Gas Chromatographic Optimization Studies on the Side Chain and Ring Regioisomers of Methylenedioxymethamphetamine

Laura Aalberg[1,2], **Jack DeRuiter**[1], **Erkki Sippola**[2], and **C. Randall Clark**[1,*]
[1]Department of Pharmacal Sciences, School of Pharmacy, Auburn University, Auburn, AL 36849 and [2]National Bureau of Investigation, Crime Laboratory, 01370 Vantaa, Finland

Abstract

Gas chromatographic (GC) optimization studies are conducted for the 10 methylenedioxyphenethylamine regioisomeric substances related to the drug of abuse 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy). These 10 compounds, having the same molecular weight and equivalent major mass spectral fragments, are not completely resolved using typical GC–mass spectrometry screening methods for illicit drugs. MDMA coelutes with at least one nondrug regioisomer under standard drug screening conditions. Separation of the 10 regioisomers is studied using stationary phases of varying polarities. Resolution optimization shows that very slow program rates give the best separation for the nonpolar stationary phases, requiring analysis times of as much as 85 min. Narrow-bore columns containing the same nonpolar stationary phases improve the analysis time to approximately 29 min. The polar stationary phase DB-35MS allows high-temperature programming rates, yielding complete resolution of all 10 compounds in less than 7 min. Temperature program optimization studies on the DB-35MS phase allow the separation time to be reduced to approximately 4.5 min.

Introduction

The mass spectrum is often the confirmatory piece of evidence for the identification of drugs of abuse in the forensic laboratory. Although the mass spectrum is often considered a specific "fingerprint" for an individual compound, there may be other substances capable of producing very similar or almost identical mass spectra. In a previous study (1), the mass spectra for ten regioisomeric compounds, the drug of abuse 3,4-methylenedioxymethamphetamine (MDMA), and nine unique regioisomeric substances (Figure 1) were reported, showing that all the major fragment ions and the molecular ion occur at equivalent masses. Furthermore, the previous report (1) pointed out the similar gas chromatographic (GC) retention properties for some of these compounds. Indeed, 3,4-MDMA was observed to coelute with a nondrug regioisomer under some common analytical conditions. A compound having similar retention properties (coelution) as the drug of abuse and the same mass spectrum would represent a serious analytical challenge. If the forensic scientist does not have retention data on the nondrug substances, the potential for coelution could not be easily eliminated. Furthermore, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of abuse (2–5).

The methylenedioxyamphetamines, such as 3,4-methylenedioxyamphetamine (MDA) and MDMA, are novel psychoactive compounds with structural similarities to both amphetamine

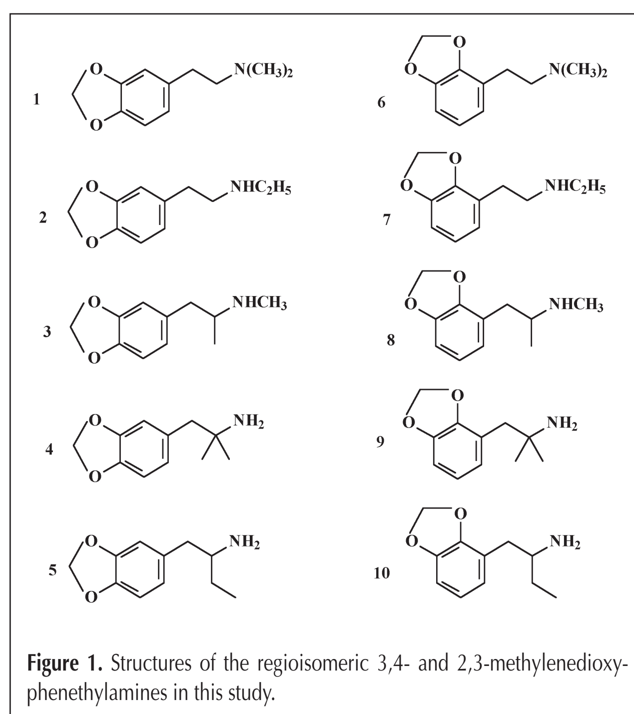

Figure 1. Structures of the regioisomeric 3,4- and 2,3-methylenedioxyphenethylamines in this study.

* Author to whom correspondence should be addressed.

