Comparison of Mass Spectrometric Ionization Techniques for the Analysis of Phenethylamines

Anthonie M.A. Verweij* and **Peter J.L. Lipman**

Department of General Chemistry, Forensic Science Laboratory of the Ministry of Justice, Volmerlaan 17,2288 GD Rijswijk, The Netherlands

Abstract

It is predicted that lower limits of detection can be obtained for some amphetamines—3,4(methylenedioxy)amphetamine (MDA), 3,4-(methylenedioxy)methylamphetamine (MDMA), and 3,4-(methylenedioxy)-*N*-ethyl amphetamine (MDEA)—with the use of liquid chromatography coupled to mass spectrometry by thermospray, electrospray, and atmospheric pressure chemical ionization interfaces. The lowest detection limits are obtained with the thermospray interface; when a tandem mass spectrometric selected reaction monitoring technique is used in the product ion mode, the limits of detection are in the low picogram range for the amphetamines.

Introduction

Currently, atmospheric pressure ionization methods like electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are important (1–3) in the estimation of molecular weights and in the elucidation of the structures of biopolymers and other high molecular weight compounds. These substances can be nonpolar or polar. With the use of ESI and APCI methods, ions can be generated at atmospheric pressure conditions (2,3) by applying an electric field (ESI) or by raising the temperature (APCI) during nebulization of the liquid that flows from a chromatographic column into the ion source.

To date, to our knowledge, data on the application of these ionization methods to the analysis of compounds of low molecular weight (< 1000) are scarce (4) as compared with the information available for compounds of high molecular weight. On the contrary, the thermospray (TSP) ionization method is used mainly in the ionization of low molecular weight compounds of nonpolar to moderately polar nature.

We compared of the limits of detection that could be obtained with the use of the TSP, ESI, and APCI ionization methods for some amphetamines: 3,4-(methylenedioxy)amphetamine (MDA), 3,4-(methylenedioxy)methylamphetamine (MDMA), and 3,4(methylenedioxy)-*N*-ethylamphetamine (MDEA). For these phenethylamines, limits of detection of 1–10 ng/mL were obtained with more conventional methods like gas chromatography (5), high-pressure liquid chromatography (HPLC) (6,7), and gas chromatography–mass spectrometry (GC–MS) (8).

Experimental

Materials

Pure MDA, MDMA, and MDEA (Figure 1) were obtained as hydrochloric salts from different sources within this laboratory. Acetonitrile and methanol were of HPLC and glass-distilled grade (Rathburn; Walkerburn, U.K.). Water was purified with the Milli Q/Organex Q system (Millipore; Milford, MA). All other reagents were of analytical grade.

Equipment

HPLC

A Waters 600 MS programmable pump (Milford, MA) equipped with a U6K injector was used to pump a mixture (25:75, v/v) of acetonitrile and ammonium acetate in water (100 mmol NH_4Ac in water) through a Waters 4-µm Nova-Pack C18 HPLC cartridge column (3.9 × 150 mm). Under TSP conditions, a flow rate of 0.40 mL/min was used. Postcolumn, 100 mmol NH_4Ac was added at a flow rate of 0.6 mL/min by a Waters 590-MS isocratic pump for enhancement of ionization in thermospray applications. Under ESI conditions, a mixture of acetonitrile, 100 mmol NH_4Ac in water, and water (40:5:55) was pumped through the column at a flow rate of 0.4 mL/min. Under APCI pumping conditions, a mixture of acetonitrile, 100 mmol NH_4Ac in water, and water (40:5:55) was pumped through the column at a flow rate of 0.7 mL/min.

Mass spectrometry

A Finnigan MAT TSQ 700 tandem quadrupole mass spectrometer (MS) (San José, CA) coupled to a Digital DEC-2100

* Author to whom correspondence should be addressed.

station (Palo Alto, CA) was used. The liquid chromatograph was connected either by a Finnigan MAT TSP-2 or a Finnigan MAT API module with possibilities for ESI and APCI sources to the MS. The operating parameters for the different interfaces were all optimized and are given below. MS–MS experiments in the daughter ion mode (9,10) were done using argon (99.999%) as the collision gas for the $(M+H)^+$ or adduct ions generated by the different interfaces. To obtain the optimum sensitivity and selectivity for the amphetamines under study, the selected reaction monitoring (SRM) technique (9,10) was applied. Collision offset voltage, argon pressure, and a correction factor for increasing the transmission of the ions in the MS–MS mode (MSMSC factor) were optimized under the different conditions for the ions generated by the ionization methods to be present in the first quadrupole. The most intense ions in the MS–MS spectra were chosen for SRM experiments (Tables I and II).

