, and also for the determination of impurities and enantiomeric composition has increased, nevertheless research in this area is not common in Polish research centers. As previously mentioned this drug is relatively new to Poland but on the increase. Many other countries and international organizations are also concerned about this substance. However, the knowledge about the impurity of MAMP obtained by Emde as well as the characteristics of its chloro-intermediates is still poor and do not provide complete information about the drug, its intermediates and impurities, especially in terms of chirality. Moreover, recently analytical procedures which are carried out for these purposes, are laborious and time-consuming, requiring the use of large quantities of toxic and expensive solvents and are often characterized by a low coefficient of enrichment. Therefore it is vital to focus on this problem and develop appropriate analytical procedures as tools to obtain reliable analytical information, that are helpful in further investigations.

# I THEORETICAL PART

## 1. Amphetamine – type compounds as psychoactive stimulants

Psychoactive substances, more commonly known as psychoactive drugs, are chemical substances, derived from plants or synthesized, that cross the blood-brain barrier and act primarily upon the central nervous system (CNS) where its affect brain function [1]. When a psychoactive substance is taken, it has the ability to change an individual's consciousness, mood or thinking processes.

Psychoactive drugs can be classified in different ways, such as common effects, chemical structure, addiction liability, or Drug Enforcement Administration schedules, however, commonly-accepted classifications include stimulants, depressants and hallucinogens [2].

Stimulants are drugs that stimulate the CNS, speeding up the communication between the brain and body, affecting alertness and physical activities. The effects of stimulants abuse are similar to those of the body's natural hormones, adrenaline, however, unlike natural hormones, stimulants have a negative impact to the body, causing serious harm and disease [3]. Due to subjective changes in consciousness and mood (e.g. increased alertness or euphoria) after stimulant consumption, many stimulants are abused and used excessively, despite the health risks or negative consequences.

There are many compounds belonging to the stimulant group including legal substances such as caffeine or nicotine as well as illegal compounds such as cocaine, khat, and the most popular and highly abused within this class, amphetamine-type stimulants [4].

Amphetamine – type stimulants (ATS) are a class of substances that are structurally derived from β-phenethylamine [5]. This group of stimulants has the potential to make people feel energetic, confident with a high sense of positive feelings like love, happiness and gratitude. Amphetamines can also increase self esteem, make more sociable, and enhance performance. However, in a large amounts, amphetamines can lead to hallucinations, bizarre or erratic behavior, restlessness, weight loss, and paranoia [6, 7].

ATS are sometimes used for the medical purposes in the treatment of Attention Deficit Hyperactivity Disorder (ADHD) as well as obesity and narcolepsy, however, due largely to the risks associated with sustained use, the ability for amphetamines to lead to addiction, they are not widely accepted for use [6, 7]. Apart from the medical reason of ATS consumption,

there are a number of different purposes of use these substances by human. The motive for non-medical ATS use may be [8]:

- psychological:
  - to attain physical pleasures such as states of euphoria or fantasy,
  - to soothe emotional pain or psychological discomfort,
  - to escape from unpleasant reality out of curiosity,
  - to derive sexual pleasures;
- sociological:
  - status-seeking peer pressure,
  - substance oriented society,
  - the news media;
- family background.

Regardless of the reason why the ATS are abused, these drugs can lead to health and social issues and can ruin every aspects of a person's life.

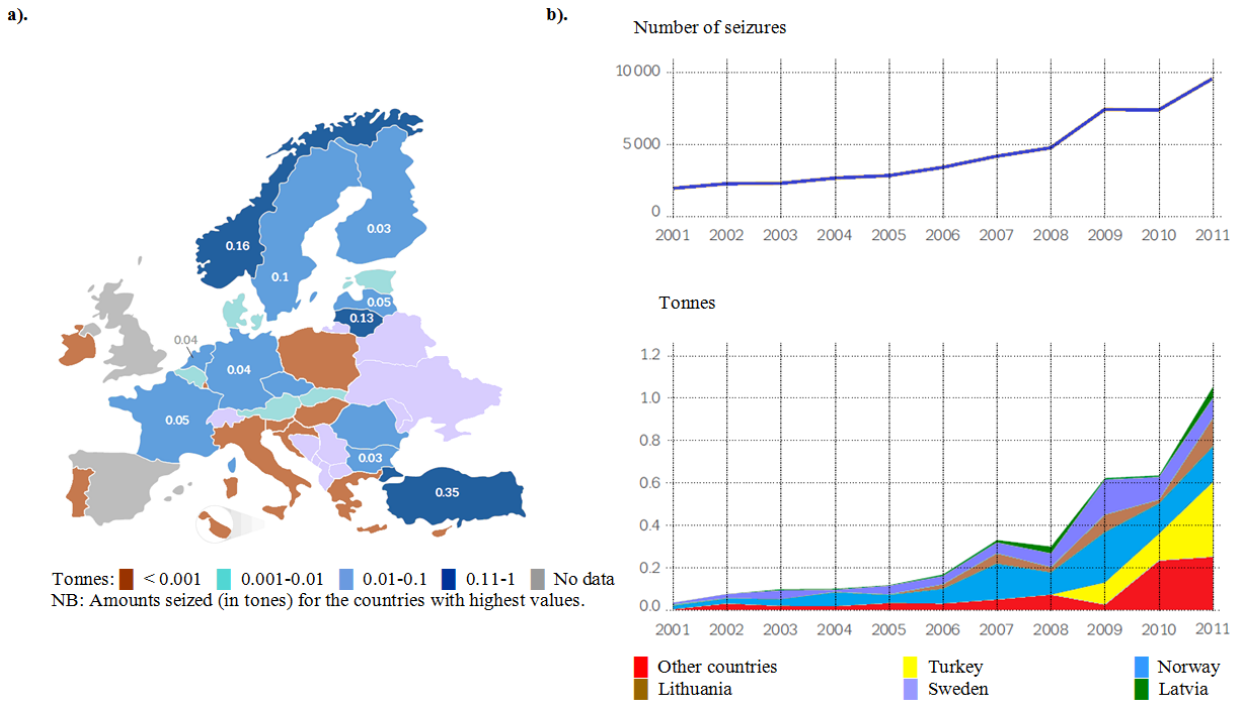



**Figure 1**. a). Quantity of methylamphetamine seized in Europe, 2011; b). Number of methylamphetamine seizures and quantity seized, 2001–2011. From [1] with permission.

The principal members of ATS include amphetamine (AM) and methamphetamine (MAMP) [6]. Amphetamine has always been very popular in Europe, however, currently

there are signs of the increasing availability of methylamphetamine [9]. Although the number and quality of methylamphetamine seizures is still small, an increased over the period of 2001-2011 has been recorded (Figure 1). Therefore it has became a global threat. Also in Poland, where methylamphetamine is illegal and strictly controlled, increase of consumption as psychoactive substances, is apparent.

### 1.1. Methylamphetamine – abused drug of the early 21st century

According to IUPAC, *N*-methyl-1-phenylpropan-2-amine, commonly called methamphetamine, ice or meth is a harmful, synthetic member of the amphetamine type stimulants that acts stimulating role on the CNS releasing massive amounts of the neurotransmitter dopamine [10]. Although, it is produced under license as a medicinal product, primarily it is also said to be the most widely illicitly manufactured drug on a global scale [10]. Because, methylamphetamine started to be a global threat, the background knowledge about this substance is significant for many reasons with the most important being associated with human health in terms of prediction and protection applied to reduction of drug addiction [10].

#### *1.1.1. History*

Although the abuse of MAMP has increased recently, the beginning of its history is known to take place in 1919 [11]. Methylamphetamine was synthesized from EP by Japanese pharmacologist Akira Ogata and has been demonstrated as a drug that alleviates fatigue and produces feelings of alertness and well-being [11]. In the 1920s and 1930s, the medical and paramedical consumption of MAMP increased in Europe. Although, problematic side effects of both, chronic and non-medical use of MAMP such as depression, dependence and psychiatric disturbance have been well known and documented, this drug enjoyed widespread acceptance as safe and beneficial medication not only among the public at large but also among the medical profession [11].

During World War II, MAMP as a Pervitin medication produced by Berlin-based Temmeler pharmaceutical company [12], was used extensively by the German troops to increase concentration and enhance performance [13]. Also in Japan, MAMP as a medication called Philopon was supplied to Japanese soldiers, especially pilots and workers in key war industries [14].

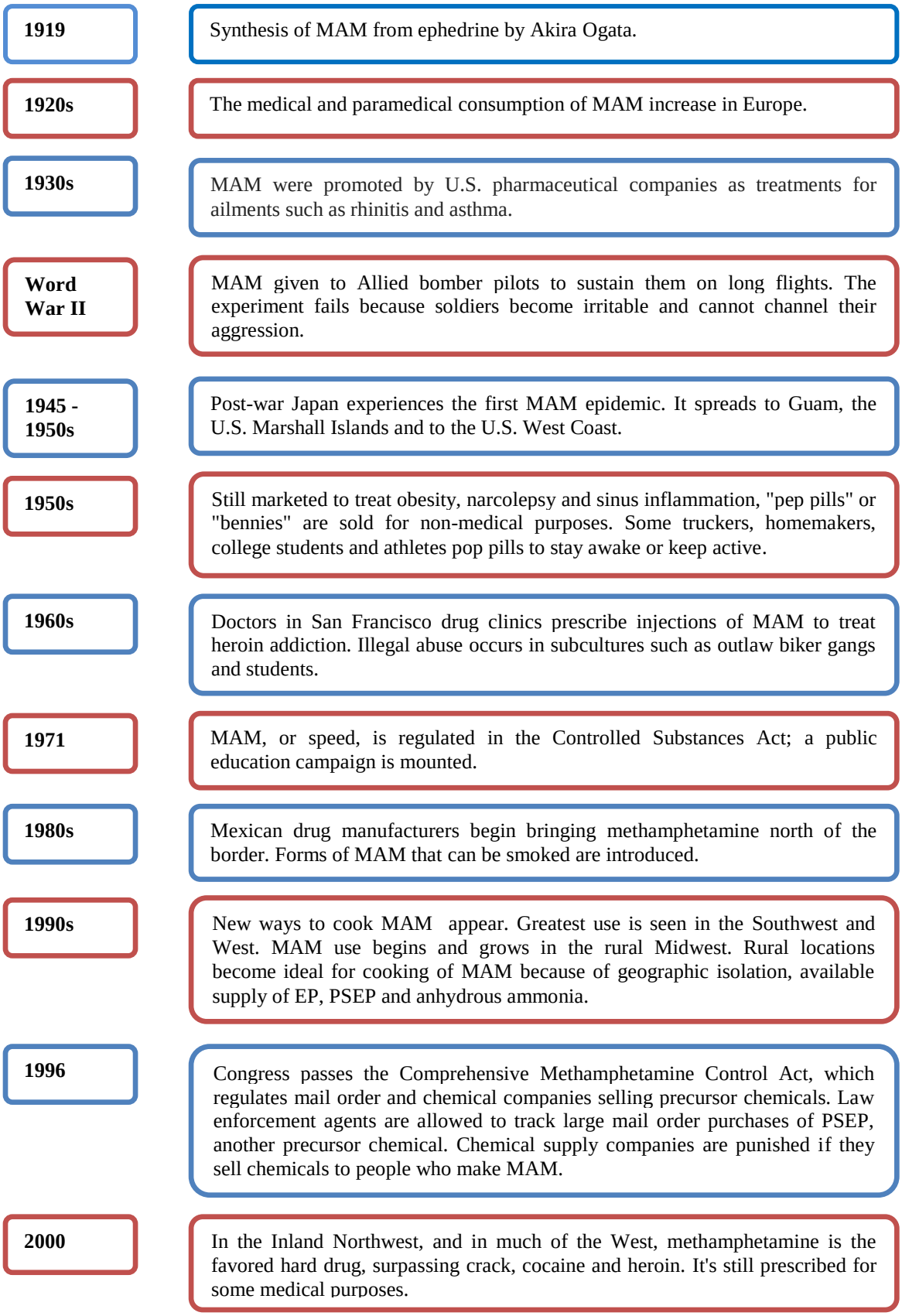



**Figure 2.** A brief history of methylamphetamine

In the Allied camp, MAMP use was not as popular as AMP use, however millions MAMP tablets were still supplied to US military [15]. In these times, MAMP also gained popularity among German and Japanese civilians.

In the 1950s and 1960s, MAMP as Obetrol become a popular medication diet pill. However, because of the extensively abuse of MAMP, The American government began strict control on the production and distribution of this drug. As a consequence, the S-enantiomer of MAMP that exhibits psychoactive properties start to be listed in schedule II of the United Nations 1971 Convention on Psychotropic Substances [10, 16]. From this time, MAMP is a strictly controlled drug [16]. The milestones in development of methylamphetamine are presented in Figure 2.

### *1.1.2. Characterization*

Methylamphetamine as with amphetamine is a simple synthetic derivative of β-phenylethylamine, which differs only in possessing two methyl group, one attached to the side chain and the second attached to the nitrogen atom. The chemical structure of these substances is presented in Figure 3.

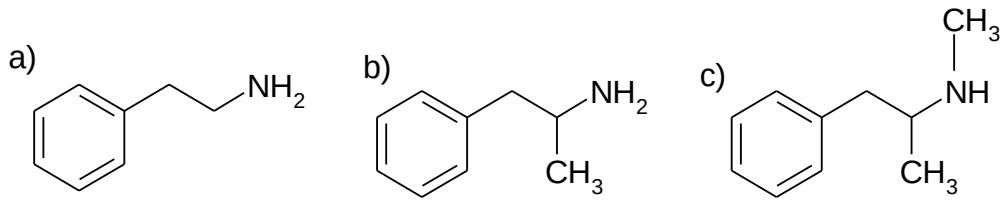



**Figure 3.** Chemical structures of: a) β-phenylethylamine, b) amphetamine, and c) methylamphetamine

Methylamphetamine is a hygroscopic liquid forming azeotrope with alkaline water. It darkens in the air due to absorbption of carbon dioxide and has a characteristic, fairly intense smell. Because of these properties, for commercial purposes, it is converted to salts, such as methylamphetamine hydrochloride [17]. In the laboratories MAMP is usually applied as a salt, since this form of amine storage ensures its low reactivity, and thus the relative stability. The hydrochloride salt of MAMP readily crystallizes by precipitation of hydrogen chloride in anhydrous diethyl ether to the free base of MAMP to form crystals. Street methamphetamine can be colorless, yellow, orange or brown [17]. It is usually sticky, with the consistency of

honey (due to its hygroscopicity and difficulty in crystallization). The properties of methylamphetamine hydrochloride are summarized in Table 1.

Methylamphetamine contains a chiral atom of carbon, and therefore it exist as two enantiomers, (S)-(+)-methylamphetamine and (R)-(-)-methylamphetamine or D-methylamphetamine or L-methylamphetamine, respectively. Although these optical isomers have identical chemical reactions, melting points as well as solubilities, they differ in their opposite rotations of plane-polarized light.

**Table 1.** Properties of methylamphetamine hydrochloride

| **Parameter** | **Description** |
|---|---|
| Appearance | Colorless, odorless, white powder |
| Molecular wejght | 129.4 |
| Chemical formula | $C_{10}H_{15}N \cdot HCl$ |
| Melting point | 173 $^{o}$C |
| Solubility | Soluble in water, methanol, ethanol, chloroform; insoluble in ether |
| Metabolism | Liver enzyme, glucuronidation, and N-demethylation |
| Average distribution time = max onset of action | 20 – 40 min |
| Average plasma-half-time = average duration of action | 7 - 34 h |
| Therapeutic dose | 3 - 9 mg |

As a result, the molecules fit chiral protein receptors differently and therefore have different biological activities [6, 17]. Biological systems are mainly chiral and this is the reason for the different reactions towards each component of an enantiomer pair. The (S)-(+)-MAMP is a highly addictive CNS stimulant that produces a euphoric high followed by restlessness, agitation, dysphoria, paranoia and in extreme cases, psychosis and is widely abused [18, 19]. It is also found in medication Desoxyn and is a metabolite of Didrex (benzphetamine). The (R)-(-)-MAMP lacks CNS activities and has a low abuse potential, but is an effective vasoconstrictor and is contained in the over-the-counter medications such as nasal inhaler (Vicks, Cincinnati). It is also a metabolite of the selegiline (e.g. Emsame, Eldepryl, Zelapar), medication used for the treatment of early-stage Parkinson's disease, depression and dementia [20]. The structure of the optical isomers of methylamphetamine are presented in Figure 4.

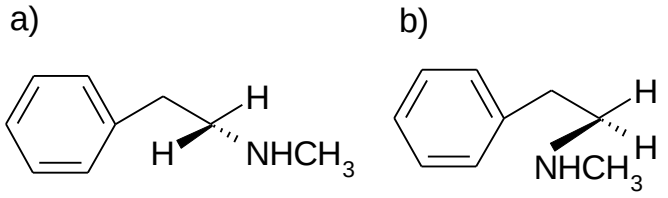



**Figure 4.** Chemical structure of: a) (S)-(+)-methylamphetamine, and b) (R)-(-)-methylamphetamine

### *1.1.3. Pharmacology*

Methylamphetamine is a synthetic substance that increases dopamine levels by stimulating presynaptic release of the neurotransmitter [17]. Higher level of dopamine has the effect of stimulating regions of the brain linked with vigilance and the action of the heart [17]. Therefore, the use of methylamphetamine affects neurochemical mechanisms responsible for regulating body temperature, heart rate, blood pressure, attention, appetite, mood as well as responses associated with alertness or alarm conditions [17]. Summary of the pharmacological effects of methylamphetamine consumption as well as summary of the different effects inducted by methylamphetamine are presented in Figure 5 and Table 2, respectively.

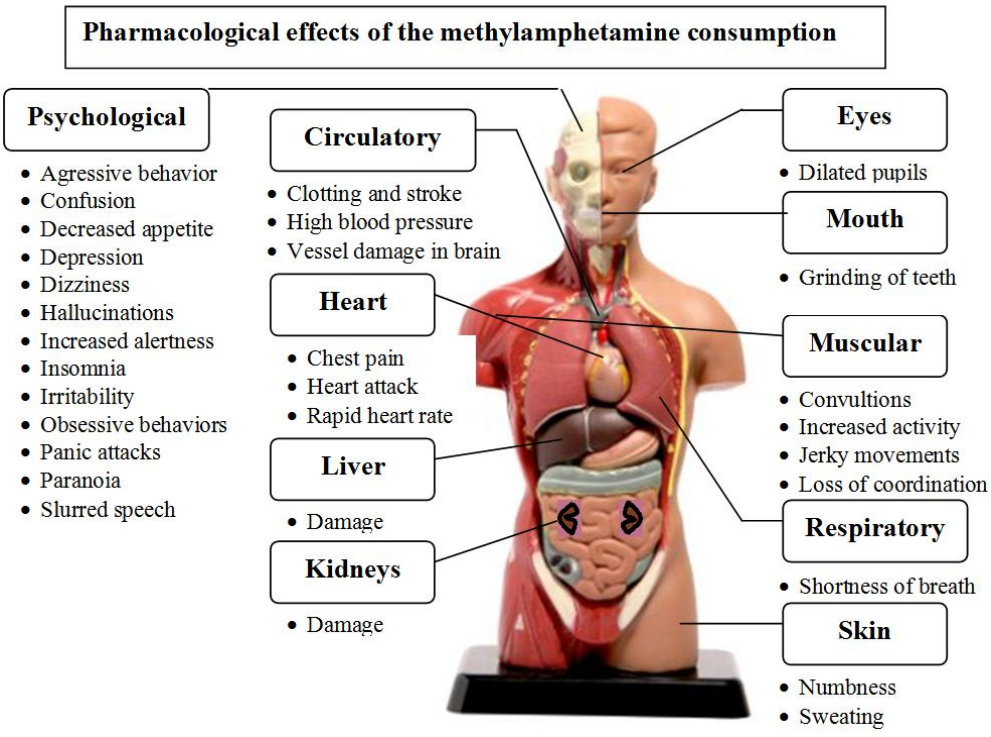



**Figure 5.** Pharmacological effects of methylamphetamine consumption

**Table 2.** Positive, negative and neutral effects of methylamphetamine abuse

| **Effects with habitual use** |
|---|
| • Anxiety reaction<br>• Deep or disturb sleep lasting up to 48 h<br>• Extreme hunger<br>• Fatal kidney and lung disorders<br>• Liver damage<br>• Lowered resistance to illnesses<br>• Permanent psychological problem<br>• Possible brain damage<br>• Psychotic reaction<br>• Stroke |
| **Positive effects** |
| • Physical stimulation<br>• Decreased need for sleep<br>• Euphoria<br>• Increased energy and alertness<br>• Increased sexuality |
| **Negative effects** |
| • Aggressiveness<br>• Anxiousness and nervousness<br>• Disturbed sleep patterns<br>• Excessive excitation, hyperactivity<br>• False sense of confidence and Power<br>• Involuntary body movements (uncontrollable movements and/or twitches of fingers, facial and body muscles, lip-smaking, tongue protrusion, grimacing, etc.)<br>• Itching, welts on skin<br>• Loss of appetite (anorexia), leading to poor nutrition and weight loss with heavy use<br>• Loss of interest in sex, over time<br>• Moodiness and irritability<br>• Nausea, vomiting, diarrhea<br>• Panic and paranoia<br>• Reduced enjoyment of eating<br>• Severe depression, suicidal tendencies<br>• Shortness of breath |
| **Neutral effects** |
| • Excessive talking<br>• Sweating<br>• Visual and auditory hallucinations<br>• Weight loss |

### *1.1.4. Methylamphetamine synthesis routes*

Clandestine laboratories have manufactured MAMP since the 1960s and possibly before, but the problem has become much more widespread in the last two decades, mainly

because of methamphetamine's growing popularity. There are several motives why MAMP has become so popular, but one of the main reason is that the synthetic routes of this drug are described not only in the literature, including 'underground literature', but also in the Internet. In addition, the synthetic routes are relatively simple and cheap processes, and the precursor chemicals are easy to obtain [21]. Moreover, the effects of MAMP last several hours which is longer than many other illicit drugs and therefore desirable for people whose abused drugs.

Although, MAMP is synthesized in clandestine laboratories by a variety of routes as is presented in Figure 6, globally, ephedrine (EP) and pseudoephedrine (PSEP) are the main precursors used for MAMP synthesis [21-24]. However, the substance can also be manufactured from 1-phenyl-2-propanone (P-2-P). In general, the price and, particularly, the availability of precursors greatly determines the choice of synthetic route used in clandestine manufacturing. The world legitimate trade of P-2-P is more restricted compared with ephedrine type precursors. Secondly, EP and PSEP are commonly obtained from medications, many of which are available over-the-counter. Another way to obtain these precursors is yeast fermentation of dextrose in the presence of benzaldehyde and this way is currently the most common fermentation. An alternative source of EP/PSEP is the naturally occurring primitive stalky plant *Ephedra sinica*. Thus, the most popular MAMP synthetic routes employ EP or PSEP as a precursor and the reaction are generally done by one of the following reduction mechanism:

- heterogeneous two-step catalytic reduction (Emde method) [25, 26],
- direct 'non metal' reduction (Nagai method) [25, 27],
- disolving metal reduction (Birch method) [27].

The information of the most common synthetic routes of methylamphetamine are described in Table 3.

One of the most widely applied illegal laboratory conversions of EP or PSEP to MAMP involve first converting these precursors to the chloro-analog by reaction with $SOCl_2$, $POCl_3$, $PCl_3$, or $PCl_5$. Then, the chloro-intermediate is hydrogenated over a palladium or platinum catalyst to yield MAMP [28].

Although, there are many prescriptions of MAMP via CEP/CPSEP synthesis that can be found in the literature [23, 29-31] including 'underground' sources and the internet, these recipes generally differ in terms of:

- the conditions of carrying out the synthesis which can be varied in a wide range of temperatures,

- time of synthesis;
- chemicals (including solvent) used,
- recrystallization used.

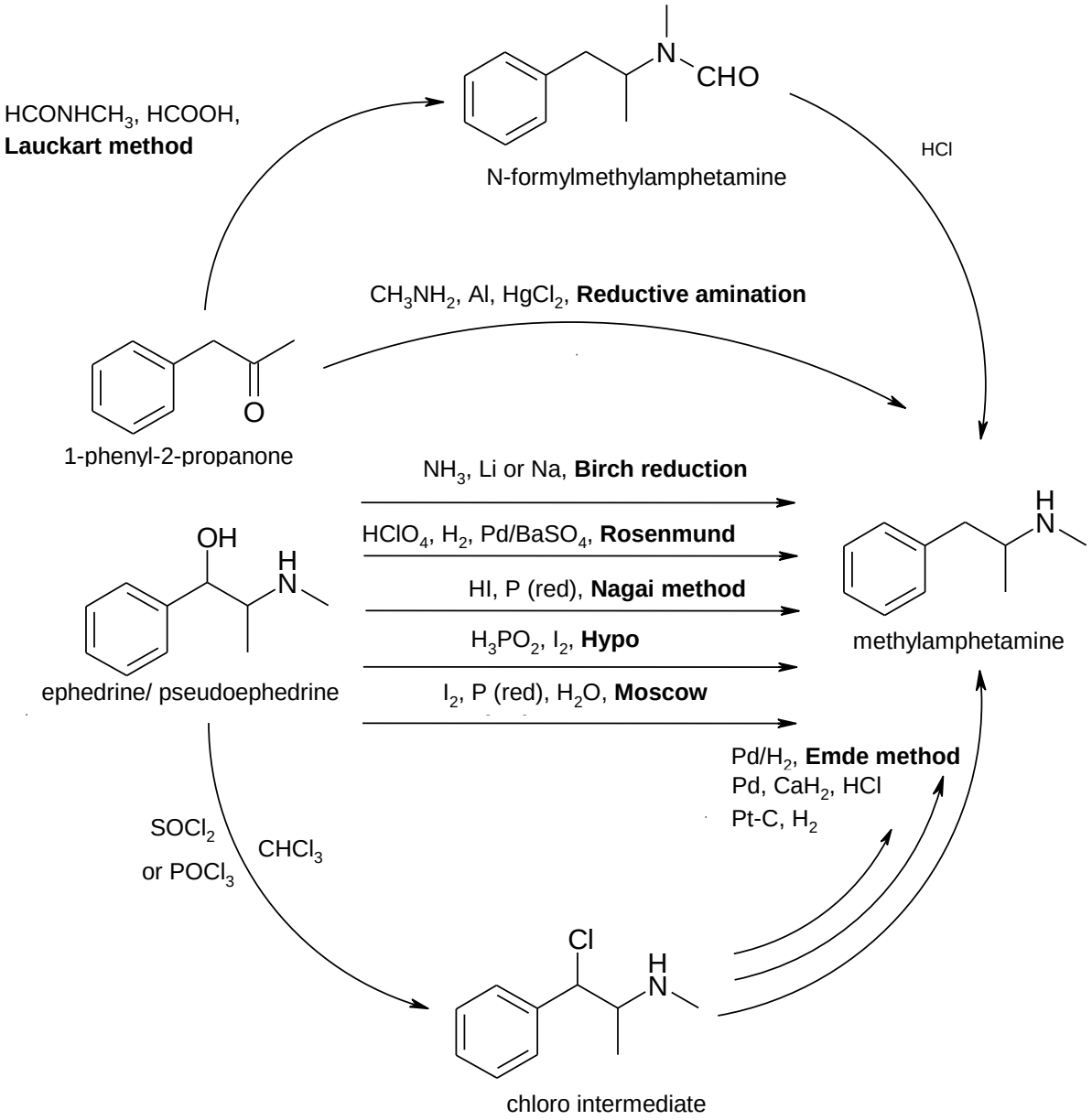



**Figure 6.** The most common methods used for illicit methylamphetamine manufacture

**Table 3.** Information on the most common synthetic routes of methylamphetamine

| Synthetic route | Precursor used | Mechanism used | Impurities occured | Notes | Ref. |
|---|---|---|---|---|---|
| Leuckart reaction; Leuckart-Wallch reaction | P-2-P and its analogs | Reductive alkylation of an amine with formic acid acting as the reducing agent. The resulting formylamphetamine is hydrolysed in aqueous acid to produce the MAMP. | α-Benzyl-N-methylphenethylamine, α, α'-dimethyldiphenethylamine, N- α, α'-trimethyldiphenylamine, N-acetylmethamphetamine | The reaction was first described by Leuckart in 1885, who used ammonium formate or formamide, and was then elaborated upon in 1893 by Wallach who used ammonium formate in the presence of excess formic acid. | [21] [32] [33] |
| Reductive amination | P-2-P and its analogs | Reaction involves the treatment of one of the P-2-P with a methylamine or ammonia to form a hemiaminal species that is then converted to an intermediate imine. Reducing agents such as sodium cyanoborohydride, sodium borohydride or aluminium in the presence of mercury chloride catalyst are used to reduce the imine to the MAMP. | 1-Phenyl-2-propanol, amphetamine, 1,3-diphenyl-2-methylaminopropane, N-cyanomethyl-N-methyl-1-phenyl-2-propylamine | Dissolving metal reduction using aluminum, zinc or magnesium amalgams is the one of most commonly used reductive amination method. | [21] [32] |
| Nagai, Hypo and Moscow methods | EP, PSEP | Non metal reduction the precursor to MAMP via iodoephedrine or iodopseudoephedrine. The Nagai method employs hydriodic acid and red phosphorus, whereas the others generate hydriodic acid in situ using iodine and red phosphorus (Moscow method or iodine and either hypophosphorous acid or phosphoric acid (Hypo method). | (2E)-N-Methyl-3-phenyl-N-(1-phenylpropan-2-yl)prop-2-enamide, iodoephedrine, N-methyl-N-(α-methylphenyl)amino-1-phenyl-2-propanone, (Z)-N-methyl-N-(α-methylphenylethyl)-3-phenylpropanamide, cis-3-phenyl-2-methylaziridines, trans-3-phenyl-2-methylaziridines | Routes most commonly used in Asia and the USA. | [21] [34] [35] [36] |
| Birch reduction | EP, PSEP | Proceeds via a dissolving metal (sodium or lithium) reduction of EP or PSEP in the presence of anhydrous ammonia. | 1-(1,4-Cyclohexadienyl)-2-methylaminopropane | Metallic lithium is more commonly used in place of metallic sodium as the former can be easily extracted from lithium batteries while the latter is more difficult to source and is more dangerous. | [21] [37] |
| Emde method | EP, PSEP | A two-step reaction in which EP/PSEP is reacted with thionyl chloride to produce the chloro-substituted intermediate, followed by a hydrogenation reaction in the presence of a catalyst. | Chloroephedrine/chloropseudoephedrine, methylephedrine, N-formylephedrine, N-acetylephedrine, N,O-diacetylephedrine, N-acetylamphetamine, cis-3-phenyl-2-methylaziridines, trans-3-phenyl-2-methylaziridines | In place of thionyl chloride other chlorinating agents such as phosphorus pentachloride, phosphorus oxychloride or phosphorus trichloride can be used. | [21] [29] |

Taking into account these differences, the final products may have different composition. It is reported that MAMP manufactured using the Emde route of synthesis contains the following by-products (compounds that arise from reactions that complete with the formation of the desired product) [23, 26, 32]:

- ephedrine or pseudoephedrine (precursors used),
- chloroephedrine (CEP) or chloropseudoephedrine (CPSEP) (intermediates occurring during synthesis),
- methylephedrine,
- N-formylephedrine,
- N-acetylephedrine,
- N,O-diacetylephedrine,
- N-acetylamphetamine,
- cis- and trans-3-phenyl-2-methylaziridines.

These by-products are largely dependent on inefficient purification procedures. The quality of the final product may also be affected by such factors as precursors, solvents and other reagents used, contamination from the laboratory, and the stage of wrapping and packing the final product. The knowledge of these specific contaminants occurring in the final product is of particular importance because they can facilitate identification of the synthetic route, origin of precursors and may suggest information as to the location of manufacture of these illicit drugs. Contaminant profiling can provide vital intelligence for investigations in which linking seizures or identifying the synthetic pathway is essential [32].

### *1.1.5. Importance of stereochemistry of the Emde route of methylamphetamine synthesis*

It is reported that (S)-(+)-MAMP has the same configuration of chiral carbon as (1R,2S)-(-)-EP and (1S,2S)-(+)-PSEP and their derivatives so these compounds can be used as starting material to manufacture of the abused drug [28, 30]. This argument also applies to (R)-(-)-MAMP which can be synthesized from (1S,2R)-(+)-EP and (1R,2R)-(-)-PSEP their derivatives [28, 30]. As was previously mentioned, in the Emde method ephedrine or pseudoephedrine are halogenated and then hydrogenated. The important question is how hydroxyl group in the precursor can be replaced by chloride in the first step of Emde route?

There are three possible mechanism that can be proposed for the reaction of alcohol with thionyl chloride [28]. First of all, the reaction of alcohols with thionyl chloride proceeds via an internal nucleophilic substitution ($S_Ni$, Figure 7a) to yield the corresponding alkyl

chlorides. This method involves a two-step and is known as Darzan's process. Firstly, dissociation of the chlorosulfite into an ion pair take place and thereafter immediate attack by chloride on the carbocation from the front side. Therefore, $S_Ni$ results in final products which retain configuration of the precursor used for the synthesis [24, 28].

A second proposed mechanism is also two-step reaction. Firstly, thionyl chloride attacks oxygen and nitrogen and freeing chloride ion into solution. This free ion attacks the carbon in bimolecular nucleophilic substitution ($S_N2$) mode, resulting in cleavage of the C-O bond with inversion of configuration (Figure 7b) [24, 28].

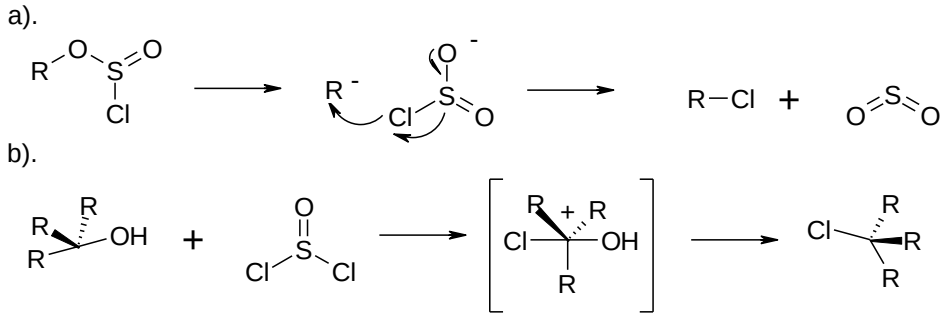



**Figure 7.** Reaction of alcohol with thionyl chloride: a). $S_Ni$ mechanism; b). $S_N2$ mechanism

The last mechanism is known as neighboring group mechanism. Here, two successive $S_N2$ reactions, each with inversion of configuration, take place. Firstly, nitrogen attacks and forces out the leaving group, while staying in its own position in molecule and in this way first $S_N2$ occurs. The second inversion of configuration occurs after attack of chloride [24, 28]. The final stereochemistry is retention.

Therefore, four different stereoisomers of chloro-derivatives of methylamphetamine could be expected when ephedrine and pseudoephedrine enantiomers are used for the synthesis by Emde route.

It is reported, that the stereochemical relationship of (S)-(+)-MAMP to its initial precursor (1R, 2S)-(-)-ephedrine or (1S, 2S)-(+)-pseudoephedrine is achieved by detection of (1S, 2S)-(+)-chloropseudoephedrine or cis-1,2-dimethyl-3-phenylaziridine, and (1R, 2S)-(-)-chloroephedrine or trans-1,2-dimethyl-3-phenylaziridine, respectively [26]. Because enantiomeric ratio of MAMP is closely related with the optical activity of starting materials used for the production of drug as well as the intermediates and impurities occurred during synthesis, this knowledge can provide useful information concerning the origins and synthetic methods used for clandestine manufacture [38]. Thus, knowledge of enantiomeric

composition of MAMP synthesized by Emde is important and may be a useful in investigations of manufacturing sources, providing information for sentencing guidance for certain drug-related offences, and determining whether the drug of concern is derived from a controlled substance. Identification of the enantiomeric composition in MAMP samples may help identify the drug's precursor material and supply valuable information about the conditions and the chemicals used in the clandestine laboratories, which can provide information about the original source of the sample [39]. Moreover, the detection of a racemic mixture of methamphetamine may indicate illicit use; the same observation can also derive the from of prescription use of a racemic precursor molecule such as furfenorex or legitimate use of (S)-(+)-MAMP along with the concurrent use of (R)-(-)-MAMP (Vicks InhalerR) or deprenyl [6].

Because the U.S. Food and Drug Administration, in 1992, issued a guideline that for chiral pharmaceuticals only its therapeutically active isomer be brought to market, and that each enantiomer of the drug should be studied separately for its pharmacological and metabolic pathways, therefore the enantiomeric composition of drugs is also important in pharmaceutical industry [40]. In addition, a rigorous justification is required for market approval of a racemate of chiral drugs. Presently, a majority of commercially available drugs, e.g. methylamphetamine, are both synthetic and chiral. Nevertheless, to avoid the possible undesirable effects of a chiral drug, it is imperative that only the pure, therapeutically active form be prepared and marketed. Hence there is a great need to develop the technology for analysis and separation of racemic drugs [40].

### *1.1.6. Methylamphetamine – necessary to control*

According to a new report published by EMCDDA (European Monitoring Centre for Drugs and Drug Addition), the number of people who abuse MAMP is increasing every year [10]. Although historically, the use of MAMP has been confined largely to the Slovakia and Czech Republic, recent signs of the spread of MAMP linked to different European countries have sparked further investigation of this topic [41].

In 2011, the UNODC received 350 reports of dismantled methamphetamine production sites from European countries, most of these reported by the Czech Republic. Europol has identified two main producer regions: one in central Europe, focused around the Czech Republic and neighbouring countries, Slovakia and Germany, and the other in the Baltic area. Manufacturing of MAMP in the Czech Republic is mainly performed in small-

scale so-called kitchen laboratories and the drug is principally distributed within the country. Another situation is in Lithuania where production tends to take place in medium-sized laboratories, which produce the drug 'on demand', and therefore, the drug is exported to Nordic countries and the United Kingdom [10, 41]. Recent data on dismantled production sites indicate that MAMP has also been produced on a small scale in other parts of Europe, including Belgium, Bulgaria, Greece, Hungary, the Netherlands, Poland, Serbia and the United Kingdom [41].

In this situation the following information is important:

- properties of MAMP and its metabolites,
- method of MAMP synthesis,
- analytical procedures to determine purity of drug as well as impurity and chirality profiling,
- detection and identification of MAMP enantiomers, its metabolites and impurities in biological samples collected from the people who abuse this drug.

Therefore background knowledge of MAMP is significant for many reasons with the most important being associated with human health in terms of prediction and protection applied to reduction of drug addiction. To gain this knowledge, the methodology for determination of MAMP and its metabolites, both, in the material collected by police and customs, as well as in the biological samples derived from people who take this drug are of great importance [42]. Many analytical methodologies used for the detection and determination of MAMP in a properly prepared samples of the collected materials are known. However, the most commonly used for the final determination are chromatographic, spectroscopic and immunological techniques.

