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Confirmation of Ephedrines - Comparison between GC-MS and LC-MS/MS

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Introduction

Stimulants such as ephedrines are prohibited according to the rules of the International Olympic Committee (IOC), and traditionally they have been quantitated and confirmed in our laboratory with a complex procedure based on gas chromatography-mass spectrometry (GC-MS). In contrast, the identification and quantification with liquid chromatography interfaced to a tandem mass spectrometer (LC-MS/MS) needs very little effort of sample preparation. Hence, in the last two years, all urine samples suspicious for ephedrine concentrations exceeding the established cut-off level have been confirmed with both analytical techniques in order to determine differences in sample handling and results.

Experimental

Sample preparation.

To 3 mL of urine, 25 µg of synthesized $^{2}H_3$-ephedrine are added and the sample is mixed thoroughly. Then, an aliquot of 100 µL is transferred to an HPLC vial for LC-MS/MS analysis and the remaining volume is prepared for GC-MS measurement. A volume of 0.3 mL of 5 M KOH (pH 14), 2 mL of *tert.*-butylmethyl ether and 2 g of anhydrous sodium sulfate are added to the urine specimen, the sample is shaken for 20 min, centrifuged for 5 min at 600 *g*, the organic layer is transferred to a 2 mL vial, and 3 µL are injected into the GC-MS system for quantitation purposes.

For qualitative analysis, the remaining ether is evaporated at room temperature overnight, 60 µL of a mixture of acetonitrile/ trifluoroacetic acid/ methylorange (60/40/8, v/v/w) are added and the sample is fortified with MSTFA until a change of colour from red to yellow is observed (approximately 100 µL). An excess of 10 µL of MSTFA is added, the sample is incubated at 80°C for 30 min, 15 µL of MBTFA are added, the sample is incubated for another 15 min at 80°C , and after cooling to ambient temperature, a qualitative identification

of the *O*-TMS - *N*-TFA derivative of ephedrine or any of its analogues is performed by GC-MS.

GC-MS parameters.

GC-MSD system:	Agilent 5890/5973
Carrier gas:	Helium (1mL/min flow), split ratio 1:10
Analytical column:	HP-5 MS capillary column, 0.25 mm i.d., 0.25 µm film thickness, length: 17m
Temperature program:	100°C, 20°C/min, 320°C, 1 min constant temp.

LC-MS/MS parameters.

Instrument:	Applied Biosystems API 2000	
Ion source:	APCI, 450°C	
Ionization/Acquisition:	positive/MRM	
Collision gas:	Nitrogen, 1.8e-5 torr	
Collision energy:	optimized for each product ion	
Ion transitions:	Ephedrine/Pseudoephedrine	166-148 (169-151)
		166-117 (169-117)
		166-115 (169-115)
	Cathine/Norephedrine	152-134
	Methylephedrine	180-162
Column:	Purospher Star 18e, 4.6x55mm, particle size 5µm	
Eluents:	A = 5mM Ammonium acetate in H_2O, 0.1% AcOH, pH = 3.5	
	B = acetonitrile	
Flow:	1.25 mL/min, isocratic at 97%A	

Results and Discussion

Since 2001, the GC-MS and LC-MS/MS procedures for qualitative and quantitative analyses of the ephedrines cathine, phenylpropanolamine, ephedrine, pseudoephedrine and methylephedrine, were compared with 21 positive doping control samples. In all analyses, immediate qualitative determination of analytes was accomplished by means of three diagnostic ion transitions, and the average deviation of quantitation results was 8%. In Figure

1, a typical chromatogram of a GC-MS analysis of a urine sample fortified with cathine, phenylpropanolamine, ephedrine and pseudoephedrine is presented, demonstrating the chromatographic separation of analytes of interest after derivatization.

