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Note

Micromethod for the determination of indalpine in mouse plasma using high-performance liquid chromatography with electrochemical detection

Y. JOULIN, L. DOARE and B. DIQUET*

Laboratoire de Pharmacocinetique Clinique (Professor P. Simon), CHU Pitie-Salpetriere, 91 Boulevard de l'Hôpital, F 75654 Paris Cedex 13 (France)

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Indalpine, [(indolyl-3)-2-ethyl]-4-piperidine, is a specific inhibitor of the reuptake of 5-hydroxytryptamine (5HT) [1, 2] and is used as a tool for pharmacological studies in animals. It has also been proposed as an antidepressant drug in man, but it is no longer used on the basis of adverse reactions occurring at a therapeutic dose.

A high-performance liquid chromatographic (HPLC) assay with fluorimetric detection [3] has been described. However, this method demands a large volume of plasma (2 ml) and cannot be applied to the determination of drug concentration in small animals. Therefore, we have developed a highly sensitive micromethod for the measurement of plasma levels of indalpine and its metabolite in mice. The method we propose is HPLC with amperometric detection, which requires only a 200-μl sample.

EXPERIMENTAL

Animals and blood-sampling procedure

Male NMRI mice weighing 20—25 g, originating from homogeneous breeding, (R. Janvier, Genest St. Isle, France) were used. Blood was drawn from the orbital sinus and collected in heparinized tubes. Animals were sacrificed and brains were immediately removed. Plasma and brains were frozen and kept at —20°C until assay.

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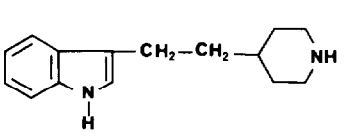
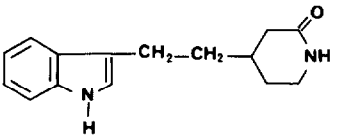
Chemicals and drugs

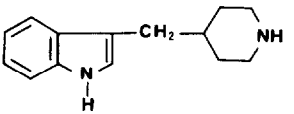
Indalpine, the metabolite 4-[2-(3-indolyl)ethyl]-2-piperidinone and the internal standard (methoxy-5-indolyl-3-methyl)-4-piperidine (Pharmuka, Gennevilliers, France) were used (Fig. 1).



INDALPINE



METABOLITE



INTERNAL STANDARD

Fig. 1. Chemical structures of indalpine, metabolite and internal standard.

Standard solutions of indalpine, metabolite and internal standard were prepared by dissolution in methanol at concentrations ranging from 1 to 10 μg/ml.

All reagents used were of analytical grade: methanol, dichloromethane, sodium hydroxide (Prolabo, Paris, France), acetic acid (Labosi, Paris, France), hexanesulphonic acid (low UV; Waters Assoc., Milford, MA, U.S.A.).

Apparatus

The chromatographic system consisted of a Model 114M solvent delivery pump (Beckman Instruments, Berkeley, CA, U.S.A.) and a Rheodyne sample valve fitted with a 50-μl loop. The column was a Nova-Pak C_{18}, 5 μm particle size, 15 cm × 3.9 mm I.D. (Waters Assoc.).

A Metrohm electrochemical detection system composed of a 641 VA detector and an Ag/AgCl reference electrode was used to oxidize the compounds at a potential of 1000 mV. The sensitivity was set at 10 nA full scale. All chromatograms were recorded on a Servotrace recorder (Sefram, Paris, France) at a chart speed of 5 mm/min.

Mobile phase

The mobile phase consisted of water—methanol—$5 \cdot 10^{-3}$ *M* hexane sulphonic acid (48:52:2), pH 4.2. The solvent mixture was thoroughly degassed and filtered through a 0.2-μm filter disk (Millipore, Bedford, MA, U.S.A.). The flow-rate was kept constant at 0.8 ml/min, corresponding to a pressure of ca. 160 bar.

Extraction procedure

To 200-μl of mouse plasma were added 10 μl of a 1 μg/ml solution of internal standard, 50 μl of 1 *M* sodium hydroxide and 4 ml of dichloromethane. The mixture was shaken for 20 min using an alternating agitator (Realis Labo, Villejuif, France). The solution was then centrifuged for 15—20 min at 2000 *g*, at −4°C. The lower organic phase was transferred to a clean tube and then evaporated to dryness in a water bath at 38°C under a gentle stream of nitrogen. The residue was dissolved in 100 μl of the mobile phase, 50 μl of which were injected into the chromatograph.

