Forensic Science International 199 (2010) 18–28

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

GC–IRD methods for the identification of some tertiary amines related to MDMA

Hadir M. Maher [a], Tamer Awad [b,c], Jack DeRuiter [c], C. Randall Clark [c,*]

[a] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt
[b] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Suez Canal University, Ismaillia 41552, Egypt
[c] Division of Medicinal Chemistry, Harrison School of Pharmacy, Auburn University, Auburn, AL 36849, USA

ARTICLE INFO

Article history:
Received 22 July 2009
Received in revised form 5 February 2010
Accepted 21 February 2010
Available online 3 April 2010

Keywords:
MDMA
2-Dimethylamino-1-(methoxyphenyl)ethanone
N,N-dimethyl-2-(methoxymethylphenyl)ethanamine
N,N-dimethyl-2-(2,3- and 3,4-methylenedioxyphenyl)ethanamine
Regioisomers
Isobaric substances
GC–IRD

ABSTRACT

Gas chromatography with infrared detection (GC–IRD) provides direct confirmatory data for the identification of the drug of abuse; 3,4-MDMA and its regioisomer; 2,3-MDMA, from a set of seven tertiary amines which have an isobaric or regioisomeric relationship with the MDMAs. These compounds include three ring substituted regioisomers of 2-dimethylamino-1-(methoxyphenyl)ethanone, two ring regioisomers of N,N-dimethyl-2-(methoxymethylphenyl)ethanamine in addition to N,N-dimethyl-2-(2,3- and 3,4-methylenedioxyphenyl)ethanamine. The major mass spectral fragments for each of these unique isomers occur at equivalent mass and all have equal molecular weight. Thus, gas chromatography with mass spectrometry detection (GC–MS) does not provide sufficient information for the confirmation of identity of any one of these isomers to the exclusion of the other compounds. The infrared spectra for these compounds allow for identification of any one of these amines. This differentiation is accomplished without the aid of chemical derivatization. The IR spectra served to divide the studied compounds into four groups depending on their absorption bands in the region 2700–3100 cm^{-1}. Moreover, compounds with different ring substitution pattern within each group can be differentiated by several bands in the 700–1700 cm^{-1} region. These regioisomeric substances are well resolved by GC on Rtx-1 stationary phase and the vapor-phase infrared spectra clearly differentiate among this set of compounds.

1. Introduction

The controlled substance 3,4-methylenedioxymethamphetamine (MDMA), also known as Ecstasy, belongs to the phenethylamine group of frequently abused drugs. MDMA appears to act through the enhancement of serotonin mediated neurotransmission producing feelings of euphoria, energy and desire to socialize [1]. However, its abuse is risky and severe even fatal intoxications have been reported [1,2]. Determination and characterization of MDMA in biological and forensic samples has been the focus of many studies over the past years [3–5]. Gas chromatography–mass spectrometry (GC–MS) is the most widely used technique in the analysis of controlled substances in forensic laboratories [6–15]. The differentiation of 2,3- and 3,4-methylenedioxyphenylalklamines by GC–MS–MS has been described [16].

Regioisomeric and isobaric substances are considered a significant challenge for the analytical techniques used to identify specific substances. This is extremely important when some of these molecules are legally controlled drugs of abuse or controlled precursor substances [6–16]. While the mass spectrum is often considered a specific "fingerprint" for an individual compound, there are other substances that produce very similar or almost identical mass spectra. Many of these compounds that yield the same mass spectrum are of a regioisomeric or an isobaric relationship to the drugs of abuse. Such compounds having mass spectral equivalency and similar elution properties, perhaps co-elution represent a serious analytical challenge. In these cases, identification by gas chromatography (GC)–mass spectrometry (MS) must be based primarily upon the chromatographic system's ability to separate the entire set of substances. Those substances co-eluting in the chromatographic system could be misidentified. A complete set of standards must be available for a thorough method validation study and to exclude the possibility of co-elution of combinations of the regioisomeric and/or isobaric molecules. Furthermore, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target molecules.

Previous reports [6–15] in this series have described the analytical properties of a group of compounds that have unique regioisomeric or isobaric equivalence to the drug of abuse 3,4-methylenedioxymethamphetamine (3,4-MDMA) or its regioisomer 2,3-methylenedioxymethamphetamine (2,3-MDMA). These compounds are likely to produce major mass spectral fragment ions of equivalent mass to 3,4-MDMA and provide a significant

* Corresponding author at: Department of Pharmacal Sciences, Division of Medicinal Chemistry, Harrison School of Pharmacy, Auburn University, Auburn, AL 36849, USA. Tel.: +1 334 844 8326; fax: +1 334 844 8331.
E-mail address: clarkcr@auburn.edu (C.R. Clark).

0379-0738/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2010.02.022

challenge for chromatographic resolution. Differentiation of regioisomers and isobaric substances is a significant issue in forensic drug chemistry and has been addressed in a number of drug categories [6–16].

The compounds involved in this study (Fig. 1) are the ring substituted regioisomers of 2-dimethylamino-1-(methoxyphenyl)ethanone (compounds 1–3), ring regioisomers of N,N-dimethyl-2-(methoxymethylphenyl)ethanamine (compounds 4 and 5) in addition to N,N-dimethyl-2-(2,3-methylenedioxyphenyl)ethanamine (compound 6) and N,N-dimethyl-2-(3,4-methylenedioxyphenyl) ethanamine (compound 7). All these compounds have the potential to produce almost identical mass spectra as 3,4-MDMA (Fig. 2). These isobaric/regioisomeric compounds have the same molecular weight and are expected to yield MS fragments of the same elemental composition $(C_3H_8N)^+$ for m/z 58 ion as that observed for 2,3- and 3,4-MDMA. Additionally, the various isobaric ring substituted methoxymethylbenzyl $(C_9H_{11}O)^+$ and isomeric methoxybenzoyl $(C_8H_7O_2)^+$ fragments have the same mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation observed in the MDMAs and occurring at m/z 135. Thus these compounds have the strong possibility of being misidentified as 3,4-MDMA by some commonly used analytical methods. Since all these seven related compounds are tertiary amines, they do not form stable amide derivatives. Thus, differentiation of these related compounds using chemical derivatization is not possible.

Methods for molecular individualization without the need for reference standards of every regioisomeric/isobaric compound have significant advantages in forensic drug chemistry. While nuclear magnetic resonance (NMR) can be a useful method for differentiation of regioisomers, it is not a technique with direct

