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Note

Convenient gas chromatographic method for the determination of the proportions of the two racemic modifications present in labetalol hydrochloride

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Labetalol hydrochloride is the British Approved Name for the equimolecular mixture of the diastereoisomeric racemers of 2-hydroxy-5-{1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl}benzamide hydrochloride (Fig. 1), the active constituents of the various pharmaceutical presentations of the anti-hypertensive drug Trandate®. This drug has a particularly favourable profile of action characterised by both α- and β-adrenoreceptor blockade[1] with these two activities being largely related one to each of the two racemers.

To ensure constancy in the ratio of α- and β-blockade, an important requirement of quality assurance of labetalol hydrochloride is the ability to determine the

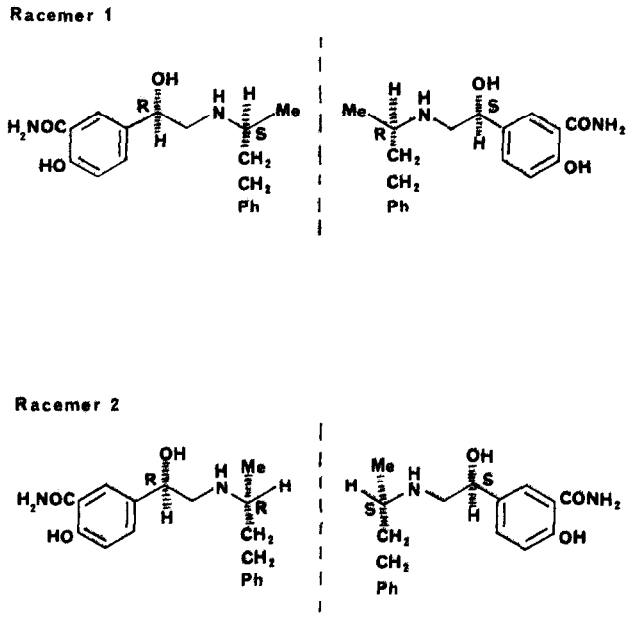

Fig. 1. Two racemers of labetalol. Me = methyl, Ph = phenyl.

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constancy of the 1:1 ratio of *RS/SR* (racemer 1) and *RR/SS* (racemer 2) racemic modifications. The recent appearance of a paper[2] reporting the gas chromatographic (GC) separation of the racemers of labetalol isolated from urine via conversion into the *n*-butylboronate derivatives has prompted us to report our development and validation of a rapid, sensitive and reliable method for the drug substance. This procedure, which is superior to an earlier GC method[3] involving conversion of the carboxamide function of labetalol into the nitrile and formation of the O-*tert*.-butyldimethylsilyl, N-trifluoroacetyl derivatives, has been validated by ^{1}H NMR.

Historically, the presence of diastereoisomers of labetalol was first shown in these laboratories by ^{1}H NMR. It was found that the spectrum of a solution in pyridine showed two overlapping methyl doublets which, at 60 MHz, were only separated by about 1 Hz. Raising the temperature to 90°C sharpened the peaks sufficiently to enable an estimate of diastereoisomer content to be made by comparison with mixtures prepared from the pure isomers. Variation in the measured signal ratio over the range 40–60:60–40 was considered at the time to reflect a 1:1 racemer ratio. No splitting of any other signals was observed.

No improvement in the separation of the signals could be obtained through use of other solvents although a small range of magnetically anisotropic solvents, which would allow sufficient solubility of the drug for the low sensitivity then available, was investigated. With the greater sensitivity of a 90 MHz instrument, the range of possible solvents was extended and better signal separation was obtained using d_6-benzene–d_4-methanol (1:2). This still remains the best solvent mixture and operation at 250 MHz gives complete separation of the methyl doublets at room temperature as shown in Fig. 2. Consequently increased precision is achieved using peak areas after resolution enhancement has been performed on the spectrum.

The expectation that increased ring currents of polycyclic hydrocarbons might give greater differences in shielding was not realised. Use of naphthalene with d_4-methanol produced about the same separation as did benzene but anthracene was insufficiently soluble.

^{19}F NMR spectra of the trifluoroacetylated derivatives was also investigated at one stage but, while the trifluoroacetamide peaks were well separated, there were complications of further multiplicity due to restricted rotation. Raising the temperature sufficiently to remove this caused decomposition of the derivative.

Finally and recently, it was found that the ^{13}C NMR broad band spectrum of labetalol hydrochloride in d_4-methanol shows complete resolution of the methyl carbons in the two diastereoisomers and consequently the ratio can be measured by integration. In addition, several other carbon atoms are also resolved between the diastereoisomers.

