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SIMULTANEOUS DETERMINATION OF METOPROLOL AND METABOLITES IN URINE BY CAPILLARY COLUMN GAS CHROMATOGRAPHY AS OXAZOLIDINEONE AND TRIMETHYLSILYL DERIVATIVES

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SUMMARY

A method for the determination of metoprolol and its main metabolites in urine is presented. The method comprises derivatization of the aminopropanol side-chain with phosgene at alkaline pH and isolation in an organic phase at acidic pH. After trimethylsilylation, separation and quantification are performed by capillary column gas chromatography with flame ionization detection.

The reaction is performed at pH 12 with 60 μl of 2 *M* phosgene in toluene added in three portions. Diethyl ether—dichloromethane is used as extraction medium and bis(trimethylsilyl)acetamide as silylating agent.

With spiked samples linear standard curves were obtained for metoprolol and three of its main metabolites with a detection limit varying between 4 and 20 μmol/l of urine. The method was applied to urine samples from a normal individual who had taken 292 μmol of metoprolol as tartrate.

INTRODUCTION

Metoprolol is mainly metabolized by oxidative deamination and O-dealkylation with subsequent oxidation and aliphatic hydroxylation [1]. These metabolites account for 85% of the dose in man [1]. A survey of the metabolism is given in Fig. 1.

The metabolic hydroxylation of drugs by individuals can in several instances be divided into poor and extensive hydroxylators [2]. The first and most well known example is debrisoquine [3]. This drug is commonly used to classify individuals into rapid or poor hydroxylators, the property being genetically controlled. Papers have appeared that indicate that this might also

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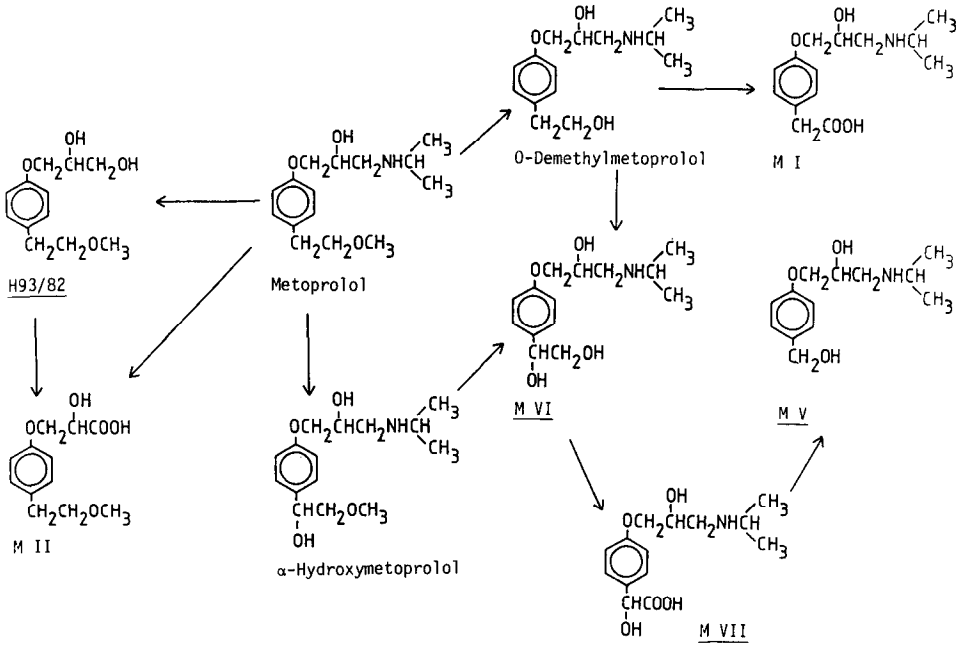


Fig. 1. Identified metabolites of metoprolol. Underlined metabolites have only been detected in the rat.

be the case for metoprolol [4, 5], but contradictory data have been published recently [6]. Benzylic hydroxylation only accounts for about 10% of the total metoprolol dose recovered in urine in man [1]. It has therefore been suggested that the formation of the acid metabolite M I (Fig. 1, H 117/04) by O-dealkylation and subsequent oxidation is under genetic control as well [4]. To enable a study of this hypothesis we have developed a method for the simultaneous determination of metoprolol and its main metabolites. Previous methods for their determination have been based on work with radioactively labelled metoprolol [1]. A mass fragmentographic method is only capable of measuring the basic compounds [7]. This is also true for some liquid chromatographic methods [8—10]. Different methods have been used in the same study [11, 12] for the quantification of basic and acidic metabolites. A simultaneous method would therefore be attractive.

