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Journal of Chromatography B, 850 (2007) 259–266
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Sensitive and specific LC–ESI–MS/MS method for the determination of a styrylquinoline, BA011FZ041, a potent HIV anti-integrase agent, in rat plasma

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Received 9 May 2006; accepted 21 November 2006
Available online 8 December 2006

Abstract

A LC–MS/MS method was validated for the determination of BA011FZ041, a styrylquinoline derivative. After addition of BA011FZ055 as internal standard (IS), the method involved solid phase extraction (SPE), LC separation with an ether-phenyl column and quantification by MS/MS after positive ESI. The calibration curve, ranging from 1 to 500 ng/mL was fitted to a $1/x$-weighted quadratic regression model. Lower limit of quantification (LLOQ) was 1 ng/mL using 100 μL of plasma. Intra- and inter-assay precision and accuracy values were within the regulatory limits. The method was successfully applied to the determination of BA011FZ041 in rat plasma and PBMCs after i.v. dosing.

Keywords: HIV; Anti-integrase; LC–MS/MS; Plasma; PBMCs

1. Introduction

Styrylquinoline derivatives (SQLs) belong to a new class of potent HIV-1 integrase inhibitors [1] and may provide lead compounds for the development of novel antiretroviral agents for AIDS therapeutics. Their *in vitro* antiviral activity and the mechanism of integrase binding inhibition have been well described [2–4]. BA011FZ041, a lead compound of this new class of inhibitors, recently showed synergism with both strand transfer inhibitors and reverse transcriptase inhibitors, suggesting that it could be used in combination with other antiretroviral drugs within multidrug therapy and illustrating the interest in developing an alternative class of integrase inhibitors. To our knowledge, no method has yet been developed for styrylquinoline assay in biological fluid. However, analytical methods for determination of similar compounds like quinoline and derivatives in plasma or blood have been previously described. These LC systems with UV and fluorescence detection [5–7] reach a lower limit of quantification (LLOQ) in the range 25–100 and 5 ng/mL for the most recently developed [8,9] and electrochemical detection [10], while with mass spectrometry (MS) and MS/MS detection the LLOQ ranges between 0.1 and 10 ng/mL [11–14]. The contribution of LC–MS/MS to the bioanalysis of xenobiotics and particularly of other HIV reverse transcriptase and protease inhibitors in plasma and in intracellular media is now well established [15–18], in terms of fast development, runtime reduction, sensitivity and specificity enhancement, which are major parameters in multidrug therapy. The aim of this work was to develop and validate a LC–MS/MS assay of BA011FZ041 in rat plasma and to use it in an exploratory phase to determine BA011FZ041 in peripheral blood mononuclear cells (PBMCs). SPE extraction, LC separation and MS/MS detection were optimized in order to obtain the best combination of recovery with a low matrix effect, short runtime and high sensitivity. The method was successfully applied to the quantification of BA011FZ041 in rat plasma and PBMCs after intravenous administration of BA011FZ041.

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doi:10.1016/j.jchromb.2006.11.038

2. Experimental

2.1. Chemicals and reagents

BA011FZ041, BA011FZ041 ethanolamine salt and internal standard (IS) BA011FZ055 were supplied by BioAlliance Pharma (France). Chemical structures are given in Fig. 1. Acetonitrile HPLC gradient grade and dimethylsulfoxide (DMSO) were obtained from Sigma–Aldrich (Saint-Quentin-Fallavier, France). Methanol HPLC gradient grade and formic acid were from V.W.R. (Nogent sur Marne, France). Ultrapure water (18.2 MΩ) was produced by a Maxima II system (Elga, Antony, France).

2.2. Preparation of calibration standards and quality controls (QC)

Calibration standards and QCs were prepared from two separate master stock solutions (2.5 mg/mL in DMSO) of BA011FZ041. A master stock solution of BA011FZ055 (internal standard) was prepared identically. These solutions were stored in clear glass vials with screw caps and were stable for at least 2 months at −20 °C. Appropriate dilutions in blank rat plasma (from blood collected in sodium heparin) were made to obtain calibration standards at concentrations of 1.00 (LLOQ), 2.00, 100, 200, 300, 400 and 500 (ULOQ) ng/mL and QCs at 1.00 (LLOQ), 3.00 (low QC), 250 (middle QC) and 450 (high QC) ng/mL. Standards and QCs were aliquoted and kept frozen at −20 °C for at least 15 days.

