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Low Picogram Determination of Ro 48-6791 and its Major Metabolite, Ro 48-6792, in Plasma with Column-switching Microbore High-performance Liquid Chromatography Coupled to Ion Spray Tandem Mass Spectrometry

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A coupled liquid chromatography/tandem mass spectrometry assay was developed for simultaneous determination of Ro 48-6791 and its secondary amine metabolite in human plasma samples with a quantification limit for both compounds of 1 pg/mL using a 1 mL plasma aliquot. The method exploits the enhanced mass sensitivity of a microbore (300 μm i.d.) reversed-phase capillary column coupled to an ion spray probe combined with tandem mass spectrometry. A straightforward column-switching system was utilized to focus the analytes onto a microbore trapping column following solid-phase extraction of a 50 μL plasma sample extract from liquid/liquid extraction. Backflushing of the retained analytes from the trapping column onto the microbore capillary column provided the requisite high peak concentration for high sensitivity. The inter-assay precision and accuracy for Ro 48-6791 and its metabolite, at 10 pg/mL, were found to be 3.4%, and 105%, and 9.1%, and 99.9%, respectively. The calibration curves were linear over the range 1 to 1000 pg/mL. The method proved to be sufficiently rugged for analysis of samples.

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The trend in drug discovery to design novel drugs, with increasing potency, demands analytical quantification methods of ever-increasing sensitivity. This requirement, along with the need for high throughput methods, has moved liquid chromatography, coupled with atmospheric pressure ionization and tandem mass spectrometry, into the forefront of analytical techniques for the quantitative analysis of drugs and their metabolites in biological fluids.[1–7] The unique features of ion spray (pneumatically-assisted electrospray), combined with tandem mass spectrometry, furnish the basis for ultra-sensitive detection without compromising specificity. Since the ion spray interface behaves like a concentration-sensitive device,[8] sensitivity can be increased by using microbore analytical high-performance liquid chromatography (HPLC) columns in conjunction with mass spectrometric detection.[9–11] This improvement is based on the fact that the peak concentration of an analyte is inversely proportional to the square of the column radius. To make use of this improved mass sensitivity, the limitations of a microbore HPLC capillary (300 μm i.d.) relating to injection volume can be nicely circumvented by employing a trapping column-switching approach.[12,13] Column-switching HPLC has long been used for on-line solid-phase extraction of pesticides from environmental samples and drugs from biological fluids using conventional detectors.[14,15] Basically, the same set up can be used for microbore HPLC by making all components compatible with the much lower flow rates.[9,16] Beside the on-line trace enrichment of analytes from a liquid sample, the trapping column of a column-switching system also acts as a means for focusing the analytes. The use of specifically designed trapping columns, combined with analytical columns, relating to dimensions and type of packing materials, has opened up new ways for the quantitative analysis of drugs, e.g. by using on-line immunoaffinity extraction.[17–19]

In support of pharmacodynamic and pharmacokinetic evaluation of Ro 48-6791 {3-(5-dipropylaminomethyl-1,2,4-oxadiazol-3-yl)-8-fluoro-5-methyl-5,6-dihydro-4*H*-imidazo[1,5-a] [1,4]benzodiazepin-6-one} and its major metabolite, a very sensitive LC/MS assay was required to characterize concentration–time profiles in plasma. Ro 48-6791 was under clinical trial investigation as a short-acting agent for conscious sedation. This drug is a new water soluble full agonist which exerts its action as a ligand at the benzodiazepine receptor (BZR) in the brain. Following intravenous administration to man, the drug undergoes rapid biotransformation to form the monopropyl derivative Ro 48-6792, as the major metabolite. Owing to the high potency of the drug, doses as low as 0.1 mg were administered to man in the first tolerance study. Extrapolations suggested that concentration levels down to at least 10 pg/mL should be determined for pharmacokinetic profiling of the drug and its metabolite. Therefore, the goal was to develop an LC/MS assay for the concentration range 1–1000 pg/mL, along with a reliable quantification limit of 1 pg/mL for both analytes. To cope with this

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challenge, an analytical methodology was devised that was capable of combining automated on-line solid-phase extraction, using a single micro-trapping (0.8 mm i.d.) column-switching system, with a microbore (300 μm i.d.) analytical column coupled to an ion spray probe of an atmospheric pressure ionization (API) tandem mass spectrometer.

EXPERIMENTAL

Chemicals, reagents and materials

Ro 48-6791 and Ro 48-6792, as well as their tetra-deuterated structural analogues, were supplied by F. Hoffmann-La Roche Ltd., Basel (Fig. 1). Methanol, formic acid (>98%) and ammonium formate (all of analytical-reagent grade) were purchased from Merck (Darmstadt, Germany) and used without further purification. Ultrapure water was produced using a Milli-Q^{Plus} purification system from Millipore (Volketswil, Switzerland. Human plasma was isolated by centrifugation of ethylene diamine tetra-acetic acid (EDTA)-treated blood. Pure nitrogen (>99.999) and ultrapure argon (>99.9997) were supplied by Carbagas (Basel, Switzerland).

