

GC/MS and GC/FTIR as Powerful Tools for Identifying Bioactive Compounds

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Abstract: Amphetamine-type stimulants (ATS) are a group of substances, mostly synthetic in origin, that generally stimulate the central nervous system. Chemical modification of their molecular structure results in a practically unlimited number of pharmacologically active compounds, some of which are more potent stimulants than others. The gas chromatography combined with mass spectrometry (GC/MS) and, more recently gas chromatography combined with Fourier transform infrared spectrometry (GC/FTIR) have made a substantial contribution in the identification of drugs abuse in clinical and forensic toxicology. In this paper, we have analyzed some of the most popular stimulant amphetamines by the GC/MS and GC/FTIR techniques. We have found that the most characteristic fragment ions for the stimulant amphetamines are phenyl ($m/z = 77$), benzyl ($m/z = 91$), cyclopentadienyl ($m/z = 65$) and cyclopropenyl ($m/z = 39$) cations. The GC/FTIR technique allows us to

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characterize the isomers of studied chemical compounds, and thus to distinguish between compounds with the same molecular structure, but with different biological activity.

Keywords: amphetamines, GC/FTIR, GC/MS .

Introduction

Amphetamine-type stimulants (ATS) are part of the psychostimulant group of drugs and include meth/amphetamine, ecstasy, cocaine and some pharmaceuticals (such as dexamphetamine and Ritalin). All stimulants work by increasing dopamine levels in the brain—dopamine is a brain chemical (or neurotransmitter) associated with pleasure, movement, and attention. The therapeutic effect of stimulants is achieved by slow and steady increases of dopamine, which are similar to the natural production of the chemical by the brain. ATS stimulate central nervous system activity, producing euphoria, a sense of wellbeing, wakefulness and alertness. ATS use is, however, associated with a range of potentially negative health consequences. ATS use results in increased heart rate, blood pressure and body temperature, sleeplessness and reduced appetite. The increases in blood pressure and heart rate can affect organs and can contribute to stroke, heart problems and kidney failure.^{1,2}

Mass spectrometry is usually used for the identification of drugs in forensic and other regulatory laboratories. However, there are many compounds (isomers) with essentially equal mass spectra among the substituted phenethylamines making their differentiation a challenge in many analytical situations. In forensic drug chemistry there is a need for the identification of the different isomeric compounds and regioisomeric

differentiation has been addressed in a number of drug categories.³⁻⁶

Infrared spectroscopy is considered a confirmation method for the identification of organic compounds due to the uniqueness of infrared spectra for very similar organic molecules. Gas chromatography coupled with transform Fourier infrared spectrometry is capable of obtaining infrared spectra from the peaks as they elute from the capillary columns thus combining the separation power of gas chromatography with the identification power of infrared spectrometry.⁷ The GC/FTIR method has been successfully used in the identification of amphetamine isomers⁸ as well as methamphetamine and phentermine (phenyl-tertiary-butylamine).⁹ Recently, GC-IRD (gas chromatography with infrared detection) was used for the differentiation of 10 different rings and side chain substituted phenethylamines having a regioisomeric and/or isobaric relationship to the controlled substance 3,4-MDMA.¹⁰

In this paper, we have analyzed five stimulant amphetaminic compounds (amphetamine, dextroamphetamine, metamphetamine, *N*-ethylamphetamine and *N-n*-propylamphetamine) using two hyphenated GC/MS and GC/FTIR techniques. We have found that in the case of isomeric compounds i.e. amphetamine and dextroamphetamine, the GC/MS spectra have not discrimination power while the GC/FTIR spectra achieve successfully the identification of these compounds. For the following compounds: metamphetamine, *N*-ethylamphetamine and *N-n*-propylamphetamine, which have an increasingly longer lateral chain, the GC/MS spectra present as base peaks a fragment ion with increasing mass i.e. $m/z = 58$ for metamphetamine, $m/z = 72$ for *N*-ethylamphetamine and $m/z = 86$ for *N-n*-propylamphetamine. In this case the GC-MS technique achieves a good discrimination of the compounds, while the GC/FTIR

technique comes to confirm this result. In addition, we have presented a spectroscopic analysis of the GC/MS and GC/FTIR spectra for studied compounds.