As the main metabolite of interest (M I) is an amino acid (Fig. 1), liquid chromatography would be a possible separation system. However, even if the expected urinary concentrations of some metabolites might be in the higher μmol/l range, direct injection of the sample onto a reversed-phase column with ultraviolet (UV) detection lacks selectivity. To isolate the amino acid by extraction requires blocking the amino group or the carboxylic group.

Carbonic dichloride (phosgene) was recently shown to be a convenient reagent for the cyclization of metoprolol to an oxazolidineone derivative suitable for gas chromatography with nitrogen-selective detection [13]. A two-phase system was used to minimize plasma interference in the derivatization step.

The strategy in the present work was to block the functional groups in the

aminopropanol side-chain by oxazolidineone formation and to isolate the neutral or acidic derivatives by extraction into an organic phase at low pH prior to silylation.

EXPERIMENTAL

Apparatus

Gas chromatography. A Varian 3700 gas chromatograph equipped with flame ionization and thermionic detectors was used. The glass column (120 × 0.2 cm I.D.) was filled with 3% Hi-EFF-8BP (cyclohexanedimethanol succinate). The carrier gas was nitrogen at a flow-rate of 45 ml/min. The injector, column and the detector temperatures were maintained at 300°C, 240°C and 300°C, respectively.

The instrument was also adapted for capillary columns [14]. The capillary columns were of borosilicate glass (25 m × 0.37 mm I.D.) and fused silica (25 m × 0.31 mm I.D.). Both were siloxane-deactivated and coated with SE-54. Helium was used as carrier gas with an inlet pressure of 50 kPa giving a linear velocity of 30 cm/sec. The split flow-rate was approximately 50 ml/min, with make up gas at 30 ml/min. The temperature of the oven was 180°C for 1 min after injection and then increased to 240°C at 10°C/min. The injector and the detector temperatures were 280°C and 300°C, respectively.

The gas chromatograph was also equipped with an automatic injection unit, a Varian 8000 autosampler. The air inlet pressure was kept at 45 kPa and μ-vials were used to allow two injections of each sample. The system was occasionally flushed with ethyl acetate at higher pressure to prevent clogging of the waste exit by solid silylation reaction products.

The peak areas were evaluated by a 3390A Hewlett-Packard integrator.

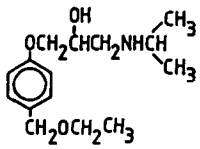
Liquid chromatography. The system consisted of an Altex 110 A pump, a Rheodyne injection valve fitted with a sample loop (100 μl) and a Cecil 212 variable-wavelength UV detector. The column (stainless steel 150 × 4.5 mm I.D.) was filled with 5-μm LiChrosorb RP-8 (E. Merck, Darmstadt, F.R.G.). The mobile phase was 0.01 *M* tetrabutylammonium, 9% acetonitrile in phosphate buffer pH 2 (I = 0.1). The flow-rate was 1 ml/min and the UV absorption of the eluate was measured at 272 nm. Prior to injection, samples were flushed with nitrogen to remove any trace of toluene which would otherwise interfere with the UV detection.

Mass spectrometry. Mass spectra of the derivatives were recorded on a Finnigan MAT 44 S gas chromatograph—mass spectrometer upon electron impact at an ionization energy of 70 eV using a packed OV-17 column. The mass spectra were acquired by a Finnigan MAT SS 200 data system followed by normalization and background subtraction.