Instrumentation

Liquid chromatography system. The HPLC system was set up to perform both isocratic and gradient runs.[9] An Hitachi L-6200A pump (A) from Merck (Dietikon, Switzerland) was used in the isocratic mode to supply the microbore trapping column (Inertsil ODS-3; 0.8 mm i.d. × 5 mm; LC Packings, Amsterdam, The Netherlands) with eluent (Fig. 2). Two LC-10AD pumps (B) from Shimadzu (Burkard Instrumente AG, Geroldswil, Switzerland) were used to simultaneously supply a dynamic mixing chamber (stirred), of 75 μL volume (LabSource, CH-4153 Reinach, Switzerland), with eluent, each at a flow rate of 100 μL/min. Under isocratic conditions, the same eluent was delivered from both pumps. The product was then split, after the mixing chamber, utilizing a high-pressure microbore mixing tee (3 μL dead volume; Valco Europe, Schenkon, Switzerland) to supply the packed-capillary column (fused-silica, Inertsil ODS-3, 5 μm; 300 μm i.d. × 150 mm; supplied by LC-Packings) with eluent at a flow rate of approximately 5 μL/min. The split ratio was adjusted by using a restriction capillary made of fused-silica tubing. Its length and internal diameter (50 μm i.d.) were predetermined to accommodate the desired split ratio of about 40:1. This arrangement allowed the system to be used in gradient or isocratic mode without any hardware modification.

A membrane degasser (LabSource) was used for on-line degassing of the eluents. An AS-4000 autosampler (Merck) equipped with a 50 μL sample loop and a high-speed electrically-driven microbore switching valve (Cheminert C2) from Valco (LabSource) were used. A low-pressure in-line filter (0.2 μm disposable filter, Whatman Inc., Sterico AG, Dietikon, Switzerland) was placed between the mobile phase reservoir and the pump.

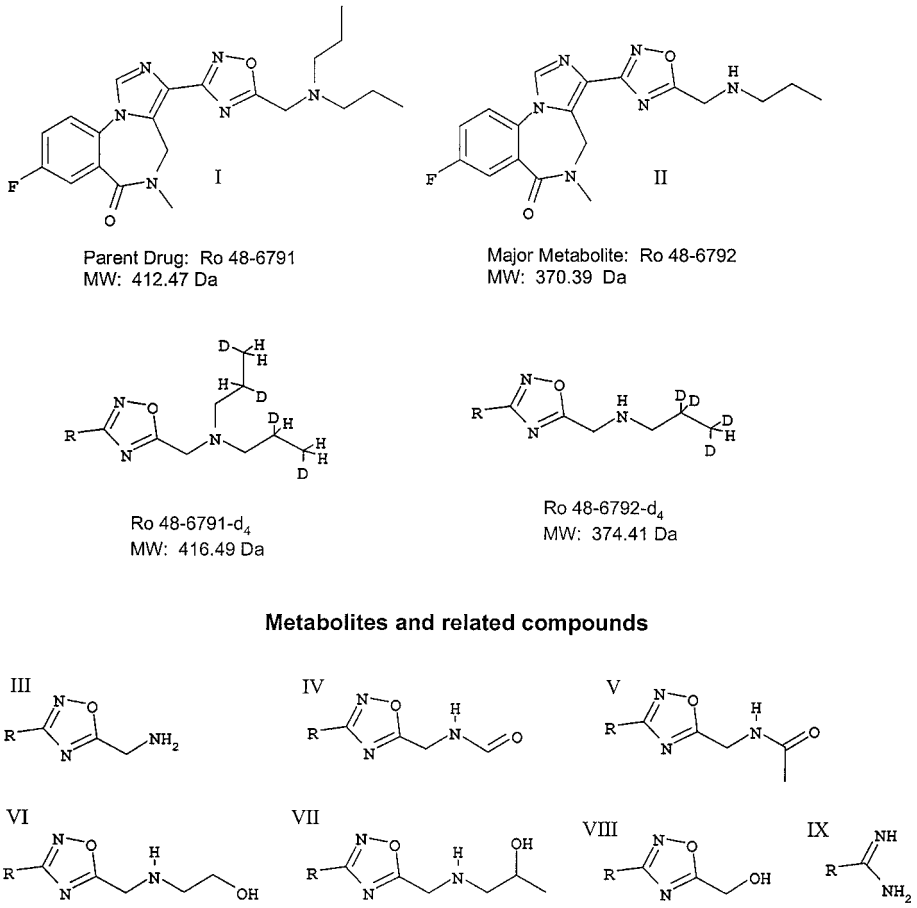

Figure 1. Structural formulae of compounds under investigation.

