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Development and validation of ultra high performance liquid chromatography–mass spectrometry method for LBH589 in mouse plasma and tissues

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ABSTRACT

An ultra high performance liquid chromatography tandem mass spectrometry method (UHPLC-MS/MS) was developed and validated for the quantitation of LBH589, a novel histone deacetylase inhibitor (HDACi), in mouse plasma and tissues (liver, spleen, kidney and lung). Tobramycin was employed as the internal standard. Separation was performed on an Acquity UPLC™ BEH column, with a mobile phase consisting of 10% water (with 0.1% of trifluoroacetic acid) and 90% methanol (with 0.1% trifluoroacetic acid). LBH589 and tobramycin were determined using an electrospray ionization (ESI) interface. Detection was performed on electrospray positive ionization mass spectrometry by multiple reaction monitoring of the transitions of LBH589 at m/z 349.42 → 157.95 and of tobramycin at 468.2 → 163. Calibration curves for the UHPLC method (0.0025–1 μg/mL for plasma and tissue homogenates, equivalent to 0.0357–14.2857 μg/g for tissue samples) showed a linear range of detector responses ($r > 0.998$). Intra-batch and inter-batch precision expressed as coefficient of variation (CV) ranged from 0.92 to 8.40%. Accuracy expressed as bias, ranged from −2.41 to 2.62%. The lower limit of quantitation (LLOQ) was 0.0025 μg/mL for both plasma and tissue homogenate samples, equivalent to 0.0357 μg/g tissue. This method was successfully applied to quantify LBH589 in plasma and tissue samples obtained after the intraperitoneal administration of a single dose of 20 mg/kg of LBH589 in BALB/c mice.

1. Introduction

The acetylation status of histones and non-histone proteins is determined by histone deacetylases (HDACs) and histone acetyltransferases (HATs) that play an important role in transcription regulation of eukaryotic cells. HDAC inhibitors (HDACi) selectively alter gene transcription, in part, by chromatin remodelling and by changes in the structure of proteins in transcription factor complexes and are promising molecules for the treatment of a number of diseases. Indeed, LBH589 is a novel HDACi that inhibits proliferation of different tumor cell lines (Hodgkin's lymphoma, acute myeloid leukemia, chronic myelogenous leukemia, and prostate, breast, colon and pancreatic cancer cell lines) [1,2].

HDACi are a diverse group of anticancer agents currently in clinical development, which can be divided into two groups: Class I-specific and pan-deacetylase inhibitors [3]. LBH589 has potent inhibitory activity at low nanomolar concentrations against all Class I, II and IV purified recombinant HDAC enzymes, suggesting true pan-DAC activity. In studies using enzymatic assays, LBH589 IC50 values (half maximal inhibitory concentration) were in the low nanomolar range (613.2 nM) for all HDAC enzymes, with the exception of HDAC4, HDAC7 and HDAC8, which had values in the mid nanomolar range (203–531 nM) [2].

LBH589 inhibits the activity of HDAC6, which makes it particularly effective in killing primary leukemia and lymphoma cells and established tumor-derived cell lines *in vitro* [4,5]. Hyperacetylation of H3, H4, and heat shock protein 90, an increase in p21 levels and induction of cell cycle G_1 phase accumulation are associated with exposure of cells to LBH589 [6]. Treatment with LBH589 also inhibits the DNA binding activity of signal transducers and activators of transcription-5 and induces apoptosis in imatinib-refractory leukemia cells [6].

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LBH589 is a promising candidate for leukemia treatment. Nevertheless, little information about LBH589 pharmacokinetics and biodistribution in animal models is available. Such studies are essential to set the administered dose, the route of administration and the pharmaceutical formulation in pre-clinical pharmacodynamic studies. In order to perform these studies, it is necessary to develop specific sensitive analytical methods for the quantitative determination of LBH589 in biological samples. To date, only a single analytical HPLC–tandem mass spectrometry method after LBH589 liquid–liquid extraction has been published for the quantization of LBH589 in plasma, with a limit of quantization of 2 ng/mL [7]. Using this method, the authors performed a primary evaluation of the pharmacokinetic profile of LBH589 in mice.

Ultra high performance liquid chromatography (UHPLC) can be considered an advanced tool for obtaining faster and more sensitive liquid chromatography. When this technology is coupled with high-speed-acquisition mass spectrometers, the main advantages are in the areas of resolution, speed and sensitivity for analytical determination [8]. During the last 5 years, several papers have focused on comparing previously developed HPLC methods with this newly introduced UHPLC [9–14]. In general, these methods have resulted in higher sensitivity, shorter analysis times with narrow peaks, and minimization of matrix effects.