LC-MS/MS identification of ephedrine and pseudoephedrine (in addition to cathine, phenylpropanolamine and methylephedrine) was accomplished by chromatographic separation of both stereoisomers (Figure 2) and subsequent determination of the product ions *m/z* 148, 117 and 115 (Figure 3) generated from the protonated molecule. For quantitation purposes, the suspicious sample as well as blank urine samples and those fortified with 10 or 25 µg/mL of ephedrine or pseudoephedrine, respectively, were prepared in triplicate. All samples were analyzed twice, and the resulting ratio of analyte and ISTD (d_3-ephedrine) areas at the ion transition *m/z* 166-148 and 169-151, respectively, was used to calculate the urinary concentration of the ephedrines. Characteristics of the collision-induced dissociation of ephedrine and pseudoephedrine enable the unambiguous identification of these compounds in human urine, as their fragmentation scheme consists of a loss of water (-18 Da) and the elimination of methylamine. Thus, the fragment ion at *m/z* 148 is shifted to *m/z* 151 in case of triply deuterated ephedrine, but the ions at *m/z* 117 and 115 remain unaffected (Figure 4).

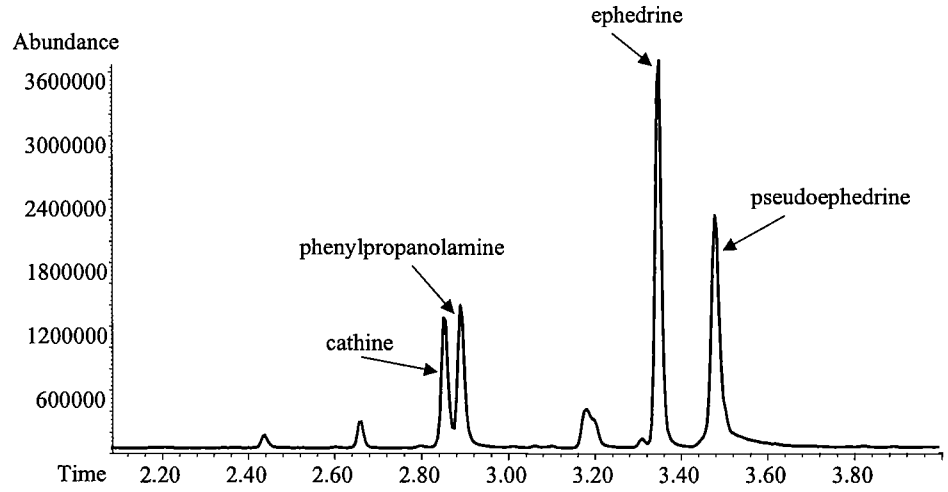

Figure 1: GC-MS chromatogram of the *O*-TMS - *N*-TFA derivatives of ephedrine and its analogues cathine, phenylpropanolamine and pseudoephedrine.

Conclusion

The quality of chromatographic separation of ephedrines by GC (after derivatization) and LC is comparable as demonstrated in the Figures 1 and 2. Advantages of the LC-MS/MS method

are a very short sample preparation, and the combined qualitative and quantitative determination of ephedrines in a single run.

