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Short communication

Identification of ephedrines as their carbon disulfide derivatives

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Abstract

A method for the separation and identification of the diastereoisomers ephedrine and pseudoephedrine and the diastereoisomers norephedrine and norpseudoephedrine is presented. The compounds were derivatised by reaction with carbon disulfide in the presence of alkali. These derivatives and their trifluoroacetic anhydride derivatives were subjected to gas chromatography with nitrogen-selective detection as well as mass-selective detection and high-performance liquid chromatography with ultraviolet detection. The results showed that ephedrine and pseudoephedrine can easily be differentiated by gas chromatographic analyses of their carbon disulfide derivatives. Norephedrine and norpseudoephedrine can be differentiated by the different chromatographic retention times of their carbon disulfide derivatives and by the fact that norephedrine yielded two products and norpseudoephedrine only one product when reacted with carbon disulfide under the same conditions. Trifluoroacetylation of the latter compounds gave a more pronounced differentiation.

1. Introduction

Ephedrine, pseudoephedrine, norephedrine and norpseudoephedrine are all sympathomimetic amines known to have central nervous system stimulating properties and are therefore classified as banned substances in sport. These ephedrines are ingredients of various medicines commonly used for colds, sinusitis, rhinitis, hay fever and appetite suppressants and may be bought over the counter without a prescription from a physician. Therefore they are often detected in the urine obtained from sportspersons. The Medical Commission of the International Olympic Committee (MC-IOC) has defined concentrations in urine above which these ephedrines are considered as a positive doping case. These concentrations are 5 μg/ml for ephedrine and norpseudoephedrine and 10 μg/ml for norephedrine and pseudoephedrine.

Ephedrine and pseudoephedrine are diastereoisomers that are resistant to simple chromatographic separation but can easily be separated after derivatization. The diastereoisomers norephedrine and norpseudoephedrine are more resistant to chromatographic separation even after derivatization. For confirmation purposes the actual ephedrine present in urine should be identified.

Since it is well known that primary and secondary amines react with carbon disulfide, to form isothiocyanates and dithiocarbamic acids,

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respectively [1–9], this reaction was used to develop a method to differentiate the four ephedrines, especially norephedrine and norpseudoephedrine.

2. Experimental

2.1. Materials

Ephedrine-HCl, pseudoephedrine-HCl, norephedrine-HCl, norpseudoephedrine-HCl and methaqualone were all from our departmental reference substance collections. Hexane, tetrahydrofuran and carbon disulfide were all reagent grade (Merck, Darmstadt, Germany).

2.2. Apparatus

Gas chromatography

Gas chromatography (GC) was carried out on a Hewlett-Packard 5890 instrument equipped with a nitrogen-selective detector and linked to a HP 3365 Series II chemstation (Hewlett-Packard, Avondale, PA, USA). A Hewlett-Packard crosslinked 5% phenylmethyl silicone fused-silica column (25 m × 0.32 mm I.D., 0.52 μm film thickness) was used at a column head pressure of 135 kPa. Helium was used as carrier gas while the injector and detector temperatures were at 250°C and 280°C, respectively. Splitless injections (time-purge 0.5 min) were made at an initial column temperature of 80°C and the temperature was programmed to rise to 300°C at 40°C per minute and maintained there for 2 min.

Gas chromatography–mass spectrometry

Combined gas chromatography–mass spectrometry (GC–MS) was carried out using a Hewlett-Packard 5970 Series mass-selective detector linked to a Hewlett-Packard 5890 gas chromatograph. A Hewlett-Packard crosslinked methyl silicone fused-silica column (12.5 m × 0.2 mm I.D.; 0.33 μm film thickness) was used. Helium was used as carrier gas. Injection port and transfer zone temperatures were maintained at 280°C. Splitless injections (time-purge 0.5 min) were made at an initial column temperature of 80°C. The temperature was then increased to 300°C at 20°C per minute and maintained there for 5 min. The ion-source temperature was 250°C and the ionizing beam at 70 eV.

High-performance liquid chromatography

A modular high-performance liquid chromatographic system consisting of a Model SP8800 Spectra-Physics pump with a Model SP8500 solvent mixer (Spectra-Physics, San Jose, CA, USA) an ultraviolet detector (Waters, Milford, MA, USA) and a Vectra computer (Hewlett-Packard). Separation was performed at ambient temperature on a Hewlett-Packard Hypersil silica column, 5-μm particle size, 100 × 2.1 mm I.D. A constant flow-rate of 0.2 ml/min was maintained through the HPLC column and the column effluent was monitored at 254 nm.