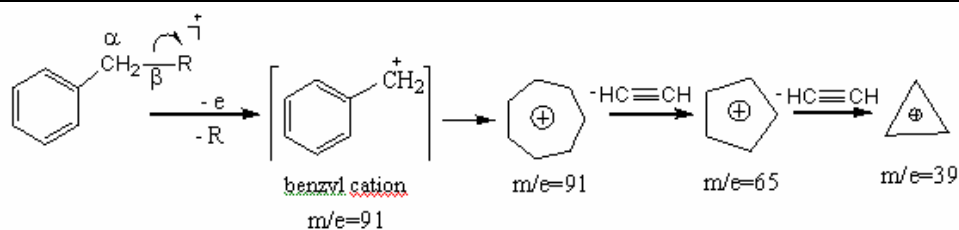
Results and discussions

Spectroscopic analysis of GC-MS spectra

A characteristic of these spectra is that they contain a basic ion relative abundance of 100% in spectrum which corresponds to a fragment ion with a mass less than the molecular mass of the compound. Molecular ion spectral line's corresponding to the molecular weight of analyte is of primordial importance in determining the structure of the compound to be analyzed, but has very low abundance in the spectrum or may be absent due to advanced fragmentation of molecule. Because these compounds are small molecular structures, their mass spectra are very poor in lines (fragments).^{11, 12}

For all amphetamines, the most important scission is breaking the C-C link neighboring with the nitrogen atom, where the rule of the preferential scission with the formation of the largest and most stable fragment is valid. Fragmentations of a molecule in mass spectrometry are governed by the formation of ions and radicals as stable as possible, and also by neutral stable particle removal.

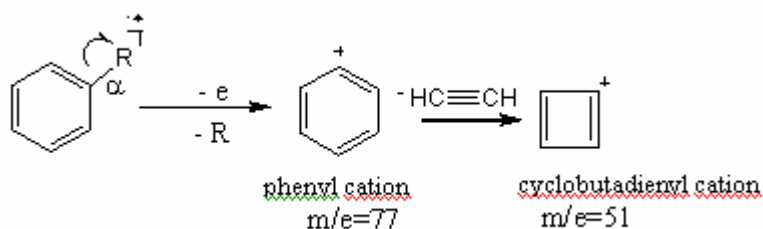
The aromatic hydrocarbons with an aliphatic side chain give type β cleavages. The tropylium cation ($m/z = 91$) stabilized by the aromatic conjugation can go over into neutral acetylene and cyclopentadienyl cation ($m/z = 65$) and, then into cyclopropenyl cation ($m/z = 39$) after a new elimination of neutral acetylene:



Scheme 1

For all studied stimulants (see Figs. 1-4) after the β cleavage it obtained a benzyl cation and a conjugated alkylamine fragment which is base ion, i.e. ethylamino cation ($m/z = 44$) for amphetamine and dextroamphetamine, *N*-methylethylamino cation ($m/z = 58$) for methamphetamine, diethylamino cation ($m/z = 72$) for *N*-ethylamphetamine and *N*-ethylpropylamino cation ($m/z = 86$) for *N*-*n*-propylamphetamine.

By α cleavage of aromatic hydrocarbons with an aliphatic side chain results a phenyl cation ($m/z = 77$) which still can transform into neutral acetylene and cyclobutadienyl cation ($m/z = 51$):



Scheme 2

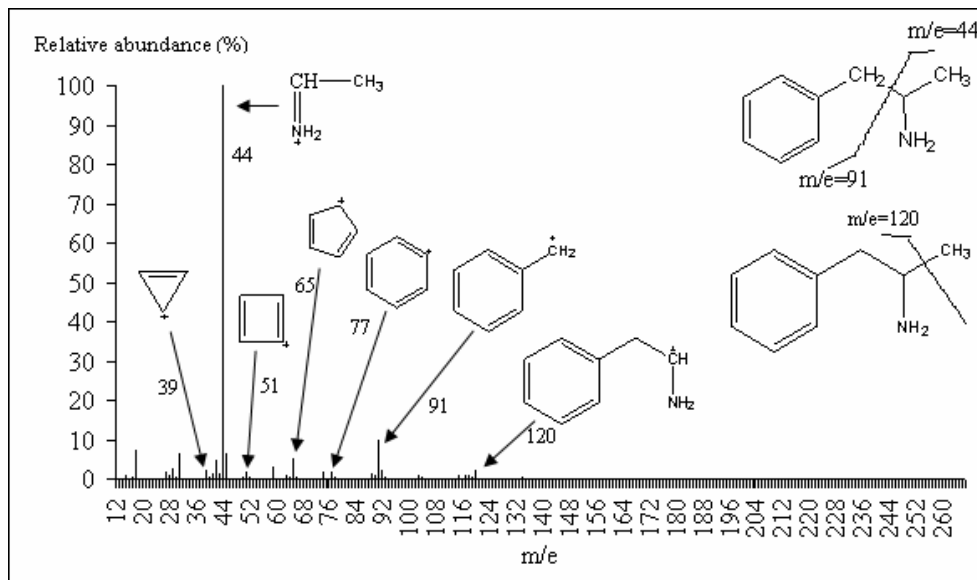


Figure 1 – The GC-MS spectrum and fragment ions of amphetamine and dextroamphetamine (C1 and C2).

