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Automated analysis of drugs in urine

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Abstract

A totally automated procedure has been developed for the preparation and analysis of 34 basic and neutral drugs in urine samples using an integrated HP 7686 PrepStation–HP 6890 gas chromatographic system. The automated preparation of the sample consisted of a liquid–liquid extraction of 250 μl urine at alkaline pH with 100 μl of methyl *tert.*-butyl ether. After phase separation the organic solvent was automatically placed in the injector of the gas chromatograph and analysed. High recoveries of extraction were obtained. The limits of detection of most of the drugs were less than 0.5 μg/ml. The method, which allows the preparation and analysis of the samples to be completely sychronised, showed good accuracy and precision.

Keywords: Amphetamine; Codeine; Ephedrine; Ethylamphetamine; Fencanfamine; Methylephedrine; Pethidine; Phenmetrazine

1. Introduction

Gas chromatography (GC) is a technique frequently used for screening of drugs in biological samples [1]. Using a nitrogen–phosphorus–flame ionization detector (NP–FID), GC is adopted in doping control procedures for screening of nitrogen-containing drugs which are excreted unconjugated in urine [2,3]. Sample preparation is usually based on a liquid–liquid extraction with an organic solvent at alkaline pH [4].

The improvements made in the area of robotics have offered great possibilities for the automation of analytical procedures. In most cases, laboratory robots have been designed to prepare samples automatically. When connected to the necessary analytical equipment, they provide a fully automated analytical process. As a result, the intervention of the analyst is reduced, the process itself is speeded up, reproducibility is improved and sample throughput is increased [5,6]. All these factors are important when carrying out routine screening in doping control.

Although various analytical methods based on automated sample preparation are available in the literature [7,8], none of them deals with the liquid–liquid extraction of multiple drugs from urine samples.

The aim of this study was to develop a totally automated method for the preparation of urine samples and the following analysis by GC–NP–FID. Urine samples from sports competitors were used. They were prepared using a PrepStation (Hewlett-Packard) able to carry out all the necessary pro-

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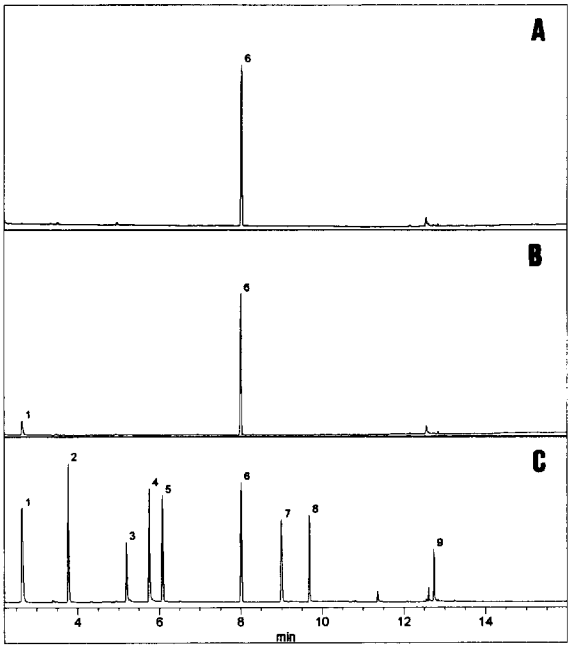

Fig. 1. Chromatograms obtained after the extraction and analysis with an integrated HP 7686 PrepStation–HP 6890 gas chromatographic system of (A) extracted blank urine containing 5 μg/ml I.S. (peak 6), (B) urine sample of an athlete who has taken amphetamine (peak 1, where the estimated concentration level was 0.6 μg/ml) and (C) standard spiked urine whith (1) amphetamine, (2) ethylamphetamine, (3) ephedrine, (4) methylephedrine, (5) phenmetrazine, (6) diphenylamine (I.S.), (7) fencanfamine, (8) pethidine, (9) codeine, each at a concentration of 5 μg/ml.

cedures for the extraction and to place the extract in the gas chromatographic injector for its subsequent analysis.

2. Experimental

2.1. Reagents

Amphetamine, benzphetamine, caffeine, cathine, cocaine, codeine, ephedrine, ethylmorphine, fenproporex, fentanyl, hydrocodone, levorphanol, methadone, methamphetamine, methylephedrine, methylphenidate, nikethamide, pethidine, phendimetrazine, phentermine, phenylpropanolamine, prolintane, pseudoephedrine and strycnine were obtained from Sigma (St. Louis, MO, USA). The other drugs were kindly provided by Dr. A. Vega, Laboratorio de Criminalistica de la Policia (Madrid, Spain) Diphenylamine (internal standard), methyl *tert.*-butyl ether, NaCl and NaOH were purchased from Merck (Darmstadt, Germany). All other reagents and solvents were of analytical reagent grade.

