ELSEVIER

Journal of Chromatography B, 664 (1995) 317–327

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Influence of the injection technique on the thermal degradation of cocaine and its metabolites in gas chromatography

M.L. González, M. Carnicero, R. de la Torre*, J. Ortuño, J. Segura

Department of Pharmacology and Toxicology, Institut Municipal d'Investigació Mèdica (IMIM), Universitat Autònoma de Barcelona, Doctor Aiguader 80, Barcelona 08003, Spain

First received 29 April 1994; revised manuscript received 16 September 1994

Abstract

Thermal degradation of some substances due to high temperature at the injection port and/or the type of injection technique used may limit the usefulness of gas chromatography with conventional detectors or coupled to mass spectrometry. To minimize thermal degradation of cocaine and its metabolites, chromatography was performed using two different insert liners and a cool on-column inlet. When using a packed liner, marked degradation of all compounds was observed. The degradation process was reduced by the use of an open liner and, when the cool on-column inlet was employed, essentially no degradation occurred.

1. Introduction

An increasing consumption of cocaine is noted in the United States and Europe, either alone or in association with other drugs, mainly alcohol. Different surveys have shown that a significant percentage of the population uses cocaine and alcohol concurrently [1–3]. Recent toxicological findings, such as the metabolic interaction between cocaine and alcohol [4,5], have increased the interest in the development of analytical techniques for the detection of cocaine as well as its main metabolites and other minor biotransformation products including cocaethylene, norcocaine and norcocaethylene.

A wide range of methods for the analysis of cocaine in clinical samples has been developed. Although different high-performance liquid chromatographic (HPLC) procedures are available [6,7], gas chromatographic (GC) techniques are extensively used for the simultaneous detection of cocaine, cocaethylene and their metabolites in biological fluids [8–12]. Gas chromatography–mass spectrometry (GC–MS) is usually preferred because of its high specificity and sensitivity [13,14]. However, it has been shown that ecgonidine methyl ester–a cocaine metabolite [11]–may also be formed as a thermal degradation product of cocaine, either during the free-base inhalation of the compound [15,16] or during GC analysis [17]. The thermal degradation product is generated by pyrolysis involving a debenzoylation [18]. Other thermal degradation products can also be generated from cocaine metabolites, such as ecgonidine methyl ester from ecgonine methyl ester, ecgonidine

* Corresponding author.

0378-4347/95/$09.50
SSDI 0378-4347(94)00484-6

from benzoylecgonine, ecgonidine ethyl ester from cocaethylene, and norecgonidine methyl ester from norcocaine (Fig. 1). All these ecgonidine-related substances are found as thermal degradation products when analyzing spiked urines or methanolic solutions of cocaine and its main metabolites, including cocaethylene, using a conventional gas chromatographic setup [17]. Since the degradation products have structures identical to those of several substances identified as cocaine metabolites [11], erroneous interpretations may occur when assessing pharmacokinetic and urinary metabolic data. In order to minimize formation of these products, a cool on-column injection system was applied and the results were compared with those obtained using a conventional splitless injection mode either with an open tube or packed liners.

2. Experimental

2.1. Materials

Ecgonine methyl ester, benzoylecgonine, cocaine and cocaethylene were purchased from Radian Corporation (Austin, TX, USA). Norcocaine was provided by Research Triangle Institute (Durham, NC, USA). Pure reference standards were supplied as 1 mg/ml solutions in N,N-dimethylformamide (cocaine), methanol (ecgonine methyl ester, benzoylecgonine, norcocaine) and acetonitrile (cocaethylene). Dilutions of solutions were checked by UV spectrophotometry and stored at −20°C. Methanol was HPLC grade and purchased from Carlo Erba (Milano, Italy). Chloroform, ethyl acetate, hydrochloric acid and potassium dihydrogen phosphate were reagent grade (Merck, Darmstadt, Germany). Isopropyl alcohol and ammonium hydroxide, 25% reagent grade, were supplied by Scharlau (Barcelona, Spain). 1,1,1,3,3,3-Hexafluoro-2-propanol (spectroscopy grade) was supplied by Merck. Pentafluoropropionic anhydride was provided by Supelco (Bellefonte, PA, USA). Bond Elut Certify columns were provided by Analytichem International (Harbor City, CA, USA) and the Visiprep vacuum manifold for solid–liquid extraction was provided by Supelco. Deionized water was prepared in house with a MILLI Q System (Millipore, Mulheim, France).