It is worth to emphasize the fact that the GC/MS spectra can discriminate the stimulant amphetamines in the case when their side chain becomes more voluminous and thus the base ion moves to bigger masse. In the case of compounds which have optic isomers (amphetamine and dextroamphetamine), the GC/MS technique cannot discriminate them and then another analysis is necessary.

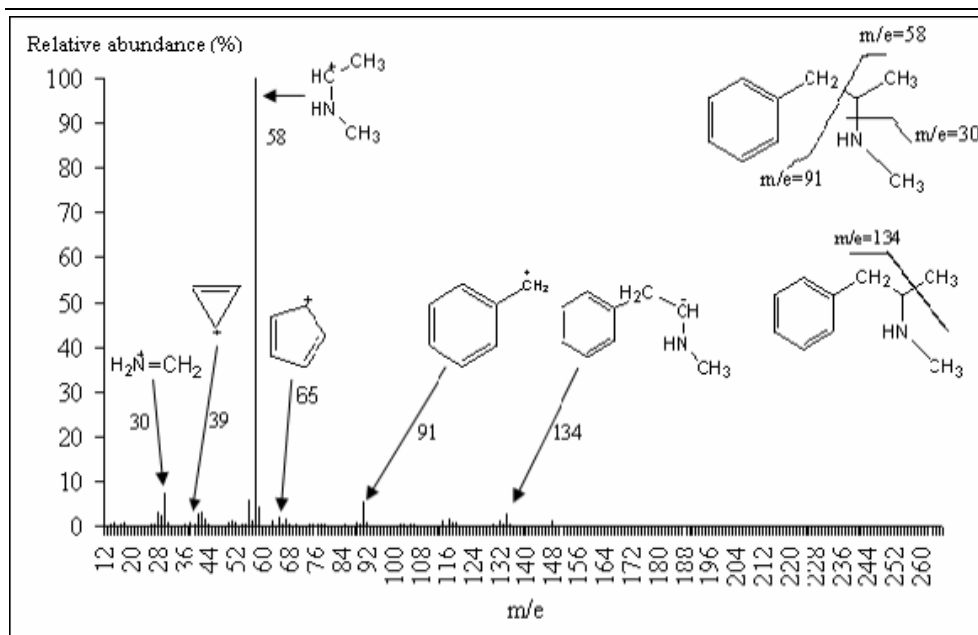


Figure 2 – The GC-MS spectrum and fragment ions of methamphetamine (C3).

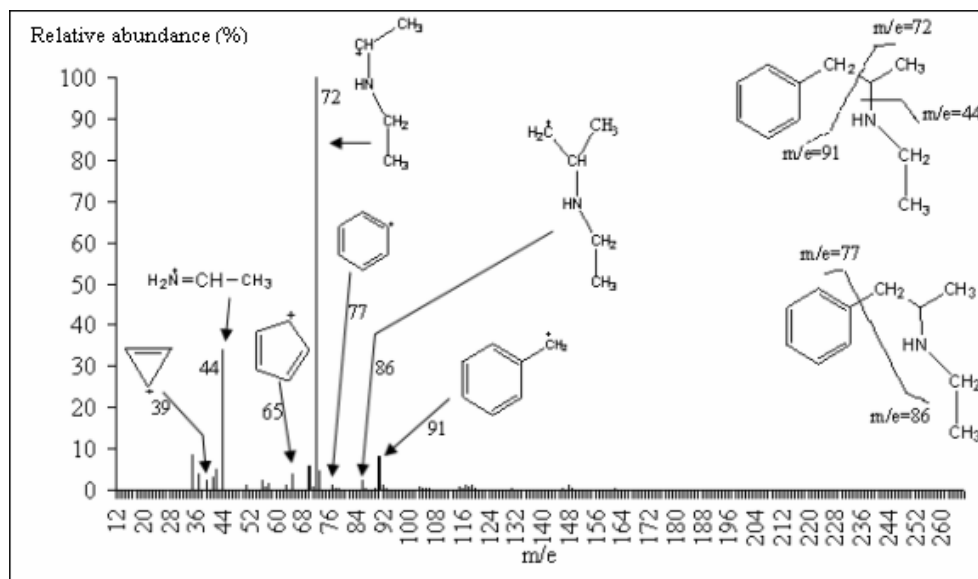


Figure 3 – The GC-MS spectrum and fragment ions of N-ethylamphetamine (C4).