The objective of the present study was to develop, optimize and validate an ultra high performance liquid chromatography tandem mass spectrometry method (UHPLC-MS/MS) for the determination and quantitation of LBH589 in mouse plasma and tissues. Pharmacokinetics and biodistribution of LBH589 were studied in mouse models by using the method that had been developed.

2. Experimental

2.1. Chemicals and reagents

LBH589 was provided by NOVARTIS (Basilea, Switzerland). Tobramycin was obtained from Normon (Madrid, Spain). Trifluoroacetic acid, tert-butyl methyl ether and methanol were obtained from Merck (Barcelona, Spain). Other reagents and solvents employed for analysis were analytical grade. Type I deionized water (18.2 MΩ resistivity) was obtained using a water purification system (Wasserlab, Pamplona, Spain). Nitrogen gas (ultra-pure, >99%) was produced by a Whatman model 75-72-K727 nitrogen generator (Haverhill, MA, USA) and by a Domnick Hunter LCMS series (Madrid, Spain). Argon gas (ultra-pure, >99.9%) was provided by Praxair (Madrid, Spain). Chemical structures of LBH589 and tobramycin are shown in Fig. 1.

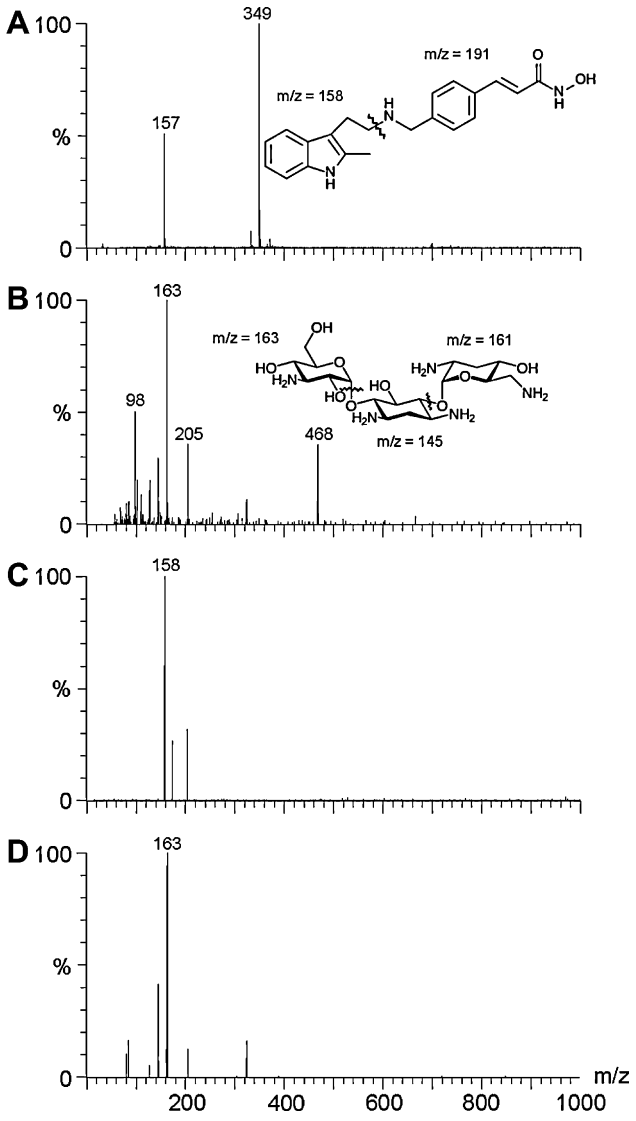

Fig. 1. Full scan ion spectra of (A) LBH589, (B) tobramycin and daughter spectra of (C) LBH589 and (D) tobramycin.

2.2. Instruments and analysis conditions

2.2.1. UHPLC–MS/MS method

The UHPLC system was composed of an Acquity UPLC™ system (Waters Corp., Milford, MA, USA) with thermostatized autosampler and column compartment. Separation was carried out on an Acquity UPLC™ BEH column (50 mm × 2.1 mm, 1.7 μm; Waters Corp., Milford, MA, USA) with isocratic elution using a mobile phase composed of 10% of water and 90% methanol. 0.1% of trifluoroacetic acid was added to both aqueous and organic phases. Column temperature was maintained at 20 °C. The flow rate was set at 0.25 mL/min. The analysis time was 2 min. The autosampler was thermostatized at 4 °C and the injection volume was 5 μL.

