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Note

High-performance liquid chromatographic method for the simultaneous determination of iothalamate and *o*-iodohippurate

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The accurate evaluation of renal blood flow (RBF) and glomerular filtration rate (GFR) is often necessary to help physicians, physiologists and pharmacologists interpret clinical and experimental data [1]. To determine these parameters in patients it is preferable to make use of non-invasive and safe methods to avoid unjustified risks and complex diagnostic procedures.

The most widely used methods are those based on the "clearance" of some substance that is completely filtered or excreted by the kidneys [2]. Unfortunately, there are no endogenous substances suitable for accurate evaluation, and it is therefore necessary to administer a suitable compound to the patient before determining GFR and RBF [3].

Inulin and *p*-aminohippuric acid were used for many years before radioactive substances became available. Now, with radioisotopes, the procedure has become simpler and faster [3], but their diagnostic use has increased the risks for patients [4], and requires expensive equipment, more complex facilities and more highly specialized staff. Furthermore, for the simultaneous determination of GFR and RBF, two different isotopes are needed and often it is not easy to distinguish between them [5].

Some methods are described in the literature [6, 7] which outline the procedure for determining sodium iothalamate concentration. However, the sensitivity of these methods is insufficient for an accurate evaluation of GFR unless large amounts of iothalamate are given.

A high-performance liquid chromatographic method for *o*-iodohippurate (OIH) determination has been reported [8], but only for pharmaceutical formulations and not for diagnostic RBF evaluation. Thus we decided to evaluate the feasibility of a new simultaneous method for the determination of GFR and RBF using non-radioactive iothalamate and *o*-iodohippurate.

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EXPERIMENTAL

Chemicals and reagents

Sodium iothalamate (3-acetamido-2,4,6-triiodo-5-methylcarbamoylbenzoic acid, sodium salt) and iodamide (3-acetamido-5-acetamido-methyl-2,4,6-triiodobenzoic acid) were kindly supplied by Bracco (Milan, Italy). *o*-Iodohippurate (OIH) and hippuric acid were obtained, respectively, from Sorin (Saluggia, Italy) and Carlo Erba (Milan, Italy). All other solvents were reagent grade except acetonitrile (LichroSolv) from Merck (Darmstadt, G.F.R.).

Instrumentation

A Perkin-Elmer Series 3B liquid chromatograph with Rheodyne valve was used equipped with a Perkin-Elmer ODS-HC Sil-X-1 reversed-phase column (25 × 0.26 cm, particle size 10 μm). The flow-rate was 1.0 ml/min; the detector was a Perkin-Elmer LC-75 variable-wavelength detector set at 235 nm.

Mobile phase preparation

The mobile phase was prepared by mixing water, acetonitrile and 85% phosphoric acid (960:40:0.3). The solution was filtered through a 0.22 μm Millipore filter (Millipore Corp., Bedford, MA, U.S.A.).

Extraction procedure

Plasma. Plasma samples (0.5—1 ml) were pipetted into glass stoppered tubes and internal standards (18 μg of iodamide and 2 μg of hippuric acid) were added. The samples were acidified with 1 ml of 1 *N* HCl and extracted twice by shaking for 10 min with 5 ml of ethyl acetate each time. After centrifugation, the organic layers were concentrated in a water bath at 60°C under a stream of nitrogen, and then 3 ml of 0.1 *N* NaOH were added. The samples were shaken for 10 min and centrifuged; the organic layer was then discarded. The aqueous layer was acidified with 0.5 ml of 1 *N* HCl and extracted twice with 5 ml of ethyl acetate each time repeating the previous step, and the organic layer was dried in a water bath at 60°C under a stream of nitrogen. The residue was reconstituted with 100 μl of mobile phase and an aliquot of 10—20 μl was injected.

Urine. To 1 ml of urine, 1 mg of iodamide (10 μl of a 10% aqueous solution) was added. Then 100 μl were transferred to a glass stoppered tube containing 1 ml of 1 *N* HCl and extracted twice with 5 ml of ethyl acetate each time. The organic layers were dried, the residue was reconstituted with 400 μl of mobile phase and 10—20 μl were injected.

RESULTS AND DISCUSSION

The retention times of iothalamate, iodamide, hippuric acid and *o*-iodohippurate are 2.0, 2.5, 5.6, 11.9 min, respectively, and a typical chromatogram of plasma spiked with known amounts of drugs is shown in Fig. 1. No interferences were observed in the plasma blank.