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission.

and the psychedelic phenethylamine, mescaline (6,7). There are nine other methylenedioxy-substituted phenethylamines with the potential to produce a mass spectrum essentially the same as 3,4-MDMA (Figure 1). The mass spectrum of phenethylamine drugs of abuse including 3,4-MDMA is characterized by a base peak formed by an α-cleavage reaction involving the carbon-carbon bond of the ethyl linkage between the aromatic ring and the amine. In 3,4-MDMA (*M*W = 193), the α-cleavage reaction yields the 3,4-methylenedioxybenzyl fragment at mass 135/136 (for the cation and the radical cation, respectively) and the substituted imine fragment at *m/z* 58. 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 (Figure 2).The individual mass spectra for compounds 1–10 are reported in reference 1. In a direct comparison of the 3,4-regioisomers versus the 2,3-regioisomers with the identical side chain, the major difference is the greater relative abundance of the radical cation at *m/z* 136 for the 3,4-substitution pattern in most cases. Thus, the differentiation between these drug and nondrug regioisomeric substances must be based on chromatographic retention properties. This paper reports the results of our efforts to maximize the GC resolution for this group of uniquely regioisomeric compounds.

Experimental

GC–mass spectrometry (MS) analyses were performed with an HP 6890 GC coupled with an HP 5973 mass selective detector (Hewlett-Packard, Little Falls, DE). The MS was operated in the electron impact (EI) mode utilizing an ionization voltage of 70 eV and a source temperature of 230°C. The samples were dissolved in pH 8.9 Trizma base buffer (1 mg/mL), extracted with iso-octane (1 mL), and introduced (1.0 µL) into the MS via the GC equipped with an HP 7673 automatic injector. The separation was carried out on a 25 m (l) × 0.20 mm i.d., coated with 0.11 µm 5% phenyl methyl silicone (Ultra-2) purchased from Hewlett-Packard. The injection was carried out using splitless mode with an injector temperature at 250°C, where the split purge valve opened after 1 min. The injection volume was 1 µL/column. The temperature program started with 1 min isothermal hold at 60°C, followed by a linear ramp (8°C/min) to 180°C, and then to the final temperature 300°C at a rate of 30°C/min with a hold time of 10 min. The helium carrier gas was adjusted to 25 cm/s at 60°C in the constant flow mode.

GC analyses were performed with an HP 6890 GC equipped with split/splitless inlet, HP 7683 automatic injector, and flame ionization detector (FID) (Agilent, Little Falls, DE). ChemStation software Rev. A.08.03 (Agilent) was used for data acquisition and processing. The carrier gas (hydrogen) was adjusted at 60°C to give average velocity to the optimum, 50 cm/s. Inlet pressure was converted according to the constant flow mode, and the total flow was 60 mL/min. The injection was in the split mode with an injector temperature at 260°C. The sample preparation was carried out by dissolving samples in Tris buffer (pH 8.9) and extracting with iso-octane.

The temperature program optimization was carried out using DryLab 2000 chromatography optimization software, v. 3.00.06 (LC Resources, Walnut Creek, CA).

Capillary columns used

The conventional columns used were: 100% methyl silicone (Ultra 1), 25-m (l) × 0.2-mm i.d., d_f 0.33 µm; 5% phenyl methyl silicone (Ultra 2) 25-m (l) × 0.2-mm i.d., d_f 0.33 µm; 35% phenyl methyl silicone (DB-35MS) 25-m (l) × 0.2-mm i.d., d_f 0.33 µm; 50% phenyl methyl silicone (HP-50+) 25-m (l) × 0.2-mm i.d., d_f 0.33 µm; 50% phenyl methyl silicone (DB-17MS) 30-m (l) × 0.25-mm i.d., d_f 0.25 µm; and 14% cyanopropylphenyl methyl silicone (HP-1701) 25-m (l) × 0.2-mm i.d., d_f 0.20 µm. The narrow bore columns used were: 100% methyl silicone (HP-1) 10-m (l) × 0.1-mm i.d., d_f 0.1 µm; 5% phenyl methyl silicone (HP-5) 10-m (l) × 0.1-mm i.d., d_f 0.17 µm; 50% phenyl methyl silicone (SPB-50) 10-m (l) × 0.1-mm i.d.,

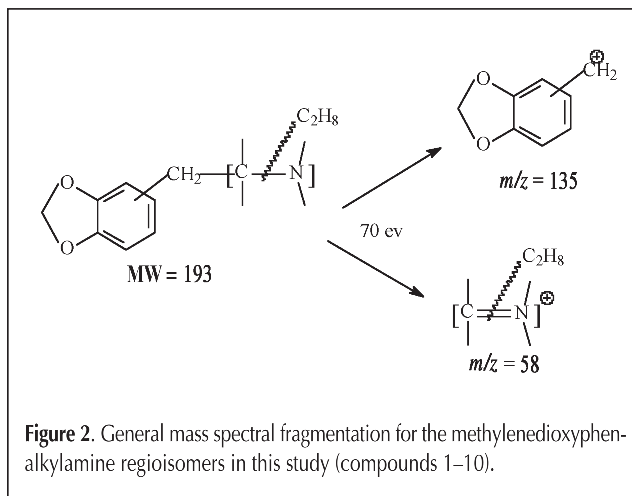

Figure 2. General mass spectral fragmentation for the methylenedioxyphenalkylamine regioisomers in this study (compounds 1–10).