Mass spectrometric detection was performed on an Acquity™ TQD (Triple Quadrupole Detector) mass spectrometer (Waters Corp., Milford, MA, USA) with an electrospray ionization (ESI) interface. The mass spectrometer operated in positive mode. The *m/z* 349.42 → 157.95 and 468.2 → 163 transitions were selected for LBH589 and tobramycin (used as internal standard, I.S.), with the dwell time of 0.1 s per transition. Data acquisition and analysis were performed using the MassLynx™ NT 4.1 software with QuanLynx™ program (Waters Corp., Milford, MA, USA).

2.3. Preparation of standard and quality control (QC) samples

2.3.1. Plasma

The LBH589 stock solution was prepared in methanol at a concentration of 100 μg/mL. These stock solutions were diluted further with methanol in order to obtain working solutions: 0.025, 0.050, 0.1, 0.3, 0.5, 1, 3, 5 and 10 μg/mL. The stock solutions of the I.S. were prepared in water at 0.1 μg/mL. Drug-free blood samples from BALB/c mice were collected in EDTA-K3 surface-coated tubes and then centrifuged at 2000 × *g* for 10 min (4 °C) to separate the plasma. Calibrator samples were prepared by adding 10 μL of the standard solutions to 90 μL of blank mouse plasma. Therefore, the effective concentrations of LBH589 were 0.0025, 0.005, 0.01, 0.03, 0.05, 0.1, 0.3, 0.5 and 1 μg/mL. The concentrations of LBH589 quality control (QC) samples were 0.0025, 0.0075, 0.4 and 0.8 μg/mL. QC samples were prepared in the same way as calibrator samples. The

spiked plasma samples (calibrators and QC) were then processed following the extraction procedure described in Section 2.4.

2.3.2. Tissues

An amount of 70 mg of tissue (spleen, liver, lung and kidney) was homogenized using silica particles and a Mini-bead Beater (BioSpect Products, Inc., Bartelsville, OK, USA) with 1 mL of ultrapure water, achieving a final concentration of 70 mg tissue/mL. Silica particles were separated by centrifugation at 3500 × *g* for 10 min, and the homogenate was stored frozen (−80 °C). Calibrator samples were prepared by adding 10 μL of the standard solutions to 90 μL of tissue homogenates. Therefore, the effective concentrations of LBH589 were 0.0025, 0.005, 0.01, 0.03, 0.05, 0.1, 0.3, 0.5 and 1 μg/mL tissue homogenate (equivalent to 0.0357, 0.0714, 0.1428, 0.4285, 0.7142, 1.4285, 4.2857, 7.1428 and 14.2857 μg/g tissue). The concentrations of LBH589 QC samples were 0.0025, 0.0075, 0.4 and 0.8 μg/mL tissue homogenate (equivalent to 0.0357, 0.1071, 5.7142 and 11.4284 μg/g tissue). QC samples were prepared in the same way as calibrator samples. The spiked tissue samples (calibrators and QC) were then processed following the extraction procedure described in Section 2.4.

2.4. Plasma and tissue sample preparation

100 μL of plasma or tissue calibrator, quality control and test samples were transferred to 1.5 mL tubes. Then, 100 μL of Tris solution (0.025 M, pH 12) was added and the mixture was vortex-mixed at room temperature for 2 min. Next, 600 μL of tert-butyl methyl ether were added, and the mixture was vortexed again for 2 min. After centrifugation at 9200 × *g* for 10 min, the organic phase was separated and evaporated for 30 min at 37 °C, using a vortex-evaporator (LABCONCO, Kansas City, USA). Finally, 100 μL of methanol were added to the tubes and then 5 μL of tobramycin (0.1 mg/mL, used as I.S.) were spiked. The samples were stirred during 1 min and 5 μL aliquots were injected into the UHPLC system.

2.5. Method validation

Plasma and tissue samples were quantified using the I.S. method. Standard curves were calculated using linear least squares regression between the ratio of the chromatographic peak area of LBH589 to that of the I.S. and the nominal LBH589 concentration on calibrator samples. To evaluate linearity, calibrator samples were prepared and analyzed in duplicate on 5 separate days.

