Simultaneous determination of (−)- and (+)- propranolol by gas chromatography-mass spectrometry using a deuterium labeling technique

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Propranolol used for the treatment of hypertension is a mixture of (+)- and (−)-isomers, where only the (−)-isomer has β-blocking activity.

Separate administration of the enantiomers to man and dogs has revealed a shorter plasma half-life for the (+)-isomer (George, Fenyvesi & others, 1972). Studies of the enantiomers after administration of the racemic drug is however of more clinical interest as the liver blood flow and thus the rate of metabolic elimination is specifically decreased by the (−)-isomer (Branch, Shand & others, 1973).

Isotope labelling with ^{3}H, ^{14}C (Iversen, Jarrott & Simmonds, 1971) or ^{2}H (McMahon, Sullivan & Due, 1974; Weinkam, Callery & others, 1974) has recently been used for studies of enantiomers in racemic mixtures.

This paper gives a technique for the determination of the ratio of the propranolol enantiomers using ^{2}H labelling in combination with g.c.–m.s. and its application to establish the enantiomeric ratio in urine after oral administration of racemic propranolol to dogs.

The m.s. analysis was carried out on an LKB *9000* S instrument equipped with an accelerating voltage alternator using an ionizing electron energy of 20 eV. The glass g.c. column (3 feet × 5 mm i.d.) was packed with 5% OV-17 on 80/100 mesh Gas-Chrom Q. The helium flow was 15 ml min^{-1} with a column temperature of 200°.

Propranolol was derivatized with trifluoroacetic anhydride (Walle, 1974) before analysis by g.c.–m.s.

^{2}H-(+)-Propranolol was synthesized by mixing 0·5 g of (+)-propranolol with 20 ml of 5% v/v $^{2}H_2SO_4$ in $^{2}H_2O$ (cf. Werstiuk & Kadai, 1974). The mixture was heated for 30 min at 100°, made alkaline with NH_3 and extracted with diethyl ether. The ether was removed by evaporation and the residue recrystallized from ethanol–water (yield: 90%). No racemization occurred as checked by polarimetric measurements. Two atoms of ^{2}H are rapidly incorporated into the naphthalene ring (Table 1). The labelling is stable in 0·01 M HCl at 37° for at least 6 h. An equimolar mixture of ^{2}H racemic and non-labelled racemic propranolol given orally to dogs (see below) revealed no metabolic isotope effects (isotope ratio was determined in the urine by g.c.–m.s.).

Table 1. *^{2}H labelling of propranolol.*

	Ratio 146
Time (min)	144+146
0	0·04
5	0·33
15	0·81
30	0·96
60	0·94

Reaction mixture: 1 mg ml^{-1} of propranolol in 5% v/v $^{2}H_2SO_4$ in $^{2}H_2O$. Reaction temperature: 100°. The ratio was determined by g.c.–m.s. after alkalization (NH_3), extraction into benzene and derivatization with trifluoroacetic anhydride.

* Naphthol fragment (Garteiz & Walle, 1972).

200 μl of equimolar amounts of ^{2}H (+)-propranolol and non-labelled (−)-propranolol (as bases) in ethanol (200 mg ml^{-1}) was given orally to two dogs (5 kg) and 48 h urine was collected. 20 ml of the urine was made alkaline with NH_3 and extracted with 20 ml of benzene. Re-extraction into 2 ml of 0·01 M HCl was followed by making the solution alkaline with NH_3 and extraction with 2 ml of benzene. The benzene was evaporated to 200 μl and mixed with 100 μl of trimethylamine (M) in benzene and with 10 μl of trifluoroacetic anhydride and left at room temperature for 10 min. The benzene phase was extracted with phosphate buffer pH 7 and 3 μl of the organic phase was injected into the g.c.–m.s. The m.s. was focused at *m/e* 144 and 146 (naphthol fragment, Garteiz & Walle, 1972) and the ratio of the peak heights was determined. The ratio was the same as (0·90, 0·92) that of the equimolar mixture (0·90) indicating no differences in the rate of metabolic elimination of the two isomers.

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The occurrence of two polymorphs of D-penicillamine

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D-Penicillamine, which may be chemically described as D-3,3-dimethylcysteine or D-3-mercaptovaline, is the required enantiomer for pharmaceutical preparations. The United States Pharmacopeia Nineteenth Revision, 1975, requires that D-penicillamine conforms to the Code of Federal Regulations, Title 21, Food and Drugs, part 440,51, 1974. These regulations include an infrared identity test where a comparison is made with an authentic specimen, samples being prepared as paraffin oil mulls. The British Pharmacopoeia, 1973 has no infrared identity test or other requirement for an authentic specimen of D-penicillamine.

Infrared absorption spectra were recorded for samples from the known commercial sources on a Perkin-Elmer 157G grating infrared spectrophotometer, the samples being prepared both as paraffin oil mulls and potassium bromide discs. These spectra were found to be consistent from a given source but showed significant differences between sources, the differences being independent of the method of sample preparation. As all samples were of satisfactory quality when tested to the British Pharmacoeia specification, it appeared that the spectral behaviour was due to the occurrence of different crystal forms.

All spectra could be classified as arising from one or other of two distinct crystal forms or from mixtures of these two forms. The form corresponding to that of the United States Food and Drug Administration authentic specimen is referred to as polymorph II and the other as polymorph I. Although seven significant spectral differences were observed between 700 and 1350 cm^{-1}, one feature was diagnostic.

This was at 1092 cm^{-1} where polymorph II had an absorption peak that was more intense than the adjacent peaks at about 1050 and 1160 cm^{-1} while polymorph I had a minimum between peaks at 1078 and 1101 cm^{-1} that were less intense than the adjacent peaks at about 1050 and 1160 cm^{-1}. In the compilation by Pouchert (1970) the infrared spectrum recorded for D-penicillamine is of polymorph II.

Under the polarizing microscope both polymorphs were seen as anisotropic crystals, I as needles and II as plates. Samples selected on the basis of the infrared classification were then examined on a focussing, Guinier-type, X-ray powder diffraction camera and the resulting patterns confirmed that forms I and II were different crystalline phases.

Thermal methods failed to distinguish between the polymorphs. In capillaries using a heated block, or on a microscope hot stage under liquid paraffin, extensive decomposition and volatilisation rather then melting was observed. Differential thermal analysis using a DuPont 900 Thermal Analyzer was applied to samples in aluminium pans in atmospheres of air or nitrogen. Heating at 10° min^{-1} from room temperature gave one endotherm only for either polymorph with an onset at 185° which corresponded to the onset of decomposition in the hot stage preparations.

Penicillamine formulations on the United Kingdom market have been found to contain one or other or a mixture of these polymorphs. As reported by Kang, Kendall & Lee (1974), tablet and capsule excipients did not interfere with the identification or classification of the drug substance by infrared spectroscopy. Dosage form stability in the case of compressed tablets of 250 mg has shown no dependence on the penicillamine crystal form over the prescribed shelf life, taking into consideration the appearance, penicillamine content and disintegration time. From the available evidence it appears that the two polymorphs alone or in admixture are equally acceptable in pharmaceutical dosage forms.

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