2.2. Instrumentation

Two different GC–MS instruments (A and B) equipped with two different injection systems (conventional and cool on-column) were used. GC–MS instrument A was a Hewlett-Packard (HP) (Hewlett-Packard, Palo Alto, CA, USA) 5890 A Series II Model gas chromatograph fitted with a HP 7673 A autosampler and coupled to a quadrupole HP 5971 A mass-selective detector. The instrument was equipped with a conventional injection port operated in splitless mode (valve activation time at 30 s after injection) using a liner packed with glass silanized beads and wool or an open liner (empty silanized tube). The injector temperature was 280°C and the injection volume was 1 μl. GC–MS instrument B also was a HP 5890 A Series II gas chromatograph coupled to a quadrupole HP 5989 A mass-selective detector. This instrument was equipped with a programmable cool on-column inlet with electronic pressure control (EPC) and temperature programme. The injector was operated at a temperature of 100°C and the injection volume was 0.5 μl. The inlet pressure was programmed at a first rate from 135.0 kPa to 194.2 kPa at 9.8 kPa/min, and a second rate from 194.2 kPa to 275.5 kPa at 31.0 kPa/min. The final time was 2.20 min.

In both instruments, the separation was carried out using crosslinked capillary columns (Ultra 2-HP) 12 m × 0.2 mm I.D., 5% phenylmethyl silicone gum (0.33 μm film thickness). Helium was used as carrier gas at a flow-rate of 0.65 ml/min (measured at 180°C). The oven temperature was programmed with two consecutive rates: first rate, from 100°C to 200°C at 20°C/min; second rate, from 200°C to 280°C at 30°C/min; and final time was 3 min. The interface was operated at 290°C. The mass spectrometers were both operated in the electron-impact ionization mode (EI, 70 eV) and in the selected-ion monitoring (SIM) acquisition mode.

2.3. Study design

Two different concentration levels of each compound (ecgonine methyl ester, benzoylecgonine, cocaine, cocaethylene and norcocaine) were added to drug-free urines (negativity checked by FPIA,TDx, Abbott Laboratories, IL, USA and GC-NPD [9]). Urine concentrations usually found in subjects using cocaine and alcohol [5] were selected for each compound. The lowest and highest concentrations were 200 and 1000 ng/ml for ecgonine methyl ester, 400 and 2000 ng/ml for benzoylecgonine and cocaine, and 50 and 500 ng/ml for cocaethylene and norcocaine, respectively. All concentrations were in the linear range of the method [19]. The recoveries, within- and between-day coefficients of variation have been previously described [19].

Urines were analyzed in a single clean-up step by reversed solid-phase with cation-exchange extraction and a single derivatization procedure using 1,1,1,3,3,3-hexafluoro-2-propanol (for esterification of carboxylic acid groups) and pentafluoropropionic anhydride (for acylation of hydroxyl and secondary amine groups) [11,12]. Both concentrations of each compound were separately injected at random on six different occasions.

2.4. Evaluation of degradation rate

In the absence of reference substances for the degradation products, some of the assumptions made for the estimation of the degradation rates were based on mass spectral data reported in the literature [11,16,18]. Table 1 summarizes the ions selected for the SIM acquisition mode of the derivatized compounds. The extent of degradation (degradation product/total product) was estimated from the ratio between the base peak of the degradation product vs. the sum of the base peak of the degradation product and the original compound [i.e. ratio m/z 152/(152 + 182) for ecgonidine methyl ester/O-PFP-ecgonine methyl ester (Fig. 1a) and for ecgonidine methyl ester/cocaine (Fig. 1c), ratio m/z 288/(288 + 318) for COO-HFIP-ecgonidine/COO-HFIP-benzoylecgonine (Fig. 1b), ratio m/z 166/(166 + 196) for ecgonidine ethyl ester/cocaethylene (Fig. 1d)]. For N-PFP-norcocaine and its degradation product (N-PFP-norecgonidine methyl ester) the same ion was selected (m/z 313) (Fig. 1e). Since the base peak for each original compound is derived from the loss from the molecular ion of a benzoate radical in the same way the base peak in the degradation product is formed, it was assumed that the compounds gave similar response factors in the detector. Interpretation of the structure of the base peak was based on previous reports for ecgonidine methyl ester, ecgonidine and norecgonidine methyl ester [11]. In the case of ecgonidine ethyl ester and following the proposal of Zhang and Foltz [11] for the formation of the

Table 1
Monitored ions and gas chromatographic relative retention times for cocaine, its metabolites and degradation products analyzed as HFIP and PFP derivatives

Compound	m/z	RRT
COO-HFIP-ecgonidine	288[a] 317[b]	0.24
Ecgonidine methyl ester	152[a] 181[b]	0.33
O-PFP-ecgonine methyl ester	182[a] 345[b]	0.33
Ecgonidine ethyl ester	166[a] 195[b]	0.35
N-PFP-norecgonidine methyl ester	313[a,b] 284	0.37
COO-HFIP-benzoylecgonine	318[a] 439[b]	0.73
Cocaine	182[a] 303[b]	1.00
Cocaethylene	196[a] 317[b]	1.07
N-PFP-norcocaine	313[a] 435[b]	1.09

[a] Ions used for percentage degradation values in Table 2.
[b] Molecular ions of studied compounds.
HFIP = hexafluoroisopropionyl derivative; PFP = pentafluoropropionyl derivative.