Thermospray conditions were as follows: repeller, 90 V; vaporizer temperature, 130°C; source temperature, 200°C; filament, off. Electrospray conditions were as follows: spray tip voltage, 3.5 kV; capillary temperature, 200°C; sheath gas, approximately 50 torr; auxillary gas, on; capillary voltage, 11.7 V; tubelense voltage, 40 V, octapole offset, –3.0 V. APCI conditions were as follows: vaporizer temperature, 400°C; capillary temperature, 150°C; auxillary gas, on; sheath gas, approximately 50 torr; capillary voltage, 11.7 V; tubelense voltage, 40 V; octapole offset, –3.0 V. MS conditions were as follows: multiplier voltage, 1500 V; dynode power, 15 kv; scan time, 1.2 s; MSMSC factor, 10; collision offset, –17.5 V; argon pressure, 1 mtorr.

Results and Discussion

GC–MS of the amphetamines under study with the use of chemical ionization with isobutane as the reaction gas was hampered by adsorption of the amphetamines in the system; detection in the subnanogram range was impossible. For these reasons, liquid chromatography was investigated in an attempt to improve the detection limits obtained previously (5–7). Separation of the amphetamines could be achieved with a C18 column and a mixture of acetonitrile and water as an eluent. To obtain as favorable conditions as possible for ESI and APCI, the percentage of acetonitrile in the eluent was increased as much as possible without influencing the quality of the chromatographic separation. Optimum values for the acetonitrile percentage in the eluent were given in Experimental. Under TSP conditions, lower percentages of acetonitrile could be used in water.

It is well known that TSP ionization is enhanced by the addition of relatively large quantities of salts that evaporate in the eluent like ammonium acetate or formate. In ESI and APCI, it seemed that the quantity of ammonium acetate in the eluent had to be substantially reduced to obtain optimum ionization. Actually, in ESI and APCI, the amount of ammonium acetate in the eluent had to be lowered 25–50 times as compared with the amount present in the TSP method to obtain optimum ionization. A small amount of acetate (approximately 3 mmol) had to be added. No ionization was attained when acetate was omitted, but the amounts of acetate as used in thermospray decreased the signal substantially. In particular, APCI appeared to be sensitive in this respect.

Figure 2 shows the separation of the amphetamines under APCI conditions. In Tables I and II, the results are tabulated for the different ionization methods. It can be seen that the thermospray method is preferable to the other ionization methods because the lowest detection limits are reached with this method. With the SRM method in the product ion scan mode (9),which is also extremely specific with noise suppression, the expected increase in detection limit over the values obtained in the full scan mode is achieved (about 10–25 times).

The sensitivity obtained is of the same order as reached in the case of benzodiazepines (11), some explosives (12), and some analgesics and tranquilizers (13). Preferential masses to

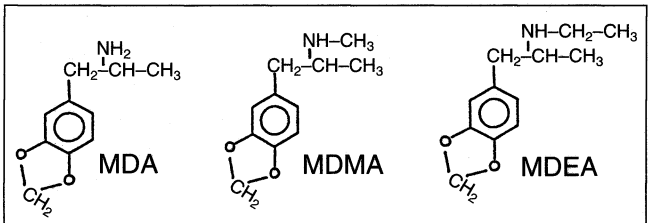

Figure 1. Structural formulas of the amphetamines under study.

Table I. On-Column Detection Limits*

	Full scan		MS–MS (SRM)	
Compound	Limit (pg)	Ion (*m/z*)	Limit (pg)	Ion (*m/z*)
Thermospray				
MDA	1000	180	30	180–163
MDMA	500	194	20	194–163
MDEA	100	208	10	208–163
Electrospray				
MDA	1000	221	100	221–163
MDMA	1000	235	500	235–163
MDEA	1000	249	100	249–163

* Signal-to-noise ratio was approximately 3.

