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Note

Qualitative gas chromatographic and gas chromatographic–mass spectrometric screening for β-blockers in urine after solid-phase extraction using Extrelut-1 columns

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Recently, β-blockers have been added to the list of forbidden substances as issued by the International Olympic Committee (I.O.C.) Since 1987, these substances also figure on the list of doping agents prohibited by the Flemisch Community Executive. Indeed, these drugs can be abused by some athletes in order to reduce sympathetic activity in these cases were high blood pressure and stress could result in bad performances [1].

Screening studies for β-blockers are rather scarce, except for the work by Maurer and Pfleger [2] and the study of Martineau et al. [3]. In the latter work [3] β-blockers were screened by gas chromatography–mass spectrometry (GC–MS) after derivatization with trifluoroacetic acid anhydride (TFAA). Monitoring a specific ion allowed the detection of ca. 80% of all β-blockers. Although excellent results were obtained, the methodology is rather lengthy (ca. 30 min for one urine sample) owing to a preliminary purification step and several centrifugations.

Solid-phase extraction is gaining wider acceptance in analytical work [4] and Extrelut in particular has already been used in doping analysis [5–7]. A method describing this rapid technique for the screening of some β-blockers by GC–MS and gas chromatography (GC) is reported here.

EXPERIMENTAL

Reagents and chemicals

Diethyl ether, *n*-propanol and ethyl acetate p.a. were purchased from Merck (Darmstadt, F.R.G.). Extrelut-1 columns were either obtained from Merck or prepared in our laboratory with equivalent amounts of Extrelut [7].

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TABLE I

DETECTION TIME PERIODS AND DETECTION LIMITS OF SEVERAL β-BLOCKERS AND THEIR MAJOR METABOLITES BY GC AND GC–MS WITH EXTRELUT-1 SOLID-PHASE EXTRACTION

Sample size: 1 ml of urine.

β-Blocker and major metabolite(s)	Registered name and dose (mg)	Detection time* (h)		Detection limit (ng/ml)	
		GC	GC–MS	GC	GC–MS**
Propranolol	Inderal®, 20	6	24	500	10
(4-hydroxypropranolol)		N.D.***	9		
Atenolol	Tenormin®, 50	24	48	500	10
Oxprenolol	Trasicor®, 40	6	48	500	20
(4-hydroxyoxprenolol)		6	48		
(5-hydroxyoxprenolol)		6	48		
Pindolol	Visken®, 5	9	24	1000	20
Metoprolol	Seloken®, 50	6	48	500	20
(α-hydroxymetoprolol)		6	48		
Alprenolol	Aptine®, 25	12	24	500	10
(4-hydroxyalprenolol)		12	48		

*Time period after which the drug is still detectable.
**Monitoring *m/z* 308 and 266.
***N.D. = not detected.

Helix pomatia juice (SHP) containing β-glucuronidase (100 000 Fishman units/ml) and sulphatase (1 000 000 Roy units/ml) was from IBF (Villeneuve, France).

The following β-blockers and some of their metabolites were supplied by their respective manufacturers: alprenolol, metoprolol and oxprenolol, Ciba-Geigy (Basle, Switzerland); atenolol, propranolol and 4-hydroxypropranolol, ICI (Destelbergen, Belgium); 4-hydroxyalprenolol, the major metabolites of metoprolol and oxprenolol, Hassle (Mölndal, Sweden); and pindolol, Sandoz (Basle, Switzerland).

Gas chromatography

A Varian (Walnut Creek, CA, U.S.A.) 3400 gas chromatograph equipped with a nitrogen-specific detector and interfaced to an IBDH data processor was used. The column was a 25 m × 0.22 mm I.D. fused-silica column coated with CP Sil 5-CB (Chrompack, Antwerpen, Belgium) at a film thickness of 0.11 μm. The oven temperature was programmed as follows: initial temperature 140°C, initial hold 1 min, temperature programming rate 10°C/min, final temperature 280°C, final hold 5 min. Injector and detector temperatures were 250 and 300°C, respectively. Helium was used as carrier gas at an inlet pressure of 0.11 MPa. Detector make-up flow-rate was 30 ml/min. Injections were made in the split mode (split ratio 1:10).

Gas chromatography–mass spectrometry

Mass spectra were recorded on a Hewlett Packard (Avondale, PA, U.S.A.) 5993 mass spectrometer. The GC–MS system was fitted with the same fused-silica column. Samples were injected with an all-glass moving needle (Chrompack). The column was directly coupled to the MS source. Injector, interface, source and analyser temperatures were 240, 260, 250 and 250°C, respectively. Helium was used as carrier gas. Ion monitoring data were acquired at an ionization potential of 70 eV. The electron multiplier voltage was set at the autotune value.

