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Note on the estimation of ibufenac in serum

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A method for the estimation of ibufenac (4-isobutylphenylacetic acid) in serum is described. The acidified serum is extracted with ether and the drug estimated by quantitative paper chromatography.

THE use of ibufenac (4-isobutylphenylacetic acid) in the treatment of rheumatoid arthritis has recently been described by Thompson, Stephenson & Percy (1963) and Chalmers (1963); and Adams, Cliffe, Lessel & Nicholson (1963) have reported some of its pharmacological properties. The drug is metabolised but the metabolites are inactive.

A means of estimating the concentration of the drug in serum was required and the physical properties of the drug were such that it seemed possible a method similar to that reported for the determination of fatty acids by Lederer & Lederer (1957), could be applied. We report the method which we have developed, which estimates the drug but not its metabolites.

Experimental

MATERIALS

Bromocresol purple reagent. Bromocresol purple (0·1 g) was dissolved in warm ethanol (20 ml) and made up to approximately 80 ml with water. The colour was adjusted to a reddish purple with 0·1N sodium hydroxide solution (about 10 ml required) and the volume made up to 100 ml with water.

Serum. Human serum was used. It can be stored at −15 to −40° for up to one week before the estimation.

EXTRACTION PROCEDURE

Serum (2 to 5 ml) containing the drug, n-octanol (0·025 ml), diethyl-ether (100 ml) and N hydrochloric acid (0·5 or 1 ml) were measured into a 250 ml separatory funnel and shaken gently for 20 min. The inclusion of octanol usually prevented emulsion formation but occasionally with certain sera an emulsion formed; then the mixture was centrifuged in a stoppered tube. An aliquot (approximately 80 ml) from the ether layer was evaporated to dryness in a round-bottomed tube (125 mm × 38 mm) on a steam-bath. The size of the tube is important since smaller tubes give a low recovery of drug. The residue was transferred to a 15 ml conical centrifugal tube in three lots of 90% ethanol (2, 1, 0·5 ml). This solution was evaporated in a vacuum dessicator over P_2O_5 and the residue was taken up in 90% ethanol (0·2 to 0·4 ml).

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CHROMATOGRAPHY

Whatman No. 1 paper was used and single 5 μl spots of standard solutions of ibufenac in 90% ethanol (12, 6, 3 and 1·5 mg/ml) were run on every sheet. The alcoholic serum extracts were concentrated on the paper by applying 25 to 100 μl in 5 μl volumes to the same position. The paper was dried under a draught of warm air between each application. The solvent system was n-butanol (AR):ammonia (AR; wt/ml 0·88): water, in the proportions 30:5:15 by volume. The papers were run for 16 hr by descending chromatography at 22°, then thoroughly dried and sprayed with a solution of 0·1% (w/v) bromocresol purple solution.

Ibufenac appeared as a yellow spot on a blue background (Rf 0·66) and the colour remained for 2 to 5 min. During this time an assessment of the amount of the compound in the unknown spots was made by comparing their area and intensity with that of the standards. At least two observers made each assessment, and each unknown sample was measured on two or three separate occasions.

RECOVERY OF IBUFENAC ADDED TO HUMAN SERUM

A solution of the sodium salt of ibufenac not exceeding 0·5 ml was added to 5 ml serum. The serum concentrations so produced covered the range of those found in rheumatoid patients receiving ibufenac treatment which was from 1 to 4 mg/100 ml.

Results and discussion

As shown in Table 1, on one occasion a control sample of serum gave an apparent concentration of 0·5 mg/100 ml (25 μg in 5 ml). In the total of 13 determinations the mean recovery was 99% with a standard error

TABLE 1. RECOVERY OF IBUFENAC FROM HUMAN SERUM

Five ml serum was used in each instance

Amount added (μg)	Concentration in /serum (mg100 ml)	Serum batch	Amount found (μg)	Recovery (%)	Mean recovery (± s.e.)
0	0	1	25	—	—
		2	0	—	
		2	0	—	
		3	0	—	
		6	0	—	
50	1	2	63	126	113 (±15)
		2	65	130	
		3	42	84	
100	2	1	113	113	99 (±9)
		2	100	100	
		3	83	83	
200	4	1	220	110	97 (±6)
		1	180	90	
		3	152	76	
		4	195	98	
		4	220	110	
400	8	3	284	71	—
500	10	5	500	100	—

of 5% and the values fell within the range 71 to 130%; the mean recovery did not vary significantly with the ibufenac concentration in the serum.

Light petroleum (b.p. 40–60°) was not satisfactory when used in place of diethyl-ether because emulsions formed more readily and low values were obtained when estimations were made after adding known amounts of ibufenac to serum. The time of shaking was also varied; extraction was virtually complete after 10 min but we have preferred to use 20 min to ensure complete extraction.

Phenylmercuric nitrate added to serum at a final concentration of 1 in 5000 was ineffective as a preservative when the serum was stored at room temperature for two days, although the estimation of added drug was not significantly affected. Samples awaiting estimation were therefore stored and transported at −15 to −40°.

The estimation of compounds by quantitative paper chromatography is now widely used and various claims have been made of the accuracy of the determinations. Reid & Lederer (1950) measured fatty acids with an accuracy of 2 to 5% by a method based on determinations of spot area. In our experience the latter measurements are too time-consuming to warrant the extra accuracy. Douglas, Ludwig, Ginsberg & Berger (1950) were able to measure the metabolites of mebutamate by a visual comparison of spots on paper chromatograms with a precision of ±15%. The present results show that ibufenac in serum can be estimated with a similar accuracy.

The estimation described needs to be made under carefully controlled conditions. For instance, an acid or alkaline atmosphere causes serious interference with colour contrast on spraying the papers. The chief disadvantage with this method is that although the bench time is reasonably short, an estimation requires a minimum of 48 hr for completion.

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