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Note

High-performance liquid chromatographic determination of imidazole in biological fluids

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Since the discovery of the role of thromboxane A_2 [1] and prostacyclin PGI_2 [2] in the regulation of platelet function, there has been considerable interest in the property of imidazole derivatives that inhibit thromboxane synthetase activity [3]. Consequently a series of 1-alkyl or alkylaryl imidazoles have been studied [4—6]. Most of these derivatives are active at low concentrations, and determination of the plasma levels requires the use of sensitive methodology such as a labelled compound [7] or high-performance liquid chromatography (HPLC) [8].

Imidazole (I) itself is a competitive inhibitor of thromboxane synthetase with a K_i of 10^{-4} *M* [9]. Imidazole 2-hydroxybenzoate (II, see Fig. 1) is a derivative which may exert this activity when administered, because of its content of I, and it has shown anti-inflammatory activity in animal experiments [10]. It therefore became interesting to have a method of determination of I in biological fluids as a tool for the study of its pharmacokinetics and metabolism.

The high solubility of I in water does not allow the use of a two-phase extraction (water and organic solvent) for the isolation of I from biological fluids and, to our knowledge, no methodology for analysis in biological materials has been reported in the literature. In addition, the same methodology should be

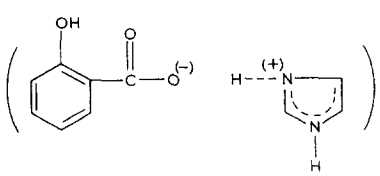

Fig. 1. Chemical structure of imidazole 2-hydroxybenzoate (II).

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useful for the determination of other drugs that can produce I as a metabolite.

Because of the low content of I expected in biological fluids after the administration of II, many of the analytical procedures in the literature [11—13] are unsuitable due to their low sensitivity. We report a sensitive and specific method based on HPLC for monitoring I in biological fluids.

MATERIALS AND METHODS

Analytical grade reagents and spectrophotometric grade eluents were used. Imidazole and salicylic acid were purchased from Merck (Darmstad, F.R.G.) and II was prepared following the procedure previously described [14].

HPLC determination

The HPLC apparatus was a Jasco Model Twincle equipped with a 25-μl loop injector and an ultraviolet (UV) detector (Uvidec 100, equipped with an 8-μl cell). Several columns packed with C_{18} or C_8 reversed phases were assayed with different t_R responses for I.

Using plasma extracts, the Finepack Sil C_{18} column (250 mm × 4.6 mm I.D., 10 μm, reversed-phase C_{18}, Jasco) gave the highest efficiency and was therefore used in determining I in the biological fluid extracts.

Elution times of I were found to be strongly dependent on the pH and ionic strength of the eluent. Satisfactory results were obtained with 0.003 *M* potassium phosphate buffer—methanol (60:40, v/v) titrated at pH 7.2 with phosphoric acid.

The UV absorbance maximum reported for I is at 207 nm (with log ϵ = 3.70), thus the detector was set at 210 nm for monitoring.

Extraction procedure

To 1 ml of plasma, 2.5 ml of acetonitrile were added. The sample was vortexed to favour the precipitation of the protein moiety and after centrifuging at 750 *g* for 10 min, the clear supernatant solution (A) was quantitatively transferred to a conical flask and evaporated to dryness at 35°C under a nitrogen stream. To the residue 200 μl of eluent were added and the opalescent solution was centrifuged at 750 *g* for 5 min; 25 μl of the solution were injected into the HPLC apparatus. When the concentration was higher than 10^{-4} *M*, 25 μl of solution A were used for analysis. Urine samples were diluted twenty times with eluent and a 25-μl volume was injected.

Calibration curves

The calibration curve was made with 1 ml of plasma sample containing up to 10 μg of I. The samples were treated following the above procedure.

The calibration curve for urine was made with 1 ml of centrifuged urine diluted twenty times with the eluent and containing up to 3 μg of I.

Plasma levels and renal excretion

The applicability of the HPLC method for determining I in biological fluids was checked after administration of II to rats. Animals weighing about 250 g were used, and 50 mg/kg of II were administered per os in aqueous solution. Blood was collected at 15, 30, 60, and 120 min.

The same experiment was made on another group of animals and the urine was collected over the period of 0—1, 1—4, 4—8, and 8—24 h after administration. The plasma and urine samples could be stored at −20°C until analysed.

Following a similar blood collection schedule, plasma samples were obtained from human adult volunteers who had taken one 750-mg tablet of II.