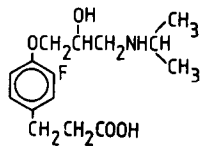
Reagents and chemicals

Metoprolol tartrate, internal standard 1 (IS 1, H 87/31), metabolite I (M I, H 117/04) and internal standard 2 (IS 2, H 177/56) as hydrochlorides, O-demethylmetoprolol (H 105/22), α-hydroxymetoprolol (H 119/66) and metabolite VI (M VI, H 119/72) as 4-hydroxybenzoates, metabolite V (M V, H 119/68) and metabolite VII (M VII, H 119/77) as neutral salts with sodium

acetate, and metabolite II (M II, H 104/83) and metoprolol oxazolidineone (H 151/38) were synthesized at the Department of Organic Chemistry, AB Hässle. The structures are given in Figs. 1 and 2. Tetrabutylammonium hydrogen sulphate was from the same source.



IS 1



IS 2

Fig. 2. Structures of internal standards.

Diethyl ether (Ph. Eur. grade) was from Standard Färg (Dörsjö, Sweden), dichloromethane, HPLC grade, from Rathburn (Walkerburn, U.K.) and acetonitrile from Reagenta (Uppsala, Sweden). Phosgene 2 *M* in toluene purum was from Fluka (Buchs, Switzerland), bis(trimethylsilyl)acetamide (BSA) from Macherey and Nagel (Düren, F.R.G.).

Buffers were prepared from sodium phosphates (Merck).

The oxazolidineone of M I was prepared by reacting 225 mg of the compound dissolved in 100 ml of water and 2 ml of buffer pH 12 with phosgene added in 100-μl portions with vigorous magnetic stirring. The pH of the solution was maintained by the aid of a pH meter and additions of 1 *M* sodium hydroxide. A total of 3 ml of phosgene in toluene was added. The reaction was followed by injecting 1-μl volumes into the liquid chromatographic system. The formed derivative was then isolated by extracting three times with 40 ml of diethyl ether at pH < 3. The collected ether fractions were dried with sodium sulphate and the solvent evaporated. White crystalline needles were obtained.

A similar approach was used for the synthesis of the oxazolidineones of α-hydroxymetoprolol and propranolol. Here the derivatives were extracted at neutral pH with dichloromethane, which was washed with dilute acid and then water prior to evaporation.

Stock solutions were prepared in 0.01 *M* hydrochloric acid: IS 1 550 μmol/l, M II 830 μmol/l, metoprolol and α-hydroxymetoprolol 750 μmol/l and M I 1.5 mmol/l.

The extraction solvent with marker was prepared by dissolving 8.8 μmol of propranolol oxazolidineone in 300 ml of diethyl ether and 200 ml of dichloromethane.

Buffer pH 12 was prepared and tested as follows: 0.5 *M* trisodium phosphate was adjusted with 5 *M* sodium hydroxide until the pH was 12 after mixing two parts of buffer with two parts of urine and one part of 0.01 *M* hydrochloric acid.

Determination of metoprolol and metabolites in urine

A 1-ml volume of urine sample was mixed with 1 ml of buffer pH 12, 0.5 ml of 0.01 *M* hydrochloric acid and 200 μl of internal standard solution IS 1 550 μmol/l. The mixture was agitated; a 20-μl portion of phosgene in toluene

was added and the agitation continued for at least 30 sec. The procedure was repeated twice. The solution was then made acid, pH < 3, by the addition of 0.5 ml of 1 *M* sulphuric acid and extracted with 5 ml of the extraction solvent (diethyl ether—dichloromethane, 3:2) containing the marker. After centrifugation, 4 ml of the organic upper phase were taken to dryness by a stream of nitrogen. The residue was dissolved in 30 μl of BSA. The reaction time was at least 30 min at room temperature.

Before injection, or loading the automatic sampling vials, 120—170 μl of dichloromethane were added. Two 1—2 μl injections of each sample were made in the split mode. By automatic injection each cycle took 25 min.

Standard samples were prepared by adding 0.5 ml of a solution of the compounds to be analysed to 1 ml of blank urine. Samples at four concentrations were analysed in duplicate. Standard curves were constructed by plotting the area ratios to the marker versus the concentration of the compound to be measured. In a typical experiment the standard concentrations of the standard samples were in the ranges 4—26 (M II), 5.5—37 (metoprolol), 18—71 (α-hydroxymetoprolol) and 56—300 (M I) μmol/l of urine.