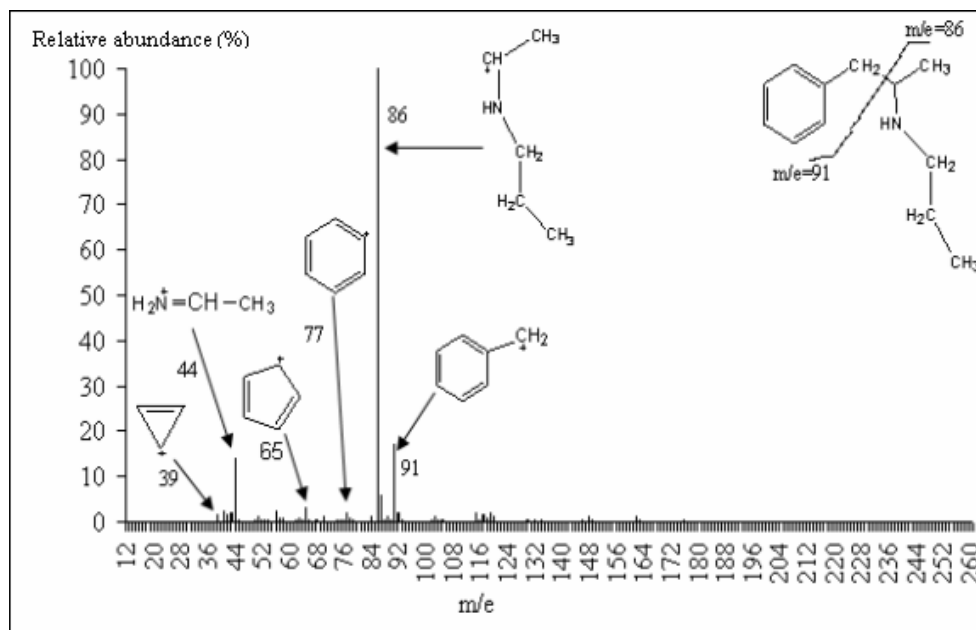


Figure 4 – The GC-MS spectrum and fragment ions of N-n-propylamphetamine (C5).

Spectroscopic analysis of GC-FTIR spectra

Analyzing the GC/FTIR spectra of stimulants (see Figs. 5-9) studied in this paper, we found there are four important spectral domains: the first spectral domain which, in its turn, is composed of other spectral regions (3027 – 3039 cm⁻¹, 3053 – 3078 cm⁻¹, 680-1000 cm⁻¹), contains the characteristic absorptions of aromatic ring, the second spectral region (680 – 900 cm⁻¹) refers to the type of substitution of the aromatic ring, the third spectral region (2830 – 3000 cm⁻¹) confirms the presence of aliphatic side chain and the fourth spectral domain (3050 – 3500 cm⁻¹) shows us that there

is an amino group in the side chain. The first spectral domain contains the absorption bands attributed of the C-H stretching and deformation vibrations and the C-C stretching vibrations of the non-substituted phenyl ring.¹³⁻¹⁶ For the majority of studied amphetamines (C1, C3, C4 and C5) the 3035 cm^{-1} and 3075 cm^{-1} absorption bands correspond to the C-H stretching vibrations of the aromatic ring and have almost the same intensity for a given compound.

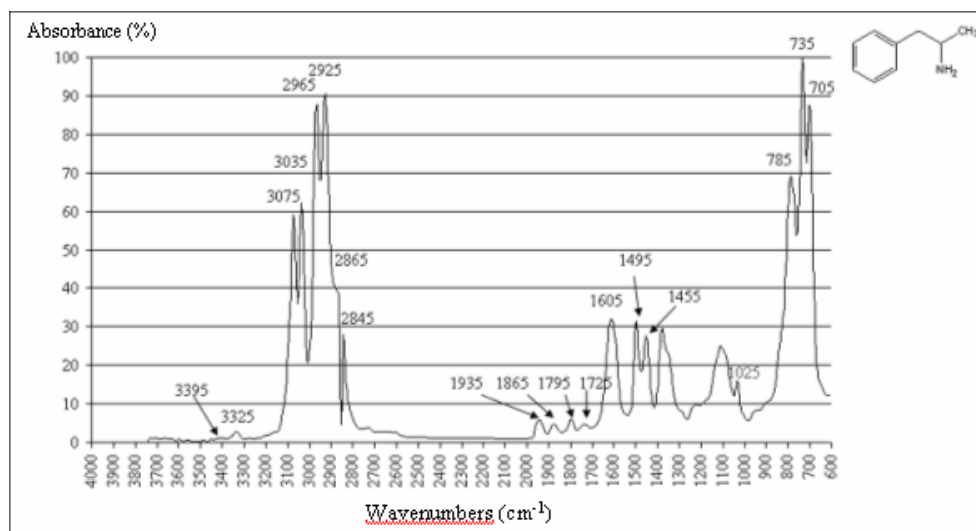


Figure 5 – The GC-FTIR spectrum of amphetamine (C1).

