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Note

Simultaneous determination of metoprolol and deuterium-labelled metoprolol in human plasma by gas chromatography—negative-ion mass spectrometry

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The simultaneous administration of a drug and the same molecule labelled with a stable isotope is used in pharmacokinetic studies [1—3] to avoid most of the pitfalls caused by intra-individual variability. Both the unlabelled and the labelled compound must be assayed in the same sample. This assay is performed by gas chromatography—mass spectrometry (GC—MS).

Since the appearance of the first assay method for metoprolol using gas chromatography with electron-capture detection (ECD) by Ervik [4], several other methods have been published [5—8]. A GC—MS assay of metoprolol and its main metabolites in plasma has also been reported [9].

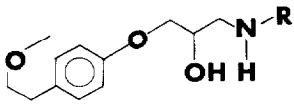
The present paper describes the simultaneous determination of metoprolol and [$^2H_6$]metoprolol by GC—MS in the negative-ion mode using [$^{13}C_3$]-metoprolol as internal standard.

EXPERIMENTAL

Chemical and reagents

[$^2H_6$]Metoprolol hydrochloride (Fig. 1) was synthesized in our laboratories according to the method of Chaudhuri and Ball [10] from [$^2H_6$]isopropylamine. The latter was obtained by the method of Colombini et al. [11] from [$^2H_6$]acetone (CEA, Saclay, France). [$^{13}C_3$]Metoprolol was prepared similarly from [$^{13}C_3$]acetone (Prochem, London, U.K.).

A buffer solution (1 l) was prepared by dissolving 168 g of potassium hydroxide (Merck 5033) and 360 g of tripotassium phosphate (Merck 5013) in distilled water. Heptafluorobutyric anhydride (HFBA) was purchased from Ventron (Ref. PCR, 1300-3; Ventron, Karlsruhe, F.R.G.).



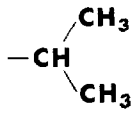
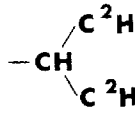
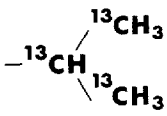
R	Compound
	Metoprolol
	[$^2H_6$]Metoprolol
	[$^{13}C_3$]Metoprolol

Fig. 1. Chemical structures of the labelled compounds.

Equipment

The glassware was washed using Extran (Merck) as detergent, silanized by immersion in a toluene bath containing hexamethyldisilazane (HMDS), chlorotrimethylsilane and pyridine at a concentration of 1% each for 15 min. It was rinsed twice with methanol, immersed in an ultrasonic bath of methanol for 15 min and dried in an oven at 100°C.

The GC—MS instrument was a Ribermag Model 10-10 equipped for negative-ion detection. The software of the on-line PDP8/A computer was only used for the measurement of the chromatographic peaks. Further calculations were made on a Wang MVP computer.

The column was a 2 m × 2 mm I.D. glass tube. The tube, treated at room temperature with a 5% HMDS solution in toluene for about 1 h, was packed with 3% OV-1 on 100—120 mesh Gas-Chrom Q. The column was operated with a helium flow-rate of 20 ml/min. Before use it was silanized at 80°C (by injecting HMDS) and conditioned at 300°C. The injection port, the column and the interface temperatures were 250°C, 230°C and 300°C, respectively. The retention time of metoprolol was about 1.4 min and the total run time about 2 min.

The mass spectrometer was operated in the negative-ion chemical-ionization mode with nitrous oxide as reagent gas (3 ml/min). The masurements were performed at *m/e* values 488, 494 and 491 for metoprolol, [$^2H_6$]- and [$^{13}C_3$]-metoprolol, respectively.