Assay Procedure

Preparation of calibration- and QC-samples. Initially, calibration samples, made up in human control plasma, were prepared in the range 0.020 to 20 ng/mL by spiking 10 mL of plasma with an appropriate volume (25 μL) of the working standard solutions (prepared in ethanol) containing Ro 48-6791 and Ro 48-6792. These calibration samples were further fortified with control plasma to yield a 20-fold dilution covering the range 1–1000 pg/mL in serial steps. The quality control samples were prepared using the same procedure as for the calibration samples, but by a different analyst, using independently prepared stock and working solutions.

Sample work-up. Aliquots of the plasma sample (1.0 mL) were transferred to glass culture tubes (Corning Inc., NY, USA) and the aqueous solution of the tetradeuterated internal standards (250 pg in 25 μL) added to the plasma. The sample was made alkaline with 1 M sodium hydroxide (50 μL) and remixed before the samples were extracted with *n*-butylchloride + 4% dichloromethane (5 mL) for 10 min on a rotary inversion mixer. Following centrifugation (680 g, 5 min), the aqueous phase was quick-frozen by immersing the tube in a dry ice–isopropanol bath. The organic phase was then decanted into a tapered glass tube and evaporated to dryness. The residues were reconstituted in 70 μL of 5 mM aqueous ammonium formate + methanol, (8:2, v/v) and centrifuged at approximately 10 000 g.

HPLC. The column-switching system (Fig. 2) consisted of a microbore trapping column and a packed fused-silica capillary column (referred to as the analytical column) all supplied by LC Packings. These were connected by a microbore switching valve (Valco). The transfer lines from the switching valve to the microbore trapping column were made of polyetheretherketone (PEEK) tubing (60 μm i.d., LC-Packings). The effluent of the analytical column was passed to the ion spray interface via fused-silica tubing (50 μm i.d.) to facilitate connection to the sprayer needle of the ion spray interface and to avoid extracolumn band broadening.

A 50 μL aliquot of the reconstituted extract was transferred by an autosampler onto the microbore trapping column at a flow rate of 100 μL/min. The eluent used was 5 mM aqueous ammonium formate (E1, pH 3.6). After a 2 min loading time, the microbore switching valve was reversed, so that eluent E2 (1% formic acid + methanol; 50:50, v/v), isocratically delivered by pump B, backflushed the retained analytes from the trapping column onto the HPLC capillary column at a flow rate of about 5 μL/min for separation of the analytes. Following a transfer time of 3 min, the switching valve was turned back to the initial position allowing the equilibration of the trapping column with eluent E1. All columns were operated at ambient temperature. Under these conditions, Ro 48-6791 and Ro 48-6792 eluted after 6.7 and 5.9 min following injection. The overall run time was 12 min.

Mass spectrometry

Mass spectrometric detection was carried out using a PE Sciex API III+ triple-quadrupole mass spectrometer (PE-SCIEX, Concord, Canada), equipped with an ion spray interface (pneumatically assisted electrospray). The ion spray probe was modified in-house by using an electropolished stainless-steel sprayer needle (25 μm i.d., 50 μm o.d.). The outlet of the HPLC capillary was taken via a fused-silica capillary (50 μm i.d.) and by a joint connection to the sprayer needle. The potential of the sprayer needle was maintained at +5600 V and the ion sampling orifice was kept at +50 V. Positive ions formed at atmospheric pressure were