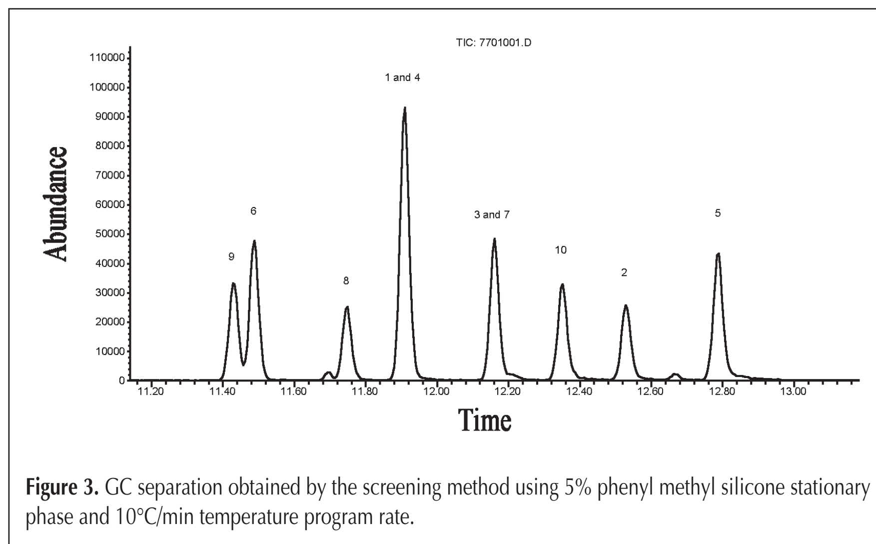

Figure 3. GC separation obtained by the screening method using 5% phenyl methyl silicone stationary phase and 10°C/min temperature program rate.

d_f 0.17 µm; and 50% phenyl methyl silicone DB-17 10-m (l) × 0.1-mm i.d., d_f 0.2 µm.

Results and Discussion

When other compounds exist that have the potential to produce the same or nearly identical mass spectrum as the drug of interest, the separation of the "nondrug regioisomers" from the actual drug of abuse is of the utmost importance. MS alone does not provide sufficient information to distinguish between MDMA and the nine regioisomeric equivalents (1). These 10 compounds have the same molecular weight (MW = 193) and yield major fragment ions at equivalent masses (*m/z* 58 and 135/136). Therefore, the identification by GC–MS must be based primarily upon the ability of the chromatographic system to separate these ten regioisomeric substances. The goal of this project was to evaluate a series of GC stationary phases of varying polarity in an attempt to maximize the resolution for these 10 methylenedioxyphenethylamine regioisomers.

The chromatogram in Figure 3 clearly demonstrates the potential problems associated with interference from regioisomeric compounds in the specific identification of controlled substances. Figure 3 was obtained using a 5% phenyl methyl silicone column [25-m (l) × 0.20-mm i.d., d_f 0.33 µm] and a temperature program rate of 10°C/min, a method commonly employed in the forensic analysis of illicit drug substances. The method does not separate all 10 regioisomers, and 2 pairs of compounds coelute in this chromatogram, *N,N*-dimethyl-3,4-methylenedioxyphenylethanamine (1) and 3,4-methylenedioxyphentermine (4), as well as 3,4-MDMA (3) and *N*-ethyl-2,3-methylenedioxyphenyl-2-ethanamine (7). Screening methods of this variety are commonly employed in forensic drug analysis, and the coelution of 3,4-MDMA with an uncontrolled regioisomer would be considered a major drawback. Such a situation could yield a false-positive analytical result. The separation of 3,4-regioisomers (compounds 1–5) from the corresponding 2,3-regiosiomers (compounds 6–10) was achieved by the temperature program used to generate Figure 3, with the 2,3-regioisomers eluting much earlier than the 3,4-isomers of the same side chain structure.

