Journal of Chromatography, 525 (1990) 401–409
Biomedical Applications
Elsevier Science Publishers B V., Amsterdam — Printed in The Netherlands

CHROMBIO 5067

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF IOMEPROL IN PLASMA AND URINE

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(First received July 17th, 1989; revised manuscript received October 6th, 1989)

SUMMARY

A high-performance liquid chromatographic method for assaying the radiographic contrast agent iomeprol in plasma and urine samples is described. Before reversed-phase chromatography, the biological fluids are treated with ion-exchange resins and iopamidol is added as internal standard. The compounds are monitored during elution by ultraviolet–visible spectrometry at 245 nm. The method shows good precision and accuracy and gives similar results to X-ray fluorescence analysis.

INTRODUCTION

Iomeprol, N,N′-bis(2,3-dihydroxypropyl)-5-[(hydroxyacetyl)methylamino]-2,4,6-triiodo-1,3-benzenedicarboxamide (Fig. 1), is a non-ionic, water-

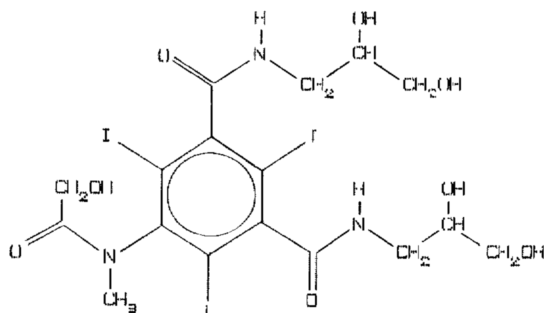

Fig 1. Structure of iomeprol.

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soluble, radiographic contrast agent used for urography and angiography [1]. Concentrations of this class of iodinated diagnostic agents in biological samples can be determined assaying the total iodine content by spectrophotometric techniques [2], X-ray fluorescence (XRF) analysis [3,4] or radiochemical methods [5]. Although these methods are satisfactory if one can be certain that all the iodine has remained in the original chemical species, they are not adequate when this is in doubt and then an assay specific for the compound is required. The high-performance liquid chromatographic (HPLC) method described here allows the selective determination of iomeprol in urine and plasma samples.

EXPERIMENTAL

Apparatus

A Merck–Hitachi chromatographic system was used, consisting of a 655 A-11 liquid chromatograph, controlled by an L-5000 LC gradient programmer, fitted with a variable-wavelength UV detector and linked with a D-2000 integrator. Analyses were performed on a reversed-phase column (Merck Hibar LiChrosorb RP-18, 25 cm×4 mm I.D., particle size 5 μm) inside a thermostated oven. A precolumn (Merck LiChroCART, 3 cm×4 mm I.D., filled with LiChrosorb RP-8, particle size 7 μm) was used to avoid degradation of the analytical column.

Materials

Iomeprol and iopamidol, (*S*)-N,N′-bis-[2-hydroxy-1-(hydroxymethyl)-ethyl]-5-[(2-hydroxy-1-oxopropyl)amino]-2,4,6-triiodo-1,3-benzenedicarboxamide, used as an internal standard, were synthesized by Bracco (Milan, Italy). The ion-exchange resins used for desalting and pH correction of the samples were Amberlite IR-45 and Amberlite IR-120 (Rohm and Haas), but other equivalent ion-exchange resins could be used. The weak anion-exchange resin Amberlite IR-45 was converted to the basic form and washed and the strong cation-exchange resin Amberlite IR-120, acid form, was washed only with water. Excess of water in both were eliminated by short suction filtration. The solvents used for HPLC were 0.0125 M KH_2PO_4 solution filtered through a Millipore filter (0.45 μm pore size) and acetonitrile (HPLC grade). All other chemicals were of analytical-reagent grade.

Biological samples

The samples were prepared from the following biological fluids: human plasma obtained by heparinization and centrifugation of whole blood taken from healthy subjects maintained on a normal diet; human urine obtained from healthy subjects maintained on a normal diet; human plasma and urine taken

from patients dosed intravenously with iomeprol solution (816.4 mg/ml) at a dose of 2.041 g/kg.