The quantitative determination of urinary *o*-iodohippurate and iothalamate was performed by adding known amounts of the drugs, in the range 0—2.0

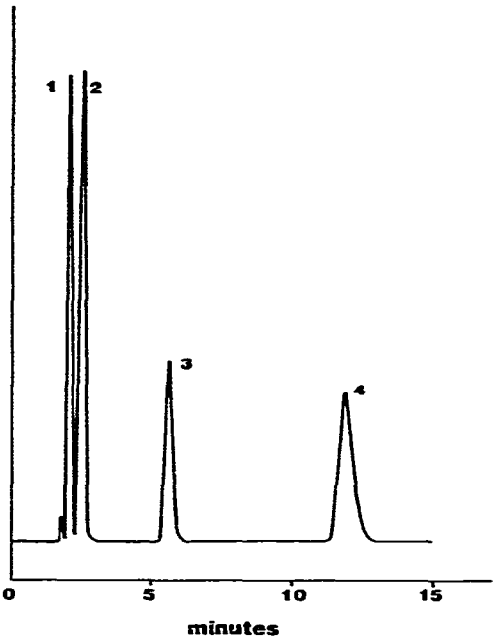


Fig. 1. Chromatogram of an extract of a 1-ml plasma sample spiked with 1 = iothalamate 16 μg; 2 = iodamide 18 μg; 3 = hippuric acid 2 μg; 4 = *o*-iodohippurate 10 μg. The chromatogram was recorded at 0.64 a.u.f.s. for peaks 1 and 2 and at 0.16 a.u.f.s. for peaks 3 and 4.

mg/ml, to urine using iodamide as internal standard for both substances. For plasma determination two internal standards (iodamide and hippuric acid) were used because greater accuracy was obtained using hippuric acid as internal standard for *o*-iodohippurate evaluation. It was not possible to use hippuric acid in urine determinations because it is an endogenous component of urine.

To analyze each 20-point calibration curve, regression analysis was first performed and the correlation coefficient r was obtained (0.999 for both curves). To test for linearity an analysis of variance was performed to isolate the following sources of variation: between concentration (linearity plus deviation from linearity), and within concentration (residual). For iothalamate the F value for deviation from linearity was 2.30 and for OIH it was 1.53. Both values were not significant as the tabled value for $F_{6,12}$ is 3.00. The average coefficient of variation from five standard curves was 6.0% for iothalamate and 6.9% for OIH. The sensitivity of the method was 0.5 μg/ml for iothalamate and 1.0 μg/ml for OIH. The reproducibility of the procedure was evaluated by analyzing replicate plasma samples to which known amounts of drugs had been added. The results are shown in Tables I and II. Good reproducibility was found and the average coefficient of variation was 0.6% for iothalamate and 1.3% for OIH.

The reliability of the method was tested in 15 normal subjects by plasma determination of the two compounds following intravenous administration. An example of the kinetics of the two substances with related parameters is shown in Figs. 2 and 3.

TABLE I

REPRODUCIBILITY AT A GIVEN PLASMA CONCENTRATION FOR IOTHALAMATE

$n = 5$ in all cases.

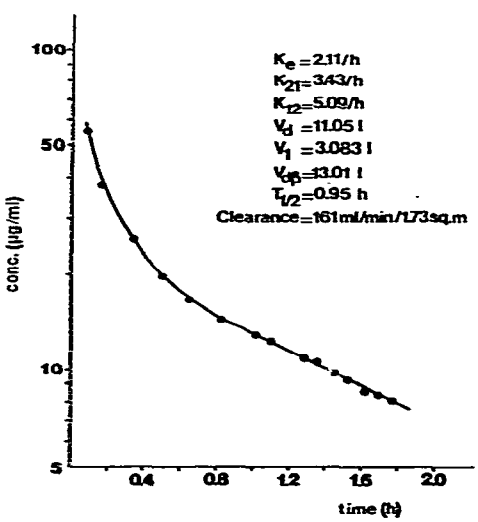
Iothalamate (μg/ml)	C.V. (%)
5	0.6
9	0.4
15	0.3
19.5	1.1
Mean	0.6

TABLE II

REPRODUCIBILITY AT A GIVEN PLASMA CONCENTRATION FOR *o*-IODOHIPPURATE

$n = 5$ in all cases.

Concentration (μg/ml)	C.V. (%)
1.5	1.2
5	0.8
10	1.0
15	2.1
Mean	1.3



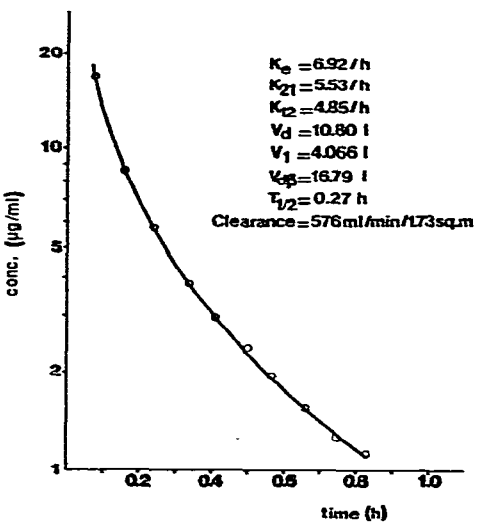


Fig. 2. Serum concentration curve following intravenous administration of iothalamate (364 mg) to a subject with normal renal function.

Fig. 3. Serum concentration curve following intravenous administration of *o*-iodohippurate (149 mg) to a subject with normal renal function.

The method described here has sufficient sensitivity and reproducibility to be used in determining simultaneously plasma concentrations of iothalamate and *o*-iodohippurate following single intravenous injections. Knowing the plasma concentrations of both substances and using the same pharmacokinetic model proposed for radioactive compounds, it is possible to calculate glomerular filtration rate and renal blood flow.

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