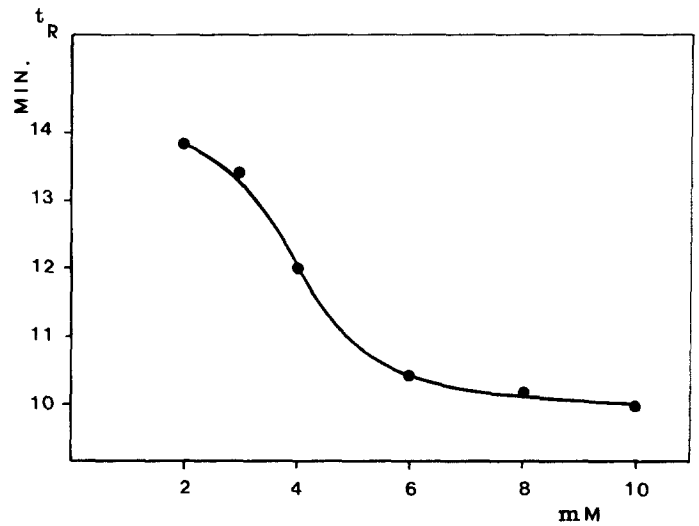

Fig. 2. Dependence of t_R of imidazole on ionic strength. Eluent, phosphate buffer (different molarities)—methanol titrated at pH 7.2 with phosphoric acid; flow-rate, 0.9 ml/min; detector, UV 210 nm.

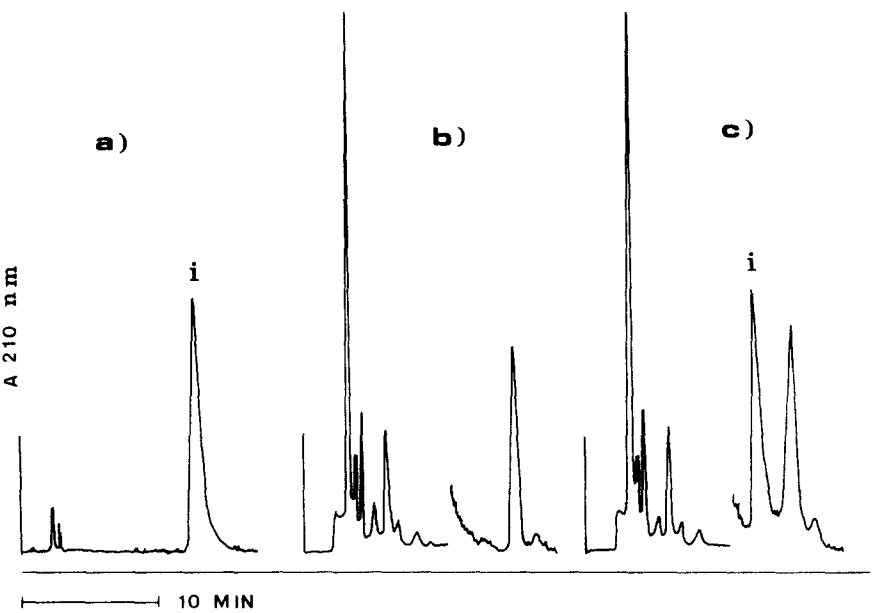

Fig. 3. Chromatograms of imidazole (a), control serum (b) and control serum with 1 μg/ml imidazole added (c). Eluent, phosphate buffer (0.003 *M*)—methanol (60:40, v/v) titrated at pH 7.2 with phosphoric acid; flow-rate, 0.9 ml/min; detector, UV 210 nm.

RESULTS AND DISCUSSION

I can be detected by HPLC using UV absorption in the far region of the UV spectrum where a strong absorption of other plasma components can interfere. Therefore the selectivity of the determination is based mainly on the efficiency of the chromatographic phase and the eluent. The t_R of I was dependent on the pH and ionic strength of the eluent. As Fig. 2 shows, I can be eluted between 10 and 14 min by varying the molarity of the buffer. This allowed the choice of the appropriate molarity of buffer to "drive" the elution of imidazole in the region where the plasma components have low interference. Useful conditions for the plasma samples were found to be 3 m*M* phosphate buffer, as shown in Fig. 3.

The lowest detectable amount was 10 ng for pure imidazole and 25 ng for imidazole in plasma.

Calibration curve

The determination of I added to plasma showed a linear response up to 100 μg/ml with a recovery of 85 ± 5% in the range 0.3—3 μg/ml and 97 ± 7% in the range 10—100 μg/ml.

Plasma levels and renal excretion

The plasma levels of I, after oral administration of II, in rats (50 mg/kg) and in man are exemplified in Fig. 4a and b. The two curves show that I is rapidly absorbed and is largely excreted in unmodified form as Table I illustrates.