The intensities of these peaks diminish as the amine is substituted with a longer chain, i.e. the C₁ compound has in the side chain a primary amine, the C₃ compound has the amino group monosubstituted with the – CH₃ group, the C₄ compound has the amino group monosubstituted with the –

C_2H_5 group and the C_5 compound has the amino group monosubstituted with the $-C_3H_7$ group (see Figs. 6-10).

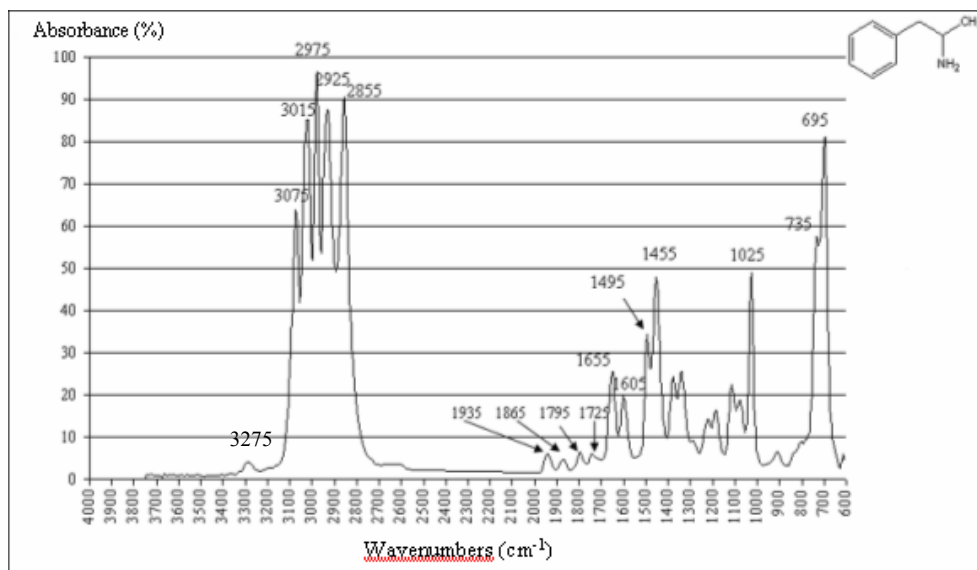


Figure 6 – The GC-FTIR spectrum of dextroamphetamine (C2)

In the case of dextroamphetamine the absorption bands associated with the aromatic C-H stretching vibrations have very different intensities (see Fig. 6) and their wavenumbers are 3015 cm^{-1} and 3075 cm^{-1} .

For the substituted derivatives of benzene, the deformation vibrations of C-H bonds in the aromatic ring produce strong peaks in the $680\text{-}1000\text{ cm}^{-1}$ spectral region. The deformation vibrations in phase of hydrogen atoms linked by the aromatic ring imply the movement of closing and opening an umbrella. The band attributed to these vibrations is

accompanied by a strong band associated with the out-of-plane deformation of aromatic ring.¹³⁻¹⁵ In the GC/FTIR spectra of studied stimulants it is an absorption band at 735 cm^{-1} which is associated with aromatic C-H deformation vibrations. This band is accompanied by a strong band (at 695 cm^{-1} for C2, C4 and C5 compounds, at 705 cm^{-1} for the C1 compound and at 685 cm^{-1} for the C3 compound) attributed to the out-of-plane deformation of non-substituted phenyl ring.

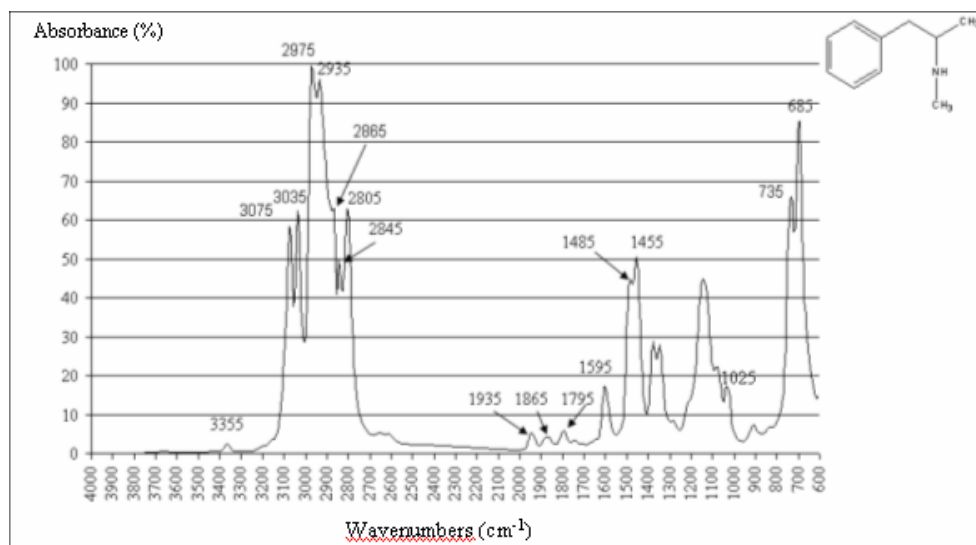


Figure 7 – The GC-FTIR spectrum of methamphetamine (C3).