Calibration curves

The calibration curves were obtained by adding known amounts of indalpine and metabolite to mouse control plasma. The final concentrations were: 25, 50, 125, 250 and 500 ng/ml. These standards were extracted under the experimental conditions as described above. The peak heights were measured and the peak-height ratios of indalpine and metabolite to internal standard were plotted against concentration.

Extension of the method to brain tissue

This method has been extended to brain tissue from mice. The extraction is processed as follows: 25 μl of a 1 μg/ml solution of internal standard were added to a whole mouse brain; the brain was homogenized with a potter apparatus (Ultraturax, Paris, France) in 1 *M* perchloric acid (3 ml per brain); after centrifugation at 2000 *g* for 15 min, the supernatant was transferred to a clean tube and adjusted to pH 12 with 1 *M* sodium hydroxide and then processed as before.

RESULTS

Fig. 2A and B illustrates typical chromatograms obtained after extraction of blank and spiked plasmas of mice. The respective retention times for indalpine, metabolite and internal standard are 5.8, 7.2 and 3.6 min (capacity coefficients $k' = 3.83$, 5.0 and 2.0) respectively. Fig. 2C illustrates a chromatogram obtained from plasma of mice collected 60 min after intraperitoneal administration of 1 mg/kg indalpine.

Fig. 3 shows a chromatogram obtained after extraction of brain tissue from mice collected 60 min after administration of a 1 mg/kg intraperitoneal dose of indalpine.

The calibration curve is linear over the range 0—500 ng/ml (correlation coefficient 0.9967 ± 0.007). The equations of the curves for indalpine and metabolite are given by $y = 0.0423x \pm 0.015$ and $y = 0.088x \pm 0.036$, respectively.

Precision

The reproducibility of the method was checked at plasma concentrations of 25, 50 and 100 ng/ml for indalpine and 12.5, 25 and 50 ng/ml for the metabolite. Ten determinations of each were made on the same day. The day-to-day reproducibility was assessed at 25, 50 and 100 ng/ml for indalpine and

