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High-performance liquid chromatographic determination of the magnetic resonance imaging contrast agent gadobenate ion in plasma, urine, faeces, bile and tissues

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Abstract

The gadobenate ion is an intravascular paramagnetic contrast agent for magnetic resonance imaging. An HPLC method for assaying gadobenate ion in plasma, urine, faeces, bile and tissue samples is described. The analysis is based on the reversed-phase chromatographic separation of gadobenate ion from the endogenous components of biological matrices and detection by UV absorption at 210 nm. The selectivity of the method was satisfactory. The mean absolute recovery was greater than 95%. The precision and accuracy of the analytical methods were in the range 0.1–6.5% and −12 to +9.3%, respectively. The detection limits in plasma (0.1 ml), urine (0.05 ml), dried faeces (200 mg suspended in 4 ml water), bile (0.5 ml), and dried liver tissue (100 mg suspended in 1 ml water) were, respectively, 0.24, 0.47, 2.6, 0.63 and 2.8 nmol ml^{-1} (corresponding to 0.16, 0.31, 1.7, 0.42 and 1.9 $\mu g\ ml^{-1}$).

Keywords: Gadobenate ion

1. Introduction

Gadobenate dimeglumine (Gd-BOPTA-Dimeg), is (4*R*,*S*) [4-carboxy-5,8,11-tris (carboxymethyl)-1-phenyl-2-oxa-5,8,11-triazatridecan-13-oato(5-)] gadolinate (2-) dihydrogen compound with 1-deoxy-1-(methylamino)-D-glucitol (1:2) (Fig. 1) 1.60 [1,2]. This compound is an intravascular paramagnetic contrast agent for magnetic resonance imaging [3,4]. The gadobenate ion (Gd-$BOPTA^{2-}$) is the moiety of

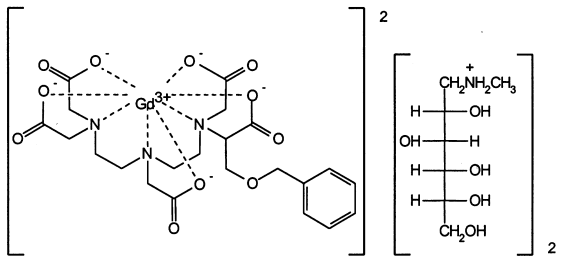

Fig. 1. Structural formula of gadobenate dimeglumine (Gd-BOPTA/Dimeg). Gd-BOPTA/Dimeg relative molecular mass, 1058.17; gadobenate ion (Gd-$BOPTA^{2-}$) relative molecular mass, 665.72.

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gadobenate dimeglumine responsible for contrast enhancement.

Concentrations in biological samples of diagnostic agents containing gadolinium can occasionally be determined radiochemically [5] or by using spectrometric techniques such as atomic absorption spectrometry [6], inductively coupled plasma atomic emission spectrometry [7] and X-ray fluorescence [8]. However, these techniques are unable to distinguish the contrast agent and the various chemical species of gadolinium potentially present in the sample (parent compounds and metabolites).

An high-performance liquid chromatographic (HPLC) method for assaying gadobenate ion in plasma, urine and bile by using the internal standard technique have already been described [9]. The purpose of this study was to validate a new HPLC method which permits the selective determination of gadobenate ion also in faecal and tissutal matrices and the addition of being more sensitive, easier applicable and less costly.

The analysis is based on the reversed-phase chromatographic separation of gadobenate ion from the endogenous components of the biological matrices and its detection during elution by ultraviolet light absorption at 210 nm. The validation process was performed on plasma, urine and faeces of humans and bile of rats. Due to ethical reasons the method validation regarding the tissue samples, which requires large amount of biological matrix, was performed on cow liver. However the method applicability was also investigated by using different tissues such as liver, kidney, heart, spleen and brain taken from rats.

Although the plasma, faeces, bile and tissues samples required specific pretreatment, the use of an internal standard technique was avoided [10]. For all assays an external standard method of calibration was adopted.

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 LiChrospher 100 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 (2.5 cm×4 mm I.D., particle size 7 μm) (Merck) was used to prevent contamination of the analytical column.

2.2. Materials

Gadobenate dimeglumine solution (0.5 *M*) was prepared by Bracco (Milan, Italy). Purified water was obtained with a Millipore Milli-Q water purification system (Bedford, MA, USA). Analytical grade glacial acetic acid, chloroform, and HPLC-grade acetonitrile were obtained from Merck, and *n*-octylamine from Fluka (Buchs, Switzerland). Sodium heparin solution (5000 IU ml^{-1}) was obtained under the name of Liquemin® from Hoffman-La Roche (Wyhlen, Germany).

2.3. Biological samples

Biological samples were of human, rat and cow origin. Blank samples of human plasma, urine and faeces were obtained from healthy subjects. Test samples of human plasma, urine and faeces containing the gadobenate ion were taken from subjects previously administered with a solution of 0.5 *M* gadobenate dimeglumine at a dose of 0.3 mmol kg^{-1}. 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^{-1}) at a ratio of about 1:50 (v/v) with blood and then centrifuged (10 min at 3500 *g*). Blank and test samples of bile were obtained, respectively, from control CD®(SD)BR rats (Charles River, Lecco, Italy) and from CD®(SD)BR rats to which a 0.25 *M* solution of gadobenate dimeglumine had been administered at a dose of 0.1 mmol kg^{-1}. The bile samples were obtained in both instances after cannulation of the bile duct. Blank liver was taken from a cow (Ultrocchi Carni S.p.A., Milan, Italy). Other blank tissue samples including liver, kidney, heart, spleen and

brain were obtained from control CD®(SD)BR rats. These various tissue samples were also taken from CD®(SD)BR rats which received a 0.5 *M* solution of gadobenate dimeglumine at a dose of 4.0 mmol kg^{-1}.