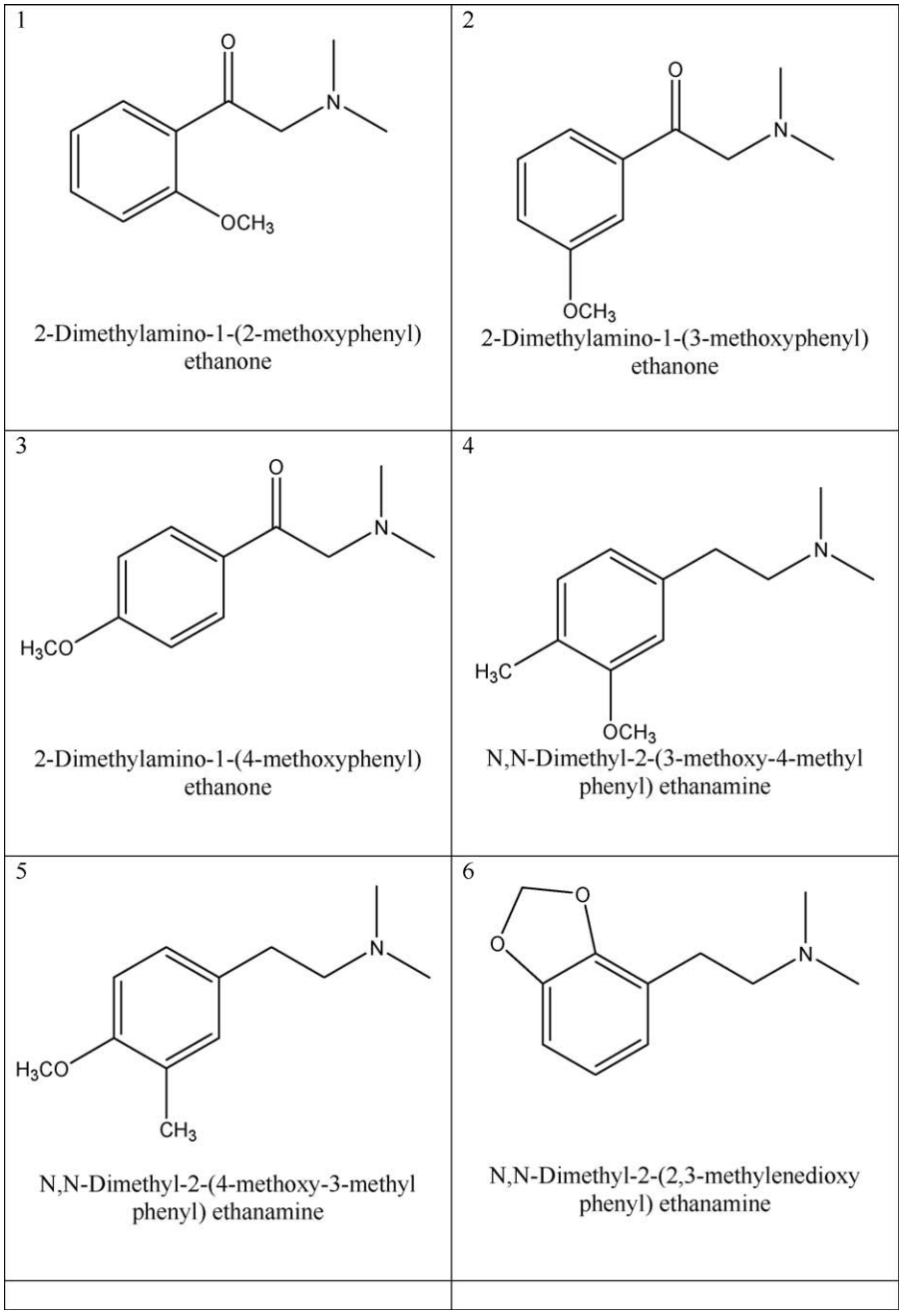

Fig. 1. Structures of compounds 1–9.

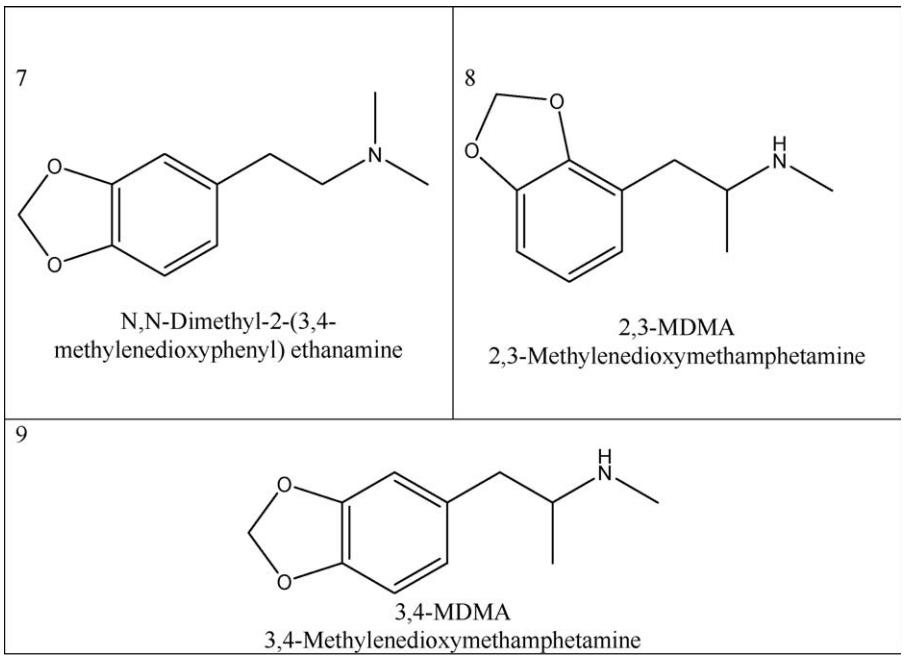

Fig. 1. (*Continued*).

application for all areas of regulatory analysis. Most forensic drug samples are not of sufficient purity for direct NMR analysis and NMR is not usually applicable to the analysis of drugs in biological samples. Thus, the analysis of these drugs must depend heavily on chromatographic methods.

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. GC–FTIR spectroscopy is characterized by scanning quickly enough to obtain IR spectra of peaks eluting from the capillary columns. Thus this technique combines the separation power of GC with the identification power of IR. The GC–FTIR has been successfully applied to the confirmation of drug identification in forensic drug chemistry [17,18]. GC–IRD has been applied in previous studies to the differentiation of various ring and side chain substituted regioisomeric phenethylamines related to the drug of abuse, 3,4-MDMA [18,19].

The aim of this study is to evaluate gas chromatography with infrared detection for the discrimination and characterization of these seven positional isobaric/regioisomeric tertiary amines related to 2,3- and 3,4-MDMA.

2. Experimental

2.1. Instrumentation

GC–MS analysis was performed with an HP-5890 GC coupled with a HP-5970 mass selective detector (Hewlett-Packard, Palo Alto, CA). The MS was operated on the electron impact (EI) mode using ionization voltage of 70 eV. The GC was operated in splitless mode with a helium (grade 5) flow rate of 0.7 mL/min and the column head pressure of 10 psi. The injector temperature was set at 250 °C and the transfer line was maintained at 280 °C. Samples were dissolved in high-performance liquid chromatography-grade acetonitrile (Fisher Scientific, Fairlawn, NJ) and manually introduced (1 μL) individually and in a physical mixture using a 10-μL Hamilton syringe (Hamilton Co., Reno, NV).

GC–MS chromatographic separations were carried out on three different types of stationary phases; a column (30 m × 0.25 mm i.d.) coated with 0.25 μm 100% dimethyl polysiloxane (Rtx-1), a column (30 m × 0.25 mm i.d.) coated with 0.50 μm 50% phenyl–50% methyl polysiloxane (Rxi-50) and a relatively polar column (30 m × 0.25 mm i.d.) coated with 0.50 μm crossbond, trifluropropyl-methylpolysiloxane (Rtx-200). All the three columns were purchased from Restek Corporation (Bellefonte, PA). All separations were generated using three temperature programs. Program 1 consisted of an initial hold at 70 °C for 1 min, ramped up to 150 °C at a rate of 7.5 °C/min and held at 150 °C for 2 min, then ramped to 250 °C at a rate of 10 °C/min and held at 250 °C for 20 min. This program was used to separate a physical mixture of compounds 1, 2, 3, 6, 7, 8 and 9 on (Rtx-1) column. Program 2 was used to separate a physical mixture of compounds 4, 5, 6, 7 and 9 (Rtx-1 column). This program consisted of an initial hold at 70 °C for 1 min, ramped up to 150 °C at a rate of 5 °C/min and held at 150 °C for 2 min, then ramped to 250 °C at a rate of 10 °C/min and held at 250 °C for 20 min. Finally, program 3 was used to separate a physical mixture of compounds 4, 5, 6, 7, 8 and 9 on the three types of stationary phases; Rtx-1, Rxi-50 and Rtx-200. This program consisted of an initial hold at 70 °C for 1 min, ramped up to 150 °C at a rate of 2.5 °C/min and held at 150 °C for 3 min, then ramped to 250 °C at a rate of 15 °C/min and held at 250 °C for 20 min.