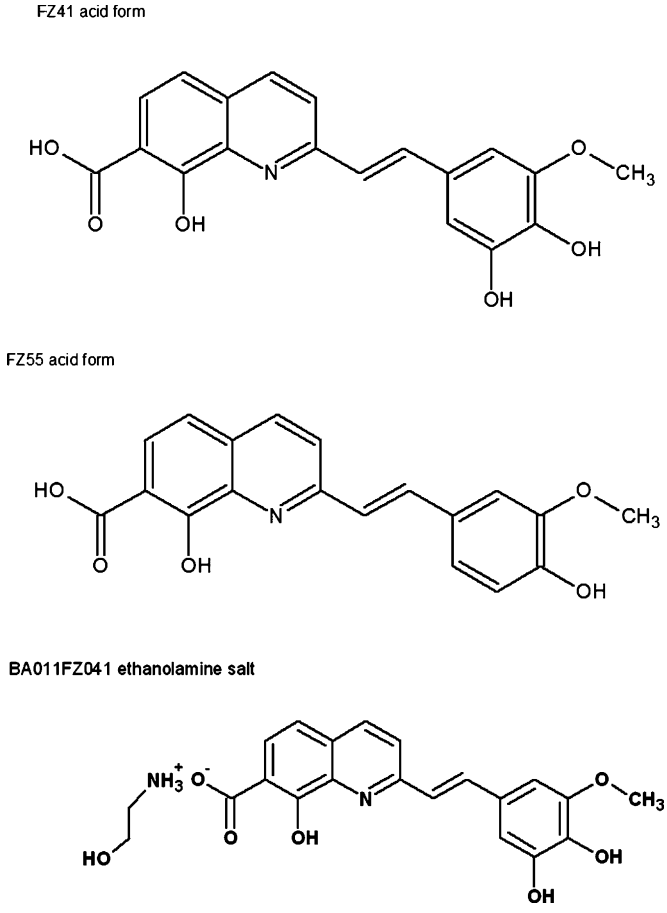

Fig. 1. Chemical structures of BA011FZ041 (FZ41), BA011FZ041 ethanolamine salt and BA011FZ055 (FZ55:IS).

2.3. Solid phase extraction (SPE) method for plasma samples

Samples were thawed at room temperature, vortex-mixed and centrifuged for 10 min at 2500 × *g* on a CR-312 centrifuge (Jouan, Saint-Herblain, France). One hundred microliter of internal standard solution (250 ng/mL in water/methanol/formic acid 93/5/2, v/v/v) were added to an aliquot of plasma (100 μL) in a polypropylene tube and vortex-mixed. The extraction steps were performed on Oasis Max cartridges (1 cc/30 mg, mixed mode anion exchange polymeric sorbent) from Waters (Saint-Quentin-en-Yvelines, France) using a positive pressure processor Speedisk 48 (Mallinckrodt Baker, Paris, France). The sorbent was conditioned with 1 mL of methanol followed by 1 mL of water. The sample was then applied under low pressure. Cartridges were washed with 1 mL of methanol and dried under a stream of nitrogen for 30 s. Analytes were eluted with 4 × 500 μL of acetonitrile/formic acid (98/2, v/v) in polypropylene collection tubes containing 20 μL of DMSO. The extract sample was evaporated at 40 °C with a nitrogen stream in a Turbovap LV evaporator (Zymark, Roissy, France) until the DMSO remained (*ca.* 20 μL). The extracts were completed with 80 μL of methanol and after vortex-mixing were transferred into polypropylene HPLC vials.

2.4. PBMC sample preparation and treatment

For this exploratory phase, the method development was based on our experience in this field and our knowledge of the collection, preparation and treatment of PBMCs [19].

Briefly, blank rat PBMCs were prepared from drug-free rat blood in order to obtain samples for calibration and QC samples. Two hundred milliliters of blood were treated and gave 480×10^6 cells (i.e., 2.4×10^6 cells/mL of blood). The cells were dispatched in conical tubes in fractions of around 10×10^6 cells and stored at about −80 °C. Five individual rat PBMC samples were prepared in order to have samples for specificity experiments.

After spiking PBMC samples with 500 μL of methanol containing BA011FZ041 and IS, the cells were lysed by vortexing and scraping the tube. After centrifugation, the supernatant was treated using the SPE extraction described above.

2.5. LC–MS/MS conditions

The LC–MS/MS consisted of an Alliance 2695 liquid chromatograph (Waters, Saint-Quentin-en-Yvelines, France) connected to a triple quadrupole mass spectrometer Quattro LCZ (Micromass, Manchester, UK) operating in positive electrospray ionization (ESI) mode. The system was piloted by Masslynx 4.0 from Micromass. We used a Synergi Polar-RP (ether linked phenyl) 4 μm, 50 mm × 2 mm i.d. column from Phenomenex

(Paris, France), protected by a guard column, 4 mm × 2 mm, packed with the same sorbent and thermostated at 40 °C. Mobile phase A consisted of water/formic acid (99.5/0.5, v/v) and mobile phase B of methanol/formic acid (99.5/0.5, v/v). The gradient conditions ramped from 5% B to 70% B between 0 and 0.01 min, and to 100% B between 0.01 and 2 min, maintained up to 3 min, ramped to 5% between 3 and 4 min and then maintained during 4 min. The flow rate was established at 300 μL/min with a backpressure of about 30 bar. The injection volume was 20 μL and run time was 8.0 min.

Compounds were detected using the multiple reaction monitoring (MRM) mode. Tuning parameters were optimized by infusing a 1 μg/mL solution of each compound in a mixture of both mobile phase solvents (1/1, v/v). The precursor → product ion transitions were monitored at 354.2 → 336.0 and 338.2 → 320.1 for BA011FZ041 and IS, respectively. Each transition was alternatively monitored with a dwell time of 0.5 s. The capillary voltage was set at 3 kV, the cone voltage was set at 25 V and the collision energy was set at 20 eV for both BA011FZ041 and IS. Argon was used in the collision cell with a pressure set at 2.10^{-3} mbar. Nitrogen was used for nebulization (200 L/h), cone gas (85 L/h) and desolvation (600 L/h). The source and desolvation temperatures were set at 100 and 300 °C, respectively.