2.4. Stock standard solutions

Gadobenate ion stock standard solutions were prepared by diluting a 0.5 *M* gadobenate dimeglumine solution in purified water. For assays in plasma the stock gadobenate ion concentration range was from 0.0786 to 15.8 m*M* (0.0523–10.5 mg ml^{-1}); for the assays in urine the range was from 0.159 to 15.9 m*M* (0.106–10.6 mg ml^{-1}); for the assays in faeces the range was from 0.384 to 15.3 m*M* (0.256–10.2 mg ml^{-1}); for the assays in bile the range was from 0.306 to 15.8 m*M* (0.204–10.5 mg ml^{-1}); for the assays in tissues the range was from 0.311 to 16.0 m*M* (0.207–10.6 mg ml^{-1}). 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 10 μl of the desired gadobenate ion stock standard solution to 100 μl of blank human plasma. To this sample 100 μl of acetonitrile were added to precipitate the plasma proteins. After agitation and subsequent centrifugation (10 min at 4000 *g*), the supernatant was diluted 1:1 (v/v) with purified water. Thirty μl of the clear solution were injected into the chromatograph. To determine the gadobenate ion content in the plasma samples taken from healthy volunteers treated with the gadobenate dimeglumine formulation (0.3 mmol kg^{-1}), 10 μl of purified water rather than the equivalent volume of gadobenate ion stock standard solution were added to 100 μl of plasma samples. 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 blank human urine previously diluted 1:20 with purified water and centrifuged (15 min at 4500 *g*). Ten μl of the clear solution were used for the chromatographic analysis. To determine the gadobenate ion content in urine samples collected from the healthy volunteers treated with the gadobenate dimeglumine formulation (0.3 mmol kg^{-1}), 100 μl of purified water rather than the equivalent volume of gadobenate ion stock standard solution was added to 1 ml of diluted and centrifuged urine. Ten μl of the clear solution were injected into the chromatograph.

2.7. Preparation of faeces samples

Faeces were first dried by means of a freeze drying process (shelf temperature ranging from −40 to 30°C; chamber pressure 0.1 mbar) and then homogeneized. Two hundred mg of faecal powder were accurately weighed and suspended in 4 ml of purified water. The 5% (w/v) suspension was agitated (15 min at room temperature) and centrifuged (15 min at 3500 *g*). The supernatant was filtered through Millipore Millex-HV filters (0.45 μm pore size) and an 0.5 ml aliquot of the resulting filtrate was processed as described below.

Each calibration standard solution was prepared by adding 50 μl of the desired gadobenate ion stock standard solution and 0.5 ml of purified water to 0.5 ml of supernatant obtained from a blank faeces sample. Twenty μl of the clear solution were injected into the chromatograph. To determine the gadobenate ion content in faeces samples collected from healthy volunteers treated with the gadobenate dimeglumine formulation (0.3 mmol kg^{-1}), 50 μl of purified water rather than the equivalent volume of gadobenate ion stock standard solution and a further 0.5 ml of purified water were added to 0.5 ml of supernatant derived from these samples. Twenty μl of the clear solution were injected into the chromatograph.

2.8. Preparation of bile samples

Each calibration standard solution was prepared by adding 50 μl of the desired contrast medium stock standard solution to 0.5 ml of blank bile. To this sample 100 μl of glacial acetic acid and 0.75 ml of chloroform were added. The mixture was shaken for

30 min at room temperature (20 to 22°C) and then centrifuged (10 min at 3500 *g*). Twenty μl of the aqueous layer solution were taken for the chromatographic analysis. To determine the gadobenate ion content in bile samples obtained from animals treated with the gadobenate dimeglumine formulation (0.1 mmol kg^{-1}), 50 μl of purified water rather than the equivalent volume of gadobenate ion stock standard solution was added to 0.5 ml of bile. The sample was then processed as described above.

2.9. Preparation of tissue samples

Tissues were first dried by means of a freeze drying process (shelf temperature ranging from −40 to 30°C; chamber pressure 0.1 mbar) and then homogeneized.

Each calibration standard solution was prepared by suspending 100 mg of blank tissutal powder, which were accurately weighed, in 0.9 ml of purified water and 100 μl of the desired gadobenate ion stock standard solution. The suspension was stirred (15 min at room temperature) and centrifuged (10 min at 6200 *g*) obtaining the relative supernatant. To 0.5 ml of this were added 0.5 ml of acetonitrile. After agitation and subsequent centrifugation (10 min at 6200 *g*), the supernatant was diluted 1:1 (v/v) with purified water. Twenty-five μl of the clear solution were injected into the chromatograph. To determine the gadobenate ion content in tissues samples collected from rats treated with the gadobenate dimeglumine formulation (4.0 mmol kg^{-1}), 100 mg of tissutal powder were suspended in 0.9 ml of purified water and other 100 μl of purified water rather than the equivalent volume of gadobenate ion stock standard solution. The sample was then processed as described above.

