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Short communication

Determination of a trace amount of cocaine on a bank note by gas chromatography–positive-ion chemical-ionization mass spectrometry

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Abstract

The determination of a trace amount of cocaine on a bank note is helpful to convict cases of drug abuse. An extremely sensitive gas chromatographic (GC)–positive-ion chemical ionization (PCI) mass spectrometric (MS) method has been developed to identify and quantitatively determine amounts of cocaine particles as low as 1 ng on a U.S. bank note. The detection limit for identification with GC–PCI scan mass spectrometry was found to be 50 ng/ml. With quantitative analysis by GC–PCI selective-ion monitoring at *m/z* 304 a detection limit of 0.5 ng/ml can be reached with a signal-to-noise ratio >5. The quantitation limit is set at 1 ng/ml. Halazepam is used as internal standard for quantitative analysis.

Keywords: Forensic analysis; Cocaine; Halazepam

1. Introduction

Cocaine abuse is very common in the U.S. Many cocaine users like to use a wrapped bank note to sniff this drug. Some cocaine users or drug dealers routinely contaminate bank notes with cocaine through handling. Therefore, establishing the presence of cocaine on bank notes is useful to convict cases of drug abuse.

Apart from the determination of cocaine in blood, there are numerous methods published to test the presence of cocaine in urine [1–3], hair [4,5], saliva [6], and the brain [7–10] by gas chromatography (GC), high-performance liquid chromatography (HPLC), or gas chromatography–mass spectrometry (GC–MS). Other techniques such as liquid chromatography–mass spectrometry (LC–MS) [11] and thin-layer chromatography [12] have been used to test the presence of cocaine in various biological fluids. However, none of these methods is suitable to establish the presence of cocoaine on a bank note. Contaminants such as ink, grease, oil, cosmetics, etc. on the note result in huge peaks in the chromatogram, overlapping the cocaine peak. Therefore, a high selectivity to separate the cocaine peak from those of interferences, and a high sensitivity to determine trace amounts of cocaine on the bank note, are key points for a suitable method.

In the present paper, a positive-ion chemical ionization (PCI)–scan mass spectrum of cocaine generated in this laboratory was used to identify cocaine by matching the spectrum with that of an unknown sample. Quantitative analysis was done in the PCI–selective ion monitoring (SIM) mode: monitoring the ion at *m/z* 304 for $[\text{cocaine}+\text{H}]^+$ and

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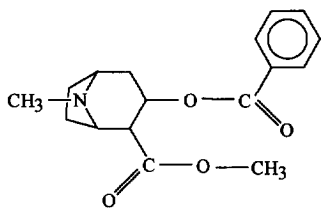
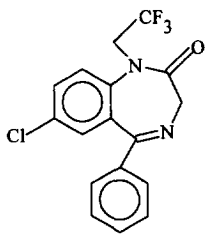


Cocaine M.W.=303



Halazepam
M.W.=352

Fig. 1. The molecular structures of cocaine and the internal standard halazepam.

m/z 353 for the ion of the internal standard, $[\text{halazepam}+\text{H}]^+$. Halazepam is used as internal standard because of its stability and because it has a retention time close to that of cocaine. The structures of cocaine and the internal standard halazepam are shown in Fig. 1.

2. Experimental

2.1. Chemicals

Pure cocaine was purchased from Sigma Chemical. Chloroform (analyzed HPLC reagent) was from J.T. Baker. Ethyl acetate (high-purity solvent grade) was obtained from Baxter Health Care. Halazepam was from Hoffman–Roche (Nutley, NJ, USA).

2.2. Sample preparation

A sample bank note was placed in a 50-ml Pyrex tube. Chloroform was used as extraction solvent because of the high solubility of cocaine in this liquid. Enough chloroform was added to the tube to completely submerge the note in the solvent. The tube was capped and vortex-mixed on a VWR Multitube vortexer for 20 min. The extraction solvent was transferred to a 15-ml centrifuge tube and centrifuged for 10 min at 750 g. The greenish, clear upper layer was transferred to a clean 16×100 mm culture tube and evaporated to dryness at 60°C under nitrogen. In the case of a very dirty solution, i.e. particles are visible, the solution was filtered through 100-mesh filter paper before centrifugation. The residue was reconstituted in 50 μl of ethyl acetate. An aliquot of the reconstituted sample was pipetted into a GC vial for analysis.