Unit resolution was applied for the two quadrupoles Q1 and Q3.

2.6. Calibration curves

Seven levels of calibration standards were used to establish the calibration curves which were fitted to a 1/*x*-weighted quadratic regression model. The calibration concentrations were back-calculated from the peak response, evaluated on the basis of the corresponding calibration curve, and deviations from the theoretical concentrations were required to be within ±20% for the LLOQ and within ±15% for other concentrations.

2.7. Recoveries

2.7.1. Ionization recovery

The ionization recovery (ion suppression also called matrix effect) was assessed by comparison of the responses obtained from analysis of extracts of a blank sample spiked with reference solutions and of reference solutions. It is given as the ratio of the average peak areas obtained from the spiked extracts relative to the average peak areas obtained from analysis of reference solutions. This ionization recovery was assessed by triplicate analysis at low level and high level QCs for BA011FZ041 and at the working concentration for IS.

2.7.2. Extraction recovery

The extraction recovery was assessed by comparison of the responses obtained from analysis of extracted rat QC samples and of extracted blank sample spiked with a reference solution. BA011FZ055 (IS) was added as internal standard just before injection for BA011FZ041 recovery measurements in order to normalize the response in case of variation of the injected volume in the LC–MS/MS system. BA011FZ041 was used as IS in the same way for BA011FZ055 recovery measurements. The response was the ratio of the peak area of the compound of interest versus the peak area of the compound used as IS. The recovery is given as the ratio of the average response obtained from extracted QCs relative to the average response obtained from analysis of spiked blank plasma extracts. This extraction recovery was assessed by triplicate analysis at low level and high level QCs for BA011FZ041 and at the working concentration for IS.

2.8. Selectivity and LLOQ intra-run accuracy and precision

The selectivity and the lower limit of quantification intra-run accuracy and precision of the analytical method were investigated by preparing and analyzing blank samples from six different batches of rat plasma (five for PBMCs) and spiked samples at the LLOQ level in the same six batches of rat plasma.

It is required that no interference be seen at the retention time of BA011FZ041 and IS in the six samples and that each LLOQ calculated concentration does not differ by more than 20% from the nominal value. The percentage precision (%CV) should not exceed 15%.

2.9. Intra- and inter-run accuracy and precision

A limited validation format was performed for this preliminary study at an early phase of the drug development. Accuracy and precision of the method were assessed by analyzing the intra- and inter-run accuracies and precisions of QCs analyzed together with the calibration standard samples on each day. Four QCs at concentrations of approximately 1.00 (LLOQ), 3.00 (low QC), 250 (middle QC) and 450 (high QC) ng/mL were analyzed on one analysis day, with five replicates (see Section 2.8. for LLOQ intra-run experiments) for intra-run and on 3 analysis days, with one determination for inter-run. Intra-run accuracy and precision were calculated as the mean accuracy and precision. Accuracy was required to be within ±15% and precision was required not to exceed 15% (see Section 2.8. for LLOQ intra-run accuracy and precision). Inter-run accuracy and precision were calculated as the mean accuracy and precision. Accuracy was required to be within ±20% for the LLOQ and ±15% for other QCs and %CV was required not to exceed 20% for the LLOQ and15% for other QCs.

2.10. Dilution test

A high QC out of the calibration range was prepared at a final concentration of 1031 ng/mL and diluted 1/4 to measure it in the median part of the calibration curve. This procedure was repeated three times. Accuracy was required to be within ±15% and the %CV was required not to exceed 15%.

2.11. Stability

The stability of BA011FZ041 and in some cases of IS was investigated by analyzing QC rat samples and stock solutions,

which were stored under various conditions, in triplicate together with freshly prepared calibration standard samples at the low and high level concentrations for plasma samples and at the middle equivalent level for stock solutions (i.e., 250 ng/mL). The stability experiments were performed, in plasma samples at room temperature, at +4 °C and at −20 °C. The effect of freeze-thaw cycles was also investigated. The stability of processed samples was also determined in elution solvent at room temperature and in injection solvent (i.e., auto sampler) at +4 °C. BA011FZ041 was considered to be stable in plasma or extracts when accuracy was within ±15%. The stability of BA011FZ041 and IS was evaluated in stock solutions stored at −20 °C. BA011FZ041 and IS were considered to be stable when the relative bias was within ±15%.