The mobile phase was hexane–tetrahydrofuran (85:15, v/v).

2.3. Reactions

Standard reference compounds (10 mg) as free bases were dissolved in 100 ml of toluene. Internal standard solution was prepared by dissolving methaqualone (20 mg) in 100 ml of toluene.

An aliquot (100 μl) of the standard reference compound solution was transferred to a 2-ml glass ampoule together with internal standard solution (10 μl), 20% NaOH solution (20 μl) and carbon disulfide (100 μl). The ampoule was sealed. For ephedrine and pseudoephedrine the reaction was carried out at room temperature for 4 h and for norephedrine and norpseudoephedrine the reaction was done at 60°C for 3 h. The mixture was frozen, the organic layer transferred to another 2-ml ampoule and then evaporated to dryness at 40°C under a stream of high purity nitrogen. The residue was dissolved in 100 μl of toluene, 1 μl subjected to GC and GC–MS analysis and 20 μl injected onto the HPLC column.

An aliquot of this toluene solution (50 μl) was transferred to a 2-ml glass ampoule containing 20 μl trifluoroacetic anhydride (TFAA). The ampoule was sealed, heated at 30°C for 60 min and

then evaporated to dryness at 40°C under a stream of high purity nitrogen. The residue was dissolved in 100 μl of toluene and 1 μl was subjected to GC and GC–MS analysis.

2.4. Extraction procedure

Blank urine spiked with one of the ephedrines or urine obtained from a positive doping case were extracted as follows: To 3 ml of urine was added 0.3 ml of 20% NaOH solution and 20 μl of methaqualone solution (20 mg/100 ml toluene as internal standard). The mixture was extracted with 4 ml of distilled diethyl ether by shaking horizontally for 5 min on a mechanical shaker. After centrifuging at 1200 *g* for 5 min the organic phase was transferred to a 5-ml ampoule and evaporated to dryness. To the residue in the ampoule was added 20 μl of 20% NaOH solution and 100 μl of carbon disulfide and the reaction done as described above for pure solutions.

3. Results and discussion

The reaction of the ephedrines with CS_2 was studied under various reaction conditions (e.g. reaction time, reaction temperature and reaction with and without NaOH). The conditions described gave the best results for identification purposes.

When ephedrine was reacted with carbon disulfide in the presence of NaOH the GC analysis of the derivatives yielded two peaks with relative retention times (RRT) of 0.962 and 0.909, respectively, relative to methaqualone, the latter in minor quantities only (Fig. 1A). GC analysis of the derivatives formed when pseudoephedrine was reacted under the same conditions yielded a single peak with an RRT of 0.890 (Fig. 1B). When norephedrine was reacted with carbon disulfide in the presence of NaOH two products were detected by GC analysis with an RRT of 0.927 and 0.984, respectively (Fig. 1D). Norpseudoephedrine yielded only one product (RRT 0.919) under the same reaction conditions (Fig. 1C). Although the RRTs of the carbon disulfide derivatives from norephedrine and norpseudoephedrine differ only slightly (0.927 and 0.919) separation could be observed when the two reaction mixtures were injected simultaneously, especially when the oven temperature was raised at a slower rate (30°/min).

Electron-impact (EI) mass spectra were obtained for these derivates during capillary GC–MS. The two CS_2 derivatives of ephedrine gave molecular ions under EI ionization of *m/z* 223 and *m/z* 207, respectively. The main product of ephedrine (RRT 0.962; *m/z* 223; product 2 in Fig. 1A) corresponds to the dithiocarbamic acid derivative $[M^+ - H_2O]$ (Fig. 2B). The sole CS_2 derivative of pseudoephedrine gave a molecular ion of *m/z* 207 under the same conditions (Fig. 2A). For the CS_2 products of ephedrine and pseudoephedrine with *m/z* 207 a substituted isothiocyanate structure is postulated (Fig. 3), but this structure is still under investigation. The sole derivative of pseudoephedrine and the major derivative of ephedrine gave different RRTs and mass spectra and ephedrine and pseudoephedrine can thus be differentiated as their CS_2 derivatives.

The two CS_2 derivatives of norephedrine gave molecular ions under EI ionization of *m/z* 193 and *m/z* 209, respectively. The sole CS_2 derivative of norpseudoephedrine gave a molecular ion of *m/z* 193 under similar conditions. The derivatives of norpseudoephedrine and norephedrine with *m/z* 193 (Fig. 2C) correspond to the isothiocyanate compound while the derivative of norephedrine with *m/z* 209 corresponds to the dithiocarbamic acid $[M^+ - H_2O]$ (Fig. 2D). Norephedrine and norpseudoephedrine can thus be differentiated only by slightly different GC RRTs and by the fact that norephedrine yielded two products and norpseudoephedrine only one product when reacted with CS_2 under the same conditions.