## 2. Gas and Liquid Chromatography used for the enantioseparation of methylamphetamine

Differences in toxicological, pharmacokinetic and therapeutic properties of the enantiomer pharmaceuticals provide a strong case for the development of optically active drug separation techniques. Due to differences in affinity to the receptor, absorption, distribution and protein binding of enantiomers, the development of analytical powerful techniques to chiral separations, quantifying minor pollutants in chiral drugs, and preparing enantiomerically pure drugs has been possible [43].

Resolution and determination of enantiomers of methylamphetamine and its analogs can be approached using a variety of analytical techniques (Figure 8). However, the most

commonly used by analytical scientists are gas and liquid chromatography (GC, LC) as well as capillary electrophoresis (CE) and supercritical fluid chromatography (SFC) coupled with various detection devices (mass spectrometry (MS), ultraviolet-visible variable wavelength detector (UVD), flame-ionization detector (FID), fluorescence detector (FLD) and diode-array detector (DAD) [6, 7, 43, 44]. Enantioseparation of MAMP using these techniques is mainly performed by using a variety of chiral stationary phases (CSPs) as well as chiral derivatizing reagents. The derivatization affords greater selectivity and sensitivity in enantiomeric separation [45].

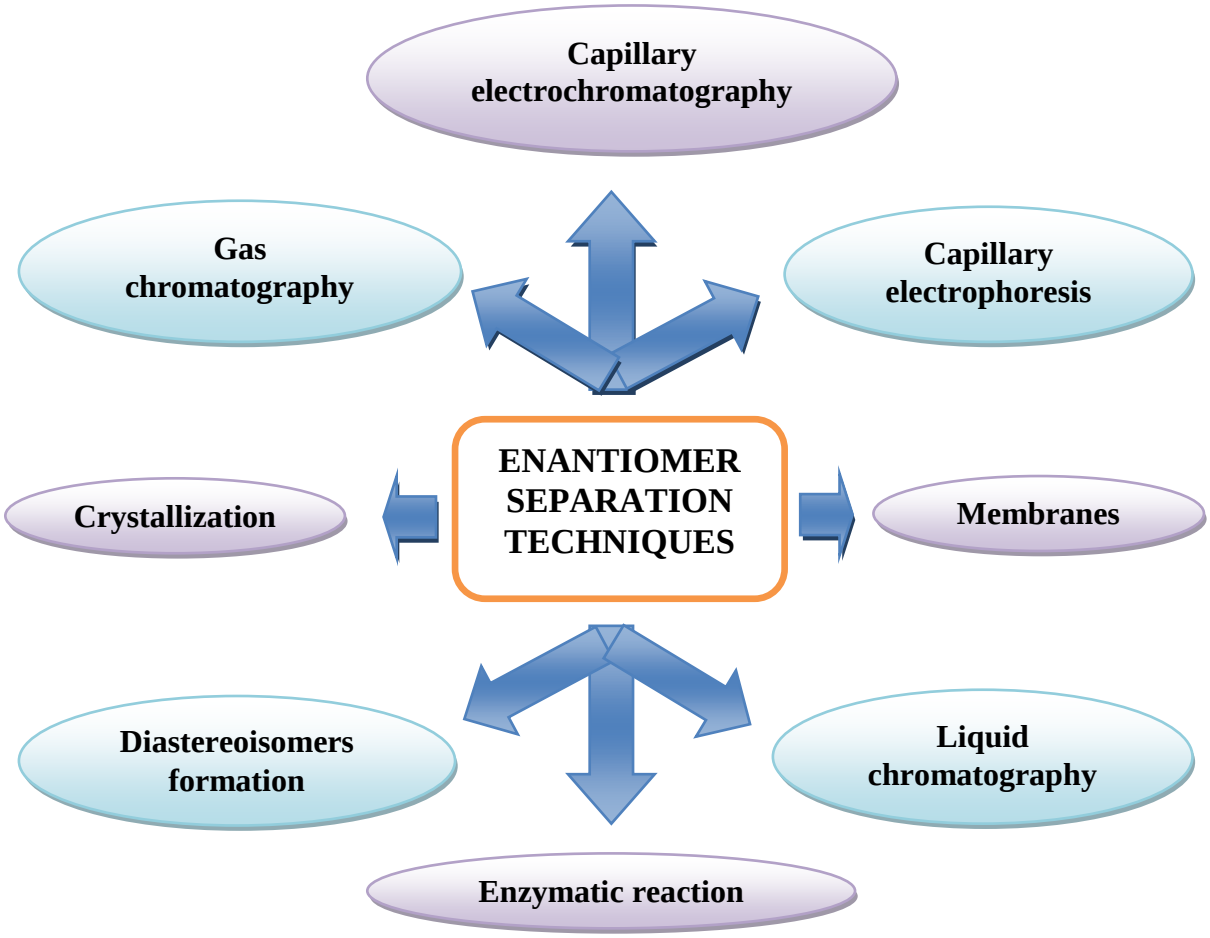



**Figure 8.** Enantioseparation techniques

### 2.1. Gas chromatography as a technique of choice for MAMP enantioseparation

Among the current analytical methods for chiral analysis, one of the most popular used for enantioseparation of MAMP is GC in combination with MS detection. Chiral separations of MAMP can be achieved with GC technique through the following approaches [6]:

- indirect separation of diastereoisomers, formed by the reaction of the analyte of interest with an enantiomerically pure chiral selector (CS), using achiral stationary phases (the diastereomers formed have different physico-chemical properties and are therefore resolvable);
- direct separation of racemates to their corresponding enantiomers using CSPs; or
- separation of chiral derivatives, formed by the reaction with non-chiral derivatizing reagents, using CSPs.

Before analysis, an appropriate sample preparation is an important precondition for analysis by chromatographic techniques. It is obvious that highly pure reagents as well gentle sample treatment are required.

### *2.1.1. Indirect separation of MAMP diastereomers using CSs and achiral stationary phase*

In recent years, indirect separation of MAMP diastereomers, formed by the reaction with appropriate, enantiomerically pure chiral selector, with using achiral stationary phase has been one of the most popular approaches for the enantiomeric analysis of MAMP followed by GC separation [46]. During the derivatization reaction, specific functional group coming from derivatizing chiral agent is introduced to analyte of interest, to form diastereomers of each enantiomeric analyte [47]. Therefore, the diastereoisomers having different physical and chemical properties can be separated from each other by an achiral chromatographic method [47]. Chiral derivatization brings not only possibility to create diastereomers that can be separated by GC but also brings the retention time of the determined analytes to a more desirable range [47]. Numerous different derivatizing reagents and procedures for MAMP existed with the most common used for MAMP are described below.

One of the most commonly chiral derivatizing reagent used for MAMP is N-trifluoroacetyl-l-prolyl chloride (l-TPC, Figure 9a). This reagent couples with MAMP to form corresponding diastereomers which can be separated on GC columns as it increases the sample volatility. This agent brings many advantages including effectiveness, providing good resolution, and commercial availability [48]. On the other hand, as is true of other chiral derivatizations, it contains enantiomeric impurity that increases error on analysis [6]. Many articles describe derivatization with 1-TPC and present satisfactory results. For example, Hensley et al [49] describes a procedure for amphetamine type compounds derivatization with l-TPC and determination of these derivatives in urine. In another research, Wang et al. [50] coupled derivatization of MAMP using l-TPC with liquid-liquid extraction (LLE). Table 4 summarizes many others of these studies.

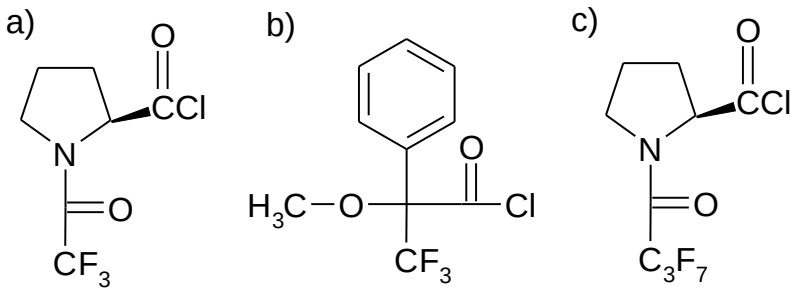



**Figure 9.** Structures of the common chiral derivatizing reagents used for the amphetamine compounds: a) l-TPC; b) MTPA; c) l-HPC

The other popular chiral reagent used for MAMP derivatization is *(l)*- and *(d)*-α–methoxy-α-(trifluoromethyl)phenylacetyl chloride (l-MTPA and d-MTPA, Fig. 9b), also known as Mosher's acid. In the reaction the hydroxyl group on the MTPA molecule is lost in the derivative formation [48]. This chiral agent can be applied to different types of matrices, from biological material (urine, blood and human plasma), environmental samples (water, air), to pharmaceuticals (deprenyl medication). D-α-methoxy-α-(trifluoromethyl)phenyl acetic acid (d-MTPAA) is a slightly modified version of d-MTPA agent and it was used for enantiomeric determination of MAMP and its precursors (EP, PSEP) by LeBelle et al. [51] with satisfactory results.

A similar derivatizing reagent (S)-(-)-N-(heptafluorobutyryl)prolyl chloride (l-HPC, Figure 9c) is also well suited to chiral derivatization of MAMP. A simple procedure is used to obtain this agent (mixing (S)-(-)-proline with heptafluorobutyric anhydride in aqueous alkaline conditions.

Other commonly used reagents for chiral derivatization of MAMP are 4-carbethoxyhexafluorobutyryl chloride (CB), (2S, 4R)-N-heptafluorobutyryl-4-heptafluorobutyloxy-prolyl chloride (l-HFBOPCl) and N-methyl-bis(trifluoroacetamide) (MBTFA). Information on chiral derivatization and GC procedures for enantiomeric analysis of MAMP and related compounds are given in Table 4.

**Table 4.** Information on chiral derivatization and GC procedures for enantiomeric analysis of MAMP and related compounds

| Analyzed compound | Matrice | Derivatizing reagent | Stationary phase | Detection | Ref. |
|---|---|---|---|---|---|
| MAMP, AM | U | l-TPC | HP-5MS 30 m x 0.20 mm x 0.33 µm (Hewlett-Packard) | EI-MS | [50] |
| AM, MAMP, MDA, MDMA, MDEA | B | l-MTPA | HP-5MS 30 m x 0.25 mm x 0.25 µm (Hewlett-Packard) | EI-MS | [52] |
| MAMP, AM | U | l-TPC | HP-5MS 25 m x 0.20 mm x 0.25 µm (Hewlett-Packard) | MS | [53] |
| AM, MAMP, MDA, MDMA, MDEA, MBDB | H | l-TPC | 5 % phenyl-methylsilicone capillary column<br>17 m x 0.20 mm x 0.33 µm<br>(G&W Scientyfic Agilent Technologies) | MS | [54] |
| AM, MAMP, NEP, EP, PEP, MDA, MDEA, MBDB | U | l-HPC | DB-5 fused-silica capillary column<br>15 m x 100 µm x 0.1 µm (Agilent Technologies) | MS | [55] |
| MAMP, AM | U | l-TPC, l-HPC | Rtx-5 capillary column 30 m x 0.25 mm x 0.25 µm (Restec) | EI-MS | [56] |
| AM, MAMP, MDA, MDMA, MDEA | H | l-HFPOPCl | HP-5MS crosslinked 5 % phenylmethylpolysiloxane<br>30 m x 0.25 mm x 0.25 µm (Hewlett-Packard) | MS-NCI | [57] |
| MAMP, AM | S | MBTFA + 1% TBDMCS<br>BSTFA + 1% TMCS | HP-5MS fused-silica capillary column<br>12 m x 0.20 mm x 0.33 µm (Hewlett-Packard) | PCI-MS | [58] |
| MAMP | S | l-TPC | Rtx-1 100 % dimethyl polysiloxane<br>30 m x 0.25 mm x 0.25 µm (Restec) | EI-MS | [59] |
| MAMP | S | l-TPC | Rtx-1 100 % dimethyl polysiloxane<br>15 m x 0.32 mm x 50 µm (Restec) | IRD | [59] |
| MAMP | S | l-TPC | Rtx-1 100 % dimethyl polysiloxane<br>2.5 m x 0.32 mm x 1.05 µm (Restec) | IRD | [59] |
| AM, MAM, HMA, HAM, NEP, EP | H, U | TFA | β-Dex 225 30 m x 0.25 mm x 0.25 µm<br>(β-CD stationary phase; Supelco/Sigma Aldrich Co.) | SIM-MS | [60] |
| MAMP, EP, PSEP | S | TFAA | Chiraldex GP-N (γ-cyclodextrin; Supelco/Sigma Aldrich) | SIM-MS | [61] |
| AM, MAMP | S | l-TPC | 2,6-di-O-pentyl-3-O-trifluoroacetyl-γ-CD<br>2,6-di-O-pentyl-3-O-propionyl-γ-CD<br>2,6-di-O-pentyl-3-O-trifluoroacetyl-β-CD<br>20 m or 30 m x 0.25 mm | FID | [46] |
| AM, MAMP, selegiline | U, P | PFP | Chiralsil Dex-CB 25 m x 0.25 mm (Chrompack) | FID, MS | [62] |

### *2.1.2. Direct separation of MAMP using CSPs*

Direct separation of racemates to their corresponding enantiomers using CSPs is the next commonly used approach for the enantiomeric analysis of MAMP followed by GC. The direct approach utilizes a non-racemic CSP as a selector, which forms transient diastereomeric intermediates with the chiral selectand, whereby the interaction occurs both rapidly, via fast kinetics, and reversibly, via distinct thermodynamics [59].

Numerous CSPs have been developed and optimized for enantioseparation of MAMP by using gas chromatography coupled to the appropriate detector, mainly mass spectrometer. Direct separation mode brings many advantages. First of all, it is not necessary to form diastereomeric derivatives. Moreover, it is a simple and effective method. However, in many cases it is necessary to used achiral derivatization to improve the chromatographic separation. In addition, commercially available chiral column are very expensive in comparison with normal phases.

Three principles are thoroughly investigated to carry out separation of enantiomers by GC using CSPs, as follows [59]:

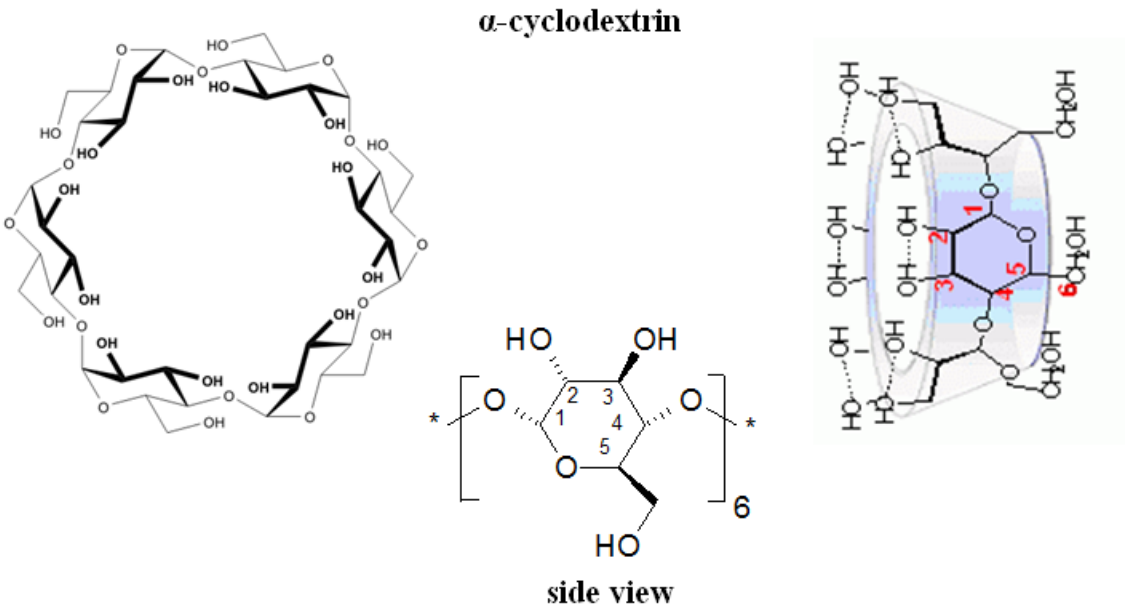
- chiral separation on non-racemic chiral metal coordination compounds via complexation;
- chiral separation on non-racemic chiral amino acid derivatives via hydrogen-bonding; and
- chiral separation on biogenic cyclodextrin derivatives via (*inter alia*) inclusion.

Cyclodextrins (CDs) (Figure 10) are the CSPs most often used in the separation of MAMP enantiomers as well as related compounds. CDs make up a family of homochiral, nonionic, cyclic oligosaccharides composed of six to twelve D-(+)-glucopyranose units connected through α-1, 4-linkages. They have ability to form inclusion complexes with different staility constants for diastereomers. The most commonly used α-, β- and γ-CDs contain six, seven or eight glucopyranose units, respectively.

Derivatized CDs were introduced in enantioselective capillary GC in 1987 [63]. Since this time they have begun to play an important role in chiral separation of different kind of compounds including amphetamine type stimulants. In order to coat capillary columns, the stationary phase must be fluidic. They are two developed approaches:

- dissolution of peralkylated CDs in a moderately polar polysiloxane [64, 65],

- application of low-melting point CD derivatives containing n-pentyl groups as undiluted liquid stationary phases coated on Pyrex glass capillary columns and fused silica capillary column [66].




| Information on α-, β-, and γ-CDs | | | |
|---|---|---|---|
| Isomer | Glucoses unit | Inside diameter [Å] | Cavity volume [Å$^3$] |
| α | 6 | 5 | 174 |
| β | 7 | 6.2 | 262 |
| γ | 8 | 7.9 | 427 |

**Figure 10.** Structure of α-cyclodextrin and information on α-, β-, and γ-CDs

The use of derivatized α-, β, and γ-CD when dissolved in polysiloxanes bears a numbers of merits [63]:

- the unique physicoselectivity of polysiloxanes in maintained and combined with the inherent chemoselectivity of CDs;
- the universal coating properties of polysiloxanes are used for producing high resolution, high efficiency and thermally stable capillary columns by GC;
- high melting points or phase transitions of derivatised CDs are not determental to column performance and can be disregarded;
- multicomponent CD-based stationary phases can be employed.

Numerous capillary columns coated with derivatised CDs are commercially available and extensively used in MAMP enantioresolution, however the most popular are named

Chiraldex (the trademark for permethylated-2-hydroxypropyl- and trifluoroacetyl-diphenyl α-, β, and γ-CDs produced by Astec). In Table 4 a summary of some information on CDs as CSPs and their applications that have been developed and studied to date.

*2.1.2.1 Separation of MAMP enantiomers using non-chiral derivatizing reagents and CSPs*

Although, enantioseparation of MAMP using CSPs is very advantageous, it also has some drawbacks among which the biggest is need for derivatization process. This additional step is an undesirable process by analytical chemist, due to the fact that it constitutes a further stage of preparing the sample for analysis, which may affect the loss of the analytes and the introduction of additional impurities. Moreover, derivatization process extends the entire procedure. An additional challenge is to carry out derivatization process in accordance with the principles of green chemistry [67] and green analytical chemistry [68, 69], which arise directly from the principles of sustainable development. However, derivatization of MAMP is required to increase volatility, speed of analysis and good peak shapes, as well as to provide suitable functions for detection, hyphenation and improved chirality recognition. The derivatization strategy should also assist the simultaneous enantioseparation of MAMP without extensive peak overlapping.

When cyclodextrins are used as CSP, many derivatizing reagent can be chosen for MAMP chemical conversion into its derivatives, however the most popular are trifluoroacetic acid (TFA), pentafluoropropionic acid (PFP) and their anhydrides (Table 4).

## 2.2. Liquid chromatography as a technique of choice for MAMP enantioseparation

Another analytical separation technique that is extensively used for the separation of MAMP enantiomers is high performance liquid chromatography (HPLC). Three ways to achieve enantioseparation of MAMP by HPLC are known [70]:

- using a chiral derivatizing reagents and non-chiral HPLC system;
- using a chiral selector immobilized on the stationary phase or
- using a chiral derivatizing reagent as an additive to the mobile phase.

### *2.2.1. Indirect separation of MAMP diastereomers using CSs and achiral stationary phase*

Because MAMP is mainly insensitive to common HPLC detectors such as fluorimetric or UV, a derivatization process with a detector-sensitive reagent is necessary, especially for

the trace level analysis of MAMP. Chemical derivatization is also proposed as a stereospecific method for enantiomeric analysis.

In the derivatization reaction, appropriate chiral agent reacts with each MAMP enantiomer to create two corresponding diastereomers that have different physicochemical properties such as stability and stereochemistry and these differences are used in enantioseparation. The four methods based on the formation of diastereomeric derivatives are known [70]:

- homogeneous-solution derivatization;
- derivatization with solid-phase reagents;
- solid-support-assisted derivatization methods, and
- on-column derivatization methods.

Many derivatizations, based on the use of *pre-column* derivatization with a detector-sensitive chiral reagent, can react with the amino group of methylamphetamine, are known however the most often used are [6]:

- Marfey's reagent (Figure 11a)
- chiral chloroformates (l-(9-fluorenyl)ethyl chloroformate (l-FLEC, Figure 11b); 9-fluorenylmethyl chloroformate-L-proline (FMOC-L-Pro, Figure 11c));
- O-phthalaldehyde and its derivatives (OPA, Figure 11d; O-phthalaldehyde-N-acetyl-L-cysteine, OPA-NAC, Figure 11e);
- (1S,2S)-N-[(2-isothiocyanato)-cyclohexyl)] pivalinoyl amide ((S,S)-PDITC, Figure 11 f);
- 2,3,4,6-tetra-O-acetyl-b-D-glucopyranosyl isothiocyanate (GITC, Figure 11g); and,
- 2,3,4-tri-O-acetyl-a-D-arabinopyranosyl isothiocyanate (AITC, Figure 11h).

Pre-column derivatization methods that used before mentioned derivatizing reagents provide a good solution for the enantiomeric analysis of MAMP due to sensitivity and high level of specificity of chromatographic resolution . Moreover, the range of limit of detections (LODs) is typically 50–100 ng/mL, which is a big advantage. However, these methods also have some disadvantages including laborious handling of the sample, the need for multiple liquid-liquid extraction process (to eliminate the excess of reagents or to isolate the analyte from the sample matrix), time consuming step. Recently, several attempts were made to simplify the analytical procedures [6].

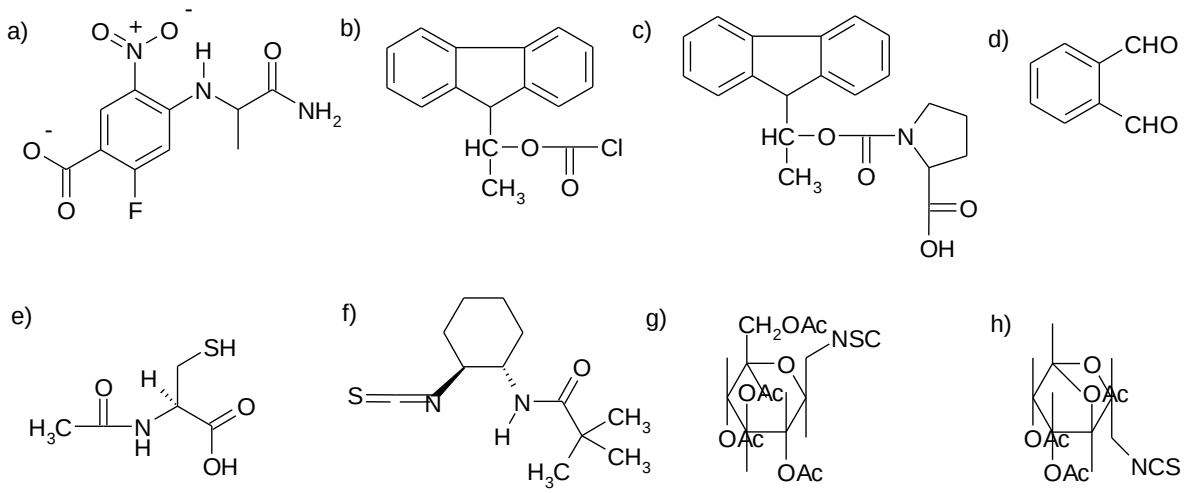



**Figure 11.** Chemical structures of derivatizing agents can be used to the formation of amphetamine diastereomeric derivatives: a) FDAA; b) FLEC; c) FMOC-l-Pro; d) OPA; e) NAC; f) PDITC; g) GITC; and, h) AITC

One of the suggested methodology for simplifying derivatizations of MAMP involves the use of solid-phase reagents [6, 70]. This method is based on the immobilization of an appropriate reagent on a solid support. Thereafter, the resulting product can be used as a packing for a trapping column or for a solid phase extraction (SPE) cartridge. Next, it can be integrated into the chromatographic system. The solid-phase reagent effects size exclusion, while the sample is passed through, enabling derivatization of small molecules. It need to be remembered that chemical conversion of large molecules is hindered. Afterwards, analyte derivatives are released by flushing with the correct solvent. Next, the derivatized analytes of interest are collected for chromatography. In the case when solid-phase reagent has been integrated into the chromatographic system, analyte derivatives are transferred to the analytical column for separation [6, 70]. A solid support can be used incorporating such materials as alumina, silica or polymer. This type of derivatization brings many advantages including sample clean-up, simply execution, unnecessary elimination of the excess of derivatizing reagent, and reduction of derivatizing reagent consumption. Moreover, after derivatization with the solid-phase reagent, the resulting chromatograms are improved, as peaks corresponding to the unreacted reagent are avoided [6, 70]. The utility of this methodology has been demonstrated for a variety of reagents, including OPA and FMOC-L-Pro.

Despite so many advantages, this methodology has not been widely accepted. This is mainly due to the need for synthesis of solid-phase reagents because they are not commercially available. Moreover, synthesized solid-phase reagents present limited stability.

**Table 5.** The formation of diaestereomeric derivatives and HPLC procedures for enantiomeric analysis of MAMP and related compounds

| Compound analyzed | Matrice | Derivatizing reagent | Stationary phase | Mobile phase | Detection | Ref. |
|---|---|---|---|---|---|---|
| **Derivatization mode-homogeneous solution** | | | | | | |
| AM, MAMP | U | Marfey's reagent | C18 (Alltech Associates) | $H_2O$ /MOH | UV (340 nm) | [71] |
| AM, MAMP | U | FLEC | C18 (Alltech Associates) | $H_2O$ / MOH | FLD | [71] |
| AM, MAMP | Saq | PDITC, GITC | Hypersil ODS 125 mm x 4 mm x 5 µm | ACN/ammonium 45/55 v/v | UV | [72] |
| AM, MDA, MDEA | S | AITC | Acquity C18 BEH 50 mm x 2.1 mm x 1.7 µm | 55/45 v/v MOH / $H_2O$ | UV (254 nm) | [73] |
| AM, MAMP, EP, PSEP, MDA, MDMA | U | FLEC | LiChrospher 100 RP C18 125 mm x 4 mm x 5 µm (Merck) | ACN + Ac.B; MOH + Ph.B; MOH + Ac.B | FLD, DAD-UV | [74] |
| AM, EP, PSEP | T | FMOC-L-Pro | LiChrospher 100 RP C18 125 mm x 4 mm x 5 µm (Merck); Hypersil ODS-C18 1 125 mm x 4 mm x 5 µm (Merck) | ACN / $H_2O$ 60/40 v/v | FLD, DAD-UV | [75] |
| **Derivatization mode-solid-phase reagents** | | | | | | |
| AM | B | FMOC-L-Pro | C18 (Alltech Associates) | ACN / $H_2O$ | FLD | [76] |
| **Derivatization mode-solid-support assisted derivatization** | | | | | | |
| AM, MAMP, MDA | Saq | SPME coated with CW-TPR, 50 µm (Supelco) + FMOC | LiChrosper 100 RP18 125 mm x 4 mm x 50 µm (Merck); precolumn: Hypersil C18 (Hewlett-Packard) 20 mm x 2.1 mm x 30 µm | ACN / $H_2O$ | FLD | [77] |
| AM, NEP, MDA | Saq, U | SPME coated with CW-TPR, 50 µm(Supelco) + OPA-NAC | LiChrosper 100 RP18 125 mm x 4 mm x 50 µm (Merck); precolumn: Hypersil C18 20 mm x 2.1 mm x 30 µm (Hewlett-Packard) | Ac.B/MOH/ACN 48/48/6 v/v | FLD | [78] |
| **Derivatization mode-on-column derivatization** | | | | | | |
| AM, MAMP, EP, PSEP, NEP | U | FMOC | LiChrosper 100 RP18 125 mm x 4 mm x 50 µm (Merck); precolumn: Hypersil ODS-C18 20 mm x 2.1 mm x 30 µm (Hewlett-Packard) | ACN / $H_2O$ | FLD | [79] |
| AM | U, Ph | OPA-NAC | LiChrospher 100 RP18 125 mm x 4 mm x 50 µm (Merck); precolumn: Hypersil ODS-C18 20 mm x 2.1 mm x 30 µm (Hewlett-Packard) | Ac.B/MOH/ACN 46/48/6 v/v | FLD | [80] |

Another method used to integrate sample clean-up, enrichment and derivatization of analytes is solid-support-assisted derivatization. This methodology is also used in chiral derivatization. Several reagents are applied for MAMP enantiomers however the most often used is OPA-NAC. This concept can be extended to solid-phase microextraction (SPME) with on-fiber derivatization, which provides simplicity and reduces solvent consumption.

Another way of creating diastereomeric derivatives is on-column derivatization. In these cases, derivatization is carried out by incorporating the reagent into the mobile phase, which greatly simplifies it, as no off-line manipulation is required [6, 70].

Information on the modes of the formation of diastereomeric derivatives and HPLC procedures for enantioseparation of MAMP and related compounds are provided in Table 5.

### *2.2.2. Direct separation of MAMP using CSPs*

Resolution of MAMP enantiomers is often carried out using HPLC CSPs. However, this methodology brings some difficulties associated with the synthesis and functioning of CSPs for HPLC. The synthesis mechanism of CSP is as follows:

- immobilization of one enantiomer of chiral molecule on the surface of a solid support,
- selective retention of the enantiomers of analytes by immobilized selector during the elution process.

Because the resulting diastereomers have different stabilities, this is an enantioselective method [70]. There are five groups of CSPs used in HPLC. Taking into account the forces for stereoisomeric retention, these CSPs are classified as illustrated in Figure 12.

A variety of CSPs have been developed and optimized for the enantioresolution of MAMP using the HPLC technique including Pirkle-type, protein-based, crown ether, cellulose-based and the most common used phases based on β-cyclodextrin. Information on chiral selectors used for enantioseparation of amphetamine type compounds and information on the application of these selectors are summarized in Table 6 and Table 7, respectively.

β-cyclodextrins are the most common used chiral selector in the separation of MAMP enantiomers as well as related compounds using HPLC. CSPs with immobilized CDs were firstly used for the enantioseparation of MAMP and its related compounds by Rizzi et al. [81] and this concept has been used by many researchers [82-86]. Neutral CDs and modified CDs (charged CDs) are known [7]. The use of modified CDs in chiral separation by CE can

increase resolution in comparison with neutral CDs. Charged CDs also provide much greater flexibility in optimizing separation conditions. Moreover, analysis is relatively simple which is advantageous when simplicity instead of sensitivity is required (e.g., analysis of enantiomeric composition of clandestine preparations). The big advantages of the CDs are that the solubility and the selectivity can be improved by derivatization [7].

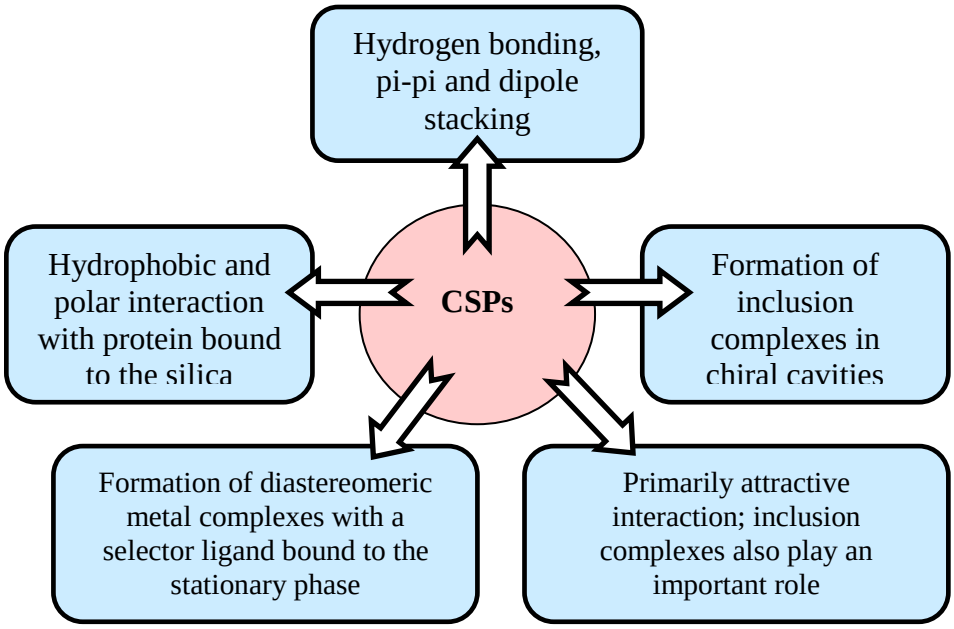



**Figure 12.** Category of chiral stationary phase depending on interaction for stereoisomeric retention

Pirkle-type CSPs contain a low-molecular-weight chiral molecule (chiral selector) covalently bound to the silica gel surface and resolve racemates as a result of enantioselective interactions between the CSP and the analytes. This CSP requires π- π interaction between stationary phase and solute, and interactions are favored in non-polar solvents. Pirkle-type CSPs are mainly performed in the normal-phase mode, however, reverse phase separation may be used for highly polar analytes. These CSPs offer many advantages including [43]:

- enantiomer separation on a wide variety of compound groups;
- column durability resulting from covalent phase bonding;
- ability to invert elution order;
- availability of analytical- to preparative-sized columns and bulk packing material;
- universal solvent compatibility

and have found applications for enantioseparation of MAMP and related compounds by chromatographic techniques using HPLC.

**Table 6.** Information on chiral selectors used for enantioresolution of MAMP and related compounds

| Type of selector | Chiral recognition mechanism | Commercial name | Chiral selector | Particle size (µm) | Manufactured (example) |
|---|---|---|---|---|---|
| Polysaccharides | Compounds form hydrogen bonds with the carbamate linkages between the side chains and the polysaccharide backbone. A compound can form multiple hydrogen bonds with the linkages of different side chains, greatly expanding the possible interactions. The polysaccharide backbone exists in a helical conformation, giving rise to steric restrictions that may inhibit access of one enantiomer to hydrogen-bonding sites. | Chiralpak AD | Amylase tris(3,5-dimethylphenyl) carbomate, coated | 10 | Daicel |
| | | Chiralpak AD-H | Amylase tris(3,5-dimethylphenyl) carbomate, coated | 5 | Daicel |
| | | Chiralpak AS | Amylase tris[(S)-α-methylbenzylcarbomate], coated | 10 | Daicel |
| | | Chiralpak IA | Amylase tris(3,5-dimethylphenyl) carbomate, coated | 5 | Daicel |
| | | Chiralcel OD | Cellulose tris(3,5-dimethylphenyl) carbomate, coated | 10 | Daicel |
| | | Chiralcel OD-H | Cellulose tris(3,5-dimethylphenyl) carbomate, coated | 5 | Daicel |
| | | Chiralcel OJ | Cellulose tris(3,5-dimethylphenyl) carbomate, coated | 10 | Daicel |
| Cyclodextrins (CDs) | The cyclodextrin cavity is composed of the glucoside oxygen and methylene hydrogen, giving it an apolar character. Therefore, chiral recognition is based on inclusion of the bulky hydrophobic group of the analyte into the hydrophibic cavity of the CD and on lateral interactions of the hydroxyl groups, such as dipole-dipole interactions, hydrogen bonds or dispersion forces, with the analyte. | β-Cyclose-2-OH | Mono-2-O-pentenyl- β-CD, sulfone linkage | 5 | Chiralsep |
| | | β-Cyclose-2-OH-T | Mono-2-O-pentenyl- β-CD, thioether linkage | 5 | Chiralsep |
| | | β-Cyclose-6-OH | Mono-6-O-pentenyl- β-CD, sulfone linkage | 5 | Chiralsep |
| | | β-Cyclose-6-OH-T | Mono-6-O-pentenyl- β-CD, thioether linkage | 5 | Chiralsep |
| Pirkle-type | These CSPs are based on the ionic or covalent attachment of one enantiomer of an amino acid to aminopropyl silica. Transient diastereomeric complexes involve electron donor-acceptor π-π interactions, hydrogen bonding and dipole-dipole interactions. | Chirex 3005 | N-(3,5-dinitrobenzoyl)-(1-naphthyl) glycine amide | 5 | Phenomenex |
| | | Whelk O1 | 4-(3,5-dinitrobenamido) tetrahydrophenanthrene, covalently bonded through monofunctional linkage | 5, 10 | Regis Technologies |
| | | Whelk O2 | 4-(3,5-dinitrobenamido) tetrahydrophenanthrene, covalently bonded through monofunctional linkage | 5, 10 | Regis Technologies |

**Table 6.** Cont.

| | | | | | |
|---|---|---|---|---|---|
| Macrocyclic antibiotic | The multiple chiral atoms and several functional groups allow multiple interactions with the analytes to enanble chiral recognition. The primary interaction include hydrogen bonding, dipole-dipole and π-π interactions, hydrophobic interactions, and steric repulsion. | Chrobiotic R | Ristocetin A, bonded | 5 | Astec |
| | | Chrobiotic T | Teicoplanin, bonded | 5 | Astec |
| | | Chrobiotic V | Vancomycin, bonded | 5 | Astec |
| | | Chrobiotic TAG | Teicoplanin aglycone, bonded | 5 | Astec |
| Polymeric | Recognition mechanism depends on polymer used. | DPEVB | N,N'-[(1R,2R)-1,2-diphenyl-1,2-ethanediyl]-bis-4-vinylbenzamide, bonded | 5 | --- |
| | | DEABV | trans-9,10-dihydro-9,10-ethanoanthracene-(11S,12S)-11,12-dicarboxylic acid bis-4-vinylphenylamide, bonded | 5 | --- |
| | | P-CAP | N,N'-(1S,2S)-1,2-cyclohexanediyl-bis-2-propenamide, bonded | 5 | Astec |
| | | P-CAP-DP | N,N'-[(1R,2R)-1,2-diphenyl-1,2-ethanediyl]-bis-2-propenamide, bonded | 5 | Astec |
| | | Kromasil CHI-TBB | O,O'bis(4-tert-butylbenzoyl)-N,N'-diallyl-l-tartar diamide, bonded | 5, 10, 16 | Eka Chemicals |

**Table 7.** Information on the chiral stationary phases used in LC and HPLC procedures for enantiomeric analysis of methylamphetamine, their metabolites, and related compounds

| Compound analyzed | Matrice | Derivatizing reagent | CSP | Detection | Ref. |
|---|---|---|---|---|---|
| AM, MAMP, MDE, MDMA, 4-MA, 2,5-DMA, 4-HA | Saq | PITC, NITC, AQC, Marfey's reagent | β-CD 250 mm x 4 mmx 5 µm Chiraldex (Merck) | UV | [81] |
| MDA, MDE | P | - | β-CD LiChroCart Superspher 60 RP-select B column 240 mm x 4 mmx 5 µm using a guard column LiChrospher 60 RP-select B 4 mmx 4 mm x 5 µm (Merck) | Fd | [82] |
| HMA | P | - | Chiral CBH 150 mm x 4 mm x 5 µm using a Chiral CBH guard column 10 mm ·x 3 mm (ChromTech) | El.D. | [82] |
| MDA, MDE, HMA | U, P | - | Chiral CBH 150 mm x 4 mmx 5 µm using a Chiral CBH guard column 10 mm ·x 3 mm (ChromTech) | Fd | [83] |
| EP, EP-HCl, MEP, PSEP, PSEP-HCl, MPEP | S | TFA | β-CD, LiChroCART ChiralDex 250 mm x 4 mmx 5 µm (Merck) | UV | [84] |
| | | | CE- β-CD, CM- β-CD, HP- β-CD, LiChrospher 100 RP C18 125 mm x 4 mm x 5 µm (Merck) | | |
| AM, MAMP, PCA, PMA, PMMA, MDMA, MDA, MDEA, NEP | Saq | - | β-CD, Cyclobond I 2000 150 mm x 4.6 mm x 5 µm (Advanced Separation Technologies Inc.) | DAD-ESI-MS/MS | [85] |
| | | | Macrocyclic antibiotic vancomycin Chirobiotic V 150 mm x 4.6 mm x 5 µm (Advanced Separation Technologies Inc.) | | |
| AM, MAMP | S | - | HP- β-CD, Cyclobond I 2000 RSP | UV | [86] |
| Selegiline | | | S-naphthylethylcarbamate functionalized β-CD Cyclobond I 2000 SN | | |
| EP | S | - | tBuCQN (SCX), Chiralpak QN-AX 150 mm x 16 mm x 15 µm (Chiral Technologies) | UV-VIS | [87] |

Other CSPs used in this area are brush-type strong cation-exchange CSPs (SCX), polysacsharides and macrocyclic antibiotics. Information on chiral stationary phases used in LC procedures for enantiomeric analysis of methylamphetamine, their metabolites, and related compounds are presented in Table 7.

