Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1045 (2004) 259–262
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Phenmetrazine or ephedrine? Fooled by library search

Sarah M.R. Wille, Willy E.E. Lambert*

Laboratory of Toxicology, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium

Received 9 April 2004; received in revised form 1 June 2004; accepted 14 June 2004

Available online 10 July 2004

Abstract

Chemical and/or thermal conversion of analytes in a sample and/or GC injector can mislead the identification of analytes in a toxicological screening. In addition, library search can even more complicate the identification. The risk for false positive identification of phenmetrazine in an ephedrine-containing sample analysed by GC–MS is described. Ephedrine reacted with formaldehyde contamination in solvents to a compound with a similar mass spectrum as phenmetrazine. High injection temperatures influenced the formation speed.

Keywords: Temperature effects; Forensic analysis; Libraries, MS; Phenmetrazine; Ephedrine

1. Introduction

In gas chromatographic–mass spectrometric toxicological analysis, identification of compounds is mainly based on the spectral data generated by the mass analyser. This gives more reliable information than detection techniques based only on retention behaviour. While large MS libraries make it even more interesting and time saving, the criticism of the user when interpreting mass spectrometric data in combination with a library is still of the utmost importance. Due to extensive ionization using electron impact, molecular ions can have a low abundance or are even sometimes not detectable, leaving unspecific fragments as the base peak. An additional problem concerns chemical and/or thermal conversion of analytes in the sample and/or in the GC injector, as discussed below. All this can mislead the identification of unknown compounds in an analytical toxicological procedure. In the past, ephedrine has already been reported to cause false positive methamphetamine identification in urine samples after carbethoxyhexafluorobutyryl chloride derivatization and high-temperature injection [1]. As discussed in this note, phenmetrazine, a central nervous system stimulant, currently abused as anorectic agent and reported to cause death after overdose [2,3], was the best hit by library search after injecting a methanolic extract of an ephedrine-containing powder on different GC–MS instruments. With this note we draw the attention to this conversion and interpretation difficulties, as it could lead to false judicial conclusions. Phenmetrazine is a controlled substance as described in the UN Convention of Psychotropic Substances 1971 [4], while ephedrine and pseudoephedrine are used in over-the-counter medications as a vasoconstrictor. However, very recently (February 2004), the US Food and Drug Administration (FDA) issued a final rule prohibiting the sale of dietary supplements containing ephedrine alkaloids because such supplements present a health risk [5].

2. Experimental

2.1. Instrumental

A HP 6890 GC system equipped with a HP 5973 mass-selective detector and a HP 7683 on-column auto injector was used (Agilent Technologies, Avondale, PA, USA). A 5 m × 0.32 mm i.d. intermediate-polarity deactivated guard column (Interscience, Louvain-la-Neuve, Belgium) was used, followed by a 30 m × 0.25 mm i.d.,

* Corresponding author. Tel.: +32 9 264 81 35; fax: +32 9 264 81 83.
E-mail address: willy.lambert@ugent.be (W.E.E. Lambert).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.06.025

0.25 μm Varian factorFOUR VF-5ms column (Varian, Middelburg, The Netherlands). The retention gap and column were combined using a glass universal press-fit connector (Alltech, Lokeren, Belgium).

The second configuration was a Varian 3400 GC equipped with a Finnigan Magnum ion-trap mass detector and a split-injector (Varian). The column was a CP-SIL 5CB 30 m × 0.25 mm i.d., 0.5 μm (Varian).

2.2. GC conditions

On-column injection conditions were as follows: the initial temperature was set at 60 °C for one min, ramped at 20 °C/min to 120 °C, whereafter the temperature was ramped again at 10 °C/min to 200 °C during another 5 min. The injection temperature was 60 °C and the Helium flow was held constant at 1.2 ml/min. For some experiments the injection temperature was elevated to 250 and 300 °C, respectively. Under these conditions, the temperature program was followed as for the split injection. The injection volume was 1 μl.

Using the split injection the conditions were as follows: the initial oven temperature of 80 °C was held for 2 min, followed by a heating at 9 °C/min to 280 °C where it was held for 2 min.

The injector temperature was set at 250 °C in the split mode and the helium flow was held constant at 13 psi inlet pressure (1 psi = 6894.76 Pa). The split ratio was 1/10 and 1 μl of the sample was injected.

2.3. MS-conditions

The mass-selective detector conditions for the on-column-configuration were set at 300 °C for the transferline, 230 °C for the source and 150 °C for the quadrupole. The spectra obtained were measured in scan mode from 45 until 650 u. The commercial library Pfleger–Maurer–Weber was used for identification of the compounds.

Mass-selective detector conditions for the split-injector ion-trap configuration were set at 280 °C for the transferline. The spectra were obtained measuring in scan mode from 45 until 450 u. The National Institute of Standards and Technology (NIST) library was used for identification of the compounds.

In both configurations the electron-impact mode with an electron voltage of 70 was used.

