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High-performance liquid chromatographic determination of the X-ray imaging contrast agent, iofratol, in plasma and urine

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Abstract

Iofratol is currently under evaluation as a potential X-ray contrast medium for angiography and myelography. An HPLC method for assaying iofratol in rat and human plasma and urine samples is described. The analysis is based on the reversed-phase chromatographic separation of iofratol and the internal standard (iopamidol) from the endogenous components of biological fluids, and detection by UV absorption at 242 nm. The selectivity of the method was satisfactory. The mean absolute recovery was greater than 90%. The precision and accuracy of the analytical methods were in the range 0.8–7.4 and −7.8 to +9.7%, respectively. The detection limits in plasma (0.1 ml) and urine (0.5 ml) were 0.1 and 0.4 μg (iofratol)/ml, respectively. The analyte was stable in the different biological matrices when stored at room temperature (20°C) for at least 1 day, 4°C for 1 month and −20°C for 1 year. © 1997 Elsevier Science B.V.

Keywords: Iofratol

1. Introduction

Iofratol is [*S*-(*R**,*R**)]-*N*,*N*″-(2-hydroxy-1,3-propanediyl)bis[*N*′ - [2-hydroxy-1-(hydroxymethyl)-ethyl]- 5 -[(2-hydroxy-1-oxopropyl)amino]-2,4,6-triiodo-1,3-benzenedicarboxamide] (Fig. 1). This dimeric iodinated compound is currently under evaluation as a potential X-ray contrast medium for angiography and myelography [1].

Concentrations of diagnostic agents containing iodine in biological samples can be determined by ultraviolet spectrophotometry [2], by assaying the total iodine content by radiochemical methods [3], including neutron activation [4–6], or by catalytic determination based on the Sandell Kolthoff reaction [7,8]. Other methods include inductively coupled plasma-atomic emission or mass spectrometry [9,10], X-ray fluorescence [11], laser spectroscopy [12] and direct potentiometry by sensitive iodine electrode [13]. Despite their reliability, these techniques are not molecular specific; they are unable to distinguish

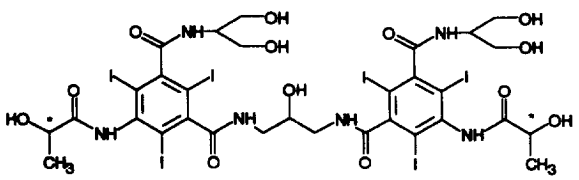

Fig. 1. Structural formula of iofratol molecular mass, 1462.08 u.

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the contrast agent and the various chemical species of iodine present in the sample (parent compounds and potential metabolites).

The high-performance liquid chromatographic (HPLC) method described here enables the selective determination of iofratol in plasma and urine samples of rat and human origin. Because the assays in plasma and urine required sample pretreatment, an internal standard method of calibration was employed using iopamidol as an internal standard (I.S.). The analysis is based on the reversed-phase chromatographic separation of iofratol and I.S. from the endogenous components of the biological fluids and their detection during elution by ultraviolet light absorption at 242 nm.

2. Experimental

2.1. Apparatus

The assays were performed on a Merck–Hitachi (Tokyo, Japan) liquid chromatograph which consisted of a Model L-7100 pump and a Model L-7200 autosampler. The chromatographic system was fitted with a Model L-4500A diode array UV–Vis detector (10 mm flow-cell path-length) linked to a Merck–Hitachi work station. Analyses were performed on a LiChrosorb RP-8 reversed-phase column (25 cm×4 mm I.D., particle size 5 μm) (Merck, Darmstadt, Germany) housed in a thermostated oven. A LiChrosorb RP-8 precolumn (3 cm×4 mm I.D., particle size 7 μm) (Merck) was used to prevent contamination of the analytical column.

2.2. Materials

Iofratol and iopamidol, chemically (*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 I.S., were synthesized by Bracco (Milan, Italy). Purified water was obtained with a Millipore Milli-Q water purification system (Bedford, MA, USA). Analytical-grade potassium dihydrogenphosphate, perchloric acid (35–36%, v/v), glacial acetic acid and HPLC-grade acetonitrile were obtained from Merck. The ion-exchange resins Duolite A-30B weak base anion exchanger, chloride form, and Amberlite IR-120 strong acid cation exchanger, acid form, were obtained from Rohm & Haas (Philadelphia, PA, USA) and Merck, respectively. The weak anion-exchange resin Duolite A-30B was first converted into the basic form by washing with 5 *M* sodium hydroxide solution and then washed with water. The strong cation-exchange resin Amberlite IR-120 was washed with water only. Excess water was eliminated from both resins by short suction filtration. Sodium heparin solution (5000 IU/ml) was obtained under the name of Liquemin® from Hoffman-La Roche (Wyhlen, Germany).