2.3. Method of analysis

Analysis was performed on an HP 5890 II gas chromatograph with a DB-17 column (30 m×0.25 mm I.D., 0.25 μm film thickness) and an HP 5989 MS Engine equipped with a Wiley138 mass library (containing 138 000 EI mass spectrum entries for over 80 000 compounds), HP 59940 ChemStation and 59944C (Rev. C.01.01) HP-UX ChemSystem (Hewlett-Packard, NJ, USA).

Helium was used as GC carrier gas, with a column head pressure of 30 kPa at ca. 25°C and a flow-rate of 1 ml/min. The injector temperature was 280°C. The oven program was set from an initial temperature of 100°C to a final temperature of 300°C at a step rate of 24°C/min. The injection volume was 2 μl. The run time was 15 min. The mass scan range was from m/z 50 to m/z 350. The temperatures of the ion source and the quadrupole were 150 and 100°C, respectively, for both the electron impact ionization (EI) and the PCI mode. For PCI in the SIM mode, the monitored ion was m/z 304 for cocaine and m/z 353 for halazepam. The energy of the ionizing electrons and the voltage of the electron multiplier (EM) were set at 70 eV and 2000 V, respectively, for the EI mode and at 230 eV and 2000 V for the PCI mode. The chemical ionization gas (CI gas) was methane, and the optimum operating pressure was found to be 0.25–0.3 kPa.

Quantitation was based on the ratio of the peak height of cocaine to that of halazepam.

Standard samples were prepared, in duplicate, by pipetting 25 μl of respective working solutions, each containing 1, 10, 50, 100, 500, and 1000 ng cocaine,

plus 25 μl of halazepam working solution (concentration 2 μg/ml) into respective test tubes with 1 ml chloroform and then evaporating to dryness under nitrogen. Then each residue was reconstituted with 50 μl of ethyl acetate and transferred to a GC vial for GC–MS analysis. Halazepam was used as internal standard and the concentration was set at 50 ng/ml.

Quality control (QC) samples were run in duplicate at three different concentration levels (QC_L=30, QC_M=250 and QC_H=550 ng/ml) to assure the accuracy of analysis.

3. Results and discussion

3.1. Mass spectrum and GC–MS chromatograms

A mass spectrum of pure cocaine in GC–PCI-scan-MS mode was generated in our laboratory, and is shown in Fig. 2. A typical EI mass spectrum (from Wiley138 mass library entry no. 74902) of cocaine is also shown in Fig. 2, for comparison. Fig. 3 is a GC–PCI-scan-MS chromatogram of a bank note sample. Peak '*' (t_R = 8.998 min) was identified as cocaine, the mass spectrum of which was matched with that of pure cocaine in Fig. 2. The mass scan range was from m/z 50 to 350. The amount of cocaine was found to be 600 ng on this bank note by PCI–SIM-MS. The chromatogram for quantitation of cocaine is also shown in Fig. 3.

With the help of the mass spectrum library Wiley138, we have identified all other components on another bank note. A typical GC–EI-MS chromatogram of a bank note sample is shown in Fig. 4. In addition to peak 12 (t_R = 9 min), identified as the cocaine peak, all other peaks are listed for those interested in what materials can be found on a bank note.

Good separation is crucial in testing for cocaine on a bank note. The cocaine peak in Fig. 4 could not be seen when a 12-m HP-5 capillary column was used, since in that case the cocaine peak was hidden by large peaks from contaminants on the bank note.