2.10. Chromatographic conditions

Elution was carried out isocratically using mixtures of *n*-octylamine 0.18% (v/v) aqueous solution and acetonitrile [72:28 (v/v) for plasma; 73:27 (v/v) for urine and bile; 74:26 (v/v) for faeces and tissues], at a flow-rate of 1 ml min^{-1}. The aqueous solution was filtered through a 0.45 μm Millipore filter (HVLP) and the mobile phase (pH 6) degassed before use. The temperature of the thermostated oven containing the column was set at 40°C. The UV detection wavelength was 210 nm. The injection volume was in the range from 10 to 30 μl. The area of the chromatographic peak relative to the gadobenate ion was integrated and used as analytical response.

2.11. Data processing

2.11.1. Selectivity

The selectivity of the chromatographic method was evaluated by checking for interference from drug-free plasma, urine, faeces of human, bile and various tissues (liver, kidney, heart, spleen and brain) of rat and cow liver [11]. Furthermore selectivity was verified by analysis of peak purity which was performed by comparison of three U.V. spectra recorded for samples taken at the beginning, apex and end of the gadobenate ion elution.

2.11.2. Stability

As described in previous analytical work, gadobenate ion can be considered stable for at least 3 months in plasma, urine and bile samples stored at −19°C in darkness [9]. The stability of gadobenate ion either in dried faeces and dried tissue samples stored at 4°C for 1 month in darkness or in processed samples of plasma, urine, faeces, bile and tissues stored for 24 h in the autosampler at room temperature (20°C) was investigated.

The stability study of processed samples was performed on five replicates using standard solutions at the following concentrations: 0.0158, 0.676 and 1.35 m*M* for plasma, 0.0393, 0.394 and 1.59 m*M* for urine, 0.0393, 0.394 and 1.59 m*M* for faeces, 0.0394, 0.676 and 1.35 m*M* for bile, and 0.159, 0.679 and 1.36 m*M* for tissues. The stability was assessed by means comparison in analytical response between stored and original (t_0) samples considering a degradation of 10% pharmacokinetically relevant [12].

2.11.3. Recovery

The recovery study for those analyses that required sample pretreatment was performed on five replicates using two standard solutions at the following concentrations: 0.0394 and 1.35 m*M* for plasma, 0.0768 and 1.53 m*M* for faeces, 0.0394 and 1.35 m*M* for bile, and 0.159 and 1.36 m*M* for tissues. The

recovery study was not performed for urine since no sample extraction was involved. Absolute recovery was measured as a mean percentage of the response for pure standard which had not been subjected to sample treatment [11].

2.11.4. Linearity

Linearity was evaluated with nine standard solutions for each biological matrix over the following concentration ranges: 0.0079 to 1.58 m*M* in plasma, 0.0159 to 1.59 m*M* in urine, 0.0384 to 1.53 m*M* in faeces, 0.0306 to 1.58 m*M* in bile and 0.0311 to 1.60 m*M* in tissue. For the analyses of each biological matrix the analytical response (y) of the gadobenate ion peak area was measured and plotted for each concentration (x) relative to each calibration standard solution. A least-squares linear regression was then performed [13]. 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 [13]. Punctual estimates of concentrations were determined by inverse interpolation.

2.11.5. Precision and accuracy

The evaluation of precision and accuracy of the analytical systems was performed using nine standard solutions for each biological matrix in the ranges previously cited for the linearity study. The assays were repeated five times on three different days separated by variable amounts of time ranging from 1 to 30 days. To evaluate the instrumental and sample preparation variability without introducing statistical error due to the calibration model, the analytical response instead of the interpolated concentration was adopted. Precision was expressed as the percentage standard deviation ($s_r(\%)$) of the analytical responses (peak area) [13]. Accuracy was evaluated by calculating the percentage difference between the estimated and the true concentrations of gadobenate ion solutions [13]. For each day, the range and the mean of absolute values were determined.

2.11.6. Detection limit

The detection limits, expressed as concentration c_L, for gadobenate ion in plasma, urine, faeces, bile and liver, were estimated as described by IUPAC [14,15], using the equation

$$c_{L=k\cdot sb/S}$$

where k is a constant corresponding to the 95th percentile of the Student's t distribution with $(n-2)$ d.f., sb is the blank standard deviation calculated experimentally by analyzing ten 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 gadobenate ion peak height (y) against concentration (x) of each calibration standard solution.

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

3. Results

For all biological matrices the chromatographic parameters of the gadobenate ion peak were calculated according to USP [16,17] and are reported in Table 1. Table 2 shows data for the absolute

Table 1
Chromatographic parameters for gadobenate ion peak in plasma, urine, faeces, bile and liver tissue

Biological matrix	Retention factor ($t_0=2.0$ min)	Plate number per metre ($\times 10^4$)	Peak symmetry
Plasma	4.2 (C.V. 0.58%, $n=90$)	2.7	1.2
Urine	5.8 (C.V. 0.85%, $n=90$)	2.4	1.2
Faeces	6.0 (C.V. 1.9%, $n=45$)	2.9	1.2
Bile	6.0 (C.V. 1.8%, $n=90$)	2.3	1.3
Liver tissue	7.8 (C.V. 0.91%, $n=45$)	2.3	1.3

Table 2
Absolute recoveries for gadobenate ion in plasma, faeces, bile and liver tissue

Biological matrix	Concentration tested mM (μg ml^{-1})	Mean absolute recovery ($n=5$), (%)
Plasma	$3.94\cdot10^{-2}$ (26.2)	102.5
	1.35 (900)	101.5
Faeces	$7.68\cdot10^{-2}$ (51.1)	99.2
	1.53 ($1.02\cdot10^{3}$)	102.1
Bile	$3.94\cdot10^{-2}$ (26.2)	100.0
	1.35 (900)	98.9
Liver tissue	$1.59\cdot10^{-1}$ (106)	97.0
	1.36 (904)	97.7

recoveries. 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. Detection limits of gadobenate ion are reported in Table 4.

