Journal of Chromatography B, 891–892 (2012) 20–26

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

A LC–MS/MS method to evaluate the hepatic uptake of the liver-specific magnetic resonance imaging contrast agent gadoxetate (Gd-EOB-DTPA) *in vitro* and in humans

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ARTICLE INFO

Article history:
Received 27 September 2011
Accepted 12 February 2012
Available online 18 February 2012

Key words:
Gadoxetate
Gd-EOB-DTPA
LC–MS/MS
OATP1B1

ABSTRACT

Gadoxetate (Gd-EOB-DTPA, Primovist®) is a frequently used liver-specific magnetic resonance imaging (MRI) contrast agent which disposition is so far not fully understood in humans. Here, we describe the development and validation of a selective and sensitive quantification method to measure cellular *in vitro* concentrations as well as human serum concentrations of gadoxetate. The drug was measured after protein precipitation with acetonitrile and ethyl acetate-mediated sample concentration using amoxicillin as internal standard and liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) for detection. Hydrophilic interaction chromatography (HILIC) was performed by using the column Atlantis® HILIC Silica (2.1 mm × 100 mm), a step-elution gradient with acetonitrile and ammonium acetate (5 mM, pH 3.8) as mobile phases and a flow rate of 200 μl/min. The MS/MS detection was done in the negative multiple reaction monitoring (MRM) mode by monitoring the *m/z* transitions 681.3/635.2 for gadotrexate and 363.8/222.7 for the internal standard. The method was validated between 5 and 4000 ng/ml in serum and between 1.25 and 500 ng/ml in cell lysates. The method was shown to possess sufficient specificity, accuracy, precision and stability without any matrix effects, thereby fulfilling current bioanalytical guidelines. The developed assay was successfully applied to quantify gadoxetate in cellular uptake studies in OATP1B1-transfected cell lines and to monitor serum concentrations-time profiles from a clinical pilot study performed in healthy volunteers carrying the wild-type or the functionally relevant variants T521C (*5) and A388G (*1b) of the hepatic uptake transporter OATP1B1.

1. Introduction

Gd-EOB-DTPA (Primovist®, gadoxetate, gadolinium-ethoxybenzyl-diethylen-triaminpentaacetic acid, disodium; Fig. 1), a synthetic gadolinium-containing chelate complex, is a novel and frequently used contrast agent for magnetic resonance imaging (MRI). In contrast to other so far available MRI contrast agents as Gd-DTPA (Magnevist®) or Gd-DTPA-BMA (Omniscan®), gadoxetate is characterized by a high liver specificity which is beneficial for detection of focal liver lesions and hepatic tumors [1]. However, the underlying mechanisms of the hepatic uptake of gadoxetate in humans remain uncertain. Considering its high molecular weight (679.7 g/mol) and the anionic charge, gadoxetate is not expected to undergo passive hepatic uptake by diffusion but is most likely a substrate of hepatic uptake transporters such as the *organic anion transporting polypeptides* (OATPs). These carriers are highly expressed at the basolateral membrane of human hepatocytes and facilitate the hepatic uptake of several drugs, xenobiotics and endogenous compounds such as statins, sexual hormones and bilirubin [2].

Experimental studies in rats clarified that the Oatp 1 is involved in the selective hepatic uptake of gadoxetate, which could be inhibited by the potent Oatp inhibitor rifampicin [3,4]. Moreover, *in vitro* uptake studies in oocytes and stably transfected cell lines demonstrated that gadoxetate is a substrate for the human liver-specific uptake carriers OATP1B1 and OATP1B3 [5,6]. There is also convincing clinical evidence that these transporters are critical determinants for the hepatic uptake of gadoxetate [7–9].

Functionally relevant OATP1B1 gene polymorphisms may be the reason for the high inter-subject variability in hepatic imaging using gadoxetate [1]. These variants such as the *5 or *15 variants were shown to affect the hepatic uptake and serum concentrations of several OATP1B1 substrates in a significant manner [2].