2.3. Biological samples

Rat and human plasma and urine samples were prepared. The plasma samples in both instances were prepared from whole blood which had been collected in test tubes containing sodium heparin solution (5000 IU/ml) at a ratio of about 1:50 (v/v) with blood and then centrifuged (10 min at 3500 *g*). Blank samples of plasma and urine were obtained from control CD® (SD) BR rats (Charles River, Lecco, Italy) and from CD® (SD) BR rats to which a 300-mg (iodine)/ml solution of the iodinated contrast medium had been administered at a dose of 300 mg(iodine)/kg. Samples of blank human plasma and urine were obtained from healthy subjects. Test samples of human plasma and urine were prepared by adding aliquots of contrast medium stock solution to the desired blank samples. Method development was carried out in biological fluids taken from rats and humans while the validation study was performed on rat species only. The stability of iofratol in plasma and urine was investigated both in rat and human samples.

2.4. Stock standard solutions

Iofratol stock standard solutions were prepared by dissolving iofratol powder with purified water to give concentrations ranging from 0.0030 to 6.7 mg/ml for assays in plasma and from 0.065 to 20 mg/ml for assays in urine.

Aqueous solutions of iopamidol at concentrations of 2.0 and 5.0 mg/ml were used as I.S. for the assays. Stock standard solutions were stored in darkness at room temperature (20°C). Under these conditions, they were stable for at least 2 months.

2.5. Preparation of plasma samples

Each calibration standard solution was prepared by adding 30 μl of the desired stock standard solution and 30 μl of iopamidol (I.S.) at concentrations of 2.0 mg/ml to 100 μl of blank plasma from rats or humans. To this sample 30 μl of perchloric acid (35–36%) was added to precipitate the plasma proteins. After agitation and subsequent centrifugation (10 min at 3500 *g*), 10 μl of the clear supernatant was injected into the chromatograph. To determine the iofratol content in the plasma of rats treated with the contrast medium formulation (300 mg(iodine)/kg), 30 μl of purified water rather than the equivalent volume of contrast medium stock solution and 30 μl of the I.S. solution were added to 100 μl of plasma. The sample was then processed as described above.

2.6. Preparation of urine samples

Each calibration standard solution was prepared by adding 100 μl of the desired contrast medium stock standard solution to 1 ml of rat or human urine previously diluted 1:2 with purified water and centrifuged (15 min at 4500 *g*). To this sample, 100 μl of I.S. solution (iopamidol 5.0 mg/ml), 100 μl of glacial acetic acid and portions of ion-exchange resins (1 g Duolite A-30B and 0.9 g Amberlite IR-120) were added. The suspensions were diluted to 5 ml with purified water and, after agitation for 30 min at room temperature, centrifuged (5 min at 3500 *g*). Ten μl of each supernatant were used for the chromatographic analysis. To determine the iofratol content in the urine of rats treated with the contrast medium formulation (300 mg(iodine)/kg), 100 μl of the I.S. solution and 100 μl of purified water rather than the equivalent volume of contrast medium stock solution was added to 1 ml of centrifuged urine. This was then treated as described above.

2.7. Chromatographic conditions

The chromatographic conditions were the same for both the plasma and urine samples. Elution was carried out isocratically with a 95:5 (v/v) mixture of 0.05 *M* KH_2PO_4 (pH 4.5) and acetonitrile at a flow-rate of 1 ml/min. The mobile phase was filtered through a 0.45-μm Millipore filter (Millex-HV) and degassed before use. The temperature of the thermostated oven containing the column was set at 45°C. The UV detection wavelength was 242 nm. The injection volume was 10 μl. The areas of the chromatographic peaks relative to iofratol and iopamidol (I.S.) were integrated and used to calculate the analytical response ratio.

2.8. Sample preparation for stability study

The stability of iofratol was tested in rat and human plasma and urine over the range of concentrations of pharmacokinetic relevance. The samples required for the stability study were prepared by adding aliquots of the aqueous stock solutions to the blank biological fluids from rats and humans such that the added volume did not exceed 1% (v/v) of the total. Blank plasma and urine samples were spiked with iofratol stock solutions to give four concentrations ranging from 10 to 2.0×10^3 and from 30 to 2.0×10^3 μg/ml, respectively. These samples were stored in similar plastic test-tubes to those in which freshly collected samples are usually kept. The standard samples were prepared from a freshly made up stock solution using the same substance batch and the same kind of biological fluid as used in the preparation of test samples.

Stability tests were assessed by means of the HPLC method previously described, after storing the samples under the following conditions: room temperature, at about +20°C for 24 h in light, 4°C for 1, 7, and 30 days in darkness, and −19°C for 1, 3, 7, and 12 months in darkness. The stability of the contrast agent was determined by comparing the analytical data obtained for the stored samples with those of freshly prepared standards. The application of a statistical procedure that takes into account the precision of the analytical method and the level of degradation that is pharmacokinetically relevant enabled the stability profile of iofratol to be defined.