3.1. Assay of gadobenate ion in plasma

Fig. 2 shows representative chromatograms of (a) a pre-dose plasma sample taken from a healthy volunteer, and (b) a plasma sample taken from a healthy volunteer to which gadobenate dimeglumine had been administered. No interfering peaks at the retention time of the gadobenate ion were detected in the chromatograms of ten blank human plasma samples. The stability study indicated no significant degradation of gadobenate ion in plasma processed samples stored 24 h at room temperature in darkness in the autosampler. The absolute recovery of gadobenate ion, which was obtained for the concentrations

Table 4
Detection limits (c_L) for gadobenate ion in plasma ($\geq$0.1 ml), urine ($\geq$0.05 ml diluted to 1 ml with purified water), dried faeces ($\geq$200 mg suspended in 4 ml), bile ($\geq$0.5 ml) and dried liver tissue ($\geq$100 mg suspended in 1 ml)

Biological matrix	c_L (nmol ml^{-1})	c_L (μg ml^{-1})
Plasma	0.24	0.16
Urine	0.47	0.31
Faeces	2.6	1.7
Bile	0.63	0.42
Liver tissue	2.8	1.9

Table 3
Linearity of standard curves for gadobenate ion in plasma, urine, faeces, bile and liver tissue

Biological matrix	Day	$a\pm s_a$ [a]	$b\pm s_b$ [b]	r [c]
Plasma	1	$6.3\cdot10^{-3}\pm1.2\cdot10^{-3}$	$145.54\cdot10^{-4}\pm0.59\cdot10^{-4}$	0.9996
	2	$5.7\cdot10^{-3}\pm1.4\cdot10^{-3}$	$144.22\cdot10^{-4}\pm0.58\pm10^{-4}$	0.9996
	3	$-9.11\cdot10^{-3}\pm0.80\cdot10^{-3}$	$143.11\cdot10^{-4}\pm0.67\cdot10^{-4}$	0.9995
Urine	1	$15.3\cdot10^{-3}\pm3.9\cdot10^{-3}$	$182.62\cdot10^{-4}\pm0.75\cdot10^{-4}$	0.9996
	2	$7.1\cdot10^{-3}\pm3.4\cdot10^{-3}$	$178.75\cdot10^{-4}\pm0.72\pm10^{-4}$	0.9996
	3	$4.9\cdot10^{-3}\pm2.4\cdot10^{-3}$	$181.24\cdot10^{-4}\pm0.23\cdot10^{-4}$	0.9999
Faeces	1	$-24.1\cdot10^{-3}\pm9.2\cdot10^{-3}$	$185.44\cdot10^{-4}\pm0.40\cdot10^{-4}$	0.9999
	2	$-26.9\cdot10^{-3}\pm5.1\cdot10^{-3}$	$188.05\cdot10^{-4}\pm0.61\cdot10^{-4}$	0.9997
	3	$54.2\cdot10^{-3}\pm5.3\cdot10^{-3}$	$188.90\cdot10^{-4}\pm0.26\cdot10^{-4}$	0.9999
Bile	1	$-11.27\cdot10^{-2}\pm0.88\cdot10^{-2}$	$298.79\cdot10^{-4}\pm0.30\cdot10^{-4}$	1.0000
	2	$-12.33\cdot10^{-2}\pm0.62\cdot10^{-2}$	$326.9\cdot10^{-4}\pm1.2\cdot10^{-4}$	0.9997
	3	$-12.04\cdot10^{-2}\pm0.46\cdot10^{-2}$	$327.83\cdot10^{-4}\pm0.92\cdot10^{-4}$	0.9998
Liver tissue	1	2.22 ± 0.55	1731.4 ± 3.1	0.9999
	2	0	1729.4 ± 6.4	0.9997
	3	0	1731.2 ± 3.8	0.9999

Forty-five observations for each curve: analysis days 1, 2 and 3 were separated by variable amounts of time.
Equation from linear regression: $y=a+bx$.
[a] Intercept$\pm$standard deviation, [b] slope$\pm$standard deviation, [c] correlation coefficient.

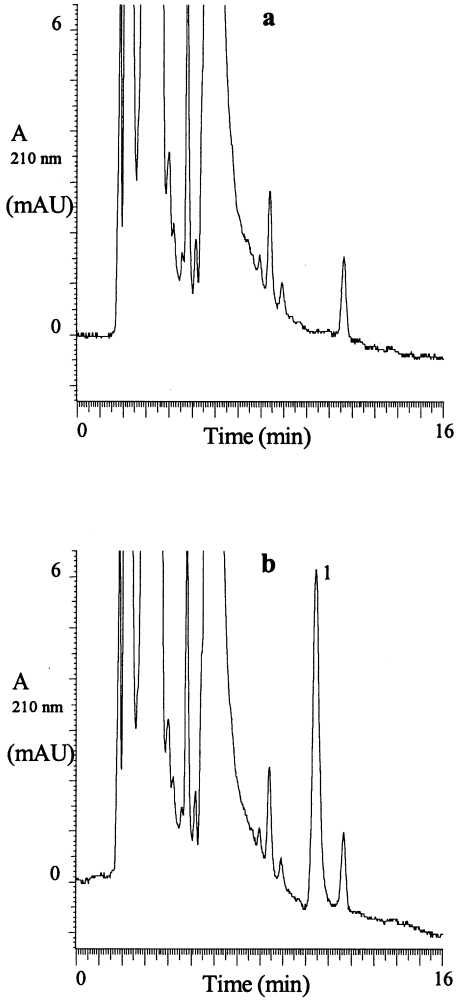

Fig. 2. High-performance liquid chromatograms of human plasma extract monitored at 210 nm. Peak 1 is gadobenate ion and all other peaks are due to undefined substances remaining in plasma after sample preparation. (a) Drug-free plasma extract from a healthy subject; (b) extract of a plasma sample from a healthy volunteer 8 h after intravenous administration of gadobenate dimeglumine (dose 0.3 mmol kg^{-1} body weight). The gadobenate ion concentration 0.037 m*M* (24.7 μg ml^{-1}) was estimated by interpolation on the standard curve.