2.4. Chemicals

Ephedrine base was purchased from Flandria (Ghent, Belgium). Phenmetrazine HCl was a kind donation from the Laboratory of Toxicology of the Catholic University of Leuven (Belgium). Methanol (analytical grade) and formaldehyde were purchased from Merck (Darmstadt, Germany). Herbal capsules containing ephedrine (according to the manufacturer) were confiscated by the Federal Police and transmitted to the laboratory for analysis. The capsules, containing a light grey powder, were red, had a black tiger paw logo and the citation JAG. They were manufactured by D&E Pharmaceuticals. The capsules are promoted on the net and abused in nightlife and for slimming.

3. Results and discussion

An aliquot of the methanolic extract of the powder in a herbal capsule, made by adding 1 ml of methanol to 20 mg of the powder, was injected in split mode after centrifugation. At the same retention time of ephedrine a peak was observed and identified by library search as phenmetrazine. Phenmetrazine, a central nervous system stimulant abused as an anorectic compound, has a molecular ion at *m*/*z* 177 with low intensity, showing up in the ion-trap configuration with an *m*/*z* value of 178, and a base peak of 71. These ions were also seen in the MS spectrum of the peak in the chromatogram of the ephedrine-containing powder. Injection of an ephedrine standard in methanol (stored for at least 6 months at −18 °C) using the split-injector, yielded a peak at the same retention time and with the same mass spectrum. As can be seen in Fig. 1 the mass spectra B and C are almost identical and all give phenmetrazine as best hit in library search. When using the ion-trap configuration, the NIST library was used in the identification process. Searching the library for compounds with a molecular ion of *m*/*z* 177 in combination with ion at *m*/*z* 71 lead to phenmetrazine as best match. When using the on-column quadrupole-mass-detector configuration, the Pfleger–Maurer–Weber library was used and gave a 78% match for phenmetrazine (the phenmetrazine standard giving a match of 91%).

After literature search [6], our findings revealed that a formaldehyde contamination in solvents such as methanol can result in conversion of ephedrine-like compounds. This reaction was described by Lewis et al. with pseudoephedrine being converted to 3,4-dimethyl-5-phenyl-1,3-oxazolidine. We investigated this by analyzing a standard of ephedrine dissolved in a methanol–formaldehyde (1:1, v/v) mixture (Fig. 1). Again the phenmetrazine-like spectrum was clearly seen in split- as well as in on-column mode.

Based on spectral information it is difficult to discriminate phenmetrazine from the above mentioned conversion product of ephedrine. The fragmentation pattern is similar because both chemical structures differ only in the position of one carbon unit (either in or out the ring structure) [6] (Fig. 2). An option to counter the reaction with formaldehyde could be the derivatization of ephedrine. When the amino and hydroxyl functions are derivatized, formaldehyde contamination could not result in the formation of the artefact because the functions are no longer available. However, derivatization of ephedrine, can also lead to false identifications as described by Wu et al. [1].

Table 1
Retention times (min) of ephedrine, the ephedrine artefact and phenmetrazine in the different injection modes

Injection temperature (°C)	Ephedrine (min)	Ephedrine artefact (min)	Phenmetrazine (min)
OC 60	8.07	8.04	10.23
OC 250	10.08	10.01	11.20
OC 300	10.09	10.00	11.16
Split 250	12.31	12.40	13.75

OC: on-column injection mode.