Modern Fourier transform (FT) NMR instrumentation allows rapid and accurate quantification of isomer ratios as shown in labetalol hydrochloride. However, for routine quality assurance purposes a less sophisticated technique has to be used. Hence the GC method involving separation of boronate derivatives has been developed and is now reported.

Earlier, a method involving cyclic boronate derivatives was examined and reported[4] but, under the conditions used, appeared to give irreproducible results. However, re-examination of this method has allowed the identification of reproducible conditions of derivatisation and appropriate columns which permit the definition of the method described.

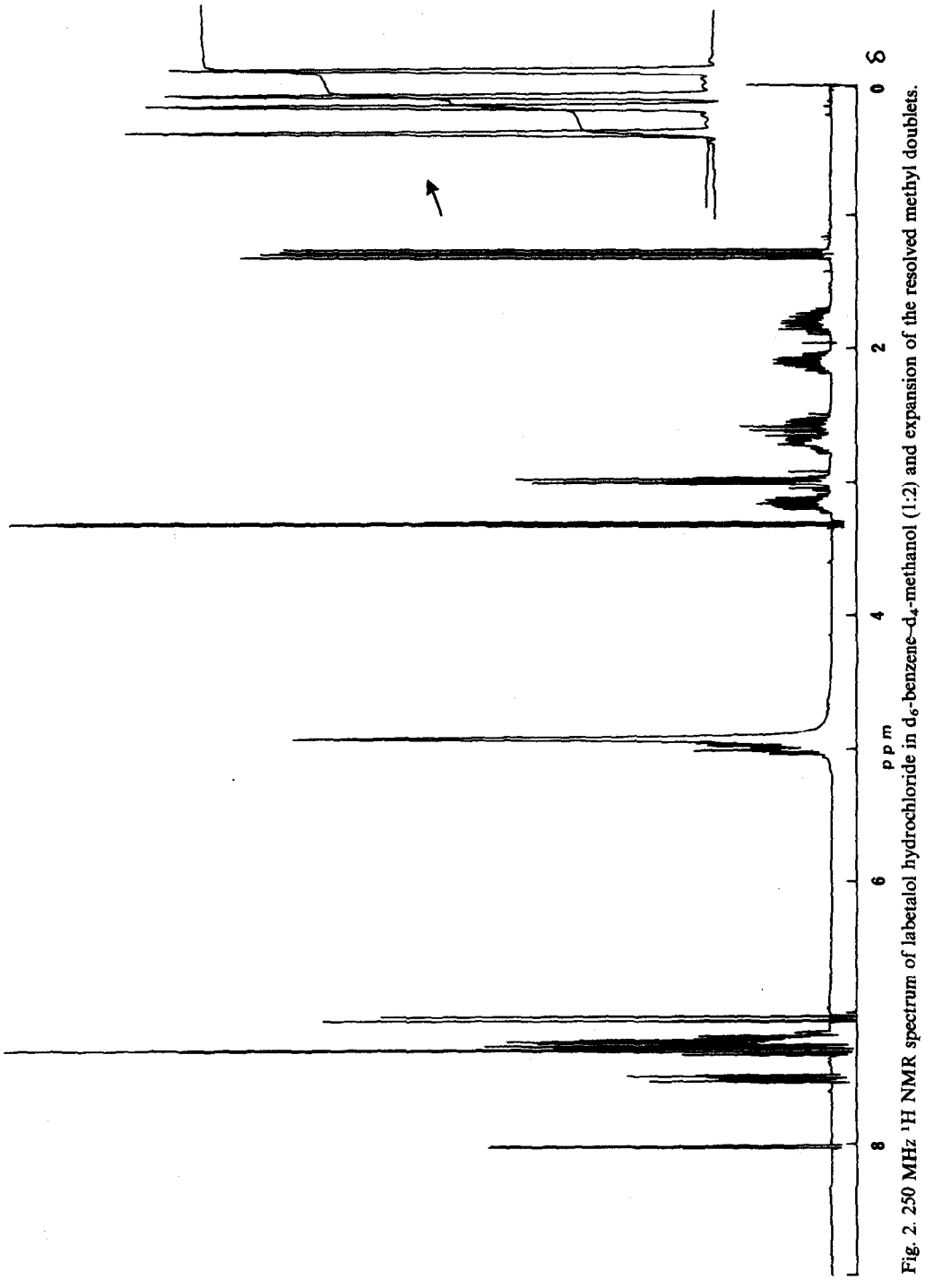
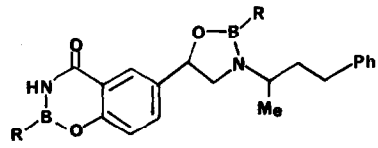
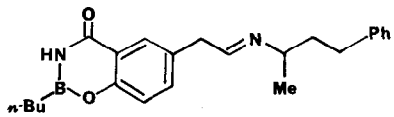


Fig. 2. 250 MHz ^1H NMR spectrum of labetalol hydrochloride in d_6-benzene–d_4-methanol (1:2) and expansion of the resolved methyl doublets.