GC–IRD studies were carried out on a Hewlett-Packard 5890 Series II gas chromatograph and a Hewlett-Packard 7673 auto-injector coupled with a IRO-II Infrared detector (Analytical Solutions and Providers, Covington, KY, USA). The vapor-phase infrared detector (IRD) spectra were recorded in the range of 4000–550 cm^{-1} with a resolution of 8 cm^{-1} and a scan rate 1.5 scans/s. The IRD flow cell temperature as well as the transfer line was 280 °C and the GC was operated in the splitless mode with a carrier gas (helium grade 5) flow rate of 0.7 mL/min and a column head pressure of 10 psi. The column used was (Rxi-50). The temperature program involved in this study consisted of an initial temperature of 100 °C for 1 min, ramped up to 230 °C at a rate of 20 °C/min followed by a hold at 230 °C for 15 min.

In both GC–MS and GC–IRD analyses, samples were dissolved and diluted in high-performance liquid chromatography-grade acetonitrile (Fisher Scientific, Fairlawn, NJ) and introduced via the auto-injector using an injection volume of 1 μL.

2.2. Drugs and reagents

The nine isomeric compounds (Fig. 1), used in this study were synthesized in our lab. All laboratory reagents and chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI) or Fisher Scientific (Atlanta, GA).

2.3. General synthetic methods

The N,N-dimethyl ring substituted methoxy ethanones (compounds 1–3) were prepared by reacting the appropriately substituted aldehydes with methyl magnesium bromide to yield the secondary alcohol which is then oxidized to the corresponding ketone by pyridinum chlorochromate. The ketones were then halogenated in the alpha position through reaction with N-bromosuccinimide and benzoyl peroxide. Halogenated ketones were then converted to the corresoponding amines through reaction with N,N-dimethylamine.

The N,N-dimethyl isomers of the ring substituted methoxymethylphenyl (compounds 4 and 5) and 2,3- and 3,4-methylenedioxyphenyl substitution patterns (compounds 6 and 7) were prepared via the condensation of the appropriately substituted aldehydes with N-butyl amine followed by reaction with nitromethane to give the corresponding nitroalkene. Reduction of the nitroalkene with lithium aluminum hydride gave the corresponding phenethylamine which upon reductive amination with formaldehyde and sodium cyanoborohydride gave the desired methylamines.

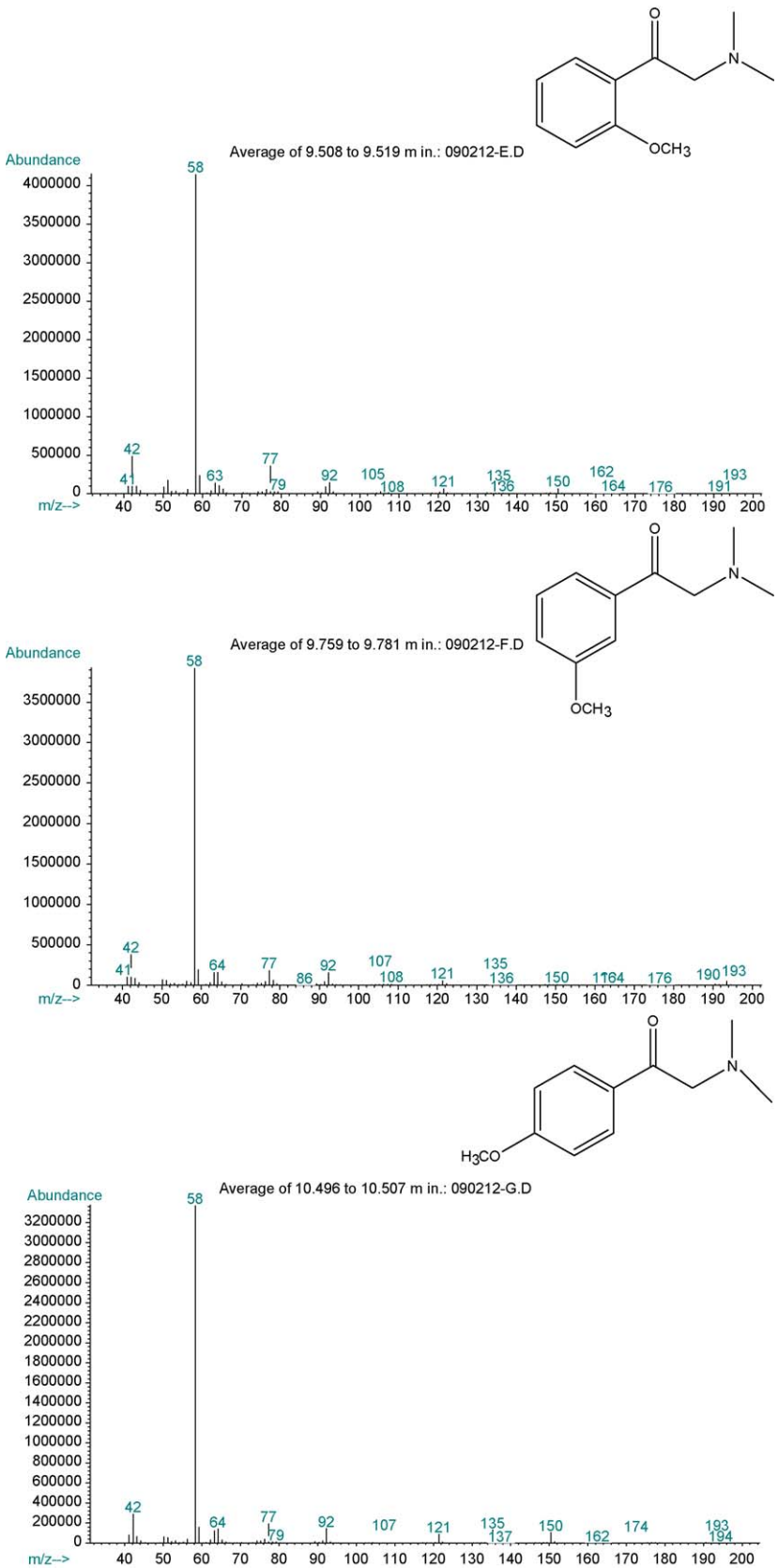

Fig. 2. Mass spectra of compounds 1–9.