However, to evaluate the certain meaning of OATP1B1 in the disposition and hepatic uptake of gadoxetate, we performed in a first

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doi:10.1016/j.jchromb.2012.02.014

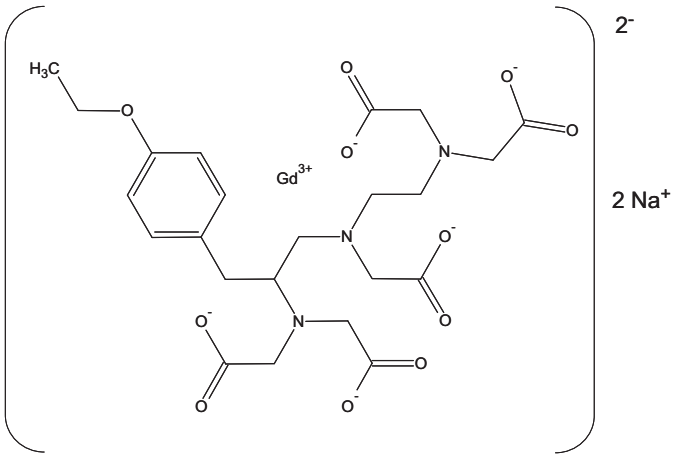

Fig. 1. Structural formula of gadoxetate (Gd-EOB-DTPA).

step *in vitro* uptake studies using OATP1B1-transfected HEK293 cells overexpressing the wild-type protein and its genetic variants to study the *in vitro* uptake of gadoxetate. In order to evaluate the *in vivo* relevance of OATP1B1 (*i.e.* serum pharmacokinetics), we initiated in a second step a clinical pilot study in healthy subject which were carriers of these functionally relevant OATP1B1 gene polymorphisms. In parallel to this, the hepatic enhancement was monitored by MRI (Nassif et al., 2012, under revision).

To quantify gadoxetate in cell lysates and in human serum samples, it was essential to develop a selective and sensitive quantification method for gadoxetate.

In the methods published so far, inductively coupled plasma-atom emission spectroscopy (ICP-AES) was almost exclusively used for detection [10–13]. These methods do not detect the intact chelate complex; instead, the gadolinium ions are monitored only. Consequently that it cannot be distinguished between unbound gadolinium the intact complex which is required for hepatic uptake and subsequent MRI. The same limitation is true for a recently published ICP-mass spectrometry method [14]. Furthermore, the described methods lack adequate sensitivity (limits of quantification: 0.1–1 μM for Gd^{3+}, *i.e.* 68–680 ng/ml for Gd-EOB-DTPA) to measure cellular uptake *in vitro* and to monitor the full elimination slope in human serum after an intravenous standard dose of 25 μM/kg [12]. There were also HPLC methods with UV detection reported [13,15]. However, neither details of these methods or validation data were provided. Finally, an electrospray-MS method was published to investigate non-covalent binding of gadoxetate to serum proteins [16]. This assay was also not useful for our purpose because no chromatographic separation was performed and no analytical details or validation data were given. Thus, there was the need to develop a new and more sensitive assay to measure gadoxetate in biological samples.

This paper describes the development and validation of this method according to the FDA guideline on bioanalytical method validation and its successful application to analyze samples from *in vitro* studies and a clinical trial in humans [17].

2. Material and methods

2.1. Reagents

Acetonitrile was purchased in LC–MS quality (Chromasolv®, Sigma–Aldrich, Taufkirchen, Germany). Deionized water (conductance: ≤0.055 μS/cm, pH 5.0–6.0) was generated with the system SG 2800 (S.G. Wasseraufbereitung und Regenerierstation GmbH, Barsbüttel, Germany). Gadoxetate (Primovist® injection solution) was obtained from Bayer Schering Pharma (Berlin, Germany) and the internal standard amoxicillin from Sigma–Aldrich.

Ammonium acetate and ethyl acetate and were obtained from Merck (Darmstadt, Germany). Blank human serum was kindly provided by the Department of Transfusion Medicine of the University Medicine of Greifswald, Germany. Stock solutions of gadoxetate and amoxicillin were prepared by appropriate dilution with deionized water and stored at −20 °C. Working solutions for both compounds were made weekly from stock solutions by adequate dilution and stored at 4 °C.

2.2. Sample preparation

Serum samples and cell lysates arrived at the laboratory deeply frozen at −20 °C. All sample preparations were carried out in one step at room temperature and did not exceed 1 h. For determination of gadoxetate in human serum, 100 μl serum were mixed with 100 μl deionized water and 25 μl of the internal standard solution (amoxicillin, 1 μg/ml).