2.12. Application to in vivo plasma and PBMC samples

2.12.1. Plasma kinetic

A preliminary study was performed on male Sprague Dawley rats supplied by Elevage Janvier (Le Genest-St-Isle, France). Four rats were used for single i.v. injection. The IV solution extemporaneously prepared with BAO11FZ041 was a 0.8 mg/mL solution in hydroxypropyl β cyclodextrine in a pH 7.4 phosphate buffer. These vehicle and pH have been chosen because they allow the solubilization of the BA011FZ41 at least 0.8 mg/mL. Dose was around 0.5 mg/kg for i.v. dosing of BA011FZ041. Blood sampling was as follows: pre-dose, 5 min, 0.25, 0.5, 0.75, 1, 2, 4, 8, 24 and 48 h. At the chosen time of blood sampling, about 250 μL of venous blood (femoral vein) was taken in a heparinized syringe (sodium heparin). After centrifugation (3500 rpm/10 min) plasma was collected and stored at around −20 °C until analysis.

2.12.2. PBMC kinetic

Fourteen other male rats (seven in duplicate) were used for single i.v. injection in order to collect PBMCs. The IV solution extemporaneously prepared with BAO11FZ041 ethanolamine salt was a 0.8 mg of BAO11FZ041/mL solution in a pH 7.4 phosphate buffer. Blood sampling for PBMCs collection was as follows: pre-dose, 0.25, 0.5, 1, 2, 4 and 6 h. At the chosen time of blood sampling, about 10 mL of blood (abdominal aorta) was taken in a heparinized syringe (sodium heparin). After centrifugation, plasma was collected and the remaining white and red cells were resuspended in the same volume (equal to plasma) of 0.9% NaCl solution and this sample was used to collect and prepare the PBMCs [19].

The assay method was applied to both types of samples in order to determine BA011FZ041 concentration versus time in plasma and PBMCs.

3. Results and discussion

3.1. LC–MS/MS system

Tuning detection was performed by infusing the compounds in MS scan mode in order to establish the more favorable mobile phase and ionization mode. Despite the presence of a carboxylic acid function, the higher intensity was observed in

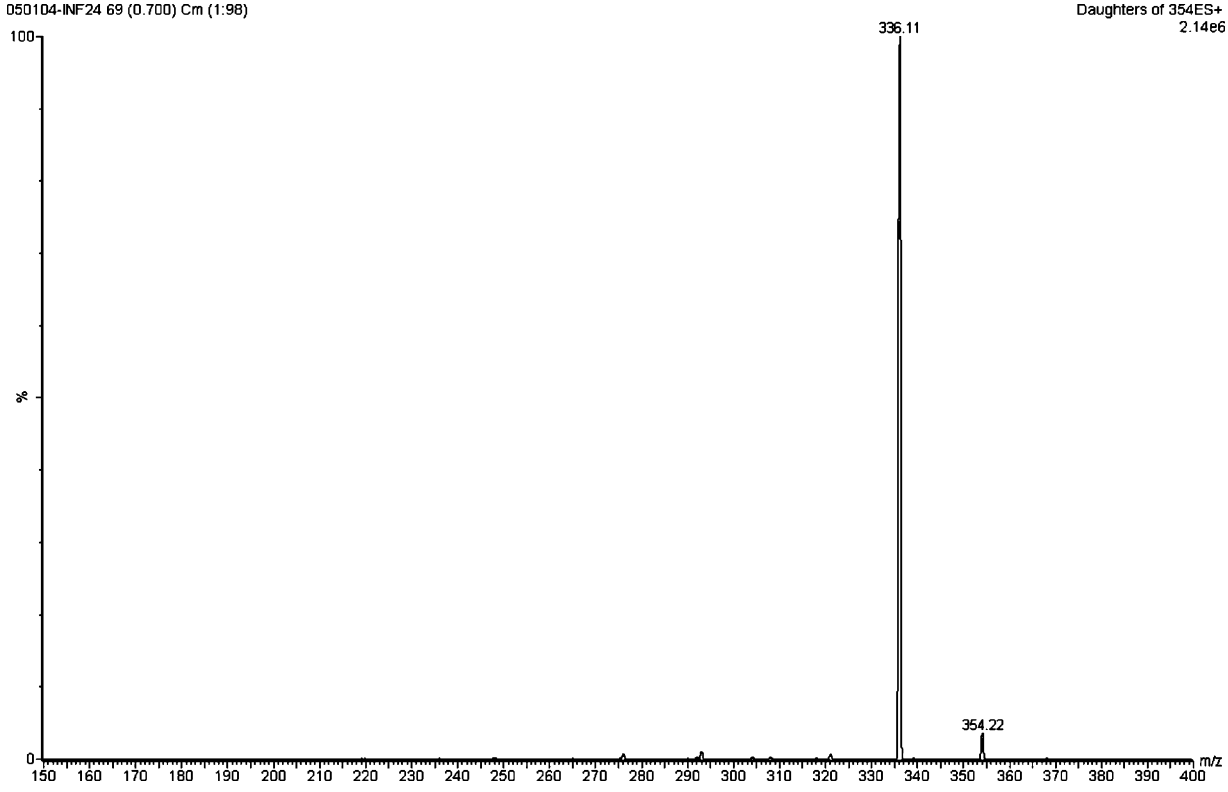

Fig. 2. Product ion spectrum of BA011FZ041 (collision energy = 20 eV).

positive ionization mode with a mixture of water/acetonitrile or methanol/0.05% formic acid showing a major ion $[M+H]^+$ at *m/z* 354. In the product ion spectrum of BA011FZ041 (Fig. 2), the main product ion was observed at *m/z* 336. It was estimated that it was produced by the loss of a water molecule. The same observations were made for BA011FZ055 (IS) since for the protonated species $[M+H]^+$ at *m/z* 338, the main product ion was observed at *m/z* 320. Not being certain of the high specificity of this ion, a second product ion, less intense, was also monitored for BA011FZ041 at *m/z* 276 with higher collision energy during the development and pre-validation phase. After this stage, only the first transition 354 → 336 was chosen because of better results for sensitivity, precision and accuracy. Due to the very similar structure of the two compounds, it was checked that no ion at *m/z* 338 (corresponding to BA011FZ055) was present in the BA011FZ041 MS scan spectrum.