### *2.2.3. Active mobile phase – chiral derivatizing reagent as an additive*

Another possibility for chiral separation of enantiomers is the transient diastereomeric complex, which is formed between the two enantiomers and the chiral component in the mobile phases (chiral selector). It is required to ensure the high enantiomeric purity of the chiral selector. The chiral mobile-phase-addictive mode comprises a CSP and an addictive mobile phase [6]. The formation of three types of transient diastereomeric complex is as follows [39]:

- inclusion complexes,
- ion pairs, and
- transition-metal-ion complexes.

The mechanism of enantioseparation based on differences in the stability constants of the two inclusion complexes produced between the enantiomers and the chiral selector with the enantiomer giving the most stable diastereomer being eluted first [70]. This approach has been applied to the enantio-resolution of MAMP and related compounds, especially for the employment of β-CDs (Table 8), however other chiral phases are also applied.

**Table 8.** Applications of chiral additives to the mobile phase for enantioseparation of MAMP and related compounds

| Compound analyzed | Matrice | Chiral additive | Chromatographic conditions | Detection | Ref. |
|---|---|---|---|---|---|
| MAMP, MDA, MDEA | P | β-CD | ChiraDex 250 mm x 4 mm x 5 µm<br>Mobile phase: ACN/Ph.B<br>LiChroCart Superspher 60 RP-select B column<br>240 mm x 4 mm x 5 µm using a guard column<br>LiChrospher 60 RP-select B<br>4 mm x 4 mm x 5 µm (Merck) | FLD | [82] |
| HMA | P | Chiral-CBH | Chiral CBH 150 mm x 4 mm x 5 µm using a Chiral CBH guard column<br>10 mm x 3 mm (ChromTech)<br>Ph.B/NaEDTA-propan-2-ol | El.D. | [82] |
| EP, EP-HCl, MEP, PSEP, PSEP-HCl, MPEP | P | native β-CD, M-β-CD, CE-β-CD, CM-β-CD, HP-β-CD | LiChrospher 100 RP C18 150 mm x 4 mm x 5 µm (Merck)<br>Mobile phase:<br>Ac. B/MOH | UV | [84] |

## 3. Other techniques used for chiral separation of MAMP

Although gas chromatography and liquid chromatography are the most common techniques used for chiral separation of MAMP, there are three other techniques combined with reliable detectors that could provide good enantiomeric resolution, including capillary electrophoresis, thin layer chromatography (TLC) and nuclear magnetic resonance.

The analysis of enantiomers of MAMP can be easily achieved by CE [88]. Both, indirect and direct modes are used for successful separation [89]. Several chiral selectors have been employed in CE in order to modify the electrophoretic mobilities of the enantiomers selectively [89]. In direct mode the most commonly used are selectors such as CDs (native and modified), antibiotics, linear polysaccharides, proteins and chiral micelles. Also, many chiral reagents used in the indirect method have been tested for the forensic applications, especially in drugs analysis and biological samples. Using CE, direct separation is usually preferred. Capillary enantioseparation offers many advantages even over chromatographic techniques (from the molecular recognition point of view) including [88]:

- high separation factor due to small thermodynamic selectivity of recognition;
- very fast screening of analyte-chiral selector interactions (especially analyte-CD);
- very flexible from the point of view of adjusting the enantioseparation factor and,
- high peak efficiency that allows observation of enantioselective effects in selector-selectand interactions that could not be visible by other techniques.

Additionally, CE is a powerful, fast, economical, and effective method. High-resolution separation is also reported. These advantages are very important in analyzing drugs of abuse, including MAMP [88].

Thin layer chromatography is another option for the enantioseparation of MAMP and related drugs. This technique is simple, rapid and inexpensive. Recently, TLC has been modernized because new adsorbents, plates and automated sample applicators are commercially available [90]. However, the lack of quantitative results without significant effort and coupling to other techniques is a big disadvantage of TLC [91]. Chiral stationary phases are usually used to separate enantiomers. However, different chiral selectors are also used as mobile-phase additives.

Nuclear magnetic resonance is another technique of choice for enantio-resolution of MAMP and related compounds. NMR determination of enantiomers is usually conducted in a chiral environment by adding the chiral derivatizing reagent or chiral solvating agent to the NMR solution of the substances (e.g., GITC or CDs) [92]. In NMR, direct separation is

usually preferred over indirect [92]. NMR is powerful, fast, and effective. Moreover, NMR provides a multiple set of data based on a single experiment, after which it is possible to obtain all information about the stereochemistry of enantiomers [93].

Although NMR techniques provide some advantages, data in the literature about its application to enantioseparation of AM-type compounds are still limited. NMR techniques are not therefore as commonly used as CE or other chromatographic techniques, and are usually complementary to chromatographic approaches.

## 4. Chemometric as a useful tool in analytical chemistry

As complexity, variety, growing importance of quality and omnipresent uncertainty mark today's chemistry, a statistical approach to experimental design is almost inevitable [94]. That is why the application of multivariate experimental design techniques is becoming increasingly common in many scientific areas including analytical chemistry [94]. Multivariate designs allow the simultaneous study of several control variables, and in comparison to conventional univariate approaches are faster to implement and more cost-effective [94].

Since chemometrics is a science which is multidisciplinary in nature and involves multivariate statistics, mathematical modeling and information technology [95]. It is applied to solve both descriptive and predictive problems in experimental life and physical sciences (Figure 13), especially in chemistry.

There are many chemometric techniques which are heavily used in analytical chemistry. The development of improved chemometric methods of analysis also continues to advance the state of the art in analytical methodology and instrumentation [96]. It is an application driven discipline, and while the standard chemometric methodologies are very widely used industrially, academic groups are dedicated to the continued development of chemometric theory, method and application development [96].

The description and applicability of advanced statistical chemometric techniques such as cluster analysis (CA), canonical discriminant analysis (DA), factor analysis (FA), linear discrimination analysis (LDA), and principal component analysis (PCA) are provided in Table 9. In analytical chemistry, these statistical techniques appeared to be highly useful tools in solving many environmental problems. They are also helpful in the classifications of different features of the examined environmental parameters and in the identification of existing pollution pattern. The application of the chemometric techniques is helpful for a

deeper understanding of the situation of selected environmental parameters. The chemometric approach is a powerful tool to solve problems in many analytical areas such as method development and optimization, evaluation of quality of the foodstuff, air, environmental water, and quality control of pharmaceuticals, characterization of environmental or biological samples, and many other applications.

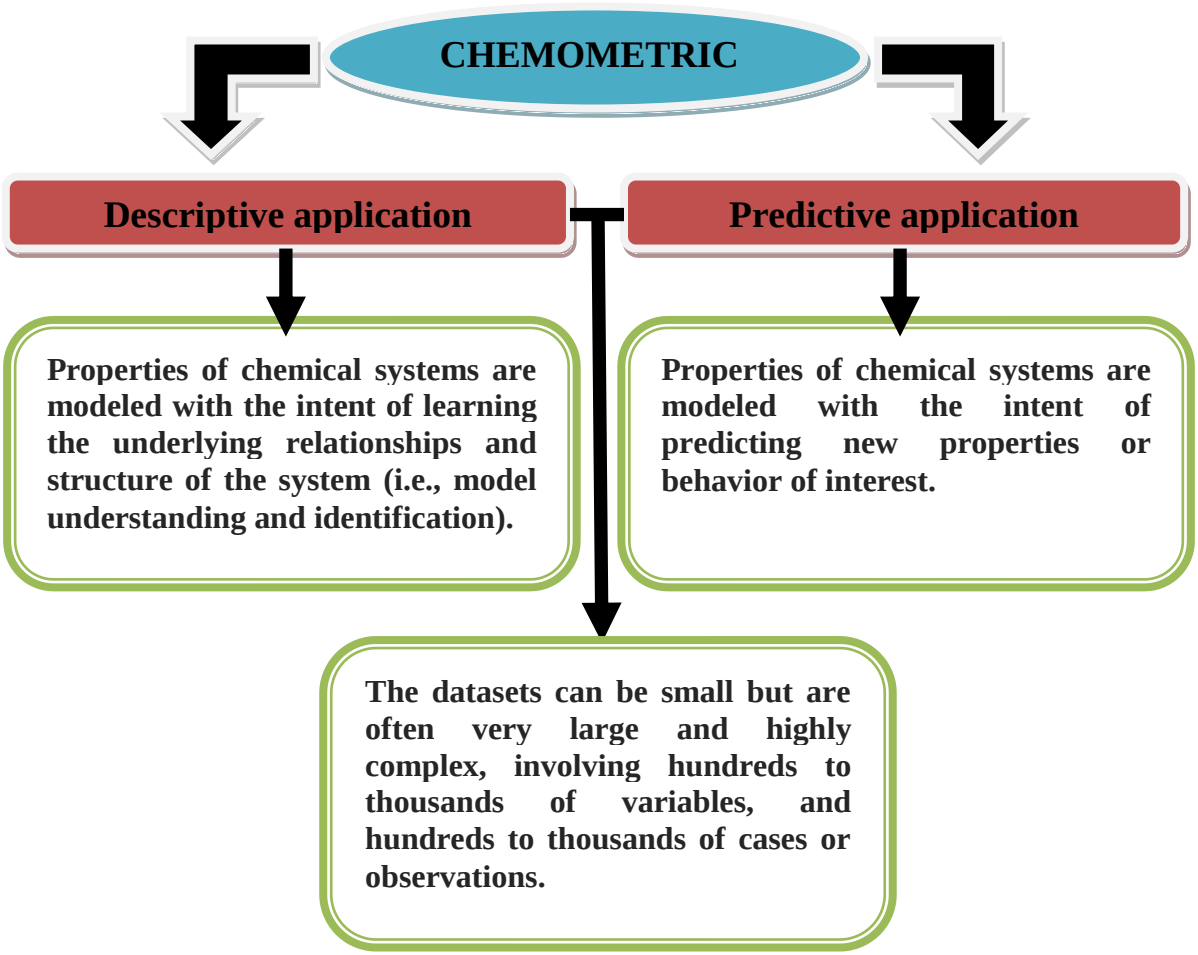



**Figure 13.** Chemometric – descriptive and predictive application

**Table 9.** Information on chemometric techniques widely used in analytical chemistry

| Chemometric technique | Description | Ref. |
|---|---|---|
| CA | An exploratory data analysis tool for solving classification problems, based on unsupervised learning. CA enables objects stepwise aggregation according to the similarity of their features. As a result hierarchically or non-hierarchically ordered clusters are formed. A single cluster describes a group of objects that are more similar to each other than to objects outside the group. A reliable grouping and comparison of experimental conditions can be achieved. | [97] |
| DA | DA is a statistical technique that allows the user to investigate the differences between multiple sets of objects across several variables simultaneously.<br>Discriminant analysis has two main goals:<br>• discrimination (construct a classifier to separate the distinct set of observations from all observations in a known population); | [98] |

**Table 9.** Cont.

| | | |
|---|---|---|
| | • classification (separate unlabeled observations into labeled groups using a classifier). | |
| FA | A statistical method used to describe variability among observed, correlated variables called factors. The main application of FA are:<br>• to reduce the number of variables,<br>• to detect structure in the relationship between variables, that is to classify variables. | [99] |
| LDA | A method used in statistics, pattern recognition and machine learning to find a linear combination of features which characterizes or separates two or more classes of objects or events. The resulting combination may be used as a linear classifier, or, more commonly, for dimensionality reduction before later classification.<br>LDA works when the measurements made on independent variables for each observation are continuous quantities. | [98] |
| PCA | A statistical procedure that uses orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. The number of principal components is less that or equal to the numer of original variables. This transformation is defined in such a way that the first principal component has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the contstraint that it is orthogonal to (i.e., uncorrelated with) the preceding components. Principal components are guaranteed to be independent if the data set is jointly normally distriuted. PCA is sensitive to the relative scaling of the original variables. | [97] |

## 5. Summary

Impurity analysis for drug profiling has been used to enable the identification of the synthetic route for methamphetamine manufacture from ephedrines. For example, route-specific impurities can be detected by HPLC and GC-MS [100]. Chiral analysis of seized methamphetamine may also be useful, for example whether the enantiomeric composition suggest the starting materials were extracted from pharmaceutical product (single enantiomers), the plant ephedra (mixture of enantiomers) or perhaps illicit ephedrine synthesized by fermentation processes [100]. The stereospecific separation of methylamphetamine can be approached using a variety of analytical techniques. The most commonly used are chromatography techniques: GC, LC, HPLC, and CE. There have been several reports that the use of chiral stationary phases such as cyclodextrins and chiral derivatizating reagents greatly facilitates these methods [6]. The analysis of compound by NMR is also potentially useful for drug profiling [101]. NMR spectroscopy has an enormous potential for investigating conformations and configurations in organic compounds. 1D and 2D hetero and homonuclear NMR experiments enable complete assignment and structural information, therefore can be useful to chiral profiling of methamphetamine and its derivatives [101].

To create comprehensive characterization of methamphetamine synthesized by the Emde method in terms of impurity profile and chirality profile, which will provide a link between starting materials and the illicit methamphetamine synthesized by the clandestine chemist, reference substances such as ephedrine derivatives as well as chloro-intermediates of methamphetamine are required. Presently, pure enantiomers of ephedrine and pseudoephedrine are commercially available but the four enantiomers of chloro-intermediates are not available, meaning that the preparation of these compounds is required and thereafter separation into single enantiomers is necessary.

## II THE PURPOSE OF THE RESEARCH

Because the number of people who abuse MAMP is increasing every year, it became a global threat. Therefore background knowledge about MAMP is significant for many reasons with the most important being associated with human health in terms of prediction and protection applied to reduction in drug addiction.

This project aims to develop and optimize the new analytical methodologies in order to create a comprehensive characterization of methylamphetamine in terms of impurity and chirality profile, and also enantiomeric characterization of chloro-intermediates of methylamphetamine that are not commercially available, and necessary as reference materials for the further analysis.

The research program is based upon implementation of the following tasks:

a) organic synthesis:

- chloro analogs of ephedrine which are necessary as substance standards in further analysis; they represent also specific impurities of MAMP synthesized by Emde method;

b) investigation of the stereochemical course of part I of the Emde:

- one- and multidimension NMR analysis of the synthesized samples;
- GC-MS analysis of the synthesized samples;

c) enantioseparation of methylamphetamine, its chloro intermediates and impurities:

- optimization of the derivatization procedures:
  - *chemometric analysis*;
- GC-MS analysis:
  - *selection of the chiral stationary phase,*

- *optimization of temperature- and flow-programmed chromatographic conditions,*
- *validation of optimized chromatographic conditions,*
- *application of developed method to the analysis of MAMP samples;*

d) determination and interpretation of the impurities found in MAMP samples.

The implementation of the above tasks of research will lead to the creation of characterization of MAMP synthesized by Emde in terms of impurity profile and enantiomeric composition of the examined substances.

## III EXPERIMENTAL PART

### 1. Standards, chemical reagents and materials

Information on the chemical reagents and standards used during the research are summarized in Table 10.

**Table 10.** Information on the standards, chemical reagents and materials used during the research

| Reagent name | Supplier |
|---|---|
| (1S,2R)-(+)-Ephedrine HCl | Sigma-Aldrich (Poole, UK) |
| (1R,2S)-(−)-ephedrine HCl | Sigma-Aldrich (Poole, UK) |
| (1S,2S)-(+)-pseudoephedrine | Sigma-Aldrich (Poole, UK) |
| (1R,2R)-(−)-pseudoephedrine | Sigma-Aldrich (Poole, UK) |
| (S)-(+)-methylamphetamine HCl | Sigma-Aldrich (Poole, UK) |
| (R)-(−)-deoxyephedrine | Sigma-Aldrich (Poole, UK) |
| thionyl chloride | Sigma-Aldrich (Poole, UK) |
| 5A molecular sieve | Sigma-Aldrich (Poole, UK) |
| acetone | Fisher Scientific (Loughborough, UK) |
| chloroform | Fisher Scientific (Loughborough, UK) |
| diethyl ether anhydrous | Fisher Scientific (Loughborough, UK) |
| methanol | Fisher Scientific (Loughborough, UK) |
| ethyl acetate (HPLC grade) | Fisher Scientific (Loughborough, UK) |
| deuterated chloroform containing 1% (v/v) TMS | Acros Organic |
| trifluoroacetic anhydride ≥ 99 % | Sigma-Aldrich (Poole, UK) |
| pentafluoropropionic anhydride ≥ 99 % | Sigma-Aldrich (Poole, UK) |
| chlorodifluoroacetic anhydride ≥ 99 % | Sigma-Aldrich (Poole, UK) |
| 3-(Trifluoromethyl)phenethylamine HCl | Sigma-Aldrich (Poole, UK) |
| helium (CP grade, 99.999 %) | BOC (Guildford, UK) |
| nitrogen (oxygen free) | BOC (Guildford, UK) |

### 2. Equipment used

Information on laboratory equipment and chromatographic columns used during the research are provided in Table 11.

IR, NMR and GC-MS analysis were performed using the equipment as follows:

- IR spectra were obtained on a Perkin-Elmer FT IR spectrometer;
- resonance spectra were recorded on a Bruker DPX 400 and Avance 400 NMR;
- GC–MS analysis was performed using an Agilent 6890 GC connected to 5973 MSD (Hewlet-Packard, Palo Alto, CA, USA).

**Table 11.** Information on the laboratory equipment and chromatographic column used during the research

| | Laboratory equipment | **Supplier** |
|---|---|---|
| **ORGANIC SYNTHESIS** | multiple neck adapters | SciLabware (Stoke-on-Trent, UK) |
| | reduction adapters | |
| | still head | |
| | stopcock adapter with cone | |
| | screwthread | |
| | low form beakers | |
| | watch glasses | |
| | filter / sinter discs | |
| | filter holder | |
| | condenser | |
| | baskets with hinged lid | |
| | crystallising with spout | |
| | glass non-vacuum dessicators | |
| | boiling flasks | |
| | narrow neck erlenmeyers | |
| | Büchner type flasks | |
| | flask stands | |
| | Büchner filters | |
| | dropping funnels | |
| | filter funnels | |
| | general purpose funnels | |
| | analytical scale | Sigma-Aldrich (Poole, UK) |
| | funnel support and stand | SciLabware (Stoke-on-Trent, UK) |
| | cone joints | |
| | weighing vessel | |
| | spatulas | |
| | tweezers / forceps | |
| | stirrer bars | |
| | thermometers | |
| | thermometer addapters | |
| | hot plate with magnetic stirrer | Sigma-Aldrich (Poole, UK) |
| **NMR ANALYSIS** | NMR tubes | |
| | analytical scale | |
| **DERIVATIZATION** | block heater | |
| | analytical scale | |
| | thermostat | |
| | blocks | |
| | vials, screw top with solid green Melamine cap, 2 mL | |
| | vials, screw top, clear glass, 1.5 mL | |
| | Eppendorf pippets (up to 20, 50, 100, 200 µL, 1mL) | |
| | tipps for Eppendorf pippets | |
| | thermometers | SciLabware (Stoke-on-Trent, UK) |

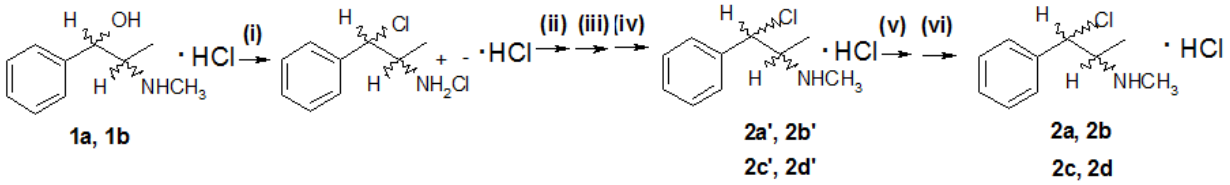
**Table 11.** Cont.

| | | |
|---|---|---|
| **GC-MS ANALYSIS** | Eppendorf pippets (up to 20, 50, 100, 200 µL, 1 mL) | Sigma-Aldrich (Poole, UK) |
| | tipps for Eppendorf pippets | |
| | vials, screw top, clear glass (1.5 mL), with liners (200 µL) | |
| | vial stands | |
| | TR-5MS<br>(30 m x 0.25 mm x 0.25 µm film thickness) | Thermo Scientific<br>(Cheshire, UK) |
| | Astec Chiraldex™ G-PN<br>(30 m x 0.25 mm x 0.12 µm film thickness) | Supelco (Bellefonte, PA) |
| | Astec Chiraldex™ G-TA<br>(30 m x 0.25 mm x 0.12 µm film thickness) | |

## 3. Analytical procedures

### 3.1. Synthesis of chloro analogs of MAMP

Both, conventional and modified procedure of synthesis are presented in Figure 14. Analysis were in agreement with published data for IR [30] and melting point [102]. Crystals obtained from conventional procedure were used for investigation of the stereochemical course of part I of the Emde method, while crystals obtained during modified procedure were used as standards in further study.




**1a** (1R,2S)-(-)-ephedrine, **1b** (1S,2S)-(+)-pseudoephedrine
**2a'** (1S,2S)-(+)-chloropseudoephedrine, **2b'** (1R,2S)-(-)-chloroephedrine – obtained by typical procedure
**2c'**(1S,2S)-(+)-chloropseudoephedrine, **2d'** (1R,2S)-(-)-chloroephedrine – obtained by modified procedure
**2a, 2b, 2c, 2d** final products after recrystallisation

Typical procedure [103]
(i) Step 1, $CHCl_3$, $SOCl_2$ (ii) Step 2, 70 °C, 5 h (magnetic stirring system) (iii) Step 3, 20 °C, evaporation to final volume of 50 mL (iv) Step 4, $Et_2O$, filtration (v) Step 5, MeOH, $(CH_3)_2CO$, 70 °C, recrystallisation (vi) Step 6, -5 °C, 24 h, recrystallisation

Modified procedure
(i) Step 1, $SOCl_2$ (ii) 100 °C, 20 mins (boiled water bath) (iii) Step 3, 20 °C (iv) Step 4, $Et_2O$, filtration (v) Step 5, MeOH, $(CH_3)_2CO$, 70 °C, recrystallisation (vi) Step 6, -5 °C, 24 h, recrystallisation

**Figure 14.** Synthesis of chloroephedrine and chloropseudoephedrine by typical and modified method

### 3.2. Recrystalization procedure

Recrystallisation procedure was carried out as follows [104]. The crystals of chloro-amines were crushed and washed with ice cold dry acetone. Then, chloroephedrines were dissolved in hot denaturated methanol. Next the alcohol was evaporated. Five drops of acetone were added. The samples were stored in a freezer. After 24 hours the crystals were filtrated quickly.

### 3.3. IR analysis

Spectra of chloro analogues of EP and PSEP enantiomers have been recorded in the region 4000−400 $cm^{-1}$. The range (100−0 % T) was correct.

### 3.4. NMR analysis

Crystals of chloroephedrine in 1 mL of $CDCl_3$ containing 1 % of TMS solution were dissolved. The solution was transferred to an NMR tube, and the spectra were recorded under conventional conditions.

### 3.5. Achiral GC-MS analyses of chloro-intermediates of MAMP

The mass spectrometer was operated in full-scan mode (m/z 40–400) in the electron ionization mode at 70 eV. Chromatography was undertaken on an Astec Chiraldex$^{TM}$ G-PN and Astec Chiraldex$^{TM}$ G-TA with helium as the carrier gas. A 1 μL volume of sample was injected into GC-MS in splitless mode with a purge time of 0.75 to 2.0 min and purge flow of 15.0 to 40.0 mL/min. A solvent delay time of 2.0 min was used. Electron ionization was used with the ion source at 230 $^{o}$C

Separation was achieved with a non-polar capillary column TR-5MS, with helium as the carrier gas at a constant flow rate of 1.8 mL/ min. The oven temperature program started at 70 °C for 2 min, was increased to 250 °C at a rate of 10 °C/min, and then held for 2 min. A 1 μL aliquot of the derivatized sample (0.05 μg/mL) was injected in the splitless mode with a purge time of 3 min. The injector and the GC interface temperatures were maintained at 200 and 250 °C, respectively. Mass spectra were obtained in the full scan mode over the range of 40–500 m/z.

### 3.6. Preparation of stock solutions

Standard stock solutions were prepared by dissolving enantiomers of EP, PSEP, CEP, CPSEP and MAMP in methanol at the concentration 0.001 g/mL and stored at 4 °C. Working solution of standards containing 50 μg/mL of each target compound and IS were prepared and calibration standard solutions (the concentration range of the analytes from (0.001 to 0.1 μg/mL) were prepared by diluting standard stock solutions containing each of target compounds in the appropriate amounts of methanol and stored in the dark at 4 °C.

### 3.7. Optimization of the derivatization procedures

The working standard solutions (50 μL) were transferred to 1.5 mL reaction vials and evaporated to dryness at appropriate temperature under a gentle stream of nitrogen. The dry residues were derivatized under the conditions stated in Table 12. The conformation of derivatives randomly selected compounds using multidimentional NMR analysis was checked. Derivatization reaction during conditions tested did not change the stereochemistry of the analytes of interest. The derivatives were analysed by GC-MS (in three replicates) under conditions presented in Table 13. Each derivatization was performed in three replicates.

**Table 12.** Different conditions of derivatization process used for the chemical conversion of the target compounds

| $No_{experiment}$ | DR | Volume of DR [μl] | Volume of EA [μl] | Temperature [$^{o}$C] | Time [min] |
|---|---|---|---|---|---|
| 1A | TFAA | 50 | 50 | 50 | 10 |
| 2A | | | | | 15 |
| 3A | | | | | 20 |
| 4A | | | | | 25 |
| 1B | TFAA | 50 | 50 | 55 | 10 |
| 2B | | | | | 15 |
| 3B | | | | | 20 |
| 4B | | | | | 25 |
| 1C | TFAA | 50 | 50 | 60 | 10 |
| 2C | | | | | 15 |
| 3C | | | | | 20 |
| 4C | | | | | 25 |
| 1D | TFAA | 50 | 50 | 65 | 10 |
| 2D | | | | | 15 |
| 3D | | | | | 20 |
| 4D | | | | | 25 |
| 1E | PFPA | 50 | 50 | 50 | 10 |
| 2E | | | | | 15 |
| 3E | | | | | 20 |
| 4E | | | | | 25 |

**Table 12.** Cont.

| | | | | | |
|---|---|---|---|---|---|
| 1F | PFPA | 50 | 50 | 55 | 10 |
| 2F | | | | | 15 |
| 3F | | | | | 20 |
| 4F | | | | | 25 |
| 1G | PFPA | 50 | 50 | 60 | 10 |
| 2G | | | | | 15 |
| 3G | | | | | 20 |
| 4G | | | | | 25 |
| 1H | PFPA | 50 | 50 | 65 | 10 |
| 2H | | | | | 15 |
| 3H | | | | | 20 |
| 4H | | | | | 25 |
| 1I | CDFA | 50 | 50 | 50 | 10 |
| 2I | | | | | 15 |
| 3I | | | | | 20 |
| 4I | | | | | 25 |
| 1J | CDFA | 50 | 50 | 55 | 10 |
| 2J | | | | | 15 |
| 3J | | | | | 20 |
| 4J | | | | | 25 |
| 1K | CDFA | 50 | 50 | 60 | 10 |
| 2K | | | | | 15 |
| 3K | | | | | 20 |
| 4K | | | | | 25 |
| 1L | CDFA | 50 | 50 | 65 | 10 |
| 2L | | | | | 15 |
| 3L | | | | | 20 |
| 4L | | | | | 25 |

**Table 13.** Chromatographic conditions for the analysis of TFAA, PFPA and CDFA derivatives of target analytes

| **TFAA/PFPA** | |
|---|---|
| Mode | Splitless |
| Heater | 230 $^{o}$C |
| Purge flow to split vent | 20 mL/min at 2 min |
| Injector temperature | 200 $^{o}$C |
| Ion source temperature | 230 $^{o}$C |
| Injection volume | 1 µl |
| Oven temperature | 65 $^{o}$C (5 min) to 145 $^{o}$C (20 min) at 10 $^{o}$C/min |
| Flow rate | 1.5 mL/min |
| Analysis time | 33 min |
| **CDFA** | |
| Mode | Splitless |
| Heater | 230 $^{o}$C |
| Purge flow to split vent | 20 mL/min at 2 min |
| Injector temperature | 200 $^{o}$C |
| Ion source temperature | 230 $^{o}$C |
| Injection volume | 1 µl |
| Oven temperature | 65 $^{o}$C (5 min) to 145 $^{o}$C (45 min) at 10 $^{o}$C/min |
| Flow rate | 1.5 mL/min |
| Analysis time | 58 min |

### 3.8. Effectiveness of derivatization

The effectiveness of derivatization was expressed by relative response factors (RRFs), calculated according to the following equation:

$$RRF = \frac{A_S \; x \; C_{IS}}{A_{IS} \; x \; C_S} \qquad (1)$$

where $A_{IS}$ is the internal standard peak area (3-(Trifluoromethyl)phenethylamine, TFMPA), $C_S$ is the target analyte concentration (μg/mL), $A_S$ is the target analyte peak area, $C_{IS}$ is the internal standard concentration (μg/mL).

### 3.9. Chemometric analysis of derivatization procedure

The goal of the intelligent data analysis was to reveal specific links between the experimental conditions used for determination of RRFs of three different derivatization agents for several compounds. Links between compounds are also sought and discussed.

CA and PCA were used as multivariate statistical methods in the data interpretation. PCA is used in this study in order to interpret the latent data structure and to find out factors determining the similarity between the optimized experimental conditions. The data for the three derivatization agents (TFAA, PFPA and CDFA) were treated separately, so input matrices of dimension [10x16] were constructed for ten compounds (five compounds in two stereo isomeric forms) and 16 different experimental conditions (combination of 4 temperatures and 4 various times of experimentation coded as variables $T_i t_i$).

### 3.10. Chiral GC-MS analyses

The mass spectrometer was operated in full-scan mode (m/z 40–400) in the electron ionization mode at 70 eV. Chromatography was undertaken on an Astec Chiraldex$^{TM}$ G-PN and Astec Chiraldex$^{TM}$ G-TA with helium as the carrier gas. A 1 μL volume of sample was injected into GC-MS in splitless mode with a purge time of 0.75 to 2.0 min and purge flow of 15.0 to 40.0 mL/min. A solvent delay time of 2.0 min was used. Electron ionization was used with the ion source at 230 $^{o}$C. Experimental conditions for enantioseparation such as the oven temperature program and velocity of gas flow were varied for method optimization: details are provided within the Results and Discussion Section. The chromatographic conditions of the derivatization products analysis are presented in Table 13.

### 3.11. Method validation

In order to obtain a calibration curve, the standard stock solutions of all standards were prepared and three repeated analyses with the aforementioned method of derivatization and GC-MS. The guidelines published by the United Nations Office on Drugs and Crime [105] for the analysis of seized materials by GC were used for the validation of the developed method.

## IV RESULTS AND DISCUSION

### 1. Synthesis of chloro intermediates of MAMP and investigation of the stereochemical course of part I of the Emde method

### 1.1. Synthesis of CE and CPSEP enantiomers

Reactions play an essential role in organic synthesis and one of the ideology of Green Chemistry is development of new chemical reaction conditions that may theoretically provide benefits for both, environment and chemical syntheses especially in terms of health and environmental safety, but also in terms of energy and resource efficiency, operational simplicity and product selectivity [106].

Because of the strong interest of methamphetamine amongst scientists, the synthesizes of MAMP and related compounds are carried out on a large scale in chemical laboratories. Unfortunately, the manufacture of methamphetamine requires many chemical reaction steps and large amounts and a number of organic solvents with varying degrees of toxicity and purity. Looking at the interest of these synthesis from aspects of sustainable development, environmentally friendly solutions are desirable.

In order to address these issues, this study investigated a modified method of CEP and CPSEP synthesis and compared with typical procedure.

The following changes were made to improve the environmental performance of the synthesis:

- the removal of precursor dissolving step (Figure 14, step 1 without chloroform). Chloroform is a hazardous, carcinogenic and toxic solvent;
- the reduced time of heated step from 5 hours to about 20 mins; the changed type of heating (Figure 14, step 2).

**Table 14.** Yields and melting points for the synthesis of CEP and CPSEP compounds from (-)EP and (+)PSEP as an example

| Entry | Precursor | $m_0$ [g] | Product | m [g] | Yield [%] | $m_p$ [°C] | $m_{p, lit}$ [°C], [102] |
|---|---|---|---|---|---|---|---|
| **TYPICAL PROCEDURE** | | | | | | | |
| 1 | (-)EP | 1.2260 | 2a | 0.1348 | 10.07 | 197-198 | 200 |
| 2 | (+)PSEP | 1.2131 | 2b | 0.4802 | 36.27 | 190 | 187-188 |
| **MODIFIED PROCEDURE** | | | | | | | |
| 3 | (-)EP | 1.3848 | 2c | 0.7658 | 54.38 | 198 | 200 |
| 4 | (+)PSEP | 1.1205 | 2d | 0.5954 | 42.70 | 187-188 | 187-188 |

These changes not only shortened synthesis, but also saved energy consumption. The results fromboth the typical and modified procedure are shown in Table 14.

## 1.2. Identification of final products. Investigation of the stereochemical course of the part I of Emde synthesis

Before comparison of both methods the confirmation correctness of synthesis was necessary. The identification of the products was ascertained by melting point, IR and NMR spectra. Comparing the melting point of obtained chloro intermediates with literatures values [102] (Table 14) confirms correctness of synthesis. It has to be mentioned that literatures values refer to the data of products obtained from the classical method. IR spectra (Table 15) also confirms conversion from EP/PSEP enantiomers to chloro analogs of EP and PSEP. The resulting products do not contain hydroxyl groups but contain the characteristic peak originating from the chlorine which indicates that the reaction had occurred.

**Table 15.** Information on the IR $\nu_{max}$ of obtained products

| Product | IR $\nu_{max}$ [$cm^{-1}$] |
|---|---|
| 2a | 2961.36, 2699.92, 2472,82, 1610.03, 1593.10, 1379.59, 761.84 |
| 2b | 2961.42, 2699.99, 2472,91, 1610.13, 1593.15, 1379.62, 761.88 |
| 2c | 2961.77, 2698.75, 2473.11, 1609.79, 1592.80, 1379.45, 761.18 |
| 2d | 2961.72, 2698.75, 2473.04, 1609.71, 1592.75, 1379.41, 761.09 |

### *1.2.1. NMR analysis*

For the synthesis of chloro-intermediates of MAMP, four stereoisomers of EP as starting materials were used to manufacture CEP derivatives by chlorination with thionyl chloride. The two chiral centres in these phenethylamines give rise to four stereoisomers. By convention the enantiomers with opposite stereochemistry around the chiral centres (1R,2S and 1S,2R) are designated CEP, while CPSEP exhibits the same stereochemistry around the chiral carbons (1R,2R and 1S,2S).

Taking into account three mechanisms for the reaction of alcohols with thionyl chloride which are described in Chapter I, four different stereoisomers could be expected during experiment. To differentiate the structure of chloro-intermediates of MAMP manufactured by first step of Emde one- and multi-dimensional NMR techniques have been used.

*1.2.1.1. Analysis of $^{1}H$ NMR spectra*

$^{1}H$ NMR spectra analysis was used to probe the conformations of the chloroephedrine derivatives (**2a, 2b, 2c, 2d**) in solution. In spectra obtained for **2a** and **2b** there were six proton signals (Table 16). Considering these signals the structure presented in Figure 15 of sample analyzed can be deducted.

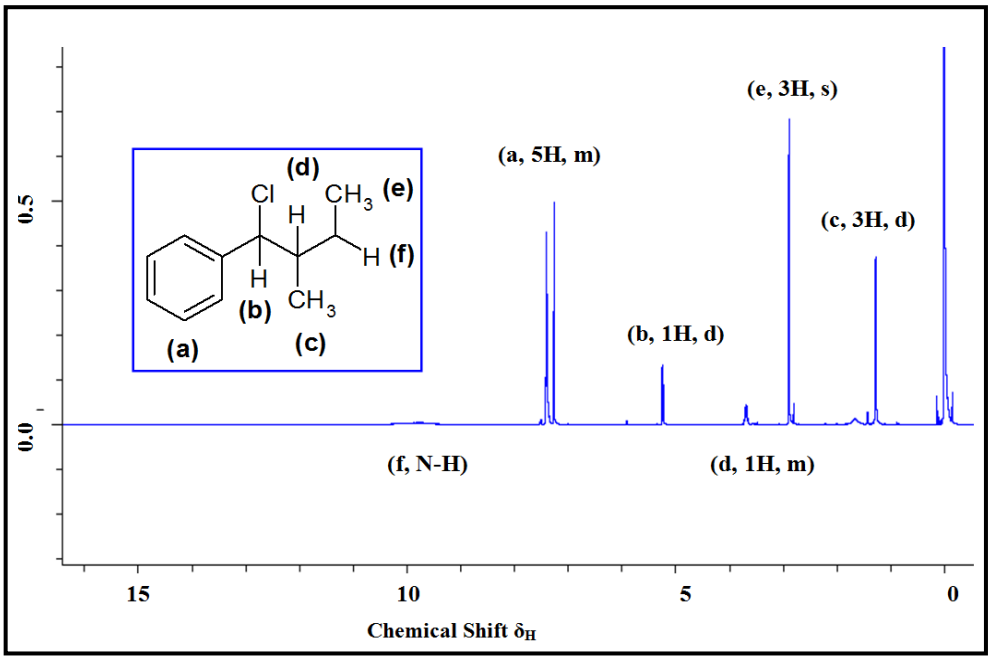



**Figure 15.** $^{1}H$ NMR spectra of chloro analog obtained from (1R, 2S)-(-)-ephedrine

The spectra obtained for 2c and 2d shown a different situation. Each of proton signal is doubled what means that two stereoisomers are presented in the sample. The signals of appropriate compounds were as presented in Table 16.