The initial sample preparation of the cell lysates differed markedly due to the presence of sodium dodecyl sulfate (SDS). To remove this strong anionic detergent, 25 μl barium acetate solution (0.3 M) were added for precipitation. To remove non-volatile barium ions which may cause substantial ion suppression in the mass spectrometer, ammonium sulfate was added (25 μl, 0.3 M). Thereafter, cloudy samples were centrifuged at 10,500 × *g* for 3 min.

Serum samples and the supernatants after centrifugation of the cell homogenates were mixed with 400 μl ice-cold acetonitrile for protein precipitation and subsequently centrifugated for 3 min at 10,500 × *g*. The supernatant was added to 1 ml of ethyl acetate in a separate vessel, which was subjected to intensive vortexing for 30 s and centrifugation at 10,500 × *g* for 3 min. We added ethyl acetate due to the following reasons: (1) to remove the precipitation reagent acetonitrile from the samples which otherwise would not have been compatible with our HPLC method (~60% acetonitrile in the samples *vs.* 10% at the beginning of our HPLC gradient); (2) to remove lipophilic compounds from the samples that were not removed by protein precipitation (*e.g.* lipids); (3) to concentrate the samples because ethyl acetate is not only capable to extract acetonitrile but also substantial amounts of water from the samples.

The organic supernatant was discarded and approximately 100 μl of the remaining aquatic residue were transferred into sample vials of which 10 μl were injected into the chromatographic system.

2.3. LC–MS/MS analysis

The LC–MS/MS analysis was done with the Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled with the triple quadrupol tandem mass spectrometer API4000 (AB Sciex, Darmstadt, Germany).

The chromatography was performed with a step-elution gradient using the Atlantis® HILIC silica column (2.1 mm × 100 mm, particle size 3 μm, Waters, Milford, USA) and acetonitrile (A) and ammonium acetate (5 mM, pH 3.8 adjusted with formic acid, B) as mobile phases at a flow rate of 200 μl/min. The elution steps were as follows: equilibration for 3 min at 90% A/10% B; 0–0.1 min, 90% A/10% B; 0.1–3 min; 10% A/90% B; 3–10 min, 90% A/10% B (% means in each case v/v). The column oven and the autosampler rack were temporized at 40 °C and 5 °C, respectively. To avoid contamination by particles, the chromatographic flow was filtered through a 0.5 μm filter device (PEEK, Supelco, Bellefonte, USA).

The mass spectrometer was equipped with the electro spray ionization (ESI) TurboIon® interface and operated in the negative ion mode to monitor the *m*/*z* transition 681.3–635.2 for gadoxetate

Table 1
Gas- and mass spectrometry parameters for the determination of gadoxetate and amoxicillin using the API4000 mass spectrometer. Nitrogen was used as nebulizer, heater, curtain and collision gas (1 psi = 6894.8 Pa). CAD, collision-activated dissociation.

Gas parameters		MS/MS parameters	Gadoxetate	Amoxicillin
Curtain gas	20 psi	Q1/Q3 mass	681.3/635.2	363.8/222.7
CAD gas	8 psi	Declustering potential (V)	−110	−70
Nebulizer gas	60 psi	Entrance potential (V)	−15	−5
Heater gas	60 psi	Collision energy (V)	−40	−16
Temperature	350 °C	Collision cell exit potential (V)	−10	−10
Needle voltage	−4500 V	Dwell time (ms)	250	250

and 363.8–222.7 for the internal standard amoxicillin. Ions were detected by an electron multiplier integrated into the API4000 system. The optimized gas and MS parameters are given in Table 1. All chromatograms were evaluated with the Analyst 1.4 software (AB Sciex) using the internal standard method and peak-area ratios for calculation (linear regression, $1/x$ weighting).

2.4. Validation

The developed method was validated according to the current FDA guideline for bioanalytical method validation [17]. Selectivity of the LC–MS/MS method was confirmed by analyzing six different human blank serum samples or six different aliquots of our HEK 293 cell lines in comparison to the respective biological matrix spiked with either the internal standard, gadoxetate or both.

After definition of the required concentration range (*e.g.* 5–4000 ng/ml in serum), linearity of the method, *i.e.* the correlation between the analyte concentration and the analytical signal, was evaluated by analyzing six individual calibration functions, which were prepared and measured on different days.