Liquid chromatography was tested on columns containing phenyl functions. For the mobile phase, methanol was chosen in order to have a higher retention than with acetonitrile and 0.5% formic acid was added for better sensitivity. The Synergi Polar RP from Phenomenex was selected because of its higher resolution and better separation of the two compounds in case of interference (not observed). Different LC analyses in isocratic mode with various proportions of methanol showed that chromatographic peaks quickly broadened as the methanol percentage decreased. We then used a gradient method to stack the compounds in the first part of the column with mobile phase containing little methanol and then rapidly increased the methanol proportions to obtain sharper chromatographic peaks symmetrical in shape allowing better sensitivity. Finally, retention times were around 3.40 and 3.55 min for BA011FZ041 and IS, respectively. With this very fast gradient using methanol, the main objective was to prepare the sample efficiently, eliminating most endogenous compounds and so resulting in a lower matrix effect for the LC–MS/MS analysis.

3.2. SPE optimization

Each step should be carefully optimized to obtain the most efficient methodology. However, in this dynamic process, some steps may have synergistic or antagonistic effects. We therefore, used simple factorial design experiments to evaluate simultaneously many parameters, such as sample acidification and dilution, solvent nature and volume for both the washing and elution phases.

Different phases were tested, like Focus (10 mg), OASIS HLB (10 mg), OASIS HLB (30 mg) and OASIS MAX (30 mg) according to the standard procedure of the supplier. The best recovery was obtained with OASIS MAX. This mixed-mode anion exchange and reverse phase sorbent is well suited to compounds with carboxylic acid functions and allows the use of both ionic and hydrophobic interaction modes. The plasma sample was first diluted and slightly acidified to break the peptide bonds and loaded on the SPE cartridge. The hydrophobic interactions were strong enough to allow the retention of the compounds of interest in the stationary phase and anion exchange allowed to perform a 100% methanol washing step resulting in a clean sample by elimination of numerous endogenous compounds. This was particularly beneficial to achieving low ion suppression

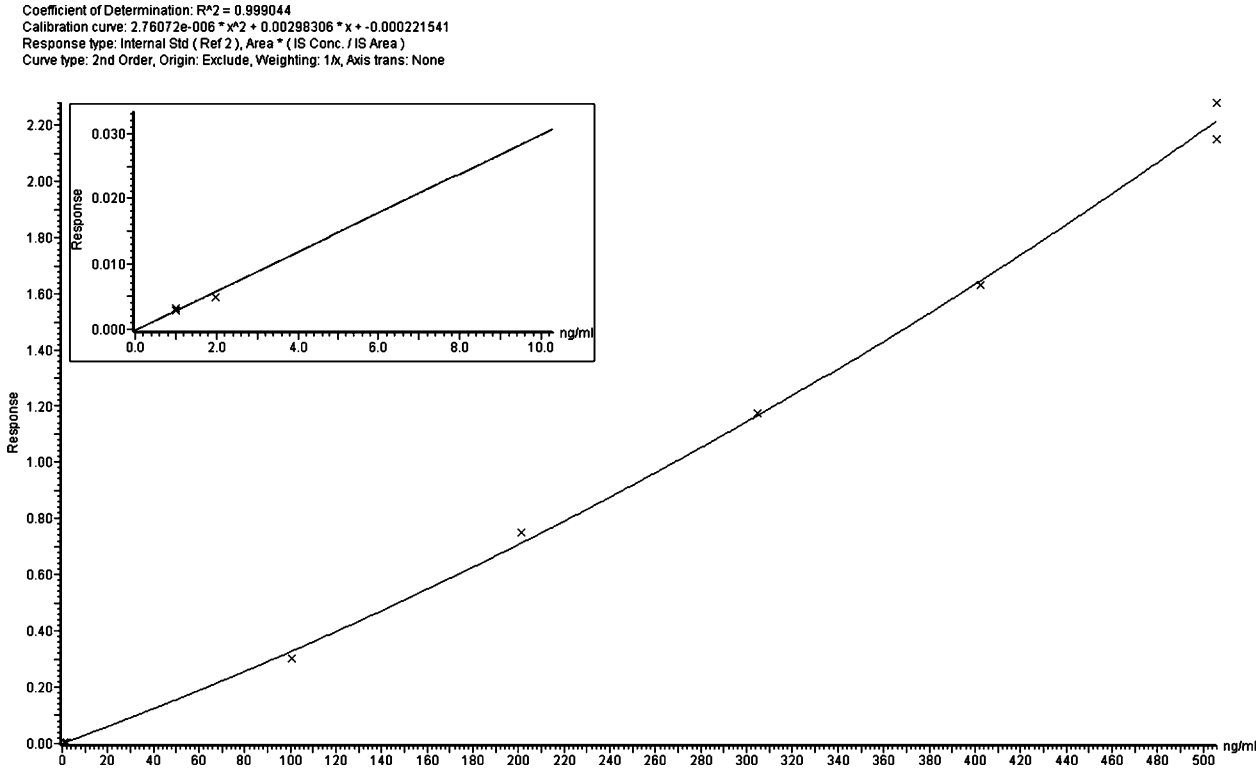

Fig. 3. Typical calibration curve in plasma.