0.0394 and 1.35 m*M*, was 101.5 and 102.5%, respectively. The best correlation between the peak area and the concentration of gadobenate ion was obtained over the range from 0.0079 to 1.58 m*M*. Table 5 reports data for the precision and accuracy of some estimated concentrations for analyses performed on three different days.

3.2. Assay of gadobenate ion in urine

Fig. 3 shows representative chromatograms of (a) a pre-dose urine sample taken from a healthy volunteer, and (b) a urine sample taken from a healthy volunteer to which gadobenate dimeglumine had been administered. No interfering peaks at the re-

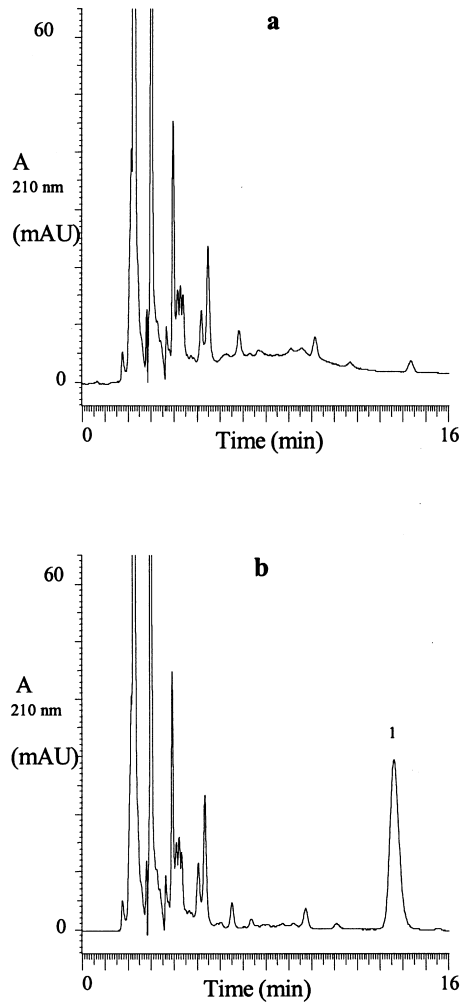

Fig. 3. High-performance liquid chromatograms of human urine monitored at 210 nm. Peak 1 is gadobenate ion and all other peaks are due to undefined substances remaining in urine after sample preparation. (a) Drug-free urine extract from a healthy subject; (b) extract of a urine sample from a healthy volunteer 16–24 h after intravenous administration of gadobenate dimeglumine (dose 0.3 mmol kg^{-1} body weight). The gadobenate ion concentration 0.186 m*M* (124 μg ml^{-1}) was estimated by interpolation on the standard curve.

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

Concentration mM (μg ml^{-1})	Day	Precision ($n=5$) s_r (%)	Accuracy ($n=5$)	
			Range (%)	Mean of absolute values (%)
$7.9\cdot10^{-3}$ (5.2)	1	2.6	−6.9+0.68	3.4
	2	5.4	−10+3.4	5.0
	3	2.9	+5.0+12	7.3
$78.6\cdot10^{-3}$ (52.3)	1	0.74	+1.7+3.5	2.6
	2	1.4	−1.9+1.4	1.1
	3	3.6	−4.9+4.2	3.0
1.58 ($1.05\cdot10^{3}$)	1	1.6	−0.18+4.1	2.0
	2	1.8	−0.17+5.0	2.4
	3	1.4	−2.5+1.1	0.92

tention time of the gadobenate ion were detected in the chromatograms of ten blank human urine samples. After 24 h at room temperature in darkness there was no sign of degradation in the processed urine samples stored in the autosampler. The best correlation between the peak area and the concentration of gadobenate ion was obtained over the range from 0.0159 to 1.59 m*M*. Table 6 reports data for the precision and accuracy of some estimated concentrations for analyses performed on three different days.

3.3. Assay of gadobenate ion in faeces

Fig. 4 shows representative chromatograms of (a) a pre-dose faeces sample taken from a healthy volunteer, and (b) a faeces sample taken from a healthy volunteer to which gadobenate dimeglumine had been administered. No interfering peaks at the retention time of the gadobenate ion were detected in the chromatograms of ten blank human faeces samples. The stability study indicated no significant degradation of gadobenate ion in freeze-dried faeces samples stored at 4°C for 1 month and in processed samples stored 24 h at room temperature in darkness in the autosampler. The absolute recovery of gadobenate ion, which was obtained for the concentrations 0.0768 and 1.53 m*M*, was 99.2 and 102.1%, respectively. The best correlation between the peak area and the concentration of gadobenate ion was obtained over the range from 0.0384 to 1.53 m*M*. Table 7 reports data for the precision and accuracy of some estimated concentrations for analyses performed on three different days.

