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POSITIVE CONFIRMATION OF IDENTITY OF DOPING AGENTS USING GAS CHROMATOGRAPHY–MASS SPECTROMETRY WITH FOURIER TRANSFORM INFRARED SPECTROMETRY

M.I. SELALA*, J.J. JANSSENS, V. COUCKE, S. ANDRIES and P.J.C. SCHEPENS

Toxicological Center, Department of Pharmaceutical Sciences, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Wilrijk (Belgium)

SUMMARY

During the past two decades, the use of retention times in gas chromatography has been augmented by mass spectrometric data. By providing both the retention indices and spectrometric data, this technique has greatly improved gas chromatographic identification analysis. However, although gas chromatography–mass spectrometry has become pre-eminent, several drawbacks still remain. The mass spectral library often gives erroneous identifications when concentrations near the detection limit are analysed, when gas chromatographically interfering substances are present, or when structural isomers or compounds exhibiting identical retention behaviour are analysed. Linked with gas chromatography–mass spectrometry, Fourier transform infrared spectroscopy can be a powerful complementary technique in peak identification analysis. Some spectral data to illustrate this point are presented.

INTRODUCTION

The International Olympic Committee (IOC) and the International Amateur Athletic Federation (IAAF) have issued a comprehensive list of substances prohibited in human sport and horse racing. These doping agents are defined according to their pharmacological activity, but the descriptions in the list often include inexplicit terms such as "related compounds" and even "chemically or pharmacologically related compounds" [1]. In numerous instances, the identification of such substances often produces conflicting results. It is therefore extremely important to establish a method to identify these prohibited agents with the highest possible confidence.

Since the introduction of combined gas chromatography–mass spectrometry (GC–MS) with electron impact (EI) or chemical ionization (CI), controversial results in toxicological analysis are scanty. GC–MS has excelled over other chromatographic techniques because of its relatively high sensitivity, the MS library

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data and the ease of operation. Unfortunately, several problems are still encountered both in forensic and general toxicological screening and in doping control. For example, the identification of doping agents by GC–MS can be difficult, particularly when the compounds being analysed coelute or have a relatively unspecific base peak and small molecular ion signals that tend to disappear when the concentration is low or when GC- interfering substances are present. The MS library search under such conditions often yields erroneous identifications.

In this work, independent GC–Fourier transform infrared (FTIR) spectroscopic and GC–MS techniques were used in the analysis of a few doping agents with central stimulatory and sympathomimetic activity. The objective of the study is to illustrate the complementary role of FTIR in GC–MS peak identification analysis when the MS library fails.

EXPERIMENTAL

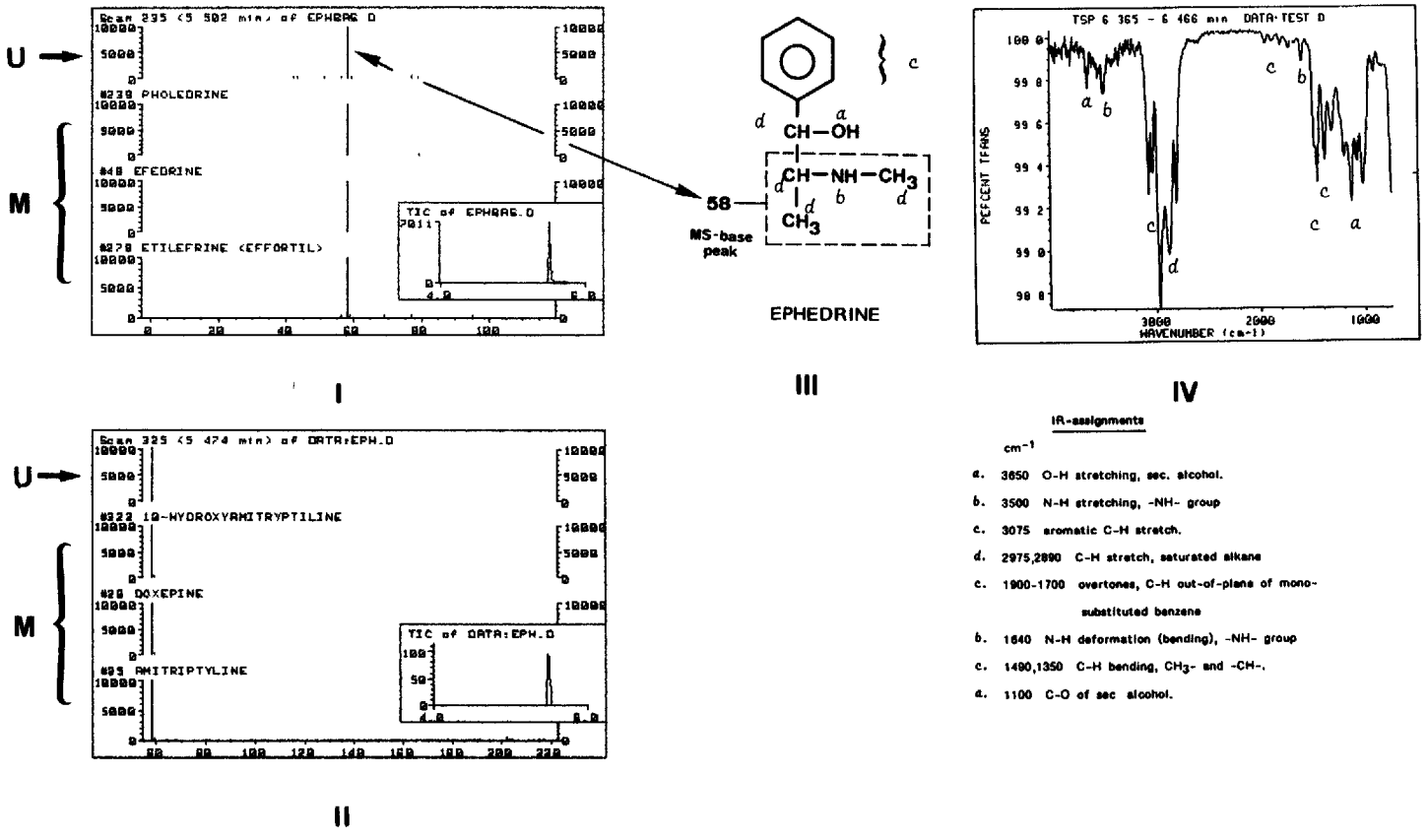
GC–MS analyses were performed on an HP 5970B mass-selective detector (all HP equipment by Hewlett-Packard, Palo Alto, CA, U.S.A.). GC–FTIR spectra were obtained from an HP 5965A infrared detector. Both detectors were independently coupled to an HP 5890 GC system equipped with a split/splitless injector. A 12.5 m × 0.203 mm I.D. (0.2 μm film thickness) HP-1 (cross-linked methyl silicone) capillary column (HP 1901-60010) was used. The oven temperature was programmed from 90 to 250 °C at 10 °C/min. The injection temperature and light pipe temperature for FTIR analysis were ranged from 200 to 300 °C, depending on the nature of the sample. Samples were injected in the split mode in both analyses.