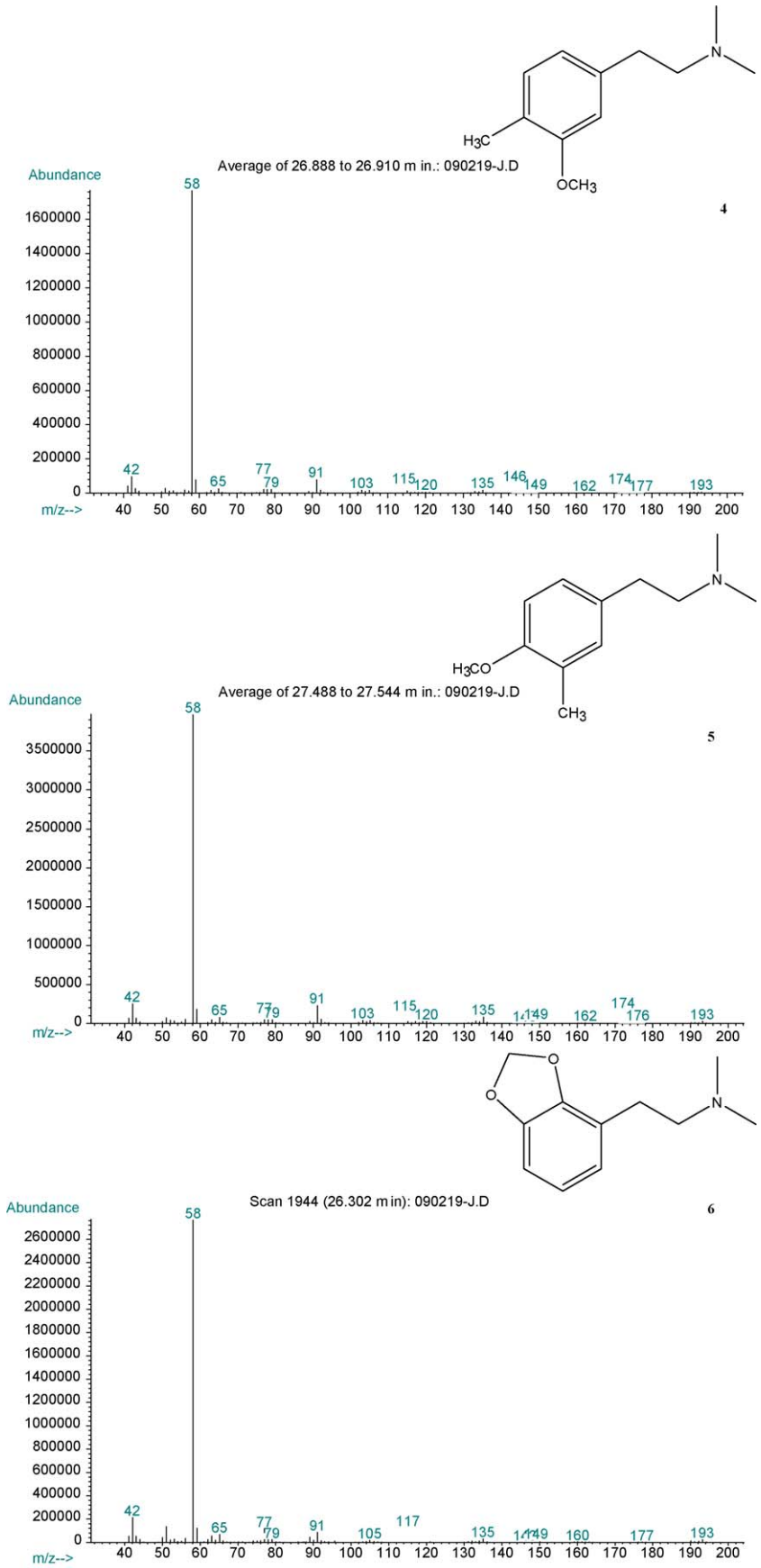

Fig. 2. (*Continued*).

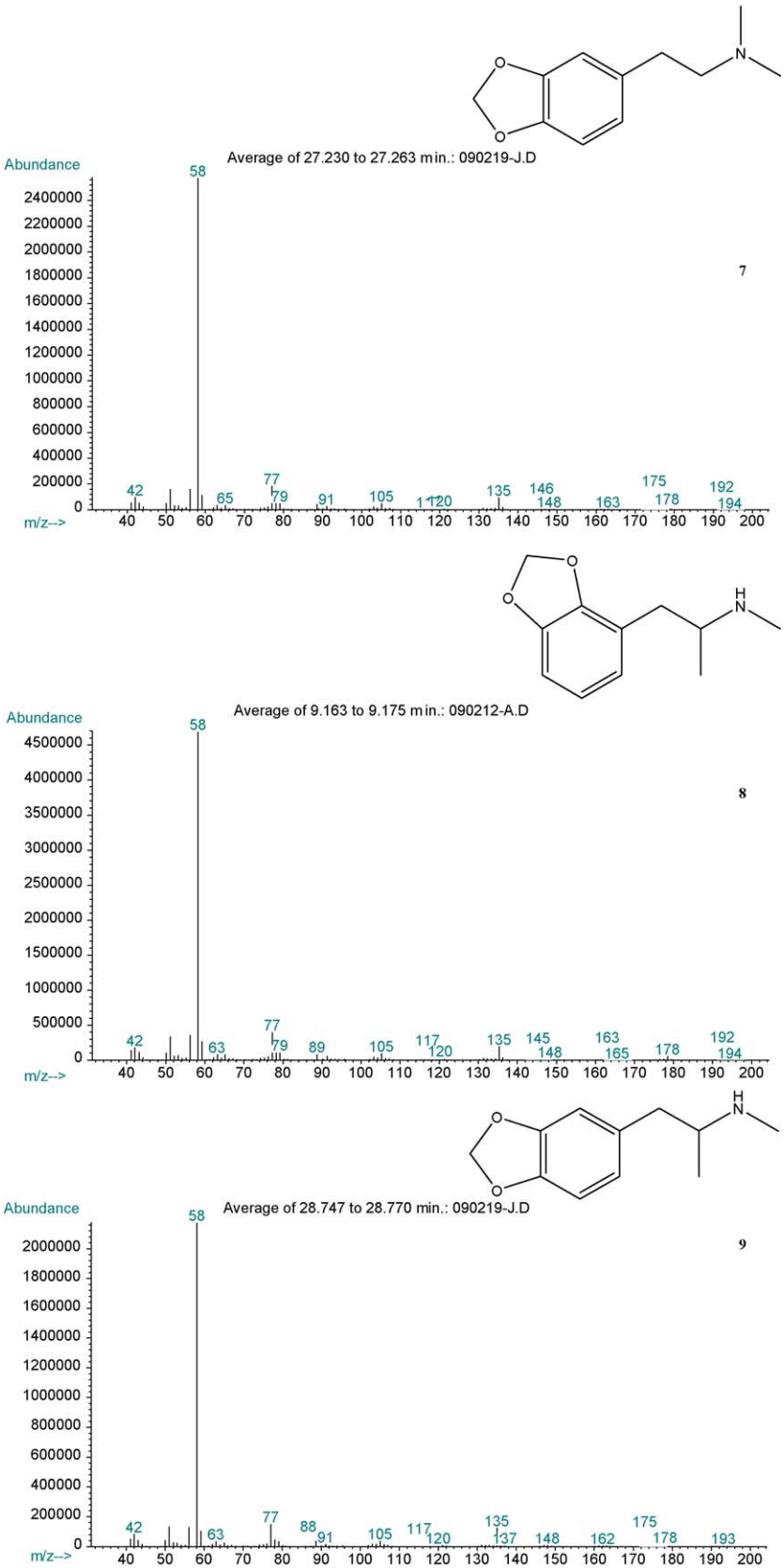

Fig. 2. (*Continued*).

The methods for the preparation of the 2,3- and 3,4-MDMA have been described in previous reports [6,8]. The general procedure for the synthesis of these compounds begins with the appropriate aldehyde, 2,3-methylenedioxybenzaldehyde and 3,4-methylenedioxybenzaldehyde (piperonal), as starting materials. The preparation of 2,3-methylenedioxybenzaldehyde has been reported previously [6,8]. Condensation of the appropriate aldehyde with nitroethane under basic conditions yields the 2-nitroalkenes, which can be reduced to the primary amines or hydrolyzed to the corresponding ketones and reductively aminated. All amines were converted to their hydrochloride salts using gaseous HCl.