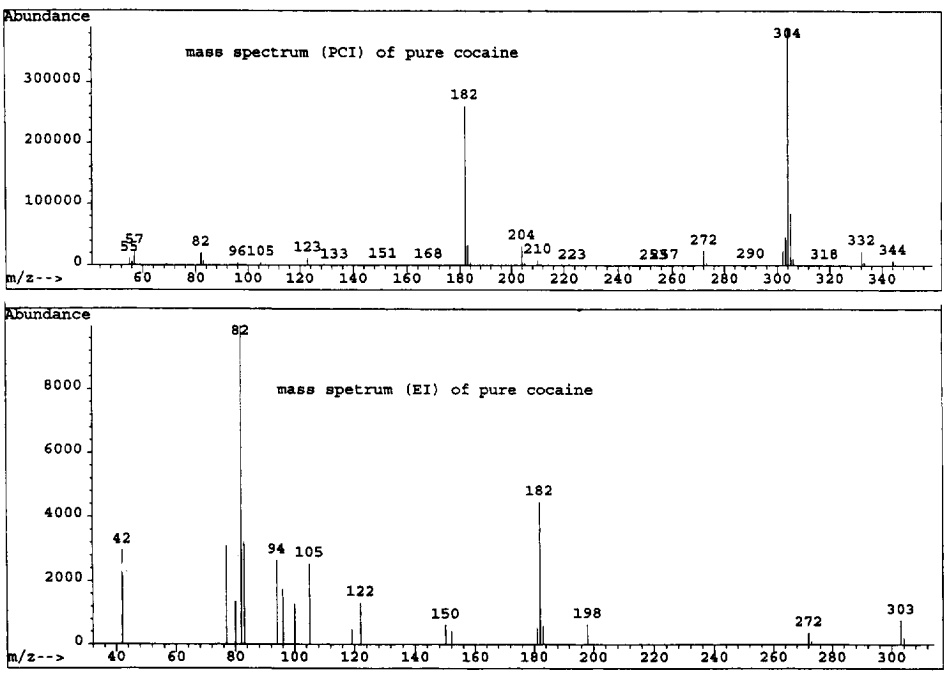


Fig. 2. The mass spectra of pure cocaine by positive chemical ionization (upper panel) and by electron impact ionization (bottom panel) (m/z 50 to 350 in both cases).

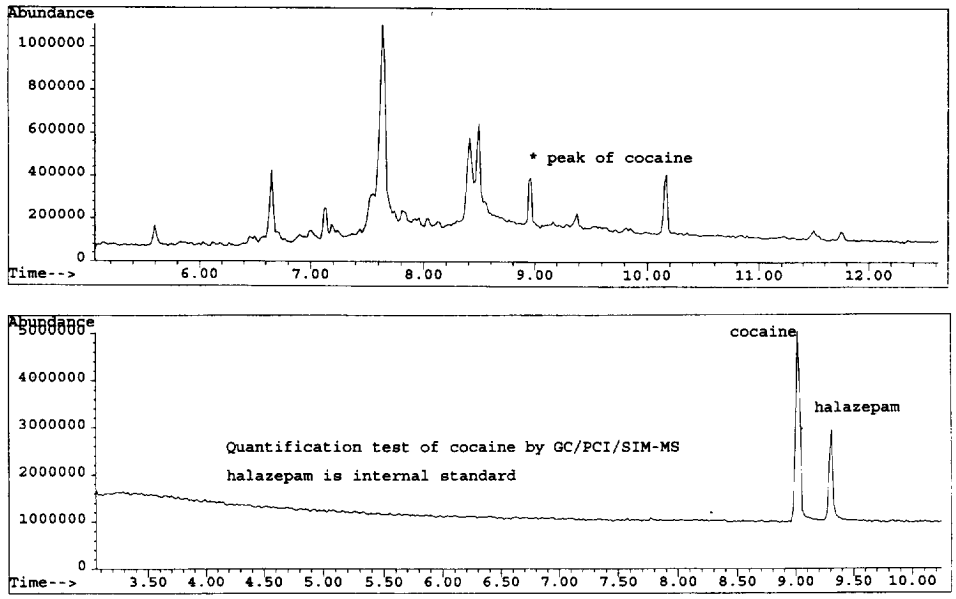


Fig. 3. (top) GC–PCI-scan-MS chromatogram of a bank note sample. Peak '*' (t_R = 8.998 min) was cocaine. (bottom) GC–PCI-SIM-MS chromatogram for quantitation of cocaine on the bank note sample.