RESULTS AND DISCUSSION

Using the selected-ion monitoring mode, a considerable gain in GC–MS sensitivity can be achieved by focusing the most abundant ion. However, many doping agents (and numerous other commonly used drugs, e.g. antihistamines, local anesthetics, some β-blocking agents, etc.) have similar EI mass spectra showing a common base peak and weak molecular ion signals. Although many elute over a wide range of retention times, some have closely related Kovats retention indices. Most amphetamines and sympathomimetic amines, for instance, have closely comparable mass spectra with a large base peak commonly occurring at m/z 44, 58, 72 or 86. These base peaks originate respectively from the very stable nitrogen-containing $C_2H_6N^+$, $C_3H_8N^+$, $C_4H_{10}N^+$ and $C_5H_{12}N^+$ fragment ions. Furthermore, the molecular and other fragment ions are usually weak in comparison with the base peak.

Figs. 1 and 2 compare the quality of data obtained by GC–MS and GC–FTIR for ephedrine, phentermine, and propylhexedrine (all on the IOC banned list) in relation to their chemical structures.

In Fig. 1, 100 and 40 ng/μl ephedrine (free base) were injected in split mode (uppermost spectra, U). The computer search of the MS library gave more than five 'matches' with a correlation of 99% and more [I and II, M are the best three



IR-assignments

	cm^{-1}	
a.	3650	O-H stretching, sec. alcohol.
b.	3500	N-H stretching, -NH- group
c.	3075	aromatic C-H stretch.
d.	2975,2890	C-H stretch, saturated alkane
e.	1900-1700	overtones, C-H out-of-plane of mono-substituted benzene
b.	1640	N-H deformation (bending), -NH- group
c.	1490,1350	C-H bending, CH_3- and -CH-.
a.	1100	C-O of sec alcohol.

Fig. 1. GC–MS spectra of ephedrine injected in split mode at 100 and 40 ng/μl (I and II, U , respectively). M, in I and II, displays the spectral 'matches' from the MS library. III and IV show the structure of ephedrine and its IR spectrum from GC–FTIR. The different IR vibrations and the corresponding functional groups are matched in italics.