Extraction, derivatization and detection

A 1 ml aliquot of plasma spiked with 50 μl of the internal standard solution

(16 μmol/l aqueous solution), 1 ml of the buffer solution and 4 ml of dichloromethane—diethyl ether 1:4 were shaken mechanically at 300 rpm for 15 min. After centrifugation, the organic phase was removed and evaporated to dryness under a nitrogen flow at 40°C. Then 1 ml of a mixture of hexane—pyridine (97:3) was added. The tube was shaken for about 15 sec; 10 μl of HFBA were added and the tube was shaken again for 15 sec. The derivatization was allowed to proceed at room temperature for 15 min, then 1 ml of a saturated aqueous solution of potassium dihydrogen phosphate was added. The mixture was shaken for about 15 sec, and 1—2 μl of the upper phase were injected.

Correction of the data for isotopic contributions

Due to isotopic impurities, the measurements at *m/e* values 488, 494 and 491 were not specific for metoprolol, [$^2H_6$]- and [$^{13}C_3$]metoprolol, respectively. As shown by Fig. 2, chromatographic peaks were recorded at the various *m/e* values after separate injection of each of the three compounds.

For the estimation of the isotopic contributions, three aqueous solutions containing either metoprolol, [$^2H_6$]- or [$^{13}C_3$]metoprolol at a concentration around 1.5 μmol/l were processed as described for the plasma samples except for the addition of the internal standard which was omitted. After separate injection, the recorded areas at *m/e* 488, 494 and 491 were used for the estimation of the isotopic contributions and correction of the measured peak areas as described [12].

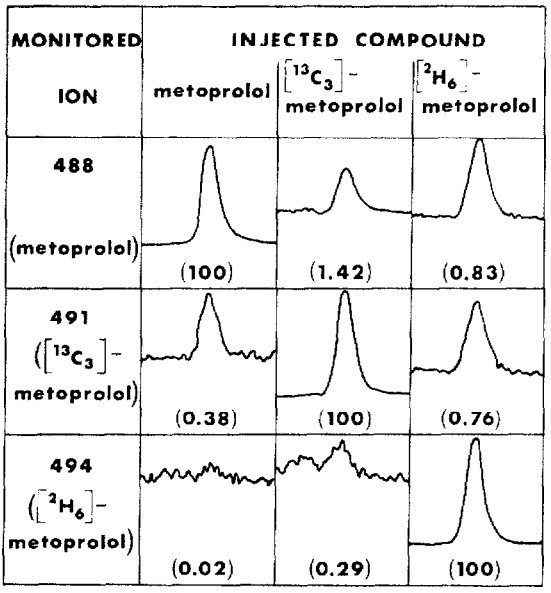


Fig. 2. Interferences for each substance from the other two substances. Unlabelled, [$^2H_6$]- and [$^{13}C_3$]metoprolol derivatives were measured at *m/e* values of 488, 491 and 494, respectively. The peaks and the related areas (arbitrary units) after separate injection of each substance are shown.

Calibration

Solutions of equal concentrations of metoprolol and [$^2H_6$]metoprolol in

the concentration range 0.25—40 μmol/l were prepared using the internal standard solution as solvent. Blank human plasma aliquots (1 ml) were spiked with 50 μl of each of these solutions resulting in final concentrations in the range 12.5—2000 nmol/l. These calibration samples were processed as described above. The calibration curves were fitted to the results as the weighted linear least-squares regression using the inverse of the squared concentration as the weighting factor [13].

RESULTS AND DISCUSSION

The fragmentation of the heptafluorobutyryl derivatives in the conditions used for the analysis resulted in a base peak ($C_3F_7COO^-$, m/e = 213), common to all three derivatives. The monitored ion, whose probable structure is displayed in Fig. 3, is specific for the side-chain of metoprolol with the isopropylamino group. The back-extraction into an aqueous phase required by GC—ECD methods could be omitted. This, and a reaction time for the derivatization reduced to 15 min, resulted in a fast analytical technique.