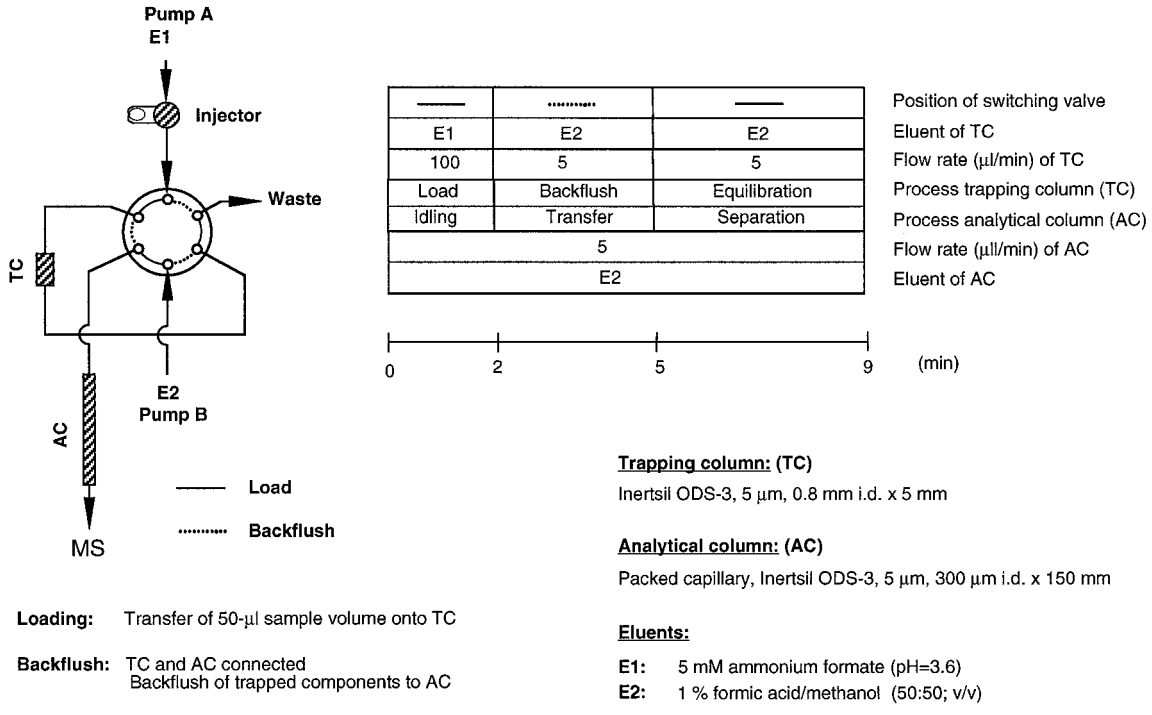

Figure 2. Set-up and time sequence of a single trapping column-switching system.

sampled into the quadrupole mass filter through this sampling orifice. Ultrapure nitrogen was used as the nebulizing and curtain gas at flow rates of 0.5 and 0.6 L/min, respectively. The electron multiple setting was –5200 V and the detector electronics was set to count every pulse. In order to optimize the position of the sprayer needle, relative to the orifice, under the conditions used for analysis of the samples (flow rate approx. 5 μL/min), flow injection of a standard solution containing the aforementioned compounds was performed. The sprayer needle position yielding the highest ion current signal was maintained throughout the analysis of samples.

The mass-analysing quadrupoles (Q_1 and Q_3) of the tandem mass spectrometer were individually calibrated daily up to *m/z* 600 at unit mass resolution, by infusion, in acetonitrile, of a standard solution containing tributylmethylammonium bromide and tetrabutylammonium up to tetradecylammonium bromides (200 fmol/μL) into the ion spray interface. Infusion was performed at 10 μL/min, with a syringe pump, (Harvard, Model 55-1111, Southnatick, Mass, USA).

Selected-reaction monitoring (SRM) was used for detection of the analytes. Two periods for data acquisition were employed to allow adaptation of the dwell times. During the first period, the transitions monitored were *m/z* 371 –> 300 (Ro 48-6792), and *m/z* 375 –> 300 (Ro 48-6792–d_4). The dwell time for each transition was 200 ms. During the second period, the target ions of Ro 48-6791 and its internal standard Ro 48-6971-d_4 were used in the form of transitions *m/z* 413 –> 114 and *m/z* 417 –> 118, respectively, at a dwell time for each transition of 400 ms. The singly protonated molecular ions of the analytes were fragmented by collision-induced dissociation (collision energy 10 eV) with ultrapure argon at a collision gas thickness of approximately 3.0×10^{15} molecules/cm^2.

Acquisition and treatment of data

Data acquisition and integration of SRM chromatograms were performed using, respectively, the proprietary software packages RAD (Routine Acquisition and Display program) and MACQUAN, for PE-SCIEX. The calibration curves were established by linear least-squares regression, weighted to the quadratic reciprocal of concentration ($1/x^2$), versus the measured peak-area ratios of the respective analyte/internal standard pair using MACQUAN.

Validation procedures

The LC/MS assay was validated in terms of inter-assay accuracy and precision for the analytes from human plasma, by the quantification limit under routine use, and by specificity of the methodology. The accuracy and precision of the method were assessed by analysing replicates of quality control samples on at least four separate occasions; the samples were spiked at different concentrations for each analyte in human plasma. The concentrations were calculated from the linear regression equation of the daily calibration curve.

RESULTS AND DISCUSSION

Method development

The goal of the method development was to achieve ultimate sensitivity (1 pg/mL) without compromising selectivity. Since the ion spray interface behaves like a concentration-dependent device, the peak concentration C_{max} is inversely proportional to the square of the column radius.[10] Therefore, when switching from a narrow-bore 2 mm i.d. column to a 300 μm i.d. capillary, a theoretical gain in sensitivity of 44 can be expected, provided the same amount of compound is injected and the separation efficiency of the columns is comparable. However, the injection volume of a capillary column (300 μm i.d.) is reduced by the same factor compared with a narrow-bore column. Usually, volumes in the range of 50 to 200 nL can be injected without causing loss in column efficiency due to band broadening. Since our goal was to achieve a quantification limit of 1 pg/mL we could not afford to inject nL or μL volumes, but had to use a 1 mL plasma sample. It is obvious that the direct injection of a 1 mL sample onto a microbore column would last more than 200 min, provided a flow rate of 5 μL/min was applied. In addition, efficient peak focusing had to be employed by using a solvent strength of the sample well below that of the eluent used. Apart from this time-consuming procedure, direct injection of plasma would likely ruin the column's performance. Therefore, a work-up procedure was required which provided both an efficient clean-up and a reconcentration of the analytes.