The monosubstituted compounds of benzene have two degenerate double vibrations (1485 cm^{-1} and 1585 cm^{-1}) attributed to the aromatic C – C bond.¹³ In our case the first degenerate double vibration appears in the GC/FTIR spectra of stimulants as two peaks at the following wavenumbers: 1495 cm^{-1} for the C1, C2, C4 (shoulder) and C5 (shoulder) compounds and

1485 cm^{-1} for the C3 compound in the case of the first peak and 1455 cm^{-1} for C1, C2, C3 and C4 stimulants and 1465 cm^{-1} for the C5 compound in the case of the second peak.

The second degenerate double vibration of the aromatic C – C bond appears for the majority of studied stimulants (C1, C3, C4 and C5) as a single absorption band excepting the dextroamphetamine which has two absorption peaks at 1655 cm^{-1} and 1605 cm^{-1} wavenumbers. For the C3, C4 and C5 stimulants the absorption band associated with the aromatic C – C stretching vibration is at 1595 cm^{-1} and for the amphetamine (C1) is at 1605 cm^{-1} .

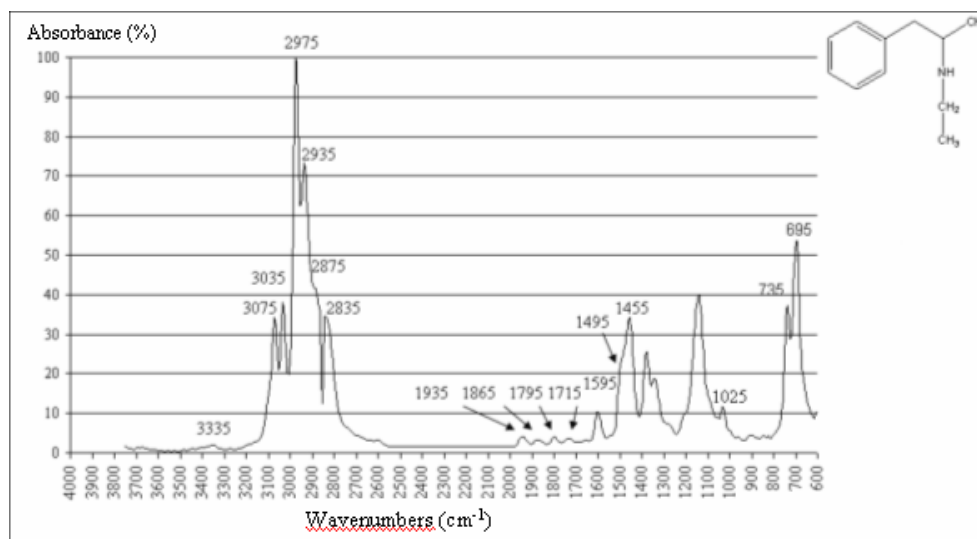


Figure 8 – The GC-FTIR spectrum of N-ethylamphetamine (C4)

For the benzene molecule there are overall vibrations of the carbon atoms which compose the aromatic ring known under the name of *ring vibrations* or *breathing vibrations*. Because of molecular symmetry the ring vibrations of benzene are inactive in IR.¹³ In the case of monosubstituted compounds of benzene e.g. stimulant amphetamine, the *breathing vibrations* are active in IR and have in GC/FTIR spectra a very stable band but with low intensity (under 20% absorbance) at 1025 cm^{-1} wavenumber (Figs. 6, 8-10). Only for dextroamphetamine (Fig. 6) this absorption peak is pronounced and has a higher intensity (about 50% absorbance).