(1) R = Me

(2) R = *n*-Bu



(3)

Fig. 3. Structures of the bis-alkylboronate derivatives of labetalol (1 and 2) and possible by-product (3) in the reaction with *n*-butylboronic acid. Me = methyl, Bu = butyl, Ph = phenyl.

Gas chromatographic–mass spectrometric (GC–MS) studies[5] confirm the structures of the cyclic bis-methylboronate (1) and bis-*n*-butylboronate (2) (Fig. 3).

EXPERIMENTAL

Apparatus

GC was performed using either a Hewlett-Packard HP5880 gas chromatograph fitted with a flame ionisation detector using a 25-m fused-silica Chrompak wall-coated open-tubular CPSil5 capillary column at 265°C (methylboronate) or 285°C (butylboronate) with a helium flow (through the column) of 1 ml/min and a split ratio of 70:1, a Perkin-Elmer F30 gas chromatograph fitted with a flame ionisation detector using a 2 m × 5 mm I.D. glass column packed with 2% OV-17 on 100–120 mesh Gas-Chrom Q at 280°C (methylboronate) with a nitrogen flow of 20 ml/min, or a Pye GCV gas chromatograph fitted with a flame ionisation detector using a 5 ft. × 4 mm I.D. glass column packed with 5% SE-30 on 80–10 mesh Gas-Chrom Q at 250°C (methylboronate) with a nitrogen flow of 60 ml/min.

^{1}H and ^{13}C NMR spectra were obtained on a Bruker WM250 spectrometer at 250.13 and 62.90 MHz respectively.

Materials

Methylboronic acid was obtained from Alpha Chemicals and *n*-butylboronic acid was obtained from Fluka. Boronic acid reagents were prepared by dissolving methyl or *n*-butylbornic acid (12 mg/ml) in pyridine dried over molecular sieve 4A overnight.

RESULTS AND DISCUSSION

Serial injections (Table I) were performed in order that the stability of the methylboronate derivative both upon standing and on the column could be checked. Munro *et al.*[4], who used a column of Gas-Chrom Q AW HMDS (100–120 mesh) coated with OV101, suggested that unreliable results obtained could arise from instability either on or off the column. Our findings indicate this is not the case when a CPSil5 capillary column is used. No significant change in the racemer ratio was apparent and the reproducibility was good with a coefficient of variation of 0.54%.

TABLE I

RACEMER RATIO IN LABETALOL HYDROCHLORIDE AT VARIOUS TIME INTERVALS AFTER ADDITION OF THE METHYLBORONIC ACID REAGENT USING A CPSil5 CAPILLARY COLUMN

Time (min)	*Racemer 2 (RR/SS)*	*Racemer 1 (SR/RS)*
30	50.8	49.2
40	50.2	49.8
50	50.6	49.4
65	50.4	49.6
75	50.3	49.7
90	50.2	49.8
115	50.5	49.5
135	50.0	50.0
215	50.0	50.0
255	49.9	50.1
270	50.2	49.8
285	50.0	50.0
340	49.9	50.1
390	50.4	49.6
410	50.4	49.6
Mean	50.25	49.75
S.D. ($\sigma - 1$)	0.27	0.27
C.V. (%)	0.53	0.54

Furthermore no decomposition products were apparent in the chromatogram even after standing one month. A typical chromatogram is shown in Fig. 4. Munro *et al.* also suggested that excess reagent could lead to further reactions with labetalol to give involatile products or react with the stationary phase and cause chemisorption of the solute. However, multiple derivatisation (Table II) performed in order to test the efficiency of the derivatisation method using a smaller excess of reagent gave reproducible racemer ratios [coefficient of variation (C.V.) = 0.62%].

The racemer ratio on one sample of labetalol hydrochloride as its methylboronate derivative was also determined on a packed system using 2% OV-17 and gave a ratio of 49.7:50.3 in good agreement with that obtained on the capillary system of 49.5:50.5.