**Table 16.** $^{1}H$ NMR data for compound 2a, 2b, 2c and 2d

| Product | δ ppm |
|---|---|
| **2a** | 1.2582-1.3237 (d, J=3.0089, 3H), 2.9258 (s, J=3.1022, 3H), 3.5591-3.7342 (m, J=0.9966, 1H), 5.2554-5.2913 (d, J=1.0000, 1H), 7.2634-7.5183 (m, J=3.3102, 5H); 9.8975-10.0197 (m, J=3.0029, 1H) |
| **2b** | 1.2561-1.3719 (d, J=5.7363, 3H), 2.9464 (s, J=5.3905, 3H), 3.5433-3.7303 (m, J=1.7499, |

**Table 16.** Cont.

| | |
|---|---|
| | 1H), 5.2584-5.2933 (d, J=1.7309, 1H), 7.2524-7.5061 (m, J=3.5802, 5H); 9.8984-10.0189 (m, J=3.0279, 1H) |
| **2c** | 1.2441-1.2734 (d, 6.0356, 3H), 1.2534-1.2902 (d, 2.7702, 3H), 2.8455 (s, J=7.3001, 3H), 2.8949 (s, J=4.0400, 3H), 3.4899-3.7000 (m, J=0.8967, 1H), 3.7183-3.8824 (m, J=1.9866, 1H), 5.2348-5.3688 (d, J=1.8955, 1H), 5.2578-5.2912 (d, J=1.0309, 1H); 7.1982-7.3883 (m, J=1.3322, 5H); 7.2524-7.5062 (m, J=1.0012, 5H); 9.8875-9.9989 (m, J=3.5329, 1H), 9.8975-10.2089 (m, J=3.4479, 1H) |
| **2d** | 1.2242-1.3388 (d, 7.2440, 3H), 1.3372-1.502 (d, 4.4235, 3H), 2.9387 (s, J=7.3021, 3H), 2.9464 (s, J=4.0501, 3H), 3.4901-3.7243 (m, J=1.4786, 1H), 3.7144-3.8615 (m, J=2.3216, 1H), 5.2849-5.2888 (d, J=2.2965, 1H), 5.2524-5.2901 (d, J=0.9999, 1H); 7.1991-7.3873 (m, J=1.3362, 5H); 7.2538-7.5102 (m, J=1.0980, 5H); 9.8911-9.9984 (m, J=3.5362, 1H), 9.8975-10.2099 (m, J=3.4489, 1H) |

*1.2.1.2. Analysis of $^{13}C$ NMR and $^{13}C$ NMR with DEPT spectra*

The $^{13}C$ NMR and $^{13}C$ NMR with DEPT data for obtained compounds are provided in Table 17. The appearance of eight carbon atoms in 2a and 2b products is confirmed by $^{13}C$ NMR. The carbon signals in obtained spectra are indicated as follows (Figure 16):

- two methyl group protons signals,
- five methine signals, and
- additional one quaternary carbon signal coming from the aromatic ring.

The $^{13}C$ NMR with DEPT revealed the same carbon signals for eight carbon atoms. The assignation of appropriate region is as follows:

- region from 127 to 137 ppm - the carbon resonance of aromatic region, which is overlapped, with the carbon resonances (Cm, Cp, Co, Ci) of aromatic region (C unit);
- signals at 14 and 30.5 - the methyl groups (C-3, $CH_3$-TS);
- signals at 61 and 63 - the methine carbon (C-2, C-1).

$^{13}C$ NMR and $^{13}C$ NMR with DEPT spectra for products obtained from PSEP enantiomers present a different situation. It can be deducted that these products are not single enantiomers. The $^{13}C$ NMR with DEPT revealed sixteen carbon atoms indicated as follows:

- four methyl group protons signals,
- ten methine signals, and
- two quaternary carbon signals.

It be concluded that two chloro-analogs of ephedrine are present in the sample.

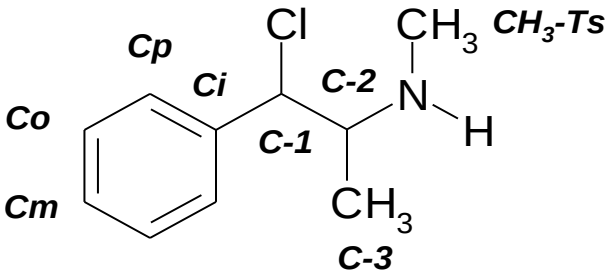



**Figure 16.** Structure of chloro-intermediates of MAMP with marked carbon atoms

**Table 17.** $^{1}$C NMR and $^{1}$C NMR with DEPT data for compound 2a, 2b, 2c and 2d

| | **$^{13}$C NMR** | | | | **DEPT** | | | |
|---|---|---|---|---|---|---|---|---|
| | **2a** | **2b** | **2c** | **2d** | **2a** | **2b** | **2c** | **2d** |
| **C-1** | 62.8742 | 62.8653 | 62.6623; 62.8305 | 62.6659; 62.8395 | CH | CH | CH; CH | CH; CH |
| **C-2** | 61.0642 | 61.0646 | 60.8687; 61.0269 | 60.8677; 61.0368 | CH | CH | CH; CH | CH; CH |
| **C-3** | 13.9854 | 13.9829 | 10.3556; 13.9821 | 10.3713; 13.9829 | $CH_3$ | $CH_3$ | $CH_3$; $CH_3$ | $CH_3$; $CH_3$ |
| **$CH_3$-Ts** | 30.4291 | 30.4186 | 30.4684; 31.0392 | 30.4583; 31.0481 | $CH_3$ | $CH_3$ | $CH_3$; $CH_3$ | $CH_3$; $CH_3$ |
| **Ci** | 136.9013 | 136.8974 | 136.3145; 136.9113 | 136.3163; 136.9189 | CH | CH | CH; CH | CH; CH |
| **Cp** | 129.2722 | 129.2734 | 128.8705; 129.2668 | 128.8715; 1292679 | CH | CH | CH; CH | CH; CH |
| **Co** | 129.6382 | 129.6422 | 128.9950; 129.6301 | 128.9975; 129.6313 | CH | CH | CH; CH | CH; CH |
| **Cm** | 127.7558 | 127.7554 | 127.4584; 127.7645 | 127.4585; 127.7643 | C | C | C; C | C; C |

*1.2.1.3. Analysis of COSY and HSQCED spectra*

The COSY spectra for obtained compounds together with indicated correlations are given in Figure 17 and Figure 18. The proton spectrum is plotted along each axis. The COSY spectrum shows a distinct set of spots on a diagonal, with each spot corresponding to the same peak on each coordinate axis. Lines have been drawn to identify the correlations. The COSY spectra for **2c** and **2d** are more complicated. Although, the same correlation of corresponding protons can be seen, it also shows that each of proton signal is doubled indicating the presence of two stereoisomers of CEP. $^{1}$H-$^{13}$C HSQC NMR spectra were obtained to determine the direct carbon-proton bonds. Application of a $^{1}$H-$^{13}$C HSQC pulse sequence allows the user to overcome the broad overlapping peaks in a 1D proton spectra by dispersing the signals into

the second $^{13}C$ dimension. $^{1}H$-$^{13}C$ HSQC NMR spectra together with the interpretation are given in Figure 19 and Figure 20. Lines have been drawn and each hydrogen and carbon has been marks in order to facilitate the identification of correlations.

NMR data obtained for the analytes of interest leads to the conclusion that:

- sample **2a** and **2b** are single stereoisomers of chloroephedrine;
- product **2c** contains two stereoisomers of chloroephedrine and one of which is identical to sample **2a**;
- product **2d** contains two stereoisomers of chloroephedrine and one of which is identical to sample **2b**.

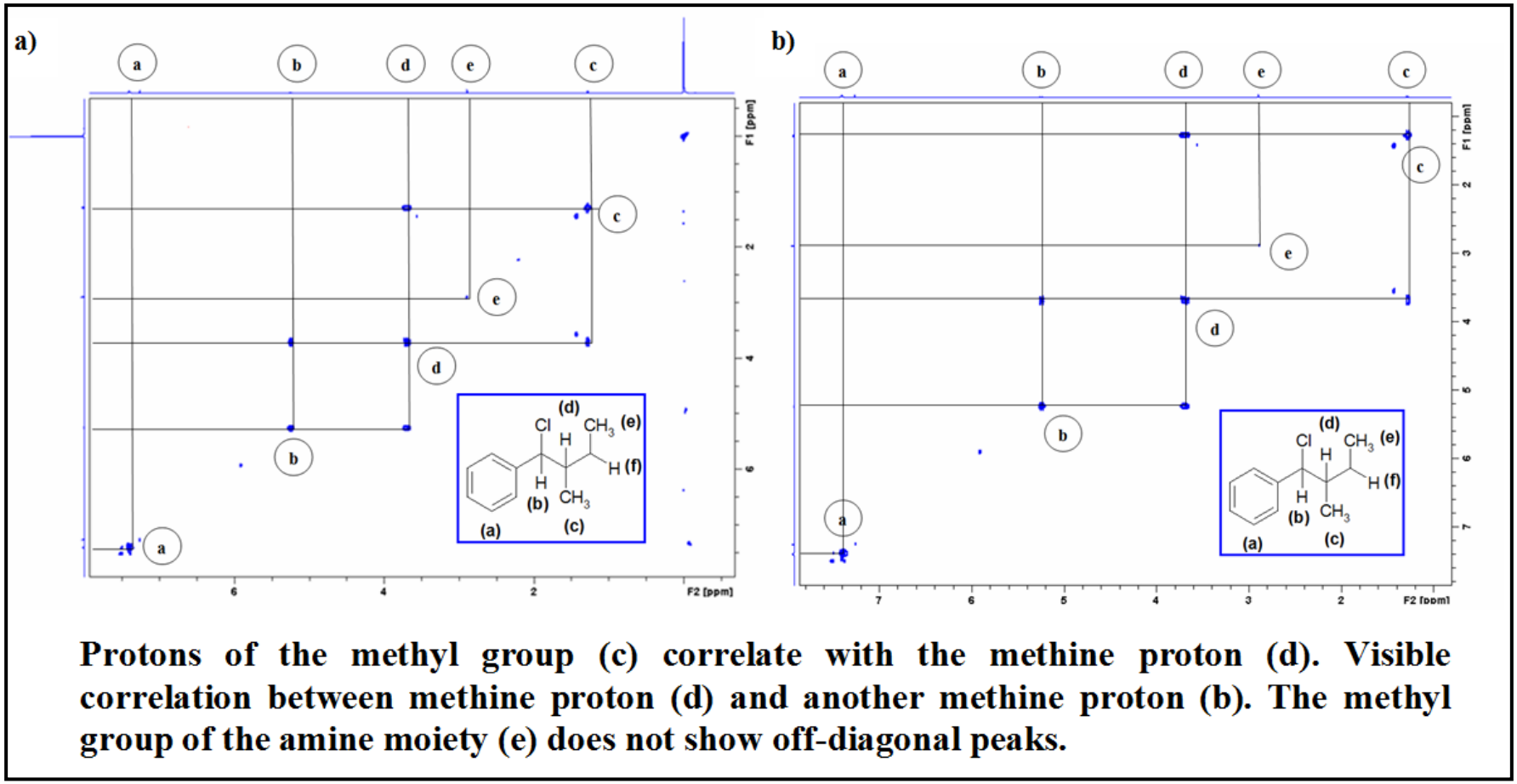



**Figure 17.** COSY NMR spectra of chloro-analog obtained from a). (-)EP and b). (+)EP

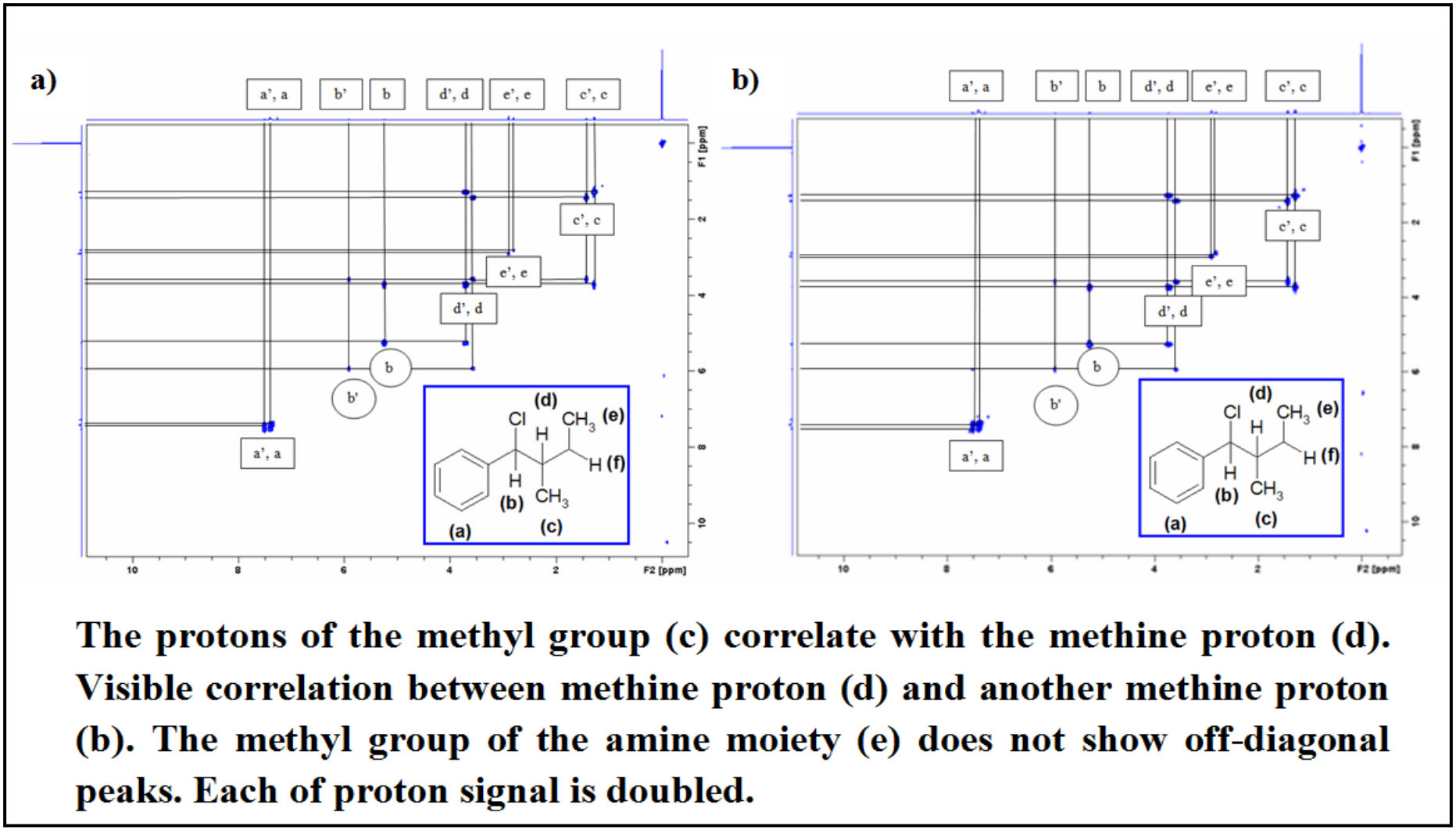



**Figure 18.** COSY NMR spectra of chloro-analog obtained from a). (+)PSEP and b). (-)PSEP

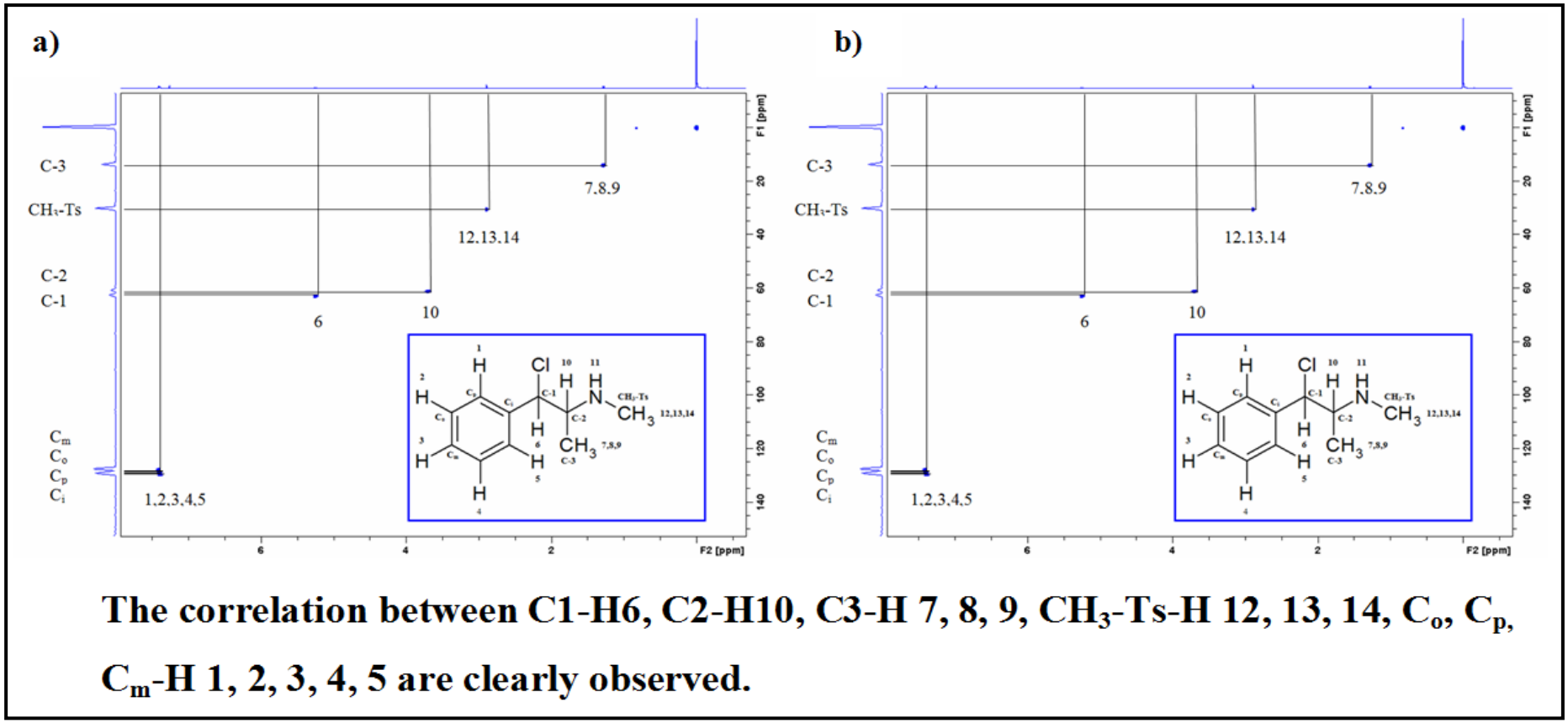



**Figure 19.** HSQC NMR spectra of chloro-analog obtained from a). (-)EP and b). (+)EP

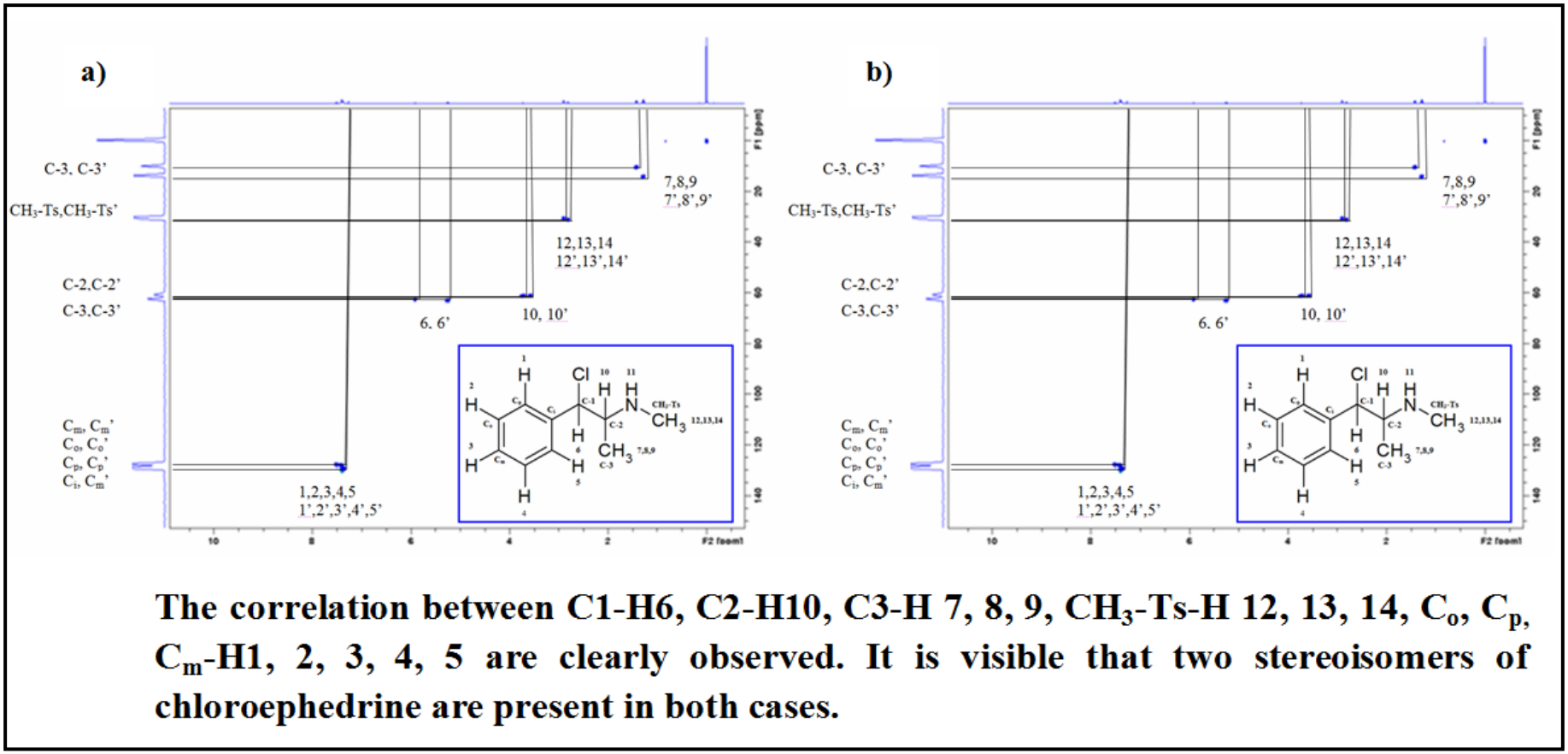



**Figure 20.** HSQC NMR spectra of chloro-analog obtained from a). (+)PSEP and b). (-)PSEP

*1.2.1.4. Analysis of 1D SELNOESY NMR spectra*

1 D SELNOESY NMR analysis of analytes of interest was performed to determine the mutual position and distance of protons in space. A SELNOESY experiment irradiates a selected signal and uses the appearance of other signals to indicate through-space interactions. *SELNOESY* experiment was run with the selected signals at:

- 2a: δ 2.9258 (s, J=3.1022, 3H), 1.2582-1.3237 (d, J=3.0089, 3H), 5.2554-5.2913 (d, J=1.0000, 1H);
- 2b: δ 2.9464 (s, J=5.3905, 3H), 1.2561-1.3719 (d, J=5.7363, 3H), 5.2584-5.2933 (d, J=1.7309, 1H);
- 2c: δ 2.8455 (s, J=7.3001, 3H), 1.2441-1.2734 (d, 6.0356, 3H), 5.2348-5.3688 (d, J=1.8955, 1H);
- 2d: δ 2.9387 (s, J=7.3021, 3H), 1.2242-1.3388 (d, 7.2440, 3H), 5.2849-5.2888 (d, J=2.2965, 1H).

(H-e, H-c and H-b, respectively in each sample) irradiated.

The correlation between appropriate protons of sample manufactured from (-)EP are as follows:

- large NOE correlation between H-e and H-c, large correlation between H-e and H-d, and week correlation between H-e and H-b, H-e proton close to amine group;
- strong NOE correlation between H-c and H-d, large correlation between H-c and H-e, and H-b, and weak correlation between H-c and H-a;
- strong NOE correlation between H-b and H-a, large correlation between H-b and H-c, weak correlation between H-b and H-e, and any NOE correlation between H-b and H-d.

These results allow the conclusion that the compound synthesized from (-)EP was (1S,2S)-(+)-chloropseudoephedrine.

Analysis of SELNOESY NMR spectra lead to the following conclusions:

- the conversion of (1R, 2S)-(-)-EP to the chloro-analogs occurred with inversion of configuration around the α atom to give one enantiomer, (1S, 2S)-(+)-CPSEP in accordance with $S_N2$ mechanism,
- the reaction of (1S, 2R)-(+)-EP with thionyl chloride also occurred in accordance with the $S_N2$ mechanism to give (1R, 2R)-(-)-CPSEP. Figure 21 presents the structure of obtained products.

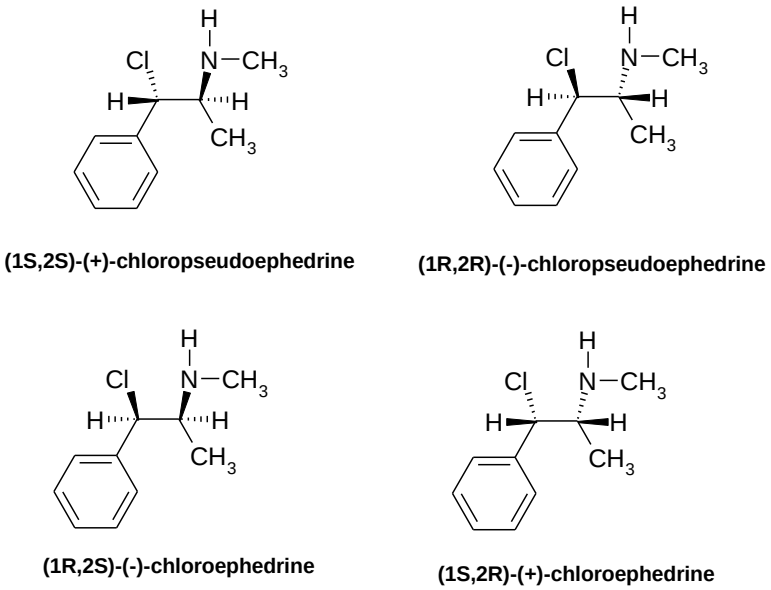



**Figure 21.** The structure of products obtained from appropriate precursors of MAMP

Taking into consideration all the NMR experiments it can be deducted that sample manufactured from pseudoephedrines contain two stereoisomers. SELNOESY NMR experiment allow to conclude that these compounds are diastereoisomers. For example, product obtained from (1S, 2S)-(+)-PSEP consists on:

- (1S, 2S)-(+)-CPSEP (data the same as for product obtained from (-)EP), and
- (1R, 2S)-(-)-CEP, because the following correlations were observed:
    - strong NOE correlation between H-e and H-c, large correlation between H-e and H-d, H-e proton close to amine group;
    - strong NOE correlation between H-c and H-d, large correlation between H-c and H-b, and weak correlation between H-c and H-e;
    - strong NOE correlation between H-b and H-a, large correlation between H-b and H-d, and weak correlation between H-b and H-c.

Analysis of SELNOESY NMR spectra lead to the following conclusions:

- the conversion of (1S, 2S)-(+)-PSEP to the chloro-analogs occurred with inversion and retention of configuration around the α atom to give two stereoisomers, (1R, 2S)-(-)-CEP and (1S, 2S)-(+)-CPSEP, respectively in accordance with $S_N2$ and $S_Ni$ mechanisms, and
- the reaction of (1R, 2R)-(-)-PSEP with thionyl chloride also occurred in accordance with $S_N2$ and $S_Ni$ mechanism to give diastereoisomers: (1S, 2R)-(+)-

CEP and (1R, 2R)-(-)-CPSEP, respectively. Figure 21 presents the structure of obtained products.

To confirm results obtained in NMR experiments, the GC-MS analysis was carried out.

### *1.2.2. Achiral GC-MS analyses of chloro-intermediates of MAMP*

GC-MS analysis was carried out for qualitative analysis of manufactured samples. Standards of MAMP, its precursors and synthesized compounds were trifluoroacetylated and analyzed individually. Retention times and mass-to-charge ratios are presented in Table 18. Chromatograms and mass spectra obtained for **2a** and **2d** are shown in Figure 22 and Figure 23.

**Table 18.** Retention time, peak area and mass-to-charge ratio of standards and chloro-derivatized of ephedrine

| | | $t_R$ [min] | m/z | Peak Area |
|---|---|---|---|---|
| **Standards TFAA** | MA | 10.831 | 154, 118, 110, 91, 69 | 678737018 |
| | EP | 10.378 | 154, 115, 110, 91, 69 | 337271504 |
| | PSEP | 11.061 | 154, 115, 110, 91, 69 | 336998756 |
| **Sample derivatized** | 2a | 11.204 | 121, 91, 77 | 1656218 |
| | | 11.898 | 154, 125/127, 117, 110, 91, 69 | 4408169 |
| | | 12.805 | 154, 125/127, 117, 110, 91, 69 | 428647672 |
| | 2b | 11.201 | 154, 121, 91, 77 | 2316076 |
| | | 11.896 | 154, 125/127, 117, 110, 91, 69 | 4858263 |
| | | 12.794 | 154, 125/127, 117, 110, 91, 69 | 356745109 |
| | 2c | 11.202 | 154, 121, 91, 77 | 9300234 |
| | | 11.905 | 154, 125/127, 117, 110, 91, 69 | 26509987 |
| | | 12.783 | 154, 125/127, 117, 110, 91, 69 | 106954998 |
| | 2d | 11.202 | 154, 121, 91, 77 | 922108 |
| | | 11.899 | 154, 125/127, 117, 110, 91, 69 | 26319547 |
| | | 12.760 | 154, 125/127, 117, 110, 91, 69 | 107455753 |

After analysis of GC-MS spectra it can be deducted that all of the batches contained two diastereomers, while NMR confirmed that mixture of diastereomers was presented in samples obtained from PSEPs. Therefore, the chlorination of EP enantiomers does not follow $S_N2$ completely as was concluded after NMR analysis, and in fact, a mixture of CPSEP and CEP was determined, implying a mixture of $S_N2$ and $S_Ni$. Although, the chlorination of PSEP enantiomers also occurred in accordance with $S_N2$ and $S_Ni$ mechanism to give mixture of CEP and CPSEP, the ratio of distereomers in the obtained products was depended on precursors used for the synthesis. And so, the conversion of ephedrine enantiomers to the chloro-analogs

occurred with inversion and retention of configuration around the α carbon atom to give mixture of: 99 % CPSEP and 1 % CEP. Conversion of PSEP enantiomers to the chloro-analogs occurred also with inversion and retention of configuration around the α carbon atom to give mixture of: 20 % CEP and 80 % CPSEP, in accordance with $S_N2$ and $S_Ni$ mechanisms. The ratio of these diastereomers is different comparing the results obtained in the research with literature values [28]. This may be due to the differences in the condition of the performed reactions (differences in the amount of reagents used, temperatures, time of synthesis, type of recrystalization). Moreover, the GC-MS data shows that one unidentified product was formed during synthesis (the mass spectral base peak of m/z 121).

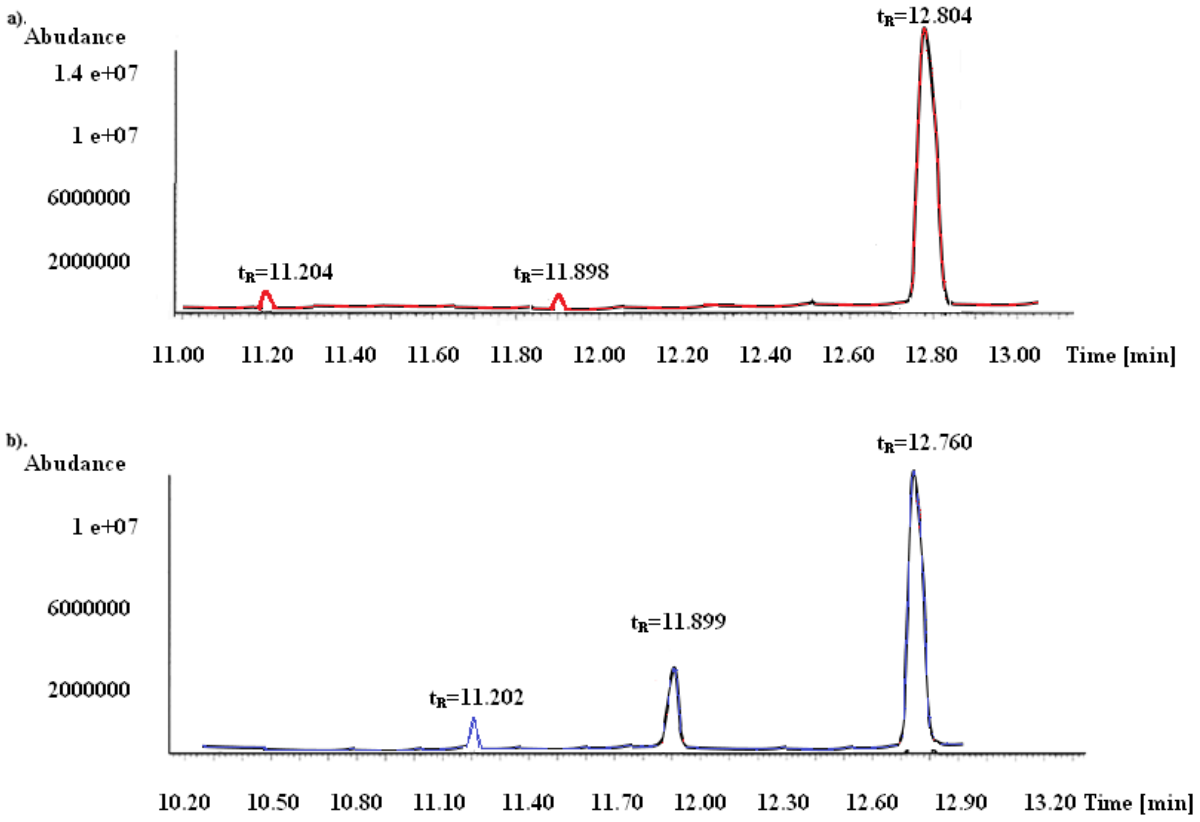



**Figure 22.** Chromatograms of analyzed samples: a). sample no 2a; b). sample no 2d

Application of both techniques, NMR and GC-MS, allowed the determination of conformation and configuration of synthesized chloro compounds, important and unavailable commercially chloro intermediates of MAMP synthesized by Emde method. The results are published in Analytical Chemistry journal [24].

NMR spectroscopy is one of the most powerful non-destructive analytical techniques that provides direct information on the structure of compounds, but is not as sensitive as the GC-MS technique, as can been seen with detection of the 1 % diastereomeric impurity produced from EP enantiomers by GC-MS. Because 2-D NMR is needed to fully elucidate

the stereoisomers, both techniques were necessary to determine chiral composition of synthesized samples.

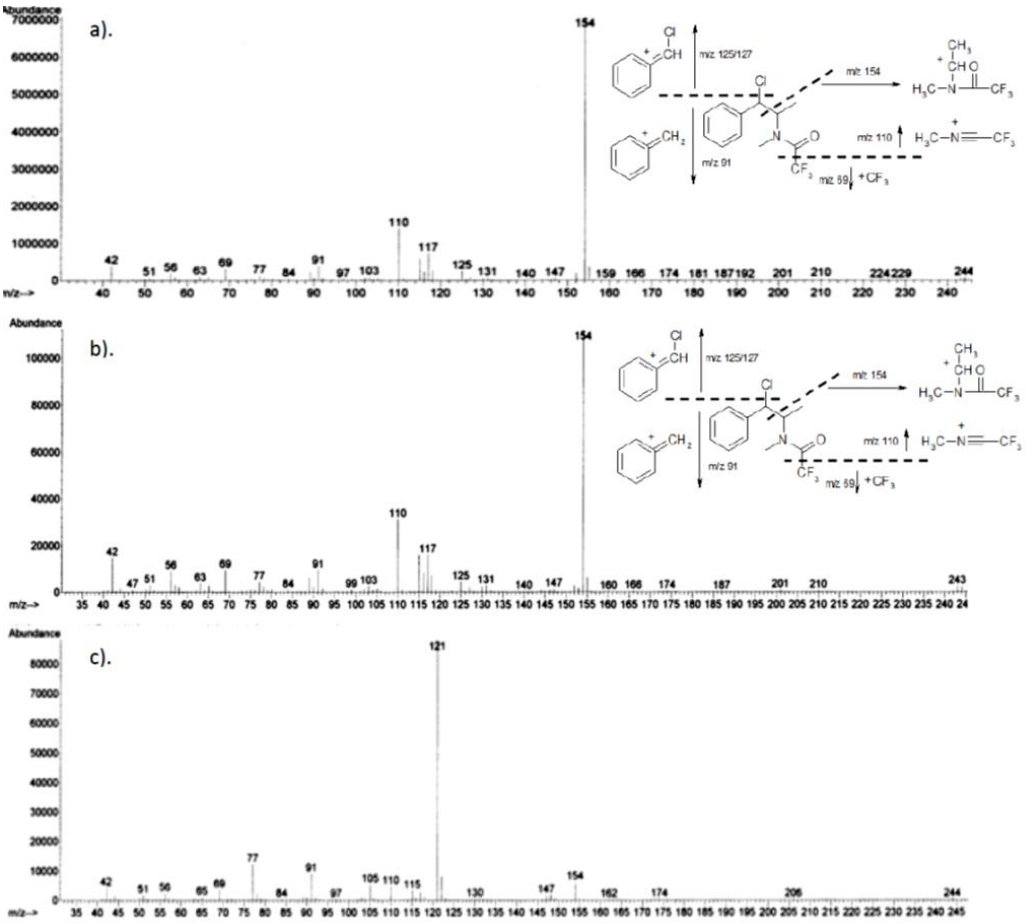



**Figure 23.** Mass spectra of compound determined in 2a-TFAA. a). (+)CEP-TFAA; b). (-)PSEP-TFAA, c). unidentified-TFAA

## 1.3. Assessment of the greenness of the synthesis

### *1.3.1. Green metrics*

A comparative quantitative assessment of the "greenness" of the new procedure proposed here, in comparison to the conventional one, was conducted using green chemistry metrics. For this the Green Chemistry Assistant [107] as well as our calculations were used. Due to conditions used in modified method (without dissolution of precursor step) particular attention was paid to environmental factor (e-factor), mass intensity (MI) and solvent and catalyst environmental impact parameter (f).