Additionally, the stability of iofratol in processed samples of plasma and urine stored for 24 h in the autosampler at room temperature (20°C) in darkness was investigated.

2.9. Data processing

2.9.1. Selectivity

The selectivity of the chromatographic method was evaluated by checking for interference from drug-free plasma and urine of rats and humans [14]. Furthermore selectivity was verified by analysis of the peak purity which was performed by comparison of three UV spectra recorded for samples taken at the beginning, apex and end of the iofratol and I.S. elutions.

2.9.2. Stability

The stability of iofratol in biological matrices of rat and human was assessed by means of a statistical procedure which is based on the percentage difference in concentration between stored and freshly prepared samples. A degradation of 10% is considered pharmacokinetically relevant. To this end, for each concentration level five replicates of stored samples were analyzed together with five freshly spiked samples representing 100% standard controls. For each time point and concentration level the percent relative difference ($D\%$) between the geometric means of responses (GM) was obtained. According to Timm et al. [15] such a difference is defined as:

$$D\% = \frac{[[\mathrm{GM(stored\ sample)} - \mathrm{GM(std\ sample)}]}{\mathrm{GM(std\ sample)}] \times 100}$$

The stability of the analytes was evaluated by computing a 90% confidence interval for $D\%$ and by adopting the conditions reported below:

(a) When 0% is included in the confidence interval:

(a1) if -10% is excluded from the confidence interval the compound is stable;

(a2) if -10% is included in the confidence interval the stability of the compound is in question.

(b) When 0% is excluded from the confidence interval:

(b1) if the interval lies within -10% and 0% the compound is stable;

(b2) if the interval includes -10% the stability of the compound is in question;

(b3) if the interval includes only values less than -10% the compound is unstable;

(b4) if the interval lies within 0% and $+10\%$ the compound is stable.

(c) When the interval includes $+10\%$, it is necessary to evaluate the possible reasons for the significant increase in the analytical response for the stored sample. If necessary, the experiment must be repeated.

2.9.3. Recovery

The recovery study was performed on three replicates using three standard solutions at concentrations ranging from 6.0 to 1.90×10^3 and from 50.0 to 1.91×10^3 μg/ml, respectively, for plasma and urine of rat. Absolute recovery was measured as the analytical response (peak area) for a processed spiked matrix standard and expressed as a mean percentage of the response for pure standard which had not been subjected to sample treatment [14]. Absolute recovery of the I.S. was assessed for nine replicates at concentrations of 600 μg/ml for plasma and 500 μg/ml for urine.

2.9.4. Linearity

Linearity was evaluated for nine concentrations ranging from 1.0 to 2.00×10^3 and from 6.5 to 2.04×10^3 μg/ml, respectively, for plasma and urine of rat. For the analyses of both biological fluids the analytical response ratio (y) of the iofratol peak area to the I.S. (iopamidol) peak area was calculated and plotted for each concentration (x) relative to each calibration standard solution. A least-squares linear regression was then performed [16]. Since the variances of peak areas at different concentrations showed strong heterogeneity, the weighting factors $1/\mathrm{var}(y|x)$ were introduced improving homogeneity of variances. A standard regression line was then fitted and the regression parameters (slope and intercept when significantly different from zero) and goodness of fit (correlation coefficient) calculated [16]. Punctual estimates of concentrations were determined by inverse interpolation.

2.9.5. Precision and accuracy

The evaluation of precision and accuracy of the analytical system was performed using five standard solutions for each biological fluid at concentrations ranging from 1.0 to 1.90×10^3 and 6.5 to 1.90×10^3 μg/ml, respectively, for plasma and urine of rat. The assays were repeated five times on three different days separated by variable amounts of time ranging from 1 to 10 days. Precision was expressed as the percentage standard deviation (s_r(%)) of the analytical method (peak area) [16]. Accuracy was evaluated by calculating the percentage difference between the estimated and the true concentrations of iofratol solutions [16]. For each day, the range and the mean of absolute values were determined.

2.9.6. Detection limit

The detection limits, expressed as concentration c_L, for iofratol in plasma and urine of rat, were estimated as described by IUPAC [17,18], using the equation