For preparation of calibration curves and quality control samples, blank human serum or lysates of HEK 293 cell samples were spiked with increasing amounts of gadoxetate to generate the following target concentrations: 5, 10, 25, 50, 100, 250, 500, 1000, 2000 and 4000 ng/ml for calibration values and 10, 250 and 4000 ng/ml for quality control samples (QC) in serum and 1.25, 2.5, 5, 10, 25, 50, 100, 250 and 500 ng/ml for calibration values and 2.5, 25 and 500 ng/ml for QC samples in cell lysates. Thereafter, the samples were handled in the same manner as described before.

For evaluation of between-day (inter-day) accuracy and precision, the measured QC samples (in each case six separately prepared sets measured on different days) were compared with the respective nominal concentrations, expressed as relative error (accuracy) and the calculation of the respective coefficients of variation (precision). Within-day (intra-day) accuracy and precision was assessed by analyzing six independently prepared QC sample sets on 1 day.

To assess matrix effects (*i.e.* ion suppression), the concentrations of gadoxetate in matrix-free samples (diluted stock solution) were compared with concentrations measured in serum which was spiked with gadoxetate after protein precipitation. Six QC sample sets (2.5, 25 and 500 ng/ml) and the respective matrix-free samples were used to determine the matrix effects. The potential matrix effect was expressed as relative error compared to matrix free samples.

Short-term, post-preparative, freeze–thaw and long-term stability was determined by using in each case six QC sample sets. Short term (bench-top) stability of spiked serum samples was investigated by comparing immediately prepared and measured samples with samples which were stored for 2 and 4 h before preparation at room temperature. Post-preparative (rack) stability was assessed by measuring sample extracts immediately and 24 h after storing in the autosampler at 5 °C. To assess freeze-and-thaw stability, the drug containing serum samples were thawed and frozen up to three times before preparation and measuring (one freeze–thaw-cycle per day). For long-term stability check, gadoxetate spiked serum samples were measured before and after storing the samples for 4 weeks at −20 °C. In each case, stability was assumed if the drug content after the given storage condition was within the acceptable range of accuracy, *i.e.* ±15%.

2.5. Measurement of biological samples

On each day of analysis, calibration curves and QC sample sets were freshly prepared for both matrices as mentioned above. QC samples represented at least 10% of all analytical samples and were measured during the entire analytical run. The criterion of acceptance for an analytical run was if at least 4 of 6 of all QC samples were within a relative error range of ±15% (±20% at the LOQ) of the nominal values as suggested by the respective FDA guideline [17].

2.6. Cellular uptake studies

HEK293-OATP1B1 cells were generated and grown as described previously [5]. Cells with variant OATP1B1 were generated by site directed mutagenesis using the vector pQCXIN-OATP1B1 and the QuickChange site-directed Mutagenisis kit from Stratagene (Amsterdam, Netherlands) according to the manufacturer's instructions. Cellular uptake of gadoxetate (500 μM) was measured after incubation of the cells at 37 °C for 10 min (each $N = 9$). Afterward, cells were lysed using 0.2% (m/m) sodium dodecyl sulfate buffer and stored at −20 °C until quantitative analysis.

2.7. Pharmacokinetic study

The pharmacokinetic study was performed according to current international and national regulations in three male healthy subjects (age 27–34 years; body mass index 23.1–25.7 kg/m^2) genotyped for the OATP1B1 (*SLCO1B1*) polymorphisms A388G (Asn130Asp) and T521C (Val174Ala) who gave informed written consent. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism analysis as recently described [18]. Haplotypes were defined as follows: *SLCO1B1*1a* as −11187G, 388A, 521T; *SLCO1B1*1b* as −11187G, 388G, 521T; *SLCO1B1*5* as −1187G, 388A, 521C and *SLCO1B1*15* as −11187G, 388G, 521C. The study was approved by the local ethical committee and the German Federal Institute for Drugs and Medical Devices (BfArM).

On the pharmacokinetic study day, a single intravenous bolus of 25 μM/kg body mass gadoxetate (Primovist®, Bayer Schering Pharma, Berlin, Germany) was administered. Venous blood (5 ml) was sampled from a forearm vein before and 0.33, 0.66, 1, 1.33, 1.66, 2, 2.33, 2.66, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 16, 24, 36 and 48 h after drug administration. Serum was stored at −20 °C until quantitative analysis. The pharmacokinetic evaluation to assess the area under the concentration–time curve between 0 and 48 h (AUC_{0-48}) and maximum serum concentrations (C_{max}) was done with standard methods.