Yield and green chemistry metrics were calculated and summarized in Table 19.

**Table 19.** Yield and green chemistry metrics of the compounds synthesized

| Entry | Compound | Yield [%] | AE [%] | AE, exp [%] | AE' [%] | RME | E-factor | MI | f |
|---|---|---|---|---|---|---|---|---|---|
| **Typical procedure** | | | | | | | | | |
| 1 | 2a | 10.07 | 69 | 15 | 7.28 | 1.00 | 2562.43 | 2563.43 | 2463.42 |
| 2 | 2b | 36.27 | 69 | 17 | 24.66 | 3.65 | 717.89 | 718.89 | 691.52 |
| **Modified procedure** | | | | | | | | | |
| 3 | 2c | 54.38 | 69 | 31 | 37.52 | 5.12 | 171.55 | 172.55 | 153.03 |
| 4 | 2d | 42.70 | 69 | 29 | 29.46 | 4.93 | 185.72 | 187.38 | 167.11 |

Clearly, focusing on chemical yields of reactions using the modified method, demonstrated an improved yield of 54 % and 43 % - compared to 10 % and 36 % yields from the conventional reaction. Moreover, in order to include the chemical yield, the selectivity towards the desired product and the mass off all reagents and solvents, etc. used in the tested reactions, the mass intensity parameter (MI), was considered. The comparison based on this metric showed that MI values of conventional method used for the synthesis of **2a, 2b** were 10 times higher (synthesis from (-)EP) than in the case of examined synthesis what also reveals the "greener" nature of modified method. The overall set of synthesizes was also evaluated through experimental atom economy (AE, exp), which is based on the actual quantities of reagents used in the experiment. In the case of modified reactions the AE, exp was two times better than in conventional synthesis. Moreover, environmental factor (e-factor), solvent and catalyst environmental impact parameter (f) were also few times lower and the data clearly indicate that if chloroform was excluded from reactions, syntheses were more friendly for environmental. Therefore, the modified method seems to be, with respect to the usage of solvents and also the waste produced, more advantageous that the conventional method. Moreover, the reactions examined here using the solvent free method were carried out in a shorter time. Instead of three hours reaction under reflux at 70 $^{o}$C, boiling water bath was replaced by a time of 15-20 mins. The rapidity of the process is important in a green chemistry procedure context in terms of reduction of energy consumption, thus, shorter time of synthesis is another advantages of the modified method. Thus, this is evident that the reduction of chloroform use is of great importance for the development of environmentally more benign process.

The graphical results shown in Figure 24 also suggest that the performance indicators of our innovative methods are considerably improved from a green chemistry point of view. This figure gives an overview at the overall process level. The figure graphically shows the actual mass amounts of the all materials used in the overall process, including solvents as well

as final product, co-products and by-products. The graph also reveals quantities of excess reactants have been used and how much of the reagents have been lost. Thus, comparing graphical overall process for **2a, 2b** synthesis with graphs obtained for **2c, 2d** it can be concluded that larger amounts of solvent and other reagents have been used in the case of **2a** and **2b** synthesis and in the same time larger quantities reagents have been lost. In each of cases big aliquots of excess reactants have been used however much more in the case of **2a** and **2b** synthesis. These differences again proved that modified method is more friendly for the environment.

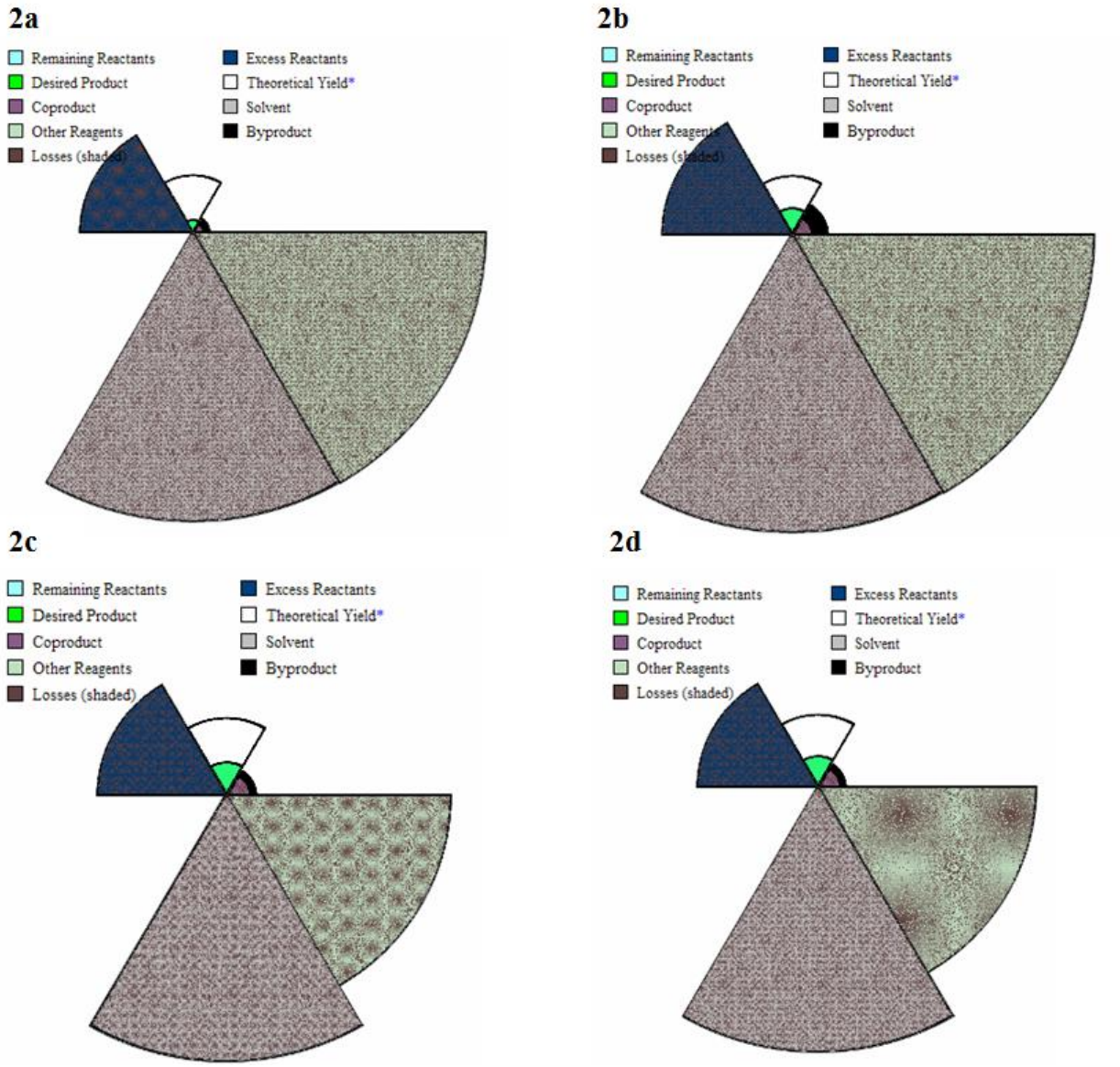



**Figure 24.** Graphical overall process for 2a, 2b, 2c and 2d obtained by the Green Chemistry Assistant

### *1.3.2. EcoScale*

EcoScale is a post synthesis analysis tool development by Aken et al. [108]. This tool evaluates the quality of the organic preparation based on yield, cost, safety, conditions and ease of workup/purification. Table 20 presents results obtained for **2a** and **2c** as an example.

**Table 20.** The penalty points for **2a** and **2c**

| **Parameter** | | **Penalty points** | |
|---|---|---|---|
| Yield: (10.07 % for **2a**; 54.38 % for **2c** | | 45 | 22 |
| Price of reaction components (to obtain 10 mmol of end product) | | 0 | 0 |
| Safety | (-)-ephedrine | 0 | 0 |
| | Chloroform (T, F,N) | 15 | - |
| | Thionyl chloride (N, F) | 10 | 10 |
| | Diethyl ether (E, F) | 15 | 15 |
| | Acetone | 0 | 0 |
| | Methanol (T, F) | 10 | 10 |
| Technical setup | Glove box | 3 | 3 |
| Temperature/Time | Heating: for **2a** > 1h, for **2c** < 1 h | 3 | 2 |
| Workup and purification | Cooling to room temperature | 0 | - |
| | Adding solvent | 0 | - |
| | Simple filtration | 0 | 0 |
| | Crystallization and filtration | 1 | 1 |
| | Sublimation | 3 | 3 |
| Penalty points total | | 105 | 66 |
| EcoScale (100 – penalty points) | | -5 | 34 |

The sum of all penalty points for **2a** obtained in classical synthesis is 105, which gives a total score of -5 on the EcoScale (an inadequate synthesis). The sum of all penalty points for **2c** obtained in modified synthesis is 66, which gives a total score of 34 on the EcoScale which is also classified as an inadequate synthesis however is less than the 39 penalty points from a typical synthesis. In both method many penalty points are given because of the hazardous nature of reagents used.

This simple tool also shows that modified method to synthesis of chloro-analogs of ephedrine is more friendly for environment however is still an inadequate synthesis which means that further development is required.

## 2. Enantioseparation of methylamphetamine, its chloro intermediates and impurities

### 2.1. Selection of the CSP and initial chromatography conditions

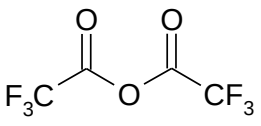
Among the current analytical methods for chiral analysis, the most popular is GC in combination with MS detection. As was mentioned in Section I, chiral separations are carried out directly on CSPs or indirectly using achiral SPs after derivatization with enantiomerically pure reagents to form diastereoisomers [109, 110]. After the literature review [48, 111-114], in this study two γ-cyclodextrin phases were taken into consideration: Chiraldex G-TA and

Chiraldex G-PN. Despite the fact that 2,6-di-O-pentyl-3-trifluoroacetyl derivative of γ-cyclodextrin phase (G-TA) was shown to be the most effective for the pharmaceutical industry, especially for the analysis of chiral intermediates and drug studies in various stages of clinical trials, it does not result in the enantioseparation of MAMP, EP, PSEP and its chloro-analogs after derivatization with any reagent tested. Superior results were obtained with the 2,6-di-O-pentyl-3-propionyl derivative of γ-cyclodextrin phase (G-PN), and therefore, the Chiraldex G-PN column was chosen for further research.

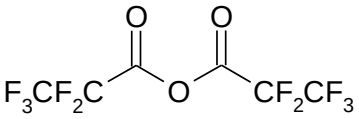
Initially the temperature program method outlined in the application note from the column manufacturer for the GC analysis of MAMP–TFA was investigated. The initial column temperature of 100 °C was held for 1 min, followed by temperature ramp of 5 °C/min to 140 °C which was kept for 20 min. However, because splitless mode has been used during research, the initial temperature had to be reduced due to boiling temperature of solvent (ethyl acetate boiling point: 77 °C). After further research, the final temperature was also changed to 145 °C because four additional compounds and IS have been analysis (enantiomers of CEP and CPSEP). A final hold time of 20 min at 145 °C was sufficient to ensure the MAMP, EP and PSEP enantiomers eluted, although this was increased to 25 min when the trifluoroacetylated TFMPA was included as internal standard. The final hold time was reduced after further analysis (Table 13).

### 2.2. Optimization of derivatization procedure

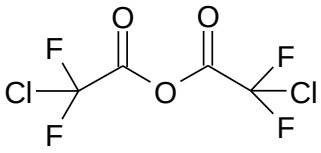
Three different commercially available acylating reagents – TFAA, PFPA, and CDFA (Figure 25) – were tested for analyte derivatization under the conditions listed in Table 12.




*Trifluoroacetic anhydride*




*Pentafluoropropionic anhydride*




*Chlorodifluoroacetic anhydride*

**Figure 25.** Structures of derivatization reagents tested

TFAA is the most reactive and most volatile of the three anhydrides. No acid byproducts are formed in derivatization reactions with TFAA [115]. The choice of these reagents was based on differences in their reactivity, selectivity and the nature of the acylation by-products as well as on literature data. The first and second reagents were extensively

applied to the determination of amphetamine-type stimulants (AMs) in different matrices [61, 116-118] and for chiral separation of methylamphetamine and its precursors [50, 119]. Despite the fact that CDFA is not as popular as TFAA and PFPA it has been described as a suitable reagent for amphetamine type stimulants determination in several articles [120].

In this study, the comparison of efficiencies of analytes derivatization using TFAA, PFPA and CDFA was made. According to our knowledge, no derivatization study for some of these compounds of interest in this research has yet been published. To this end, each of the above derivatizing reagents (DRs) was first tested during sixteen experiments performed at 50, 55, 60 and 65 $^{o}$C for 10, 15, 20 and 25 min in which the derivatizing reagent and ethyl acetate (1:1, v/v) were used (Table 12, experiments 1A–4L). The concentrations of the analytes of interest and IS were constant in each experiment. Afterwards, the effectiveness of derivatization under the different reaction conditions, expressed by relative response factors (RRFs), was calculated for the compounds of interest. Because the IS is inert to derivatization, higher RRFs indicated an increase in reaction effectiveness and this knowledge was used to compare the effectiveness of the derivatization process. During the optimization of derivatization, all determinations were carried out using the same GC-MS conditions (only the full analysis time is changed for CDFA-derivatives). The mean RRFs (n = 3) calculated from the GC-MS results of experiments of 1A–4L for the tested analytes are presented in Table 21. The relative standard deviations (RSD) of all RRFs were < 3.3 %.

**Table 21.** Relative response factors (RRFs) (mean value; n = 3; RSD < 3.3 %) obtained from the acylation of target compounds under the chromatographic conditions of experiments 1A–4L, as described in Table 12

| DR | **TFAA** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $No_{experiment}$ | 1A | 2A | 3A | 4A | 1B | 2B | 3B | 4B | 1C | 2C | 3C | 4C | 1D | 2D | 3D | 4D |
| Compound | *RRF parameters (mean value) (n = 3) [x $10^{-3}$]* | | | | | | | | | | | | | | | |
| (-)EP | 267 | 554 | 527 | 482 | 416 | 854 | 850 | 843 | 424 | 813 | 789 | 778 | 357 | 748 | 732 | 589 |
| (+)EP | 371 | 928 | 934 | 925 | 478 | 1576 | 1563 | 1319 | 603 | 1545 | 1552 | 1540 | 431 | 1216 | 1211 | 1121 |
| (-)PSEP | 413 | 949 | 945 | 941 | 379 | 1673 | 1679 | 1589 | 871 | 1634 | 1619 | 1559 | 840 | 1200 | 1194 | 1179 |
| (+)PSEP | 88 | 89 | 94 | 91 | 88 | 194 | 192 | 187 | 99 | 131 | 121 | 117 | 90 | 165 | 178 | 151 |
| (-)CEP | 113 | 292 | 365 | 35 | 134 | 521 | 538 | 535 | 407 | 460 | 489 | 451 | 393 | 611 | 552 | 436 |
| (+)CEP | 457 | 989 | 986 | 974 | 510 | 1712 | 1700 | 1621 | 818 | 1689 | 1677 | 1602 | 832 | 1227 | 1238 | 1206 |
| (-)CPSEP | 285 | 297 | 411 | 367 | 289 | 772 | 767 | 720 | 300 | 756 | 760 | 741 | 338 | 689 | 599 | 463 |
| (+)CPSEP | 287 | 650 | 662 | 631 | 349 | 873 | 868 | 841 | 654 | 879 | 861 | 857 | 439 | 906 | 882 | 825 |
| (-)MAMP | 82 | 107 | 109 | 105 | 249 | 326 | 315 | 331 | 172 | 182 | 157 | 137 | 140 | 167 | 149 | 143 |
| (+)MAMP | 127 | 915 | 896 | 889 | 737 | 1098 | 1092 | 954 | 816 | 1054 | 1051 | 1047 | 779 | 1003 | 961 | 937 |
| DR | **PFPA** | | | | | | | | | | | | | | | |
| $No_{experiment}$ | 1E | 2E | 3E | 4E | 1F | 2F | 3F | 4F | 1G | 2G | 3G | 4G | 1H | 2H | 3H | 4H |
| Compound | *RRF parameters (mean value) (n = 3) [x $10^{-3}$]* | | | | | | | | | | | | | | | |
| (-)EP | 82 | 85 | 101 | 99 | 263 | 286 | 539 | 507 | 286 | 335 | 645 | 623 | 275 | 288 | 591 | 574 |
| (+)EP | 83 | 88 | 102 | 100 | 287 | 409 | 543 | 499 | 405 | 452 | 699 | 695 | 289 | 346 | 569 | 517 |
| (-)PSEP | 98 | 110 | 125 | 123 | 655 | 669 | 775 | 724 | 814 | 815 | 856 | 855 | 754 | 762 | 846 | 848 |
| (+)PSEP | 61 | 84 | 92 | 89 | 85 | 93 | 119 | 118 | 88 | 126 | 188 | 187 | 86 | 99 | 153 | 139 |

**Table 21.** Cont.

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (-)CEP | 51 | 67 | 90 | 82 | 263 | 263 | 420 | 434 | 288 | 446 | 711 | 700 | 273 | 320 | 584 | 540 |
| (+)CEP | 106 | 112 | 129 | 124 | 686 | 724 | 814 | 775 | 744 | 815 | 876 | 874 | 775 | 828 | 853 | 849 |
| (-)CPSEP | 44 | 53 | 84 | 75 | 253 | 288 | 443 | 400 | 265 | 287 | 449 | 448 | 269 | 329 | 639 | 613 |
| (+)CPSEP | 64 | 86 | 104 | 103 | 342 | 415 | 595 | 569 | 442 | 472 | 737 | 723 | 414 | 447 | 666 | 672 |
| (-)MAMP | 46 | 83 | 95 | 87 | 96 | 120 | 180 | 155 | 139 | 189 | 196 | 195 | 105 | 185 | 191 | 190 |
| (+)MAMP | 130 | 170 | 184 | 183 | 702 | 728 | 826 | 827 | 750 | 801 | 858 | 855 | 776 | 812 | 847 | 837 |
| DR | **CDFA** | | | | | | | | | | | | | | | |
| $No_{experiment}$ | 1I | 2I | 3I | 4I | 1J | 2J | 3J | 4J | 1K | 2K | 3K | 4K | 1L | 2L | 3L | 4L |
| Compound | *RRF parameters (mean value) (n = 3) [x $10^{-3}$]* | | | | | | | | | | | | | | | |
| (-)EP | - | - | - | - | 52 | 54 | 60 | 59 | 255 | 267 | 283 | 281 | 538 | 618 | 774 | 771 |
| (+)EP | - | - | - | - | 56 | 62 | 70 | 69 | 277 | 279 | 339 | 332 | 546 | 651 | 813 | 777 |
| (-)PSEP | - | - | - | - | 87 | 97 | 99 | 98 | 682 | 739 | 833 | 832 | 805 | 821 | 864 | 866 |
| (+)PSEP | - | - | - | - | 58 | 63 | 76 | 72 | 228 | 229 | 236 | 235 | 259 | 327 | 660 | 652 |
| (-)CEP | - | - | - | - | 47 | 50 | 66 | 66 | 444 | 450 | 548 | 541 | 679 | 733 | 834 | 835 |
| (+)CEP | - | - | - | - | 103 | 105 | 111 | 111 | 658 | 676 | 738 | 740 | 814 | 815 | 857 | 856 |
| (-)CPSEP | - | - | - | - | 44 | 48 | 74 | 68 | 340 | 445 | 615 | 625 | 692 | 726 | 838 | 831 |
| (+)CPSEP | - | - | - | - | 55 | 57 | 78 | 80 | 414 | 454 | 627 | 620 | 708 | 730 | 845 | 844 |
| (-)MAMP | - | - | - | - | 46 | 49 | 72 | 73 | 234 | 241 | 261 | 257 | 233 | 271 | 295 | 285 |
| (+)MAMP | - | - | - | - | 104 | 106 | 113 | 112 | 770 | 807 | 830 | 829 | 836 | 839 | 881 | 880 |

Analysis of the mass spectra of the products showed that all the compounds were derivatized by TFAA, PFPA and CDFA. Each of the compounds yielded a single derivative under all experimental conditions tested (except experiments numbered 1I-4I where no signals were obtained). Thereafter, CA and PCA were used as multivariate statistical methods in the data interpretation. Information on the formed clusters are presented in Table 22.

**Table 22.** Information on the formed clusters for CA

| **CA of the experimental conditions** | **CA of chemical compounds** |
|---|---|
| Formed clusters for RRFs obtained for acylation of analytes with TFAA | Formed clusters for linkage between the chemical compounds using TFAA as derivatization agent |
| K1 (T2t1, T3t1, T4t1); T1t1 is an obvious outlier | K1 (+EP, -PSEP, +CEP, +MAMP) |
| K2 (T2t2, T2t3, T2t4, T3t2, T3t3, T3t4) | K2 (-CEP, -MAMP, +PSEP) |
| K3 (T1t2, T1t3, T1t4, T4t2, T4t3, T4t4) | K2* (-EP, +CPSEP, -CPSEP) |
| Formed clusters for RRFs obtained for acylation of analytes with PFPA | Formed clusters for linkage between the chemical compounds using PFPA as derivatization agent |
| K1 (T1t1, T1t2, T1t3, T1t4) | K1 (-PSEP, +CEP, +MAMP) |
| K2 (T2t1, T2t2, T3t1, T3t2,T4t1, T4t2) | K2 (-MAMP, +PSEP) |
| K3 (T2t3, T2t4, T3t3, T3t4, T4t3, T4t4) | K2* (-EP, +EP, -CEP, +CPSEP, -CPSEP) |
| Formed clusters for RRFs obtained for acylation of analytes with CDFA | Formed clusters for linkage between the chemical compounds using CDFAas derivatization agent |
| K1 (T1t1 T1t2, T1t3, T1t4) | K1 (-PSEP, +CEP, +MAMP) |
| K2 (T2t1, T2t2, T2t3, T2t4) | K2 (-MAMP, +PSEP) |
| K3 (T3t1, T3t2, T4t1, T4t2) | K3 (-EP, +EP, -CEP, +CPSEP, -CPSEP) |
| K4 (T3t3, T3t4, T4t3, T4t4) | |

### *2.2.1. Chemometric interpretation of RRFs values calculated for TFAA derivatization of analytes*

The clustering of the TFAA matrix by hierarchical agglomerative clustering gave the following results. Hierarchical dendrogram for linkage between the experimental conditions using TFAA as derivatization agent is presented in Figure 26.

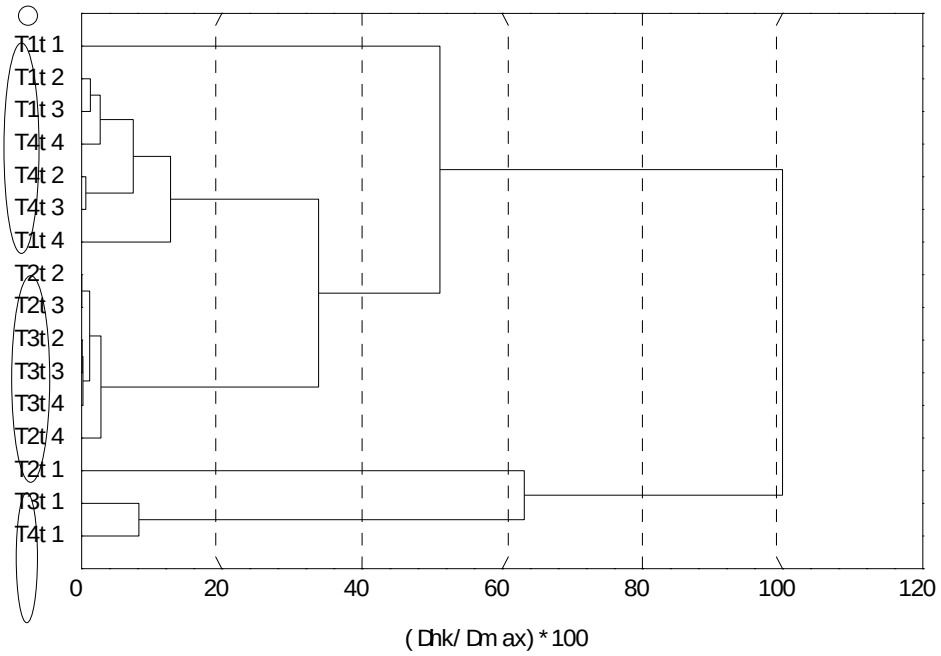



**Figure 26.** Hierarchical dendrogram for linkage between the experimental conditions using TFAA as derivatization agent

Apparently, short times of treatment despite the temperature conditions are not favorable for the experimental scheme with TFAA since the average values for RRFs in cluster 1 show low values as the lowest values belong to the outlier ***T1t1***. On contrary, the experimental conditions of cluster 2 (temperatures in the interval *55 – 60 $^{o}C$* and times in the interval *15 – 25* min) deliver the highest values of RRFs. It seems that this is the optimal range of temperature and time of treatment with TFAA since the interpretation of cluster 3 indicates that temperatures lower that 55 $^{o}$C and higher that 60 $^{o}$C do not improve the RFF values Table 23).

**Table 23.** Average RRF values for clusters 1, 2, and 3 with all compounds (TFAA)

| | **(-)EP** | **(+)EP** | **(-)PSEP** | **(+)PSEP** | **(-)CEP** | **(+)CEP** | **(-)CPSEP** | **(+)CPSEP** | **(-)MAMP** | **(+)MAMP** |
|---|---|---|---|---|---|---|---|---|---|---|
| Outlier | 267 | 371 | 413 | 88 | 113 | 457 | 285 | 287 | 82 | 127 |
| K1 | 399 | 504 | 697 | 92 | 311 | 720 | 309 | 481 | 187 | 777 |
| K2 | 821 | 1516 | 1626 | 157 | 499 | 1667 | 753 | 863 | 241 | 1049 |
| K3 | 605 | 1056 | 1068 | 128 | 382 | 1100 | 471 | 759 | 130 | 934 |

From the input data set it can be seen that all single maximal values of RRFs for the single compounds lie in the range of K2: for T2t2 five maximal RRFs are observed ((+)EP, (+)PSEP, (+)CEP, (-)CPSEP, and (+)MAMP); other two maximal values are in the next time level T2t3 ((-)PSEP and (-)CEP) as their values are very close to those registered in T2t2; two more maxima lie in the next time level T2t4 ((-)EP and (-)MAMP) as, again the RRF maximal values are very near to the values obtained by the previous two time levels of T2. It might be concluded that temperature T2 (55 $^{o}$C) is optimal for most of the compounds involved. The only exception is (+)CPSEP showing maximal RRF at T3t2 (higher temperature) but the careful consideration of the RRF values in the vicinity of the maximum (being 879) indicates very low differences to RRF at T2t2 (873) or at T2t3 (868), i.e. within the rage of the experimental error. Thus, even (+)CPSEP could be reliably treated at 55 $^{o}$C.

In Figure 27 the clustering of the compounds is presented as hierarchical dendrogram.

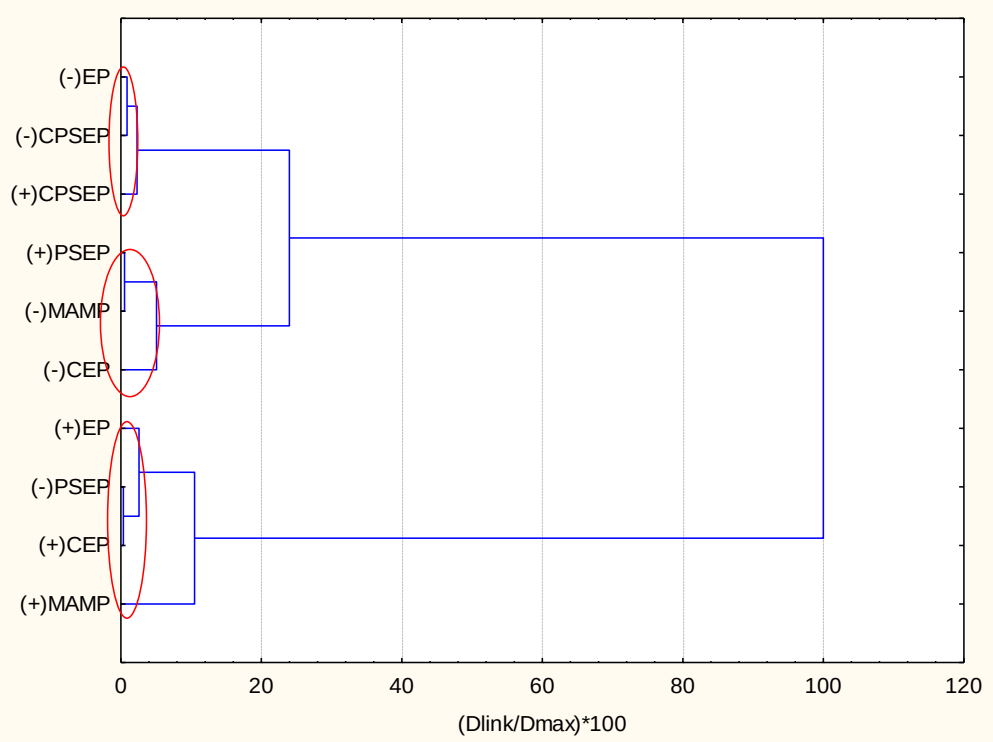



**Figure 27.** Hierarchical dendrogram for linkage between the chemical compounds using TFAA as derivatization agent

For the CA of chemical compounds two distinct clusters were formed (Table 22). This grouping is very logic since the compounds included in K1 have much higher RRFs as cluster average maximal values (above 1000) as compared to those participating in K2 (very low RRFs between 100 and 500) or in K2* (RRFs between 500 and 850).

The application of PCA to the data set of TFAA did not show any specific result. Only one hidden factor was identified which indicates that the experimental conditions do not differ significantly in achieving respective RRFs. In this sense the separation using CA seems to be much more reliable.

### *2.2.2. Chemometric interpretation of RRFs values calculated for PFPA derivatization of analytes*

The clustering of PFPA reagent experimental conditions was performed under the same rules as that of TFAA. Hierarchical dendrogram for linkage between the experimental conditions using PFPA as derivatization agent is presented in Figure 28. The formed clusters are presented in Table 22.

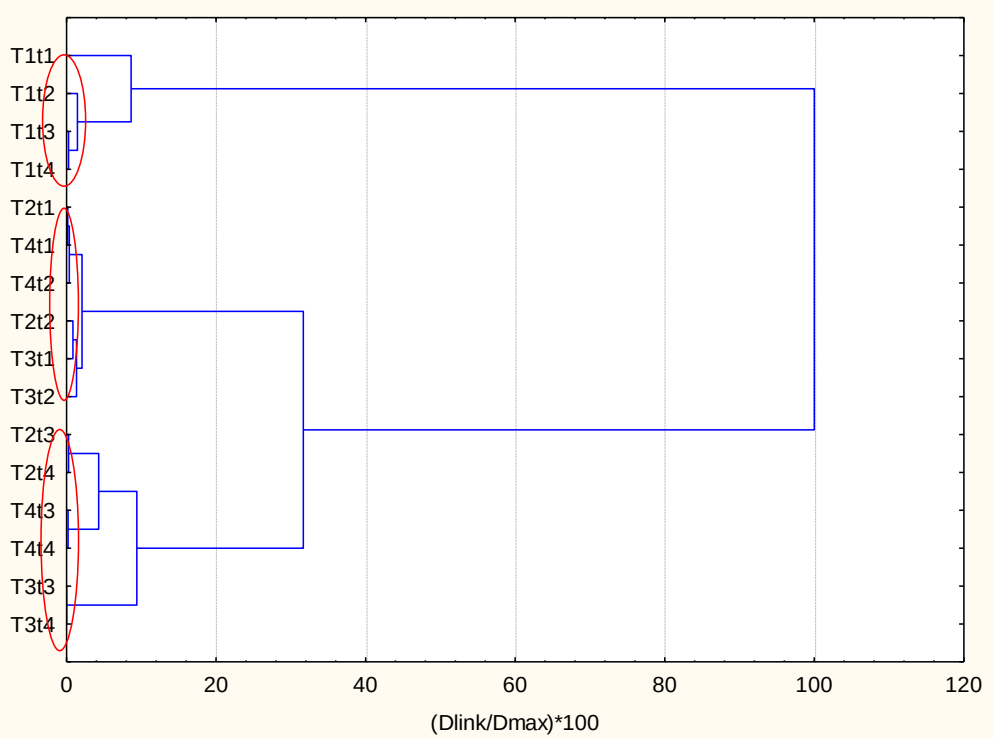



**Figure 28.** Hierarchical dendrogram for linkage between the experimental conditions using PFPA as derivatization agent

In Table 24 the averages of RFFs for all compounds and for each one of the clusters identified are presented.

**Table 24**. Averages of RFFs for all compounds and for each one of the clusters identified

| | (-)EP | (+)EP | (-)PSEP | (+)PSEP | (-)CEP | (+)CEP | (-)CPSEP | (+)CPSEP | (-)MAMP | (+)MAMP |
|---|---|---|---|---|---|---|---|---|---|---|
| **K1** | 92 | 93 | 114 | 82 | 73 | 118 | 64 | 89 | 78 | 167 |
| **K2** | 289 | 365 | 745 | 96 | 309 | 762 | 282 | 422 | 139 | 762 |
| **K3** | 580 | 587 | 817 | 151 | 565 | 840 | 499 | 660 | 184 | 833 |

Obviously, low temperature conditions create low values of RRF, so temperature of 50 $^{o}$C is not favorable. The next temperature conditions (between 55 $^{o}$C and 65 $^{o}$C) ensure higher RRFs and in this case the time parameter is a determining factor (longer times give better responses independently on the temperature of treatment). In general, PFPA works as effective derivatization agent in the temperature interval 55 – 65 $^{o}$C and in the time interval of 20 – 25 min.

Considering the single maxima of RRFs obtained by the experiment (input matrix) it might be concluded that optimal conditions for PFPA are at temperature 60 $^{o}$C in the time interval 20 – 25 min (nine out of ten compounds have maximal RRF values at these experimental conditions). The only exception is (–)CPSEP requiring higher temperature (65 $^{o}$C for 20 min). It has to be mentioned that the RRF values reached by PFPA are significantly lower as compared to those obtained by TFAA both as averages of the clusters formed and as single maxima.

In Figure 29 the clustering of the compounds derivatized by PFPA is presented as hierarchical dendrogram.

As in the case with TFAA two distinct clusters were formed (Table 22). This grouping is very logic since the compounds included in K1 have much higher RRFs (above 800 as maximal values) as compared to those participating in K2 (very low RRFs between 150 and 190 as maximal values) or in K2* (RRFs between 565 and 660 as maximal values). Again, the clustering is based on the absolute values of RRF.

In Table 25 the factor loadings are presented. As seen PC1 explains almost 60 % of the total variance of the system and includes high loadings for experimental parameters of the temperature interval above 50 $^{o}$C. The minond latent factor PC2 indicates the relationship between the experimental parameters with temperature of 50 $^{o}$C. These results correspond entirely to those from cluster analysis where even a finer separation of the experimental conditions was possible.

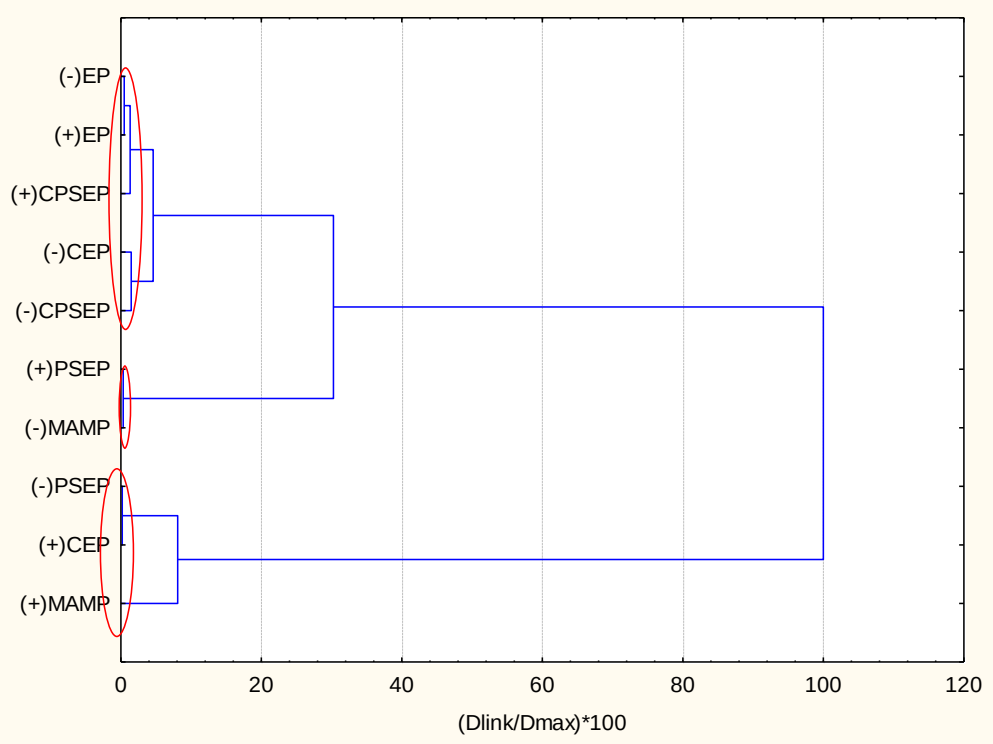



**Figure 29.** Hierarchical dendrogram for linkage between the chemical compounds using PFPA as derivatization agent

**Table 25.** Factor loadings (PFPA)

| | PC 1 | PC 2 |
|---|---|---|
| **T1t1** | 0,50 | 0,82 |
| **T1t2** | 0,25 | 0,97 |
| **T1t3** | 0,36 | 0,92 |
| **T1t4** | 0,36 | 0,92 |
| **T2t1** | 0,79 | 0,60 |
| **T2t2** | 0,81 | 0,57 |
| **T2t3** | 0,89 | 0,44 |
| **T2t4** | 0,88 | 0,47 |
| **T3t1** | 0,80 | 0,56 |
| **T3t2** | 0,82 | 0,53 |
| **T3t3** | 0,92 | 0,28 |
| **T3t4** | 0,92 | 0,29 |
| **T4t1** | 0,78 | 0,59 |
| **T4t2** | 0,78 | 0,58 |
| **T4t3** | 0,95 | 0,26 |
| **T4t4** | 0,94 | 0,29 |
| **Expl. Var %** | 59.1 | 37.3 |

### *2.2.3. Chemometric interpretation of RRFs values calculated for CDFA derivatization of analytes*

The clustering of CDFA agent experimental conditions was performed under the same rules as that of TFAA and PFPA. Hierarchical dendrogram for linkage between the

experimental conditions using CDFA as derivatization agent is presented in Figure 30. The formed clusters are presented in Table 22.

