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Short Communication

High-performance liquid chromatographic determination of iothalamic acid in human plasma and urine

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ABSTRACT

A simple, rapid and sensitive method for the determination of iothalamic acid (IA) in both plasma and urine is reported. After extraction with ethyl acetate, IA was determined by strong anion-exchange high-performance liquid chromatography with ultraviolet detection at 254 nm. The lower limit of detection was 0.5 μg/ml. The average recovery was 73 and 57% from plasma and urine, respectively. Linearity was found over the investigated concentration range (up to 500 μg/ml for plasma and up to 10.0 mg/ml for urine). The reproducibility of the technique was good (coefficient of variation less than 6%) as was the precision and accuracy (coefficient of variation less than 2.5%). No interference from endogenous substances or any of the common drugs tested was found.

INTRODUCTION

The glomerular filtration rate (GFR) is generally believed to be the best overall index of renal function in health and disease [1–4]. Rigorous assessement of the GFR requires the measurement of renal clearance, utilizing an ideal filtration marker. Inulin, a 5200-dalton polymer of fructose, fulfils the criteria for an ideal filtration marker and is the standard against which other markers are compared [5]. However, the classical inulin clearance method is not practical either in clinical practice or research as inulin is difficult to determine. As a consequence, alternative filtration markers and clearance methods have been developed and validated. The most widely used alternative filtration markers are [^{125}I]iothalamic acid (IA) and [^{99m}Tc]diethylenetriaminepentaacetic acid (DTPA) [6–9]. Both IA and DTPA are almost completely excreted by glomerular filtration. For radioactive compounds there are problems of discarding the radioactive waste and the administration of radioactive materials to humans. These problems would be avoided if methods for measuring unlabelled compounds could be developed. For these reasons a reproducible, rapid and simple high-performance liquid chroma-

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tographic (HPLC) method was developed to determine non-radioactive IA in both plasma and urine for the determination of the GFR.

EXPERIMENTAL

Reagents

IA, 3-(acetylamino)-2,4,6-triiodo-5-[(methylamino)carbonyl] benzoic acid, was kindly supplied by Bracco (Milan, Italy); ethyl acetate and hydrochloric acid were obtained from Merck (Darmstadt, Germany), dichloromethane, potassium dihydrogenphosphate and phosphoric acid from Farmitalia Carlo Erba (Milan, Italy), and HPLC-grade acetonitrile form Omnia Res (Milan, Italy).

Stock solutions of IA were prepared in doubly distilled water to yield final concentrations of 1.0 mg/ml and 100 μg/ml. The solutions were stored at 4°C.

Chromatographic conditions

A Model 342 liquid chromatograph (Beckman, Fullerton, CA, USA), equipped with a Model 160 UV detector (Beckman) operating at 254 nm, was used. A Chromatopac C-R3A integrator (Shimadzu, Kyoto, Japan) was used for the detector output signal. The attenuation of the integrator signal was set at 5 and the detector sensitivity was 0.050 a.u.f.s. The samples were injected using a Promis II autoinjector (Spark Holland, Emmen, Netherlands). The samples were chromatographed on an anion-exchange guard column (Chrompack, Middelburg, Netherlands, 10 mm × 3 mm) connected to a strong anion-exchange high-performance column (Partisil 10 SAX, 250 mm × 4.6 mm, Whatman, Clifton, NJ, USA). The mobile phase consisted of acetonitrile–25 m*M* potassium dihydrogenphosphate, pH 3 with phosphoric acid (15:85, v/v). The flow-rate was 1.0 ml/min.

Analysis of plasma and urine samples

A 500-μl volume of plasma, obtained from blood drawn from an antecubital vein of either healthy volunteers or patients with a progressive decline in renal function was pipetted into disposable polypropylene tubes and acidified with 0.5 ml of 1 *M* hydrochloric acid. The tubes were vortex-mixed for 10 s; 6 ml of ethyl acetate were then added. The tubes were vortex-mixed for a further 20 s and centrifuged at 1700 g for 10 min at 4°C. The organic (upper) layer was separated and evaporated to dryness under a gentle stream of nitrogen. The residue was redissolved in 400 μl of 25 m*M* potassium dihydrogenphosphate (pH 3) and washed with 500 μl of dichloromethane. After a gentle horizontal shaking (to prevent any emulsion forming) for 5 min the tubes were centrifuged at 1700 g for 5 min at room temperature, and 20 μl of the aqueous phase were injected into the liquid chromatograph.