Table II. On-Column Detection Limits Obtained with APCI*

	Corona on		Corona off	
Compound	Limit (pg)	Ion (*m/z*)	Limit (pg)	Ion (*m/z*)
Full scan				
MDA	1000	180	300	221
MDMA	2000	194	400	235
MDEA	2000	208	400	249
MS–MS (SRM)				
MDA	1000	180–163	1000	221–163
MDMA	5000	194–163	500	235–163
MDEA	5000	208–163	100	249–163

* Signal-to-noise ratio was approximately 3.

be used in the SRM procedure were the protonated molecular ions and *m/z* 163 (elimination of methylamine or ethylamine). There was no indication of adduct formation of the molecular ion with acetonitrile under TSP conditions.

With the ESI method, all detection limits are about 2–20 times higher than the values reached with the TSP method. Adduct formation between the protonated molecular ion and acetonitrile is obtained, and the 1:1 adduct of $(M+H)^+$ and acetonitrile was found to be present together with the protonated molecular ion. Quotients of quantities of the 1:1 adduct acetonitrile $(M+H)^+$ and the nonclustered $(M+H)^+$ varied from 1:1 to 10:1 depending on the amphetamine under study. No correlation was determined for the limits of detection and the quotients of quantities of adduct formation and protonated molecular ions. It appeared that in SRM, the best sensitivities were found when the adduct ions (*m/z* 221, 235, 249) were used as starting ions.

The situation when APCI is used is somewhat more complicated. Because evaporation of the liquid flow was assisted by heating in the vaporizer, the quotients of quantities of 1:1 adducts of acetonitrile$(M+H)^+$ and nonclustered $(M+H)^+$ were changed as compared with those obtained under ESI conditions; they varied between 5:1 and 1:2. Results given here are for the 1:1 adducts (as starting ions in the SRM method) and are somewhat better than that given for the protonated molecular ions. Furthermore, thermospray can be simulated by turning off the corona potential, which increases the sensitivity of the method. In full scan (corona off) mode, limits of detection were comparable with that found in TSP, but unfortunately, the limits of detection in MS–MS (SRM in product ion mode) were of the same order as that obtained in full scan mode.