3. Results and discussion

3.1. Mass spectrometry

Mass spectrometry is the primary method for confirming the identity of drugs and related substances in forensic samples. The mass spectra of phenethylamines are characterized by a base peak formed from an amine initiated α-cleavage reaction involving the carbon–carbon bond of the ethyl linkage between the aromatic ring and the amine. In 3,4-MDMA (MW = 193), the α-cleavage reaction yields the substituted immonium ion at *m/z* 58 and the 3,4-methylenedioxybenzyl fragment at *m/z* 135/136 (for the cation and the radical cation, respectively). Thus, the mass spectrum for 3,4-MDMA contains major ions at *m/z* 58 and 135/136 as well as other ions of low relative abundance [6]. This study deals with the ring substituted regioisomers of 2-dimethylamino-1-(methoxyphenyl)ethanone (compounds 1–3) and the ring regioisomers of N,N-dimethyl-2-(methoxymethylphenyl)ethanamine (compounds 4 and 5). These two ethanamines (compounds 4 and 5) have an isobaric relationship (same mass but different elemental composition) with the MDMAs. The studied compounds also include N,N-dimethyl-2-(2,3-methylenedioxyphenyl)ethanamine (compound 6) and N,N-dimethyl-2-(3,4-methylenedioxyphenyl) ethanamine (compound 7) which are side chain regioisomers of 2,3- and 3,4-MDMA, respectively. Thus they have the potential to yield a mass spectrum essentially equivalent to 2,3- and 3,4-MDMA. All have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at *m/z* 58 and 135/136. The mass spectra of the studied compounds are shown in Fig. 2. The isobaric methoxymethylbenzyl $(C_9H_{11}O)^+$ and the indirect regioisomeric methoxybenzoyl $(C_8H_7O_2)^+$ fragments have the same mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at *m/z* 135. In addition, they all share regioisomeric immonium ions in their electron ionization mass spectra at *m/z* 58. The fragmentation pattern and structures of the regioisomeric immonium ions for compounds 1–9 are shown in Fig. 3.

Since the studied compounds (1–7) are tertiary amines they do not form stable amide derivatives. Chemical derivatization using acylating agents cannot be used to achieve additional mass

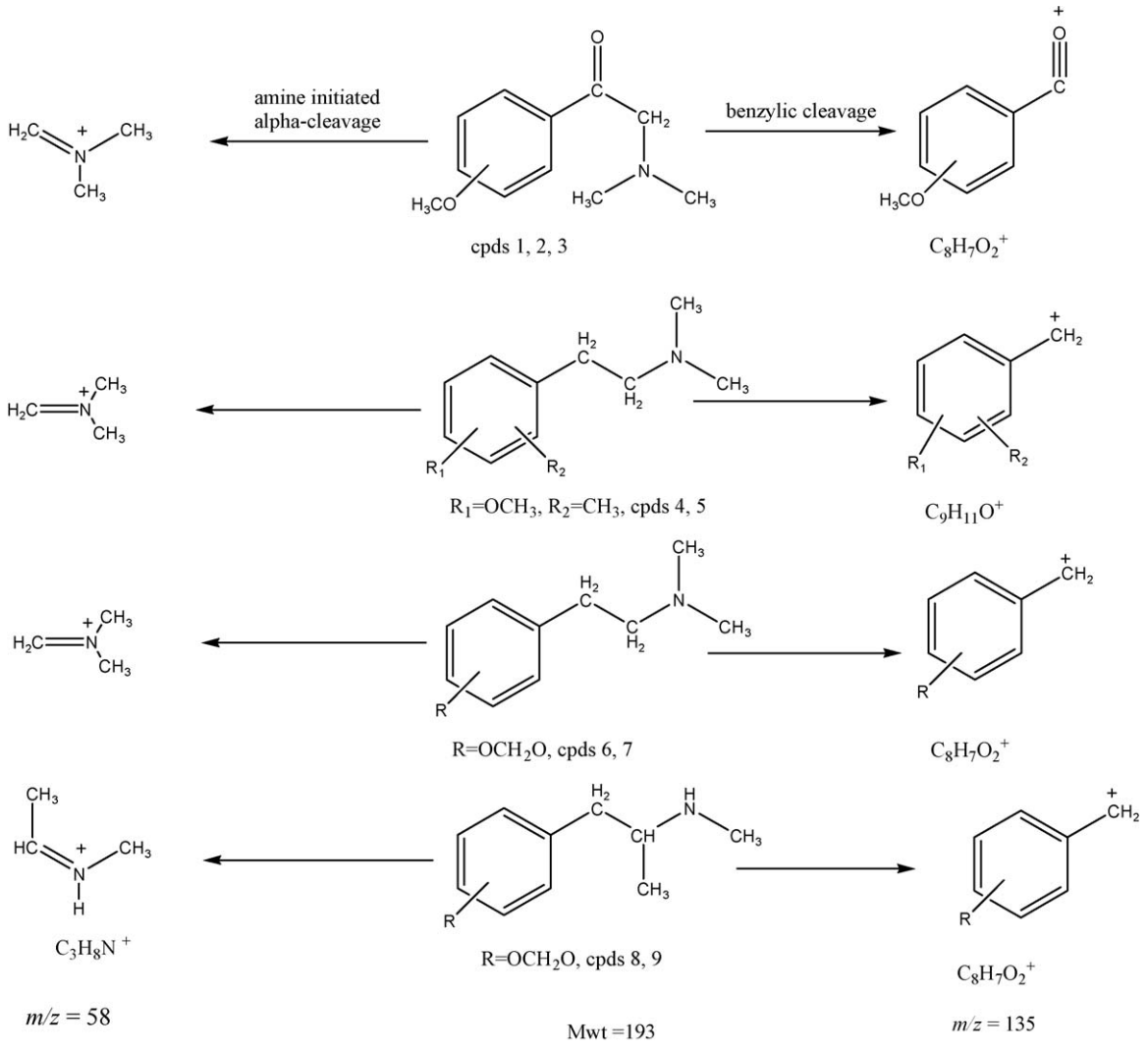

Fig. 3. Formation of the major fragment ions in the mass spectra of compounds 1–9.

spectral specificity in this set of compounds. This lack of mass spectral specificity for the isomers shown in Fig. 2, in addition to the possibility of chromatographic co-elution with 3,4-MDMA, could result in misidentification in this series of drugs and drug-like substances. The lack of available reference materials for all these compounds further complicates this situation. This constitutes a significant analytical challenge where the specific identification by gas chromatography–mass spectrometry (GC–MS) must be based primarily upon the ability of the chromatographic system to separate the regioisomeric non-drug substance from the actual drug of interest.

Therefore, analysis of these tertiary amine regioisomeric and isobaric substances by electron ionization mass spectrometry alone does not provide significant data for the specific differentiation of one of these regioisomers to the exclusion of the other compounds.

3.2. Vapor-phase infrared spectroscopy

Infrared spectroscopy is often used as a confirmatory method for drug identification in forensic drug analysis. In the second phase of this study, gas chromatography with infrared detection (GC–IRD) was evaluated for differentiation among the two reference drugs (2,3- and 3,4-MDMA) and their isobaric ring regioisomers (compounds 1–5) and side chain isomers (compounds 6 and 7) involved in the study. The vapor-phase infrared spectra should provide additional compound specificity although chemical modification of the tertiary amines is not practical. The vapor-phase infrared spectra for the nine studied compounds are shown in Fig. 4. The spectra were generated in the vapor-phase following sample injection into the gas chromatograph. Each compound shows a vapor-phase IR spectrum with absorption bands in the regions 700–1700 cm^{-1} and 2700–3100 cm^{-1}. Both regions are useful in the identification and differentiation among this set of compounds.