Fig. 4 shows chromatograms obtained when 2 μg/ml of norephedrine (Fig. 4A) and norpseudoephedrine (Fig. 4B) were spiked to urine, extracted and reacted as described. From these results it is clear that this concentration can easily be detected by this method. It is interest-

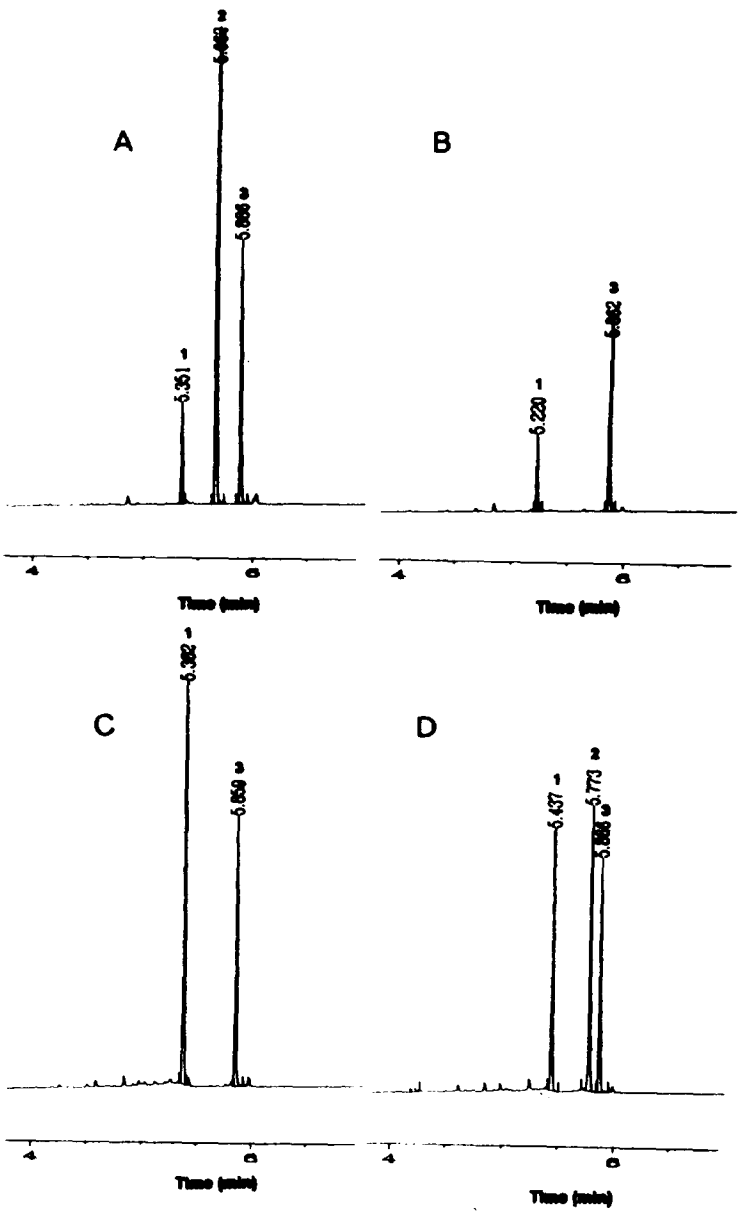

Fig. 1. Gas chromatograms of the CS_2 derivatives of ephedrine (A), pseudoephedrine (B), norpseudophedrine (C), and norephedrine (D). (1 and 2 = products; 3 = internal standard).

ing to note that for a low concentration of norephedrine less of product 2 is formed but that for a high concentration more of product 2 is formed. This happens already at a concentration of 5 μg/ml. The concentration of 2 μg/ml is well below the cut-off concentration defined by the MC-IOC for a positive doping case.