$$c_L = k \times s_b / S$$

where k is a constant corresponding to the 95th

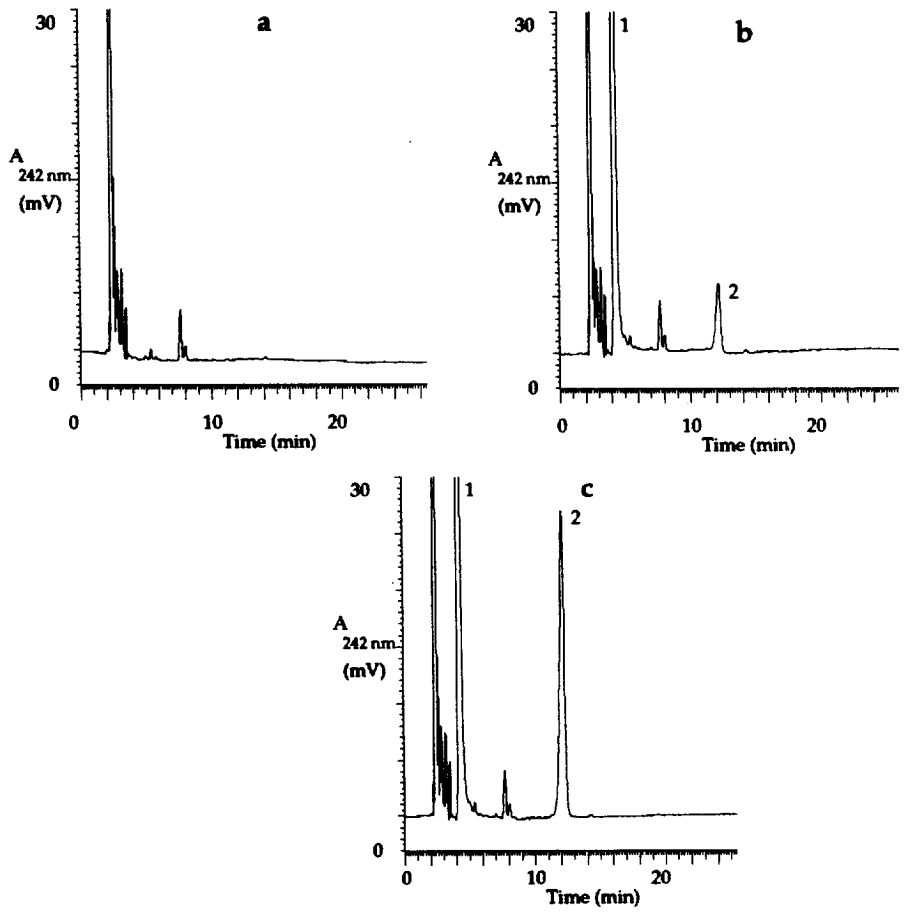

Fig. 2. High-performance liquid chromatograms of rat plasma extract monitored at 242 nm. Peak 1 is due to iopamidol (I.S.) and peak 2 is iofratol. All other peaks are due to undefined substances remaining in plasma after sample preparation. (a) Drug-free plasma extract from a rat maintained on a control diet; (b) rat plasma extract spiked with iopamidol (I.S.) and iofratol (13.5 μg/ml); (c) extract of a plasma sample from a rat 45 min after intravenous contrast media administration (dose 300 mg (iodine)/kg body weight). The iofratol concentration (60.0 μg/ml; sample diluted 20× with drug-free plasma) was estimated by interpolation on the standard curves.

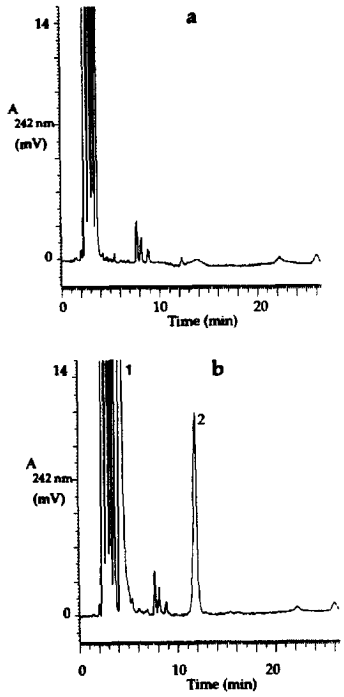

Fig. 3. High-performance liquid chromatograms of human plasma extract monitored at 242 nm. Peak 1 is due to iopamidol (I.S.) and peak 2 is iofratol. All other peaks are due to undefined substances remaining in plasma after sample preparation. (a) Drug-free plasma extract from a healthy subject; (b) human plasma extract spiked with iopamidol (I.S.) and iofratol (27.0 μg/ml).