When using off-line solid-phase extraction (SPE), the biggest problem was occasional clogging of the microbore trapping column, believed to be caused by particulate matter in the sample extract. Liquid–liquid extraction was preferred to other procedures, since it yielded very clean extracts combined with a good reconcentration effect. Initial attempts showed that reconstitution of the residues after the evaporation step with solvent volumes < 10 μL was critical due to loss of compounds. Chiefly due to this finding, direct injection of the reconstituted extract onto the HPLC capillary, utilizing solvent focusing, was discarded. The use of a simple column-switching system with a microbore trapping column (0.8 mm i.d. × 5 mm) proved to be an efficient way to load a 50 μL sample in less than 5 min onto the microbore capillary column, which is run isocratically at 5 μL/min, with unattended operation. A benefit of this approach was that the sample was further purified on the trapping column by solid-phase extraction; additionally, the trapping column also acted as a guard column for the analytical capillary column. This contributed to the ruggedness of the assay in that the longevity of the capillary column could be enhanced. The use of a micro-guard column directly attached on top of the capillary column is advisable to enhance its useful lifetime. It was also interesting to note that the injection of a 50 μL sample aliquot could be performed with a standard autosampler, while injection of volumes in the nL or lower-μL range demanded a specifically designed autoinjector.

An important point for full exploitation of the inherent sensitivity enhancement, available when using a microbore capillary compared to narrow-bore columns, was to reduce major dead volumes of the

column-switching system so as to maintain the original column efficiency. Thus, the transfer lines from the microbore switching valve to the trapping and the analytical column were kept as short as feasible. Provided the connections between the different devices were properly used, no significant loss in column efficiency could be observed (Fig. 3).

Mass spectrometric considerations

The mass assignment of the precursor and the product ions used for the assay was performed by infusion of the target analytes. In order not to jeopardize the specificity of the mass spectrometric detection, unit mass resolution was used throughout. Full-scan product-ion spectra of the protonated precursor molecules of Ro 48-6791 ($[MH]^+$ at *m/z* 413) and Ro 48-6792 ($[MH]^+$ at *m/z* 371) are depicted in Fig. 4. It is interesting to note that the most abundant product ion of Ro 48-6791 at *m/z* 114 is produced by cleavage of the tertiary amine at the alpha position to form $CH_2 = N^{\oplus}(C_3H_7)_2$. For Ro 48-6792, the same cleavage occurred, but the formation of $CH_2 = N^+(C_3H_7)$ at *m/z* 72 was not the predominant reaction pathway; rather, the complementary moiety (*m/z* 300) of the molecule, produced the most abundant product ion. As a result, the same product ion was used for Ro 48-6792 and its deuterated internal standard, for detection in the SRM mode, in contrast to the procedure for Ro 48-6791. Therefore, special emphasis was given to monitoring possible carry-over in the collision cell at *m/z* 300.

The highest abundance of the product ions was found at a collision energy of approximately 10 eV. The effluent of the capillary column was passed directly to the ion spray needle, without using a make-up solvent, to avoid any decrease in sensitivity due to dilution of the peak volume. A key factor in ultimate sensitivity was the careful examination of ion suppression effects caused by the matrix. A decrease in sensitivity, by a factor of about 2, was found for both compounds when plasma extracts, produced by liquid/liquid extraction, spiked with the same amount of compounds, were compared with a pure solution containing the same concentrations. Extracts generated by SPE showed a higher suppression factor.

Assay characteristics

Inter-assay precision and accuracy. The inter-assay precision and accuracy were evaluated by assaying QC samples in duplicate at 10 pg/mL and 100 pg/mL on 4 different days using a calibration curve from 1 to 1000 pg/mL. The mean precision and accuracy for Ro 48-6791 were found to be 4.3% and 100.8%, respectively (Table 1). The corresponding precision and accuracy for Ro 48-6792 were found to be 7.4% and 98.4%, respectively. The precision at 10 pg/mL was higher compared with that of the parent compound, probably chiefly due to the lower SRM response of the secondary amine metabolite, but the accuracy was not affected.