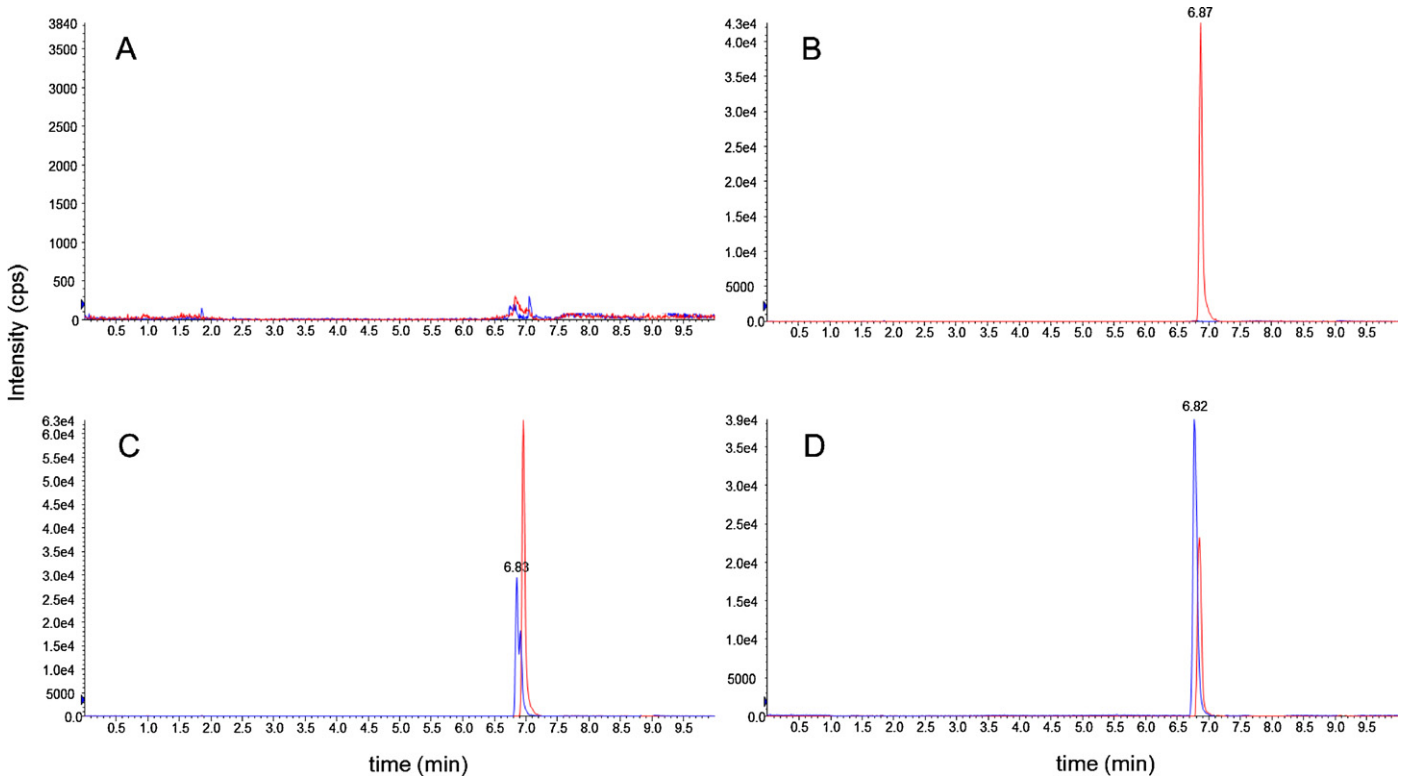

Fig. 2. Total ion chromatograms of a blank human serum (A), a serum sample spiked with the internal standard amoxicillin (B), a serum sample measured 12 h after intravenous administration of gadoxetate (25 μmol/kg body weight) (C), and a cell lysate of OATP1B1-transfected HEK293 cells after incubation of 500 μM gadoxetate for 10 min. All chromatograms were obtained by monitoring the *m*/*z* transitions 681.3/635.2 for gadotrexate and 363.8/222.7 for the internal standard in the negative ionization mode. All details of the applied method are given in Section 2.3 LC–MS/MS analysis.