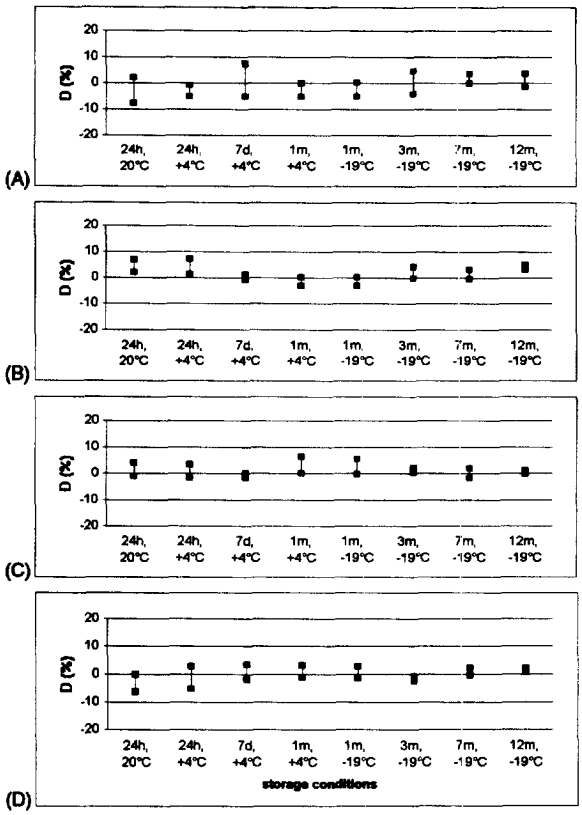

Fig. 4. Graphic representations of the results relating to the stability study performed at four concentrations of iofratol in rat plasma samples: (A) 10, (B) 100, (C) 1.00×10^3, and (D) 2.00×10^3 μg/ml. $D(\%)$ is the percentage relative difference between the geometric means of responses relative to the stored and standard samples.

percentile of the Student's t-distribution with $(n-2)$ d.f., s_b is the blank standard deviation calculated experimentally by analyzing 10 replicate blank samples, expressed as peak height and S is the sensitivity of the calibration model that corresponds to the slope (b) of the calibration line. In this case the regression line was performed by plotting the analytical response of the iofratol peak height (y) against concentration (x) of each calibration standard solution.

The software employed for statistical data processing was SYSTAT (Version 5 Edition; SYSTAT, Evanston, IL, USA, 1992) for personal computer.

Table 1
Chromatographic parameters for iofratol and iopamidol (I.S.) peaks in plasma and urine

Biological fluid	Analyte	Retention factor ($t_0=2.0$ min)	Plate number/m ($\times10^4$)	Peak symmetry	Peak resolution
Plasma	Iofratol	5.1 (C.V. 0.95%, $n=99$)	2.7	0.8	20.5
	I.S.	1.1 (C.V. 0.45%, $n=99$)	3.7	1.1	
Urine	Iofratol	5.0 (C.V. 1.5%, $n=99$)	2.6	0.8	20.3
	I.S.	1.1 (C.V. 1.1%, $n=99$)	3.9	1.1	

Table 2
Absolute recoveries for iofratol and iopamidol (I.S.) in plasma and urine

Biological fluid	Analyte	Concentration tested (μg/ml)	Mean absolute recovery (%)
Plasma	Iofratol	6.0	99.4 ($n=3$)
		951	97.2 ($n=3$)
		1.90×10^{3}	93.0 ($n=3$)
	I.S.	600	101.5 ($n=9$)
Urine	Iofratol	47.8	92.2 ($n=3$)
		956	91.5 ($n=3$)
		1.91×10^{3}	90.4 ($n=3$)
	I.S.	500	87.9 ($n=9$)

Table 3
Linearity of standard curves for iofratol in plasma and urine (33 observations for each curve): analysis days 1, 2 and 3 were separated by variable amounts of time

Biological fluid	Day	$a\pm s_a^a$	$b\pm s_b^b$	r^c
Plasma	1	$6.3\times10^{-5}\pm1.5\times10^{-5}$	$171.100\times10^{-5}\pm0.016\times10^{-5}$	1.0000
	2	0	$163.50\times10^{-5}\pm0.17\times10^{-5}$	1.0000
	3	0	$166.50\times10^{-5}\pm0.28\times10^{-5}$	0.9999
Urine	1	$-4.8\times10^{-4}\pm2.3\times10^{-4}$	$214.30\times10^{-5}\pm0.72\times10^{-5}$	0.9998
	2	$-9.4\times10^{-4}\pm1.9\times10^{-4}$	$215.6\times10^{-5}\pm1.2\times10^{-5}$	0.9995
	3	$-1.29\times10^{-3}\pm0.27\times10^{-3}$	$223.80\times10^{-5}\pm0.77\times10^{-5}$	0.9998

Equation from linear regression: $y=a+bx$.
[a] Intercept±standard deviation.
[b] Slope±standard deviation.
[c] Correlation coefficient.