Analytical procedure

Urine (2 ml) was buffered with 0.5 ml of 1 *M* sodium acetate buffer and hydrolysed overnight at 37°C after the addition of 50 μl of SHP. After cooling, the hydrolysate was made alkaline with 1 g of potassium carbonate–sodium hydrogencarbonate (1:1). After vortexing (5 s) and briefly centrifuging (1 min, 700 *g*) 1 ml of the supernate was applied to the Extrelut-1 column. After 5 min the column was eluted with 3 ml of diethyl ether–*n*-propanol (9:1), and the eluate was evaporated under nitrogen at 70°C. Afterwards, the residue was dissolved in 50 μl of ethyl acetate and derivatized with 50 μl of TFAA for 20 min at 70°C. After evaporation to dryness under nitrogen, the residue was redissolved in 200 μl of ethyl acetate, and a 1-μl sample was injected.

Detection time experiments

Healthy persons volunteered for the excretion studies. Tablets containing subtherapeutic amounts of the β-blocker were given orally (Table I). Urine was collected at 0 h and 1, 2, 3, 4, 6, 9, 12 and 48 h after administration.

Detection limit

Methanolic solutions (10 and 1 μg/ml) were used to add different amounts of each β-blocker to screw-capped tubes. After evaporation of the solvent, blank urine, buffer and SHP were added. The tubes were kept at 37°C overnight and analysed as described above. However, only the ions *m/z* 308 and 266 were monitored during the GC–MS analysis.

RESULTS AND DISCUSSION

As several β-blockers, and their hydroxylated metabolites in particular, are partially excreted as conjugates, hydrolysis of the urine is required in order to improve the recovery and the "detection time" (period after administration of a drug during which its presence can be confirmed). In the screening method of Martineau et al. [3], β-blockers are released from their conjugates by acid hydrolysis at 100°C for 30 min, while in the work of Maurer and Pfleger [2] acidified urine is refluxed for 15 min. However, atenolol and pindolol are completely decomposed by acid hydrolysis [2]. Consequently, such a hydrolysis step could result in a decreased detection for these drugs using the method of Martineau et al. [3]. In order to identify atenolol and pindolol, Maurer and Pfleger [2] pro-

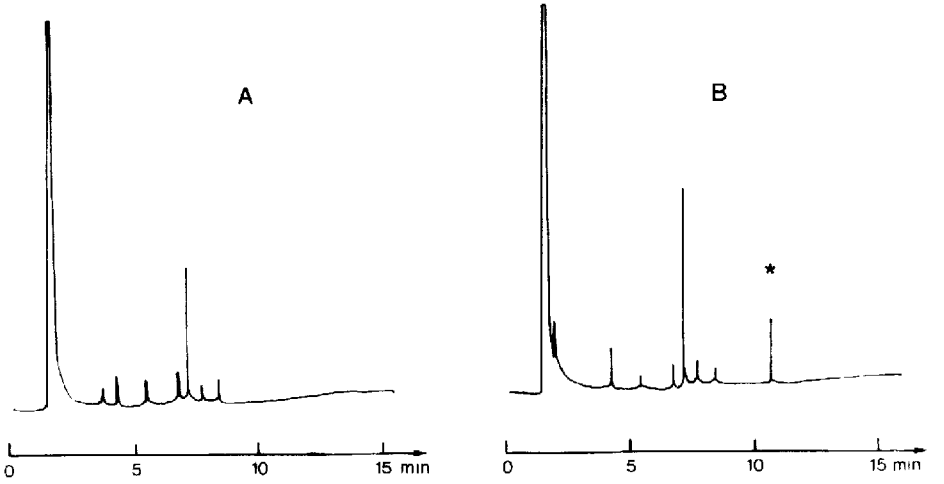

Fig. 1. Gas chromatograms of blank urine (A) and urine 3 h (B) after the intake of propranolol (asterisk).