3. Results and discussion

3.1. LC–MS/MS analysis

Since no stable-isotope labeled gadoxetate was commercially available, amoxicillin was used as internal standard because of its comparable physicochemical properties (slight water solubility, anionic compound at pH > 3.5, free carboxyl group, phenyl ring) and its very similar chromatographic behavior in hydrophilic interaction chromatography (HILIC).

In the negative ionization mode, mass peaks from the deprotonated molecular species ($[M-H]^-$) were predominately observed for both compounds. They were considerably higher than the respective peaks from the protonated compounds in the positive mode.

Gadoxetate (*m*/*z* 681.3) was fragmented to the product ions 374.0, 388.1, 522.2, 591.2 and 635.2; the latter generated the highest signal intensity. The finally monitored mass-to-charge transitions, *i.e.* 681.3/635.2 for gadotrexate and 363.8/222.7 for the internal standard, were manually optimized to obtain maximum mass peak intensities (Table 1).

Because of the hydrophilic nature of gadoxetate and the need for chromatographic separation from biological matrices to avoid ion suppression, we performed HILIC using a step-elution gradient. The resulting mean retention times were 6.8 min for gadoxetate and 6.9 min for the internal standard (Fig. 2). Interestingly, the resulting gadoxetate peak was characterized by a considerable shoulder. Although we have no clear explanation for this phenomenon, one may speculate that this may be due to the presence of two diastereomers which have been reported in the literature (ratio 65:35) [15]. Another explanation may be the presence of protonated and non-protonated forms of the acetic acid residues of gadoxetate the ratio of which is expected to be approximately 50:50 at pH 3.8 (mobile phase). One analytical run lasted 13 min which enabled a sample throughput of about 80–90 per day.

3.2. Validation

The analytical method was shown to be selective for gadoxetate as indicated by the absence of analytical signals in different blank serum samples and cell lysates and lack of interferences between the analyte and the internal standard (Fig. 2). However, we have not checked potential interferences of our method with different clinical drugs. Although detection *via* tandem mass spectrometry (including the retention time) represents so far the most selective detection, one cannot exclude influences from other compounds. Our approach to check the selectivity of our assay represents only a minimal version (*i.e.* using six different sources of blank matrix), which is also recommended by the bioanalytical method validation guidelines from the FDA and EMA. However, during an ongoing clinical study we measured serum pharmacokinetics from 36 healthy volunteers and verified that all blank serum samples from these subjects were free of any analytical signals.

As gadoxetate could not be sufficiently extracted from the biological samples by liquid–liquid extraction or solid phase extraction, we used protein precipitation with acetonitrile for sample preparation. Because this preparation leads to substantial dilution of the analytes and because it is not compatible with the chosen gradient elution (the content of acetonitrile was about 60%, v/v), acetonitrile was back-extracted by addition of ethyl acetate and the concentrated aqueous residue (~100 μl) was used for chromatographic analysis.

In order to avoid disturbances of the chromatography during the analysis of cell lysates, it was necessary to remove SDS by precipitation with barium acetate and subsequent capture of non-volatile barium ions with ammonium sulfate. This simple precipitation

Table 2
Individual calibration curves of gadoxetate in human serum and statistical evaluation (linear regression with 1/*x* weighting, *x* = concentration). Each calibration curve was prepared and measured on different days (between-day data).

No.	Concentration of gadotrexate (ng/ml)									
	5	10	25	50	100	250	500	1000	2000	4000
1	4.5	9.8	24.9	49.7	98.6	276	512	1070	1900	3990
2	4.6	9.2	24.2	50.7	97.3	284	541	1030	1890	4010
3	4.5	9.5	25.8	55.5	98.6	274	454	1030	1990	3990
4	4.2	10.0	27.2	51.2	96.4	293[a]	487	1040	1700	4230
5	5.5	10.5	26.6	46.6	89.4	252	489	949	2000	4070
6	4.5	8.6	25.3	53.1	104	278	528	988	1920	4030
Mean	4.62	9.6	25.7	51.1	97.4	273	502	1017	1900	4053
SD	0.47	0.7	1.1	3.0	4.7	12.2	31.6	42.7	108.3	91.6
RSD (%)	10.1	6.8	4.3	5.9	4.8	4.5	6.3	4.2	5.7	2.3
RE (%)	−7.6	−4.0	2.7	2.3	−2.6	9.1	0.4	1.8	−5.0	1.3

SD, standard deviation; RSD, relative standard deviation (precision); RE, relative error (accuracy).
[a] Outlier, not included in calculation.