Table 4
Precision and accuracy for the assay of iofratol in plasma: analysis days 1, 2 and 3 were separated by variable amounts of time

Concentration tested (μg/ml)	Day	Precision ($n=5$), s_r (%)	Accuracy ($n=5$)	
			Range (%)	Mean of absolute values (%)
1.0	1	4.3	−7.8 to +3.6	3.2
	2	4.8	−1.2 to +8.7	3.8
	3	2.4	−0.84 to +5.6	2.0
2.0	1	0.99	−1.1 to +1.2	0.84
	2	3.6	−3.1 to +6.6	4.5
	3	2.0	−4.5 to +0.30	1.9
50.1	1	0.84	−0.21 to −2.2	0.99
	2	2.0	−3.1 to +6.6	1.8
	3	2.4	−5.1 to +1.3	2.7
952	1	4.4	−7.7 to +4.2	3.5
	2	1.7	−0.92 to +2.5	1.3
	3	0.85	−2.3 to −0.29	0.85
1.90×10^{3}	1	1.2	−2.4 to +0.57	0.87
	2	1.1	+0.049 to +2.8	0.87
	3	1.8	−2.1 to +2.9	1.2

3. Results

3.1. Assay of iofratol in plasma

Fig. 2 shows representative chromatograms of (a) a pre-dose sample taken from a rat, (b) a spiked sample (calibration standard) of rat plasma containing iofratol, and (c) a plasma sample taken from a rat to which iofratol had been administered. Typical chromatograms of iofratol in human plasma samples are shown in Fig. 3. The chromatographic parameters of iofratol and I.S. peaks were calculated according to USP [19,20] and are reported in Table 1. No interfering peaks at the retention times of iofratol and the I.S. were detected in the chromatograms of 10 blank plasma samples from rats and humans. Evaluation of the 90% confidence interval of the percent relative difference ($D\%$), which was performed for each analyte concentration level for both the stored and standard samples, showed values which fit cases (a1) and (b4). Therefore iofratol can be considered stable in rat and human plasma. An example of graphic representation of the obtained results for rat plasma is shown in Fig. 4. After 24 h at room

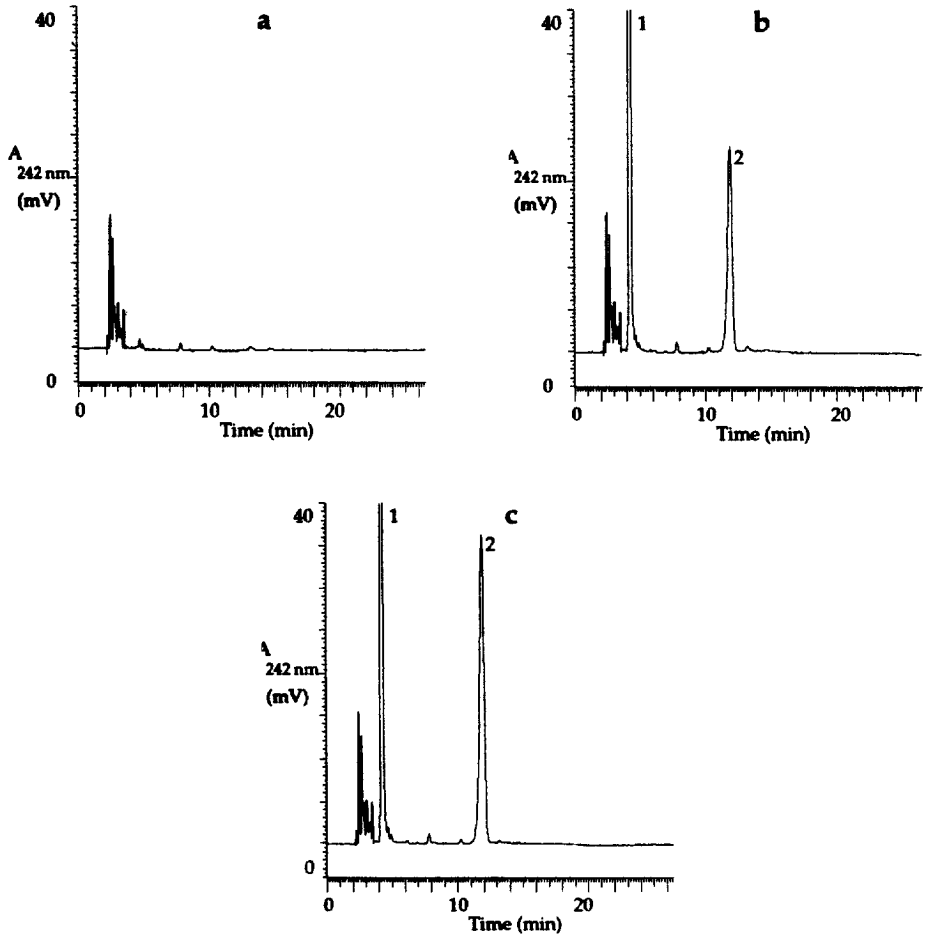

Fig. 5. High-performance liquid chromatograms of rat urine extract monitored at 242 nm. Peak 1 is due to iopamidol (I.S.) and peak 2 is iofratol. All other peaks are due to undefined substances remaining in urine after sample preparation. (a) Drug-free urine extract from a rat maintained on a control diet; (b) rat urine extract spiked with iopamidol (I.S.) and iofratol (100 μg/ml); (c) extract of a urine sample from a rat 0–45 min (collection time) after intravenous contrast media administration (dose 300 mg (iodine)/kg body weight). The iofratol concentration (156 μg/ml; sample diluted×200 with drug-free urine) was estimated by interpolation on the standard curves.