2.2. Standard solutions

Stock solutions of each drug and the I.S. were prepared in methanol at a concentration of 1000 μg/ml. They were diluted further to yield appropriate working solutions for the preparation of the calibration standards. The solutions were sealed and refrigerated at 4°C until use.

2.3. Instrument

The study was carried out using an integrated HP 7686 PrepStation/HP 6890 Gas Chromatograph with an NP-FID detector system (Hewlett-Packard, Palo Alto, CA, USA), which was controlled by a Chemstation HP 3365 series II. Bench Supervisor software was used to synchronize the gas chromatography activity and the PrepStation in order to create a continuous automated chain. The PrepStation system was equipped with a tray with capacity for 100 vials, with a communal arm with the GC, and with a bar code reader/mixer HP G1296A. The workstation was fitted with a 2.5-ml capacity syringe, capable of carrying out the whole process of extraction and pumping solvents out from eight bottles, with a block to heat the vials, and with an outlet connected to a nitrogen source in order to perform the evaporation.

The gas chromatograph was equipped with an HP fused-silica capillary column (12 m×0.2 mm I.D., cross-linked 5% phenylmethyl silicone, film thickness 0.3 μm). The carrier gas was helium at 0.8 ml/min at 90°C, splitting ratio 1:10. The column temperature was programmed from 90°C at 10°C/min to 180°C and at 30°C/min to 300°C (held for 5 min). The injector temperature was 250°C and the detector temperature 300°C.

Table 1
Recovery and limit of detection for the studied drugs in spiked urine samples ($n=5$)

Drug	t_R (min)	Recovery (%)		Approximate detection limit (μg/ml)
		Mean±S.D.	R.S.D (%)	
Amphetamine	2.58	63.0±2.3	3.7	0.3
Benzphetamine	10.36	66.8±2.8	4.2	0.6
Caffeine	10.00	27.4±1.6	5.8	0.6
Cathine	4.60	36.3±2.1	5.8	0.7
Chlorphentermine	5.04	77.6±0.6	0.8	0.4
Clorbenzorex	12.72	83.2±3.6	4.3	0.5
Clorprenaline	7.77	80.2±3.6	4.5	0.5
Cocaine	12.12	83.1±1.9	2.3	0.3
Codeine	12.68	84.5±4.3	5.1	0.6
Dihydrocodeine	12.73	87.9±1.6	1.8	0.6
Ephedrine	5.18	49.6±2.2	4.4	0.4
Ethylamphetamine	3.73	102.3±1.6	1.6	0.2
Ethylmorphine	12.86	73.4±4.2	5.7	0.3
Fencanfamine	8.94	83.8±3.4	4.1	0.3
Fenproporex	7.72	77.7±2.5	3.2	0.1
Fentanyl	14.06	98.6±4.1	4.2	0.1
Heptaminol	2.47	29.3±1.2	4.1	0.8
Hydrocodone	13.04	71.4±1.7	2.4	0.6
Levorphanol	12.26	63.9±3.3	5.2	0.4
Mefenorex	5.35	89.4±1.3	1.5	0.6
Methadone	11.96	74.4±2.9	3.9	0.5
Methamphetamine	3.18	85.2±0.9	1.1	0.4
Methoxyphenamine	5.33	78.3±1.9	2.4	0.3
Methylephedrine	5.71	71.3±1.5	2.1	0.3
Methylphenidate	9.47	62.7±1.5	2.4	0.3
Nikethamide	7.01	65.1±1.9	2.9	0.3
Pentylenetetrazole	6.78	51.3±2.1	4.1	0.4
Pethidine	9.66	70.2±2.7	3.8	0.3
Phendimetrazine	6.37	98.1±3.2	3.3	0.3
Phenmetrazine	6.00	88.5±1.7	1.9	0.3
Phentermine	2.91	73.4±2.4	3.3	0.3
Phenylpropanolamine	4.60	37.4±1.8	4.8	0.7
Pseudoephedrine	5.20	50.1±1.8	3.6	0.4
Strychnine	16.63	59.8±2.3	3.8	0.3

2.4. Sample preparation

The development of this sample-preparation method was carried out in line with the current practice in doping control laboratories. Preliminary studies served to select the most suitable solvents, optimum volumes of urine and reagents and the mixing times. Preparation of the sample was as follows: a 250-μl urine sample was added to a vial containing 100 mg NaCl which was then placed in the tray of the integrated HP 7686 Prepstation/HP 6089 GC system. Then, without any further manual intervention, 10 μl of a 1000 μg/ml diphenylamine (I.S.) solution, 20 μl of 1M NaOH solution, and 100 μl of methyl *tert.*-butyl ether (extraction solvent) were successively added. The contents of the vial were mixed for 5 min with intermittent pauses of 15 s every 45 s. Then, after waiting for 30 s for phase separation, the organic phase was aspirated from the vial and transferred to an empty 100-μl capacity vial previously sealed. The vial was then transferred to the GC injector where 2 μl of the sample were injected.