3.4. Assay of gadobenate ion in bile

Fig. 5 shows representative chromatograms of (a) a pre-dose bile sample taken from a rat, and (b) a bile sample taken from a rat to which gadobenate

Table 6
Precision and accuracy for the assay of gadobenate ion in urine, analysis days 1, 2 and 3 were separated by variable amounts of time

Concentration mM (μg ml^{-1})	Day	Precision ($n=5$) s_r (%)	Accuracy ($n=5$)	
			Range (%)	Mean of absolute values (%)
$15.9\cdot10^{-3}$ (10.6)	1	2.2	−5.0+0.015	2.1
	2	2.2	−1.4+5.1	2.5
	3	2.0	−2.8+1.1	2.0
$15.9\cdot10^{-2}$ (106)	1	1.5	−1.1+2.4	1.3
	2	0.82	+1.1+3.2	2.1
	3	1.0	+0.27+2.7	0.99
1.59 ($1.06\cdot10^{3}$)	1	0.97	−1.3+1.2	0.92
	2	0.89	+1.0+3.1	1.9
	3	0.37	+0.012+0.98	0.40

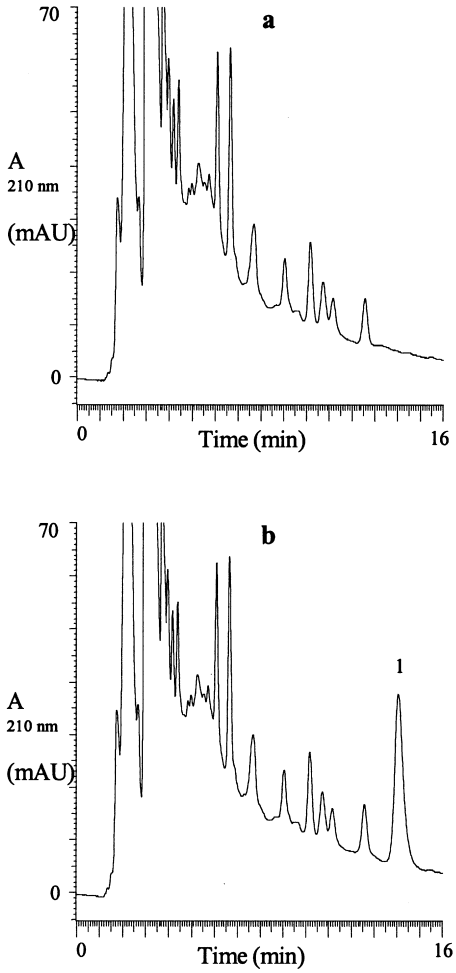

Fig. 4. High-performance liquid chromatograms of human faeces extract monitored at 210 nm. Peak 1 is gadobenate ion and all other peaks are due to undefined substances remaining in faeces after sample preparation. (a) Drug-free faeces extract from a healthy subject; (b) extract of a faeces sample from a healthy volunteer 24–48 h after intravenous administration of gadobenate dimeglumine (dose 0.3 mmol kg^{-1} body weight). The gadobenate ion concentration 0.186 m*M* (124 μg ml^{-1}), corresponding to 3.7 μmol g^{-1} of freeze-dried faeces, was estimated by interpolation on the standard curve.

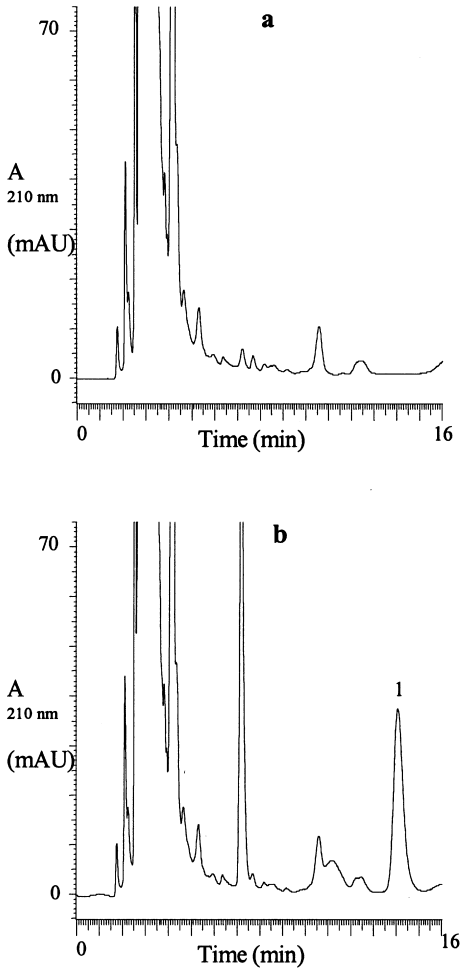

Fig. 5. High-performance liquid chromatograms of rat bile extract monitored at 210 nm. Peak 1 is gadobenate ion and all other peaks are due to undefined substances remaining in bile after sample preparation. (a) Drug-free bile extract from a rat; (b) extract of a bile sample from a rat 4–8 h after intravenous administration of gadobenate dimeglumine (dose 0.1 mmol kg^{-1} body weight). The gadobenate ion concentration ion 0.186 m*M* (124 μg ml^{-1}) was estimated by interpolation on the standard curve.

dimeglumine had been administered. No interfering peaks at the retention time of the gadobenate ion were detected in the chromatograms of ten blank rat bile samples. After 24 h at room temperature in darkness there was no sign of degradation in the processed bile samples stored in the autosampler. The absolute recovery of gadobenate ion, which was obtained for the concentrations 0.0394 and 1.35 m*M*, was 100.0 and 98.9%, respectively. The best correlation between the peak area and the concentration of gadobenate ion was obtained over the range from 0.0306 to 1.58 m*M*. Table 8 reports data for the precision and accuracy of some estimated concentrations for analyses performed on three different days.

