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Simultaneous Quantification of Ephedrines in Urine by HPLC

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Introduction

Ephedrines and their congeners pseudoephedrine, norephedrine, norpseudoephedrine and methylephedrine are ingredients of various medicines commonly used for colds, sinusitus, rhinitis, hay fever and apetite 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 IOC has set concentrations of these ephedrines in urine below which it will not be regarded as a positive sample. It is thus necessary to have an accurate and reliable method for the simultaneous quantification of these ephedrines in urine.

A fully validated HPLC method, with no derivatization or evaporation, for the simultaneous quantification of the ephedrines in urine is described.

Experimental

Extraction procedure:
To 1ml urine in a 5ml glass ampoule was added 100µl of 20% NaOH solution and 40µl of internal standard solution (ethylephedrine, 10mg/10ml methanol). The mixture was extracted with 4ml of distilled diethylether by vortexing for 30 seconds. After centrifugation the aqueous layer was freezed and the ether layer decanted to another 5ml ampoule containing 100µl 1% acetic acid. The mixture was vortexed for 30 sec., centrifuged and the aqueous layer freezed. The ether layer was discarded and the remaining ether evaporated under N_2 at room temperature. Mobile phase (100µl) was added to the acetic acid and 5µl injected onto the HPLC column.

Instrumentation:
A Series 1050 Hewlett-Packard pump and a autosampler were coupled to a 150mm x 4.6mm ID Phase Sep Spherisorb ODS 1 stainless-steel column. The compounds were detected with a Waters Model 481 UV-detector at 214nm. The results were processed on a Hewlett-Packard Model HP3396A integrator.

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Chromatography:

The mobile phase consisted of TEAP-buffer:methanol (98:2, v/v). The buffer was repared by adding a 20% solution of tetraethylammoniumhydroxide to 0.05M H_3PO_4 till a pH of 2.8 was reached. A constant flow of 1.0ml/min was maintained through the HPLC column at ambient temperature.

Results

A representative chromatogram obtained from urine extracts is shown in figure 1. The peaks of interest were well separated from any interferences while a blank urine extract showed no interfering endogenous compounds.

Validation of the method was done by preparing standards by spiking urine with known concentrations of the ephedrines in the range 0.5 to 100µg/ml. Eight validation quality control standards were prepared in fivefold in a similar fashion as the standards in the same concentration range. These standards and controls were analyzed as described.

Quantification was achieved using peak height ratios of the individual ephedrines to internal standard. The calibration curves were shown to be linear over a wide range to at least 100µg/ml, with the curves almost passing through the origin (fig I).

The recovery of each ephedrine at three different concentrations is given in table I.

The performance of the assay procedure with regard to accuracy and precision is presented in table II (for ephedrine) and table III (for norephedrine).

Conclusion

It can be concluded that the method has a fast and repeatable extraction procedure with no evaporation or derivatization. Simultaneous separation of the ephedrines is obtained which is valuable for a mixture of the ephedrines. The method has a good accuracy and precision which makes it suitable for quantification.

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Table I:

Recovery (%) of the ephedrines from urine

	40µg/ml	10µg/ml	2µg/ml
Ephedrine	100	95	97
Pseudoephedrine	95	95	96
Methylephedrine	81	88	87
Norephedrine	80	81	95
Norpseudoephedrine	86	90	88

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Table II

Within-day accuracy and presicion of the assay for ephedrine.

Spiked concentration µg/ml	Within-day (n = 5) spiked/found mean (%)	CV (%)
93	98	2,0
48	98	2,0
30	95	2,0
19	95	1,1
10	95	1,5
5	99	1,0
2	95	0,2
1	100	1,1

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Table III

Within-day accuracy and presicion of the assay for norephedrine

Spiked concentration (µg/ml)	Within-day (n = 5) spiked/found mean (%)	CV (%)
93	100	2,1
47	99	2,3
30	96	2,0
15	95	1,6
10	97	2,1
5	100	1,8
2	95	1,7
1	95	2,3