Table 3
Within- and between-day accuracy and precision for the determination of gadoxetate in human serum and cell lysates. Data are presented as relative errors (accuracy) or coefficients of variation (precision) of nominal and respective mean concentrations.

	n	Within-day data		Between-day data	
		Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
Serum, *c* (ng/ml)					
10	6	−4.8	3.8	1.9	3.8
250	6	11.5	1.7	11.0	3.9
4000	6	−3.7	2.8	1.4	4.8
Cell lysates, *c* (ng/ml)					
2.5	6	2.1	7.5	4.6	4.7
25	6	2.6	5.8	3.9	4.9
500	6	5.4	3.7	4.0	5.4

method may be an easy and cheep alternative compared to commercially available removal kits for SDS.

There was a good linear correlation between the concentrations of gadoxetate and the analytical signal among the entire validation range for serum (5–4000 ng/ml) and cell lysates (1.25–500 ng/ml). In both matrices, the linear regression model (1/*x* weighting, *x* = concentration) showed a distribution of the residuals without in-homogeneities and trends (Table 2; Fig. 3). Mean correlation coefficient (*r*) of all calibration curves (each $N = 6$) was 0.9993 ± 0.0002 in serum and 0.9988 ± 0.0013 in cell lysates.

Within-day and between-day accuracy and precision for determination of gadoxetate in serum and cell homogenates were within the stipulated range of the current bioanalytical guidelines (±15%, Table 3) [17,19].

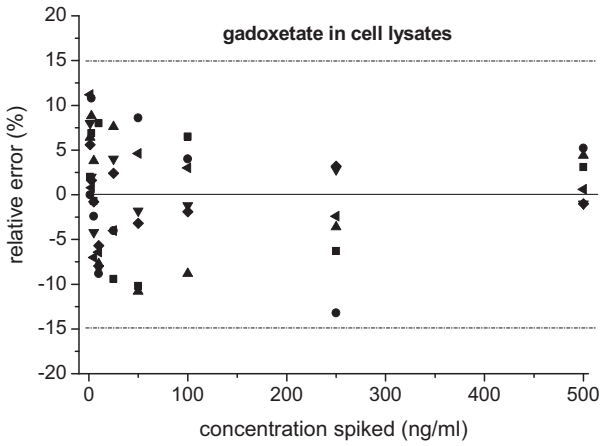

Fig. 3. Residual plot for the determination of gadoxetate in cell lysates. Data points represent individual errors from six individual calibration curves prepared and measured on different days. Values from different calibration curves are indicated by different shapes (*e.g.* circle, diamond).

We observed no significant matrix effects (Table 4), *i.e.* the signal intensity for gadotrexate obtained from matrix samples was nearly identical with the signals from matrix free samples (relative error was between −0.6% and −7.5%). Considering the well-established level of acceptance for the error of an analytical assay suggested by the FDA guideline (*i.e.* ±15%), we conclude that matrix effects are negligible in our analytical assay. The considerable retention of the analyte and the internal standard most likely contributed to this desired lack of ion suppression.

Gadoxetate was show to be stable in the biological matrices at room temperature for at least 2–4 h (98.2–108.5%, Table 4) and for up to 4 weeks when stored at −20 °C (103.3–106%). The prepared samples were also shown to be stable for at least 24 h when stored at 5 °C in the autosampler (92.2–98%). Finally, gadoxetate containing samples can undergo up to three freeze–thaw cycles without any significant influence on its stability. These stability data are in good agreement with the literature [14].

The validation data demonstrated that the developed LC–MS/MS method was specific, sensitive, precise and accurate for the determination of gadoxete in human serum and cell lysates. All quality parameters fulfilled the international criteria for bioanalytical method validation. In our assay, different to previously described methods, the intact chelate complex was exclusively measured. The sensitivity of the LC–MS/MS assay is at least 50-fold higher than of well established ICP-AES methods [10–13].

3.3. Application of the method

The analytical method enabled the quantitative determination of gadoxetate in cell lysates and human serum. The cellular uptake of gadoxetate into HEK293 cells transfected with the OATP1B1 wild-type protein (*1a) was several-fold higher compared to

Table 4
Results of stability tests and determination of matrix effects for gadoxetate in human serum. Data are given as mean values in percent of the respective initial concentrations (stability) or as relative error (RE) calculated from the difference to matrix-free samples (matrix effects). All values were calculated in each case from $N = 6$ experiments, respectively.

