New and Simple Method for Determination of 2-(3-Benzoylphenyl)propionic Acid in Body Fluid

R. BALLERINI, A. CAMBI, and P. DEL SOLDATO ˣ

Abstract ☐ A simple, fast, sensitive, and reliable method for the determination of 2-(3-benzoylphenyl)propionic acid in biological fluid is described. The method is based on a quantitative ether extraction of plasma samples followed by TLC separation, spot visualization and elution, and determination at 255 nm. The acid is detectable in amounts as low as 1 μg.

Keyphrases ☐ 2-(3-Benzoylphenyl)propionic acid—TLC analysis, plasma, rats ☐ TLC—analysis, 2-(3-benzoylphenyl)propionic acid, plasma, rats ☐ Analgesic agents—2-(3-benzoylphenyl)propionic acid, TLC analysis, plasma, rats

GC, polarographic, and colorimetric methods (1) have been used to determine the concentration of 2-(3-benzoylphenyl)propionic acid (I), a recently synthesized compound with analgesic and anti-inflammatory properties (2), in body fluids. Unfortunately, these methods are complicated, unreliable, time consuming, nonspecific, and not sufficiently sensitive. Furthermore, they are limited to the determination of I in the urine and require rather expensive instrumentation.

The thin-layer method described in this paper overcomes most of these limitations.

EXPERIMENTAL

Reagents and Materials—Compound I was synthesized and characterized by means of IR, NMR, UV, and TLC techniques[1]. All solvents were analytical reagent grade[2], and the developing solvent, ether–benzene–1-butanol–methanol (85:8:6:1), was prepared fresh daily. Silica gel plates[3], 250 μm thick, were activated at 105° for 1 hr.

Instrumentation—A UV lamp[4] (250 nm), a prism spectrophotometer[5], and 1-cm quartz microcells[6] were used.

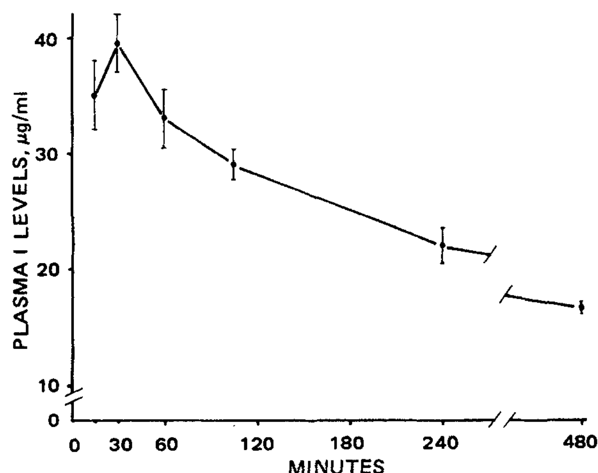


Figure 1—*Concentration of I in plasma of male rats at different time intervals after oral administration of 25 mg/kg.*

[1] Chemical Department, Menarini Laboratories, Florence, Italy.
[2] Merck.
[3] Merck 60 F 254.
[4] Mineral light UVS 11.
[5] Beckman DB.
[6] Hellma 108/2 QS.

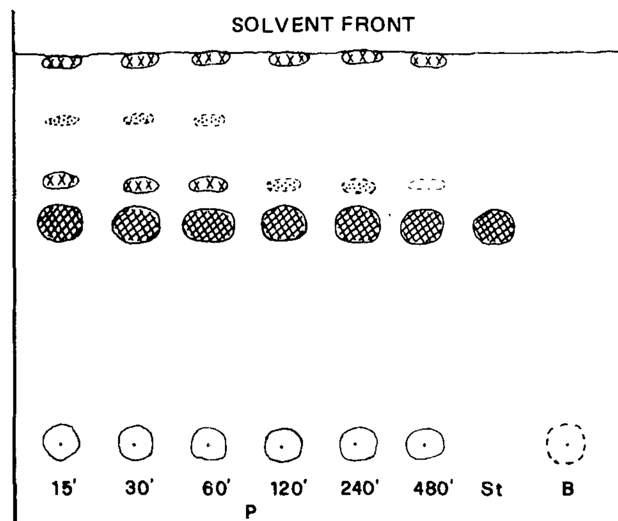


Figure 2—*Thin-layer chromatogram of I. Key: B, 0.1 ml of plasma from untreated rats; St, 0.1 ml of I (250 μg/ml in 96% ethanol); and P, plasma sample obtained at different time intervals from I-treated rats (25 mg/kg po).*

Preparation of Standards—Compound I was dissolved in 96% ethanol. Aliquots equivalent to 2.5, 7.5, 10, and 12.5 μg were placed in glass-stoppered centrifuge tubes and evaporated to dryness under a gentle stream of nitrogen. Then 0.25 ml of plasma from untreated rats was added to each tube and mixed well. These standards and the appropriate blanks were handled in the same manner as the plasma specimens.

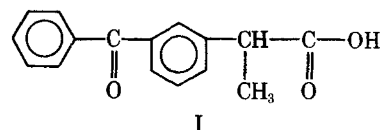
Preparation of Samples—Plasma (0.25 ml) from treated animals, 10 ml of water, and 1 ml of 1 *N* HCl were introduced into a 100-ml separator.