RESULTS AND DISCUSSION

The cyclization reaction

The aminopropanol side-chain reacts with phosgene as outlined in Fig. 3 and the derivatives formed have no basic properties. The lactic acid M II does not react under the present conditions. The hydrochloric acid formed during the reaction causes a shift in the pH of the aqueous phase towards the acid side.

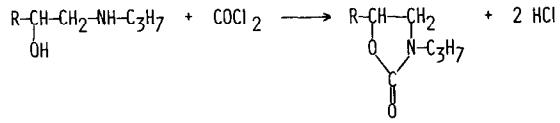


Fig. 3. Cyclization of the aminopropanol side-chain with phosgene.

Identification of formed oxazolidineone and trimethylsilyl derivatives

The structures of the formed derivatives were confirmed by mass spectrometry after gas chromatographic separation. The most important ions are listed in Table I. A complete spectrum of the metoprolol oxazolidineone has been published elsewhere [13]. In general, the oxazolidineones give weak molecular ions and have *m/z* 56 as base peak. This ion may be identical with the ion formed by fragmentation of boronate derivatives of alprenolol [15]. The trimethylsilyl derivatives have *m/z* 73 as base peak in three out of four instances. The molecular ions are weak except for IS 2 (22%). Consequently, the possibilities for mass fragmentographic quantitation using electron-impact ionization seem rather limited.

Optimum pH for the derivatization of M I

The derivatization conditions were studied by the aid of liquid chromatography. The aqueous phase could be injected directly and the formation of the derivative as well as the disappearance of the parent compound could be

TABLE I

SIGNIFICANT MASS SPECTRAL DATA OF METOPROLOL AND METABOLITES AS DERIVATIVES

Compound	Trimethylsilyl		Oxazolidineone	Relative intensity (%)					
	ether	ester		56	73 (TMS)	100	M − 45	M − 15	M⁺ (*m/z*)
M II	x	x			100		8	10	8 (384)
IS 1			x	100		38	18		15 (293)
Metoprolol			x	100		65	90		28 (293)
Propranolol			x	100		48		5	78 (285)
O-Demethyl-metoprolol	x		x	25	100	12	1	22	8 (351)
α-Hydroxy-metoprolol	x		x	20	75		100	3	— (381)
M I		x	x	18	100			5	4 (365)
IS 2		x	x	81	100	18	14	22	22 (397)

followed. To do this by gas chromatography would have required extraction and further derivatization.

In a previous paper it was shown that metoprolol in aqueous solution could be derivatized with 20 μl of 2 *M* phosgene in toluene [13]. However, with plasma present, the reaction was facilitated by using a two-phase system with a distinct organic phase present. It is probable that in both instances the reaction takes place in the organic phase. With M I being an amino acid with poor partition properties to organic solvents, the reaction might take place either in the aqueous phase, where water competes for phosgene, or in the minute organic toluene phase with poor solubility of the reactant.

Optimum pH of the reaction was found to be near pH 12 (Table II). As the reagent is consumed the pH falls. It is therefore necessary to use buffers with a high buffer capacity. This is also evident from the sample with 0.1 *M* sodium hydroxide (Table II).

TABLE II

INFLUENCE OF pH ON THE DERIVATIZATION OF M I WITH PHOSGENE AS MONITORED BY LIQUID CHROMATOGRAPHY

Method: 1.0 ml of buffer (ionic strength 1), 1.0 ml of M I (1 μmol in 0.01 *M* hydrochloric acid), 20 μl of 2 *M* phosgene in toluene, 20 μl analysed by liquid chromatography after evaporation of remaining toluene.

Initial pH of reaction solution	pH after phosgene reaction	Peak height (mm)	
		Derivative (n = 2)	Unreacted M I
7.0	n.m.**	6	275
8.1	7.3	12	293
9.4	7.4	29	273
11.0	10.4	77	n.m.
11.7	11.0	120	n.m.
12.1	11.4	104	n.m.
12.5	11.7	101	n.m.
12.6*	9.7	96	n.m.

*0.1 *M* sodium hydroxide.
**n.m. = not monitored.