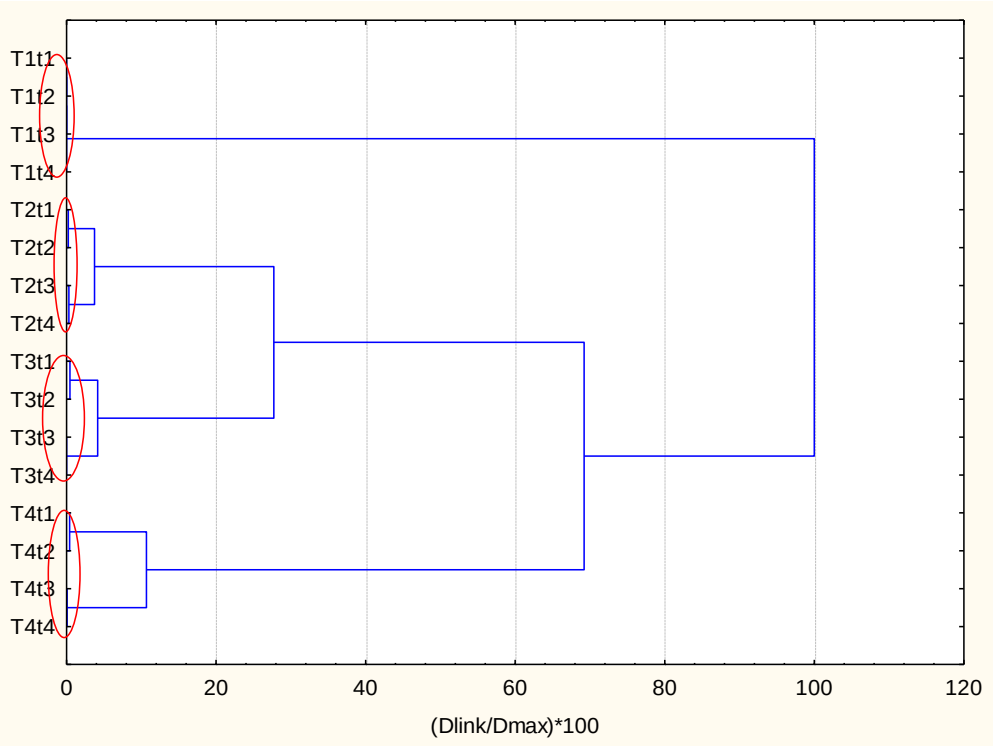



**Figure 30.** Hierarchical dendrogram for linkage between the experimental conditions using CDFA as derivatization agent

In this case a clear separation with respect to temperature is observed. It is important to note that the conditions determined by K1 are out of interest, there is no difference in RRFs values for all four cases involved. Thus, K1 is the cluster shown very low values of RRFs.
Next three clusters separated by the temperature conditions of the experiment show a constant increase in the values of RRF and a distinct temperature dependence – the higher the temperature the higher the RRFs. This trend is illustrated in Table 26 where the averages of RRF for all compounds for each one of the clusters are shown. As already mentioned K1 is out of interest.

**Table 26.** Average RRF values for clusters 2, 3, and 4 with all compounds (CDFA)

| | (-)EP | (+)EP | (-)PSEP | (+)PSEP | (-)CEP | (+)CEP | (-)CPSEP | (+)CPSEP | (-)MAMP | (+)MAMP |
|---|---|---|---|---|---|---|---|---|---|---|
| **K2** | 56 | 64 | 95 | 67 | 57 | 108 | 59 | 68 | 60 | 109 |
| **K3** | 272 | 307 | 785 | 232 | 496 | 703 | 506 | 529 | 248 | 809 |
| **K4** | 675 | 697 | 839 | 457 | 770 | 836 | 772 | 782 | 271 | 859 |

In this case, it is easy to determine that the highest temperature creates highest RRF values. In principle, the RRF values reached by the use of CDFA as derivatization agent are intermediate and lie between those obtained by TFAA and PFPA.

If the single maxima are considered it could be concluded that the optimal conditions with CDFA are at temperature 65 $^{o}$C and time of 20 min. Almost all maxima (nine out of ten compounds) are registered at these conditions and only one is found with time of 25 min.

In Figure 31 the clustering of the compounds derivatized by CDFA is presented as hierarchical dendrogram. Three distinct clusters were formed (Table 22).

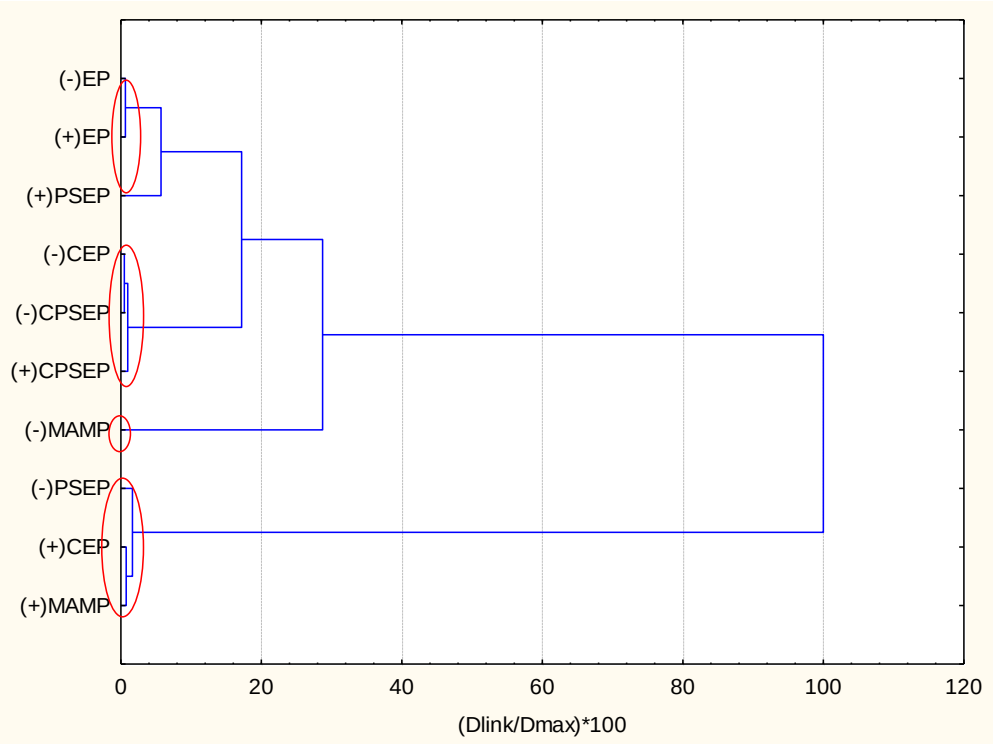



**Figure 31.** Hierarchical dendrogram for linkage between the chemical compounds using CDFA as derivatization agent

The formation of the clusters follows the scheme of separation based on the values of RRFs reached by the separate compounds. Thus, K1 includes compounds giving highest values of RRF (above 800 for the cluster with highest RRFs). K2, on contrary, comprises compounds with lowest RRFs (between 270 and 470) and K3 is for compounds with medium RRF values.

The results of PCA confirm the conclusions of CA. Two latent factors are responsible for explanation of more than 90 % of the total variance. The factor loadings are shown in Table 27. PC1 indicates the close correlation between the experimental conditions at temperature of 55 $^{o}$C and 60 $^{o}$C since PC2 reveals the role of temperature 65 $^{o}$C where optimal experimental conditions are reached.

**Table 27.** Factor loadings (CDFA)

| | PC 1 | PC 2 |
|---|---|---|
| **T2t1** | 0,93 | 0,24 |
| **T2t2** | 0,93 | 0,24 |
| **T2t3** | 0,97 | 0,20 |
| **T2t4** | 0,97 | 0,19 |
| **T3t1** | 0,84 | 0,51 |
| **T3t2** | 0,81 | 0,55 |
| **T3t3** | 0,68 | 0,67 |
| **T3t4** | 0,68 | 0,67 |
| **T4t1** | 0,45 | 0,87 |
| **T4t2** | 0,36 | 0,91 |
| **T4t3** | 0,14 | 0,95 |
| **T4t4** | 0,16 | 0,95 |
| **Expl. Var. %** | 52,3 | 42,2 |

### *2.2.4. General conclusions of chemometric data interpretation*

Based on the chemometric expertise it could be concluded that TFAA is the optimal acylating agent for six out of all 10 chemical compounds if the cluster averages are considered (Table 28). The other four compounds reach cluster averages highest RRF values by the use of CDFA (Table 28). It seems that PFPA is not appropriate for the goals of the study.

**Table 28.** Averages (maximal values) of clusters for all compounds and all agents

| | (-)EP | (+)EP | (-)PSEP | (+)PSEP | (-)CEP | (+)CEP | (-)CPSEP | (+)CPSEP | (-)MAMP | (+)MAMP |
|---|---|---|---|---|---|---|---|---|---|---|
| **TFAA** | **821** | **1516** | **1626** | 157 | 499 | **1667** | 753 | **863** | 241 | **1049** |
| **PFPA** | 580 | 587 | 817 | 151 | 565 | 840 | 499 | 660 | 184 | 833 |
| **CDFA** | 675 | 697 | 839 | **475** | **770** | 836 | **772** | 782 | **271** | 859 |

If single optimal values of RRF are considered, again, TFAA reaches maximal values of RRF even for 7 out of all ten compounds and the other three maximal values are obtained by the use of CDFA. The results are summarized in Tables 29 and Table 30.

**Table 29.** Single maxima for all compounds and all agents

| | (-)EP | (+)EP | (-)PSEP | (+)PSEP | (-)CEP | (+)CEP | (-)CPSEP | (+)CPSEP | (-)MAMP | (+)MAMP |
|---|---|---|---|---|---|---|---|---|---|---|
| **TFAA** | **843** | **1576** | **1679** | 194 | 538 | **1712** | 772 | **879** | **331** | **1098** |
| **PFPA** | 645 | 695 | 856 | 188 | 711 | 876 | 639 | 737 | 196 | 858 |
| **CDFA** | 774 | 812 | 866 | **660** | **834** | 857 | **838** | 845 | 295 | 881 |

**Table 30.** Optimal experimental conditions for the single maxima

| | (-)EP | (+)EP | (-)PSEP | (+)PSEP | (-)CEP | (+)CEP | (-)CPSEP | (+)CPSEP | (-)MAMP | (+)MAMP |
|---|---|---|---|---|---|---|---|---|---|---|
| **TFAA** | **T2t4** | **T2t2** | **T2t3** | T2t2 | T2t3 | **T2t2** | T2t2 | **T3t2** | **T2t4** | **T2t2** |
| **PFPA** | T3t3 | T3t4 | T3t3 | T3t3 | T3t3 | T3t3 | T4t3 | T3t3 | T3t4 | T3t4 |
| **CDFA** | T4t3 | T4t3 | T4t4 | **T4t3** | **T4t3** | T4t3 | **T4t3** | T4t3 | T4t3 | T4t3 |

### 2.3. Peak identification

Standards of MAMP, EP, PSEP, CEP and CPSEP enantiomers with TFMPA as internal standard were trifluoroacetylated and analyzed individually. Mass spectra of analytes and IS together with mass fragmentation patterns of the trifluoroacetylated derivatives of analytes and IS are presented in Figure 32.

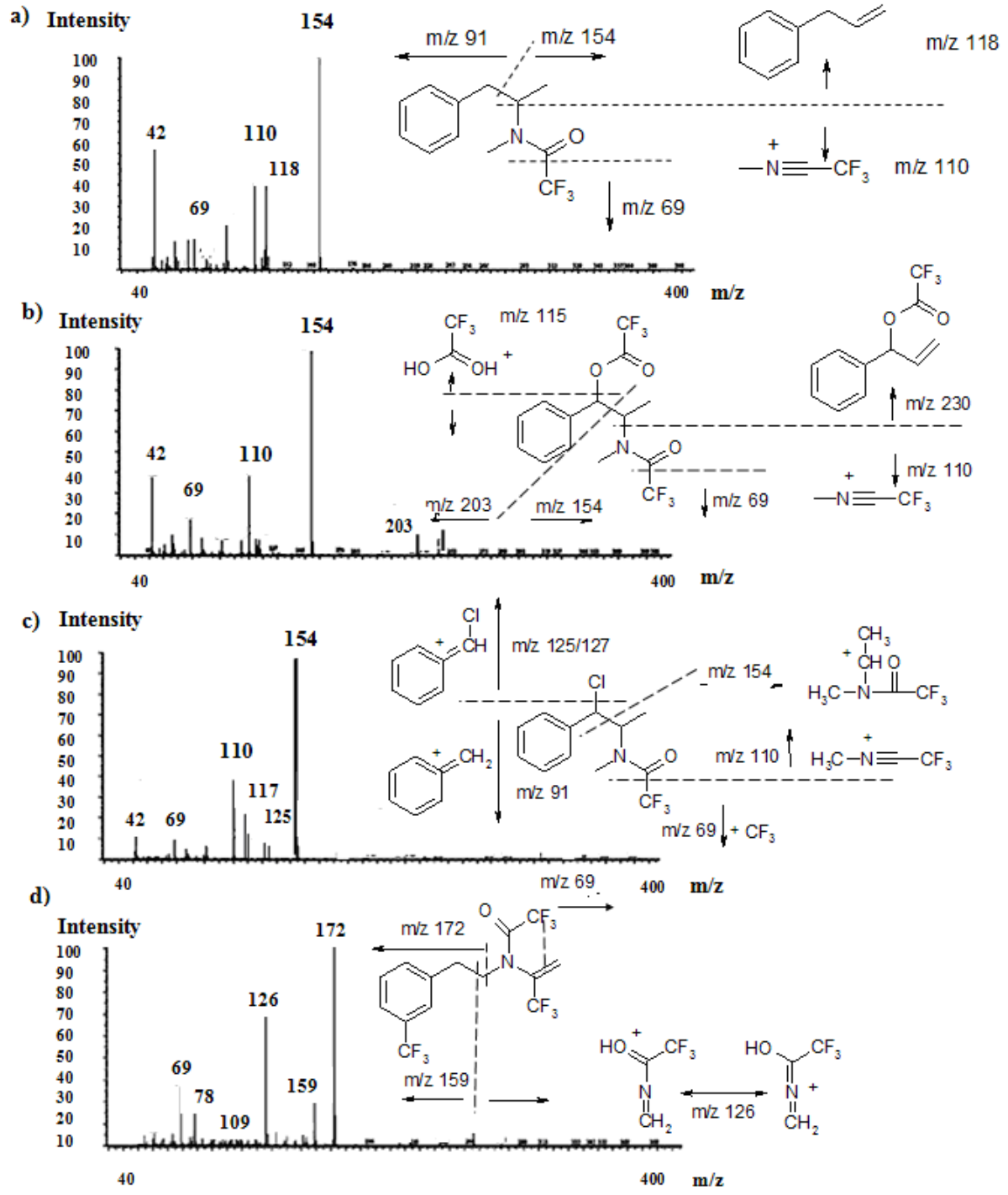



**Figure 32.** Mass spectra and mass fragmentation patterns of the trifluoroacetylated derivatives of analytes and IS: a). MAMP, b). EP and PSEP, c). CEP and CPSEP, d). TFMPA

## 2.4. Development of temperature-programmed chromatographic conditions

Initially, the temperature program method presented in Table 13 was investigated. Despite the fact that this temperature program was sufficient to determine single analytes, it was not suitable to separate all analytes of interest. Thus, the two-ramp temperature gradient with linear flow rate was investigated (Figure 33).

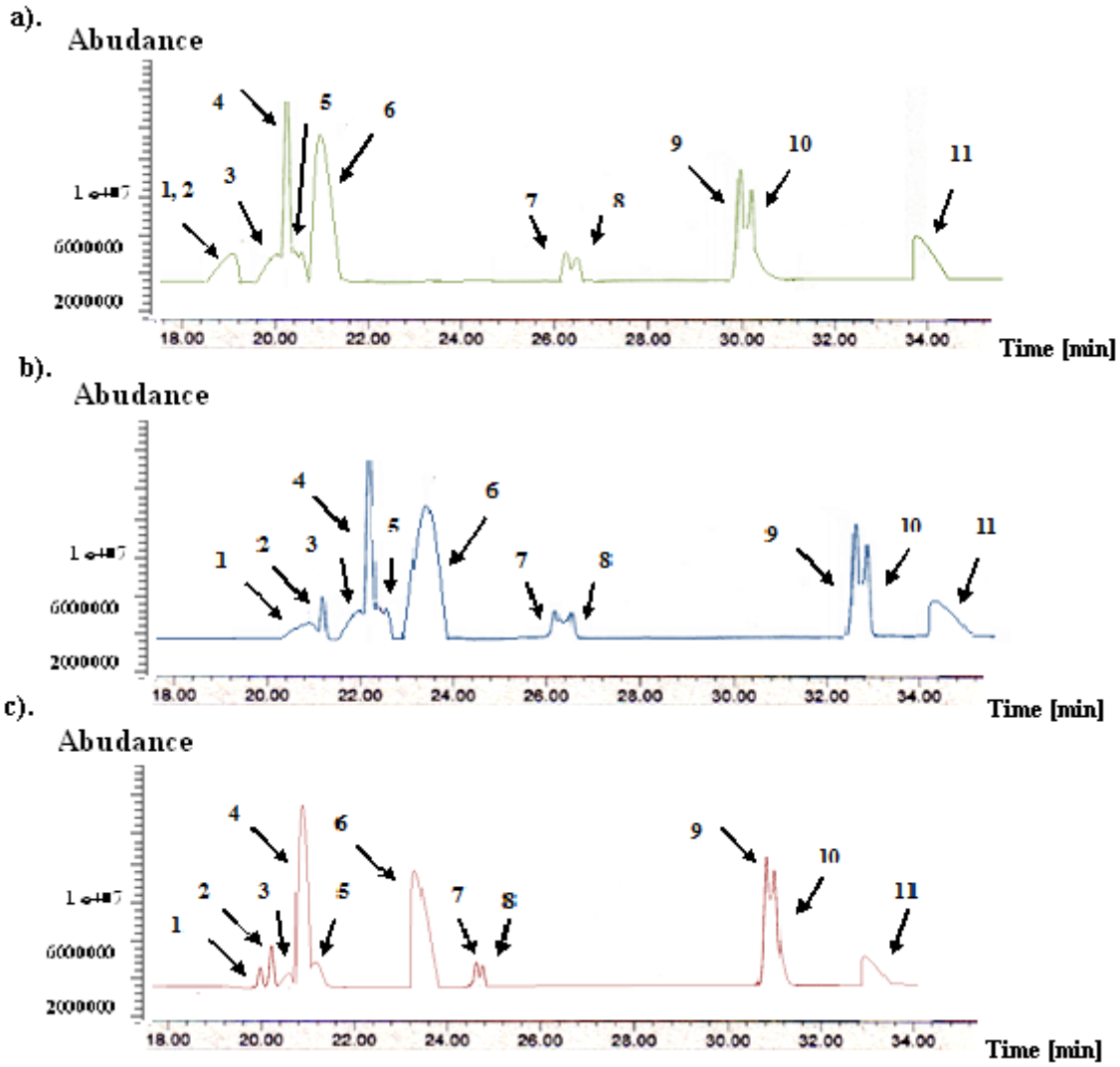



**Figure 33.** Effect of adjusting the temperature program on the separation of enantiomers by GC-MS. Linear flow rate is 101.83 cm/sec. Temperature program range is: a). 65 °C (5 min) to 145 °C (22 min) at 10 °C/min; b). 65 °C (5 min) to 120 °C (10 min) at 5 °C/min, to 135 °C (8 min) at 10 °C/min; c). 65 °C (3 min) to 126 °C (13 min) at 10 °C /min, to 140 °C (9 min) at 4 °C /min. 1. (-)EP, 2. (+)MAMP, 3. (-)MAMP 4. (+)EP, 5. (+)PSEP, 6. (-)PSEP, 7. (-)CEP, 8. (+)CEP, 9. (+)CPSEP, 10. (-)CPSEP, 11. TFMPA

It was also found that different values of flow rate affected the separation of appropriate analytes of interest. For example, while the higher flow rate allowed the separation of such compounds as (+)/(-)-MAMP, (+)/(-)-EP and (+)-PSEP, the lower value

allowed separation of CEP enantiomers. Therefore, variable flow rate was found particularly useful with regards to the separation of these compounds. The broad, tailing peak from enantiomers of CPSEP and the lengthy run time, however, lead to the temperature program being improved. The effect on the chromatography of a two-stage temperature program and flow rate program is illustrated in Figure 33 and Figure 34, respectively with chromatographic parameters provided in Table 31 for the best resolutions.

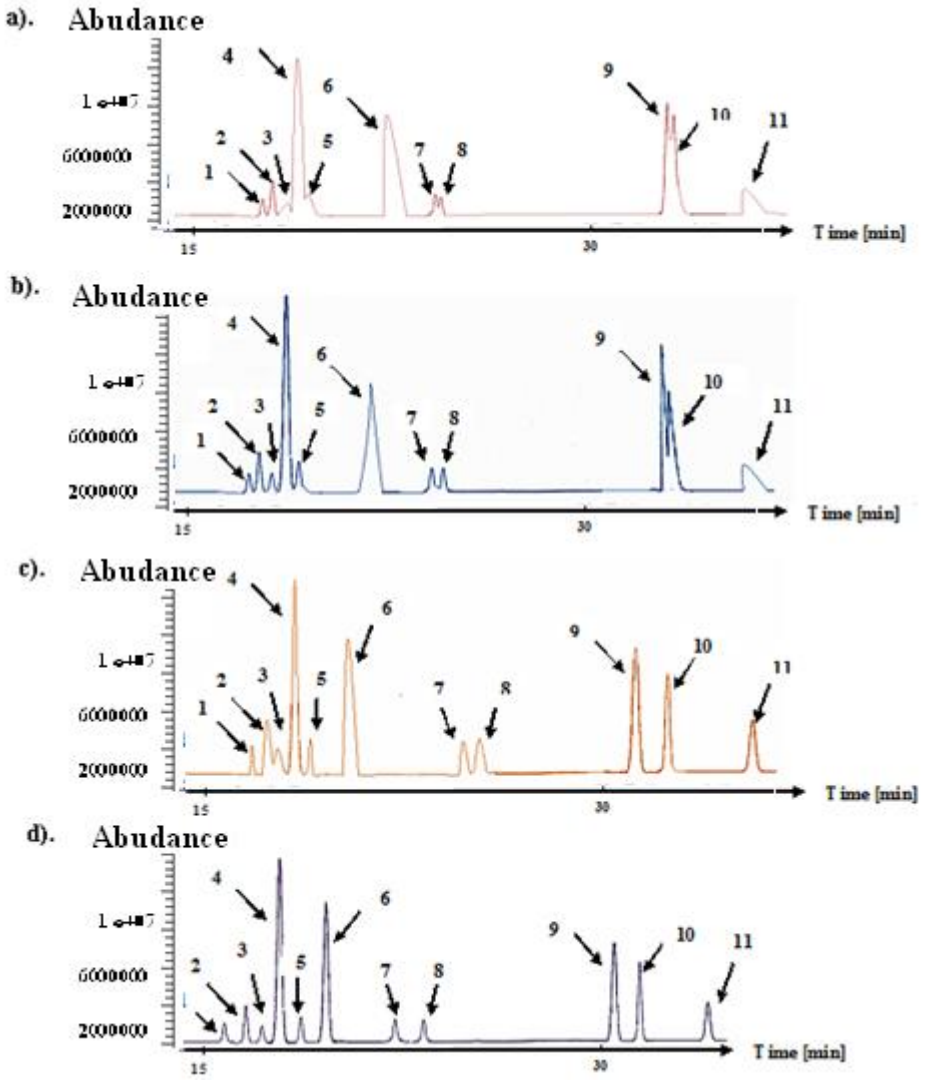



**Figure 34.** Effect of adjusting the flow rate program on the separation of enantiomers by GC-MS. Temperature program is as follows: 65 °C (3 min) to 126 °C (13 min) at 10 °C /min, to 140 °C (9 min) at 4 °C /min. Flow rate program range is: a). 3.0 mL/min (35 min); b). 2.5 mL/min (22 min) to 1.9 mL/min (5.5 min) at 1.2 mL/min, to 4.0 mL/min (6 min) at 2.1 mL/min; c). 2.7 mL/min (25 min) to 1.9 mL/min (2min) at 1.5 mL/min, to 4.5 mL/min (6 min) at 2.2 mL/min; d). 2.9 mL (23 min) to 1.8 mL (4 min) at 1.1 mL/min, to 4.5 mL/min (6 min) at 2.2 mL/min. 1. (-)EP, 2. (+)MAMP, 3. (-)MAMP, 4. (+)EP, 5. (+)PSEP, 6. (-)PSEP, 7. (-)CEP, 8. (+)CEP, 9. (+)CPSEP, 10. (-)CPSEP, 11. TFMPA

The temperature program together with the flow rate program giving the shortest overall run time (35 min) and the best chiral resolution, while maintaining the separations of the ten analytes and IS, were as follow: the initial column temperature of 65 °C was maintained for 3 min, then temperature was increased to 126 °C at 10 °C /min and held for 13 min, and the next ramp rate was 4 °C /min to 140 °C which was held for 9 min; the initial flow rate of 2.9 mL was held for 23 min, then decreased to 1.8 mL at 1.1 mL/min and held for 4 min, and finally increased to 4.5 mL/min at 2.2 mL/min and held for 6 min.

**Table 31.** Retention factors *k*, separation factors $\alpha$ and resolution factors $R_s$ for the developed method

| Analyte | *k* | $\alpha$ | $R_s$ |
|---|---|---|---|
| (-)EP | 3.93 | - | - |
| (+)MAMP | 4.03 | 1.03 | 1.52 |
| (-)MAMP | 4.44 | 1.10 | 1.63 |
| (+)EP | 4.65 | 1.05 | 1.62 |
| (+)PSEP | 4.96 | 1.07 | 1.62 |
| (-)PSEP | 5.33 | 1.08 | 1.63 |
| (-)CEP | 6.36 | 1.19 | 2.01 |
| (+)CEP | 6.49 | 1.02 | 1.43 |
| (+)CPSEP | 8.44 | 1.30 | 2.17 |
| (-)CPSEP | 8.82 | 1.05 | 1.73 |

### 2.5. Validation of optimized chromatographic conditions

Method validation and verification aims to ensure that the results produced are fit for their intended purpose. Generally accepted principles as well as the recommendation published by UNODC [105] for the analysis of seized materials by GC-MS were respected for the validation of the developed method. The optimized conditions were used for validating the developed method for quantitative analysis of enantiomers of MAMP, EP, PSEP, CEP, and CPSEP (linear range, detection limit, and precision). Information on the validation parameters are introduced in Table 32.

Working calibration standard solutions (the concentration range of the analytes from 0.001 to 0.1 μg/mL for all tested compounds, except (1R,2S)-(-)-EP and (1S,2R)-(+)-CEP, where the concentration range was 0.01-0.1 μg/mL) were prepared by diluting standard stock solutions containing each of target compounds in the appropriate amounts of methanol. Linear range for analytes of interest was studied by replicate analysis of the standard stock solutions. Linear calibration curves for all analytes over seven (five in two cases) calibration levels were constructed using 0.05 μg/mL TFMPA as IS. The linear regression values were calculated with the average peak areas of three replicate injections for each analyte. The linear regression

for each analyte with coefficient of determination in the range from 0.996 ((1R,2R)-(-)-CPSEP) to 0.999 ((S)-(+)-MAMP) what is presented in Table 33.

**Table 32.** Information on basic parameters of validation

| Parameter | Description | Note |
|---|---|---|
| Linearity and working range | Methods are described as linear when there is a directly proportional relationship between the method response and concentration of the analyte in the matrix over the range of analyte concentrations of interest (working range). | The working range was predefined by the purpose of the method. |
| Limit of detection (LOD) | The lowest analyte concentration that can be detected and identified with a given degree of certainty. | The numerical values of LOD of each analyte were calculated using the following relationship: $$LOD = 3.3 \times \frac{SD}{b} \quad (2)$$ where: SD = The standard deviation of the peak area b = The slope of the calibration curve (mean). |
| Limit of quantification (LOQ) | The lowest concentration level at which a measurement is quantitatively meaningful. | The numerical values of the LOQ of individual analytes were calculated by equation: $$LOQ = 3 \times LOD \quad (3)$$ |
| Precision (under conditions of repeatability and reproducibility) | Precision is a measure of the closeness of the analytical results obtained from a series of replicate measurements of the same measure under the conditions of the method. | Calculated as recommended by UNODC guideline. |

The calculated calibration curves showed good linearity range up to 0.1 μg/mL for all tested analytes. Coefficient of variation (CV) was the average value of different concentrations of examined compounds in the linear range and was in the range from 1.0 % ((R)-(-)-MAMP) to 3.9 % ((1R,2R)-(-)-CPSEP), which is considered good method precision. Sensitivity of the developed method was considered in terms of limit of detection (LOD). As it can be seen in Table 33 the method allows detection of the tested compounds at concentrations lower than 0.008 μg/mL. LODs were in the range from 0.002 μg/mL ((S)-(+)-MAMP) to 0.008 μg/mL ((1R,2R)-(-)-CPSEP). The limits of quantitation (LOQs) defined as 3 times the LOD were analyte-dependent and ranged from 0.006 μg/mL ((S)-(+)-MAMP) to 0.024 μg/mL ((1R,2R)-(-)-CPSEP). Precision under conditions of repeatability and reproducibility was determined as follows: Ten replicate samples were derivatized, each containing the ten enantiomers of interest at concentrations between 1.25 and 2 times the limit of detection and the internal standard at 0.05 μg/mL (quoted as mass of TFMPA freebase prior to derivatization divided by the volume of the final, derivatized sample).

**Table 33**. Basic validation parameters obtained for each analyte by using developed method (n, number of standards in three replicates; $R_t$, Retention time; $R^2$, Coefficient of determination)

| Analyte | $R_t$ [min] | Equation | n | $R^2$ | LOD [µg/mL] | LOQ [µg/mL] | Linearity range [µg/mL] | CV [%] |
|---|---|---|---|---|---|---|---|---|
| (-)EP | 16.061 | $y = (5.85 \times 10^{9})\, x - 4.09 \times 10^{7}$ | 5 | 0.997 | 0.006 | 0.018 | 0.018-0.1 | 1.5 |
| (+)EP | 18.417 | $y = (1.48 \times 10^{10})\, x - 4.61 \times 10^{7}$ | 7 | 0.997 | 0.005 | 0.015 | 0.015-0.1 | 3.4 |
| (-)PSEP | 20.648 | $y = (1.47 \times 10^{10})\, x - 3.62 \times 10^{7}$ | 7 | 0.998 | 0.004 | 0.012 | 0.012-0.1 | 1.0 |
| (+)PSEP | 19.414 | $y = (3.97 \times 10^{8})\, x - 3.21 \times 10^{5}$ | 7 | 0.998 | 0.005 | 0.015 | 0.015-0.1 | 1.1 |
| (-)CEP | 23.987 | $y = (2.63 \times 10^{9})\, x - 2.27 \times 10^{6}$ | 7 | 0.997 | 0.006 | 0.018 | 0.018-0.1 | 1.2 |
| (+)CEP | 24.415 | $y = (1.66 \times 10^{10})\, x - 1.56 \times 10^{8}$ | 5 | 0.998 | 0.005 | 0.015 | 0.015-0.1 | 2.3 |
| (-)CPSEP | 32.110 | $y = (4.09 \times 10^{9})\, x - 1.71 \times 10^{7}$ | 7 | 0.996 | 0.008 | 0.024 | 0.024-0.1 | 1.9 |
| (+)CPSEP | 30.778 | $y = (6.33 \times 10^{9})\, x - 2.81 \times 10^{7}$ | 7 | 0.997 | 0.006 | 0.018 | 0.018-0.1 | 1.7 |
| (-)MAMP | 17.730 | $y = (3.07 \times 10^{8})\, x + 9.65 \times 10^{6}$ | 7 | 0.998 | 0.005 | 0.015 | 0.015-0.1 | 1.1 |
| (+)MAMP | 16.861 | $y = (1.01 \times 10^{10})\, x - 2.41 \times 10^{7}$ | 7 | 0.999 | 0.002 | 0.006 | 0.006-0.1 | 3.9 |

The concentrations used for the MAMP, EP, PSEP, CEP and CPSEP enantiomers are provided in Table 33. After analysis, the coefficient of variation (CV) of the retention times with respect to the IS was calculated for each analyte. The UNODC guidelines state that no more than one in five samples should give a false negative result and the CV should be better than 2 %. The chromatographic method presented here meets both of these acceptance criteria for the analysis of all ten enantiomers of interest: none of the samples gave a false negative result and, as can be seen from the results presented in Table 34. The CV values were in the range from 0.01 to 0.7 which is below the maximum recommended in the UNODC guidelines.

**Table 34.** Results from testing the precision of the optimized GC-MS method ($X_{av}$, Mean relative retention time; SD, Standard deviation of the relative retention times)

| Analyte | Concentration [µg/mL] | $X_{av}$. | SD | CV [%] |
|---|---|---|---|---|
| (-)EP | $1.05 \times 10^{-2}$ | 0.4162 | $8 \times 10^{-4}$ | 0.2 |
| (+)EP | $8.75 \times 10^{-2}$ | 0.4899 | $2.1 \times 10^{-4}$ | 0.4 |
| (-)PSEP | $7 \times 10^{-2}$ | 0.5637 | $5 \times 10^{-4}$ | 0.09 |
| (+)PSEP | $9.25 \times 10^{-2}$ | 0.5230 | $1 \times 10^{-4}$ | 0.02 |
| (-)CEP | $9.9 \times 10^{-2}$ | 0.6768 | $4.8 \times 10^{-3}$ | 0.7 |
| (+)CEP | $7.5 \times 10^{-2}$ | 0.6858 | $2 \times 10^{-4}$ | 0.03 |
| (-)CPSEP | $1.4 \times 10^{-2}$ | 0.9347 | $3 \times 10^{-4}$ | 0.03 |
| (+)CPSEP | $7.8 \times 10\ 10^{-2}$ | 0.8921 | $1 \times 10^{-4}$ | 0.01 |
| (-)MAMP | $8.75 \times 10^{-2}$ | 0.4687 | $3 \times 10^{-4}$ | 0.06 |
| (+)MAMP | $3.8 \times 10^{-2}$ | 0.4120 | $3 \times 10^{-3}$ | 0.7 |

### *2.5.1. The uncertainty of measurement*

The expanded uncertainty of measurement is a quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution

of values that could reasonably be attributed to the measurand [121, 122]. The dominant parameter taken into consideration during the development of procedures was repeatability of measurements.

The value of the expanded uncertainty of measurement was calculated using the following equation:

$$U = k \times \frac{SD}{\sqrt{n}} \qquad (4)$$

where:

SD – standard deviation of the relative retention times;

n – number of measurements;

k – coverage factor (k = 2, defines an interval having a level of confidence of approximately 95 %).

On the other hand, the calculated value of the confidence interval for the series of results is described by Equation (5):

$$\Delta x = t(\alpha, f) \times \frac{SD}{\sqrt{n}} \qquad (5)$$

For the 0.05 significance level and the number of degrees of freedom $f \to \infty$, a critical parameter Student's t-test is approximately 2.

### 2.6. Application of developed method to analyze MAMP samples

The developed procedure was applied to determine the EP, PSEP, CEP, CPSEP and MAMP enantiomers in methylamphetamine synthesized in Analytical Laboratory at Faculty of Science and Technology (University of the West of Scotland) by different methods. First, a blank sample (ethyl acetate after derivatization) was analyzed using the optimized conditions, in order to verify the presence of different peaks in the corresponding chromatogram at the same retention times as the compounds being studied. Then the synthesized samples were analyzed with three replicates.

Thirty samples were analyzed. Compounds of interest had good separations. The characterization of CEP analogs synthesized by Emde method proposed by Płotka et al. [24] leads to the indication of the method of synthesis as well as which precursors were used for MAMP manufacture. For example, in the case where (1S,2R)-(+)-EP, (R)-(-)-MAMP, (1S,2R)-(+)-CEP, (1R,2S)-(-)-CEP, (1S,2S)-(+)-PSEP, (1R,2R)-(-)-CPSEP, (1S,2S)-(+)-

CPSEP were identified, it can be concluded that the Emde method was used for the synthesis of methylamphetamine and (1S,2R)-(+)-EP together with (1S,2S)-(+)-PSEP being used as precursors. Taking into account the 30 samples analyzed, 12 were classified as manufactured by Emde method.

Information on the results of MAMP samples analyzed using developed method are summarized in Table 35, while Figure 35 presents examples of chromatograms obtained for two samples by using the method developed. Results are published in Journal of Chromatography A [123].