Table 7
Precision and accuracy for the assay of gadobenate ion in faeces

Concentration m*M* (μg ml^{-1})	Day	Precision ($n=5$) s_r (%)	Accuracy ($n=5$)	
			Range (%)	Mean of absolute values (%)
$38.4\cdot10^{-3}$ (25.6)	1	6.5	−12+2.6	4.0
	2	2.0	−0.18+4.5	2.2
	3	2.0	−3.6+1.5	2.7
$36.8\cdot10^{-2}$ (245)	1	1.1	−0.30+2.4	1.1
	2	1.3	+2.0+4.8	3.2
	3	0.60	+1.5+3.0	2.0
1.53 ($1.02\cdot10^{3}$)	1	1.6	+0.87+5.3	2.6
	2	0.61	−1.4−0.0098	0.64
	3	0.32	−0.62+0.22	0.36

Analysis days 1, 2 and 3 were separated by variable amounts of time.

3.5. Assay of gadobenate ion in tissues

Fig. 6 shows representative chromatograms of (a) a pre-dose liver tissue sample taken from a rat, and (b) a liver tissue sample taken from a rat to which gadobenate dimeglumine had been administered. No interfering peaks at the retention time of the gadobenate ion were detected in the chromatograms of various tissue samples (cow liver and liver, kidney, heart, spleen and brain of rat). The stability study indicated no significant degradation of gadobenate ion in freeze-dried tissue samples stored at 4°C for 1 month and in processed samples stored 24 h at room temperature in darkness in the autosampler. The absolute recovery of gadobenate ion, which was obtained for the concentrations 0.159 and 1.36 m*M*, was 97.0 and 97.7%, respectively. The best correlation between the peak area and the concentration of gadobenate ion was obtained over the range from 0.0311 to 1.60 m*M*. Table 9 reports data for the precision and accuracy of some estimated concentrations for analyses performed on three different days.

4. Discussion

This proposed methodology can be considered an improvement on previous published analytical work [9], for, as well as introducing faecal and tissutal analysis, the method also offers (a) increased sensitivity (for the gadobenate ion assay in plasma the quantitation limit was reduced five-fold), (b) re-

Table 8
Precision and accuracy for the assay of gadobenate ion in bile

Concentration m*M* (μg ml^{-1})	Day	Precision ($n=5$) s_r (%)	Accuracy ($n=5$)	
			Range (%)	Mean of absolute values (%)
$30.6\cdot10^{-3}$ (20.4)	1	2.5	+3.6+9.3	6.1
	2	1.1	+0.76+2.8	1.8
	3	1.3	−0.92+1.8	1.3
$39.4\cdot10^{-2}$ (262)	1	2.6	−6.9–1.0	2.9
	2	2.0	−5.7–1.1	2.6
	3	1.2	−1.8+0.95	1.4
1.58 ($1.05\cdot10^{3}$)	1	0.48	+1.7+3.0	2.6
	2	0.98	+2.1+4.5	2.8
	3	0.77	+0.20+2.3	1.0

Analysis days 1, 2 and 3 were separated by variable amounts of time.

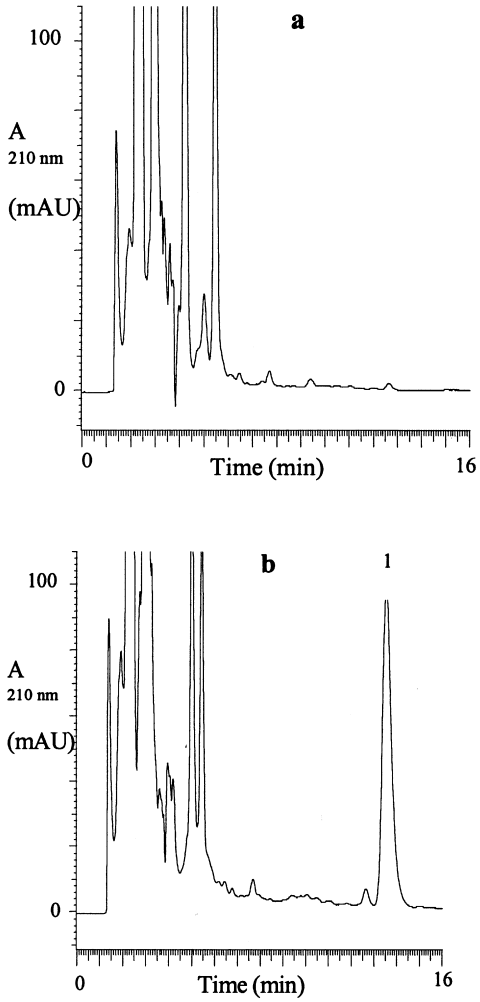

Fig. 6. High-performance liquid chromatograms of rat liver extract monitored at 210 nm. Peak 1 is gadobenate ion and all other peaks are due to undefined substances remaining in liver after sample preparation. (a) Drug-free liver extract from a rat; (b) extract of a liver sample from a rat 50 min after intravenous administration of gadobenate dimeglumine (dose 4.0 mmol kg^{-1} body weight). The gadobenate ion concentration 0.881 m*M* (580 μg ml^{-1}), corresponding to 8.81 μmol g^{-1} of freeze-dried tissue, was estimated by interpolation on the standard curve.

dundancy of the internal standard (B19106/7), (c) reduced quantities of biological samples required for assay (for plasma 0.1 ml and for bile 0.5 ml rather than 0.8 and 1 ml, respectively) and (d) reduced cost per analysis.