Optimum amount of phosgene reagent for the reaction

The yields of derivative using varying amounts of 2 *M* phosgene in toluene are illustrated in Table III. It is apparent that at least one 20-μl portion is required and even more derivative can be formed by adding further reagent as the pH is still sufficiently high. Three successive additions of the reagent with agitation periods 30 sec long in between were selected (Table IV). The pH was readjusted to 12 after the third 20-μl portion and after the second 50-μl portion (Table IV).

TABLE III

OPTIMAL AMOUNT OF PHOSGENE REAGENT FOR THE DERIVATIZATION OF M I AS MONITORED BY LIQUID CHROMATOGRAPHY

Method: see Table II.

Added 2 *M* phosgene (μl)	Peak height (mm)	
	M I derivative (n = 2)	Unreacted M I (n = 2)
50	110	28
20	108	57
10	85	118
5	36	163
2	8	119
1	4	144
0.5	1	155
0	0	30
40 (2 × 20)	163	28

TABLE IV

EFFECT OF REPEATED ADDITIONS OF THE PHOSGENE REAGENT ON THE YIELD OF DERIVATIVE OF M I

Method: see Table II, 200 μg of H 151/38 (marker, metoprolol oxazolidineone [13]). The average of the two highest yields was arbitrarily set to 100%.

Addition number	Volume (μl)									
	20		50		100		200		500	
1	66	68	74	68	73	71	60	62	60	60
2	88	83	80	92						
3	91	101	75*	79*						
4	99*	87*								

*Denotes adjustment of the pH.

Isolation of metoprolol and metabolites from urine

Dichloromethane and diethyl ether alone were not capable of extracting all compounds quantitatively (> 95%) using a phase ratio of 4:1 at acidic pH (Table V). By combining ether and dichloromethane 3:2 and a 2:1 phase volume ratio, quantitative extraction was also obtained with M II being the

most polar metabolite according to the extraction data. Metoprolol as oxazolidineone has been shown to be extracted with ease by hexane—dichloromethane 4:1 and the same phase ratio [13].

TABLE V

ISOLATION OF METOPROLOL AND METABOLITES FROM THE AQUEOUS PHASE AFTER CYCLIZATION WITH PHOSGENE

Liquid chromatography of the aqueous phase, 0.5 *M* hydrochloric acid, before and after equilibration with the organic phase. Propranolol used as marker.

Organic phase	V_{org}/V_{aq}	Recovery in the organic phase (%)			
		M II	O-Demethyl-metoprolol	α-Hydroxy-metoprolol	M I
Dichloromethane	2:1	63	95	95	95
	4:1	76	>95	n.m.*	n.m.
Diethyl ether	2:1	89	78	63	79
	4:1	90	n.m.	n.m.	90
Dichloromethane—diethyl ether (3:2)	2:1	95	>95	>95	>95

*n.m. = not monitored.

Separation and quantification system

The selectivity and the sensitivity of the present liquid chromatographic system was not sufficient to allow quantification of metoprolol and metabolites after derivatization and extraction of urine samples. The packed column with 3% Hi-EFF-8BP was not capable of separating metoprolol from α-hydroxymetoprolol but was used briefly to check silylation conditions. Instead the possibility of using capillary column gas chromatography was investigated. Flame ionization detection was chosen in order to make quantification of the lactic acid, M II, derivative possible. This was not the case with the thermionic detector. Capillary columns 25 m long coated with SE-54 were used. The temperature of the column oven was programmed in order to elute the more volatile and the least volatile derivatives within a reasonable time as well as to retain peak height. The resolution of α-hydroxymetoprolol and M I was improved by using helium instead of nitrogen as carrier gas. A chromatogram with metoprolol and most of the metabolites is shown in Fig. 4.

Trimethylsilylation before gas chromatography

Carboxylic and remaining hydroxylic groups were protected by trimethylsilylation prior to the gas chromatographic separation step. BSA [bis(trimethylsilyl)acetamide] was preferred over BSTFA (corresponding trifluoroacetamide) since the latter reagent produced several interfering peaks in the region of interest. Hexamethyldisilazane—trimethylchlorosilane was less reactive than BSA with the carboxylic acids. At least 10 μl of the BSA reagent were required to derivatize the compounds of interest isolated from a high concentration urine sample. A 30-μl volume was selected as a safe over-capacity when samples were to be analysed at a later stage. The reaction itself appeared to be