Separation of the 10 regioisomers was studied using various stationary phases, and optimization was carried out with the aid of DryLab chromatographic software. The software enables the prediction of separation with any temperature program after two initial runs using different linear temperature programs. Initially, two runs with a starting temperature of 60°C and program rates of 2°C and 20°C/min were performed. The retention data obtained from these two runs was modeled to produce resolution maps. Resolution maps describe the rela-

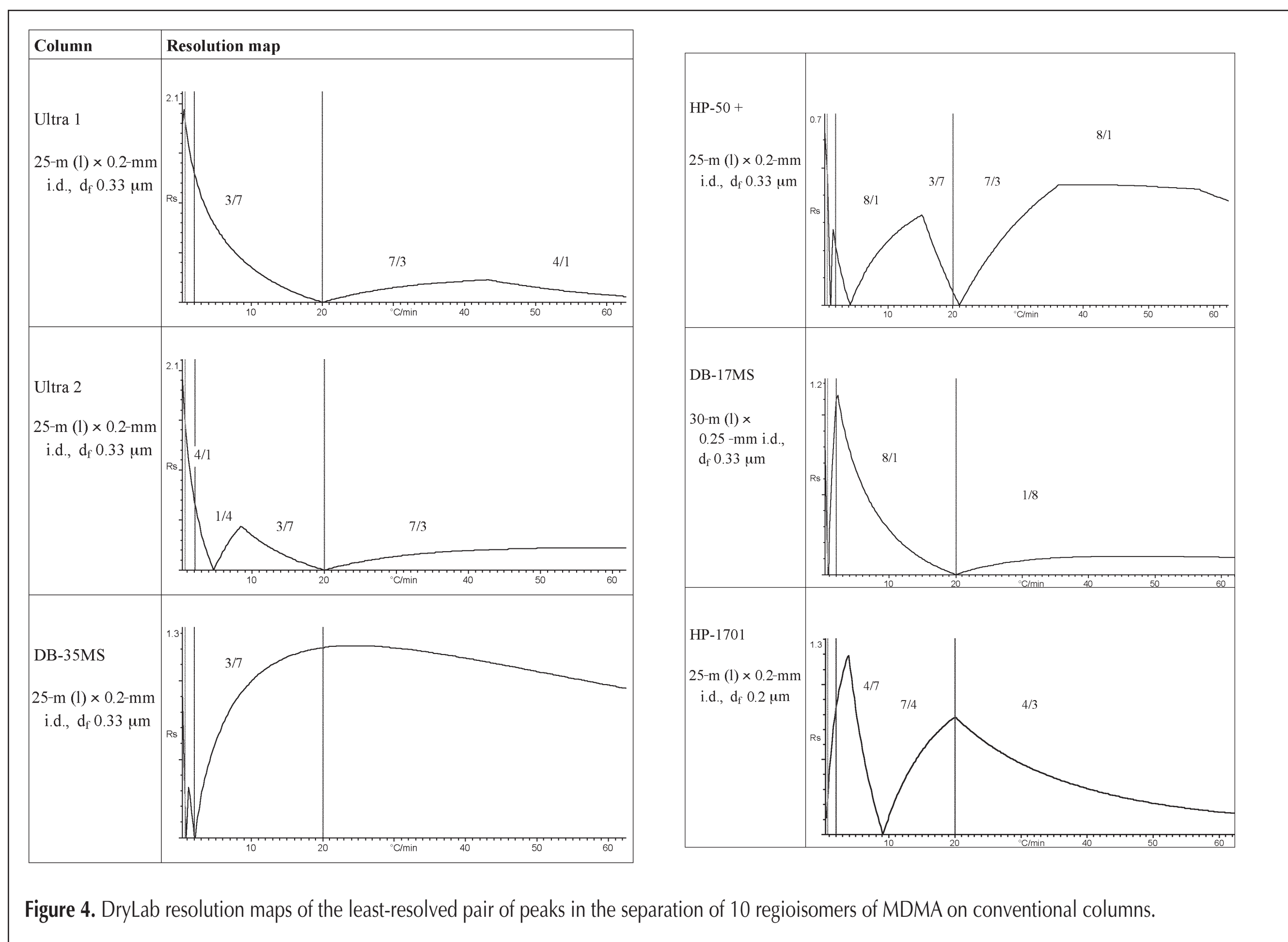

Figure 4. DryLab resolution maps of the least-resolved pair of peaks in the separation of 10 regioisomers of MDMA on conventional columns.

tionship between chromatographic resolution and a variable separation parameter; in capillary GC, the parameter is usually the temperature-programming rate. Although resolution results are obtained for each peak pair only, the critical pairs (the least-resolved pairs of peaks) are plotted because resolution for all other peaks would be greater than that shown for the critical pair.