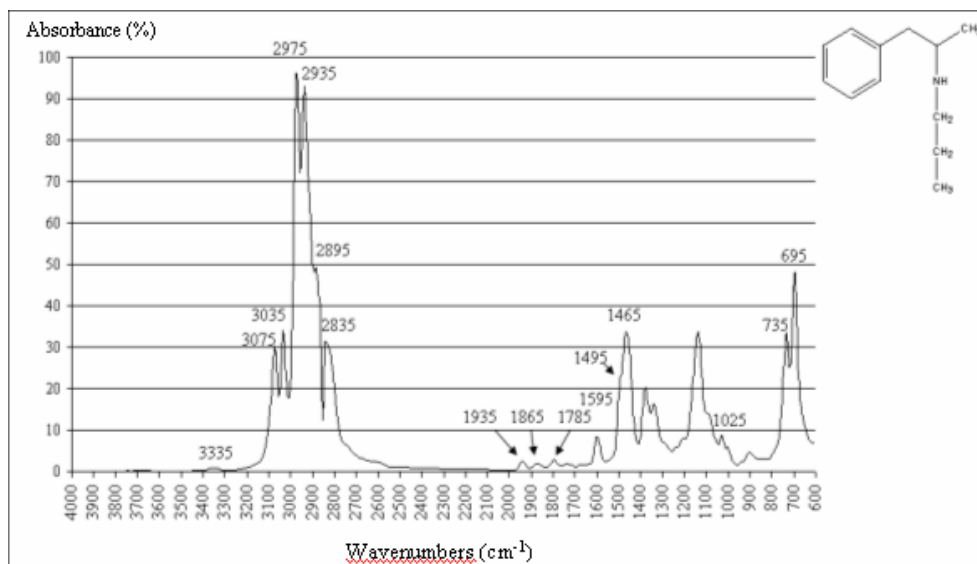


Figure 9 – The GC-FTIR spectrum of N-n-propylamphetamine (C5).

The second spectral region puts in evidence the type of substitution of aromatic ring. The presence of monosubstituted aromatic ring is

confirmed by the existence of four overtone and combination bands with low intensities in the 680 – 900 cm^{-1} spectral region.¹³⁻¹⁵ In the case of studied stimulant amphetamines these absorption bands are present in their GC/FTIR spectra (see Figs. 5-9) with the exception that for the methamphetamine (C3) and *N-n*-propylamphetamine (C5) the fourth band has very low intensity. The wavenumbers of overtone and combination bands are the following: 1935, 1865, 1795 and 1725 cm^{-1} for amphetamine and dextroamphetamine, 1935, 1865, 1795 and 1715 cm^{-1} for *N*-ethylamphetamine, 1935, 1865 și 1795 cm^{-1} for methamphetamine and 1935, 1865 și 1785 cm^{-1} for *N-n*-propylamphetamine.

The third spectral domain contains medium and strong peaks as intensities attributed to the symmetric and antisymmetric stretching vibrations of – CH_3 and – CH_2 groups present in the aliphatic side chain of the molecular structure of stimulants. These absorption bands appear in the 2830 – 3000 cm^{-1} spectral region.^{13, 14} In the GC/FTIR spectra of studied stimulants the antisymmetric stretching vibrations of aliphatic – CH_3 and – CH_2 groups lead to the appearance of two strong peaks at 2965 cm^{-1} and 2925 cm^{-1} for C1, at 2975 cm^{-1} and 2925 cm^{-1} for C2, and at 2975 cm^{-1} and 2935 cm^{-1} for C3, C4 and C5. The symmetric stretching vibrations of aliphatic – CH_3 and – CH_2 groups give in the GC/FTIR spectra two

absorption peaks: the first is a shoulder (at 2865 cm^{-1} for C1 and C3, at 2875 cm^{-1} for C4, and at 2895 cm^{-1} for C5) and the second absorption is a peak with medium intensity (2835 cm^{-1} for C4 and C5, and 2845 cm^{-1} for C1 and C3). In the case of dextroamphetamine (C2) the aliphatic $-\text{CH}_3$ and $-\text{CH}_2$ stretching vibrations present three absorption bands at the following wavenumbers: 2975 cm^{-1} , 2925 cm^{-1} and 2855 cm^{-1} .

The fourth spectral domain is composed of other two spectral regions attributed to N – H stretching and deformation vibrations. The first spectral region ($3050 - 3500\text{ cm}^{-1}$) contains absorption bands characteristic of the N – H symmetric and antisymmetric stretching vibrations.^{13, 14} In the case of studied stimulants, in the above-mentioned spectral region there is a peak with extremely low intensity ($0.03 - 0.007$) at 3325 cm^{-1} for C1, 3275 cm^{-1} for C2, at 3355 cm^{-1} for C3 and 3335 cm^{-1} for C4 and C5. Also, we observe that the type of amine, i.e. primary or secondary, cannot be determined. In the second spectral region ($650 - 1000\text{ cm}^{-1}$) an absorption band is present, associated with out-of-plane N – H deformation vibrations. As in the same spectral region there are also present bands attributed to the aromatic ring, sometimes we cannot identify the band associated with N – H deformation vibrations being overlapped with these. Only in the GC/FTIR spectrum of amphetamine (see Fig. 5) we can observe an absorption band at 785 cm^{-1} wavenumber associated with out-of-plane N – H deformation vibrations.