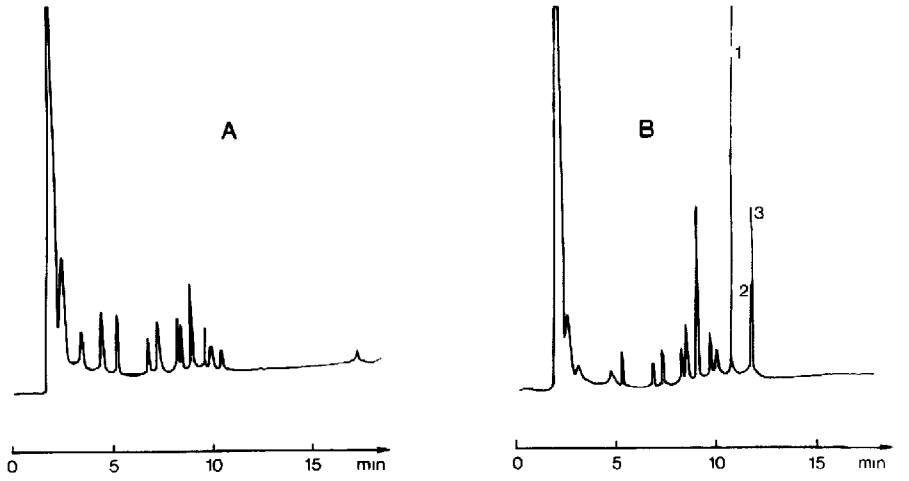

Fig. 2. Gas chromatograms of blank urine (A) and urine 4 h (B) after the administration of oxprenolol. Peaks: 1 = oxprenolol-di TFA; 2 = 5-hydroxyoxprenolol-tri TFA; 3 = 4-hydroxyoxprenolol-tri TFA.

posed a supplementary direct extraction of the urine. It should be clear, however, that supplementary extractions for one or two drugs are time-consuming and should be avoided in doping screening analysis. Although the cleavage of conjugates by acid hydrolysis can be completed more quickly than by enzymatic hydrolysis, we prefer to hydrolyse them with SHP at 37°C overnight. However, in circumstances where a rapid result is needed, the enzymatic reaction could be carried out at 50°C within 2–3 h.

Using Extrelut-1 columns the total extraction step is reduced to 5 min, and the consumption of organic solvent is decreased to 3 ml. Although the urine volume used (1 ml on column) is reduced by a factor of 5–10 compared with the other methods [2,3], the results in Table I indicate that the β-blockers and their me-

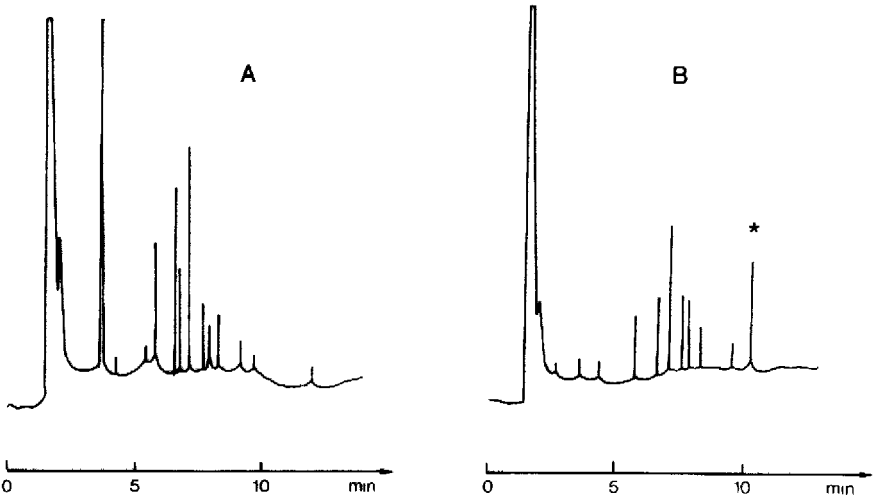

Fig. 3. Gas chromatogram of blank urine (A) and urine 24 h (B) after the intake of atenolol (asterisk).