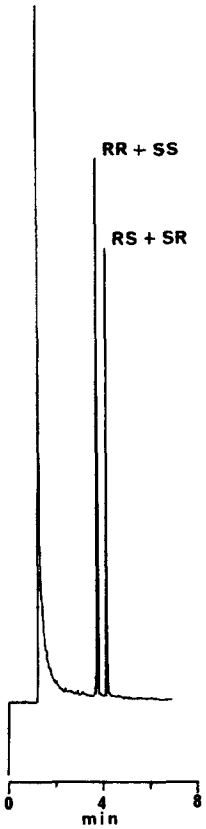


Fig. 4. Chromatogram of the bis-methylboronate derivatives of labetalol on a CPSil5 capillary column one month after derivatisation.

TABLE II

RACEMER RATIO IN LABETALOL HYDROCHLORIDE UPON MULTIPLE DERIVATISATION OF THE SAME SAMPLE USING A CPSil5 CAPILLARY COLUMN

Derivative	*Racemer 2 (RR/SS)*	*Racemer 1 (SR/RS)*
1	50.6	49.4
2	50.7	49.3
3	50.9	49.1
4	51.0	49.0
5	50.9	49.1
6	50.3	49.7
7	50.3	49.7
8	51.1	48.9
9	50.4	49.6
10	51.0	49.0
Mean	50.7	49.3
S.D. ($\sigma - 1$)	0.30	0.30
C.V. (%)	0.60	0.62

Although the method was not extensively studied, the results obtained using the *n*-butylboronate derivative are equally satisfactory. It was found however that GC conditions were somewhat more critical and if the injection temperature was too high a minor component of shorter retention time was observed. This did not appear to affect the racemer ratio and GC–MS studies[5] suggest the presence of the imine (3) (Fig. 3).

Other stationary phases[6] including 3% OV-17 on 100–120 mesh Gas-Chrom Q and 5% SE-30 on Gas-Chrom Q have proved equally successful. On the later system an excellent linearity of response is observed on application of the method to a series of synthetic mixtures of racemer 1 (*RS*/*SR*) and racemer 2 (*SS*/*RR*) of differing compositions across the range 0–100% to 100–0%, Table III.

Comparison of racemer ratios determined by GC and ^{1}H NMR methods (Table IV) shows good agreement (within 0.4%) on three batches using peak integration in both cases. ^{13}C NMR seems less reliable probably due to the less sensitive nature of this technique.

It is concluded that either bis-methyl- or bis-*n*-butylboronate derivatives of labetalol hydrochloride may be analysed by GC thus providing a rapid and sensitive method for determining the ratio of the two racemers. Excellent agreement is achieved with a modern FT ^{1}H NMR method.

TABLE III

LINEARITY OF RESPONSE BETWEEN PEAK AREA RATIO AND WEIGHT RATIO OF RACEMERS 1 AND 2 OF LABETALOL AS ITS BIS-METHYLBORONATE DERIVATIVES USING A 5% SE-30 PACKED COLUMN

Sample composition		*Area found (%)*	
Racemer 1 (%)	*Racemer 2 (%)*	*Racemer 1*	*Racemer 2*
100	0	100	0
		100	0
90.87	9.13	90.23	9.78
		90.08	8.92
72.06	27.94	72.27	27.73
		71.71	28.29
52.51	47.49	53.00	47.00
		51.66	48.34
32.15	67.85	33.65	66.35
		32.58	67.43
10.94	89.06	9.67	90.24
		9.14	90.86
0	100	0	100
		0	100
Correlation coefficient	0.9998	0.9998	
Slope	1.0013	1.0035	
Intercept	−0.2791	−0.0270	
Standard error (slope)	0.0063	0.0061	
Standard error (intercept)	0.3935	0.3719	

TABLE IV

COMPARISON OF THE RACEMER RATIO IN THREE BATCHES OF LABETALOL HYDROCHLORIDE BETWEEN GC AND NMR METHODS

Method	*Ratio racemer 2:1*		
	Batches		
	0010B	*0011B*	*0018*
GC*	48.6:51.4	45.7:54.3	48.5:51.5
^{1}H NMR**	48.2:51.8	45.9:54.1	48.5:51.5
^{13}C NMR***		47.9:52.1	

* CPSil5 as bis-methylboronate derivative. Shorter retained peak is racemer 2 (*RR/SS*).

** In d_4-methanol–$d_6$benzene (2:1). Lower field methyl doublet is associated with racemer 1 (*RS/SR*).

*** In d_4-methanol. Lower field methyl carbon is racemer 1 (*RS/SR*).

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