The IR spectra served to divide the studied compounds into four groups depending on their absorption bands in the region 2700–3100 cm^{-1}; the three regioisomers of 2-dimethylamino-1-(methoxyphenyl)ethanone (compounds 1–3), ring regioisomers of N,N-dimethyl-2-(methoxymethylphenyl)ethanamine (compounds 4 and 5), N,N-dimethyl-2-(2,3 and 3,4-methylenedioxyphenyl) ethanamine (compounds 6 and 7) and the reference 2,3- and 3,4-MDMA (compounds 8 and 9). The IR spectrum of the first group (compounds 1–3) is characterized by four absorption bands at about 2782, 2832, 2952 and 3079 cm^{-1} with the first and third bands of nearly equal intensity and the last one being a minor absorption band. Moreover, this set of three compounds is characterized by a significant absorption band in the 1700 cm^{-1} range, corresponding to the carbonyl group, which is not present in any of the remaining compounds. The second group of compounds (compounds 4 and 5) is characterized by four absorption peaks in the region 2700–3100 cm^{-1} at about 2778, 2825, 2867 and 2944 cm^{-1}, the last of which is the most intense peak and the two middle bands are of minor intensity. However, the third group of compounds (compounds 6 and 7) is characterized by having five absorption bands at 2779, 2825, 2875, 2952 and 3068 cm^{-1} with the last one being a very minor peak, the two middle peaks of relatively low intensity and the first and fourth peak of nearly equal stronger intensity. Finally, the fourth group of compounds which comprises 2,3- and 3,4-MDMA was significantly different from the other members in this limited set of compounds by the absorption band at 2801 cm^{-1} in addition to other bands at about 2879, 2929 and 2971 cm^{-1}.

Since members within the same group have the same side chain, they share almost the same IR features in the region 2700–3100 cm^{-1}. However, they can be easily discriminated from each other by some absorption bands in the region 700–1700 cm^{-1}. Concerning the first group, the three regioisomeric aminoketones (compounds 1–3) can be easily differentiated by the positions and intensities of several IR peaks in the region 700–1700 cm^{-1}. The 2-methoxy isomer (compound 1) can be distinguished by at least three IR bands of medium to strong absorption. The first is the absorption peak at nearly 752 cm^{-1} which is absent in the other two regioisomers. The second is the two characteristic peaks of nearly equal intensity in the IR spectrum of the 2-methoxy isomer at 1242 and 1285 cm^{-1} which are shifted to 1262, 1285 and 1250, 1289 cm^{-1} in the case of the 3- and 4-methoxy regioisomers, respectively. The third is the strong absorption band at 1482 cm^{-1} which is accompanied with a shoulder at about 1443 cm^{-1} for the 2-methoxy isomer. This is converted to the doublet peak at 1432 and 1482 cm^{-1} or to the small peaks at about 1416, 1466 and 1509 cm^{-1} for the 3- and 4-methoxy regioisomers, respectively. The 3-methoxy isomer (compound 2) is characterized by having an absorption band of intermediate intensity at nearly 1042 cm^{-1} which is lacking in the 2-methoxy isomer while shifted to a shorter wave number at 1034 cm^{-1} in the 4-methoxy ring isomer. Finally, the 4-methoxy regioisomer (compound 3) can be identified by the strong absorbance at 1173 cm^{-1} while the other two regioisomers do not show this peak.

The second group, the two ring regioisomers of N,N-dimethyl-2-(methoxymethylphenyl)ethanamine (compounds 4 and 5) can be distinguished from each other by at least three absorption bands in the region 700–1700 cm^{-1}. Compound 4 is characterized by the strong singlet absorption peak at 1258 cm^{-1} which is converted into the two adjacent peaks at 1223 and 1254 cm^{-1} in the IR spectrum of compound 5. On the other hand, the latter could be distinguished by the two adjacent peaks at 1466 and 1505 cm^{-1} which is converted into the relatively weak triplet peak at 1416, 1466 and 1509 cm^{-1} in the 3-methoxy-4-methyl isomer. In addition, the small absorption peak appearing in the IR spectra of compound 5 at 1613 cm^{-1} is converted into a small doublet peak at about 1582 and 1613 cm^{-1} in the case of compound 4.

Members of the third group which are N,N-dimethyl-2-(2,3 and 3,4-methylenedioxyphenyl) ethanamine (compounds 6 and 7) could also be differentiated by at least three absorption peaks of different intensities. The first is the strong absorption band at 1073 cm^{-1} which is present in the IR spectrum of compound 6. This peak is shifted to a shorter wave number at 1050 cm^{-1} in case of compound 7. The second is the relatively small broad band at 1173 cm^{-1} which is shifted to a sharp small absorption band at 1192 cm^{-1} in the IR spectra of compounds 6 and 7, respectively. The third is the strong absorption band at 1459 cm^{-1} which appears in the IR spectrum of compound 6. This absorption appears as two adjacent peaks at 1443 and 1489 cm^{-1} in compound 7.

Concerning the reference compounds, 2,3- and 3,4-MDMA (compounds 8 and 9), both compounds have the methamphetamine side chain composition; therefore they show similar IR absorption to other methamphetamine compounds in the region 2800–3100 cm^{-1}. The two MDMA regioisomers can be differentiated using two IR bands [17]. Since these two MDMAs share the same side chain composition but differ in the ring substitution pattern, like other members within the same group, they show significant differences only in the region 700–1700 cm^{-1}. The 2,3-MDMA shows a medium intensity peak at 1065 cm^{-1} while the 3,4-MDMA shows a similar peak at 1050 cm^{-1}. Another more evident IR band to differentiate these two compounds can be seen in the region 1440–1490 cm^{-1} where the 2,3-MDMA shows a strong band at 1456 cm^{-1}, the 3,4-MDMA has two adjacent peaks in this region at 1443 and 1489 cm^{-1} of which the latter is a strong absorption band.

These studies indicate that vapor-phase infrared spectra provide useful data for differentiation among these regioisomeric