The minimum resolution maps produced by DryLab optimization studies for each column are shown in Figure 4. The least-resolved pair of compounds (the "critical pair") is shown for the individual line segments in the resolution map using the same numbering system for the regioisomeric compounds as in Figure 1. For example, in Figure 4, compounds 3 and 7 (3/7) are the critical peak pair between 2°C and 20°C/min program rate on the Ultra 1 column. The vertical lines at 2°C and 20°C/min on the resolution map indicate the values from the linear temperature program input runs. The other vertical line at less than 2°C/min indicates the limits of extrapolation for modelling.

The highest resolution, obtained at very slow program rates, provides best separation power with the nonpolar stationary phases, such as 100% methyl silicone (Ultra 1) and 5% phenyl methyl silicone (Ultra 2). The critical peak pair is 3,4-MDMA (3) and *N*-ethyl-2,3-methylenedioxyphenyl-2-ethanamine (7). The optimum separation (i.e., minimum resolution ≥ 1.5 at the highest possible temperature program rate) was predicted to be a temperature program rate of 1.35°C/min for the Ultra 1 phase. The retention time of the last eluting compound would be 47 min, which is a relatively long analysis time. Similar results were obtained when an Ultra 2 column [25-m (l) × 0.2-mm i.d., d_f 0.33 µm] was used. Separation of critical compound pairs 3/7 and 1/4 can be achieved at a rate of 0.65°C/min, but the retention time of last eluting compound would be 85 min.

The resolution map for 35% phenyl methyl silicone column (DB-35MS) had a completely different shape than that for the nonpolar columns, and, in contrast, the optimum separation was predicted to be achieved at fast temperature program rates. This is indeed generally valid for polar columns in which $\Delta t_R/\Delta T$ is much more pronounced than for nonpolar columns. The highest resolution for the critical pair of compounds (3/7) was predicted at 25°C/min. The optimum linear temperature program rate (25°C/min) was applied, and the results of the predicted and "real" analyses are shown in Figures 5 and 6.

The predicted (Figure 5) and experimentally determined (Figure 6) chromatograms for the DB-35MS stationary phase are almost identical. The time of

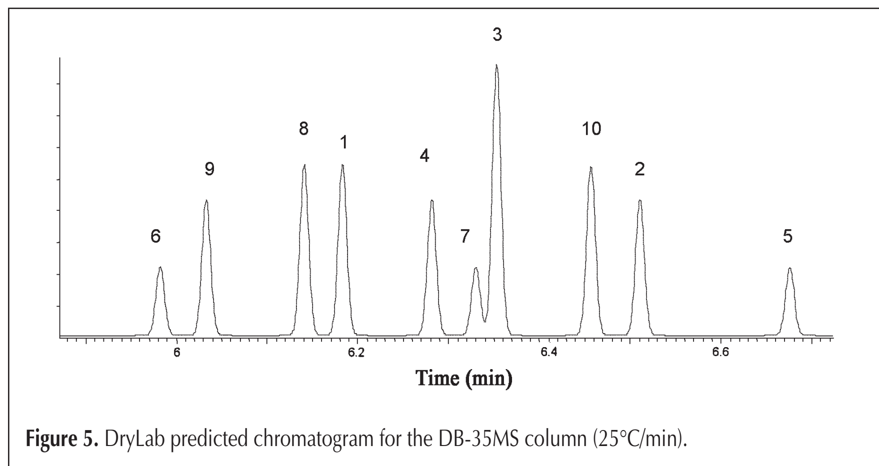

Figure 5. DryLab predicted chromatogram for the DB-35MS column (25°C/min).

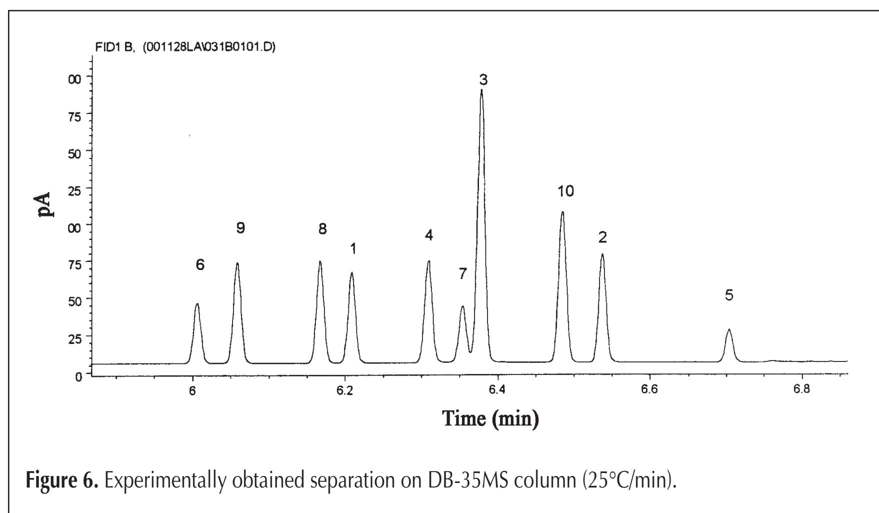

Figure 6. Experimentally obtained separation on DB-35MS column (25°C/min).