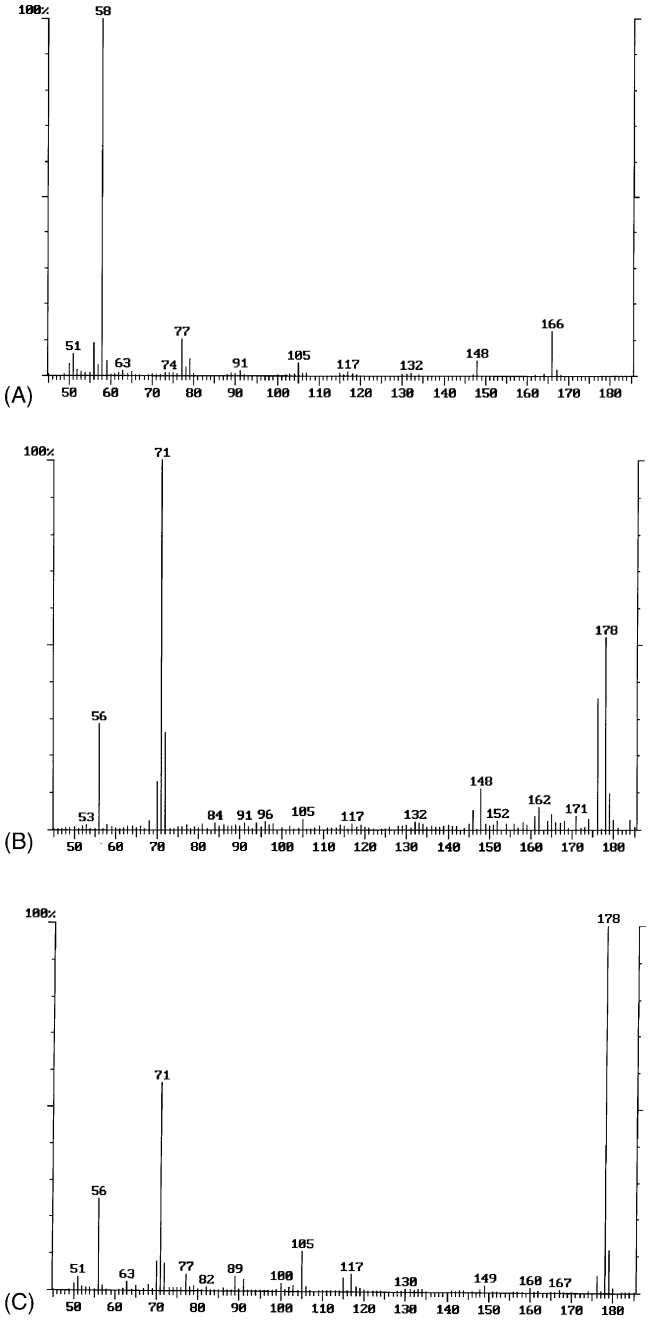

Fig. 1. Spectrum obtained after injection of (A) a "fresh" methanolic ephedrine solution; (B) an ephedrine standard solution in methanol–formaldehyde (1:1, v/v) (ephedrine artefact); (C) a methanolic phenmetrazine solution, using the split-ion-trap configuration.

Only when injecting a phenmetrazine standard a difference in retention time was observed (Table 1). Combining the spectral information and the retention time differences, a difference between the ephedrine artefact and phenmetrazine could be detected. However, as clearly demonstrated, library search alone can be misleading in this case. Due to the fact that phenmetrazine is a controlled substance, the reference standard is often difficult to obtain.

Purity of used solvents in an analysis is definitely important. Alternative solvents such as toluene were not considered in the experiments as it seemed that it was the formaldehyde contamination and not the methanol that contributed to the artefact formation. A second reason is that methanol is the first choice for extracting pills or powders in toxicological analyses.

In addition to formaldehyde, the injection temperature, however, is also critical. When injecting a freshly made ephedrine standard in methanol, the MS spectrum of ephedrine was recognized with on-column (injection temperature: 60 °C) and with split injection (injection temperature: 250 °C). The difference though was the formation of the "phenmetrazine-like" peak in the split mode injection at 250 °C. Although the formation was due to the presence of formaldehyde, it appeared faster with split-injection than with on-column injection. This can be explained by the high injection temperature applied in the split-injection mode to evaporate the injection solvent before entering the column. Normally in an on-column injection procedure, the temperature of the injector and starting temperature of the column oven should be lower than the boiling point of the solvent (methanol: 64.6 °C) to avoid backflow. However, because here there was no intention to quantify, but just to evaluate the influence of the injector temperature on the formation of the phenmetrazine-like compound, the injection temperature of the on-column injector was elevated to 250 and 300 °C, respectively. Changing the on-column injection temperature to 300 °C resulted in the formation of the phenmetrazine-like peak and a small remainder of ephedrine. This was also observed when injecting a fresh ephedrine solution in split mode (250 °C).

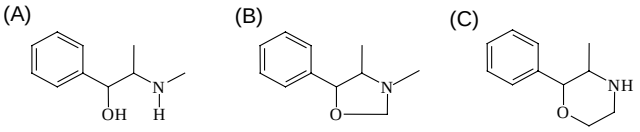

Fig. 2. Structure of ephedrine (A); the reaction product of ephedrine and formaldehyde (B); and of phenmetrazine (C).

The data demonstrate that not only the presence of formaldehyde is important in the formation of this artefact from ephedrine, in addition, the injection temperature influences the formation rate. Furthermore, library search can misidentify the artefact as phenmetrazine.

4. Conclusion

The combination of ephedrine, formaldehyde contamination in analytical reagents and high injection temperature can lead to a false identification of phenmetrazine. Library searches alone can lead to erroneous conclusions. Attention should be drawn to this phenomenon, in view of the judicial implications. Injection of a phenmetrazine standard reveals however a difference in retention time.

References

[1] A.H.B. Wu, S.S. Wong, K.G. Johnson, A. Ballatore, W.E. Seifert Jr., Bio. Mass Spectrom. 21 (1992) 278.
[2] A. Dasgupta, M.S. Handler, J.S. Nine, J. Forensic Sci. 43 (1998) 630.
[3] G. Norheim, J. Forensic Sci. Soc. 13 (1973) 287.
[4] Convention on Psychotropic Substances, 1971, http://www.incb.org/e/conv/1971/.
[5] FDA, US Food and Drug Administration, http://www.fda.gov/bbs/topics/NEWS/2004/NEW01021.html.
[6] R.J. Lewis, E.F. Huffine, A.K. Chaturvedi, D.V. Canfield, J. Mattson, J. Forensic Sci. 45 (2000) 898.