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Note

Rapid determination of ibuprofen in plasma by high-performance liquid chromatography

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In order to compare the bioavailability of *dl*-2-(*p*-isobutylphenyl)propionic acid with that of its water-soluble L-lysine salt (Saren®), it was necessary to have available an analytical method that would be sufficiently rapid, precise and specific. In addition, for a study of the metabolic pathways of the drug, an analytical procedure was desired that would not cause any changes in the nature of any metabolites that might be present.

There have been several reports in the recent literature on the pharmacokinetics of ibuprofen and its metabolites. In all of the studies cited, the quantitative assay of active products was always carried out by gas chromatography, using various derivatives, such as the methyl esters[1,2], the trimethylsilyl ethers[3], the (±)-α-phenylethylamide[4], and, in one instance, the acid itself[5].

MATERIALS AND METHODS

The sample of the L-lysine salt of *dl*-2-(*p*-isobutylphenyl)propionic acid used was a normal commercial product, with properties corresponding to those described in the analytical specifications.

Plasma was prepared from heparinized blood of mongrel dogs by centrifugation for 15 min at 2000 rpm.

Other reagents were of analytical-reagent grade, obtained from E. Merck (Darmstadt, G.F.R.).

Standard solutions

Internal standard. A solution of cinnamic acid (microanalytical standard: BDH, Poole, Great Britain) in methanol (60 μg/ml).

L-lysine salt of dl-2-(p-isobutylphenyl)propionic acid. Eight solutions in dog plasma, with concentrations corresponding to 0.5, 1, 2, 5, 10, 20, 30 and 50 μg/ml of ibuprofen, were prepared.

Extraction procedure

Aliquots of 1 ml of each standard solution of L-lysine salt of *dl*-2-(*p*-isobutylphenyl)propionic acid were placed in 10-ml centrifuge tubes. After acidification with 0.25 ml of 1 *N* hydrochloric acid, the solution was extracted with 5 ml of chloroform.

shaking the mixture for 1 min on a Rotamixer. After centrifugation at 3000 rpm, 4 ml of the chloroform phase were evaporated to dryness at 40° with a gentle stream of dry nitrogen. The walls of the test-tube were washed with 0.5 ml of chloroform, and the washings were evaporated to dryness. The residue was then dissolved in 100 μl of the methanolic solution of the internal standard.

Chromatographic assay

The chromatographic assay was carried out on a 10-μl sample of the methanolic solution of the extract, injected into a Varian 8520 high-performance liquid chromatography (HPLC) apparatus with a steel column (250 × 4,6 mm OD × $^1/_4$ in. IB.), packed with 10-μm RP-8 stationary phase as described by Majors[6].

Isocratic elution was carried out with 0.01 *M* orthophosphoric acid–methanol (3:7) as the mobile phase at a flow-rate of 120 ml/h, using a UV detector with variable wavelength (Varian Varichrom) set at 220 nm (slit bandpass = 8 nm). Under these conditions, the internal standard was eluted after 2.7 min and ibuprofen after 6.5 min (see Fig. 1).

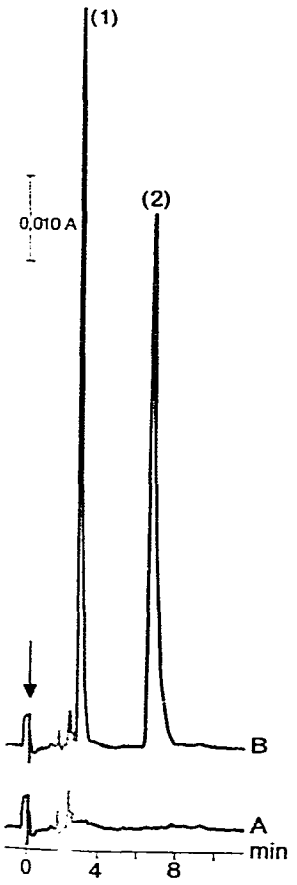

Fig. 1. HPLC of dog plasma extracts. A, Plasma blank; B, plasma containing 20 μg/ml of ibuprofen. Peaks: 1 = internal standard; 2 = ibuprofen.

RESULTS

Table I shows the results obtained in four series of determinations. The effectiveness of the method was evaluated by statistical analysis of the regression line for $\bar{R}$ (mean values of the ratio of the height of the peak for ibuprofen to that for the internal standard) *versus* concentration of ibuprofen. The following equation was found:

$$\text{Ibuprofen } (\mu g/ml) = 32.646\ \bar{R} + 0.087$$

TABLE I

ANALYTICAL RESULTS

Ibuprofen added (μg/ml)	*R**	$\bar{R}$	*Ibuprofen found (μg/ml)*		*Differences*			
			Single value	*Mean*	*Single value*		*Mean*	
					μg/ml	*%*	*μg/ml*	*%*
49.86	1.586	1.538	51.86	50.29	+2.00	1.0	+0.43	0.9
	1.569		51.30		+1.44	2.9		
	1.490		48.72		−1.16	2.3		
	1.508		49.31		−0.55	1.1		
29.92	0.882	0.890	28.87	29.13	−0.95	3.6	−0.79	2.7
	0.909		29.76		−0.16	0.5		
	0.869		28.45		−1.47	5.2		
	0.900		29.46		−0.46	1.6		
19.94	0.603	0.609	19.77	19.98	−0.17	0.9	+0.04	0.2
	0.621		20.35		+0.41	2.0		
	0.588		19.28		−0.66	3.4		
	0.627		20.55		+0.61	3.0		
9.97	0.2925	0.3006	9.63	9.89	−0.34	3.4	−0.08	0.8
	0.290		9.55		−0.42	4.2		
	0.312		10.27		+0.30	3.0		
	0.308		10.14		+0.17	1.7		
4.99	0.158	0.1545	5.24	5.12	+0.25	5.0	+0.13	2.6
	0.160		5.30		+0.31	6.2		
	0.153		5.08		+0.09	1.8		
	0.147		4.88		−0.11	2.2		
1.99	0.0678	0.0640	2.29	2.17	+0.30	15.1	+0.18	9.0
	0.0595		2.02		+0.03	1.5		
	0.0680		2.30		+0.31	15.6		
	0.0609		2.07		+0.08	4.0		
1.00	0.0332	0.3035	1.16	1.07	+0.16	16.0	+0.07	7.0
	0.0326		1.14		+0.14	14.0		
	0.0279		0.99		−0.01	1.0		
	0.0277		0.98		−0.02	2.0		
0.5	0.0143	0.0129	0.55	0.50	+0.05	10.0	0.0	0.0
	0.0159		0.60		+0.10	20.0		
	0.0116		0.46		−0.04	4.0		
	0.0098		0.40		−0.10	20.0		

* R = ratio of peak height of ibuprofen to that of internal standard.

with $s_{x_y} = \pm 0.388$, $s_b = \pm 0.27128$, $s_a = \pm 0.18381$ and $r = 0.999800$. The error was less than $\pm 5\%$ for ibuprofen concentrations in plasma between 5 and 50 μg/ml, and $\pm 20\%$ for a plasma level of 0.5 μg/ml.

CONCLUSIONS

The reversed-phase HPLC method developed for the determination of ibuprofen in plasma is applicable down to levels of 0.5 μg/ml. The method is as rapid and precise as the already known gas chromatographic procedures and seems particularly suitable for use in metabolic studies.

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