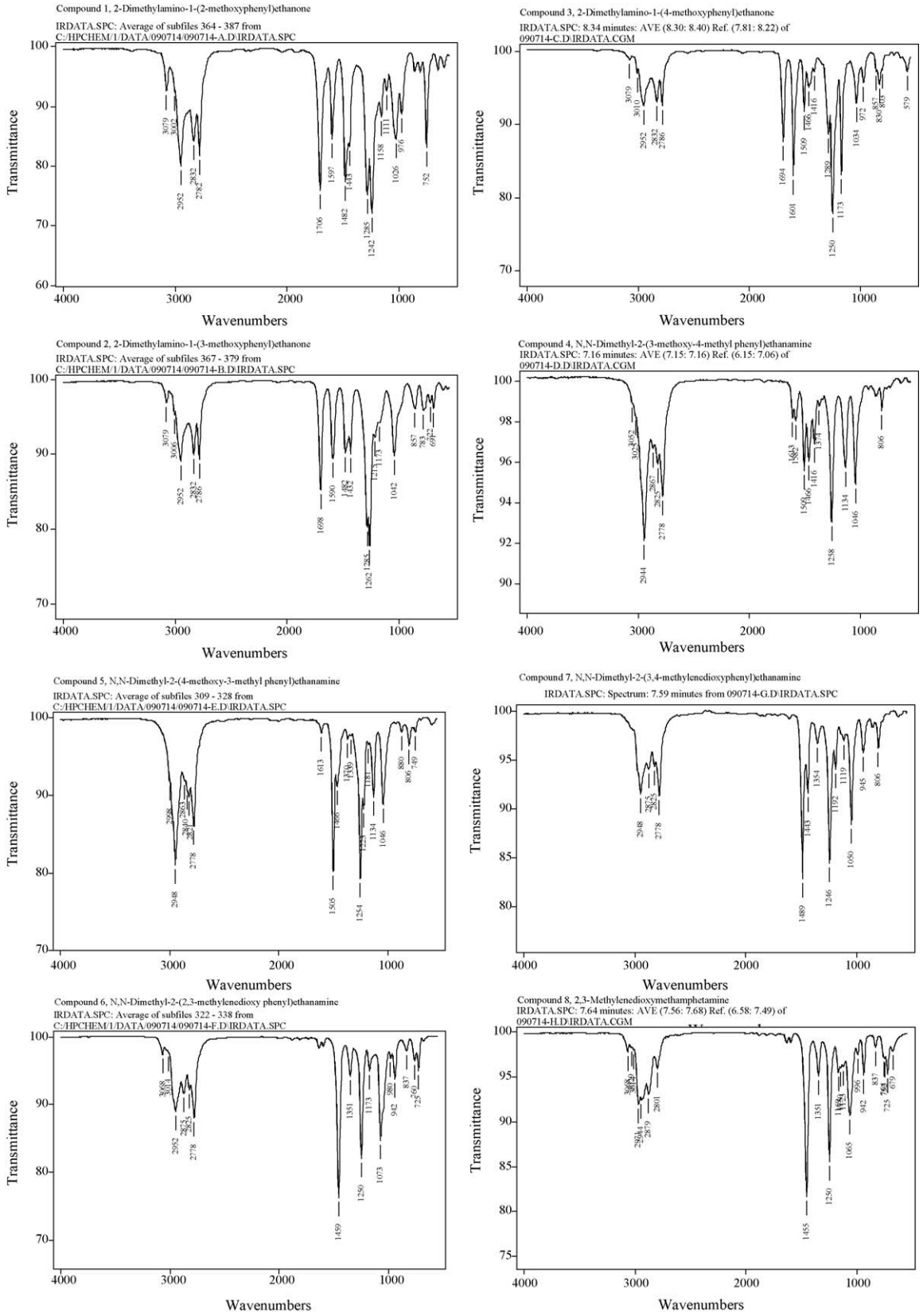

Fig. 4. Vapor-phase IR spectra of compounds 1–9.

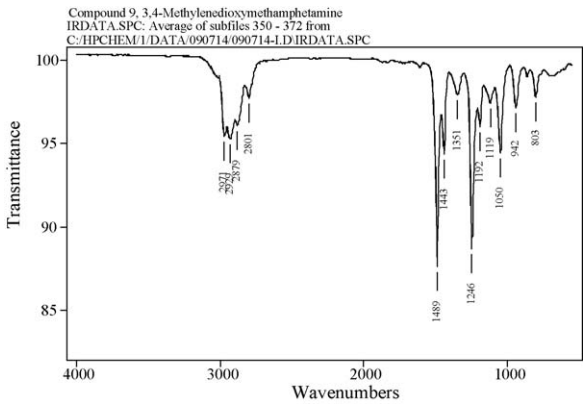

Fig. 4. (*Continued*).

and isobaric amines of mass spectral equivalence. Infrared absorption bands provide distinguishing and characteristic information to individualize the amines in this set of uniquely similar compounds which cannot be obtained from their mass spectra.

3.3. Gas chromatography

The compounds involved in this study were divided into two groups based on ring and side chain substitution pattern. The first group of compounds are the three regioisomers of 2-dimethylamino-1-(methoxyphenyl)ethanone (compounds 1–3) together with N,N-dimethyl-2-(2,3 and 3,4-methylenedioxyphenyl) ethanamine (compounds 6 and 7). This first group is made up of the direct and indirect regioisomers of MDMA. The second group is the ring regioisomers of N,N-dimethyl-2-(methoxymethylphenyl)ethanamine (compounds 4 and 5) in addition to compounds 6 and 7. The elution properties of each of these groups of compounds

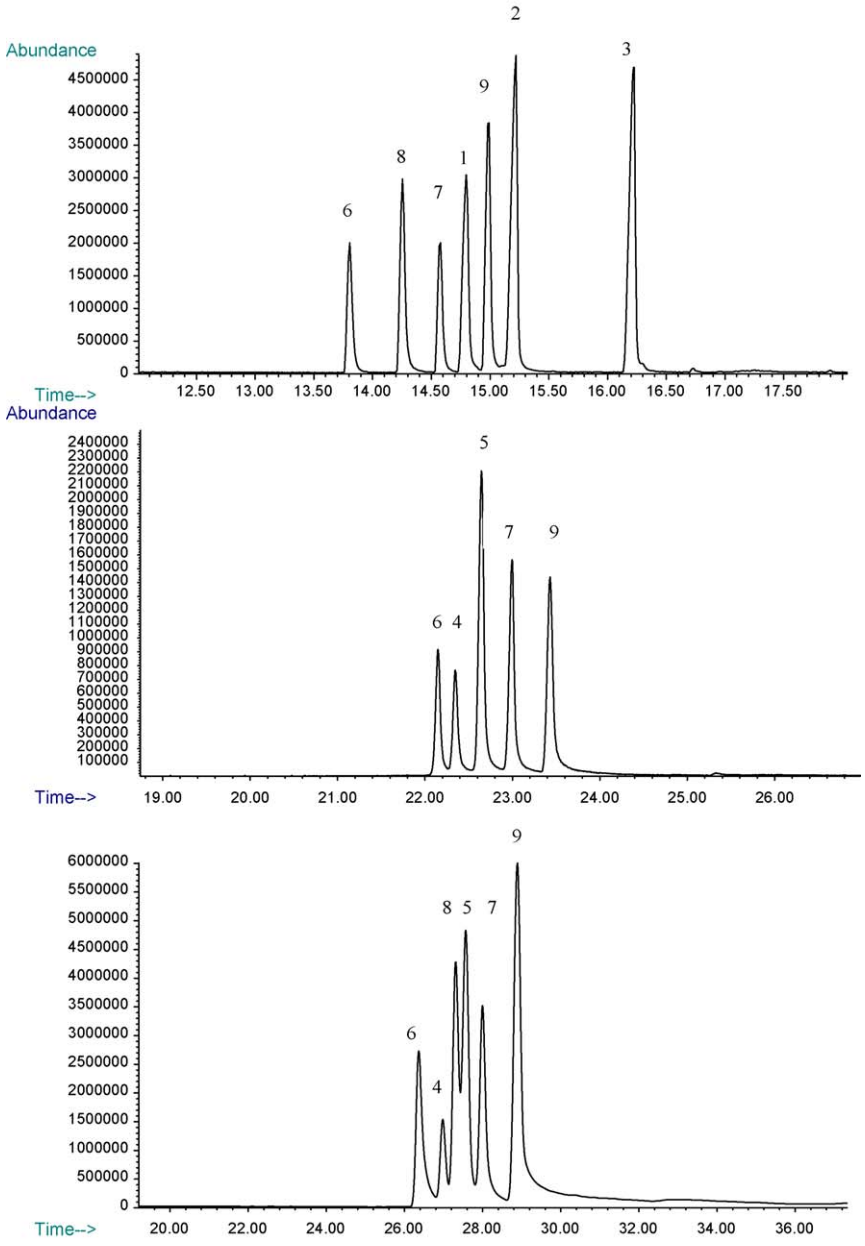

Fig. 5. Gas chromatographic separation of compounds (1–9) using (A) temperature program 1, (B) temperature program 2 and (C) temperature program 3.

were compared to 2,3- and 3,4-MDMA (compounds 8 and 9). The mixtures were compared on different stationary phases using several temperature programs, however the best compromise between resolution and analysis time was achieved on the 100% dimethyl polysiloxane (Rtx-1) column. Two temperature programs were developed. Program 1 showed the best resolution for the first mixture (Fig. 5A), while temperature program 2 offered good resolution for the second mixture (Fig. 5B) on the Rtx-1 column. Details of the temperature programs were described in Section 2.