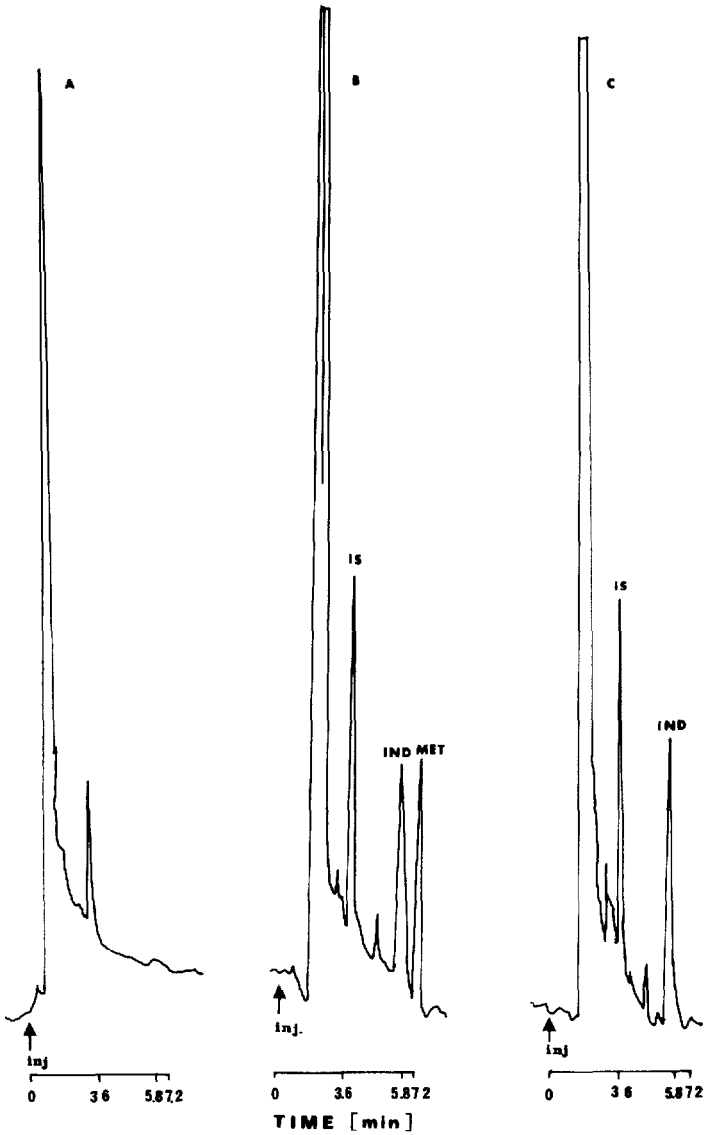
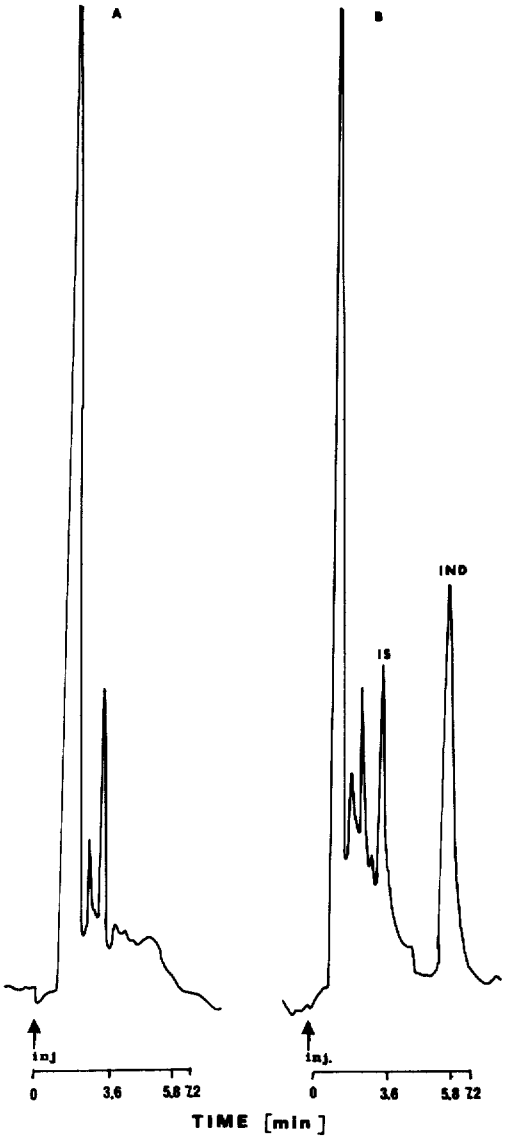


Fig. 2. Typical chromatograms obtained from mouse plasma after injection of (A) blank plasma control; (B) plasma control spiked with internal standard, 20 ng/ml indalpine and 10 ng/ml metabolite; (C) mouse plasma sample obtained 60 min after an intraperitoneal dose of 1 mg/kg. Peaks: IND = indalpine; MET = metabolite; IS = internal standard.

12.5, 25 and 50 ng/ml for metabolite over a period of five days. The coefficients of variation are shown in Table I.

Recovery

Recovery of indalpine and metabolite was estimated by comparing the peak heights after injection of a pure solution of indalpine and metabolite with those obtained after injection of extracted plasma containing the same amount of indalpine and metabolite; the recovery of the extraction procedure (mean ± S.D.) was 70 ± 5.3 and 66 ± 7.1%, respectively.



F9g. 3. Typical chromatograms obtained from brain tissue from mice after injection of (A) blank brain tissue control; (B) mouse brain tissue sample obtained 60 min after a 1 mg/kg intraperitoneal dose of indalpine. Peaks: IND = indalpine; IS = internal standard.

Sensitivity

Under the conditions described in this paper, the limit of detection of indalpine (defined as three times the signal-to-noise ratio) was 200 pg; thus, when 200 μl of plasma are used, the minimum detectable concentration is 2 ng/ml but this could easily be improved by increasing the volume of plasma.