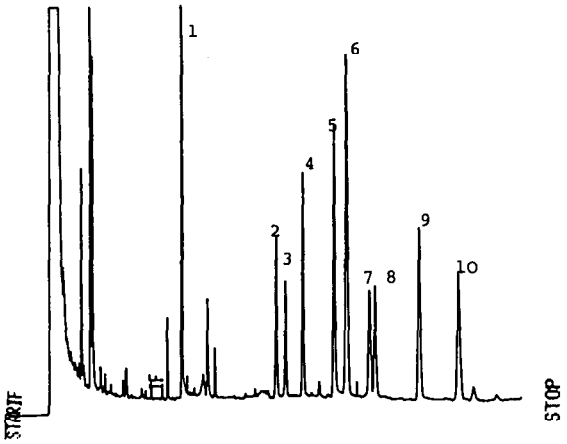


Fig. 4. Gas chromatogram of metoprolol and metabolites after phosgene derivatization, extraction and trimethylsilylation. 1 = M II, 2 = M V, 3 = metoprolol, 4 = M V, 5 = O-demethylmetoprolol, 6 = propranolol (marker), 7 = α-hydroxymetoprolol, 8 = M I, 9 = M VI, 10 = M VII. Retention time of M VII 16 min. Structures of the parent compounds are given in Fig. 1, and gas chromatographic conditions in the Experimental section.

virtually instantaneous. The reagent excess was not evaporated as the carboxylic acid trimethylsilyl esters tended to decompose in the absence of reagent. This instability was more pronounced with M II than with M I.

Selection of internal standard

Because of the variety of chemical structures of the compounds in this study, the selection of an internal standard was bound to be a compromise. At an early stage of method development, IS 2 (Fig. 2) was investigated as a potential internal standard for M I.

However, precision data were inferior to those obtained with the propranolol oxazolidineone as marker. Also the area ratios versus IS 1 spread more than versus propranolol oxazolidineone. IS 1 is an isomer of metoprolol (Fig. 2) and was used in the phosgene-based method for metoprolol mentioned [13] and is now also used in the electron-capture gas chromatographic method for metoprolol [16]. Thus, for quantitative purposes the propranolol oxazolidineone marker was preferred but IS 1 was added to check the phosgene reaction. Both were used in a fairly large amount compared with some of the compounds to be measured. Approximately 110 μmol of each were added per sample to minimize disturbances from endogenous components present.

As no related acid was available, M II, the lactic acid metabolite was also quantified by the aid of propranolol oxazolidineone despite the large difference in retention time (6 min). Stearic acid might be an alternative when M II only is studied. Its trimethylsilyl ester has only a 1 min longer retention in this gas chromatographic system. The acid can be added to the extraction medium and an excess is recommended as endogenous octadecanoic acids may be present.

Standard curves

Standard curves were constructed after analysing samples prepared by adding metoprolol and metabolites to urine to give the anticipated concentrations. A positive intercept of the slope of M II was observed and is due to chromatographic interference. In some samples this was a problem as the integrator recognized only one peak. A gas chromatogram with M II from urine is shown in Fig. 5.

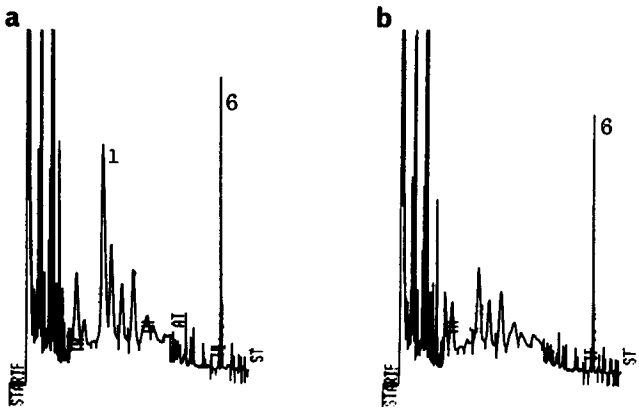


Fig. 5. Gas chromatograms of M II in urine: (a) 17 μmol/l added to urine, (b) blank urine. Peak identification as in Fig. 4, 6 = marker. Chart speed ten times higher in the region where M II elutes and the attenuation decreased × 2.