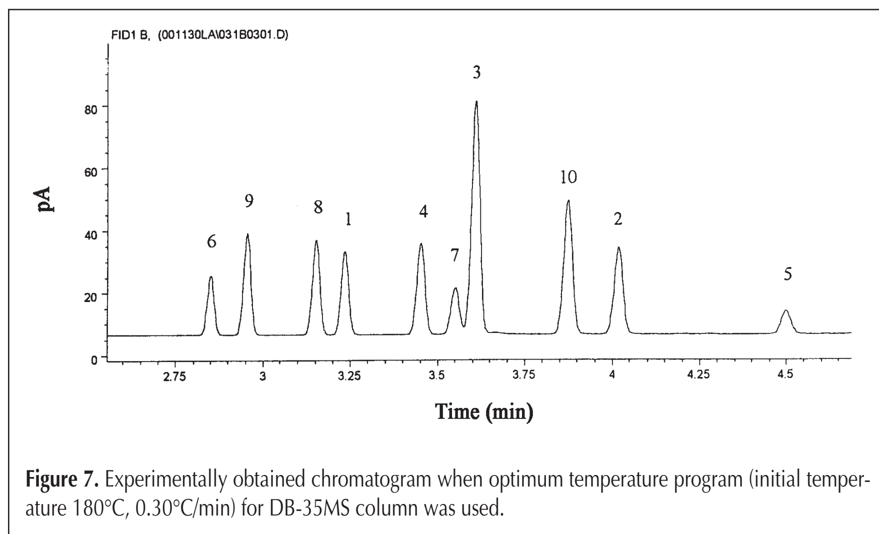

Figure 7. Experimentally obtained chromatogram when optimum temperature program (initial temperature 180°C, 0.30°C/min) for DB-35MS column was used.

the chromatographic separation was further improved by increasing the initial temperature from 60°C to 180°C and using the temperature program rate of 0.30°C/min (Figure 7). The analysis time decreased to 4.5 min, and the resolution of compounds 3 and 7 remained essentially constant at 1.32.

The more polar columns, HP-50+ and DB-17MS, both have 50% phenyl methyl silicone stationary phases. However, the selectivity of the two stationary phases is different, and, therefore, the resolution maps are somewhat different. The overall resolution in the HP-50+ column is poor, with a maximum predicted resolution of only 0.5 for the critical pair. DB-17MS shows better resolution, and the optimum separation was predicted at a temperature program rate of 2.1°C/min, suggesting a fairly long analysis time. Interestingly, the critical peak pair was 1/8 instead of 3/7, as with less polar columns. Polarity of the stationary phase seemed to improve the separation of peak pair 3/7.

The resolution map of 14% cyanopropylphenyl methyl silicone column (HP-1701) showed two resolution maxima at 3.8°C/min and 20°C/min. The critically eluting compounds were 3,4-MDMA (3), 3,4-phentermine (4), and *N*-ethyl-2,3-methylenedioxyphenylethanamine (7), and the elution order of compounds with slow program rates (less than 9°C/min) was 4, 7, and 3. When the faster program rates (more than 9°C/min) were applied, the elution order of compounds 4 and 7 changed, and, therefore, the separation of compounds 4 and 3 became the crucial one.

Basic studies of capillary GC have demonstrated that the application of narrow-bore capillary columns (i.d. < 0.1 mm) has a number of advantages (8). Decreasing the column diameter of open-tubular capillaries is an effective way to speed up the separation process. Because the optimum reduced plate heights (h_{min}) have fixed values, a decrease of d results in a proportionally decreased value of H_{min} ($H = hd$). Therefore, the column length ($L = NH$) can be decreased by the same factor, in order to yield the same plate number N. For example, a 10-m × 0.10-mm i.d. capillary column has approximately the same theoretical plate number as a 25-m × 0.25-mm i.d. column. The advantage of using narrow-bore capillary columns was applied to improve the analysis time for compounds 1–10. It was already shown that the separation of compounds 1–10 with standard nonpolar columns [25-m (l) × 0.2mm i.d., d_f 0.33 µm] gave baseline separation of the compounds using a relatively long analysis time. When using the corresponding but narrow-bore columns, the analysis time was improved significantly (Figure 8). For example, baseline separation was obtained over 29 min by using a rate of 2.1°C/min on 5% phenyl methyl silicone stationary phase column (HP-5) when the dimensions of the column were 10-m (l) × 0.1-mm i.d., d_f 0.17 µm (Figure 9). The corresponding standard column also gave baseline separation, but the slower temperature program