Application of the method

This method has been employed to determine the pharmacokinetics of

TABLE I

REPRODUCIBILITY AND ACCURACY OF HPLC WITH ELECTROCHEMICAL DETECTION FOR INDALPINE AND ITS METABOLITE

Concentration		Coefficient of variation (%)			
Indalpine (ng/ml)	Metabolite (ng/ml)	Reproducibility		Accuracy	
		Indalpine	Metabolite	Indalpine	Metabolite
5	2.5	8.6	8.0	3.1	8.3
10	5	7.9	6.7	2.8	9.2
20	10	7.0	4.1	1.6	9.5

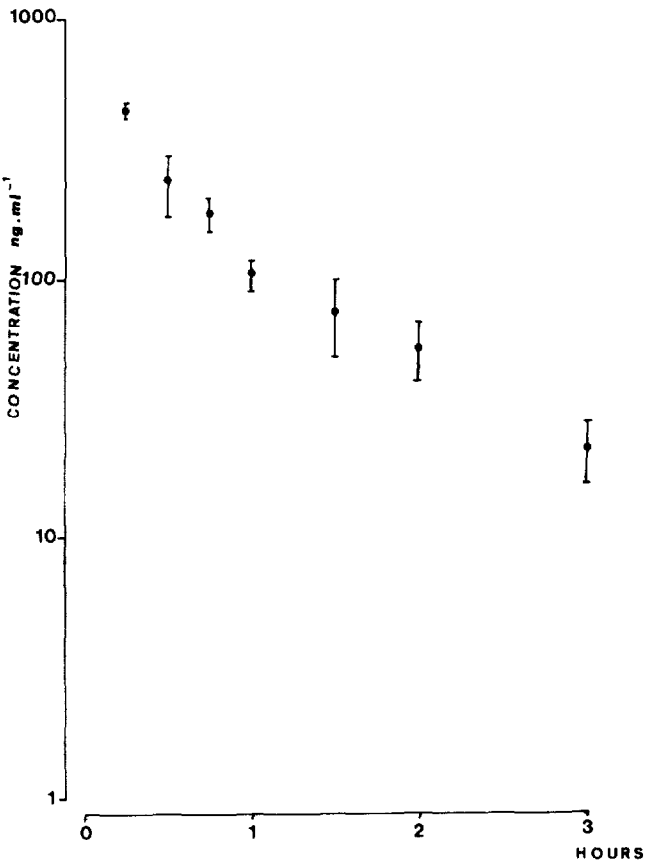


Fig. 4. Plasma levels of indalpine after administration of a 2 mg/kg intraperitoneal dose. Each point represents the mean ± S.D. of five measurements.

indalpine in plasma of mice following an intraperitoneal dose of 2 mg/kg. Intraperitoneal administration of indalpine is followed by a rapid decrease in concentration (Fig. 4); the elimination half-life found in the study is 41 min.

DISCUSSION

Indolamines are currently detected using electrochemical detection [4–6], so the structure of indalpine being related to the structure of serotonin leads us to develop a sensitive method using the same procedure.

The electrochemical method reported in this study has a limit of quantitation as low as 2 ng/ml for only 200 μl of sample, which makes it very sensitive. Compared with the fluorimetric method described by Jozefczak et al. [3], which demands large volumes of plasma, the proposed method requires only small samples and allows individual measurements of indalpine in plasma of mice, so avoiding the need to pool several samples.

In preliminary assays we have ascertained the lack of interference of endogenous compounds, which, owing to their indole structure, could have interfered with the method. So, under the chromatographic conditions described above, serotonin shows a very short retention time and is chromatographed with the unretained compounds. 1,5-Hydroxytryptophan, l-tryptophan and tryptamine eluted before the internal standard and so did not interfere with the analysis of indalpine and its metabolite, even when they were administered at large doses to the animals at the time of pharmacological experiments.

The method using ion-pairing agents has been chosen because of excellent resolution between the three analytes (internal standard, indalpine, metabolite). An ion-pairing agent such as hexanesulphonic acid gives the best resolution, consistent with a short retention time, whereas pentanesulphonic acid, despite good resolution, leads to a long analysis time.

Preliminary assays were realized with a standard μBondapak C_{18} column, but such a column gives poor resolution, the peaks appearing broad. The use of a Nova-Pak column proved to be necessary, this column showing great efficiency and resolution is suitable for the ion-pairing method, but it is not devoid of disadvantages. The lifetime of this column is shorter than a standard one and is liable to variability, which requires the composition of the solvent to be modified in order to maintain good separation.

However, this method represents a highly sensitive, reproducible assay procedure for the pharmacokinetic study of indalpine in mouse plasma and for its determination in whole brain without any additional problems.

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