Linearity and limit of quantification. The linearity of the calibration curves for Ro 48-6791 and Ro 48-6792 encompassed 3 orders of magnitude with a quantification limit of 1 pg/mL for both analytes. Fig. 5 displays the calibration curve of the secondary amine metabolite covering 1 to 1000 pg/mL. Excellent linearity was found in this calibration range for both analytes. The intercept of the calibration graph did not significantly

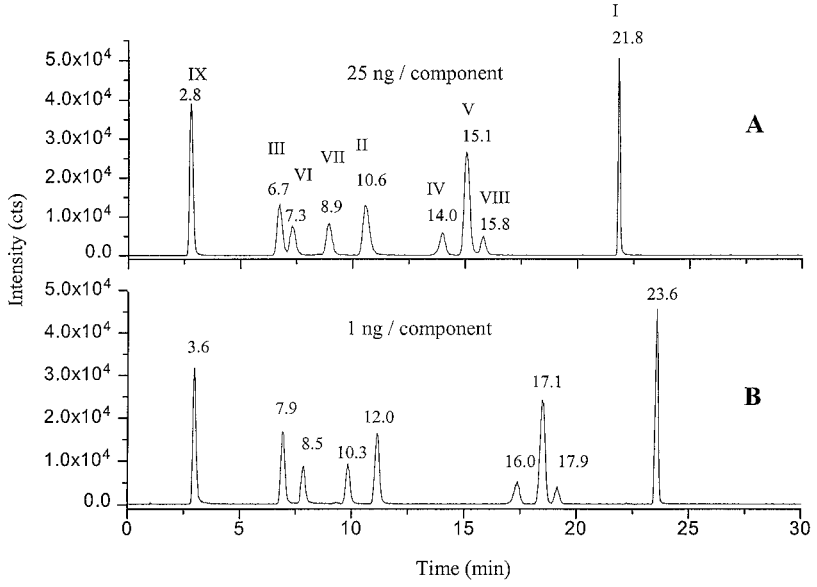

Figure 3. SRM chromatograms of Ro 48-6791 and some of its metabolites for comparison of sensitivity and separation between (a) narrow-bore (2.0 μm i.d. × 125 mm); and (b) microbore (300 μm i.d. × 125 mm); HPLC with gradient elution. The packing material was Kromasil 100 C18, 5 μm, in each case. Eluent A: 5 mM ammonium formate (pH 3.6) + methanol (90:10, v/v). Eluent B: 5 mM ammonium formate (pH 3.6) + methanol (10:90, v/v). The separation was accomplished by applying a linear solvent gradient from 10% B to 90% B in 20 min and maintaining this for an additional 5 min at a flow rate of 5 μL/min for the microbore column and 200 μL/min for the narrow-bore column. The concentration of the test solution was 5 ng/μL for each component. Injection volumes: 5 μL (a) and 200 nL (b) directly on column.

deviate from zero, underlining the excellent selectivity of the assay and the absence of the nonlabelled compound in the tetradeuterated structural analogues used as internal standard. The detection limit was well below 1 pg/mL (Fig. 6).

Sensitivity and specificity. Fig. 6 displays SRM chromatograms from a calibration sample at the quantification limit of 1 pg/mL for each of the compounds and its pertinent human control plasma using isocratic elution. The signal-to-noise ratio of Ro 48-6792 at this level was found to be about 6 (Fig. 6(a)), while that of Ro 48-6791 was better than 20, underscoring the excellent sensitivity (Fig. 6(c)). Selectivity can be assessed by comparison of the SRM chromatograms of the calibration samples with those of the human control plasma samples. At the retention times of the target analytes, minor peaks could be detected which were presumably caused by some carry-over in the chromatographic system. For Ro 48-6791 the signal-to-noise ratio at the quantification limit was therefore reduced to about 10. The biggest challenge to achieve this quantification limit was to exclude any other sources contributing to the overall blank. Specificity provided by chromatographic separation and two stages of mass selectivity is useless if the target compounds contribute to the blank due to carry-over during the assay procedure.

Features of the LC/MS assay

This LC/MS assay has been developed to demonstrate the versatility of the column-switching approach with microbore HPLC columns to achieve ultimate sensitivity. The purity of the plasma extracts turned out to be the key to success when using capillary HPLC columns. Although the stability of 300 μm i.d. capillaries is comparable to narrow-bore columns,[20] this only holds true as long as relatively clean samples are injected. For this reason, the quality of the sample clean-up prior to analysis of the extract with the capillary analytical column determines the ruggedness of the assay. Under the clean-up conditions described, batches of approximately 30 samples were analysed in a series. Flushing of the trapping column with an eluent of acetonitrile +5% aqueous buffer restored the original performance. Using this procedure, more than 100 1 mL plasma samples could be analysed utilizing the same microbore trapping and analytical column. In rare cases, clogging of the 60 μm i.d. transfer capillary, which ran from the microbore switching valve to the analytical column, was encountered.

A further benefit of this column-switching approach is its facile application for gradient elution using microbore columns. This is important for qualitative analysis but also for quantitative analysis if evidence is accumulating that isomeric metabolites are in the sample since gradient elution provides the means to chromatographically separate isomer compounds. Owing to the unique features of the trapping, column-switching approach, metabolites from samples for structure elucidation can be concentrated on the trapping column to yield the required sensitivity (Fig. 3).