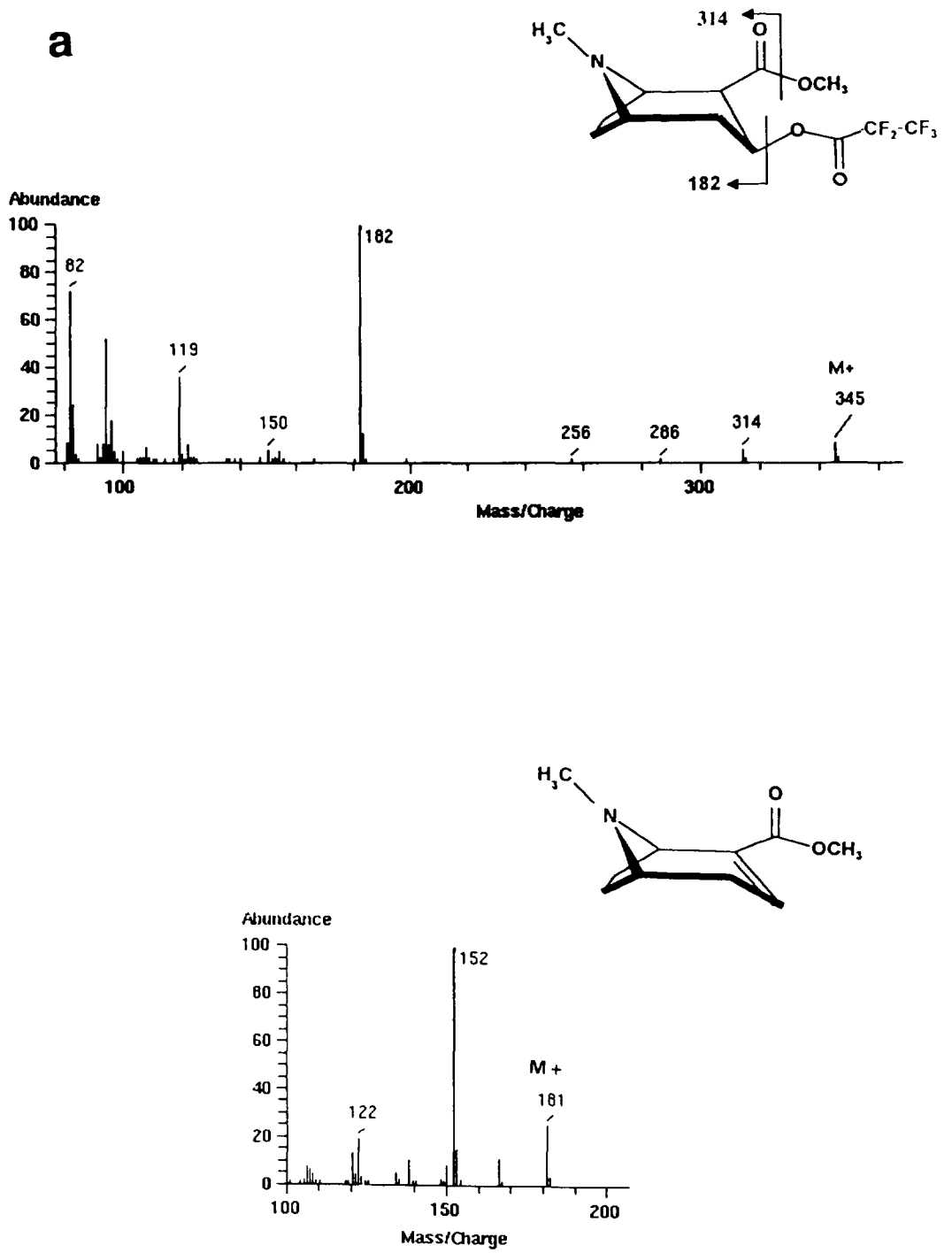


Fig. 1. Products of thermal degradation of cocaine and the GC derivatives of its main metabolites. (a) O-PFP-ecgonine methyl ester (upper) and ecgonidine methyl ester (lower); (b) COO-HFIP-benzoylecgonine (upper) and COO-HFIP-ecgonidine (lower); (c) cocaine (upper) and ecgonidine methyl ester (lower); (d) cocaethylene (upper) and ecgonidine ethyl ester (lower); (e) N-PFP-norcocaine (upper) and N-PFP-norecgonidine methyl ester (lower).