	Concentration of Gd-EOB-DTPA (ng/ml)		
	10	250	4000
Matrix effects (RE)	−7.5%	−1.5%	−0.6%
Post-preparative (rack) stability			
18 h @ 5 °C (autosampler)	95.3%	98.0%	92.2%
Freeze–thaw stability			
1st freeze–thaw cycle	93.9%	101.0%	92.7%
2nd freeze–thaw cycle	95.2%	101.6%	99.7%
3rd freeze–thaw cycle	105.4%	107.0%	96.3%
Short-term (bench-top) stability			
2 h @ room temperature	100.1%	104.4%	99.0%
4 h @ room temperature	108.5%	106.5%	98.2%
Long-term stability			
4 weeks @ −20 °C	106.0%	106.0%	103.3%

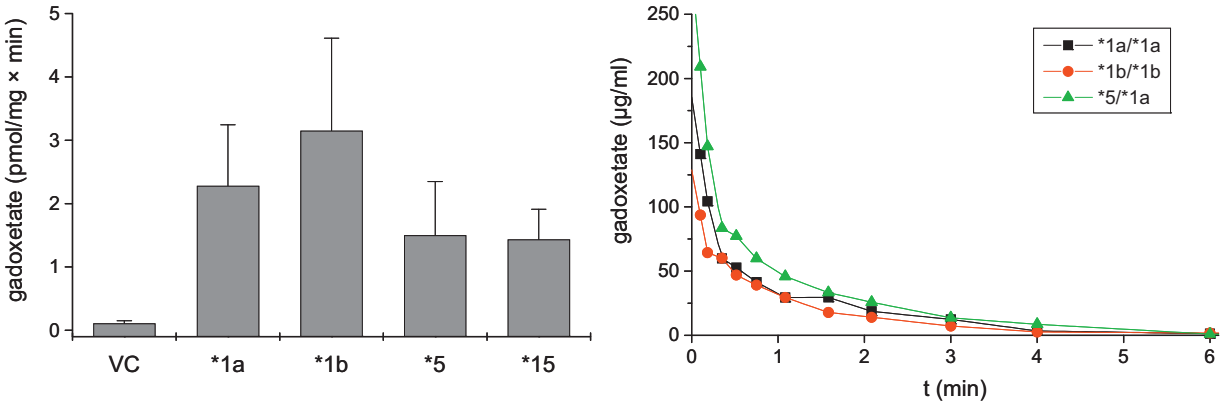

Fig. 4. Uptake of gadoxetate (500 µM) into vector control cells (VC) and OATP1B1**1a/*1b/*5* and **15* transfected HEK293 cells after incubation for 10 min (left); serum concentration-time profiles of gadoxetate after intravenous application of 25 µmol/kg in three healthy volunteers genotyped for *SLCO1B1* (right).

vector control cells (Fig. 4) as expected from previous studies [5]. The accumulation of gadoxetate in cells expressing the variant proteins OATP1B1*1b, *5 and *15 was markedly different compared to the wild-type cells (Fig. 4). The results suggest that the hepatic uptake of gadoxetate may be influenced by genetic polymorphisms of OATP1B1. In good agreement to our *in vitro* studies, carriers of variant OATP1B1 alleles (*1b, *5 and *15) showed slightly different serum concentration time profiles of gadoxetate which may be caused by different hepatic uptake of the contrast agent in these subjects.

4. Conclusions

The developed LC–MS/MS method was shown to be specific, sensitive, precise and accurate for the quantification of gadoxetate in human serum and cell lysates. The method validation also indicated stability of the analyte and good linearity over a wide concentration range. To our knowledge, this is the first LC–MS/MS assay which enables the sensitive quantification of the intact chelate complex gadoxetate in biological matrices. The new method was successfully applied to analyze cellular uptake studies and a pharmacokinetic pilot study.

Gadoxetate is a substrate of the hepatic uptake transporter OATP1B1. Genetic variants of this transporter may contribute to the substantial inter-subject variability of the drug.

Funding

This study was supported by the grant 03IP612 (InnoProfile) of the German Federal Ministry for Education and Research.

Acknowledgments

The authors express their gratitude to Gitta Schumacher, Sabine Bade and Danilo Wegner for their excellent technical assistance.

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