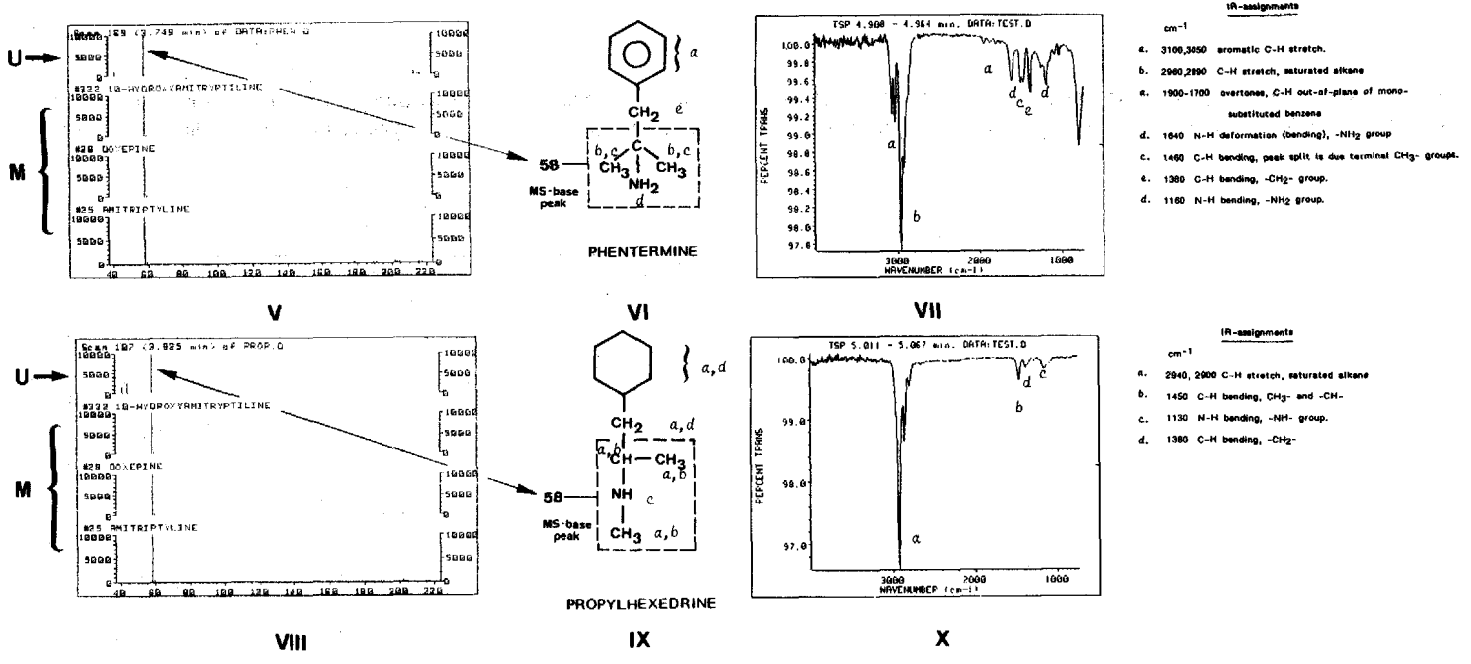


Fig. 2. GC–MS spectra and computer library searches for phentermine and propylhexedrine each injected in split mode at 50 ng/μl (V and VIII, U). VI and IX are the respective molecular structures. VII and X show the respective IR spectra obtained from GC–FTIR. IR assignments and the corresponding functional groups are matched in italics.

hits (match indices not shown)]. In both instances, the library search failed to produce ephedrine as best 'fit'. The spectra also show a large base peak at m/z 58 common to both the injected sample and the 'matched' compounds. Misidentification in II is further worsened by the low concentration injected (40 ng/μl split). It can also be seen from this spectrum that the molecular ion signal of ephedrine and other minor fragment peaks (evident in I) have disappeared.

In Fig. 2 (V and VIII, U), 50 ng/μl each of the approximately coeluting compounds phentermine and propylhexedrine were analysed. In both instances, the MS library also failed to yield these compounds as first 'hits' (V and VIII, M). The spectra also show a large base peak at m/z 58 common to both the injected and 'matched' compounds. As in II of Fig. 1, the molecular ion signals of both compounds and other peaks have also disappeared.

The ambiguity caused by such spectral data seriously limits analytical validity. The verification of results in such circumstances may lead to controversy since both the retention times and m/z values are not specific enough to allow absolute identification. Additional information, such as that derived from IR absorbance data, is therefore necessary. Visual inspection of the IR spectra, obtained from GC–FTIR [Fig. 1 (IV) and 2 (VII and X)], indicates clear differences. Assignment of the observed vibrations to the various functional groups complements the analysis and thereby yields a positive confirmation of identity.

In combination with GC–MS, FTIR can also play a vital role in the identification of structural isomers. The complementary contribution of all three techniques [GC, MS (CI–EI) and FTIR] will be of great value in this respect. MS will provide the molecular mass of the component under scrutiny, FTIR will give information on the structural attributes and geometric isomerism and the GC retention index will confirm that the peak being analysed is the same one in each case. The integrated GC–FTIR–MS system will certainly facilitate such analysis.

CONCLUSION

GC–FTIR is a promising analytical technique for the identification of structurally related compounds, such as isomers or drugs of the same group, especially when retention times are nearly identical. The combined GC–FTIR–MS system has emerged as a powerful tool in toxicological analysis: it provides a higher confidence result than either GC–MS or GC–FTIR alone. The major drawback at the moment is the sensitivity of FTIR relative to GC–MS. For amphetamine, the FTIR limit of detection is ca. 200 ng (splitless injection). A full-scan MS analysis using the same amount yields a complete mass spectrum. Thus when MS fails in the described conditions, FTIR will also fail. However, an increase of IR sensitivity by two orders of magnitude (i.e. to the 100 pg level) is believed to be possible and will make GC–FTIR–MS a unique tool in drug and doping analysis [2].

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