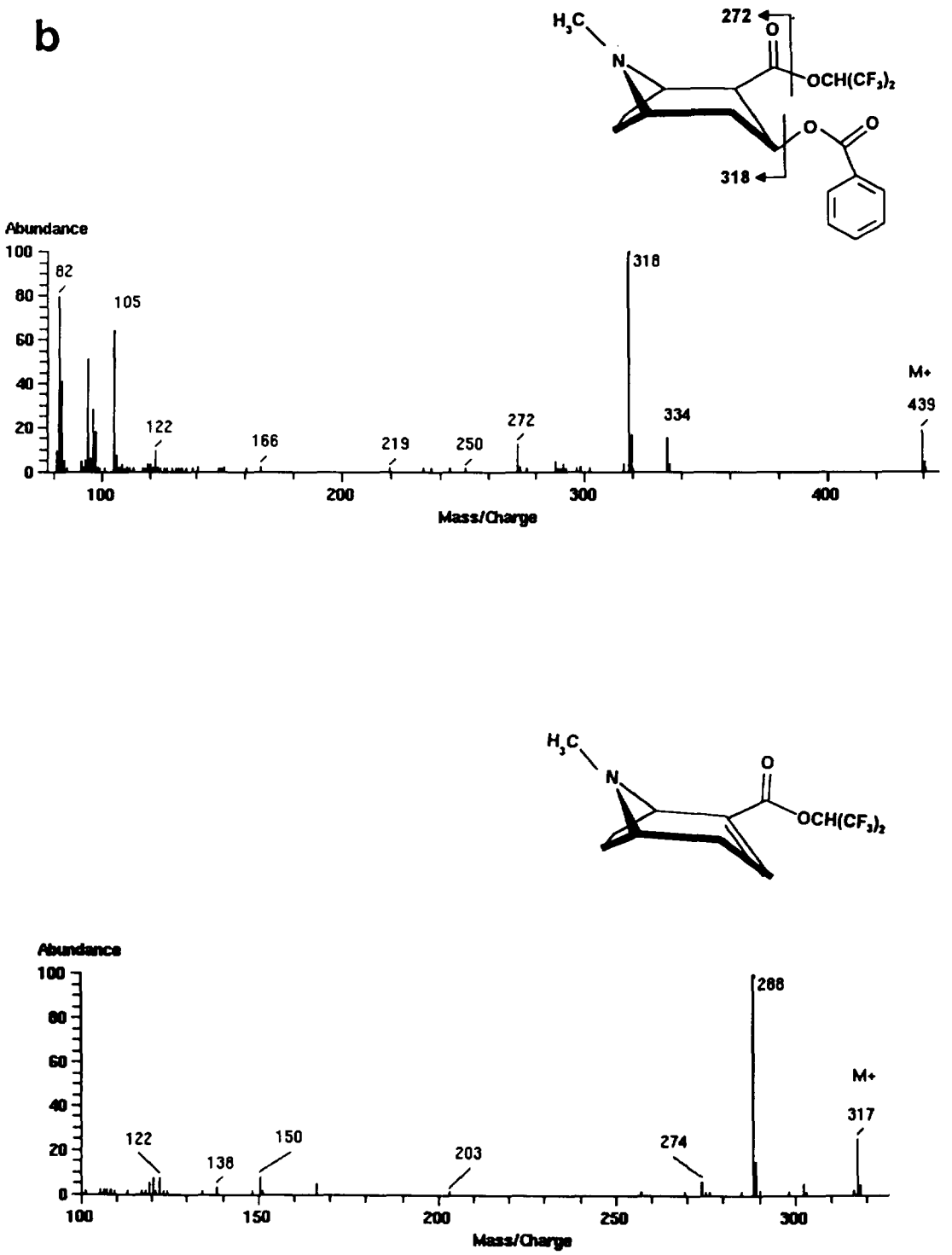
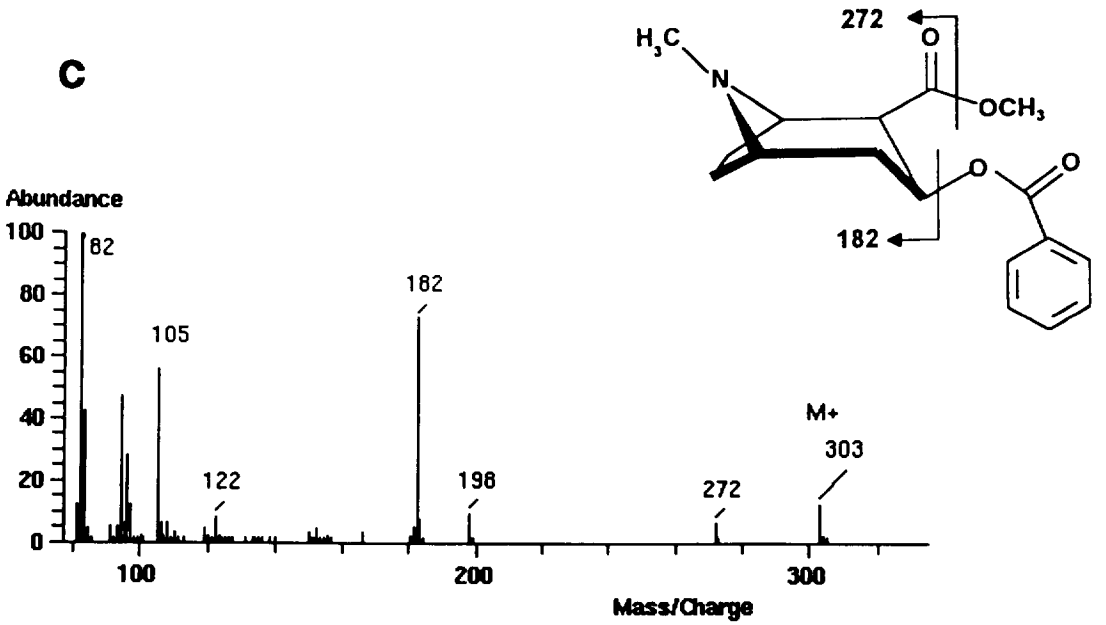