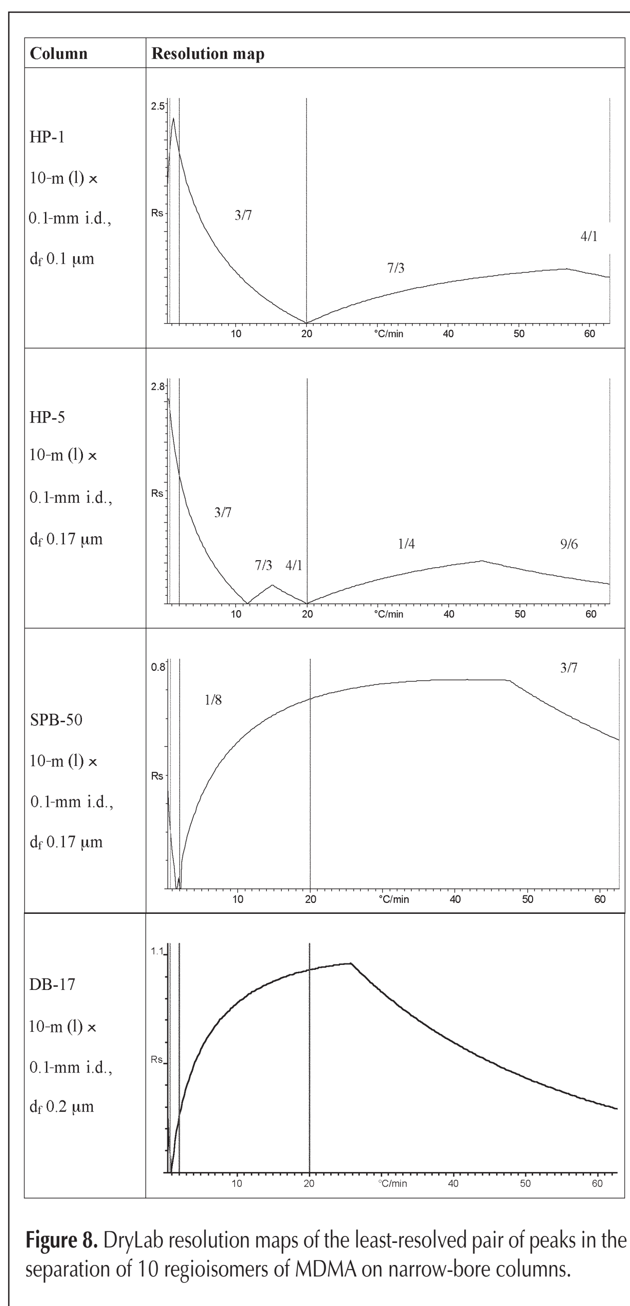

Figure 8. DryLab resolution maps of the least-resolved pair of peaks in the separation of 10 regioisomers of MDMA on narrow-bore columns.

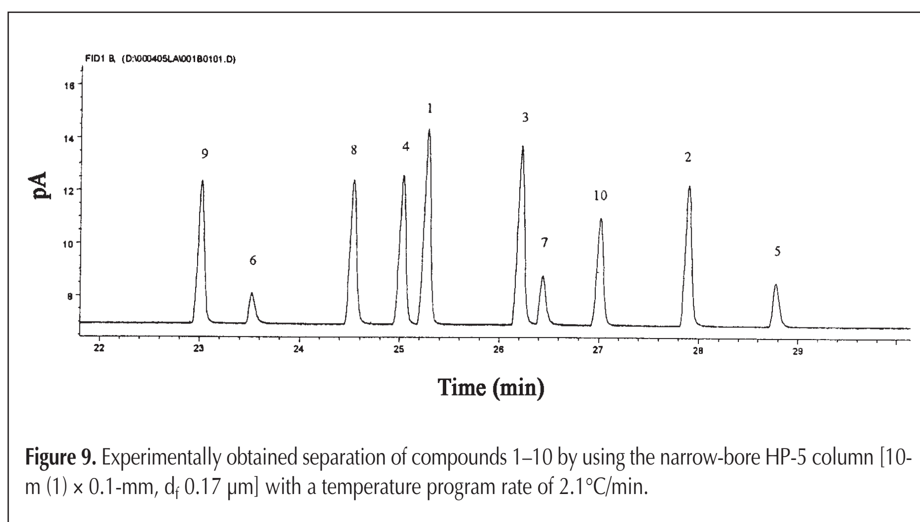

Figure 9. Experimentally obtained separation of compounds 1–10 by using the narrow-bore HP-5 column [10-m (1) × 0.1-mm, d_f 0.17 µm] with a temperature program rate of 2.1°C/min.