Standard solutions

Iomeprol stock solutions for the assay in plasma were prepared by dissolving the anhydrous compound in distilled water to obtain solutions in the concentration range 0.1–50 mg/ml. Iomeprol working standards for the assay in urine were obtained dissolving the compound directly in urine from healthy human subjects to give concentrations of 0.02–50 mg/ml. Aqueous solutions of anhydrous iopamidol at concentrations of 2 and 5 mg/ml were used as internal standard solutions for the assay in plasma and urine, respectively. The standard solutions and the biological samples were stored at 4 °C and proved stable for more than one month.

Preparation of plasma samples

Calibration standard solutions were prepared by adding 0.1 ml of each aqueous iomeprol stock solution and 0.1 ml of 2 mg/ml aqueous iopamidol solution to 1-ml aliquots of plasma taken from healthy subjects. These samples were treated with 0.1 ml of 70% perchloric acid to precipitate plasma proteins. After agitation and subsequent centrifugation (10 min at 3500 *g*), to a 1-ml portion of supernatant were added 1.2 g of Amberlite IR-45, basic form, and 0.4 g of Amberlite IR-120, acid form. The suspension was agitated for 30 min at room temperature and centrifuged for 5 min at 3500 *g*. These amounts of ion-exchange resins were sufficient to adjust to neutrality the pH of the solutions and to eliminate substances interfering with the chromatographic separation. The clear supernatant was used for chromatographic analysis. For monitoring of the iomeprol content of plasma from patients treated with the contrast agent, 0.1 ml of distilled water, instead of the same volume of iomeprol stock solution, was added to 1 ml of plasma, then the sample was processed as described above.

Preparation of urine samples

Portions of 1 ml of urine working standard solutions were supplemented with 0.1 ml of 5 mg/ml aqueous iopamidol solution and 0.05 ml of glacial acetic acid was added, followed by the ion-exchange resins (1 g of Amberlite IR-45 and 0.9 g of Amberlite IR-120). The suspension was diluted to 10 ml with distilled water and, after agitation for 30 min at room temperature, centrifuged (5 min at 3500 *g*). Also in this instance the amounts of resins were sufficient to adjust to around neutrality the pH of the solutions and to eliminate the interferences of urinary salts with the chromatographic separation. Aliquots of clear supernatant were injected into the liquid chromatograph. Urine samples from patients treated with iomeprol were processed as described above for the working standard solutions. Calibration standards and samples with io-

meprol contents above 0.2 mg/ml were diluted with water to a final volume of 20 ml in order to obtain correct peak shapes.

Chromatographic conditions

The conditions used for the chromatographic run were the same for urine and plasma samples. The volume injected was 10 μl. Elution was carried out isocratically with 0.0125 *M* KH_2PO_4 solution–acetonitrile (95:5, v/v) at a flow-rate of 1 ml/min. The temperature of the thermostated oven containing the column was set at 40°C. The detection wavelength was 245 nm.

X-ray fluorescence analysis

The chromatographic method was cross-validated by running parallel XRF assays [3] on the same samples and on the same days. The samples were prepared by diluting biological fluids with ethanol before X-ray measurements. The measurements were performed using a Philips PW 1410 wavelength-dispersion spectrometer with a chromium target X-ray tube, a LiF 200 analysing crystal and a gas-flow proportional detector.

Data processing

The peak-area ratios of iomeprol to iopamidol were calculated for standard solutions, and a least-squares regression was performed in linear and natural log–log scales on these data. The model was used to interpolate with inverse prediction the iomeprol content of clinical samples.