The whole sample-preparation time was 18 min,

Table 2
Between-day precision and accuracy for some selected drugs in spiked urine samples ($n=5$)

Drug	Concentration added (μg/ml)	Concentration found	
		Mean±S.D. (μg/ml)	R.S.D. (%)
Amphetamine	2.00	1.95±0.54	2.8
	7.00	6.50±1.50	2.0
	22.00	21.36±3.73	1.8
Codeine	2.00	1.87±0.65	3.5
	7.00	7.29±2.20	3.0
	22.00	22.80±6.25	2.7
Ephedrine	2.00	2.15±0.62	2.9
	7.00	6.52±3.40	5.2
	22.00	23.25±5.91	2.5
Ethylamphetamine	2.00	1.90±0.59	3.1
	7.00	6.80±1.80	2.7
	22.00	21.00±7.80	3.7
Fencanfamine	2.00	2.12±0.34	1.6
	7.00	6.53±1.48	2.3
	22.00	21.40±5.56	2.6
Methylephedrine	2.00	2.07±0.63	3.0
	7.00	7.10±1.67	2.4
	22.00	21.40±5.56	2.6
Pethidine	2.00	1.86±0.77	4.1
	7.00	7.50±2.06	2.8
	22.00	23.00±5.60	2.4
Phenmetrazine	2.00	1.89±0.50	2.7
	7.00	7.50±1.72	2.3
	22.00	22.34±4.56	2.0

the same as that for the GC analysis of the extract. In this way, when a batch of samples is prepared, it is possible to eliminate wasted time between preparation and analysis of the sample.

3. Results and discussion

Fig. 1 shows chromatograms produced by positive and negative samples for amphetamine which were prepared and analysed by the described method. It also shows a chromatogram of a urine sample spiked with 8 drugs each at 5 μg/ml. These drugs were chosen to evaluate the precision and accuracy of the method in the whole range of the chromatogram.

Table 1 shows the retention times, the recovery data and the limits of detection of the 34 drugs considered in our study. The recoveries were obtained by analysing in duplicate 5 aliquots of urine spiked with each compound at 5 μg/ml, and by comparing peak areas with those produced by the analysis of a known amount of the pure standard of each compound. The recovery data of caffeine, heptaminol, cathine and phenylpropanolamine show low yields, while those of ethylamphetamine and fentanyl are the highest. The limit of detection (LOD) of each drug was calculated at a signal-to-noise ratio of 3. For most drugs analysed the LOD was less than 0.5 μg/ml, while the highest LODs (0.7–0.8 μg/ml) were calculated for the drugs that showed poor recovery with our method, namely heptaminol, phenylpropanolamine and cathine.

Table 2 shows the between-day precision and accuracy of the method. Calibration was carried out using urine spiked with each drug at a concentration of 1, 5, 15 and 30 μg/ml and with 5 μg/ml of internal standard. Correlation coefficients varied between 0.9980 and 0.9999. Precision was calculated by analysing, in duplicate, 5 aliquots of spiked urine with final concentrations of 2,7 and 22 μg/ml on 5 different days during 2 weeks. The results of the between-day runs show an acceptable precision with R.S.D. values between 1.6 and 4.1% for the lowest concentrations and 1.8 to 3.7% for the highest concentrations. Accuracy, calculated as the percentage error of the difference between the expected and the observed value, was found to be between 1.4 and 7.5% for the lowest concentration of the drugs.

4. Conclusion

The automated GC–NP–FID analysis for the determination of all the compounds studied has proven to be accurate, precise, sensitive and low-cost as it uses a minimum amount of sample and of reagents to perform the microextraction. The system is capable of continuous preparation of the sample and immediate GC analysis, thus avoiding sample evaporation in the vial. The instrumentation was found to be suitable for the development of the investigation and the software program was user-friendly. We are currently using this method, with excellent results, for screening basic and neutral drugs excreted unconjugated in urine samples orig-

inating from sport competitions. The method developed is an alternative to manual techniques and reduces the need for intervention by the analyst.

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