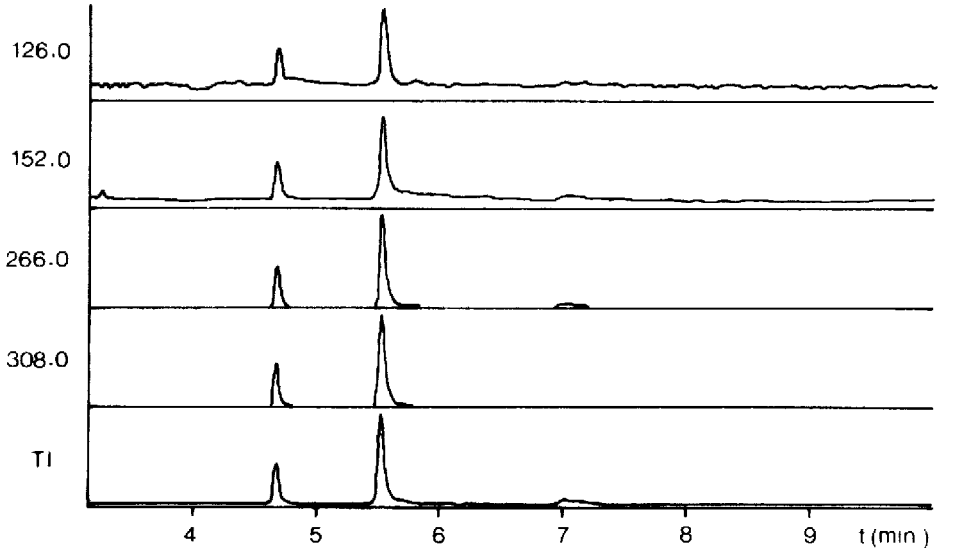

Fig. 4. Selected-ion monitoring of a urine extract 12 h after the administration of alprenolol.

tabolites were detectable by GC for at least 6 h after the oral administration of subtherapeutic amounts. Although pharmacokinetic parameters such as urinary half-life and metabolism certainly influence the period during which a drug can be detected in biological fluids, the relatively long detection periods could be also attributed to the clean extracts and chromatograms obtained after Extrelut-1 extraction, as shown in Figs. 1–3. The detection limits for these β-blockers are also included in Table I.

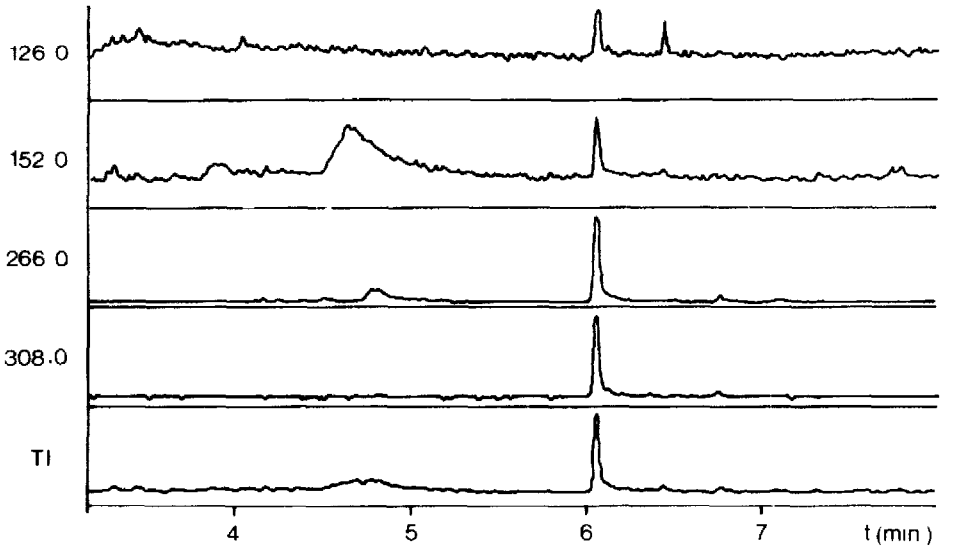

Fig. 5. Selected-ion monitoring of a urine extract 12 h after the administration of pindolol.

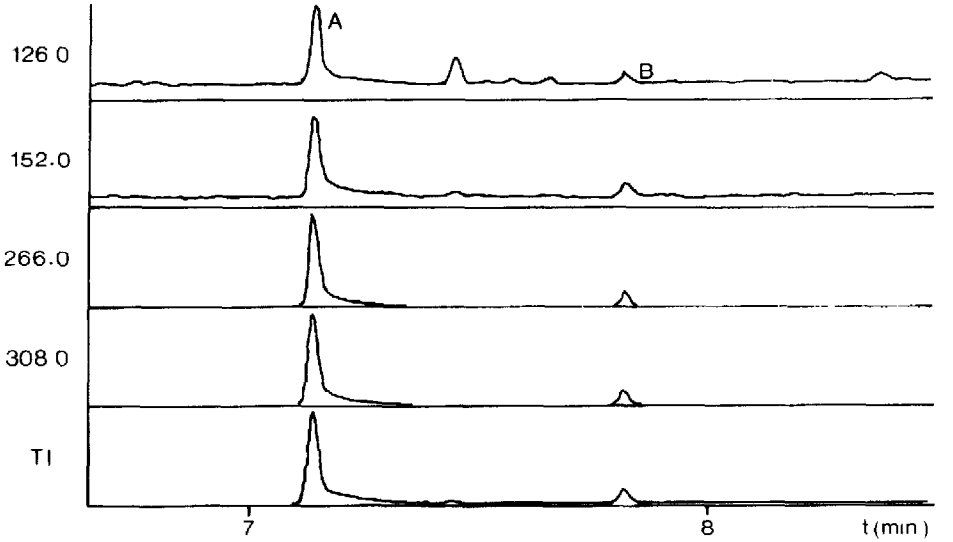

Fig. 6. Selected-ion monitoring of a urine extract 9 h after the administration of propranolol (A = propranolol-di TFA; B = 4-hydroxypropranolol-tri TFA).