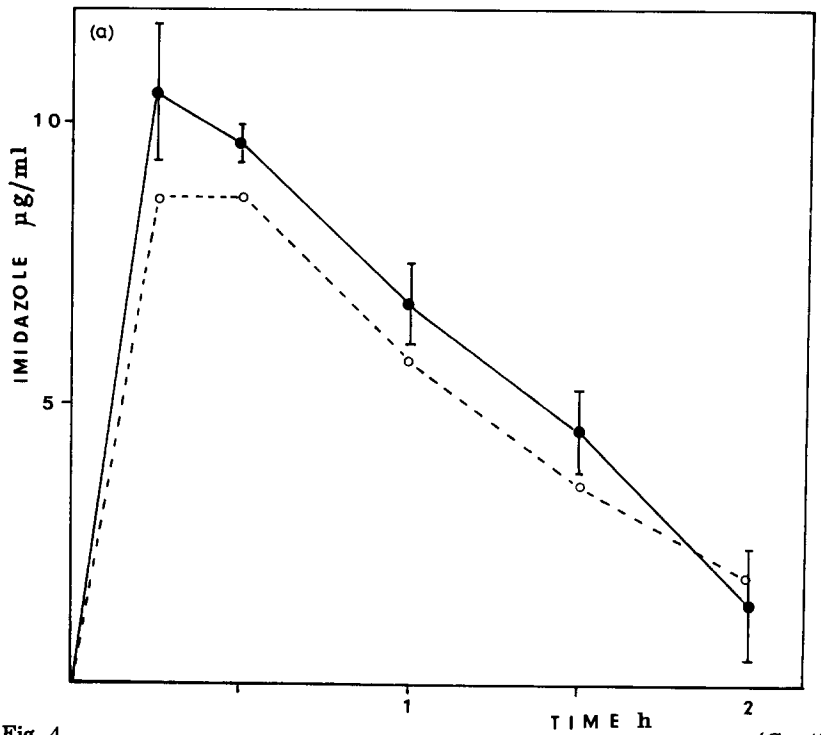

Fig. 4.

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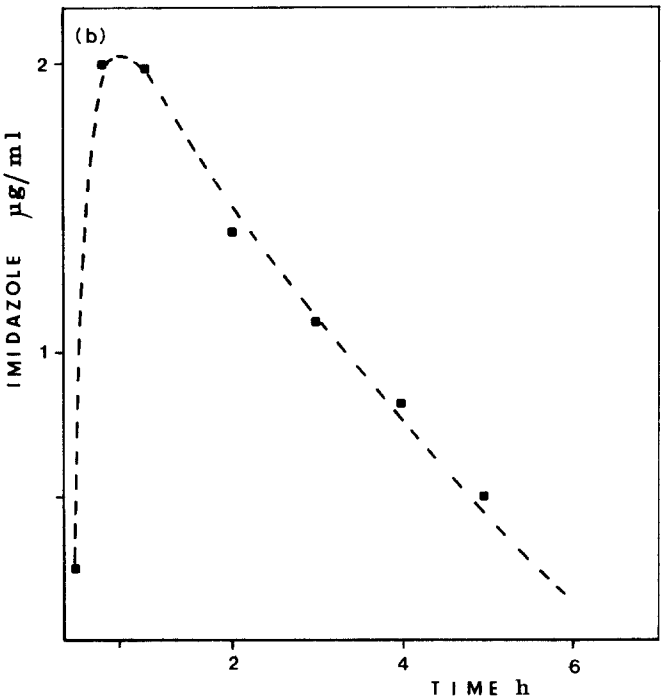

Fig. 4. (a) Plasma samples of imidazole as a function of time. (•) 50 mg/kg of II, and (○) 17 mg/kg of imidazole, administered to rats; plasma was obtained from citrated (10%) blood. Same conditions as Fig. 3. (b) Plasma levels of imidazole as a function of time. Conditions: 750 mg of II were administered to human volunteers; plasma was obtained from citrated (10%) blood. Same chromatographic conditions as Fig. 3.

TABLE 1

HPLC DETERMINATION OF IMIDAZOLE IN URINE

Dosage*	Time intervals (h)	R_1** (%)	± S.D.	± S.E.	R_2*** (%)
A	0— 1	9.08	2.89	1.67	—
A	1— 4	2.65	1.41	0.82	—
A	4— 8	9.16	6.83	3.94	—
A	8—24	1.75	2.27	1.60	22.64
B	0— 1	8.18	5.06	2.92	—
B	1— 4	8.70	0.85	0.49	—
B	4— 8	2.43	2.59	1.49	—
B	8—24	9.36	8.21	4.74	28.67

*Administration of II to rats: 50 mg/kg per os (A); 50 mg/kg intravenously (B).
**R_1 = percentage recovery for each time interval.
***R_2 = percentage recovery for 24 h

CONCLUSIONS

Several methods are reported in the literature for the determination of imidazole derivatives, utilized as drugs, that can be extracted from biological fluids by organic solvents in a two-phase system. For imidazole this procedure did not give good results. With the HPLC methodology reported here, I can be determined in plasma samples with a minimum detectable concentration of $4.5 \cdot 10^{-6}$ *M*. The determination can be applied also to biological fluids other than plasma and is useful for drugs that can give I as a product.

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