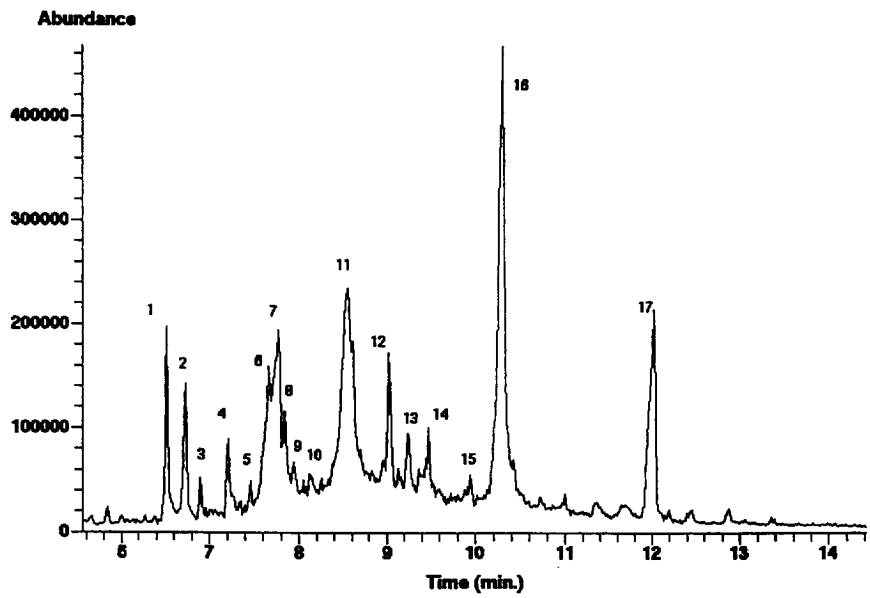


Fig. 4. GC–EI-MS chromatogram from a bank note sample. Peak 12 is cocaine. Following are possible components found on the note. Peaks: 1=2,2′-diethyl-1,1′-biphenyl; 2=ferrocene, acetyl-; 3=isopropyl myristate; 4=unknown; 5=benzoic acid, 2-(phenylthioxomethyl)hydrazide; 6=dibutyl phthalate; 7=hexadecanoic acid; 8,9,10=unknown; 11=oleic acid; 12=cocaine; 13=2-propenoic acid, 3-(4-methoxyphenyl)-; 14,15=unknown; 16=1,2-benzene-dicarboxylic acid, diisooctyl ester; 17=pentanoic acid, 1,3,3-trimethylbicyclo[2,2,1]hept-2-yl-ester.

This experiment showed that resolution is the key point in testing for trace amounts of cocaine on a bank note. The experiment also showed that a 25-m capillary column was needed to separate the cocaine peak from the peaks of other substances on the note.

3.2. Positive chemical ionization (PCI) mass spectrometry

Comparing the two mass spectra in Fig. 2, the differences between the EI and the PCI mass spectrum of cocaine are obvious. First, in the PCI mode the maximum peak is at m/z 304 and is from the ion $[\text{cocaine}+\text{H}]^+$, while in the EI mode the maximum peak is at m/z=82. Second, in the PCI mode there are much less fragments from the cocaine molecule than in the EI mode. These two major differences are exactly the advantages of the PCI mode over the EI mode. The concentration of the m/z 304 ion in the PCI mode must be much higher than that of the m/z 303 or m/z 182 ion in the EI mode. Thus, because of the fewer fragments and the higher abundance of the molecular ion $[\text{cocaine}+\text{H}]^+$, one obtains the highest sensitivity and signal-to-noise ratio with PCI–SIM at m/z 304. In all reported GC–MS techniques used for quantitation of cocaine, EI-SIM at m/z 182 was employed. The detection limit of these methods ranged from 50 ng/ml [1,8,13,14] to 12.5 [15] or 11 ng/ml [16]. This sensitivity is comparable to that of GC with nitrogen–phosphorus detection (NPD) (20 ng/ml) [17,18]. The limit of detection (LOD) of our method can reach 0.5 ng/ml at a signal-to-noise ratio >5, which is at least ten times better than that for EI with SIM monitoring at m/z 182. The sensitivity would even be higher when using EI–SIM at m/z 82. However, m/z 82 in the EI mass mode is not specific for cocaine. Many fragments from all tested molecules would give ions at m/z 82.

For identification, the mass scan mode is recommended. In our experiments, the detection limit for identification of cocaine was found to be 500 ng/ml by EI-scan (m/z 50–350) and 50 ng/ml by PCI–scan (m/z 50–350). The detection limit here is defined such that the mass spectrum generated at this lowest concentration of sample matches the spectrum of pure cocaine (EI or PCI) better than 50%.

In negative chemical ionization (NCI) mass spectrometry, the cocaine molecule fragmented into small pieces. No molecular ions such as as e.g. $[\text{cocaine}-\text{H}]^-$ (m/z 302) or $[\text{cocaine}]^-$ (m/z 303) were found. This may indicate that $[\text{cocaine}+\text{H}]^+$ is the most stable molecular ion, as found in positive chemical ionization mass spectrometry.