When the carbon disulfide derivatives of norephedrine and norpseudoephedrine were trifluoroacetylated, GC analysis yielded one new peak for norpseudoephedrine (RRT 0.758) (Fig. 5B) and two peaks for norephedrine (RRT 0.790 and 0.831, respectively) (Fig. 5A). These TFA derivatives gave a more distinct separation of the

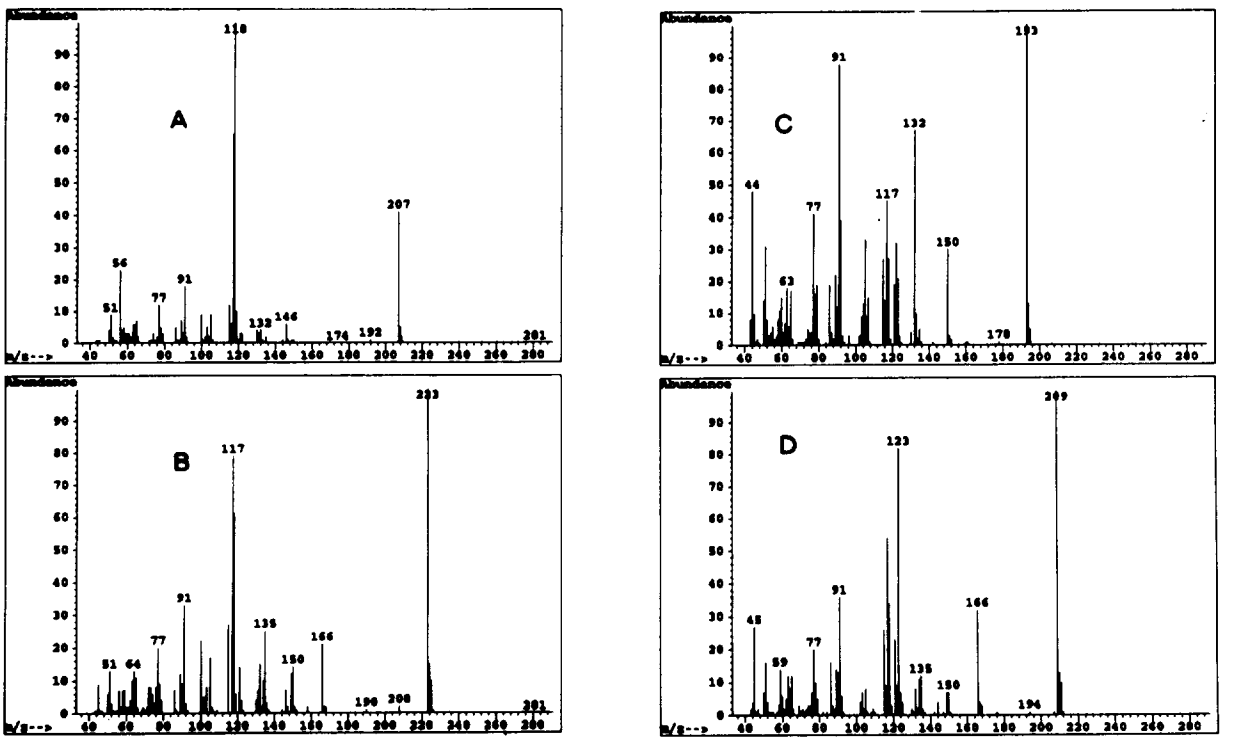

Fig. 2. Mass spectra of the CS_2 derivatives of ephedrine product 1 and pseudoephedrine (A), ephedrine product 2 (B), norephedrine product 1 and norpseudoephedrine (C) and norephedrine product 2 (D).

CS_2 derivatives of norephedrine and norpseudoephedrine.

The HPLC retention times of the CS_2 derivatives of these ephedrines are given in Table 1. From the results it is clear that there is a good separation between the CS_2 derivatives of ephedrine and pseudoephedrine as well as for norephedrine and norpseudoephedrine.

4. Conclusion

From the GC–MS data it is clear that ephedrine and pseudoephedrine gave two different products when reacted with CS_2 under the conditions described. Ephedrine gave the dithiocarbamic acid derivative as the major product while pseudoephedrine gave the substituted isothiocyanate derivative as the sole product. The GC retention times and mass spectra data can be used to unambiguously identify ephedrine and pseudoephedrine. The HPLC data can be used to give additional evidence for the presence of ephedrine or pseudoephedrine.

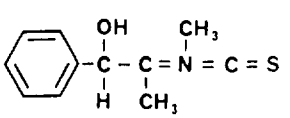

Fig. 3. Structure postulated for the CS_2 derivatives of ephedrine product 1 and pseudoephedrine.