Since the chromatographic peak of the gadobenate ion is completely separated from any other peak in plasma, urine, faeces, bile and various tissue samples, the described assays can be said to have excellent selectivity. On the basis of the results obtained, gadobenate ion can be considered stable in the original and processed samples when stored under the above conditions. Small quantity of the chelating agent (BOPTA) was found in plasma samples added with gadobenate ion and stored at −20°C. The phenomena, probably due to transmetallation of the gadolinium complex, can be prevented either by freeze-drying or analyzing the plasma samples immediately after their preparation (work in progress). For the method validation, precision and accuracy were calculated by using the same calibration standard samples adopted to generate the calibration curve in order to use the maximum available information for studying the calibration model. For the method application, precision and accuracy are checked by using quality control samples independent of the calibration standard samples. The absolute recovery, linearity, precision, accuracy and detection limits of the described methods are satisfactory for pharmacokinetic studies of gadobenate dimeglumine in large animals and man at clinically relevant doses. The maximum plasma concentration values (C_{max}) of gadobenate ion for healthy volunteers administered intravenously with gadobenate dimeglumine at doses 0.1, 0.2 and 0.3 mmol kg^{-1} are resulted 0.94, 1.3 and 2.36 m*M*, respectively. The methods are also suitable for similar studies in smaller animals and infants because the amount of plasma, urine, faeces, bile and tissue required does not limit the number of data points obtainable from individual subjects. The C_{max} values of gadobenate ion for rats administered intravenously with gadobenate dimeglumine at doses 0.1, 0.25, 0.5 and 1.0 mmol kg^{-1} are resulted 0.33, 1.08, 1.96 and 2.62 m*M*, respectively. Furthermore we can suppose that the method validated to determine gadobenate ion in tissutal matrix of animal could be also suitable for potential analytical investigations supporting the product pharmacovigilance on tissue samples obtained by human biopsy or autopsy. Finally the fact that the proposed techniques can be performed on readily available equipment and are rapid makes them suitable for routine use.

Table 9
Precision and accuracy for the assay of gadobenate ion in liver tissue

Concentration mM (μg ml^{-1})	Day	Precision ($n=5$) s_r (%)	Accuracy ($n=5$)	
			Range (%)	Mean of absolute values (%)
$31.1\cdot10^{-3}$ (20.7)	1	2.3	−4.9+1.1	1.6
	2	2.8	−4.9+0.75	2.0
	3	2.8	−3.8+2.8	2.2
$38.8\cdot10^{-2}$ (258)	1	2.3	−2.8+3.3	1.7
	2	1.6	−3.5+0.86	2.1
	3	1.3	−2.1+1.2	1.1
1.60 ($1.06\cdot10^{3}$)	1	1.3	−0.87+2.4	1.2
	2	2.3	−3.0+3.1	1.8
	3	0.71	−0.69+1.0	0.55

Analysis days 1, 2 and 3 were separated by variable amounts of time.

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References

[1] E. Felder, F. Uggeri, L. Fumagalli, G. Vittadini, US Pat., 4 916 246 (1990); Ital. Pat. 19236A/86 (1986).
[2] F. Uggeri, S. Aime, P.L. Anelli, M. Botta, M. Brocchetta, C. de Haën, G. Ermondi, M. Grandi, P. Paoli, Inorg. Chem. 34 (1995) 633.
[3] F. Cavagna, P. Tirone, E. Felder, C. de Haën, in: J.T. Ferrucci, D.D. Stark (Eds.), Liver Imaging: Current Trends and New Techniques, Andover Medical, Boston, 1991, p. 384.
[4] T.J. Vogl, W. Pegios, C. Mc Maon, J. Balzer, J. Waitzinger, G. Pirovano, J. Lissner, Am. J. Roentgenol. 158 (1992) 887.
[5] B.L. Engelstad, D.L. White, J.P. Huberty, C.S. Wynne, E.C. Ramos, M.T. McNamara, H.I. Goldberg, Invest. Radiol. 22 (1987) 232.
[6] L. Liang, P.C. D'Haese, L.V. Lamberts, F.L. Van de Vyver, M.E. De Broe, Anal. Chem. 63 (1991) 423.
[7] J.G. Crock, F.E. Lichte, Anal. Chem. 54 (1982) 1329.
[8] M. Lal, R.K. Choudhury, R.M. Agrawal, X-Ray Spectrom. 16 (1987) 23.
[9] V. Lorusso, I. Poggesi, T. Arbughi, D. Dal Fiume, P. Tirone, J. Chromatogr. B. Biomed. Appl. 656 (1994) 415.
[10] P. Haefelfinger, J. Chromatogr. 218 (1981) 73.
[11] T. Karnes, G. Shiu, P. Vinod, Pharm. Res. 8 (1991) 421.
[12] U. Timm, M. Wall, D. Dell, J. Pharm. Sci. 74 (1985) 972.
[13] IUPAC, Pure Appl. Chem., 66 (1994) 595.
[14] IUPAC, Anal. Chem., 48 (1976) 2294.
[15] Analytical methods committee Analyst 112 (1987) 199.
[16] USP XXIII ed., p. 1774.
[17] IUPAC, Pure Appl. Chem., 65 (1993) 819.