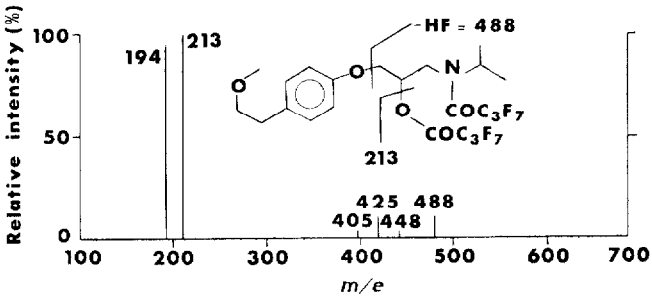


Fig. 3. Mass spectrum of metoprolol as its heptafluorobutyryl derivative (molecular weight = 659) using negative-ion detection and nitrous oxide as reagent gas.

TABLE I

MEAN PERCENTAGE RECOVERY OF METOPROLOL IN PLASMA SAMPLES SIMULTANEOUSLY SPIKED WITH METOPROLOL AND $^2H_6$-LABELLED METOPROLOL

[$^2H_6$]Metoprolol added (nmol/l)	Metoprolol added (nmol/l)				
	20	50	250	750	1500
0		97.9	101.9	103.0	100.8
20	99.1		101.4		
50		100.8	102.7	102.4	101.0
250	109.3	104.7	103.8	103.3	100.7
750		94.6	101.7	101.7	100.6
1500		99.3	102.4	102.0	101.0
Mean*	102.5	99.7	102.4	102.4	100.8
S.D.*	5.9	3.6	1.0	1.2	0.53

Mean overall recovery ± S.D.: 101.5 ± 2.78%

*Calculated on actual individual values.

TABLE II

MEAN PERCENTAGE RECOVERY $^2H_6$-LABELLED METOPROLOL IN PLASMA SAMPLES SIMULTANEOUSLY SPIKED WITH METOPROLOL AND $^2H_6$-LABELLED METOPROLOL

Metoprolol added (nmol/l)	[$^2H_6$]Metoprolol added (nmol/l)				
	20	50	250	750	1500
0		102.4	99.6	101.7	100.6
20	107.4		102.8		
50		102.7	100.9	101.8	101.7
250	117.3	104.4	101.9	101.9	101.4
750		106.0	102.3	101.2	101.9
1500		104.0	101.5	102.3	100.3
Mean*	110.7	103.9	101.5	101.7	101.2
S.D.*	5.9	1.8	1.4	0.52	1.1

Mean overall recovery ± S.D.: 103.1 ± 3.79%

*Calculated on actual individual values.

Plasma samples simultaneously spiked with variable amounts of metoprolol and [$^2H_6$]metoprolol in the concentration range 20—1500 nmol/l were used for the validation of the method. The mean recovery (Tables I and II) was in the range 99.7—103.9% for both compounds at all the investigated concentrations except for [$^2H_6$]metoprolol at the level of 20 nmol/l (110.7%). The good precision, as indicated by the low values of the standard deviation, was probably due to the use of [$^{13}C_3$]metoprolol as the internal standard which provided an efficient compensation for the loss of metoprolol and [$^2H_6$]metoprolol. The slope of the regression straight line of recovered amounts plotted versus the theoretical was 1.01 for both compounds (coefficients of correlation higher than 0.9998). The value of the intercept was 2.12 and 1.14 nmol/l for metoprolol and [$^2H_6$]metoprolol, respectively. The method described by Ervik [4] requires 2—4 ml of plasma and the internal standard is added just before injection. The other methods [5—8] have a sensitivity limit in the range 3—30 nmol/l. A sensitivity limit of 1 nmol/l was claimed for the GC—MS method [9] but without experimental evidence.

The sensitivity limit of the present technique is 20 nmol/l, but since its objective is essentially the simultaneous determination of metoprolol and [$^2H_6$]metoprolol, it cannot be compared to previously published methods.

CONCLUSION

The GC—MS method described here permits the simultaneous determination of metoprolol and [$^2H_6$]metoprolol present in the same plasma sample at concentrations in the range 20—1500 nmol/l (7 ng/ml of the tartrate salts). It is suitable for the performance of pharmacokinetic studies in which both compounds are administered simultaneously to the same individual by two different routes and/or as two different preparations.

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