Fig. 1 (*continued*)



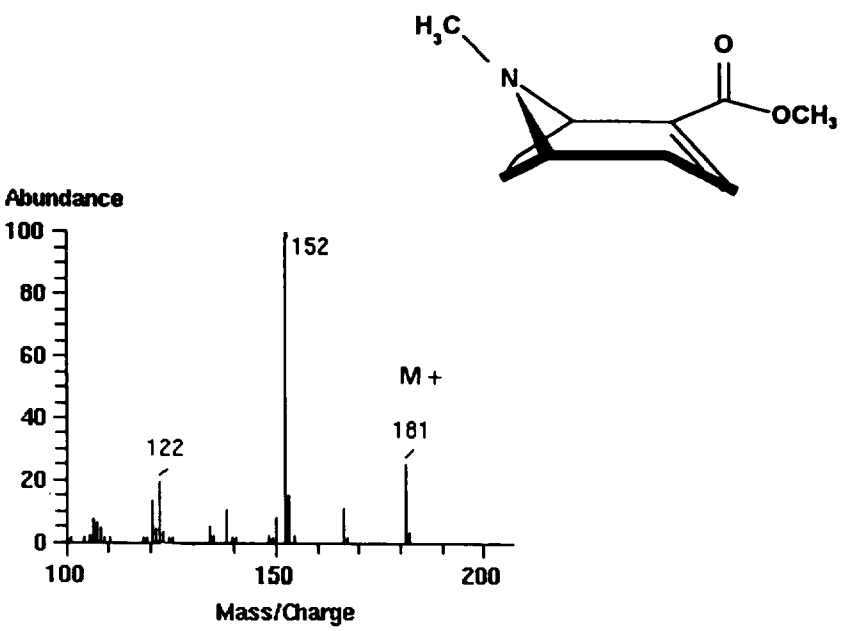


Fig. 1 (*continued*)