Within-day accuracy and precision were determined on a single batch with five replicate measurements ($n = 5$) of quality control samples at four concentration levels (0.0025, 0.0075, 0.4 and 0.8 μg/mL for plasma and tissue homogenate samples). Between-day accuracy and precision were also determined on five analytical batches with three replicate measurements for each quality control concentration level ($n = 15$). The accuracy was expressed as: (determined concentration–nominal concentration)/(nominal concentration) × 100, and the precision by the CV (%) of the measured concentration values obtained after analysis of the quality control samples with different nominal concentration values.

The absolute extraction recoveries of LBH589 at QC levels (0.0025, 0.0075, 0.4 and 0.8 μg/mL) were evaluated by measuring the samples as described above and comparing the peak areas of the LBH589 and the I.S. with those obtained from direct injection of the compounds dissolved in the extract of the processed blank plasma or tissue samples. The matrix effect of plasma and tissues at QC levels was evaluated by measuring the samples as described above and comparing the peak areas of the LBH589 and the I.S. dissolved in the extract of the processed blank plasma or tissues samples with those obtained from direct injection of the compounds dissolved in methanol.

The stability of LBH589 in BALB/c plasma and liver was assessed by analyzing replicates of QC samples at concentrations of 0.0075 and 0.8 μg/mL for plasma and liver homogenate, during the sample and storage procedure. For all stability studies, freshly prepared and stability testing QC samples were evaluated by using a freshly prepared standard curve for the measurement. The short-term stability was assessed after exposure of the samples to room temperature for 6 h. The freeze/thaw stability was determined after three freeze/thaw cycles (−80 °C to room temperature). The long-term stability was assessed after the storage of the samples for 30 days at −80 °C. LBH589 autosampler stability was evaluated at 4 °C over 48 h. The concentrations obtained from all stability studies were compared to the concentrations calculated in the beginning of the study, and the percentage concentration deviation was calculated. The analytes were considered stable when the concentration difference was <15% between the concentrations in the beginning of the study and those calculated from the stability testing samples.

2.6. Application of the method

This assay was applied to the quantitation of LBH589 in plasma samples obtained from 10 BALB/c mice treated with an intraperitoneal dose of 20 mg/kg of a LBH589 solution. Blood samples were withdrawn at 0, 0.3, 0.6, 1, 2, 5 and 8 h post-administration in EDTA-K3 surface-coated tubes and then centrifuged at 2000 × *g* for 10 min (4 °C) to separate the plasma (100 μL). Plasma was stored frozen (−80 °C) until analysis. The tissue distribution of LBH589 was also studied. The animals were sacrificed 48 h after the LBH589 administration and the spleen, liver, lungs and kidneys were collected and weighed. Tissues were homogenized as previously described in Section 2.3.2.

3. Results and discussion

3.1. Mass spectrometry

The MS/MS parameters were optimized to the maximum response for LBH589 and tobramycin. To optimize the MS parameters, standard solutions of both analyte and I.S. were infused into the mass spectrometer at a concentration of 10 μg/mL. The following optimized MS parameters were finally employed: 2.5 kV capillary voltage, 30 V cone voltage for LBH589 and 55 V for the internal standard, 150 °C source temperature and 300 °C desolvation temperature. Nitrogen was used as desolvation and cone gas at a flow rate of 550 and 50 L/h, respectively. Argon was used as collision gas. The optimized collision energy for LBH589 was 20 eV and 23 eV for the I.S. The MS/MS transition of *m/z* 349.42 → 157.95 for LBH589 and 468.2 → 163 for tobramycin was finally selected. The LBH589 fragment ion at *m/z* 157.95 was generated from the ion at *m/z* 349.42 by the loss of 174 Da (see Fig. 1). In the case of tobramycin, the product ion is a result of cleavage of the glycosidic bonds and the subsequent loss of the aminosugar rings.

3.2. Development of the analytical method

The availability of sensitive and selective analytical procedures is a critical condition for the development of preclinical studies of drug pharmacokinetics and biodistribution. To achieve this aim, it is critical to optimize the chromatographic conditions to obtain symmetrical peak shapes, with short chromatographic analysis times. Usually, the way to improve the efficiency of the chromatography separation is to decrease the particle size. However, an increase in the column pressure above the limits of traditional HPLC hardware is observed when the particle size is decreased. The development