Experimental

A Perkin Elmer (Buckinghamshire, UK) Autosystem GC was interfaced with a light pipe GC-IR System 2000 and connected to a FTIR System 2000 with a mid-infrared source and a medium band, liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. Temperature-programmed separations were carried out on a Hewlett-Packard 5Palo, CA, USA) Ultra-1 methylsilicone capillary column (25 m x 0.32 μ m i.d., 0.52 μ m film thickness). The carrier gas was helium at a flow rate of 1.8 ml min⁻¹. The analytical column outlet the light pipe inlet. Helium carrier gas was added as make-up gas at a flow rate of 1.8 ml min⁻¹ at the connection between the capillary column and the light pipe. The gold-coated light pipe (12 cm x 1 mm i.d.) was heated at a constant temperature of 270 °C. Real time spectra were obtained by addition of two scans, with a spectral resolution of 8 cm⁻¹ and 32 background scans. Chromatograms were calculated by the Gram-Schmidt vector orthogonalization method. Methanolic stock solutions (1.0 mg/ml) of the reference standards were injected into the GC-FTIR system. The value of the concentration of the stock solutions was chosen at 1.0 mg mL⁻¹ because the methanolic extracts of the street samples are in the same concentration range.

Gram-Schmidt reconstruction was performed using 10 basis vectors throughout the run. Baseline correction was performed on the reconstructed Gram-Schmidt gas-chromatogram and low-noise vapor-phase FTIR spectra were generated after co-addition. The obtained reference spectra were stored in a digital library after normalization. The scan range was from 4000 to 580 cm⁻¹. All spectra were reduced in size by eliminating the spectral windows where the compounds in the database have no IR absorptions.

Hence, data ranged from 3745 to 2555 cm^{-1} and from 1995 to 605 cm^{-1} .

The mass spectra (electron impact ionization) of the compounds studied in this paper were imported from general MS libraries (NIST mass spectral database, AAFS spectral library and an in-house-made MS library). The MS spectra range from m/z 12 to 260.

Conclusions

The GC/MS technique discriminates very well the stimulant amphetamines of different molecular masses. In the case of optical isomers e.g. amphetamine and dextroamphetamine, this technique has no discrimination power. All stimulants have as specific ion fragment the tropilium cation resulted from the preferential scission – β cleavage – which is accompanied by cyclopropenyl and cyclopentadienyl cations.

The GC/FTIR technique is a selective method for the identification of the stimulant amphetamines. It is worth to emphasize that the length of aliphatic side chain has a major influence on the shape and intensity of the absorption band and much less on their position. An exception to this remark is the (2000-1700) cm^{-1} spectral region, which is specific to overtone and combination bands very stable for all stimulants.

The optical isomers, amphetamine and dextroamphetamine, can be successfully identified by means of GC/FTIR spectra.

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References

1. Karch, S.B., *Drug Abuse Handbook*, New York, CRC Press, 1998.
2. National Institute on Drug Abuse, *Pharmacology and Toxicology of Amphetamine and Related Designer Drugs*, Washington, Research Monograph 94, 1989.
3. Bronson, M. E., Jiang W., De Ruiter J., and Clark, C. R., *Pharmacol. Biochem. Behav.*, **51**, 447 (1995).
4. Aalberg, L., DeRuiter, J., Noggle, F. T., Sippola E., and Clark, C. R., *J. Chromatogr. Sci.*, **41**, 227 (2003).
5. Thigpen, A. L, Awad, T., De Ruiter J., and Clark, C. R., *J. Chromatogr. Sci.*, **46**, 900 (2008).
6. Awad, T., Clark C. R., and DeRuiter, J., *J. Chromatogr. Sci.*, **45**, 468 (2007).

7. Smith, F. P. *Handbook of Forensic Drug Analysis*, London, Elsevier Academic Press, 2005.
8. Duncan W. and Soine, W.H., *J. Chromatogr. Sci.*, **26**, 521 (1988).
9. Kempfert, K. *Appl. Spectroscopy*, **42**, 845 (1988).
10. Belal, T., Awad, T., DeRuiter J., and Clark, C. R., *Forensic Science Int.*, **184**, 54 (2009).
11. Smith, F. P., *Handbook of Forensic Drug Analysis*, London, Elsevier Academic Press, 2005.
12. Vâjială, G. E., *Analiza substanțelor interzise în sport*, București, Editura Renaissance, 2006.
13. Avram M. and Mateescu, GH. D, *Spectroscopia în infraroșu. Aplicații în chimia organică*, București, Editura Tehnică, 1966.
14. Dyer, J. R., *Applications of absorption spectroscopy of organic compounds*, N. J., Prentice-Hall, Inc., Englewood Cliffs, 1965.
15. Bellamy, L. J., *The infra-red spectra of complex molecules*, London, Chapman and Hall Ltd, 1978.
16. Herzberg, G., *Molecular Spectra and Molecular Structure, vol. II, IR and Raman Spectra of Polyatomic Molecules*, New York, Princeton, Van Nostrand Company, 1960.