Another versatile approach[21,22] investigated was to deproteinize the plasma sample with perchloric acid, and load a 1.0 mL aliquot of the supernatant for solid-phase extraction directly onto a standard-bore trapping column (Supelcosil LC-AB2, 5 μm, 4.6 mm i.d. × 20 mm, from Supelco (Buchs, Switzerland)) at a flow-rate of 2.5 mL/min in 2 min, using the same column-switching set-up as displayed in Fig. 2. Then, instead of a 300-μm i.d. capillary column, a microbore 1 mm i.d. column was used at a flow rate of 50 μL/min. Therefore, the transfer time of the retained analytes from the standard-bore trapping column onto the analytical column could be reduced by a factor of ten, compared with the 300 μm i.d. capillary, producing acceptable run

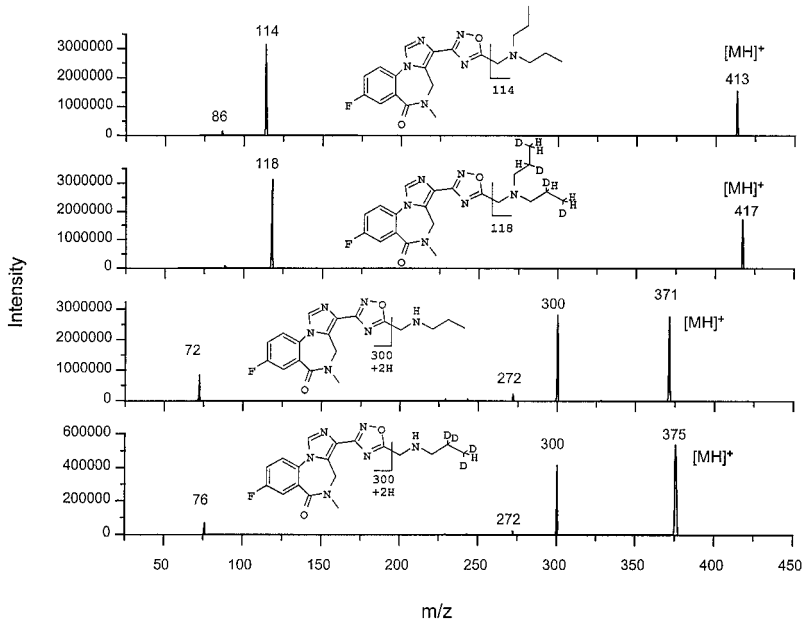

Figure 4. Product-ion spectra of (a) Ro 48-6791 and (c) Ro 48-6792 along with their tetradeuterated structural analogues (b) and (d) respectively with specified fragment ions. Positive ion mode; collision energy: 10 eV. Collision gas: argon; collision gas thickness: 3.0×10^{15} molecules/cm^2.

Table 1. Inter-assay precision and accuracy with isocratic, microbore column-switching HPLC combined with ion spray MS, operated in the SRM mode and using deuterated internal standards for the determination of the analytes in human plasma

	Ro 48-6791					Ro 48-6792			
Conc. added (pg/mL)	Mean conc. found (pg/mL)	Accuracy (%)	RSD (%)	*n*	Conc. added (pg/mL)	Mean conc. found (pg/mL)	Accuracy (%)	RSD (%)	*n*
10.0	10.5	105.4	3.4	7	10.0	9.99	99.9	9.1	7
100	96.2	96.2	5.1	8	100	96.8	96.8	5.1	8

times of about 12 min. Fig. 7 displays SRM chromatograms of the parent drug at 5 pg/mL. This assay proved to be more than 5 times less sensitive compared with the microbore trapping approach, but it offered the advantage of a reduced sample work-up combined with a ruggedness that was sufficient for routine use.

CONCLUSIONS

The microbore column-switching LC/MS/MS assay provided very good sensitivity without impairing precision or accuracy. Its ruggedness is considered sufficient to analyse moderate batches of plasma samples for special investigations. This approach demonstrates the feasibility of the sensitive quantification of drugs in very small sample volumes (< 10 μL). As a consequence, complete pharmacokinetic profiles of a drug might be assessed with one small animal without sacrificing it. Furthermore, this approach allows the