Although the amount of 4-hydroxypropranolol (free and conjugated) in urine should account for ca. 18% of the administered dose [8], this metabolite could not be detected by GC and Extrelut-1 extraction. Addition of ascorbic acid during hydrolysis [9,10] could improve the detection of this substance.

Although only ca. 50% of atenolol is available 24 h after oral administration [11,12] its GC detection time is longer than that of propranolol. This could be attributed to the higher administered dose and the fact that hydrophilic β-blockers tend to be less metabolized.

Parent drugs and major metabolites are both detectable in urine samples for times up to 6 h for oxprenolol and metoprolol and 12 h for alprenolol. In all the alprenolol samples examined, the peak area corresponding to the 4-hydroxy derivative was greater than that of the parent drug. This agrees with earlier findings [13], where alprenolol and 4-hydroxyalprenolol accounted for 18 and 42%, respectively, of the radioactivity in hydrolysed urine after oral administration of [^{3}H]alprenolol.

Trifluoroacetic acid derivatives of β-blockers with a 2-hydroxy-3-isopropylaminopropoxy side-chain give rise in their mass spectra to ions at m/z 308, 266, 152 and 126, the base peak being either m/z 308 or 266 [14]. These ions are also common to the main metabolites. The time periods during which the drugs were still detectable when these four characteristic ions were monitored are given in Table I. They are obviously longer than for GC with nitrogen-selective detection, owing to the greater sensitivity of mass fragmentography. All β-blockers indicated in Table I could be identified for periods of at least one day (Figs. 4 and 5), and even 4-hydroxypropranolol could be detected for up to 9 h (Fig. 6) post administration. It should be clear that these periods could be further extended by monitoring only the ions m/z 266 and 308. Concerning interference by other drugs, it should be clear that the monitoring of these very specific ions (m/z 266, 308) with their relative intensities and the resolving power of capillary GC using a moderate temperature programme virtually exclude such interferences.

In conclusion, the results reported here indicate that several β-blockers can easily be screened with low solvent consumption using Extrelut-1 columns. This rapid extraction technique and GC or GC–MS detection enables the detection of β-blockers and their main metabolites in 1 ml of urine during time periods sufficiently lengthy for doping analysis.

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REFERENCES

1 T. Videman, T. Sonck and J. Janne, Med. Sci. Sports, 11 (1979) 266.
2 H. Maurer and K. Pfleger, J. Chromatogr., 382 (1986) 147.
3 A. Martineau, R. Masse and R. Dugal, Cologne Workshop on Doping Analysis, April 6–11, 1986.
4 R.D. McDowall, J.C. Pearce and G.S. Murkitt, J. Pharm. Biomed Anal., 4 (1986) 3.
5 F.T. Delbeke and M. Debackere, J. Chromatogr., 161 (1978) 360.
6 T. Matsumoto, K. Kamei, M. Murata, A. Takeda, E. Suzuki, F. Takeda and A. Momose, in G.H. Johnston (Editor), Proceedings of the Vth International Conference on Control of Use of Drugs in Race Horses, Toronto, June 12–16, 1983, Canadian Section of the Association of Official Racing Chemists, Toronto, 1985, p. 152.
7 F.T. Delbeke and M. Debackere, J. Chromatogr., 278 (1983) 418.
8 B.M. Silber, N.H.G. Holford and S. Riegelman, J. Pharm. Sci., 72 (1983) 725.
9 M.W. Lo, B. Silber and S. Riegelman, J. Chromatogr. Sci., 20 (1982) 126.
10 P.M. Harrison, A.M. Tonkin, C.M. Cahill and A.J. McLean, J. Chromatogr., 343 (1985) 349.

11 W.D. Mason, N. Winer, G. Kochak, I. Cohen and R. Vell, Clin. Pharmacol. Ther., 25 (1979) 408.
12 W. Kirch, H. Koehler, E. Mutschler and M. Schaefer, Eur. J. Clin. Pharmacol., 19 (1981) 65.
13 N.O. Bodin, Life Sci., 14 (1974) 685.
14 D A. Garteiz and T. Walle, J. Pharm. Sci., 61 (1972) 1728.