for the MS detector. Finally, an elution mixture containing 2% formic acid in acetonitrile allowed to deionize the compounds of interest and thus, eliminate both ionic and hydrophobic retention. The extraction recovery was higher with fractionated elution in four equal parts of 500 μL than with twice 1 mL and also allowed a more rugged elution with better reproducibility.

First extraction recovery experiments gave very low performance. This measurement was performed after the extraction itself but also the evaporation of the eluted extract, and its reconstitution with the injection solvent before LC–MS/MS analysis. In fact the extraction step was not the main factor to blame. Due to poor compound solubility in a mixture of hydrophilic solvents, the reconstitution of the extract sample was not efficient and had to be improved. Finally, the best result was obtained with addition of 20 μL of dimethylsulfoxide to the extract before evaporation. This volume was minimized to eliminate any impact on chromatographic quality. At the end of the evaporation, only the DMSO remained with the concentrated compound. After addition of methanol, an aliquot was injected into the LC–MS/MS system. Reconstitution of the extract sample was assessed at low and high QCs for BA011FZ041 and at the working concentration for BA011FZ055. In these conditions, reconstitution recovery was complete (from 96.7 to 102.4% for BA011FZ041 and was 102.9% for IS) and allowed to performed extraction recovery measurements.

3.3. Calibration curves

A typical calibration curve is presented in Fig. 3. The calibration range was 1–500 ng/mL. The calibration curves were fitted to a 1/*x*-weighted quadratic regression model. As the ionization recovery in the electrospray ionization source was not constant but increased over the range of calibration, such a model was the most appropriate to our calibration curves. As a matter of fact, we did not observe a saturation phenomenon of the detector but quite the opposite. Mean coefficient of determination (r^2) was 0.9977 (%CV = 0.1; $n = 11$). Accuracy was −1.8% to +16.9% at the LLOQ level, −15.0% to +9.6% for the other levels.

3.4. Recoveries

3.4.1. Ionization recovery

As described above, the ionization recovery in the MS source was not constant over the entire calibration range. This phenomenon was not related to matrix effect since its amplitude was identical both in reference solutions and in plasma extract samples.

Mean recoveries were 109.6% for BA011FZ041 over the calibration range and 96.3% for IS at the working concentration. Such values close to 100% are reached when the sample preparation is well developed and then allows elimination of the major part of interfering endogenous compounds able to compete at the ionization step with the compound of interest.

Regarding the recoveries comprised between 100 and 115%, it is difficult to conclude toward a positive matrix effect since data are ranging in the normal analytical variability.

Table 1
Intra- and inter-day assay precision and accuracy of QC plasma samples for BA011FZ041

	Theoretical concentration (ng/mL)			
	1.00 (LLOQ)	3 (Low QC)	250 (Middle QC)	450 (High QC)
Intra-day ($n = 5$)[a]				
Precision (%)	13.9	8.0	4.6	1.9
Accuracy (%)	91.9	97.3	97.5	95.3
Inter-day ($n = 3$)				
Precision (%)	11.6	9.1	2.7	2.6
Accuracy (%)	101.6	99.2	98.3	91.9

[a] For LLOQ, intra-day experiments were performed with six different batches of rat plasma.

3.4.2. Extraction recovery

Mean recovery was 70.8% for BA011FZ041 over the calibration range. At the working concentration, recovery was 45.9% for IS. This value was not very high. Indeed, the extraction conditions were optimized for the analyte and unfortunately were not the best for IS. This is not a crucial point because the quantity of IS is adjusted in order to obtain a signal of similar intensity than the analyte, in the middle calibration range. Even with a poor recovery, the main question remains the precision of the response and in the case of this method the goal was reached.

3.5. Selectivity and LLOQ intra-run accuracy and precision

No interference appeared at the retention time of both BA011FZ041 and IS for the blank samples prepared from six different batches of rat plasma. Intra-day accuracy at the LLOQ level ranged from 81.7% to 115%. Intra-day mean accuracy and mean precision were 91.9% and 13.9%, respectively.

3.6. Intra-and inter-run accuracy and precision

Data are summarized in Table 1. Intra- and inter-day accuracy and precision met international requirements [20], but for this part, a more detailed validation will be needed for future work.