The urine samples were centrifuged and diluted 1:10 with distilled water, then

TABLE I

DRUGS WHICH DO NOT INTERFERE IN THE ASSAY

Furosemide	Hydrochlorthiazide	Chlorthalidone
Nifedipine	Diltiazem	Verapamil
Nicardipine	Trinitrin	Isosorbide
Dinitrate	Atenolol	Propranolol
Captopril	Enalapril	Prazosin
Hydralazine	α-Methyldopa	Clonidine
Digoxin	Digitoxin	Ibopamine
Gemfibrozil	Acipimox	Simvastatin
Aspirin	Salicylic acid	Dipyridamole
Allopurinol	*p*-Amminohippuric acid	Insulin
Inulin		

1 ml of the diluted samples was acidified with 1 ml of 1 *M* hydrochloric acid and treated as described for plasma.

All the biological samples were stored at −20°C until required for analysis.

Drug interference study

To evaluate the accuracy of the assay further, an interference study was conducted using 31 commonly prescribed drugs (Table I). All the drugs were tested at therapeutic concentrations, as given in the current literature.

RESULTS

Fig. 1 shows a typical chromatogram of (A) drug-free plasma, (B) a sample taken form a patient 30 min after the intravenous administration of 3 g of IA, (C) drug-free urine and (D) urine collected between 1 and 2 h after administration of 3 g of IA. The retention time of IA was 7.05 min and both blank plasma and urine gave a baseline that was virtually free of interfering peaks after the initial front. The limit of detection, defined as a signal-to-noise ratio of 3:1, was at least 0.5 μg/ml.

The extraction recovery was 72 ± 3% from plasma and 57 ± 5% from urine. Calibration graphs for both palsma and urine were linear over the concentration range studied (up to 500 μg/ml for plasma, and up to 10.0 mg/ml for urine) and in all instances had correlation coefficients greater than 0.998. By calculating the coefficient of variation of ten determinations at two concentrations obtained 30 and 600 min after the intravenous injection of IA, the precision of the assay was assessed as follows: 159.18 μg/ml, 1.23%; 7.06 μg/ml, 2.05%. The accuracy, 1.38%, was calculated as the percentage error from the true value by processing eight samples containing a known amount of compound.

The validity of the assay was assessed by determining the plasma concentra-

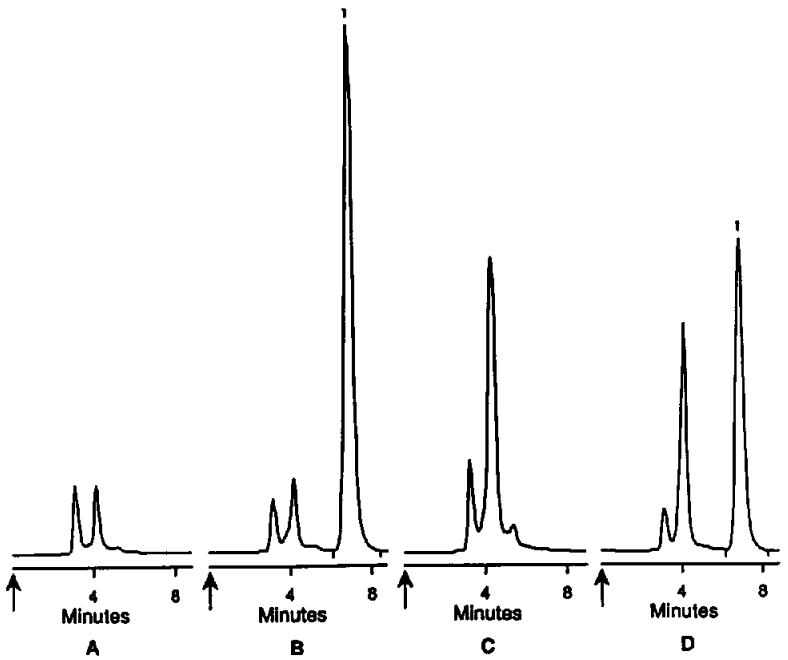

Fig. 1. Chromatogams of (A) blank human plasma, (B) plasma taken from a patient 30 min after the intravenous administration of 3 g of IA, (C) blank human urine and (D) urine collected from the same patient between 1 and 2 h after the intravenous administration of IA. The concentrations found in these samples were 191.46 μg/ml and 5.88 mg/ml IA in plasma and urine, respectively. Peak 1 = IA.

tion and the urinary excretion rate of IA (Fig. 2A) after a 3-g intravenous injection of IA to a patient enrolled in a study for the evaluation of the GFR.