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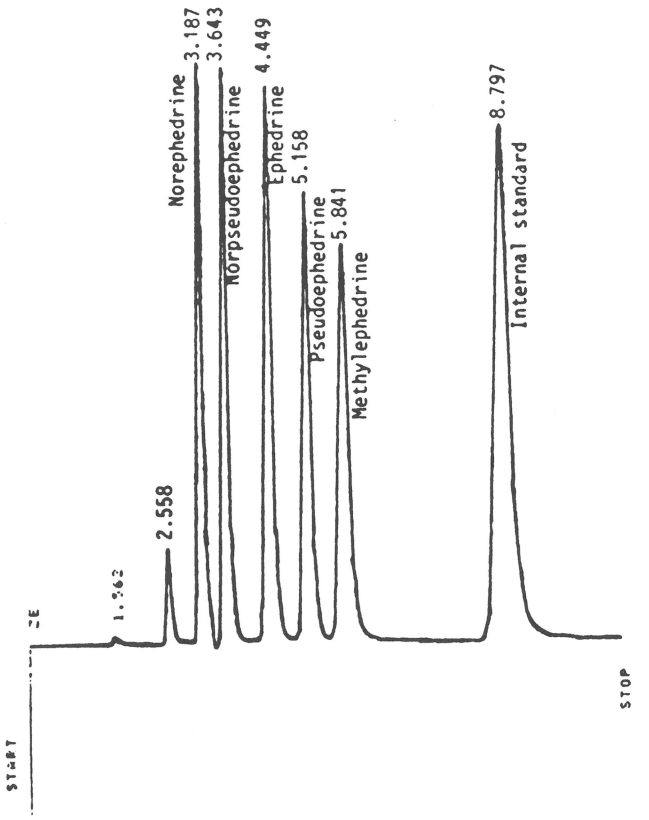

FIGURE 1: Representative chromatogram from urine extracts.

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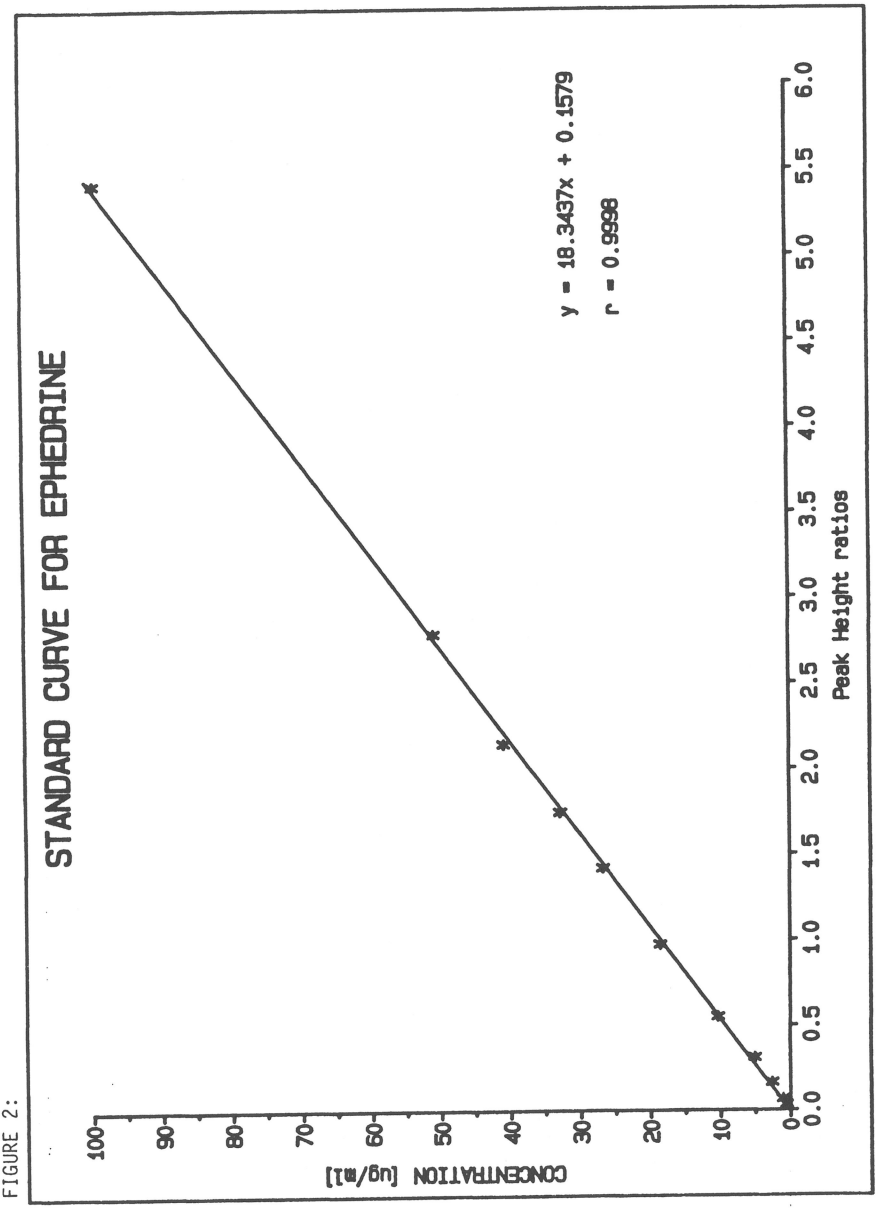

FIGURE 2:

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