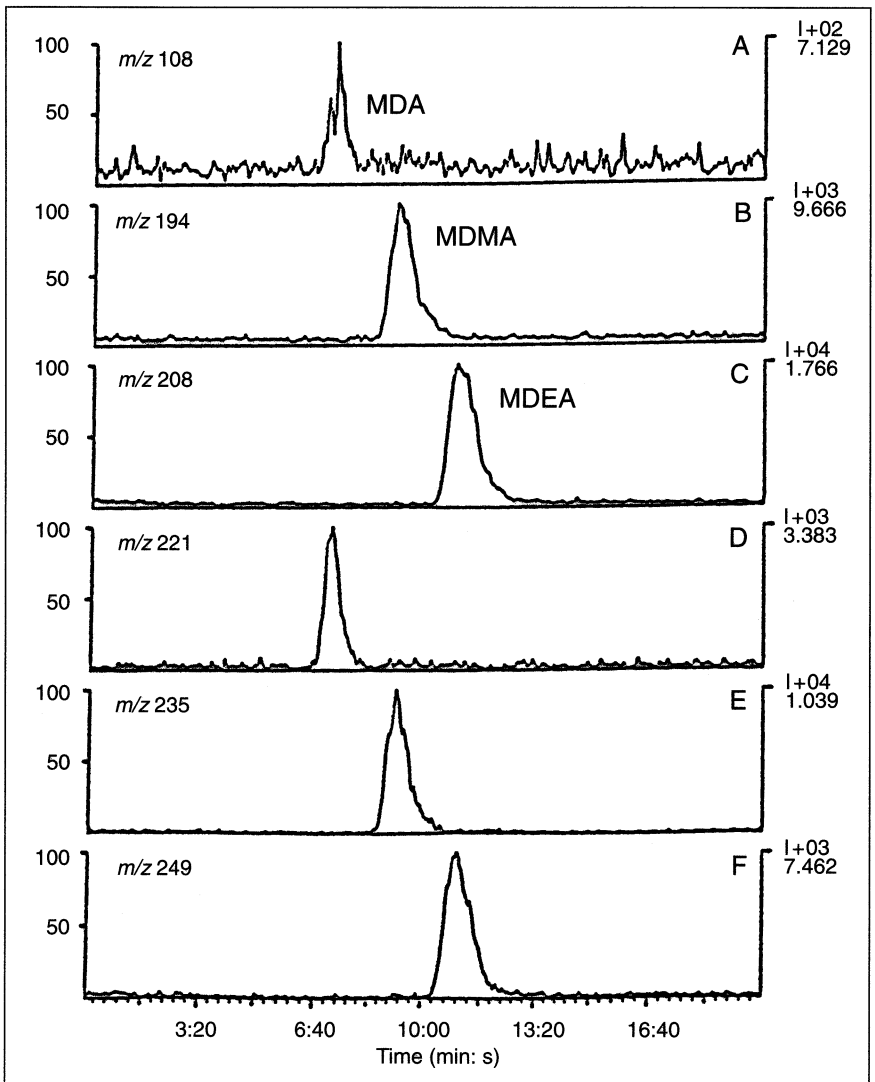

Figure 2. Chromatogram of a mixture of MDA (A) MDMA (B), MDEA (C), and acetonitrile adducts of MDA (D), MDMA (E), and MDEA (F). The chromatogram was obtained under APCI conditions in the full scan mode. Five nanograms was injected.

Conclusion

For the amphetamines under study, the use of thermospray ionization with the SRM method in the product ion mode gave far better results than those obtained with ESI and APCI ionization methods. In full scan mode, the results are more or less comparable for the different ionization methods. Preference should be given to TSP with the SRM procedure in the product ion mode because increased sensitivity and selectivity can be obtained at detection levels several times better than those reported in the literature.

References

1. R. Caprioli. In *Biological Mass Spectrometry, Present and Future.* T. Matsuo, R. Caprioli, M. Gross, and Y. Seyama, Eds. John Wiley, New York, NY, 1994
2. A.P. Bruins. Atmospheric pressure ionization mass spectrometry I: instrumentation and ionization techniques. *Trends in Anal. Chem.* **13:** 37 (1994).
3. A.P. Bruins. Atmospheric pressure ionization mass spectrometry II: applications in pharmacy, biochemistry and general chemistry. *Trends in Anal. Chem.* **13:** 81 (1994).
4. S. Bajic, D.R. Doerge, S. Lowes, and S. Preece. APCI a technique for routine LC–MS. *Am. Lab* **25:** 40B–40O (1993).
5. K.K. Midha, J.K. Cooper, D. Gagné, and K. Bailey. Detection of nanogram levels of various ring-substituted phenylisopropylamines in urine and plasma by GLC–ECD. *J. Anal. Toxicol.* **3:** 53 (1979).
6. C.R. Clark, A.K. Valaer, and W.R. Davis. Liquid chromatographic determination of *N*-hydroxy-3,4-methylenedioxyamphetamine and metabolite in plasma. *J. Liq. Chromatogr.* **13:** 1375 (1990).
7. Y. Nakahara and Y. Tahida. β-Naphtoquinone sulfonate as electrochemical labelling reagent for high sensitivity LC analysis of drugs in biological fluids. *Chromatographia* **26:** 363 (1988).

8. R.L. Fitzgerald, R.V. Blanke, R.A. Glinnon, N.Y. Yousif, J.A. Roserans, and A. Poklis. Determination of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in whole-blood. *J. Chromatogr.* **490:** 59 (1989).
9. J. Yinon. *Forensic Science Progress*, Vol 5. A. Meahly and R.L. Williams, Eds. Springer Verlag, Berlin, Germany, 1991, pp 1–29.
10. C.E.M. Heeremans. Thermospray liquid chromatography tandem mass spectrometry. Ph.D. Thesis, University of Leiden, Netherlands, 1990.
11. A.M.A. Verweij, P.J.L. Lipman, and P.G.M. Zweipfenning. Quantitative liquid chromatography, thermospray/tandem mass spectrometry analysis of some thermolabile benzodiazepines in whole-blood. *Forensic. Sci. Int.* **54:** 67 (1992).
12. A.M.A. Verweij, P.C.A.M. de Bruyn, C. Choufoer, and P.J.L. Lipman. Liquid chromatography thermospray–negative ion tandem mass spectrometry analysis of some explosives. *Forensic. Sci. Int.* **60:** 7 (1993).
13. A.M.A. Verweij, M.L. Hordijk, and P.J.L. Lipman. Quantitative liquid chromatographic thermospray–tandem mass spectrometric analysis of some analgesics and tranquilizers of the methadone, butyrophenone, or diphenylbutylpiperidine groups in whole blood. *J. Anal. Toxicol.* **19:** 65 (1995).

Manuscript accepted February 21, 1996.