Two standard solutions (0.06 and 5 mg/ml in urine and 0.06 and 2.5 mg/ml in plasma) were used to evaluate the precision and accuracy of the method. The assays were repeated five times and accuracy was calculated as the percentage difference between the mean calculated concentrations and the nominal contents iomeprol of the solutions. Precision was estimated from the relative standard deviation expressed as a percentage of the mean. Method detection limits (MDL) were generated as described by Inman and Rickard [6] by varying $n_{\mathrm{replicates}}$ in the equation

$$\mathrm{MDL} = \frac{k\sqrt{2}}{\sqrt{n_{\mathrm{replicates}}}} \cdot S_{\mathrm{m}}$$

where k is a proportionality constant, corresponding to the z value of the normal distribution for the desired confidence level, and S_{m} is the method's standard deviation, expressed as concentration and calculated on fifteen replicates of samples with concentrations of 0.004 mg/ml for both plasma and urine.

RESULTS

Assay of iomeprol in plasma

Fig. 2 shows representative chromatograms of (a) a plain human plasma extract, (b) a spiked sample (working standard) of human plasma extract containing 0.1 mg/ml iomeprol and (c) a plasma extract from a patient who had received iomeprol. The retention times for iomeprol and the internal standard (iopamidol) were 5.4 and 3.8 min, respectively.

The best correlation model between the peak-area ratios (iomeprol to iopamidol) and the concentrations of analyte was obtained by using a linear calibration in the range 0.01–0.1 mg/ml and a log–log fitting for samples with analyte contents between 0.1 and 4 mg/ml. Table I reports the precision and accuracy data of the method for analyses made on three different days. Detec-

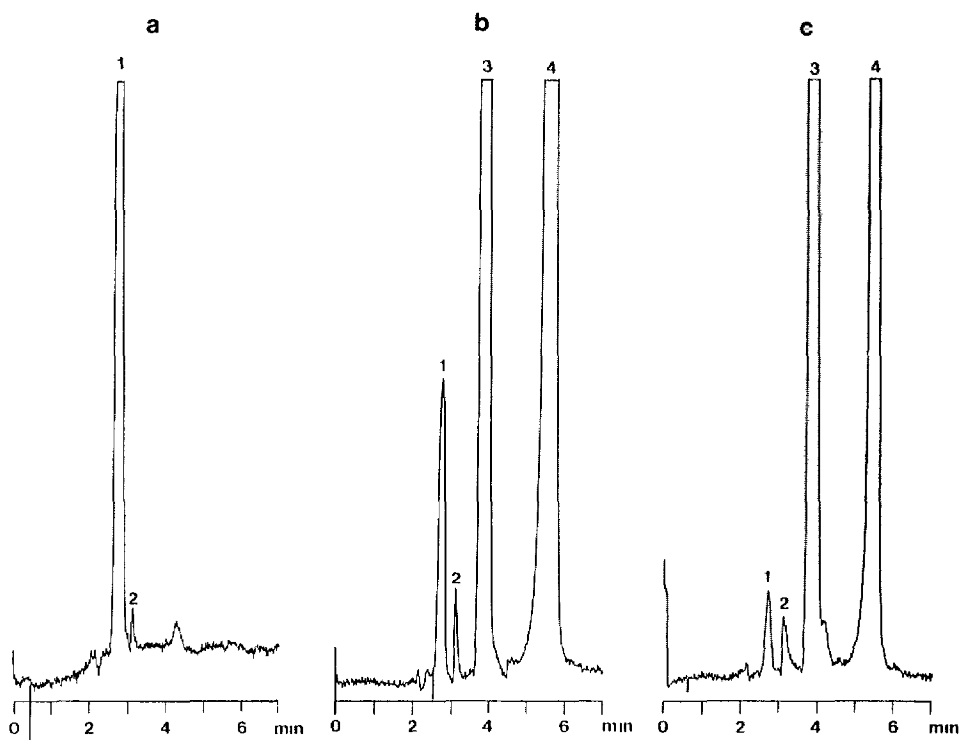

Fig 2. High-performance liquid chromatograms of human plasma extract containing iomeprol, monitored by absorbance at 245 nm Peaks 1 and 2 are due to undefined substances remained in plasma after sample preparation. Peak 3 is due to iopamidol (internal standard) and peak 4 is iomeprol (a) Human plasma extract from a healthy subject maintained on a normal diet; (b) human plasma extract spiked with iomeprol (0.1 mg/ml); (c) extract of a plasma sample from a human patient 8 h after intravenous iomeprol administration (dose 2.041 g/kg body weight); iomeprol concentration 0.057 mg/ml.