**Table 35.** Conclusions drawn from the analysis of MAMP samples using the method developed

| No | Determined analytes | Concentration [µg/mL] [(concentration ± U (k=2)) × $10^{-2}$] | Emde method ? | Used precursor(s) |
|---|---|---|---|---|
| 1 | (-)EP | 2.20 ± 0.15 | YES | (-)EP, (+)PSEP |
| | (+)MAMP | 9.70 ± 0.39 | | |
| | (-)CEP | 3.00 ± 0.18 | | |
| | (+)PSEP | 4.60 ± 0.22 | | |
| | (+)CPSEP | 5.90 ± 0.24 | | |
| 2 | (+)EP | 2.30 ± 0.11 | NO | (+)EP |
| | (-)MAMP | 8.70 ± 0.35 | | |
| 3 | (-)MAMP | 9.20 ± 0.41 | NO | (-)PSEP |
| | (-)PSEP | 3.60 ± 0.18 | | |
| 4 | (-)EP | 2.00 ± 0.16 | YES | (-)EP |
| | (+)MAMP | 9.40 ± 0.36 | | |
| | (-)CEP | 2.90 ± 0.18 | | |
| | (+)CPSEP | 8.20 ± 0.31 | | |
| 5 | (+)EP | 1.90 ± 0.15 | YES | (+)EP |
| | (-)MAMP | 8.60 ± 0.26 | | |
| | (+)CEP | 2.10 ± 0.11 | | |
| | (-)CPSEP | 5.80 ± 0.22 | | |
| 6 | (-)MAMP | 8.40 ± 0.26 | NO | (-)PSEP, (+)PSEP |
| | (+)MAMP | 9.20 ± 0.31 | | |
| | (-)PSEP | 4.30 ± 0.22 | | |
| | (+)PSEP | 5.40 ± 0.23 | | |
| 7 | (+)MAMP | 9.70 ± 0.28 | YES | (-)PSEP, (+)PSEP |
| | (-)MAMP | 8.60 ± 0.31 | | |
| | (+)PSEP | 4.30 ± 0.22 | | |
| | (-)PSEP | 3.90 ± 0.22 | | |
| | (-)CEP | 2.00 ± 0.16 | | |
| | (+)CEP | 1.80 ± 0.19 | | |
| | (+)CPSEP | 5.50 ± 0.24 | | |
| | (-)CPSEP | 4.50 ± 0.22 | | |
| 8 | (-)EP | 1.90 ± 0.15 | YES | (-)EP, (+)EP, (-)PSEP, (+)PSEP |
| | (+)EP | 1.60 ± 0.16 | | |
| | (+)MAMP | > linearity range | | |
| | (-)MAMP | 9.80 ± 0.31 | | |
| | (+)PSEP | 5.60 ± 0.25 | | |
| | (-)PSEP | 4.80 ± 0.23 | | |
| | (-)CEP | 2.00 ± 0.15 | | |
| | (+)CEP | 1.70 ± 0.14 | | |
| | (+)CPSEP | 5.90 ± 0.24 | | |

**Table 35.** Cont.

| | | | | |
|---|---|---|---|---|
| | (-)CPSEP | 5.10 ± 0.22 | | |
| 9 | (+)EP | 2.10 ± 0.15 | YES | (+)EP, (+)PSEP |
| | (-)MAMP | 8.90 ± 0.38 | | |
| | (+)CEP | 1.80 ± 0.13 | | |
| | (-)CEP | 2.00 ± 0.14 | | |
| | (+)PSEP | 3.90 ± 0.14 | | |
| | (-)CPSEP | 5.60 ± 0.23 | | |
| | (+)CPSEP | 6.20 ± 0.24 | | |
| 10 | (+)MAMP | 7.80 ± 0.27 | NO | (+)PSEP |
| | (+)PSEP | 3.70 ± 0.19 | | |
| 11 | (+)MAMP | 9.50 ± 0.32 | YES | (+)PSEP |
| | (-)CEP | 1.90 ± 0.14 | | |
| | (+)PSEP | 3.20 ± 0.17 | | |
| | (+)CPSEP | 5.80 ± 0.21 | | |
| 12 | (-)EP | 4.20 ± 0.22 | NO | (-)EP, (+)EP |
| | (+)MAMP | > linearity range | | |
| | (+)EP | 3.40 ± 0.20 | | |
| | (-)MAMP | 9.60 ± 0.29 | | |
| 13 | (-)EP | 2.10 ± 0.17 | YES | (-)EP, (+)EP, (-)PSEP, (+)PSEP |
| | (+)EP | 1.90 ± 0.15 | | |
| | (+)MAMP | > linearity range | | |
| | (-)MAMP | 8.60 ± 0.27 | | |
| | (+)PSEP | 4.80 ± 0.24 | | |
| | (-)PSEP | 4.40 ± 0.21 | | |
| | (-)CEP | 2.20 ± 0.13 | | |
| | (+)CEP | 1.90 ± 0.14 | | |
| | (+)CPSEP | 6.10 ± 0.22 | | |
| | (-)CPSEP | 4.90 ± 0.20 | | |
| 14 | (-)MAMP | 7.80 ± 0.25 | NO | (-)PSEP, (+)PSEP |
| | (+)MAMP | 9.80 ± 0.33 | | |
| | (-)PSEP | 3.80 ± 0.21 | | |
| | (+)PSEP | 5.10 ± 0.20 | | |
| 15 | (+)EP | 3.10 ± 0.12 | NO | (+)EP |
| | (-)MAMP | 9.90 ± 0.34 | | |
| 16 | (+)EP | 3.70 ± 0.21 | YES | (-)PSEP, (+)PSEP |
| | (-)MAMP | 9.70 ± 0.29 | | |
| | (+)CEP | 2.10 ± 0.17 | | |
| | (-)CEP | 2.00 ± 0.14 | | |
| | (+)PSEP | 4.70 ± 0.20 | | |
| | (-)CPSEP | 5.90 ± 0.22 | | |
| | (+)CPSEP | 7.20 ± 0.25 | | |
| 17 | (+)EP | 1.90 ± 0.13 | NO | (+)EP |
| | (-)MAMP | 9.20 ± 0.37 | | |
| 18 | (-)MAMP | 9.90 ± 0.29 | NO | (-)PSEP |
| | (-)PSEP | 2.20 ± 0.17 | | |
| 19 | (-)EP | 1.90 ± 0.12 | YES | (-)EP, (+)PSEP |
| | (+)MAMP | 9.90 ± 0.37 | | |
| | (-)CEP | 2.50 ± 0.15 | | |
| | (+)PSEP | 5.20 ± 0.27 | | |
| | (+)CPSEP | 5.40 ± 0.23 | | |
| 20 | (+)EP | 2.10 ± 0.12 | YES | (+)EP |
| | (-)MAMP | 8.90 ± 0.28 | | |
| | (+)CEP | 2.30 ± 0.17 | | |
| | (-)CPSEP | 5.10 ± 0.24 | | |
| 21 | (-)EP | 4.70 ± 0.21 | NO | (-)EP, (+)EP |
| | (+)MAMP | > linearity range | | |
| | (+)EP | 2.40 ± 0.19 | | |
| | (-)MAMP | 9.40 ± 0.26 | | |

**Table 35.** Cont.

| | | | | |
|---|---|---|---|---|
| 22 | (-)MAMP | 8.10 ± 0.23 | NO | (-)PSEP, (+)PSEP |
| | (+)MAMP | 9.60 ± 0.30 | | |
| | (-)PSEP | 4.20 ± 0.19 | | |
| | (+)PSEP | 5.60 ± 0.18 | | |
| 23 | (+)PSEP | 3.20 ± 0.19 | NO | (+)PSEP |
| | (+)MAMP | > linearity range | | |
| 24 | (+)EP | 1.90 ± 0.13 | NO | (+)EP (-)EP |
| | (-)EP | 1.90 ± 0.12 | | |
| | (-)MAMP | 8.90 ± 0.28 | | |
| | (+)MAMP | 9.90 ± 0.29 | | |
| 25 | (+)MAMP | 9.90 ± 0.36 | YES | (+)PSEP |
| | (-)CEP | 2.10 ± 0.15 | | |
| | (+)PSEP | 3.20 ± 0.20 | | |
| | (+)CPSEP | 6.20 ± 0.22 | | |
| 26 | (-)PSEP | 3.20 ± 0.19 | NO | (-)PSEP |
| | (-)MAMP | > linearity range | | |
| 27 | (+)EP | 2.10 ± 0.13 | NO | (+)EP |
| | (-)MAMP | 9.80 ± 0.32 | | |
| 28 | (-)EP | 2.10 ± 0.14 | NO | (+)EP, (-)EP, (-)PSEP, (+)PSEP |
| | (+)EP | 1.90 ± 0.13 | | |
| | (-)PSEP | 3.20 ± 0.19 | | |
| | (+)PSEP | 3.40 ± 0.18 | | |
| | (-)MAMP | > linearity range | | |
| | (+)MAMP | > linearity range | | |
| 29 | (-)EP | 2.10 ± 0.11 | NO | (-)EP, (+)PSEP |
| | (+)PSEP | 3.40 ± 0.21 | | |
| | (+)MAMP | > linearity range | | |
| 30 | (+)EP | 2.20 ± 0.12 | NO | (+)EP, (-)PSEP |
| | (-)PSEP | 4.40 ± 0.25 | | |
| | (-)MAMP | > linearity range | | |

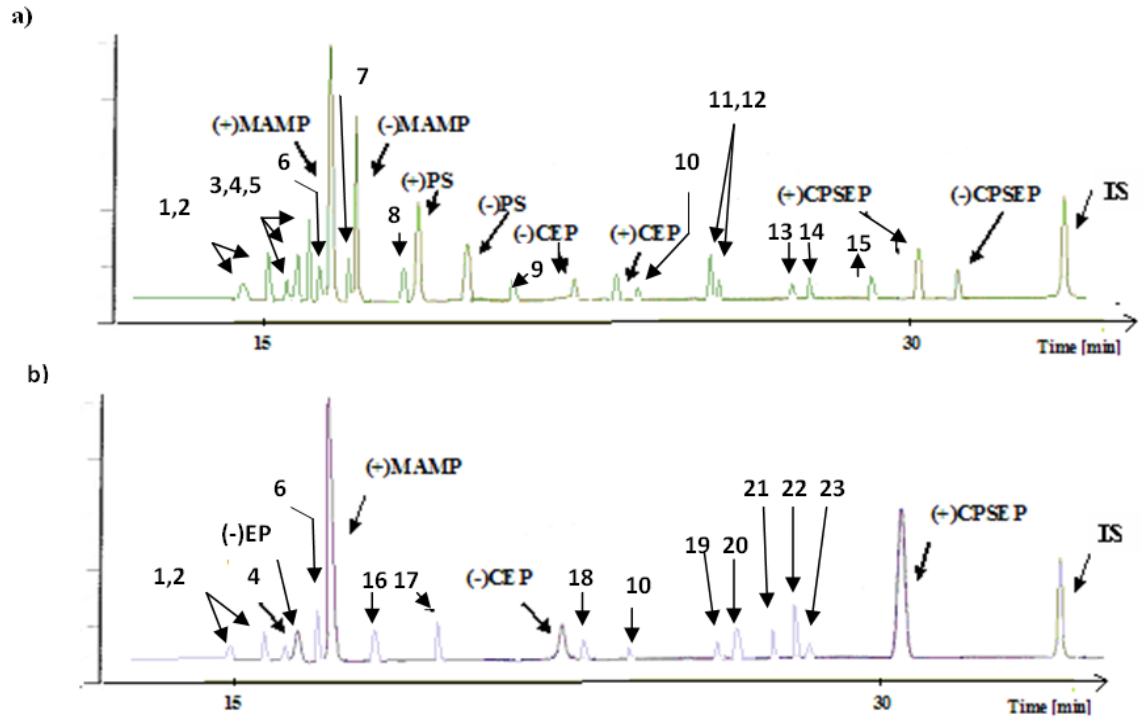



**Figure 35.** Chromatograms obtained for the sample of MAMP using developed methodology: a). sample no 7; b). sample no 4 (description in Table 34 and Table 35)

### *2.6.1. Identification of impurities of MAMP classified as synthesized by Emde method*

Impurity profile of MAMP classified as synthesized by Emde method have been investigated. Allen et al. [28, 34], Lekskulchai et al. [124] and Tanaka et al. [125] have reported on impurities such as ephedrine, chloroephedrine analogs, cis and trans-1,2-dimethyl-3-phenylaziridine, methamphetamine dimer from methamphetamine synthesized by Emde method. Stojanowska et al. reported on other impurities named: methylephedrine, N-formylephedrine, N-acetylephedrine, N,O-diacetylephedrine, N-acetylamphetamine. Puthaviriyakorn et al. [126] described two other impurities found in MAMP samples called: cis-3,4-dimethyl-5-phenyl-2-oxazoline and trans-3,4-dimethyl-5-phenyl-2-oxazoline.

Taking into account literature data and using Open Source Mass Spectrometry Tool more impurities were newly detected in this study in MAMP classified as manufactured by Emde:

- N-Methylpseudoephedrine enantiomers,
- N-Formylpseudoephedrine enantiomers,
- N-Acetylpseudoephedrine enantiomers,
- N-ethylamphetamine,
- N-ethylephedrine enantiomers, and
- N-ethylpseudoephedrine enantiomers.

Moreover, three unidentified impurities were detected.

Information on the tentative or identified impurities investigated in MAMP samples synthesized by Emde method are summarized in Table 36. Figure 36 and Figure 37 present mass spectra and proposed mass fragmentation patterns of the trifluoroacetylated derivatives of some impurities found in MAMP samples studied.

The determination of impurities in MAMP is important because contaminants can facilitate identification of the synthetic route, origin of precursors and may suggest information as to the location of manufacture of these illicit drugs. Contaminant profiling can provide vital intelligence for investigations in which linking seizures or identifying the synthetic pathway is essential.

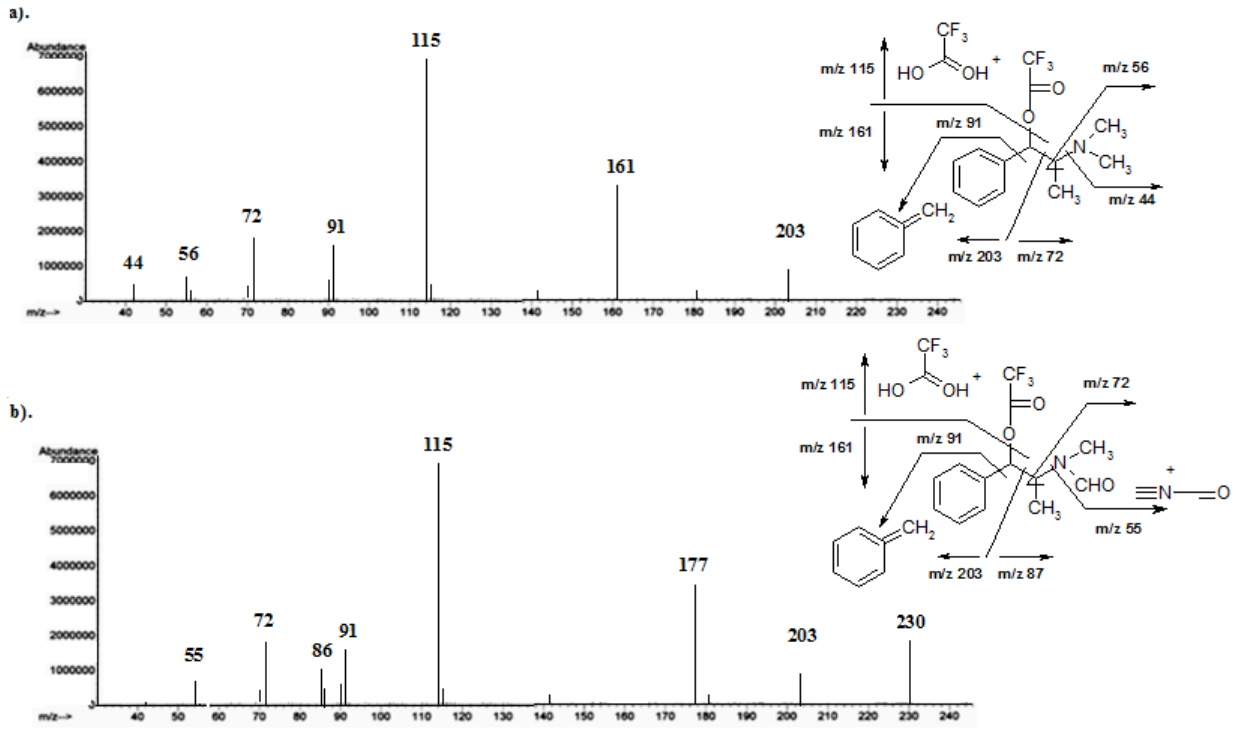



**Figure 36.** Mass spectra and proposed mass fragmentation patterns of trifluoroacetylated of: a). N-Methylephedrine (N-Methylpseudoephedrine) and b). N-Formylephedrine (N-Formylpseudoephedrine)

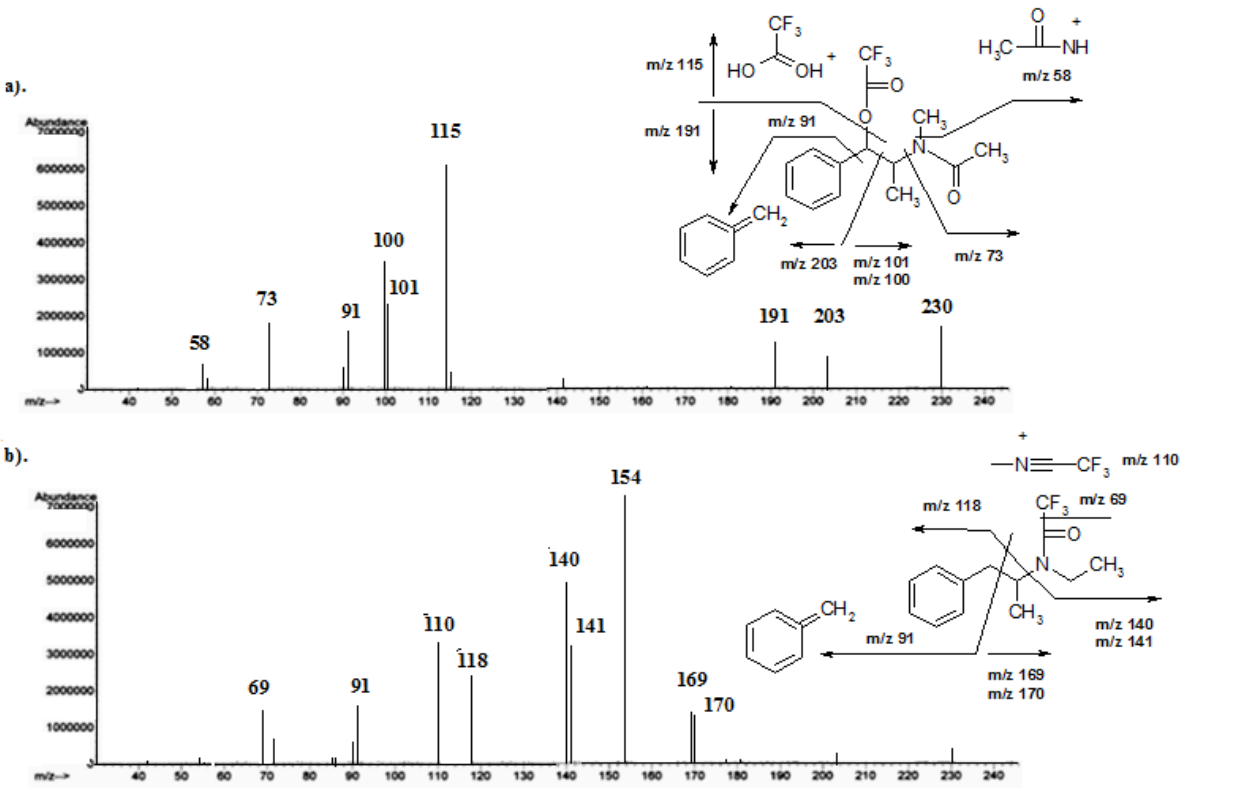



**Figure 37.** Mass spectra and proposed mass fragmentation patterns of trifluoroacetylated of: a). N-Acetylephedrine (N-Acetylpseudoephedrine) and b). N-ethylamphetamine

**Table 36.** Information on tentative and identified impurities investigated in the MAMP sample tested

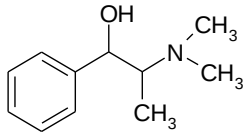
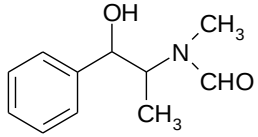
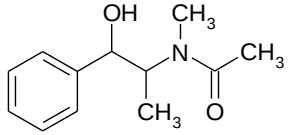
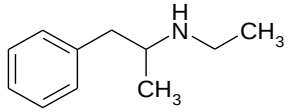
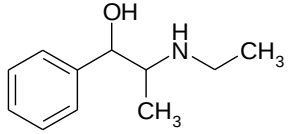
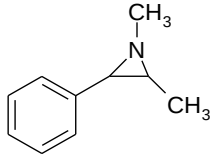
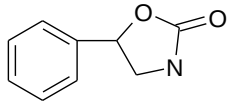
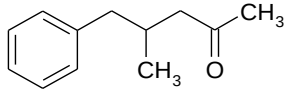
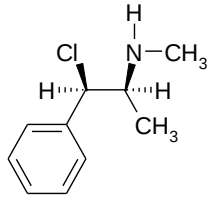
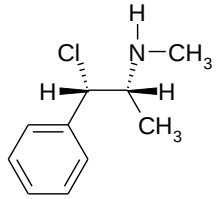
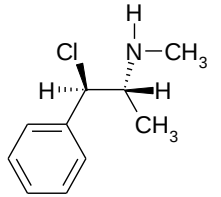
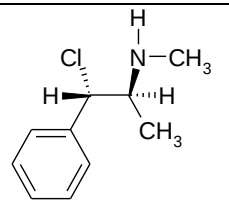
| $No_{peak}/No_{sample}$ | $R_t$ | Major m/z of trifluoroacetylated impurity | Tentative or identified compound | Structure |
|---|---|---|---|---|
| 12 / 1, 7, 8, 9, 11, 13, 16, 19, 25<br>19 / 1, 4, 8, 13, 19<br>14 / 1, 7, 8, 9, 11, 13, 16, 19, 25<br>22 / 1, 4, 8, 13, 19 | 26.130<br>26.467<br>28.567<br>28.975 | 203, 161, 115, 91, 72, 56, 44 | (1R, 2S)-N-Methylephedrine<br>(1S, 2R)-N-Methylephedrine<br>(1R, 2R)-N-Methylpseudoephedrine<br>(1S, 2S)-N-Methylpseudoephedrine |  |
| 13 / 1, 7, 8, 9, 11, 13, 16, 19, 25<br>21 / 1, 4, 8, 13, 19<br>23 / 1, 4, 8, 13, 19<br>15 / 1, 7, 8, 9, 11, 13, 16, 19, 25 | 28.017<br>28.477<br>29.020<br>29.598 | 230, 203, 177, 115, 91, 87, 86, 72, 55 | (1 R,2S)-N-Formylephedrine<br>(1 S,2R)-N-Formylephedrine<br>(1 R,2R)-N-Formylpseudoephedrine<br>(1 S,2S)-N-Formylpseudoephedrine |  |
| 7 / 1, 7, 8, 9, 11, 13, 16, 19, 25<br>16 / 1, 4, 8, 13, 19 | 17.467<br>17.999 | 230, 203, 191, 115, 101, 100, 91, 73, 58 | (1R,2S)-N-Acetylephedrine<br>(1S,2R)-N-Acetylephedrine<br>(1R,2R)-N-Acetylpseudoephedrine<br>(1S,2S)-N-Acetylpseudoephedrine |  |
| 11 / 1, 7, 8, 9, 11, 13, 16, 19, 25 | 26.114 | 154, 140, 141, 110, 118, 91, 69 | N-ethylamphetamine |  |
| 9 / 1, 7, 8, 9, 11, 13, 16, 19, 25<br>18 / 1, 4, 8, 13, 19<br>10 / 1, 4, 5, 7, 8, 9, 11, 13, 16, 19, 20, 25<br>20 / 1, 4, 8, 13, 19 | 21.765<br>24.042<br>24.769<br>26.750 | 154, 140, 125, 91, 77, 69, 56 | (1 R,2S)-N-ethylephedrine<br>(1 S,2R)-N-ethylephedrine<br>(1 R,2R )-N-ethylpseudoephedrine<br>(1 S,2S )-N-ethylpseudoephedrine |  |
| 2 / 1, 4, 5, 7, 8, 9, 11, 13, 16, 19, 20, 25<br>4 / 1, 4, 5, 7, 8, 9, 11, 13, 16, 19, 20, 25 | 15.156<br>15.848 | 244, 220, 203, 183, 154, 146, 132, 117,<br>105, 91,77,65, 51, 42 | cis-1,2-dimethyl-3-phenylaziridine<br>trans-1,2-dimethyl-3-phenylaziridine |  |
| 8 / 1, 7, 8, 9, 11, 13, 16, 19, 25<br>5 /1, 7, 8, 9, 11, 13, 16, 19, 25 | 19.207<br>15.997 | 191, 176, 147, 132, 117, 105, 91, 77, 57,<br>43 | cis-3,4-dimethyl-5-phenyl-2-oxazoline<br>trans-3,4-dimethyl-5-phenyl-2-oxazoline |  |

**Table 36.** Cont.

| | | | | |
|---|---|---|---|---|
| 1 / 1, 4, 5, 7, 8, 9, 11, 13, 16, 19, 20, 25 | 14.789 | 182, 154, 118, 110, 91, 69 | N-acethylamphetamine |  |
| 3 / 1, 7, 8, 9, 11, 13, 16, 19, 25 | 15.654 | 244, 154, 121, 110, 91, 78, 69, 56 | Unidentified 1 | - |
| 6 / 1, 4, 5, 7, 8, 9, 11, 13, 16, 19, 20, 25 | 16.272 | 244, 154, 121, 110, 91, 78, 69, 56 | Unidentified 2 | - |
| 17 / 1, 4, 8, 13, 19 | 20.266 | 244, 154, 121, 110, 91, 78, 69, 56 | Unidentified 3 | - |
| (-)CEP / 1, 4, 7, 8, 9, 11, 13, 16, 19, 25 | 23.987 | 154, 125, 127, 117, 110, 91, 69 | (1R, 2S)-(-)-chloroephedrine |  |
| (+)CEP / 5, 7, 8, 9, 13, 16, 20 | 24.415 | 154, 125, 127, 117, 110, 91, 69 | (1S, 2R)-(+)-chloroephedrine |  |
| (-)CPSEP / 5, 7, 8, 9, 13, 16, 20 | 32.110 | 154, 125/127, 117, 110, 91, 69 | (1R, 2R)-(-)-chloropseuroephedrine |  |
| (+)CPSEP /1, 4, 7, 8, 9, 11, 13, 16, 19, 25 | 30.778 | 154, 125/127, 117, 110, 91, 69 | (1S, 2S)-(+)-chloropseuroephedrine |  |

## V CONCLUSIONS AND SUMMARY

The review of literature data shown that the following information is important:

- properties of methamphetamine and its metabolites,
- method of methamphetamine synthesis,
- analytical procedures to determine purity of drug as well as impurity and chirality profiling,
- detection and identification of MAMP enantiomers, its metabolites and impurities in biological samples collected from the people who abuse this drug.

The background knowledge of MAMP is significant for many reasons with the most important being associated with human health in terms of prediction and protection applied to reduction of drug addiction. To gain this knowledge, the methodology for determination of methamphetamine and its metabolites, both, in the material collected by police and customs, as well as in the biological samples derived from people who take this drug are of great importance.

The aim of this research was to develop and optimize the new analytical methodologies in order to create a comprehensive characterization of methylamphetamine in terms of impurity and chirality profile, and also enantiomeric characterization of chlorointermediates of MAMP that are not commercially available, and necessary as reference materials for the further analysis. To realize this goal a number of studies have been made. The results of these research can be the basis for the following conclusions.

1. The determination of conformations and configurations of manufactured chloroephedrine derivatives, important and unavailable commercially chloro-intermediates of MAMP synthesized by Emde method, was possible by using the one- and multi-NMR and achiral GC-MS techniques. The results are as follows (Figure 38) [24]:
    - the conversion of EP enantiomers to the chloro-analogs occurred with inversion and retention of configuration around the α carbon atom to give mixture of: 99 % CPSEP and 1 % CEP, in accordance with $S_N2$ and $S_Ni$ mechanisms;
    - the conversion of PSEP enantiomers to the chloro-analogs occurred with inversion and retention of configuration around the α carbon atom to give mixture of: 20 % CEP and 80 % CPSEP, in accordance with $S_N2$ and $S_Ni$ mechanisms;

- the ratio of distereomers in the obtained products was depended on precursors used to the synthesis;
- one unidentified product was formed during synthesis (the mass spectral base peak of m/z 121);
- the separated compounds could be used as reference materials in further studies.

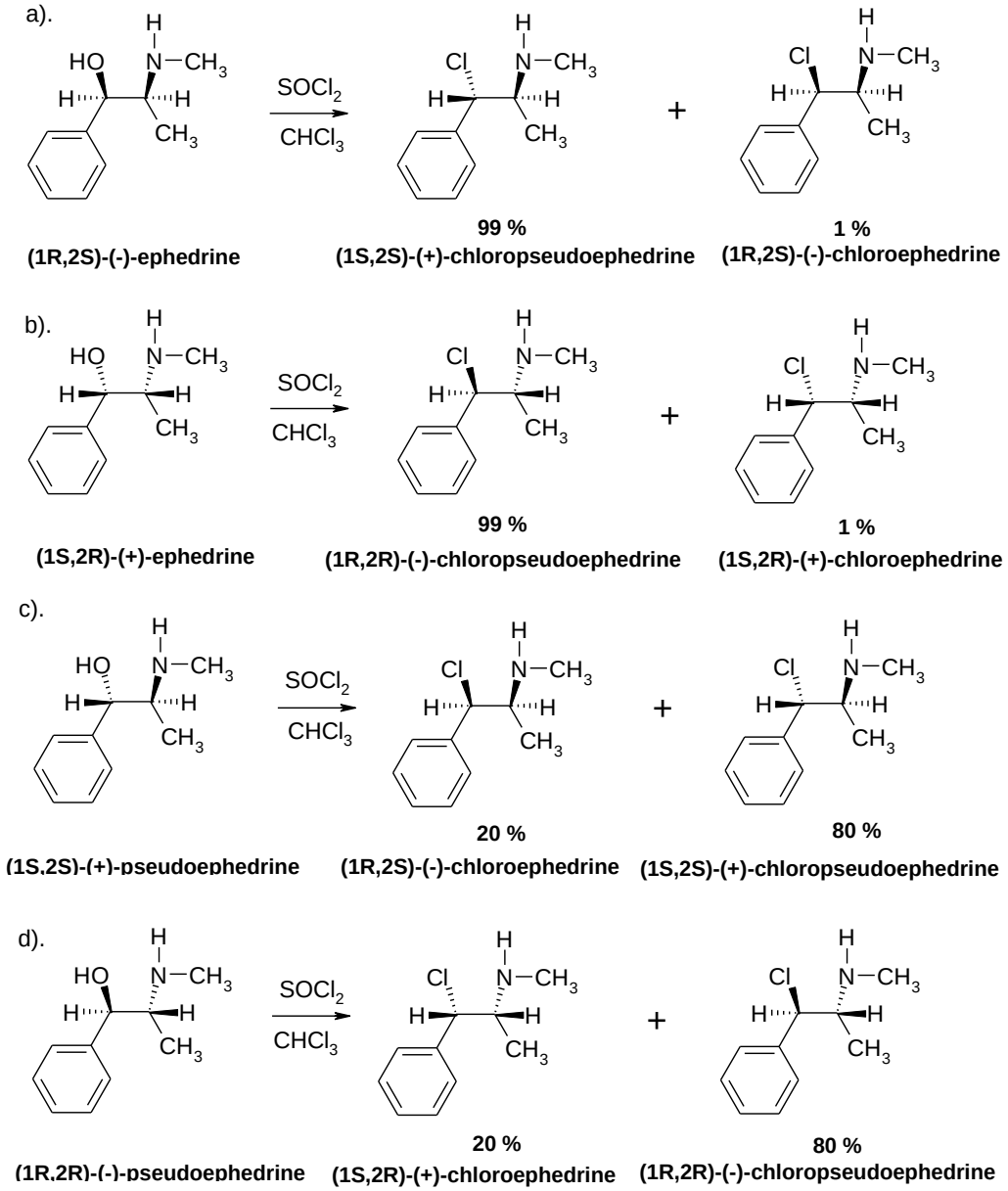



**Figure 38.** Reaction of a). (1R, 2S)-(-)-ephedrine, b). (1S, 2R)-(+)-ephedrine with thionyl chloride, c). (1S, 2S)-(+)-pseudoephedrine and d). (1R, 2R)-(-)-pseudoephedrine with thionyl chloride

2. Small changes that have been made in the conventional prescription of synthesis of MAMP chloro-intermediates gave favourable results obtained in terms of reaction speed and hence reduction of energy consumption indicate that these procedures can be considered simple, fast and eco-friendly.

3. The simultaneous enantiomeric separations of MAMP and its common precursors, EP and PSEP, as well as its chlorointermediates formed during MAMP synthesis by Emde method was possible by applied developed and validated chiral GC-MS method [123].

    - The 2,6-di-O-pentyl-3-propionyl derivative of γ-cyclodextrin phase (Chiraldex G-PN column) was found as the most effective for enantioseparation of analytes of interest.
    - Chemometric data interpretation of derivatization reactions prior to GC/MS by PCA and CA helped to establish the most effective derivatization conditions for specific multiresidue GC–MS analysis.
    - Cluster analysis was an attractive alternative to PCA for visualizing derivatization efficiency data sets.
    - Trifluoroacetic anhydride was found as the optimal acylating agent for derivatization of Analyte of interest.
    - TFMPA was found to be a suitable internal standard and the method has surpassed all the acceptance criteria required by the UNODC for the analysis of seized materials.
    - The temperature program together with the flow rate program gave the shortest overall run time and the best chiral resolution, while maintaining the separations of the ten analytes and IS.
    - Under the optimized experimental conditions, the calculated calibration curves showed good linearity range up to 0.1 μg/mL for all tested analytes. The limits of detection were in the range of 0.002-0.008 μg/mL and the coefficient of variability was between 1.0 and 3.9 %.
    - The method has the advantage of achieving excellent precision under repeatability and reproducibility conditions while detection by MS allows for the identity of analytes to be confirmed in a single analysis.

- The new methodology in comparison with other existing procedures is innovatory, because it is more economical and ecological (the chiral separation of analytes of interest is performed in a single analysis run in short time, what has not been presented in any literature data). Moreover, the new methodology is easy to apply and rapid.

4. Methylamphetamine samples were successfully analyzed using the proposed method [123].
    - Compounds of interest had good separations.
    - Several impurities were newly detected in this study in MAMP classified as manufactured by Emde. These tentative impurities are named as follows:
        - N-Methylpseudoephedrine enantiomers,
        - N-Formylpseudoephedrine enantiomers,
        - N-Acetylpseudoephedrine enantiomers,
        - N-ethylamphetamine,
        - N-ethylephedrine enantiomers, and
        - N-ethylpseudoephedrine enantiomers.
    - Three unidentified impurities were detected.

5. Developed analytical procedure and characterization of CEP analogs synthesized by Emde method leads to the indication of the method of synthesis as well as which precursors were used for MAMP manufacture indicating that this research would provide valuable information to law enforcement agencies regarding the provenance of MAMP seizures.

## VI FUTURE STUDY

Although this project brings much information on the MAMP synthesized by Emde method, there are still some issues that have to be resolved. First of all it is necessary to put attention on the impurities found in MAMP samples classified as synthesized by Emde method (e.g. aziridines). It is recommended to manufacture these contaminants and thereafter carry on the research of stereochemical configuration of them in order to confirm exactly which enantiomer occurred in the MAMP sample synthesized from appropriate precursor.

Secondly, it is recommended to develop alternative analytical procedure that could be used for enantioseparation of analytes of interest in this study without prior derivatization (HPLC, CE).

In general, future research is necessary to address knowledge gaps in regards to the manufacture of MAMP, to identify new developments in the synthesis of the drug (i.e. the identity of new precursors or new synthetic methods) and to develop knowledge in regards to newly emerging drugs.

## VII LITERATURE


[1] A.J.M. Forsyt, Psychoactive drug: the pharmacopoeia of substance use, Stationery Office, London, 2000.

[2] Metro North Mental Health – Alcohol and Drug Service, Understanding Psychoactive Drugs, InSight, Brisbane, 2013.

[3] L. Clayton, In: Amphetamines and others stimulants; L. Clayton, 4th Ed.; The Rosen Publishing Group, New York, 2001; pp. 9-19.

[4] UNODC, Recent statistics and trend analysis of illicit drug markets, In: World Drug Report 2013, United Nations, New York, 2013, pp.1-58.

[5] UNODC, Recommended methods for the identification and analysis of amphetamine, methamphetamine and their ring-substituted analogues in seized materials, United Nations, New York, 2006.

[6] J. M. Płotka, C. Morrison, M. Biziuk, Common methods for the chiral determination of amphetamine and related compounds I. Gas, liquid and thin-layer chromatography, Trends Anal. Chem., 30, 1139-1158, 2011.

[7] J. M. Płotka, C. Morrison, M. Biziuk, Common methods for the chiral determination of amphetamine and related compounds II. Capillary electrophoresis and nuclear magnetic resonance, Trends Anal. Chem., 31, 23-37, 2012.

[8] E. O. Egbochuku, O. Aluede, P. Oizimende, Analysis of the Use, Dependence and Source of Knowledge of Stimulants among Nigerian University Undergraduates, Anthropol., 11, 213-218, 2009.

[9] European Drug Report 2013: Trends and developments, EMCDDA, Lisbon, 2013.

[10] EMCDDA, Methamphetamine: a European Union perspective in the global contex, EMCDDA & Europol, Luxembourg, 2009.

[11] A. Ogata, Constitution of ephedrine-desoxyephedrine, J. Pharm. Soc. Jpn., 451, 751–764, 1919.

[12] P. Griffiths, V. Mravcik, D. Lopez, D. Klempova, Quite a lot of smoke but very limited fire: The use of methamphetamine in Europe, Drug Alcohol Rev., 27, 236–242, 2008.

[13] R. J. Defalque, A. J. Wright, Methamphetamine for Hitler's Germany: 1937 to 1945, Bull Anesth Hist., 29, 21-24, 2011.

[14] M. Tamura, Japan: Stimulant epidemics past and present, Bulletin on Narcotics, 1, 83–93, 1989.

[15] T. Zábranský, Methamphetamine in the Czech Republic, J. Drug Issues, 37, 115–180, 2007.

[16] FDA, Controlled Substance Act, Schedule II, U.S. FDA, 2013.

[17] E. Freye, J. V. Levy, Pharmacology and Abuse of Cocaine, Amphetamines, Ecstasy and Related Designer Drugs: A comprehensive review on their mode of action, treatment of abuse and intoxication, Springer, London, 2010.

[18] T. Kraemer, H. H. Maurer, Toxicokinetics of amphetamines: Metabolism and toxicokinetic data of designer drugs, amphetamine, methamphetamine, and their N-alkyl derivatives, Ther. Drug. Monit., 24, 227-289, 2002.

[19] C. C. Cruickshank, K. R. Dyer, A review of the clinical pharmacology of methamphetamine, Addiction, 104, 1085-1099, 2009.

[20] R. West, A. Pesce, C. West, C. Mikel, J. Velasco, E. Gonzales, Z. Dizon, P. Almazan, S. Latyshev, Differentiating Medicinal from Illicit Use in Positive Methamphetamine Results in a Pain Population, J. Anal. Toxicol., 11, 1-7, 2013.

[21] UNDOC: World Drug Report 2010; United Nations: New York; 2010.

[22] H. Inoue, Y. T. Iwata, K. Kuwayama, Characterization and profiling of methamphetamine seizures, J. Health Sci., 54, 615-622, 2008,

[23] B.J. Ko, S. Suh, Y.J. Suh, M.K. In, S.-H. Kim, J.-H. Kim, (1S,2S)-1-Methylamino-1-phenyl-2-chloropropane: Route specific marker impurity of methamphetamine synthesized from ephedrine via chloroephedrine, Forensic Sci. Int., 221, 92-97, 2012.

[24] J.M. Płotka, C. Morrison, D. Adam, M. Biziuk, Chiral analysis of chloro intermediates of methylamphetamine by one-dimensional and multidimentional NMR and GC/MS, Anal. Chem., 84, 5625-5632, 2012.

[25] Y. S. Lee, E. Y. Han, S. Y. Lee, E. M. Kima, Y. H. Park, M. A. Lima, H. S. Chung, J. H. Park, Analysis of the impurities in the methamphetamine synthesized by three different methods from ephedrine and pseudoephedrine, Forensic Sci. Int., 161, 209-215, 2006.

[26] W. D. Barker., W. Antia, A study of the use of Ephedra in the manufacture of methamphetamine, Forensic Sci. Int., 166, 102-109, 2007.

[27] G. Man, B. Stoeber, K. Walus, An assessment of sensing technologies for the detection of clandestine methamphetamine drug laboratories, Forensic Sci. Int., 189, 1-13, 2009.