The GC–MS data shows that norephedrine, when reacted with CS_2 under the conditions described, forms both the isothiocyanate and the dithiocarbamic acid derivatives in more or less equal quantities. Norpseudoephedrine gave only the isothiocyanate derivative under the same conditions. This data can be used to unambiguously identify norephedrine and norpseudoephedrine. Good separation can be obtained by GC when these products are trifluoroacetylated. The HPLC data can be used to give

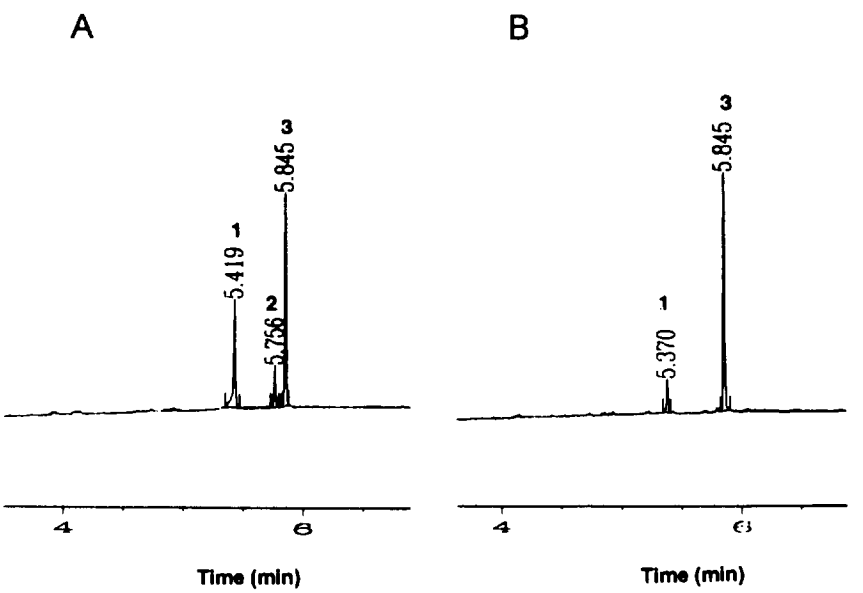

Fig. 4. Gas chromatograms of the CS_2 derivatives of norephedrine (A) and norpseudoephedrine (B), each at a concentration of 2 μg/ml.

additional evidence for the presence of norephedrine and/or norpseudoephedrine.

Positive urine samples containing norephedrine or norpseudoephedrine which were obtained from competitors in sporting events were analysed by this method and very good results were obtained. Since the concentrations of these ephedrines are usually higher than 5 μg/ml the products were easy to detect. Several positive urine samples were analysed and the method has been shown to be free from interferences from endogenous compounds in urine and from a range of drugs containing a primary or secondary amine function.

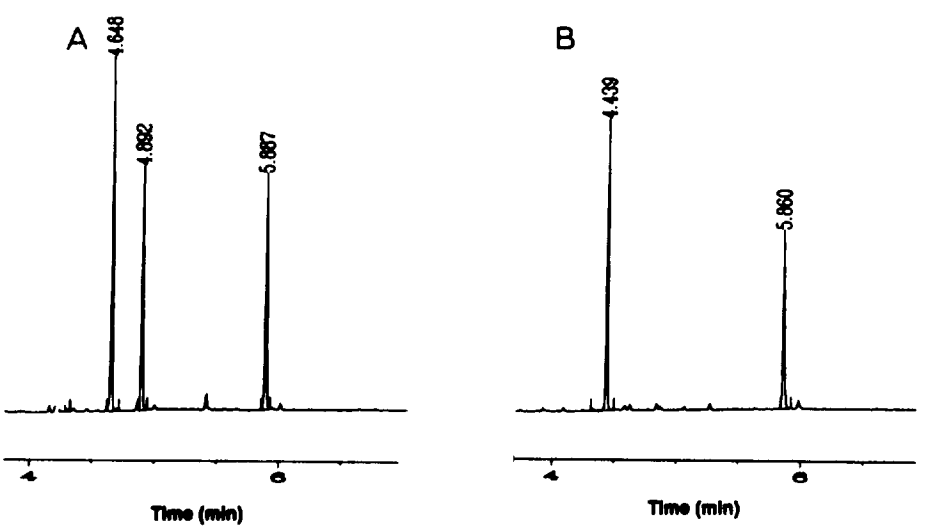

Fig. 5. Gas chromatograms of the trifluoroacetylated CS_2 derivatives of norephedrine (A) and norpseudoephedrine (B).

Table 1
HPLC retention times of the CS_2 derivatives of the ephedrines

Compound	Retension time (min)
Ephedrine product 1	3.85
Ephedrine product 2	8.51
Pseudoephedrine	5.30
Norephedrine product 1	4.35
Norephedrine product 2	12.90
Norpseudoephedrine	7.68

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