(0.65°C/min) was needed, and the analysis time was 85 min. Thus, the narrow-bore column did not only decrease the analysis time, but it also improved the separation by enabling the use of faster temperature programs.

Conclusion

The ten methylenedioxyphenethylamine regioisomeric substances related to MDMA are not completely resolved using typical GC–MS screening methods of illicit drugs. The drug of abuse MDMA (Ecstacy) coelutes with at least one nondrug regioisomer under standard drug screening conditions. Coeluting molecules having the same mass spectrum represent serious challenges for forensic drug chemistry.

Separation of the 10 regioisomers was studied using stationary phases of varying polarities, and resolution optimization was carried out with the aid of DryLab software. The highest resolution, obtained at very slow program rates, provided the best separation power with the nonpolar stationary phases, such as 100% methyl silicone (Ultra 1) and 5% phenyl methyl silicone (Ultra 2). The critical peak pair was 3,4-MDMA (3) and *N*-ethyl-2,3-methylenedioxyphenyl-2-ethanamine (7). When using the corresponding but narrow-bore columns, the analysis time was improved significantly.

The more polar stationary phase, 35% phenyl methyl silicone column (DB-35MS), gave the best separation, which was achieved at fast linear temperature program rates (25°C/min), and, therefore, the analysis time was significantly shorter (less than 7 min). The critical pair of compounds was also 3/7, but the elution order was reversed compared to less polar columns. The time of the chromatographic separation could be decreased to 4.5 min by increasing the initial temperature from 60°C to 180°C and using the much lower temperature program rate of 0.30°C/min.

Acknowledgments

We would like to thank Dr Imre Molnar (Molnar Institut Berlin) for providing the DryLab software. In addition, the authors are grateful for the financial support from the Finnish Cultural Foundation and the Academy of Finland.

Rerferences

1. L. Aalberg, J. DeRuiter, F.T. Noggle, E. Sippola, and C.R. Clark. Chromatographic and mass spectral methods of identification for the side-chain and ring regioisomers of methylenedioxymethamphetamine. *J. Chromatogr. Sci.* **38:** 329–37 (2000).
2. J. DeRuiter, P.L. Holston, F.T. Noggle, and C.R. Clark. Liquid chromatographic and mass spectral methods of identification for the regioisomeric 2,3- and 3,4-methylenedioxyphenalkylamines. *J. Chromatogr. Sci.* **36:** 131–38 (1998).
3. F.T. Noggle, Jr., C.R. Clark, S.V. Andurkar, and J. DeRuiter. Liquid chromatographic analysis of regioisomers and enantiomers of N-(chlorobenzyl)-β-methylphenethylamines: analogues of clobenzorex. *J. Liq. Chromatogr.* **13:** 763–77 (1990).
4. C.R. Clark, A.K. Valaer, F.T. Noggle, and J DeRuiter. GC–MS analysis of acylated derivatives of methamphetamine and regioisomeric phenethylamines. *J. Chromatogr. Sci.* **33:** 485–92 (1995).
5. W.H. Soine, R.E. Shark, and D.T. Agee. Differentiation of 2,3-methylenedioxy-amphetamine from 3,4-methylenedioxyamphetamine. *J. Forensic Sci.* **28:** 386–90 (1983).
6. K.M. Hegadoren, G.B. Baker, and M. Bourin. 3,4-Methylenedioxy analogues of amphetamines: defining the risks to humans. *Neurosci. Biobehav. Rev.* **23:** 539–53 (1999).
7. D.E. Nichols, A.J. Hoffman, R.A. Oberlender, P. Jacob, III, and A.T. Shulgin. Derivatives of 1-(1,3-benzodioxolyl)-2-butanamine: representatives of a novel therapeutic class. J. Med. Chem. **29:** 2009–15 (1986).
8. C.A. Cramers, H.G. Janssen, M.M. van Deursen, and P.A. Leclercq. High-speed gas chromatography: an overview of various concepts. *J. Chromatogr. A* **856:** 315–29 (1999).

Manuscript accepted May 3, 2004.