Linear-regression analysis gave correlation coefficients > 0.999 except for M I (0.987). Preliminary analysis of O-demethylmetoprolol in urine from several individuals who had taken metoprolol never gave concentrations above 4 μmol/l. Thus, standard curves were not constructed for this metabolite.

Precision and absolute yield

The precision of the method was studied by the analysis of spiked urine samples from six individuals. The results are listed in Table VI together with the absolute yield. This was obtained by comparison with solutions of synthesized derivatives and the pure compounds. From Table VI it is obvious that urine from different individuals has a negligible influence on the spread for

TABLE VI

PRECISION AND YIELD OF THE METHOD

Method: see Experimental, urines from six individuals were spiked.

	Precision (urine, R.S.D., %)	Concentration (μmol/l)	Yield (%)	
			Urine (n = 6)	Water (n = 3)
M II	11.5	42	93	86
Metoprolol	4.5	37	92	88
α-Hydroxymetoprolol	4.1	71	72	72
M I	6.4	75	56	86

TABLE VII

DETERMINATION OF METOPROLOL AND METABOLITES IN URINE FROM A HEALTHY VOLUNTEER AFTER INGESTION OF 292 μmol OF METOPROLOL TARTRATE

Fraction (h)	M II		Metoprolol		O-Demethylmetoprolol		α-Hydroxymetoprolol		M I	
	μmol/l	μmol excreted	μmol/l	μmol excreted	μmol/l	μmol excreted	μmol/l	μmol excreted	μmol/l	μmol excreted
0–6	66	16	22	5.2	<4	—	50	12	369	87
6–12	4	1	13	3.6	<4	—	30	9.3	133	38
12–24	<4	—	<4	—	<4	—	19	7.7	38	15

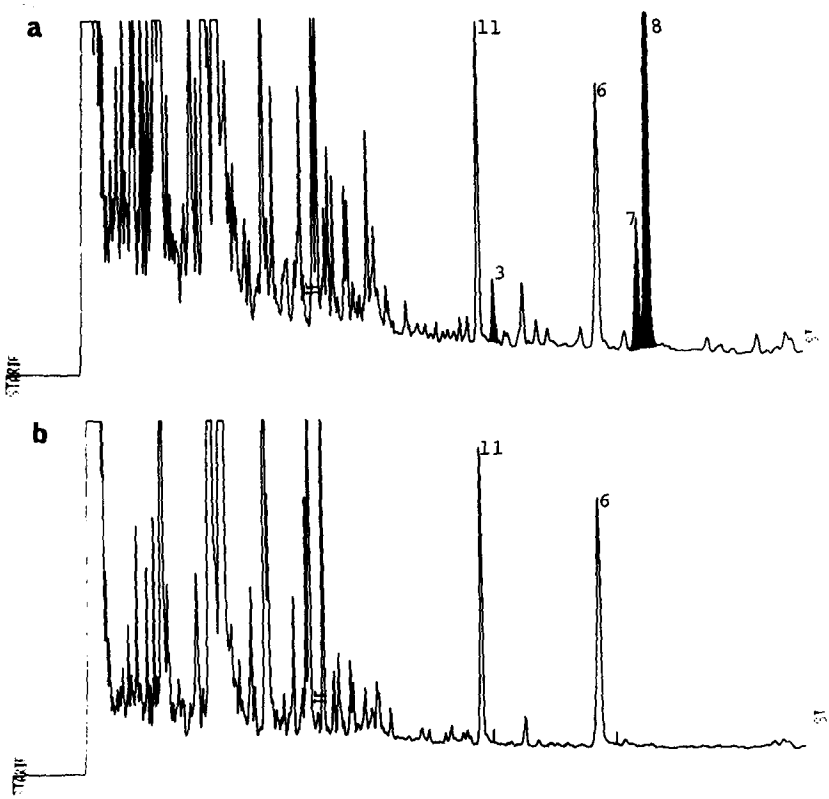


Fig. 6. Gas chromatograms of metoprolol and metabolites after the analysis of urine from an individual who had been given 292 μmol of metoprolol tartrate: (a) 0–6 h fraction, (b) blank urine from the same individual. Peak identification as in Fig. 4, 11 = IS 1. Retention time of M I 12.8 min. For gas chromatographic conditions see Experimental and for levels found see Table VII.