temperature in darkness there was no sign of degradation in the processed plasma samples stored in the autosampler. The absolute recovery of iofratol, which was obtained for concentrations from 6.0 to 1.90×10^3 μg/ml, was in the range from 93.0 to 99.4%. Table 2 shows data for the recovery of iofratol and I.S. The best correlation between the peak area ratios (iofratol to I.S.) and the concentration of iofratol was obtained over the range from 1.0 to 2.00×10^3 μg/ml. The coefficient of correlation (r) and the regression parameters of the standard curves, which were fitted to data on three different days, are shown in Table 3. Table 4 reports data for the precision and accuracy of the estimated concentrations for analyses performed on three different days. Detection limit for assays performed on plasma samples was in the range from 0.075 to 0.086 μg/ml (Table 6).

3.2. Assay of iofratol in urine

Fig. 5 shows representative chromatograms of (a) a pre-dose sample taken from a rat, (b) a spiked sample (calibration standard) of rat urine containing iofratol, and (c) a urine sample taken from a rat to which iofratol had been administered. Typical chromatograms of iofratol in human urine samples are shown in Fig. 6. The chromatographic parameters of iofratol and I.S. peaks were calculated according to USP [19,20] and are reported in Table 1. No interfering peaks at the retention times of iofratol and the I.S. were detected in the chromatograms of 10 blank urine samples of rat and human origin. Evaluation of the 90% confidence interval of the percent relative difference (D%), which was performed for each analyte concentration level for both the stored and standard samples, showed values which fit cases (a1), (b1) and (b4). Two samples of human urine containing iofratol at concentrations of 30 and 1000 μg/ml, which were stored for 7 days at +4°C and 12 months at −19°C, gave confidence intervals which may have been indicative of instability in that biological fluid (case (b2)). However, the results obtained for other concentration levels and at that particular concentration at the following time point (1 month, 4°C), verified the absence of any degradation. Iofratol in rat and human urine can thus be considered stable. After 24 h at room temperature in

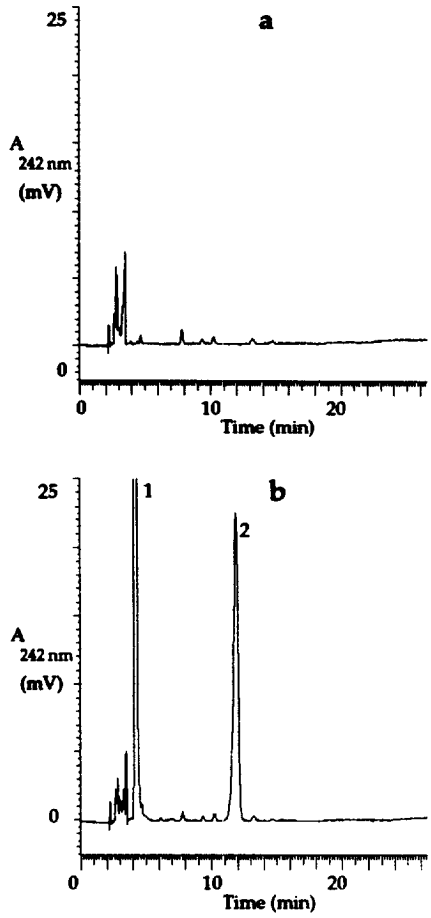

Fig. 6. High-performance liquid chromatograms of human urine extract monitored at 242 nm. Peak 1 is due to iopamidol (I.S.) and peak 2 is iofratol. All other peaks are due to undefined substances remaining in urine after sample preparation. (a) Drug-free urine from a healthy subject; (b) human urine extract spiked with iopamidol (I.S.) and iofratol (100 μg/ml).

darkness there was no sign of degradation in the processed urine samples stored in the autosampler. The absolute recovery of iofratol, which was obtained for concentrations from 47.8 to 1.91×10^3 μg/ml, was in the range from 90.4 to 92.2%. Table 2 shows data for the recovery of iofratol and I.S. The best correlation between the peak area ratios (iofratol to I.S.) and the concentration of iofratol was obtained over the range from 6.5 to 2.04×10^3 μg/ml. The coefficient of correlation (r) and the regression parameters of the standard curves, which were fitted to data on three different days, are shown in Table 3.