3.3. Quantitative analysis

Quantitative determination of cocaine on the bank note was performed by an internal standard (halazepam) method. The dynamic range of the standard curve is 1–1000 ng/ml. The results of the experiment showed that the standard curve was linear for concentrations of cocaine between 25 and 1000 ng/ml and non-linear from 1 to 25 ng/ml. Therefore the calibration graph for cocaine was generated by weighted non-linear regression ($1/Y^2$). The concentration of cocaine was calculated using the equation

$$X = (A - YC)/(B - 1) \quad (1)$$

where X is the concentration of cocaine in ng/ml and Y the ratio of the peak height of cocaine to that of the internal standard halazepam found for an experimental sample. A, B, and C are constants taken from Ref. [19].

Dilution of the sample is necessary if the sample has a very high concentration (>1000 ng/ml). Thus, the use of an expensive isotope as internal standard can be avoided, and analysis costs can be reduced. Moreover, this overcomes the disadvantages of the external standard method.

Table 1 lists the intra-assay accuracy results. The accuracy and precision were about 10%.

4. Conclusion

The method presented here can detect and determine as low as 1 ng of cocaine on a bank note. It is a powerful method to detect traces of the drug. Because of its extremely high sensitivity and selectivity, this method may be applied to determine cocaine not only on any piece of paper but also in other matrices, with different extraction procedures.

Table 1
QC sample results for cocaine

Sample	Theoretical concentration (*ng/ml*)	Concentration (mean±S.D.) (*ng/ml*)	%Theoretical	*n*
QC_L	30	27.5±1.5	91.7	5
QC_M	250	232 ±24.1	93.0	5
QC_H	550	509 ±13.3	92.6	5

Acknowledgments

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References

[1] N. De Giovanni and S. Strano Rossi, J. Chromatogr. B, 658 (1994) 69–73.
[2] G. DiGregorio, A.P. Ferko, E.J. Barbieri, E.K. Ruch, H. Chawia, D. Koehane, R. Rosenstock and A. Aldano, J. Anal. Toxicol., 18 (1994) 247–250.
[3] M. Balikova and J. Vecerkova, J. Chromatogr. B, 656 (1994) 267–273.
[4] F. Tagliaro, C. Antonioil, Z. De Battisti, S. Ghielmi and M. Marrigo, J. Chromatogr. A, 674 (1994) 207–215.
[5] G.L. Henderson, M.R. Harkey, C. Zhou and R.T. Jones, J. Anal. Toxicol., 16 (1992) 199–201.
[6] D.A. Kidwell, Gov. Rep. Announce. Index (U.S.) 1990, 90(22), Abstr. No. 058, 069.
[7] A. Hernandez, A. Antonio, H. Wilmo and W.L. Hearn, Forensic Sci. Int., 65 (1994) 149–156.
[8] S.K. Patrick, W.O. Boggan, S.R. Miller and L.D. Middaugh, J. Chromatogr., 621 (1993) 89–94.
[9] M.J. Kaufman and B.K. Madras, Synapse (N.Y.), 12 (1992) 99–111.
[10] C. Moore, S. Browne, I. Tebbett and A. Negrusz, Forensic Sci. Int., 53 (1992) 215–219.
[11] M. Nishikawa, K. Nakajima, M. Tatsuno, F. Kasuya, K. Igarashi, M. Fukui and H. Tsuchihashi, Forensic Sci. Int., 66 (1994) 149–158.
[12] M.J. Kogan, D.J. Pierson, M.M. Durkin and N.J. Willson, J. Chromatogr., 490 (1989) 236–242.
[13] S.R. Miller, A.L. Salo, W.O. Boggan and K.S. Patrick, J. Chromatogr. B, 656 (1994) 335–341.
[14] C.C. Okeke, J.E. Wyne and K.S. Patrick, Chromatographia, 38 (1994) 52–56.
[15] S.J. Mule and G.A. Casella, J. Anal. Toxicol., 12 (1988) 153–155.
[16] N. Lombardero, O. Casanova, M. Behnke, F. Eyler, B. Davis and L. Roger, Ann. Clin. Lab. Sci., 23 (1993) 385–394.
[17] G.W. Hime, W.L. Hearn, S. Rose and J. Cofino, J. Anal. Toxicol., 15 (1991) 241–245.
[18] S. Dawling, E.G. Essex, N. Ward and B. Widdop., Ann. Clin. Biochem., 27 (1990) 478–481.
[19] Drug Metabolism Laboratory Information System, programmed by G.L. Rathsmill, Penn Comp, PA, USA.