α-hydroxymetoprolol and M I at these levels. However, in the case of M II, some of the blank urines contained a peak near that of the compound of interest, thus affecting the precision in its determination. The absolute yield of M I in the organic phase was about two-thirds that obtained when a pure aqueous solution was derivatized in parallel (Table VI). In the other instances the recovery from aqueous and urine samples were the same. A further 10% of M I could be recovered if the aqueous phase was isolated and derivatized anew after readjustment of the pH. Further studies of the derivatization of M I in urine might be worthwhile. However, since the actual levels were adequate for

the present method this was not deemed necessary at the present time.

Blank urine from two individuals was spiked with M II, α-hydroxymetoprolol and M I to varying concentrations by an independent person. The eight samples were then analysed by the present method. The levels found correlate well (better than 0.997) and the slopes were between 0.90 and 1.10.

Determination of metoprolol and metabolites in urine samples

An individual known to be a normal hydroxylator was given 292 μmol of metoprolol as tartrate. Urine was collected in fractions during 24 h and was analysed according to this method. The concentrations found are presented in Table VII. A gas chromatogram of 0—6 h fraction together with a blank is shown in Fig. 6. In agreement with data reported earlier based on radioactivity measurements [1], the metabolite M I is the major urinary excretion product in man after an oral dose of metoprolol. Unchanged drug and α-hydroxymetoprolol are present in urine as well as the lactic acid metabolite M II. The method in this paper revealed that 67% of the given dose can be accounted for.

Samples with M VI and M VII (Fig. 1) were prepared to a concentration of 20 μmol/l of blank urine each. After analysis the peaks in the chromatogram were matched with peaks in the same region from the individual who had been given metoprolol. However, on the basis of retention times, corresponding peaks in the biological samples could not be detected.

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REFERENCES

1 K.O. Borg, E. Carlsson, K.-J. Hoffmann, T.-E. Jönsson, H. Thorin and B. Wallin, Acta Pharmacol. Toxicol., 36 (Suppl. V) (1975) 125.
2 M.S. Lennard, L.E. Ramsay, J.H. Silas, G.T. Tucker and H.F. Woods, Pharm. Int., 4 (1983) 53.
3 A. Mahgoub, J.R. Idle, L.G. Dring, R. Lancaster and R.L. Smith, Lancet, ii (1977) 584.
4 M.S. Lennard, J.H. Silas, S. Freestone and J. Trevethick, Brit. J. Clin. Pharmacol., 14 (1982) 301.
5 M.S. Lennard, J.H. Silas, S. Freestone, L.E. Ramsay, G.T. Tucker and H.F. Woods, N. Engl. J. Med., 307 (1982) 1558.
6 D.B. Jack, M. Wilkins and C.P. Quaterman, Brit. J. Clin. Pharmacol., 16 (1983) 188.
7 M. Ervik, K.-J. Hoffmann and K. Kylberg-Hanssen, Biomed. Mass Spectrom., 8 (1981) 322.
8 D.B. Pautler and W.J. Jusko, J. Chromatogr., 228 (1982) 215.
9 J.B. Lecaillon, C. Souppart and F. Abadie, Chromatographia, 16 (1982) 158.
10 M.S. Lennard and J.H. Silas, J. Chromatogr., 272 (1983) 205.
11 C.P. Quarterman, M.J. Kendall and D.B. Jack, J. Chromatogr., 183 (1980) 92.
12 C.P. Quarterman, M.J. Kendall and D.B. Jack, Brit. J. Clin. Pharmacol., 11 (1981) 287.
13 O. Gyllenhaal and J. Vessman, J. Chromatogr., 273 (1982) 129.
14 M. Ahnoff and G. Holm, in R.E. Kaiser (Editor), Proc. 4th Int. Symp. on Capillary Chromatography, Hindelang, May 3—7, 1981, Hüthig, Heidelberg, 1981, p. 673.
15 K.-J. Hoffmann, I. Skånberg and K.O. Borg, Xenobiotica, 9 (1979) 79.
16 L. Johansson, personal communication.