[28] A. C. Allen, W. O. Kiser, Methamphetamine from Ephedrine: I. Chloroephedrines and Aziridines, J. Forensic Sci., 32, 953-962, 1987.

[29] H. Emde, Uber Diastereomerie I. Konfiguration des Ephedrins, Helv. Chim. Acta, 12, 365–376, 1929.

[30] A. Flores-Parra, P. Suarez-Moreno, S. A. Sanchez-Ruiz,. M. Tlahuextl, J. Jaen-Gaspar, H. Tlahuext, R. Salas-Coronado, A. Cruz, H. Nöth, R. Contreras, Chlorination reactions of ephedrines revisited. Stereochemistry and functional groups effect on the reaction mechanisms, Tetrahedron: Asymmetry, 1998, 9, 1661-1671.

[31] Uncle Fester, Methamphetamine from Ephedrine and Pseudoephedrine, Amphetamine from PPA. In: Secrets of methamphetamine manufacture. (Uncle Fester) 8th ed. Gressn Bay: Festering Publications, 2009, pp. 119-176.

[32] N. Stojanovska, S. Fu, M. Tahtouh, T. Kelly, A. Beavis, K.P. Kirkbride, A review of impurity profiling and synthetic route of manufacture of methylamphetamine, 3,4-methylenedioxymethylamphetamine, amphetamine, dimethylamphetamine and p-methoxyamphetamine, Forensic Sci. Int., 224, 8-26,2013.

[33] R. Leuckart, Uber eine neue Bildungsweise von tribenzylamin, Ber. Der Detsch. Chem. Ges., 18, 2341-2344, 1985.

[34] T.S. Cantrell, B. John, L. Johnson, A.C. Allen, A study of impurities found in methamphetamine synthesised from ephedrine, Forensic Sci. Int., 39, 39–53, 1983.

[35] H.F. Skinner, Methamphetamine synthesis via hydriodic acid/red phosphorus reduction of ephedrine, Forensic Sci. Int., 48, 123–134, 1990.

[36] P. Vallely, A single step process for methamphetamine manufacture using hypophosphorus acid, J. Clandestine Lab. Investig. Chem. Assoc., 5, 14–15, 1995.

[37] A.J. Birch, Reduction by dissolving metals, J. Chem. Soc., 430–436, 1944.

[38] J. S. Lee, W. K.Yang, E. Y. Han, S. Y. Lee, Y. H. Park, M. A. Lim, H. S. Chung, J. H. Park, Monitoring precursor chemicals of methamphetamine through enantiomer profiling, Forensic Sci. Int., 173, 68-72, 2007.

[39] J.T. Liu, R.H. Liu, Enantiomeric composition of abused amine drugs: chromatographic methods of analysis and data interpretation, J. Biochem. Biophys. Methods, 54, 115-146, 2002.

[40] M. E. Y. Cabusas, Chiral Separations on HPLC Derivatized Polysaccharide CSPs: Temperature, Mobile Phase and Chiral Recognition Mechanism Studies, PhD Thesis, Virginia Polytechnic Institute and State University, 1998.

[41] EMCDDA, Exploring methamphetamine trends in Europe, EMCDDA Papers, Publications Office of the European Union, Luxembourg, 2014.

[42] K. Nakashima, High-Performance Liquid Chromatographic Analysis of Drugs of Abuse in Biologic Samples, J. Health Sci., 51, 272-277, 2005.

[43] J. M. Płotka, C. Morrison, M. Biziuk, J. Namieśnik, Pharmaceutical and forensic drug applications of chiral supercritical fluid chromatography, Trends. Anal. Chem., 56, 74-89, 2014.

[44] Y. Zhang, D.-R. Wu, D. B. Wang-Iverson, A. A. Tymiak, Enantioselective chromatography in drug discovery, Drug Discov. Today, 10, 571-577, 2005.

[45] B. L. He, Y. Shi, B. Kleintop, T. Raglione, Direct and indirect separations of five isomers of Brivanib Alaninate using chiral high-performance liquid chromatography, J. Chromatogr. B, 875, 122-135, 2008.

[46] D. Jirovsky, K. Lemr, J. Sevcık, B. Smysl, Z. Stransky, Methamphetamine--properties and analytical methods of enantiomer determination, Forensic Sci. Int., 96, 61-70, 1998.

[47] S. Shaikh, M.S. Muneera, O.A. Thusleem, Chiral chromatography and its application to the pharmaceutical industry, Pharmaceut. Rev., 7, 1371-1381,

2009.

[48] F. Orata, Derivatization Reactions and Reagents for Gas Chromatography Analysis, In: Advanced Gas Chromatography - Progress in Agricultural, Biomedical and Industrial Applications, M. A. Mohd (Ed.) INTECH: Rijeka, 83, 83-108, 2012.

[49] D. Hensley, J.T. Cody, Simultaneous determination of amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and methylenedioxyethylamphetamine (MDEA) enantiomers by GC-MS, J. Anal. Toxicol., 23, 518-523, 1999.

[50] S.M. Wang, T.C. Wang, Y.S. Giang, Simultaneous determination of amphetamine and methamphetamine enantiomers in urine by simultaneous liquid-liquid extraction and diastereomeric derivatization followed by gas chromatographic-isotope dilution mass spectrometry. J. Chromatogr. B, 816, 131-143, 2005.

[51] M.J. LeBelle, C. Savard, B.A. Dawson, D.B. Black, L.K. Katyal, F. Zreek, A.W. By, Chiral identification and determination of ephedrine, pseudoephedrine, methamphetamine and metecathinone by gas chromatography and nuclear magnetic resonance, Forensic Sci. Int., 71, 215-223, 1995.

[52] L.B. Rasmussen, K.H. Olsen, S.S. Johansen, Chiral separation and quantification of R/S-amphetamine, R/S-methamphetamine, R/S-MDA, R/S-MDMA, and R/S-MDEA in whole blood by GC-EI-MS, J. Chromatogr. B. 842, 136-141, 2006.

[53] S.M. Wang, Enatiomeric determination of amphetamines: Exploring a novel one-step solid-phase microextraction-based approach, J. Chromatogr. B, 825, 79-87, 2005.

[54] S. Strano-Rossi, F. Botre, A.M. Bermejo, M.J. Taberneo, A rapid method for the extraction, enantiomeric separation and quantification of amphetamines in hair, Forensic Sci. Int., 193, 95-100, 2009.

[55] A.A.S. Marais, J.B. Laurens, Rapid GC-MS confirmation of amphetamines in urine by extractive acylation, Forensic Sci. Int., 183, 78-86, 2009.

[56] Z. Liu, K. Hara, S. Kashimura, J. Liu, H. Furii, M. Kashiwagi, A. Miyoshi, T. Yanai, M. Kagura, Two simple methods for enantiomeric analyses of urinary amphetamines by GC/MS using deuterium-labeled L-amphetamines as internal standards, Forensic Toxicol., 24, 2-7, 2006.

[57] L.F. Martins, M. Yegles, H. Chung, R. Wennig, Sensitive, rapid and validated gas chromatography/negative ion chemical ionization-mass spectrometry assay including derivatisation with a novel chiral agent for the enantioselective quantification of amphetamine-type stimulants in hair, J. Chromatogr. B, 842, 98-105, 2006.

[58] W. Yang, A.J. Barnes, M.G. Ripple, D.R. Fowler, E.J. Cone, E.T. Moolchan, H. Chung, M.A. Huestis, Simultaneous quantification of methamphetamine, cocaine, codeine, and metabolites in skin by positive chemical ionization gas chromatography-mass spectrometry, J. Chromatogr. B, 833, 210-218, 2006.

[59] V. Schurig, Chiral separations using gas chromatography, Trends Anal.

Chem., 21, 647-661, 2002.

[60] T. Nagai, M. Kido, J. Maeda, K. Matsushima, T. Okazaki, A. Kurosu, M. Hitosugi, S. Tokudome, Stereoisomeric identification of norephedrine derived from methamphetamine or amphetamine: urinalysis results of 33 methamphetamine abusers and 1 amphetamine abuser in Japan, Anal. Chem., 79, 4177-4181, 2007.

[61] S.J. Drake, C. Morrison, F. Smith, Simultaneous chiral separation of methylamphetamine and common precursors using gas chromatography/mass spectrometry, Chirality, 23, 593–601, 2011.

[62] M. Hasegawa, K. Matsubara, S. Fukushima, C. Maseda, T. Uezono, K. Kimura, Stereoselective analyses of selegiline metabolites: possible urinary markers for selegiline therapy, Forensic Sci. Int., 101, 95-106, 1999.

[63] V. Schurig, Use of derivatized cyclodextrins as chiral selectors for the separation of enantiomers by gas chromatography, Ann. Pharm. Fr., 68, 82-98, 2010.

[64] V. Schurig, P. Nowotny, Separation of Enantiomers on Diluted Permethylated beta-Cyclodextrin by High-Resolution Gas Chromatography, J. Chromatogr., 441, 155-163, 1988.

[65] H.-P. Nowotny, D. Schmalzing, D. Wistuba, V. Schurig, Extending the Scope of Enantiomer Separation on Diluted Methylated b-Cyclodextrin Derivatives by High-resolution Gas Chromatography, J. High Resolut. Chromatogr., 12, 383-393, 1989.

[66] W.A. Köning, S. Lutz, P. Mischnick-Lübbecke, B. Brassat, G. Wenz, Cyclodextrins as chiral stationary phases in capillary gas chromatography I: Pentylated α-cyclodextrin, J. Chromatogr., 447, 193-197, 1988.

[67] L. H. Keith, L.U. Gron, J.L. Young, Green Analytical Methodologies, Chem. Rev., 107, 2695-2708, 2007.

[68] J. Płotka, M. Tobiszewski, A. Sulej, M. Kupska, T. Górecki, J. Namieśnik, Green Chromatography, J. Chromatogr. A, 1307, 1-20, 2013.

[69] A. Gałuszka, Z. Migaszewski, J. Namieśnik, The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices, Trends Anal. Chem., 50, 78–84, 2013.

[70] R. Herráez-Hernández, P. Campíns-Falcó , J. Verdu-Andres, Strategies for the enantiomeric determination of amphetamine and related compounds by liquid chromatography, J. Biochem. Biophys. Methods, 54, 147-167, 2002.

[71] B.S. Foster, D.D. Gilbert, A. Hutchaleelaha, M. Mayersohn, Enantiomeric determination of amphetamine and methamphetamine in urine by precolumn derivatization with Marfey's reagent and HPLC, J. Anal. Toxicol., 22, 265-269, 1998.

[72] O.R. Kleidernigg, W. Lindner, Synthesis of new stable aliphatic isothiocyanate-based chiral derivatizing agent and application to indirect separation of chiral amino and thiol compounds, Chromatographia, 44, 465-472, 1997.

[73] D. Guillarme, G. Bonvin, F. Badoud, J. Schappler, S. Rudaz, J.-L. Veuthey, Fast chiral separation of drugs using columns packed with sub-2 μm particles and ultra-high pressure, Chirality, 22, 320-330, 2010.

[74] R. Herráez-Hernández, P. Campíns-Falcó, J. Verdu-Andres, Enantiomeric separation of amphetamine and related compounds by liquid chromatography using derivatization with ophthaldialdehyde, Chromatographia, 57, 309-316, 2003.

[75] P. Campíns-Falcó, L.A. Tortajada-Genaro, R. Herráez-Hernández, Chiral determination of amphetamine and related compounds using chloroformates for derivatization and high-performance liquid chromatography, Analyst, 123, 2131-2137, 1998.

[76] F.-X. Zhou, I.S. Krull, B. Feibus, 9-Fluoreneacetyl-tagged, solid-phase reagent for derivatization in direct plasma injection, J. Chromatogr., A 609, 103-112, 1992.

[77] C. Chafer-Pericas, R. Herráez-Hernández, P. Campíns-Falcó, Application of solid-phase microextraction combined with derivatization to the determination of amphetamines by liquid chromatography, Anal. Biochem., 333, 328-335, 2004.

[78] C. Chafer-Pericas, R. Herráez-Hernández, P. Campíns-Falcó, Application of solid-phase microextraction combined with derivatization to the enantiomeric determination of amphetamines, J. Pharm. Biomed. Anal., 40, 1209-1217, 2006.

[79] R. Herráez-Hernández, P. Campíns-Falcó, A. Sevillano-Cabeza, Determination of amphetamine and related compounds in urine using on-line derivatization in octadecyl silica columns with 9-fluorenylmethyl chloroformate and liquid chromatography, J. Chromatogr. B, 679, 69-78, 1996.

[80] M.D. Pastor-Navarro, R. Porras-Serrano, R. Herráez-Hernández, P. Campíns-Falcó, Automated determination of amphetamine enantiomers using a two-dimensional column-switching chromatographic system for derivatization and separation, Analyst, 123, 319-324, 1998.

[81] A.M. Rizzi, R. Hirz, S. Cladrowa-Runge, H. Jonsson, Enantiomeric Separation of amphetamine, methamphetamine and ring substituted amphetamines by means of a B-cyclodextrin chiral stationary phase, Chromatographia, 39, 131-137,1994.

[82] M. Brunnenberg, K.-A. Kovar, Stereospecific analysis of ecstasy-like N-ethyl-3,4-methylenedioxyamphetamine and its metabolites in humans, J. Chromatogr. B, 751, 9-18, 2001.

[83] J. Buechler, M. Schwab, G. Mikus, B. Fischer, L. Hermle, C. Marx, G. Gro¨n, M. Spitzer, K.-A. Kovar, Enantioselective quantitation of the ecstasy compound (R)- and (S)-N-ethyl-3,4-methylenedioxyamphetamine and its major metabolites in human plasma and urine, J. Chromatogr. B, 793, 207-222, 2003.

[84] R. Herráez-Hernández, P. Campíns-Falcó, Chiral Separation of Ephedrines by Liquid Chromatography Using β-cyclodextrins, Anal. Chim. Acta, 434, 315-324, 2001.

[85] K. Pihlainen, R. Kostiainen, Effect of the eluent on enantiomer separation of controlled drugs by liquid chromatography-ultraviolet absorbance detection-electrospray ionisation tandem mass spectrometry using vancomycin and native beta-cyclodextrin chiral stationary phases, J. Chromatogr. A, 1033, 91-

99, 2004.

[86] D.W. Armstrong, K.L. Rundlett, U.B. Nair, Enantioresolution of Amphetamine, Methamphetamine, and Deprenyl (Selegiline) by LC, GC and CE, Curr. Seps., 15, 57-61, 1996.

[87] C.V. Hoffmann, M. Lammerhoffer, W. Lindner, Novel strong cation-exchange type chiral stationary phase for the enantiomer separation of chiral amines by high-performance liquid chromatography, J. Chromatogr. A, 1161, 242-251, 2007.

[88] Z. Wang, J. Ouyang, W.R.G. Baeyens, Recent development of enantioseparation techniques for adrenergic drugs using liquid chromatography and capillary electrophoresis.A review, J. Chromatogr. B, 862, 1-14, 2008.

[89] S. Fanali, Z. Aturki, C. Desiderio, New strategies for chiral analysis of drugs by capillary electrophoresis, Forensic Sci. Int., 92, 137-155, 1998.

[90] J. Pothier, N. Galand, M. El Ouali, C. Viel, Comparison of planar chromatographic methods (TLC, OPLC, AMD) applied to essential oils of wild thyme and seven chemotypes of thyme, II Farmaco, 56, 505-511, 2001.

[91] T. Kraemer, H.H. Maurer, Determination of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments in blood and urine, J. Chromatogr. B, 713, 163-187, 1998.

[92] R. Rothchild, Determining Enantiomeric Excess by Direct NMR Methods and by Indirect Methods, The Chemical Educator, 1, S1430-4171, 1996.

[93] B. Chankvetadze, Combined approach using capillary electrophoresis and NMR spectroscopy for an understanding of enantioselective recognition mechanisms by cyclodextrins, Chem. Soc. Rev., 33, 337-347, 2004.

[94] S. L.C Ferreira, W. N.L dos Santos, C. M Quintella, B. B Neto, J. M Bosque-Sendra, Doehlert matrix: a chemometric tool for analytical chemistry-review, Talanta, 63, 1061–1067, 2004.

[95] A.G. Cruz, R.S. Cadena, M.B.V.B. Alvaro, A.S. Sant'Ana, C.A.F. Oliveira, J.A.F. Faria, H.M.A. Bolini, M.M.C. Ferreira, Assessing the use of different chemometric techniques to discriminate low-fat and full-fat yogurts, Food Sci. Technol., 50, 210-214, 2013.

[96] S. Mennickent, M. de Diego, B. Schulz, M. Vega, C. G. Godoy, New Approachs in Drug Quality Control: Matrices and Chemometrics, In: Latest Research into Quality Control, M.S.F. Nezhab (Ed.), InTech: Rijeka, 215-225, 2012.

[97] D.L. Massart, L. Kaufman, The interpretation of analytical chemical data by the use of cluster analysi, J. Wiley & Sons, New York, 1983.

[98] K. Varmuza, Chemometrics in Practical Applications, InTech: Rijeka, 2012.

[99] J. Zhao, L. Shi, Automated learning of factor analysis with complete and incomplete data, Comp. Stat. Data Anal., 72, 205–218, 2014.

[100] P. L. Rogers, H. S. Shin, B. Wang, Biotransformation for L-ephedrine production, Adv. Biochem. Eng. Biotechno., 56, 33-59, 1997.

[101] T. Matsumoto, Y. Urano, Y. Makino, R. Kikura-Hanajiri, N. Kawahara, Y. Goda, T. Nagano, Evaluation of characteristic deuterium distributions of ephedrines and methamphetamines by NMR spectroscopy for drug profiling,

Anal. Chem., 80 (2008) 1176-1181.

[102] T. Taguchi, M. Kojima, The formation and chemical properties of diastereomeric 1,2-dimethyl-3-phenylaziridine, Chem. Pharm. Bull., 7, 103-107, 1959.

[103] B. J. Ko, S. Suh, Y.J. Suh, M.K. In, S. Kim, The impurity characteristics of methamphetamine synthesized by Emde and Nagai method, Forensic Sci. Int., 170, 142-147, 2007.

[104] BLUELIGHT

[available at:. http://www.bluelight.ru/vb/showthread.php?t=318209. . Accessed on May 20, 2014]

[105] UNODC. Guidance for the validation of analytical methodology and calibration of equipment used for testing of illicit drugs in seized materials and biological specimens, United Nations, New York, 2009.

[106] C.-J. Li, B. M. Trost, Green chemistry for chemical synthesis, PNAS, 105, 13197-13202, 2008.

[107] St. Olaf College Green Chemistry Assistant. http://fusion.stolaf.edu/gca/ (Accessed April 20, 2011).

[108] K. Van Aken, L. Strekowski, L. Patiny, EcoScale, a semi-quantitative tool to select an organic preparation based on economical and ecological parameters, Beilstein J. Org. Chem., 2, 3-10, 2006.

[109] Q.F. Tao, S. Zeng, Analysis of enantiomers of chiral phenethylamine drugs by capillary gas chromatography/mass spectrometry/flame-ionization detection and pre-column chiral derivatization, J. Biochem. Biophys. Methods, 54, 103-113, 2002.

[110] T. Kraemer, S.K. Roditis, F.T. Peters, H.H. Maurer, Amphetamine concentrations in human urine following single-dose administration of the calcium antagonist prenylamine-studies using fluorescence polarization immunoassay (FPIA) and GC-MS, J. Anal. Toxicol., 27, 68-73, 2003.

[111] W.A. Koenig, D. Icheln, T. Runge, I. Pforr, A. Krebs, Cyclodextrins as chiral stationary phases in capillary gas chromatography, part VII: cyclodextrins with an inverse substitution pattern—synthesis and enantioselectivity, J. High Resolut. Chromatogr., 13, 702-707, 1990.

[112] W.A. Koenig, R. Krebber, P. Mischnick, Cyclodextrins as Chiral Stationary Phases in Capillary Gas Chromatography. Part V: Octakis(3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin, J. High Resolut. Chromatogr., 12, 732-738, 1989.

[113] W.A. Koenig, R. Krebber, G. Wenz, Cycodextrins as Chiral Stationary Phases in Capillary Gas Chromatography. Part VI: Octakis(2,3,6-tri-O-pentyl)-γ-cyclodextrin, J. High Resolut. Chromatogr., 12, 790-792, 1989.

[114] W.A. Koenig, R. Krebber, G. Wenz, Enantioselective Capillary Gas Chromatography on the Basis of Host-Guest Interactions with Modified Cyclodextrins, J. High Resolut. Chromatogr., 12, 641-644, 1989.

[115] J.Y. Kim, S.H. Shin, M.K. In, Determination of amphetamine-type stimulants, ketamine and metabolites, Forensic Sci. Int., 194, 108-114, 2008.

[116] C. Jurado, M.P. Giménez, T. Soriano, M. Menéndez, M. Repetto, Rapid analysis of amphetamine, methamphetamine, MDA, and MDMA in urine using solid-phase microextraction, direct on-fiber derivatization, and analysis

by GC-MS, J. Anal. Toxicol., 24, 11-16, 2000.
[117] J. Röhrich, G. Kauert, Determination of amphetamine and methylenedioxy-amphetamine-derivatives in hair, Forensic Sci. Int., 84, 179-188, 1997.
[118] A.A.S. Marais, J.B. Laurens, Rapid GC-MS confirmation of amphetamines in urine by extractive acylation, Forensic Sci. Int., 183, 78-86, 2009.
[119] J. D'Nicuola, R. Jones, B. Levine, M.L. Smith, Evaluation of six commercial amphetamine and methamphetamine immunoassays for cross-reactivity to phenylpropanolamine and ephedrine in urine, J. Anal. Toxicol., 16, 211-213, 1992.
[120] F.N. Bin-Eisa, Diagnostic use of hair analysis for the detection of misuse of amfetamines and cannabinoids. PhD thesis, Department of Forensic Medicine and Science, University of Glasgow, Glasgow, 2007.
[121] European co-operation for Accreditation, Evaluation of the Uncertainty of Measurement in Calibration, Document: EA-4/02 M: 2013, pp. 1-75.
[122] P. Konieczka, J. Namiesnik, Ocena i kontrola jakosci wyników pomiarów analitycznych, Wydawnictwo Naukowo-Techniczne, Warszawa, 2007.
[123] J. M. Płotka, V. Simeonov, C. Morrison, M. Biziuk, J. Namieśnik, Capillary gas chromatography using a γ-cyclodextrin for enantiomeric separation of methylamphetamine, its precursors and chloro intermediates after optimization of the derivatization reaction, J. Chromatogr. A, 1347, 146-156, 2014.
[124] V. Lekskulchai, K. Carter, A. Poklis, W. Soine, GC–MS analysis of methamphetamine impurities: reactivity of (+)- or ( )-chloroephedrine and cis- or trans-1,2-dimethyl-3-phenylaziridine, J. Anal. Toxicol., 24, 602–605, 2000.
[125] K. Tanaka, T. Ohmori, T. Inoue, Analysis of impurities in illicit methamphetamine, Forensic Sci. Int., 56, 157–165, 1992.
[126] V. Puthaviriyakorn, N. Siriviriyasomboon, J. Phorachata, W. Pan-ox, T. Sasaki, K. Tanaka K, Identification of impurities and statistical classification of methamphetamine tablets (Ya-Ba) seized in Thailand, Forensic Sci. Int., 126, 105-13, 2002.

## VIII STRESZCZENIE

Zgodnie z raportem organizacji EMCDDA, liczba osób zażywających MAMP wzrasta z roku na rok, przez co stała się ona globalnym zagrożeniem. W tej sytuacji, wiedza na temat: właściwości MAMP oraz jej metabolitów, metod syntezy MAMP, procedur analitycznych do określenia zarówno czystości narkotyku jak i charakterystyki obecnych w niej zanieczyszczeń i składu enancjomerycznego, wykrywania i identyfikacji MAMP, produktów jej metabolizmu i zanieczyszczeń w próbkach materiału biologicznego pochodzącego od osób spożywających ten narkotyk, jest istotna z wielu powodów, z których najważniejszym jest ochrona zdrowia i życia ludzkiego, a co za tym idzie zapobieganie i zwalczanie uzależnienia narkotykowego.

Celem kompleksowych badań projektu jest opracowanie i optymalizacja procedury postępowania analitycznego do sporządzenie charakterystyki próbek MAMP pod względem zanieczyszczeń oraz chiralności substancji badanej, a także charakterystyki enancjomerów próbek chloropochodnych enancjomerów EP i PSEP jako produktów pośrednich do syntezy MAMP metodą Emde, niedostępnych komercyjnie, a niezbędnych jako materiały wzorcowe do planowanych analiz.

Konfiguracja chloropochodnych enancjomerów EP i PSEP została oznaczona z wykorzystaniem takich technik jak: 1D i 2D NMR oraz GC-MS. Wykazano, że reakcja chlorowani EP oraz PSEP zachodzi zgodnie z mechanizmem substytucji nukleofilowej wewnątrzcząsteczkowej oraz dwucząsteczkowej, co prowadzi do powstania mieszaniny diastereoizomerów (chloroefedryny i chloropseudoefedryny). Stosunek mas powstałych związków zależy od prekursorów reakcji.

Opracowano i zwalidowano procedurę analityczną jednoczesnego oznaczenia enancjomerów MAMP, jej prekursorów oraz chloropochodnych występujących w MAMP otrzymanej metodą Emde, po uprzedniej derywatyzacji z bezwodnikiem kwasu trifluorooctowego, z wykorzystaniem techniki GC-MS przy użyciu chiralnej fazy stacjonarnej opartej na γ-cyklodekstrynie. W celu optymalizacji procesu derywatyzacji zastosowano analizę chemometryczną (CA i PCA). Opracowana procedura, w porównaniu z już istniejącymi, pozwala na rozdzielenie wymienionych analitów w pojedynczej analizie, w krótkim czasie. Opracowaną procedurę zastosowano do analizy próbek MAMP. Wyniki analizy pozwoliły na sklasyfikowanie próbek MAMP jako zsyntezowanych metodą Emde. Ponadto, wykryto i zaproponowano strukturę innych zanieczyszczeń MAMP.

Zarówno opracowanie charakterystyki chloropochodnych MAMP pod względem chiralności oraz procedury analitycznej do rozdzielenia enancjomerów MAMP, EP, PSEP oraz ich chloro pochodnych pozwoliło na sporządzenie charakterystyki MAMP otrzymanej metodą Emde. Informacje te mogą być przydatne nie tylko w dochodzeniu źródeł wytwarzania narkotyku, ale również mogą stać się istotne dla analiz sądowych w celu rozróżnienia spożycia MAMP jako leku farmaceutycznego (selegilina) czy też jako środka odurzającego, ponieważ produkty metabolizmu tych substancji różnią się pod względem chiralności.

Reasumując, zdobyta wiedza dostarczyła cennych informacji użytecznych w wielu dziedzinach naukowych, włączając w to przemysł farmaceutyczny i laboratoria kryminalistyczne. Tym samym chemiczna identyfikacja MAMP odgrywa istotną rolę w zapobieganiu i zwalczaniu narkomanii metamfetaminowej.

## XI SCIENTIFIC ACHIEVEMENTS

### Publications

| No | Reference | IF |
|---|---|---|
| **1** | J. M. Płotka, C. Morrison, M. Biziuk, Common methods for the chiral determination of amphetamine and related compounds I. Gas, liquid and thin-layer chromatography, *Trends Anal. Chem.*, **30**, 1139-1158, 2011. | 6.351 |
| **2** | J. M. Płotka, C. Morrison, M. Biziuk, Common methods for the chiral determination of amphetamine and related compounds II. Capillary electrophoresis and nuclear magnetic resonance, *Trends Anal. Chem.*, **31**, 23-37, 2012. | 6.351 |
| **3** | J. Płotka, C. Morrison, D. Adam, M. Biziuk, Chiral analysis of chloro intermediates of methylamphetamine by one-dimensional and multidementional NMR and GC/MS, *Anal. Chem.*, **84**, 5625-5632, 2012. | 5.695 |
| **4** | J. Płotka, M. Tobiszewski, A. M. Sulej, M. Kupska, T. Górecki, J. Namieśnik, Green Chromatography, *J. Chromatogr. A*, **1307**, 1-20, 2013. | 4.612 |
| **5** | S. Narkowicz, J. Płotka, Ż. Polkowska, M. Biziuk, J. Namieśnik, Prenatal exposure to substance of abuse: A worldwide problem, *Environ. Int.*, **54** 141–163, 2013. | 6.248 |
| **6** | Płotka J., Narkowicz S., Polkowska Ż., Biziuk M., Namieśnik J., Effects of Addictive Substances During Pregnancy and Infancy and their Analysis in Biological Materials, *Rev. Environ. Contam. T.*, **227**, 55-77, 2014. | 4.125 |
| **7** | J. M. Płotka, M. Biziuk, C. Morrison, J. Namiśnik, Pharmaceutical and forensic drug applications of chiral supercritical fluid chromatography, *Trends Anal. Chem.* **56**, 74–89, 2014. | 6.351 |
| **8** | J. M. Płotka, V. Simeonov, C. Morrison, M. Biziuk, J. Namieśnik, Capillary gas chromatography using a γ-cyclodextrin for enantiomeric separation of methylamphetamine, its precursors and chloro intermediates after optimization of the derivatization reaction, *J. Chromatogr. A*, **1347**, 146-156, 2014. | 4.612 |
| **9** | J. Płotka, M. Biziuk, Metamfetamina - analityk w dochodzeniach kryminalistycznych, *Analityka*, **1**, 54-59, 2011. | - |
| **10** | J. Płotka, M. Biziuk, Метамфетамин. Аналитик в криминалистических расследованиях, *Аналитика*, **1,** 12-16, 2012. | - |
| **11** | J. Płotka, S. Narkowicz, Ż. Polkowska, J. Namieśnik, Rola łożyska ludzkiego w analityce ksenobiotyków, *Analityka*, **1,** 36-40, 2014. | - |
| **12** | M. Biziuk, B. Cieślik, J. Płotka, Badanie toksyczności termicznie stabilizowanych i cementowanych osadów ściekowych, *Analityka*, **2**, 38-42, | - |

| | |
|---|---|
| | 2014. |

**Conference Proceedings**

| No | Reference |
|---|---|
| **1** | J. Płotka, M. Biziuk, C. Morrison, The enantiomeric determination of chloro-precursors of methamphetamine, [In]: "Advances in Chemical and Mechanical Engineering", ed. C. Fijało, Gdansk University of Technology, Gdańsk, 2011, pp. 184-191. |
| **2** | S. Narkowicz, J. Płotka, Recent developments in biological sample testing methods for the confirmation of gestational exposure to tobacco smoke and drugs of abused, [In]: "Advances in chemical and mechanical engineering", ed. C. Fijało, Gdansk University of Technology, Gdańsk, Vol. II/II, 2012, pp. 131-136. |
| **3** | J. Płotka, A Green Chemistry "environmentally friendly" approach to the synthesis of chloro-intermediates of ephedrine/pseudoephedrine, [In]: "Advances in chemical and mechanical engineering", ed. C. Fijało, Gdansk University of Technology, Gdańsk, Vol. II/II, 2012, pp. 139-144. |
| **4** | J. Płotka, C. Morrison, M. Biziuk, A novel methodology for the enantiomeric resolution of methamphetamine, its precursors and intermediates by GC-MS, [In]: Proceedings of the 9th International Students Conference "Modern Analytical Chemistry", ed. K. Nesmerak, University of Prague, Praga, 2013, pp. 37-40. |

**Oral presentations**

| No | Reference |
|---|---|
| **1** | J. Płotka, M. Biziuk, C. Morrison, The enantiomeric determination of chloro-precursors of methamphetamine, [In]: 14th International Symposium of Students and Young Mechanical Engineers: "Advances in Chemical and Mechanical Engineering", 5-7.05.2011, Gdańsk. |
| **2** | J. Płotka, M. Tobiszewski, A. Sulej, T. Chmiel, J. Namieśnik, Zielona Chemia, [In]: Ogólnopolskie Sympozjum: Nauka i przemysł – metody spektroskopowe w praktyce, nowe wyzwania i możliwości, 8-10.06.2011, Lublin. |
| **3** | J. Płotka, M. Biziuk, C. Morrison, Identyfikacja składu enancjomerycznego chloropochodnych efedryny i pseudoefedryny z wykorzystaniem techniki NMR oraz GC-FID, [In]: IX Konferencja Chromatograficzna: "Chromatografia? To przecież codzienność!", 26-29.06.2011, Poznań. |
| **4** | J. Płotka, C. Morrison, M. Biziuk, K. Wilczewska, D. Adam, Enantioresolution of chloro intermediates of methamphetamine by multidimensional nuclear magnetic resonance and chiral gas chromatography, [In]: EUROanalysis: 16th European Conference on Analytical Chemistry: Challenges in Modern Analitycal Chemistry, 11- |

| | |
|---|---|
| | 15.09. 2011, Belgrade. |
| **5** | J. Płotka, M. Tobiszewski, A. Sulej, T. Chmiel, J. Namieśnik, Zielona Chromatografia, [In]: VI Konferencja Analityczne zastosowania chromatografii cieczowej, 20-21. 10.2011, Warszawa. |
| **6** | J. Płotka, S. Narkowicz, Recent developments in biological sample testing methods for the confirmation of gestational exposure to tobacco smoke and drugs of abused, [In]: *"Advances in chemical and mechanical engineering",* Gdańsk, 16th-19th May 2012, Gdansk University of Technology, Faculty of Mechanical Engineering, Chemical Faculty, Gdańsk, 2012. |
| **7** | J. Płotka, A Green Chemistry "environmentally friendly" approach to the synthesis of chloro-intermediates of ephedrine/pseudoephedrine, [In]: *"Advances in chemical and mechanical engineering",* Gdańsk, 16th-19th May 2012, Gdansk University of Technology, Faculty of Mechanical Engineering, Chemical Faculty, Gdańsk, 2012. |
| **8** | J. Płotka, M. Tobiszewski, A, Sulej, T. Chmiel, J. Namieśnik, Zielona Chemia, [In]: VI Kopernikańskie Seminarium Doktorantów, UMK, 13-16.06.2012, Toruń. |
| **9** | J. M. Płotka, C. Morrisom, M. Biziuk, Chiral Gas Chromatography together with Nuclear Magnetic Resonance as a tools for investigations into illicitly manufactured methylamphetamine, [In]: 8th International Conference on Instrumental Methods of Analysis: Modern Trends and Applications, TU01, s.125; 2013, Thessaloniki, Greece. |
| **10** | J. Płotka, A novel methodology for the enantiomeric resolution of methamphetamine, its precursors and intermediates by GC-MS, [In]: Proceedings of the 9th International Students Conference "Modern Analytical Chemistry", ed. K. Nesmerak Praga: University of Prague, Faculty of Science, 2013. |

**Poster presentations**

| No | Reference |
|---|---|
| **1** | J. Płotka, M. Biziuk, C. Morrison, Oznaczanie zanieczyszczeń metamfetaminy zsyntezowanej metodą Emde techniką GC-MS podstawą dochodzeń kryminalistycznych, [In]: IX Konferencja Chromatograficzna: "Chromatografia? To przecież codzienność!", 26-29.06.2011, Poznań. |
| **2** | J. Płotka, M. Biziuk, C. Morrison, Zastosowanie techniki NMR oraz GC-FID do oznaczania enancjomerów chloropochodnych pierwszego etapu syntezy metamfetaminy metodą EMDE, [In]: IX Konferencja Chromatograficzna: "Chromatografia? To przecież codzienność!", 26-29.06.2011, Poznań. |
| **3** | J. Płotka, C. Morrison, M. Biziuk, Strategies and approaches for illicit drug investigation using chiral analysis, [In]: EUROanalysis: 16th European Conference on Analytical Chemistry: Challenges in Modern Analitycal Chemistry, 11-15.09. 2011, Belgrad. |

**4** J. Płotka, C. Morrison, D. Adam, M. Biziuk, A chiral profile of methylamphetamine impurities synthesized by emde as a tool useful in environmental and forensic investigations, [In]: Fourth Scottish Postgraduate Symposium on Environmental Analytical Chemistry, 7.12.2012, Glasgow.

**5** J. Płotka, C. Morrison, M. Biziuk, A green chemistry "environmentally friendly" approach to the synthesis of chloro-intermediates of ephedrine/pseudoephedrine, [In]: Fourth Scottish Postgraduate Symposium on Environmental Analytical Chemistry, 7.12.2012, Glasgow.

**6** S. Narkowicz, J. Płotka, Ż. Polkowska, M. Biziuk, J. Namieśnik, Recent developments in biological sample chromatographic methods for the confirmation of gestational exposure to tobacco smoke and drugs of abused, [In]: 29th International Symposium on Chromatography, Toruń, 9-13 Wrzesień 2012, eds. B. Buszewski, J Kowalska, Uniwersytet Mikołaja Kopernika, Toruń, 2012.

**7** J. Płotka, M. Tobiszewski, A. Sulej, T. Chmiel, T. Dymerski, J. Namieśnik, Different approaches in the field of green chromatography, [In]: 29th International Symposium on Chromatography, Toruń, 9-13 Wrzesień 2012, eds. B. Buszewski, J Kowalska, Uniwersytet Mikołaja Kopernika, Toruń, 2012.

**8** J. Płotka, C. Morrison, M. Biziuk, GC-MS and multi-dimentional nmr analysis for the chiral investigation of chloro-intermediates of methylamphetamine, [In]: 29th International Symposium on Chromatography, Toruń, 9-13 Wrzesień 2012, eds. B. Buszewski, J Kowalska, Uniwersytet Mikołaja Kopernika, Toruń, 2012. (Poster was awarded)

**9** J. Płotka, C. Morrison, M. Biziuk, A Chiral Profile of Methylamphetamine Impurities Synthesized by Emde as a Tool Useful in Environmental and Forensic Investigations, [In]: 29th International Symposium on Chromatography, Toruń, 9-13 Wrzesień 2012, eds. B. Buszewski, J Kowalska, Uniwersytet Mikołaja Kopernika, Toruń, 2012.

**10** J.M. Płotka, C. Morrisom, M. Biziuk, Simultaneous chiral separation of methylamphetamine, its common precursors and intermediates using gas chromatography-mass spektrometry, [In]: 8th International Conference on Instrumental Methods of Analysis: Modern Trends and Applications, P1-23, s.110; 2013, Thessaloniki, Greece.

## Grants and scholarships

- Scholarship "InnoDoktorant – Scholarships for PhD students, Vth edition, 2013". Project co-financed by the European Union in the frame of the European Social Fund.
- Scholarships "InterPhD" in three editions: 2011/2012; 2012/2013; 203/2014; Project co-financed by the European Union in the frame of the European Social Fund: POKL.04.01.01-00-368/09.

- Scholarships for the best PhD students (2011/2012; 2012/2013; 2013/2014).
- Travelgrant “InterPhD” in 2012 for three months internship in University of the West of Scotland.
- Research grant given by National Science Center for young scientists for project entitled: Development of new methodology to enantioseparation and qulitative and quantitative determination of methamphetamine synthesized by Emde method and its impurities and intermediates in order to create of comprehesive characterization of methamphetamine sample producted by Emde method (2012/05/N/ST4/02001).