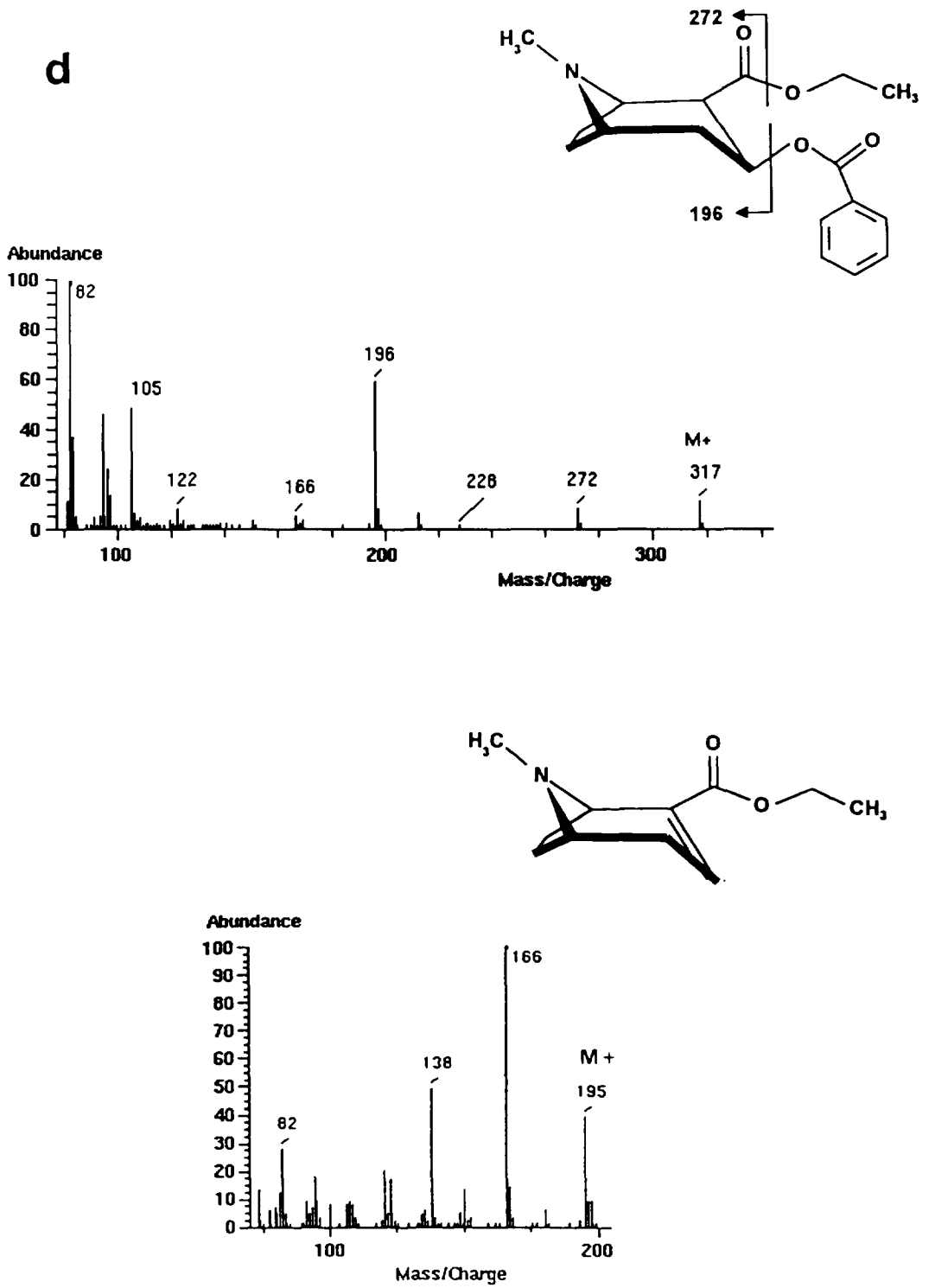
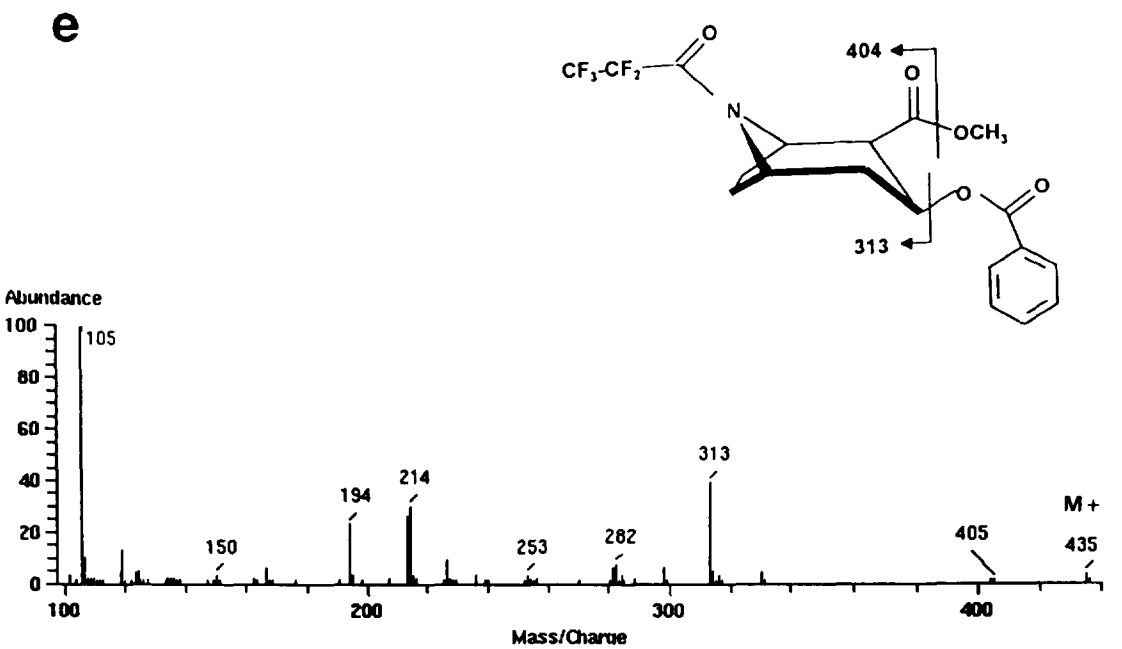


Fig. 1 (*continued*)