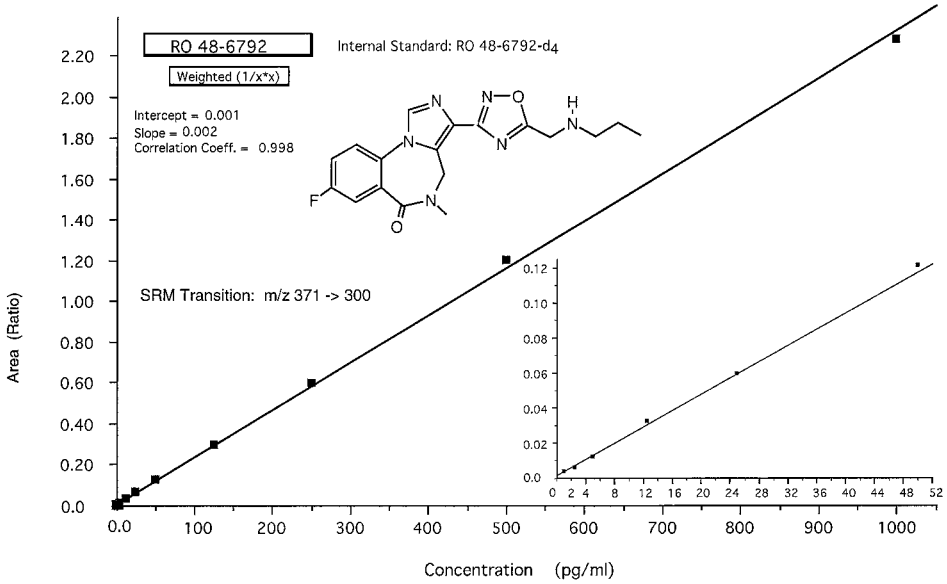

Figure 5. Calibration curve for Ro 48-6792 using column-switching microbore HPLC. SRM operation with ion spray MS. Calibration range 1–1000 pg/mL.

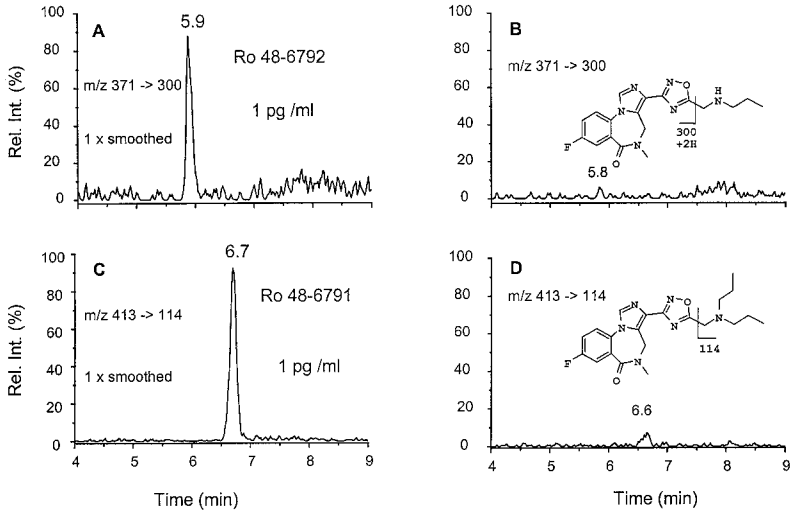

Figure 6. Traces (a) and (c) depict SRM chromatograms for Ro 48-6792 and Ro 48-6791 respectively in a human calibration sample at the quantification limit of 1 pg/mL in each case. Traces (b) and (d) correspond to the pertinent control plasma sample. Chromatographic conditions are as outlined in the experimental section.

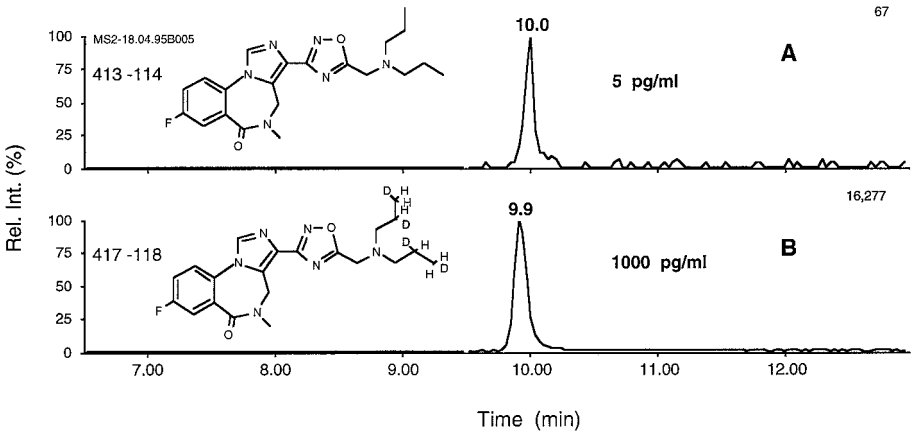

Figure 7. (a) SRM chromatogram of a human plasma sample of Ro 48-6791 at 5 ng/mL. (b) SRM trace for the tetradeuterated internal standard. Column-switching with on-line SPE, of a deproteinized plasma sample on a standard bore trapping column coupled with a 1 mm i.d. analytical column and combined with ion spray SRM detection. See text for experimental details.

efficient use of microbore capillary columns using conventional autosamplers and pumps. The possibility of using neat bile and urine for sensitive metabolite screening will be a further benefit of this approach.

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