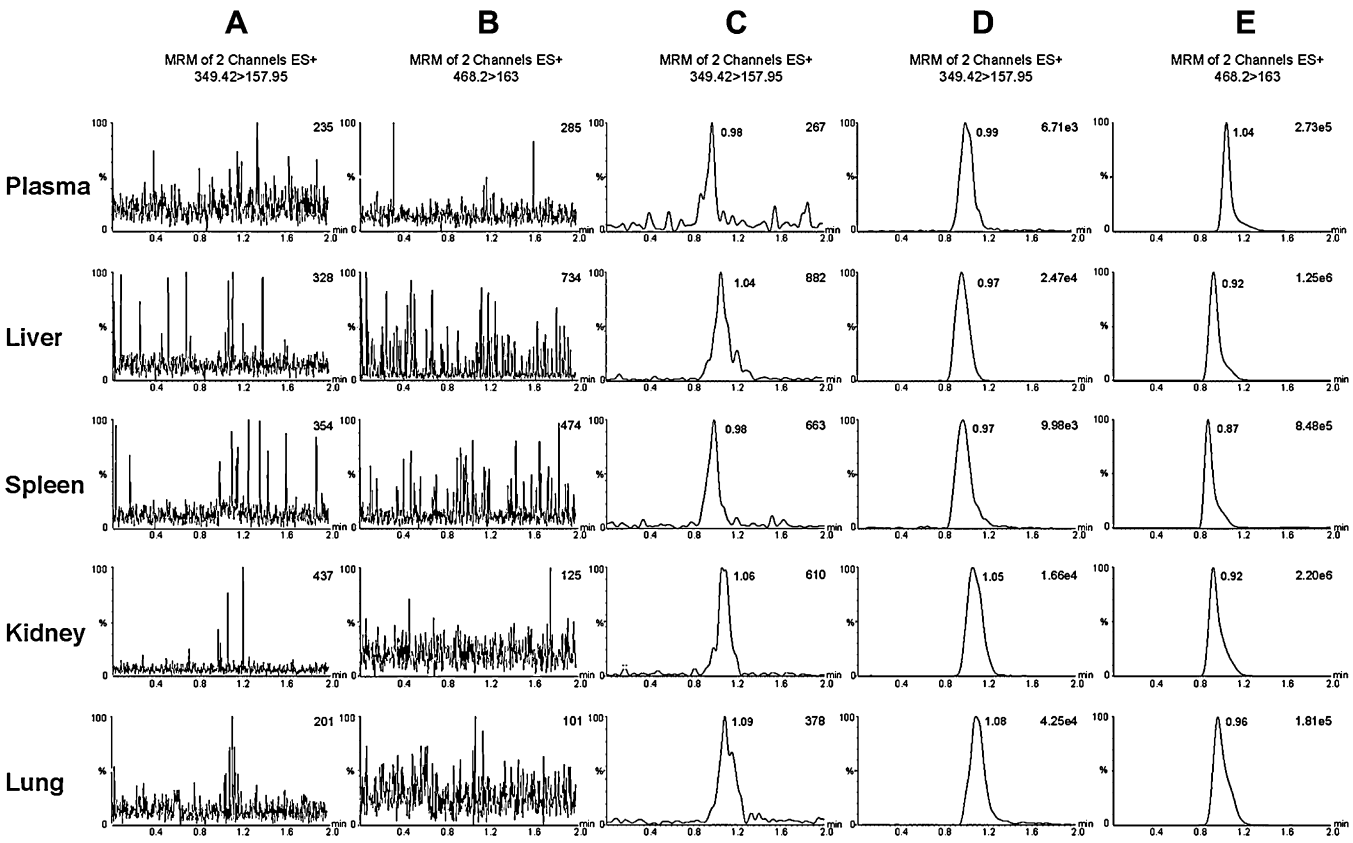

Fig. 2. MRM transitions for (A) drug-free samples (349.42 > 157.95), (B) drug-free samples (468.2 > 163) (C) LBH589 at LLOQ (0.0025 μg/mL in plasma and tissue homogenate, equivalent to 0.0357 μg/g tissue), (D) LBH589 (1 μg/mL in plasma and tissue homogenate, equivalent to 14.2857 μg/g tissue) and (E) tobramycin (0.8 μg/mL in plasma and tissue homogenate, equivalent to 11.42 μg/g tissue).

of ultra high performance liquid chromatography has made it possible to avoid this drawback, with equipment that can easily resist pressure values up to 15,000 psi [8,11].