Table 2
Stability of BA011FZ041 in plasma and processed samples (extracts)

	Theoretical concentration (ng/mL)	
	3 (Low QC)	450 (High QC)
R.T.[a]	79.9	90.0
+4 °C[b]	98.3	98.7
−20 °C[c]	101	96.6
3 Freeze-thaw cycles	85.1	88.8
Processed samples −1[d]	88.4	86.1
Processed samples −2[e]	99.5	92.2

Figures represent the mean accuracy ($n = 3$) in %.
[a] Stability in plasma for 4 h at room temperature.
[b] Stability in plasma for 4 h at −4 °C.
[c] Stability in plasma for 2 weeks at −20 °C.
[d] Stability in elution solution for 4 h at room temperature.
[e] Stability in injection solvent for 72 h at +4 °C.

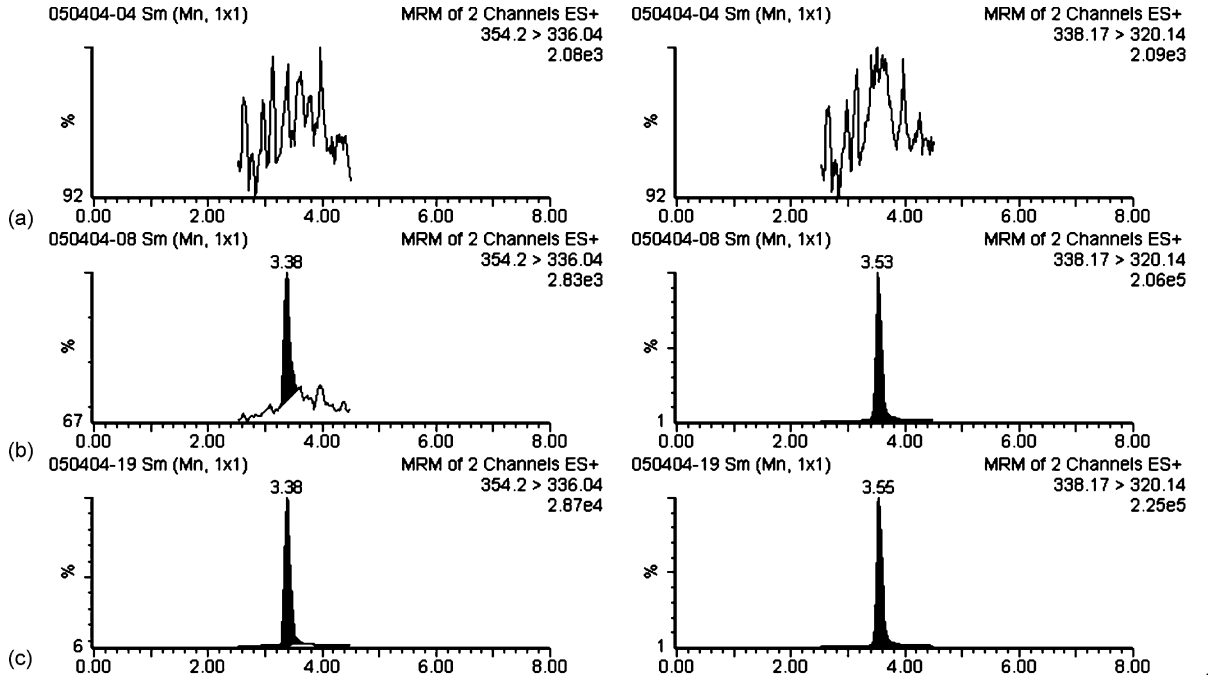

Fig. 4. Typical chromatograms of rat plasma sample: blank (a), spiked at the LLOQ level (b), 15 min after i.v. dosing (c). BA011FZ041 and IS are presented on the left and right side of the figure, respectively.