TABLE I

VARIABILITY OF PRECISION AND ACCURACY IN THE ASSAY OF IOMEPROL IN PLASMA

Concentration added (mg/ml)	Day	Concentration found (mean, $n=5$) (mg/ml)	Precision (C.V.,%)	Accuracy (%)
0.06	1	0.0586	2.29	−2.33
	2	0.0596	1.50	−0.67
	3	0.0600	2.36	0.00
2.5	1	2.5032	1.30	+0.13
	2	2.4780	0.87	−0.88
	3	2.4280	0.67	−2.88

TABLE II

METHOD DETECTION LIMIT IN PLASMA

$MDL = \frac{k\sqrt{2}}{\sqrt{n_{replicates}}} \cdot S_m$; S_m = method standard deviation = 0.9 μg/ml; $k=2$.

$n_{replicates}$	MDL (μg/ml)	$n_{replicates}$	MDL (μg/ml)
1	2.6	4	1.3
2	1.8	5	1.2
3	1.5	10	0.8

tion limits for assays performed on multiple replicates of plasma sample are reported in Table II.

Assay of iomeprol in urine

Fig. 3 shows the chromatograms of (a) untreated human urine, (b) a spiked sample (working standard) of human urine containing 0.02 mg/ml iomeprol and (c) urine from a subject treated intravenously with iomeprol. Iomeprol showed a retention time of ca. 5.3 min and the internal standard 3.7 min.

Also in this instance a double regression model was used: a linear fit for concentrations up to 0.2 mg/ml and a natural log–log fit for samples with higher iomeprol contents, in which dilution to a final volume of 20 ml was required. Table III reports the precision and accuracy data of the method for analyses made on three different days. Detection limits for assays performed on multiple replicates of urine sample are reported in Table IV.

Comparison with X-ray fluorescence analysis

The results of parallel XRF assays are reported in Table V.