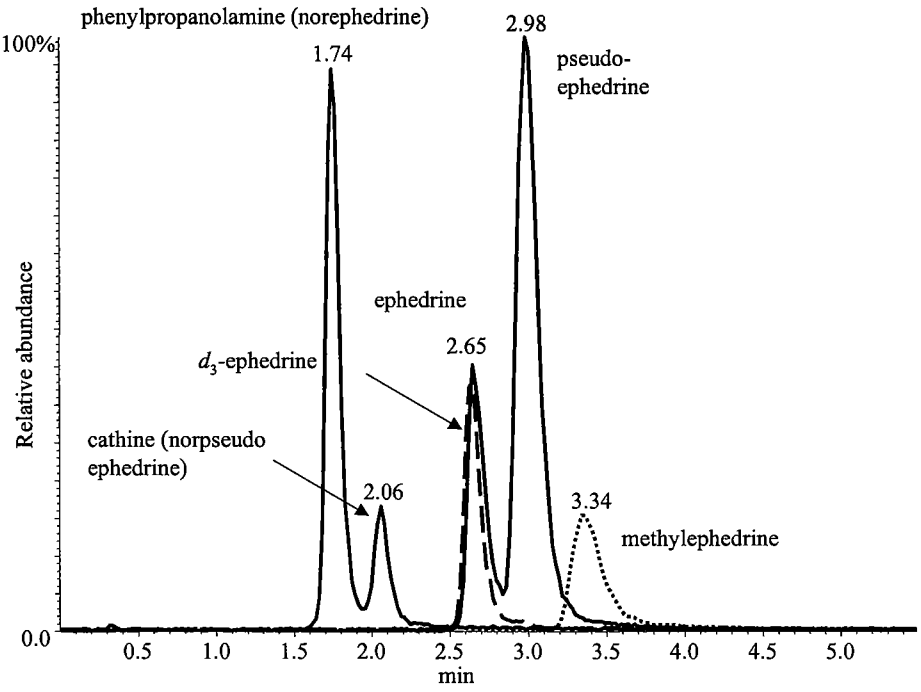

Figure 2: LC-MS/MS chromatogram of a urine sample spiked with norephedrine (25 µg/mL), cathine (norpseudoephedrine, 5 µg/mL), ephedrine (10 µg/mL), pseudoephedrine (25 µg/mL) and methylephedrine (10 µg/mL).

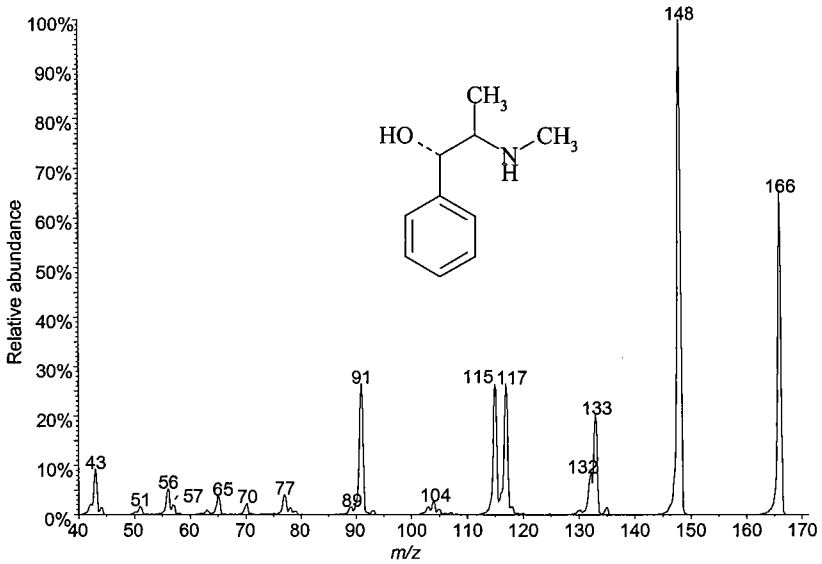

Figure 3: ESI-product ion spectrum of *m/z* 166 of ephedrine

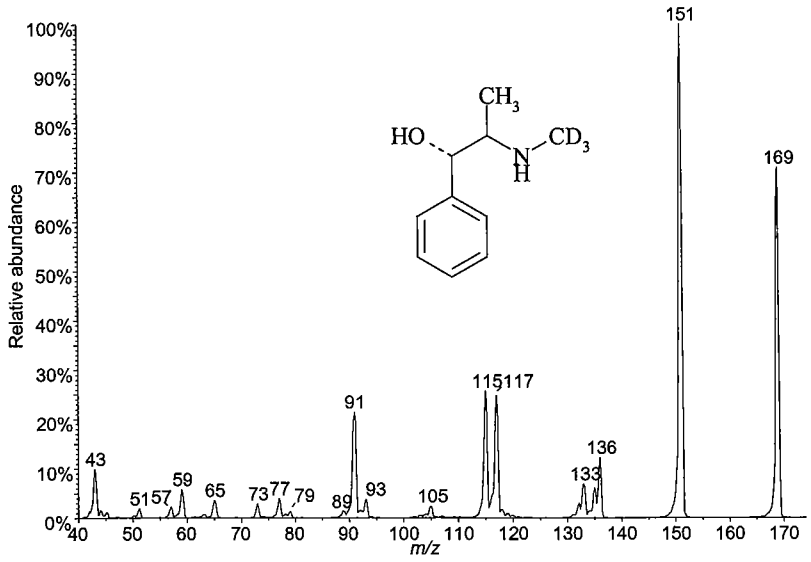

Figure 4: ESI-product ion spectrum of *m/z* 169 of d_3-ephedrine

References

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