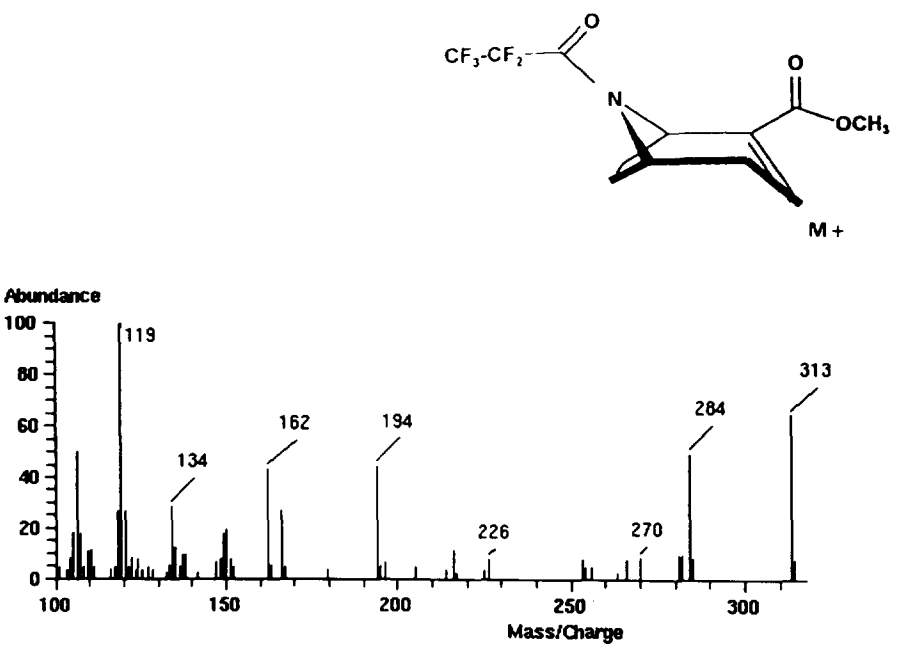


Fig. 1 (*continued*)

C_3-substituted N-methyl pyridinium ion, 15 mass units were added to the corresponding ion in the reference spectrum of the ecgonidine methyl ester.

3. Results and discussion

The present study compares the results obtained with two different insert liners in the conventional splitless injection mode and a cool on-column inlet, which was used as an alternative injection system to minimize the thermal degradation of cocaine and related compounds (Table 2).

When a packed liner was used, thermal degradation of all compounds tested was observed. Thermal degradation was particularly relevant for norcocaine (rate of 15.4%). The use of an open liner (2.7% maximal value) or a cool on-column inlet (less than 0.1%) drastically reduced the occurrence of degradation products.

When cocaine and its metabolites are analyzed by GC techniques, one has to consider that the compounds are exposed to thermal stress along the chromatographic process. The present results show that other cocaine-related compounds undergo a pyrolysis process similar to that found for cocaine [15,16,18]. The four degradation products detected in our chromatographic setup share the ecgonidine nucleus [17] and are probably subjected to the same degradation mechanism in the injection port. Pyrolysis of benzoylecgonine, cocaine, cocaethylene and norcocaine could be a *cis*-elimination process mediating a cyclic transition state and involving a debenzoylation.

When a packed liner inlet is used, the high-boiling compounds of the sample, due to multipath diffusion through the packed inlet and the longer residence time in the liner as compared with low-boiling compounds, show a slower diffusion to the column and so the thermal stress in the injection port is more pronounced. This would explain the higher degradation rate of cocaethylene and norcocaine as compared with ecgonine methyl ester (the lowest boiling compound), benzoylecgonine and cocaine. In contrast, when an open liner is used, a decrease in the degradation rate was observed. The thermal stress on the sample in the injection port is lower because the contact surface is reduced.

The use of a cool on-column injection system with EPC and temperature program allows the analysis of thermally-labile compounds. Cool on-

Table 2
Values of degradation percentage obtained for two different concentration levels of the studied compounds with packed and open inlets (conventional injection) and with cool on-column injection

Compound	Degradation percentage (mean ± S.D.)					
	Low concentration level			High concentration level		
	Conventional injection		Cool on-column injection	Conventional injection		Cool on-column injection
	Packed liner	Open liner		Packed liner	Open liner	
O-PFP-ecgonine methyl ester	n.d.	n.d.	n.d.	1.78 ± 0.50	n.d.	n.d.
COO-HFIP benzoylecgonine	2.87 ± 0.41	0.48 ± 0.02	n.d.	1.68 ± 0.23	0.21 ± 0.03	n.d.
Cocaine	2.62 ± 0.11	n.d.	n.d.	1.52 ± 0.08	0.69 ± 0.11	0.08 ± 0.04
Cocaethylene	8.64 ± 2.91	n.d.	n.d.	4.40 ± 0.27	2.74 ± 0.20	n.d.
N-PFP-norcocaine	15.42 ± 2.88	n.d.	n.d.	14.69 ± 1.62	0.44 ± 0.06	n.d.

n.d. = not detected.

column injection is not a flash vaporization technique (as opposed to the splitless injection) and the sample is directly injected as a "liquid" onto the column. Since lower injector temperatures can be used with a cool on-column inlet as compared with the conventional injection techniques, thermal degradation of the sample during the injection process is reduced. A temperature of 100°C was sufficient to prevent the degradation process. In fact, this temperature is lower than the temperatures used in some thermal degradation studies of cocaine (range 100–600°C) [16,18,20]. Moreover, as preliminary studies showed, an improvement in the resolution (peak width) was achieved by programming the pressure in such a way that a constant flow was achieved during the chromatographic analysis.

Although with the use of cool on-column inlet injection no degradation products (or extremely low amounts) were found, the conventional injection system may also yield low or undetectable amounts of degradation products if a proper insert liner (i.e. open liner) is selected. Since some variability in the degradation rates has been observed, depending on the sample concentration, the optimum conditions should be carefully controlled when analyzing biological fluids. The use of homologue deuterium-labeled compounds as internal standards allows the accurate quantification of cocaine, cocaethylene and their main metabolites (benzoylecgonine, ecgonine methylester, norcocaine, norcocaethylene) because thermal degradation of the internal standards balances the degradation processes [19].