Fig. 2B shows a plot of the rate of excretion of IA *versus* the plasma concentration at the mid point of the urine collection period: the renal clearance of IA equals the slope of the resulting straight line. The mean value was 55.14 ± 2.42 ml/min, which is in good agreement with the clearance value obtained from fitting the IA plasma concentrations (54.14 ml/min) and calculated as clearance = dose/AUC, where AUC is the area under the plasma concentration time curve, determined using the trapezoidal rule.

The results of the drug interference study showed that none of the drugs considered (Table I) interfered with the assay. Indeed, only *p*-aminohippuric acid was detectable after the extraction step and eluted as a sharp peak at about 4 min.

DISCUSSION

[^{125}I]IA has become generally accepted as an accurate marker for measuring the GFR [7,10–12]. This paper reports an analytical method for determining the

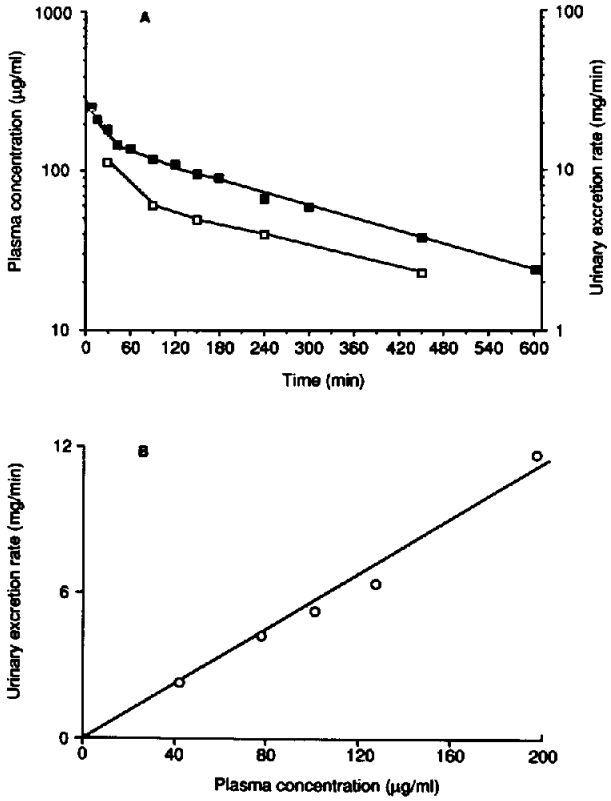

Fig. 2. (A) Plasma concentration profile (■) and urinary excretion rate (□) following the intravenous administration of 3 g of IA to a patient. (B) Relationship between urinary excretion rate of IA and plasma concentration (determined at the mid-point of each urine collection period). The renal clearance equals the slope of the resulting straight line.

unlabelled compound so that no radioactivity is given to the subjects. Although other HPLC methods for determining IA exist [13–16], this method uses an extraction step together with an elution performed by a strong anion-exchange column. This avoids the possibility of interference due to drugs co-administered to patients with renal impairment, as only neutral and acidic compounds are extracted, with the neutral drugs eluted in the solvent front. Salicylic and *p*-aminohippuric acids are extracted by ethyl acetate, but the former is removed by the washing step with dichloromethane and the latter elutes a considerable time before IA.

In all the previously published methods, which use reversed-phase columns [13–15] or a reversed-phase column combined with an ion-pairing technique [16] for the determination of IA, an internal standard was employed. A suitable internal standard was not found for this method; however, a good overall performance of the assay was obtained. Moreover, automatic injection of the samples

improved the precision of the method, although the precision was still acceptable with manual injection.

The extraction recovery was dependent on the ratio between the organic solvent and the aqueous phase and this could explain the lower value for urine compared to plasma. However, the recoveries obtained were adequate for these purposes.

The described procedure is routinely used in this laboratory, and because the extraction and drying steps can be completed in about 1.5 h and the chromatographic analysis time is about 9 min, 25–30 plasma and/or urine samples can be processed and analysed within an 8-h working day, even when manual injection is used.

It is concluded that the proposed method is adequate and has sufficient sensitivity and reproducibility for the determination of the GFR in both healthy volunteers and patients with a progressive decline in renal function following a single intravenous injection of IA.

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