Compound I was extracted with three 40-ml portions of ether. The standards and the appropriate blanks were handled in a similar manner. The pooled extracts were washed with 20 ml of 0.1 *N* HCl and 20 ml of water, dried on sodium sulfate, and flash evaporated to a volume of about 5 ml. This final amount as well as the successive ether washes was transferred to a glass-stoppered centrifuge tube and evaporated to dryness under a gentle stream of nitrogen.

TLC—Each sample was taken up in 0.2 ml of ethanol, and 100-μl aliquots were spotted 2 cm from the edge of the chromatographic plate. Aliquots equivalent to 1.25, 2.5, 5, and 6.25 μg of I standard ethanolic solution were likewise spotted on the plate.

The chromatograms were developed in an ascending system of ether–benzene–1-butanol–methanol (85:8:6:1) in a saturated atmosphere. After 30 min the solvent front was marked, plates were dried, and I spots were visualized by exposure to the UV lamp. These areas were scraped into glass-stoppered centrifuge tubes, eluted with 1.5 ml of ethanol, and shaken every 5 min for 30 min. A similar spotless area (blank) was also scraped off and subjected to the identical procedure. The test tubes were then centrifuged at 4000 rpm for 10 min, and the relative absorbance of the supernates was measured at 255 nm against a blank.

Calculation—Calibration curves were prepared by plotting absorbance at 255 nm against known concentrations of I in plasma or 96% eth-



anol. Values for unknown concentrations of I in plasma specimens were calculated from the slope of the standard curve.

Table I—Recovery of I Added to Rat Plasma or 96% Ethanol

Amount of I Added to Plasma or 96% Ethanol, μg/ml	Amount Recovvered from Plasma, μg/ml	Recovery, %	Amount Recovered from 96% Ethanol, μg/ml	Recovery, %
2.50	2.24	89.6	2.30	92.0
	2.28	91.2	2.28	91.0
	2.30	92.0	2.26	90.0
	2.29	91.6	2.28	91.0
	2.31	92.6	2.30	92.0
	2.30	92.0	2.27	90.0
7.50	6.80	90.7	6.90	92.0
	6.60	88.0	6.60	88.0
	6.75	90.9	6.80	90.7
	6.90	92.0	6.80	90.7
	6.90	92.0	6.70	89.3
	6.70	89.3	6.90	92.0
10	9.10	91.0	9.00	90.0
	9.15	91.5	8.80	88.0
	9.15	91.5	9.15	91.5
	9.12	91.2	9.10	91.0
	8.90	89.0	8.80	88.0
	9.10	91.0	8.90	89.0
		Mean ± *SD* = 90.95 ± 1.22		Mean ± *SD* = 90.35 ± 1.41

Drug Administration to Rats—Male Wistar albino rats, 300–330 g, were divided into six groups of six or seven animals each. Compound I was suspended in an aqueous vehicle consisting of 0.9% sodium chloride, 0.4% polysorbate 80, 0.5% carboxymethylcellulose sodium, and 0.9% benzyl alcohol and administered by gavage at a dose of 25 mg/kg in a constant volume of 5 ml/kg.

Blood samples were obtained by cardiac puncture by means of a heparinized syringe at time intervals ranging from 15 min up to 8 hr after drug administration. Plasma samples were obtained by centrifugation at 2000 rpm for approximately 10 min.

Table II—Precision Data from Determinations in Plasma[a]

Sample	Concentration of I at 15 min, μg/ml	Concentration of I at 480 min, μg/ml
Mean	16.5	38.4
SD	0.45	1.21
RSD	2.75	3.15

[a] Reproducibility limits are $RL = \bar{X} \pm RSD.t_{38;0.05} = \bar{X} \pm 6.01\%$ of $\bar{X}$.

RESULTS AND DISCUSSION

Known amounts of I were added either to plasma or 96% ethanol. The percentage recovery of I from plasma was identical to that from the alcoholic solution spotted directly on the plate (Table I), with an accuracy index of 100%. This method is adequately specific, since it was unaffected by endogenous substances. The values relative to plasma concentration at different time intervals after oral administration of 25 mg/kg of I to male rats are shown in Fig. 1.

Preliminary chromatograms of I added to plasma indicated that recoveries of 90 ± 1.2% were obtained following an elution time of 30 min. Prolongation of the elution time (up to 5 hr) did not improve recovery. Compound I has an R_f value of 0.75 (Fig. 2). No attempt was made to identify the chemical nature of the substances (metabolites) with an R_f greater than that of I. Due to a sufficiently high molar absorptivity ($E^{1\%}_{1\,cm}$ in 96% ethanol or plasma at 255 nm is 640), I can be determined in an amount as low as 1 μg.

To assess the precision of the procedure, 20 determinations were carried out on two plasma samples obtained at 15 and 480 min following I administration (Table II). The relative mean standard deviation was 2.95% and the reproducibility limits were ±6.01%.

The time required to determine the concentration of I in plasma varies according to number of samples, *i.e*, about 3 hr for one sample and 1 day for six samples.

The described method is fast and reliable and does not require expensive instrumentation. Furthermore, it is sensitive enough for the determination of unaltered I in the biological fluids of both experimental animals and humans.

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ˣ To whom inquiries should be directed.