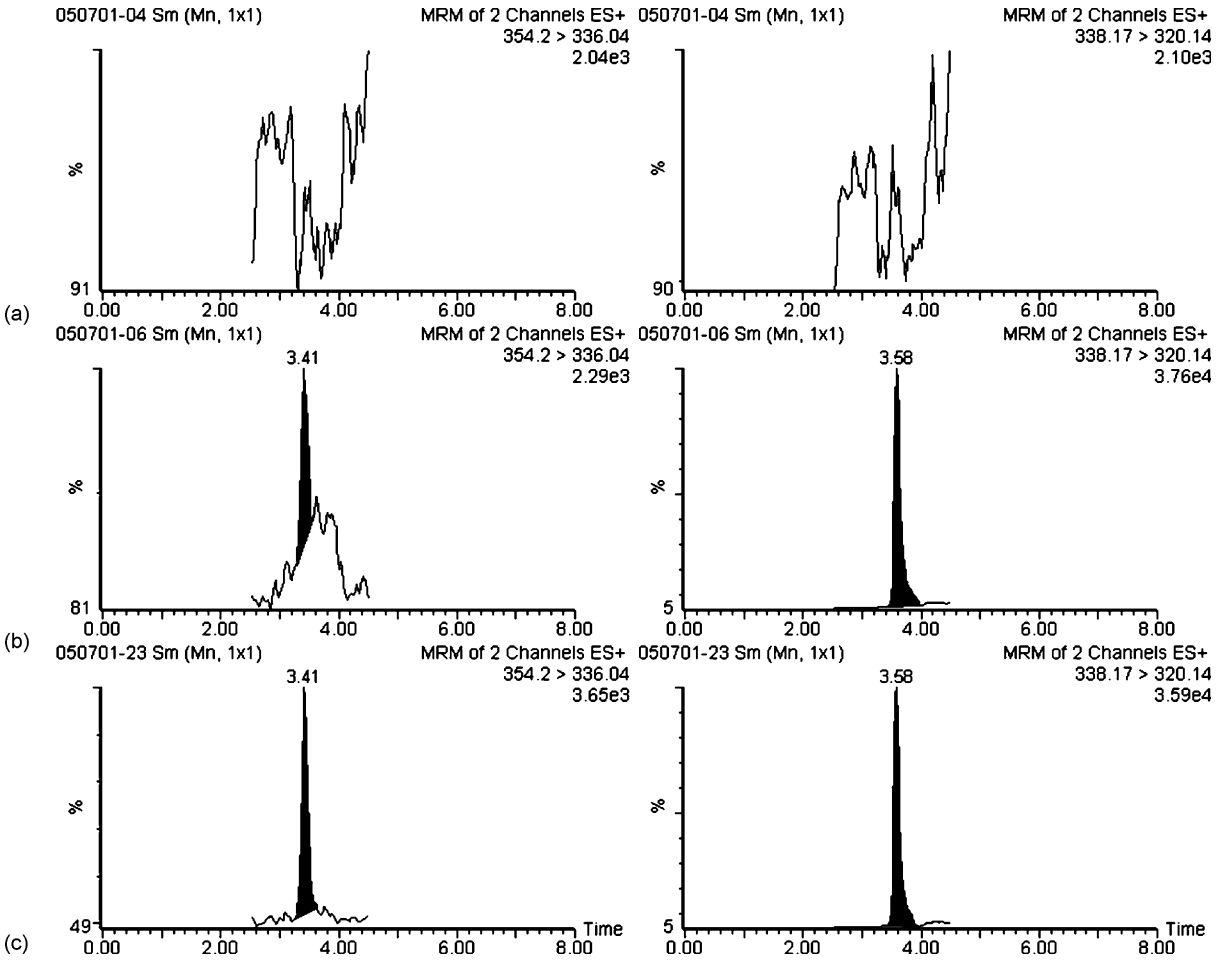

Fig. 5. Typical chromatograms of rat PBMCs sample: blank (a), spiked at the LLOQ level (b), 15 min after i.v. dosing (c). BA011FZ041 and IS are presented on the left and right side of the figure, respectively.

3.7. Dilution test

The high QC (1031 ng/mL) was diluted in a pool of three blank rat plasmas or in solvent (water/methanol/formic acid (93/5/2, v/v)). Mean accuracy and precision were 105% and 2.1% and 104% and 2.1%, respectively. The two procedures could be applied to plasma samples with high of BA011FZ041 concentrations outside the calibration range.

3.8. Stability

Results are presented in Table 2. BA011FZ041 was unstable at the low QC level at room temperature after 4 h. BA011FZ041 remained stable in rat plasma after three freeze-thaw cycles, for at least 4 h at +4 °C and 2 weeks at −20 °C. The compound was stable in elution solvent for 4 h of storage at room temperature, in the injection solvent (auto sampler) for at least 72 h at +4 °C and also in stock solutions after 2 months at about −20 °C (data not shown).

3.9. Application to in vivo plasma and PBMC samples

Typical chromatograms of blank rat plasma, rat plasma spiked at the LLOQ level and rat plasma after i.v. administration are shown in Fig. 4. On average, BA011FZ041 concentrations were followed until 4 h after i.v. dosing administration.

The assay was applied to the intracellular medium. A partial validation was therefore, performed. The calibration range was 0.1–10 ng/PBMC pellet (i.e., about 10 million cells). Mean extraction-lysis recoveries were 81.5% and 93% for BA011FZ041 and IS, respectively. Mean ionization recoveries were 98% for both BA011FZ041 and IS. Selectivity and intra-run accuracy and precision were checked by analyzing five blank extracts from five different rat PBMC samples and the same PBMC samples spiked with around 3.4 ng/PBMC pellet. Mean accuracy was 87.3% and precision was 4.7%. In these conditions, PBMC samples from different rats after intravenous administration of BA011FZ041 ethanolamine salt were analyzed. Typical chromatograms are presented in Fig. 5. Quantification of BA011FZ041 in rat PBMCs indicated concentrations around 0.17 ng/PBMC pellet. Mean cell count was found around 14×10^6 cells giving concentrations of BA011FZ041 around $0.012 \text{ ng} \times 10^6$ cells.

4. Conclusions

A sensitive LC–MS/MS method for the determination of BA011FZ041 in rat plasma was developed and validated. This method was shown to be specific, precise and accurate over a range of 1–500 ng/mL, and was successfully applied to the analysis of rat plasma samples in a preliminary pharmacokinetic study. The method was also useful for the preliminary determination of BA011FZ041 in rat PBMCs.

Acknowledgements

The authors thank Frédéric Théodoro for his help collecting and preparing PBMCs. Financial support for this study was provided by BioAlliance Pharma S.A.

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