The chromatogram in Fig. 5A shows the separation of the first group of compounds, the ring regioisomers as the side chain substitution pattern is held constant (methoxy isomers; compounds 1–3 and methylenedioxy isomers; compounds 6 and 7) together with the two reference compounds 2,3- and 3,4-MDMA. The methoxy regioisomers elution order is the 2-methoxy regioisomer (compound 1) followed by 3-methoxy (compound 2) then the 4-methoxy regioisomer (compound 3) elutes last. Also the 2,3-methylenedioxy substitution pattern elutes before the 3,4-methylenedioxy substitution for both secondary (compounds 8 and 9) and tertiary (compounds 6 and 7) amines. Moreover, among the methylenedioxy substitution pattern, the tertiary amines elute before the secondary amines, compound 6 elutes before compound 8 and also compound 7 elutes before compound 9. The chromatogram in Fig. 5B shows the separation of the methoxymethyl ring regioisomers (compounds 4 and 5) and methylenedioxy tertiary amines (compounds 6 and 7) as well as 3,4-MDMA (compound 9). In this set of compounds, compound 6 elutes first while 3,4-MDMA is the most retained compound. The 3-methoxy-4-methyl regioisomer (compound 4) is less retained compared to the 4-methoxy-3-methyl regioisomer (compound 5).

An examination of the chromatogram in Fig. 5C (temperature program 3) illustrates the value of the IR spectral differentiation coupled with chromatographic separation. The chromatogram in Fig. 5C is a duplicate of Fig. 5B with the addition of compound 8 to the mixture. Compounds 8 and 5 have similar elution properties under these chromatographic conditions resulting in a significant peak overlap. Since the mass spectra for compounds 8 and 5 are essentially equivalent (see Fig. 2), misidentification of one of these compounds is quite possible. However, the vapor-phase infrared spectra of these two substances clearly allow their differentiation.

Under optimized chromatographic conditions, a significant overlap between the peaks of compound 5 and 8 (2,3-MDMA) was observed when the separation was performed using the relatively non-polar stationary phases Rtx-1 or Rxi-50 columns. When the more polar Rxi-200 column was used, a similar overlap was observed between compounds 6 and 4.

4. Conclusion

Ring substituted regioisomers of 2-dimethylamino-1-(methoxyphenyl)ethanone, ring regioisomers of N,N-dimethyl-2-(methoxymethylphenyl)ethanamine in addition to N,N-dimethyl-2-(2,3- and 3,4-methylenedioxyphenyl) ethanamine yield major fragment ions in their mass spectra equivalent to those of 2,3- and 3,4-MDMA. GC–IRD analysis yields unique and characteristic vapor-phase infrared spectra for these nine isobaric/regioisomeric amines. These spectra allow discrimination among all the regioisomeric amines included in this study. This is especially significant since these tertiary amines do not undergo chemical derivatization. Mixtures of these amines (based on ring and side chain substitution pattern) were successfully resolved via capillary gas chromatography using a 100% dimethyl polysiloxane (Rtx-1) stationary phase.

Acknowledgements

This project was supported by cooperative agreement 2006-DN-BX-K016, U.S. Department of Justice, Office of Justice Programs, National Institute of Justice. The opinions contained herein are those of the author(s) and do not necessarily represent the official position of the U.S. Department of Justice.

The Auburn University Laboratory thanks Analytical Solutions and Providers LLC of Covington, Kentucky for the use of the GC–IRD equipment.

References

[1] G. Boatto, M. Nieddu, A. Carta, A. Pau, M. Palomba, B. Asproni, R. Cerr, Determination of amphetamine-derived designer drugs in human urine by SPE extraction and capillary electrophoresis with mass spectrometry detection, J. Chromatogr. B 814 (2005) 93–98.
[2] B.K. Logan, Amphetamines: an update on forensic issues, J. Anal. Toxicol. 25 (2001) 400.
[3] D.L. Allen, J.S. Oliver, The use of supercritical fluid extraction for the determination of amphetamines in hair, Forensic Sci. Int. 107 (1–3) (2000) 191–199.
[4] M. Laloup, G. Tilman, V. Maes, G.D. Boeck, P. Wallemacq, J. Ramaekers, N. Samyn, Validation of an ELISA-based screening assay for the detection of amphetamine, MDMA and MDA in blood and oral fluid, Forensic Sci. Int. 153 (1) (2005) 29–37.
[5] S.D. Brown, D.J. Rhodes, B.J. Pritchard, A validated SPME–GC–MS method for simultaneous quantification of club drugs in human urine, Forensic Sci. Int. 171 (2–3) (2007) 142–150.
[6] L. Aalberg, J. DeRuiter, F.T. Noggle, E. Sippola, C.R. Clark, Chromatographic and mass spectral methods of identification for the side-chain and ring regioisomers of methylenedioxymethamphetamine, J. Chromatogr. Sci. 38 (2000) 329–337.
[7] L. Aalberg, J. DeRuiter, E. Sippola, C.R. Clark, Gas chromatographic optimization studies on the side chain and ring regioisomers of methylenedioxymethamphetamine, J. Chromatogr. Sci. 41 (2003) 227–233.
[8] L. Aalberg, C.R. Clark, J. DeRuiter, Chromatographic and mass spectral studies on isobaric and isomeric substances related to 3,4-methylenedioxymethamphetamine, J. Chromatogr. Sci. 42 (2004) 464–469.
[9] T. Awad, J. DeRuiter, C.R. Clark, GC–MS analysis of acylated derivatives of the side chain and ring regioisomers of methylenedioxymethamphetamine, J. Chromatogr. Sci. 43 (2005) 296–303.
[10] T. Awad, C.R. Clark, J. DeRuiter, Chromatographic and mass spectral studies on methoxymethcathinones related to 3,4-methylenedioxymethamphetamine, J. Chromatogr. Sci. 44 (2006) 155–161.
[11] T. Awad, C.R. Clark, J. DeRuiter, Chromatographic and mass spectral studies on methoxy methyl methamphetamines related to 3,4-methylenedioxymeth-amphetamine, J. Chromatogr. Sci. 45 (2007) 468–476.
[12] T. Awad, C.R. Clark, J. DeRuiter, GC–MS analysis of acylated derivatives of the side chain regioisomers of 4-methoxy-3-methyl phenethylamines related to methylenedioxymethamphetamine, J. Chromatogr. Sci. 45 (2007) 477–485.
[13] T. Awad, J. DeRuiter, C.R. Clark, GC–MS analysis of the ring and side chain regioisomers of ethoxyphenethylamines, J. Chromatogr. Sci. 46 (2008) 671–679.
[14] T. Awad, J. DeRuiter, C.R. Clark, GC–MS analysis of acylated derivatives of a series of side chain regioisomers of 2-methoxy-4-methyl-phenethylamines, J. Chromatogr. Sci. 46 (2008) 375–380.
[15] T. Belal, T. Awad, J. DeRuiter, C.R. Clark, GC–MS studies on acylated derivatives of 3-methoxy-4-methyl- and 4-methoxy-3-methyl-phenethylamines: regioisomers related to 3,4-MDMA, Forensic Sci. Int. 178 (2008) 61–82.
[16] S. Borth, W. Hänsel, P. Rösner, T. Junge, Synthesis of 2,3- and 3,4-methylenedioxyphenylalkylamines and their regioisomeric differentiation by mass spectral analysis using GC–MS–MS, Forensic Sci. Int. 114 (2000) 139–153.
[17] S. Gosav, M. Praisler, J. Van Bocxlaer, A.P. De Leenheer, D.L. Massart, Class identity assignment for amphetamines using neural networks and GC–FTIR data, Spectrochim. Acta: Part A 64 (2006) 1110–1117.
[18] T. Belal, T. Awad, J. DeRuiter, K. Kramer, C.R. Clark, GC–IRD methods for the identification of isomeric ethoxyphenethylamines and methoxymethcathinones, Forensic Sci. Int. 184 (2009) 54–63.
[19] T. Belal, T. Awad, J. DeRuiter, K. Kramer, C.R. Clark, Comparison of GC–MS and GC–IRD methods for the differentiation of methamphetamine and regioisomeric substances, Forensic Sci. Int. 185 (2009) 67–77.