Table 5
Precision and accuracy for the assay of iofratol in urine: analysis days 1, 2 and 3 were separated by variable amounts of time

Concentration tested (μg/ml)	Day	Precision ($n=5$), s_r (%)	Accuracy ($n=5$)	
			Range (%)	Mean of absolute values (%)
6.5	1	3.9	−5.9 to +4.1	2.8
	2	2.5	−2.5 to +3.4	1.8
	3	1.5	−1.1 to +2.2	1.1
12.2	1	5.2	−3.6 to +9.7	3.0
	2	1.4	−1.7 to +1.6	1.0
	3	7.4	−8.6 to +8.7	6.8
47.8	1	1.3	−1.8 to +1.2	1.1
	2	2.2	−5.9 to −1.4	4.0
	3	1.2	−4.9 to −2.3	3.4
956	1	2.0	−3.2 to +2.3	1.4
	2	3.4	−6.4 to +1.7	2.5
	3	2.7	−6.5 to +0.78	3.2
1.91×10^3	1	1.1	−2.1 to −0.60	0.73
	2	1.4	−1.6 to +2.2	1.1
	3	0.79	−5.0 to −2.9	3.8

Table 5 reports data for the precision and accuracy of the estimated concentrations for analyses performed on three different days. Detection limit for assays performed on urine samples was in the range from 0.43 to 0.48 μg/ml (Table 6).

4. Discussion

On the basis of the results obtained, iofratol can be considered stable in the different biological matrices when stored under the above conditions. Since the chromatographic peaks for iofratol and the I.S. are completely separated from any other peak in plasma and urine samples from rats and humans, the described assays can be said to have excellent selectivity. The stability of the processed samples and the absolute recovery, linearity, precision, accuracy and detection limits of the described methods are satisfactory for pharmacokinetic studies of iofratol in large animals at clinically relevant doses. The methods are also suitable for similar studies in small animals because the amount of plasma and urine required does not limit the number of data points obtainable from individual animals. The fact that the proposed techniques are rapid and can be performed on readily available equipment makes them suitable for routine use.

Table 6
Detection limits (c_L) for iofratol in plasma (≥0.1 ml) and urine (≥0.5 ml): analysis days 1, 2 and 3 were separated by variable amounts of time

Biological fluid	Day	c_L (μg/ml)
Plasma	1	0.086
	2	0.081
	3	0.075
Urine	1	0.44
	2	0.43
	3	0.48

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References

[1] F. Luzzani, C. de Haën, Drugs Future 20 (1995) 1120.
[2] F. Medzihradsky, P.J. Dahlstrom, T.B. Hunter, J.R. Thor-

nbury, T.M. Silver, Invest. Radiol. 10 (1975) 532.
[3] O. Muratore, S. Saitta, G. Mallarini, P. Corvisiero, M. Sanzone, Experientia 39 (1983) 119.
[4] M. Langenauer, U. Kraehenbuehl, A. Wyttenbach, Anal. Chim. Acta 274 (1993) 253.
[5] M. Dermely, Z. Slejkovec, A.R. Byrne, P. Stegnar, S. Hojker, M. Porenta, G. Sestakov, Analyst 117 (1992) 443.
[6] M.M. Mason, V.L. Spate, J.S. Morris, C.K. Baskett, T.P. Cheng, C.L. Reams, L. Le Marchand, B.E. Henderson, L.N. Kolonel, J. Radioanal. Nucl. Chem. 195 (1995) 57.
[7] A.S.K. Ayiannidis, A.N. Voulgaropoulos, Analyst 113 (1988) 153.
[8] G. Aumont, J. Tressol, Analyst 111 (1986) 841.
[9] A. Agut, F. Laredo, M.A.S. Valverde, J. Murciano, M. del Carmen Tovar, Invest. Radiol. 30 (1995) 296.
[10] P. Allain, Y. Mauras, C. Dougé, L. Jaunault, T. Delaporte, C. Beaugrand, Analyst 115 (1990) 813.
[11] J. Pavel, M. Fille, U. Frey, Fresenius Z. Anal. Chem. 331 (1988) 51.
[12] V.S. Burakov, A.V. Isaevich, P. Misakov, P.A. Naumenkov, S.N. Raikov, J. Anal. At. Spectrom. 9 (1994) 307.
[13] G.A. Rechnitz, M.R. Kresz, S.B. Zamochnick, Anal. Chem. 38 (1966) 973.
[14] T. Karnes, G. Shiu, P. Vinod, Pharm. Res. 8 (1991) 421.
[15] U. Timm, M. Wall, D. Dell, J. Pharm. Sci. 74 (1985) 972.
[16] IUPAC Pure Appl. Chem. 66 (1994) 595.
[17] IUPAC Anal. Chem. 48 (1976) 2294.
[18] Analytical Methods Committee Analyst 112 (1987) 199.
[19] USP, XXIII ed., p. 1774.
[20] IUPAC Pure Appl. Chem. 65 (1993) 819.