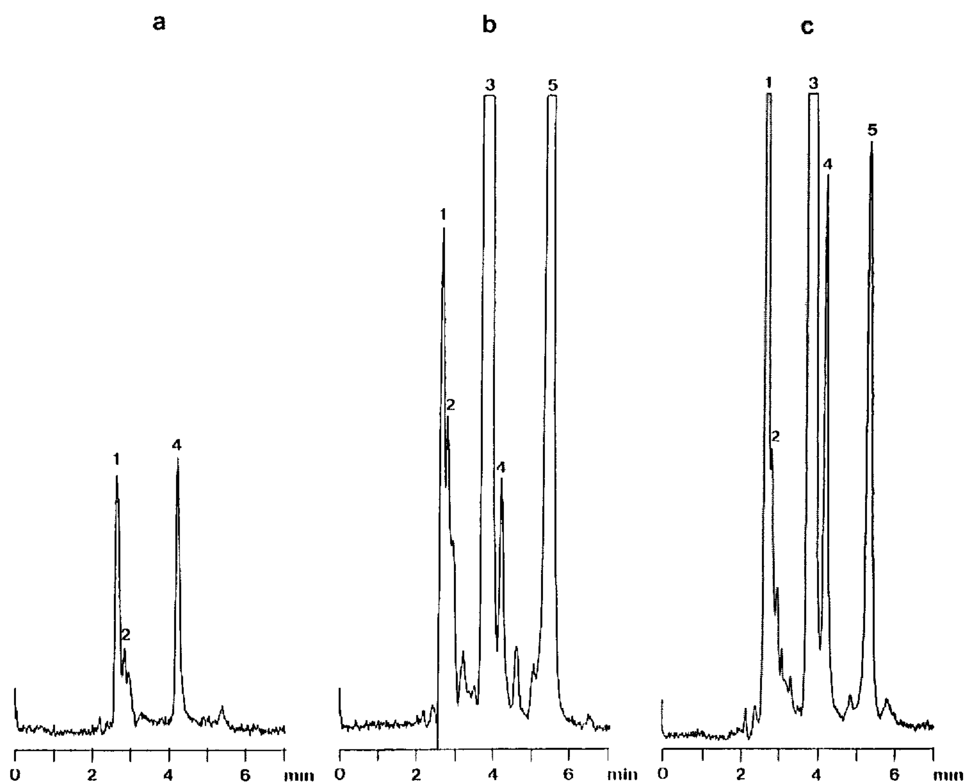

Fig. 3 High-performance liquid chromatograms of human urine extract containing iomeprol, monitored by absorbance at 245 nm. Peaks 1, 2 and 4 are due to undefined substances remained in urine after sample preparation; peak 3 is due to iopamidol (internal standard) and peak 5 is iomeprol. (a) Human urine extract from a healthy subject maintained on a normal diet; (b) human urine extract spiked with iomeprol (0.20 mg/ml); (c) extract of a urine sample from a human patient 72 h after intravenous iomeprol administration (dose 2.041 g/kg body weight); iomeprol concentration 0.032 mg/ml.

TABLE III

VARIABILITY OF PRECISION AND ACCURACY IN THE ASSAY OF IOMEPROL IN URINE

Concentration added (mg/ml)	Day	Concentration found (mean, $n=5$) (mg/ml)	Precision (C.V.,%)	Accuracy (%)
0.06	1	0.0608	1.38	+1.33
	2	0.0578	0.77	−3.67
	3	0.0606	1.48	+1.00
5	1	5.133	1.49	+2.66
	2	5.226	0.86	+4.52
	3	5.102	0.64	+2.04

TABLE IV

METHOD DETECTION LIMIT IN URINE

$MDL = \frac{k\sqrt{2}}{\sqrt{n_{replicates}}} \cdot S_m$, S_m = method standard deviation = 0.5 µg/ml; k = 2.

$n_{replicates}$	MDL (µg/ml)	$n_{replicates}$	MDL (µg/ml)
1	1.4	4	0.7
2	1 0	5	0 6
3	0 8	10	0 4

TABLE V

ASSAY OF IOMEPROL IN BIOLOGICAL FLUIDS: COMPARISON OF HPLC AND XRF

Values in parentheses are mean percentages ± S.D of the nominal concentrations.

Biological fluid	Concentration added (mg/ml)	Concentration found (mean ± S.D., n=5) (mg/ml)	
		HPLC	XRF[a]
Plasma	0.06	0 0601 ± 0.0012 (100.1 ± 2 0)	0.0598 ± 0.0013 (97.7 ± 3.9)
	2.50	2.4275 ± 0.0175 (97.1 ± 0 7)	2.4725 ± 0.0250 (98.9 ± 1.0)
Urine	0.06	0.0577 ± 0.0004 (96.2 ± 0 7)	0.0616 ± 0.0005 (102.6 ± 0.9)
	5.00	5.1350 ± 0.0750 (102.7 ± 1.5)	5.0490 ± 0.0650 (100.98 ± 1 3)

[a]XRF measurements were made under the conditions stated in the text. Iodine was by assayed measuring its $L\alpha_1$ emission line (102 95° 2θ).

CONCLUSIONS

The HPLC method for the assay of iomeprol in plasma and urine provided the specificity required for the determination of the drug in the presence of other iodinated compounds. It is rapid, precise and accurate. Parallel assays using the proposed method and XRF analysis showed the two methods to be similar in precision and accuracy. Also, the detection limits of the proposed procedure are comparable with those of XRF. The proposed technique has the advantages of being specific for iomeprol, more practical for routine analyses and using equipment more generally available.

ACKNOWLEDGEMENT

We are grateful to C. De Häen for his kind encouragement and helpful discussions.

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