No other alternative derivatization techniques (i.e. silanization) have been assayed in the thermal degradation studies. Nevertheless, the phenomenon can be generalized because thermal degradation seems to depend more on the injection technique than on the derivatization reaction applied. For example, underivatized cocaine and cocaethylene also yield ecgonidine methyl ester and ecgonidine ethyl ester, respectively, as thermal degradation products during GC–MS analysis [17,21].

It has been suggested that ecgonidine methyl ester [21] and also ecgonidine and norecgonidine methyl ester, as identified by GC–MS, are cocaine urinary metabolites [11]. However, the origin of the ecgonidine methyl ester remains unclear. When cocaine is smoked, ecgonidine methyl ester is the main pyrolysis product and it has been suggested to use this compound as a marker for the route of administration in biological fluids [15]. However, ecgonidine methyl ester can also be generated during GC analysis in the injection process [17]. Thus, when analyzing urine samples the situation can be rather complex, with the same substance appearing in GC analysis from metabolic and/or artifactual sources. These considerations regarding the ecgonidine methyl ester can be extrapolated to other ecgonidine derivatives (i.e. ecgonidine, ecgonidine ethyl ester, norecgonidine methyl ester) previously identified as cocaine metabolites [11].

In conclusion, when analyzing cocaine, cocaethylene and/or their metabolites the injection system should be optimized to minimize the loss of target compounds while producing acceptable chromatographic analyses. The rate of degradation should be monitored when performing this type of assays.

Acknowledgements

We are grateful to NIDA Supply System for providing the reference substances. The authors are indebted to J. Camí and M. Farré for valuable scientific review, and to Marta Pulido for editorial assistance and copy editing. This study was supported by grants from Fondo de Investigaciones Sanitarias (FIS 92/0152) and CITRAN Foundation (3/89, 3/90).

References

[1] J. Camí and G. Barrio, *Med. Clin.*, 101 (1993) 344.
[2] B.F. Grant and T.C. Harford, *Drug Alcohol Depend.*, 25 (1990) 97.
[3] National Institute on Drug Abuse (1987) Data from the Drug Abuse Warning System (DAWN); annual data NIDA Statistical Series No. 7: DHHS publication No. (ADM) 88-1854. Government Printing Office, Washington, D.C.

[4] R.M. Smith, *J. Anal. Toxicol.*, 8 (1984) 38.
[5] R. de la Torre, M. Farré, J. Ortuño, J. Camí and J. Segura, *J. Anal. Toxicol.*, 15 (1991) 223.
[6] P. Jatlow and H. Nadim, *Clin. Chem.*, 36 (1990) 1436.
[7] C.E. Lau, *J. Chromatogr.*, 582 (1992) 167.
[8] P. Jacob III, B.A. Elias-Baker, R.T. Jones and N.L. Benowitz, *J. Chromatogr.*, 417 (1987) 277.
[9] J. Ortuño, R. de la Torre, J. Segura and J. Camí, *J. Pharm. Biomed. Anal.*, 8 (1990) 911.
[10] D.S. Isenschmid, B.S. Levine and Y.H. Caplan, *J. Anal. Toxicol.*, 12 (1988) 242.
[11] J.Y. Zhang and R.L. Foltz, *J. Anal. Toxicol.*, 14 (1990) 201.
[12] M. Farré, R. de la Torre, M. Llorente, X. Lamas, B. Ugena, J. Segura and J. Camí, *J. Pharmacol. Exp. Ther.*, 266 (1993) 1364.
[13] S. Goenechea, G. Rücker, M. Neugebauer and U. Zerell, *Fresenius Z. Anal. Chem.*, 323 (1986) 326.
[14] S.J. Mule and G.A. Casella, *J. Anal. Toxicol.*, 12 (1988) 153.
[15] P. Jacob III, R.T. Jones, N.L. Benowitz and A.T. Shulgin, *Clin. Pharmacol. Ther.*, 45 (1989) 186.
[16] Y. Nakahara and A. Ishigami, *J. Anal. Toxicol.*, 15 (1991) 105.
[17] R. de la Torre, M.L. González, J. Ortuño, J. Camí, M. Farré and J. Segura, *Ther. Drug Monit.*, 15 (1993) 165.
[18] S.P. Jindal, T. Lutz and J.F. Sayegh, *Res. Comm. Subst. Abuse*, 14 (1993) 37.
[19] R. de la Torre, J. Ortuño, M.L. González, M. Farré, J. Camí and J. Segura, *J. Pharm. Biomed. Anal.*, (1994) in press.
[20] C.E. Cook, A.R. Jeffcoat and M. Pérez-Reyes, in G. Barnett and C.N. Chiang (Editors), *Pharmacokinetics and Pharmacodynamics of Psycoactive Drugs*, Biomedical Publications, Foster City, CA, 1985, pp. 48–74.
[21] S.P. Jindal and T. Lutz, *J. Pharm. Sci.*, 78 (1989) 1009.