Different types of stationary phase were evaluated. HPLC chromatographic phases such as Gemini NX, HSST3 and HSS C_{18} reversed phase package or hydrophilic phases such as Luna HILIC and Synergy Polar-RP were employed with discrete results. The best results were obtained with the reversed-phase Acquity UPLC™ BEH column, based on the highly efficient 1.7 μm Bridged Ethyl Hybrid (BEH) particles allowing maximum speed and sensitivity in the separation. The selectivity of the method showed a significant increase when trifluoroacetic acid was included in the mobile phase composition. As a consequence, increasing concentrations (0.1, 0.5 and 1%) of trifluoroacetic acid were assayed in order to achieve the optimal conditions for the chromatographic separation. As the trifluoroacetic acid concentration increased, better resolution was achieved; however, sensitivity was seriously compromised. The lowest tested concentration (0.1%) provided the best sensitivity results without significant decrease in resolution of chromatographic peaks. Under these conditions, LBH589 and tobramycin were eluted at 1.03 ± 0.04 and 0.94 ± 0.05 min (0.98 ± 0.03 and 1.03 ± 0.07 min in plasma, 0.99 ± 0.06 and 0.91 ± 0.05 min in liver, 1.07 ± 0.09 and 0.90 ± 0.05 min in spleen, 1.07 ± 0.06 and 0.96 ± 0.08 min in kidney, and 1.03 ± 0.07 and 0.92 ± 0.07 min in lung, $n = 6$ in all cases), with no endogenous interference at retention times of peaks of interest (Fig. 2). The mean asymmetry factors of LBH589 and tobramycin were 1.01 ± 0.07 and 1.07 ± 0.05, respectively. The run time was 2 min, a value 60% lower than that fixed by Yeo [7] with a rapid resolution reversed phase column at a similar flow rate.

3.3. Sample preparation

Liquid–liquid extraction procedure was selected as sample extraction procedure. Liquid–liquid extraction allows drug quantitation after UHPLC–MS/MS analysis without the significant matrix effects usually observed when protein precipitation is employed. For the liquid-liquid extraction, different salts (borate, Tris), pH conditions, and organic solvents were assayed. Lower extraction recoveries were obtained with acidic or neutral media (between 3.69 and 7.4% after the addition of boric acid 0.1 M pH 4, and near the 25% with 0.125 M pH 7 Tris solution). The best results were obtained when LBH589 was extracted from plasma and tissue samples with tert-butyl methyl ether after the addition of 0.025 M pH 12 Tris solution. The use of a Tris solution to adjust the pH to 12 increases the extraction efficiency with a significant reduction in the time employed for the liquid–liquid extraction, and makes it possible to obtain recovery values near to 75%, a higher value than that reported by Yeo (59–65%) [7]. Moreover, the sample extraction procedure developed also permitted LBH589 extraction from more complex matrices such as liver, spleen and lung, allowing acceptable recovery with no modification.

3.4. Method validation

3.4.1. Assay selectivity

Blank plasma and tissue samples from untreated mice were tested for the presence of endogenous components that might interfere with LBH589 and tobramycin. These samples were prepared in accordance with the sample preparation procedure, using the liquid–liquid extraction procedure. No interfering peaks were

observed in chromatograms of blank plasma and tissue samples. Fig. 2 shows the typical chromatograms of blank samples, and spiked plasma and tissues samples with LBH589, and the I.S. analyzed by the UHPLC technique.

3.4.2. Linearity

A linear range was obtained for all types of samples from 0.0025 to 1 μg/mL. The limit of quantitation in plasma and tissue homogenate was 0.0025 μg/mL, a similar value to that previously reported by Yeo [7]. For each point of calibration standards, the concentrations were back-calculated from the equation of the regression curves and relative standard deviations (%RSD) were measured. %RSD did not exceed 15% in any case. For all calibration curves, linear regression provided *r* values greater than 0.998. Results of standard curves for the LBH589 determination are given in Table 1.

Table 1
Standard calibration curves of LBH589 in plasma, liver, spleen, kidney and lung calculated by the UHPLC method.

Range	Regression equation	*r*
Plasma 0.0025–1 μg/mL	$y = 0.00978x + 3.09 \times 10^{-5}$	0.999
	$y = 0.00962x + 2.48 \times 10^{-5}$	0.999
	$y = 0.00973x + 1.83 \times 10^{-6}$	0.999
	$y = 0.00964x - 1.49 \times 10^{-6}$	0.998
	$y = 0.00963x + 1.54 \times 10^{-5}$	0.999
Liver[a] 0.0025–1 μg/mL	$y = 0.00431x + 8.47 \times 10^{-6}$	0.999
	$y = 0.00320x + 9.56 \times 10^{-6}$	0.998
	$y = 0.00478x + 7.42 \times 10^{-6}$	0.998
	$y = 0.00515x + 8.95 \times 10^{-6}$	0.999
	$y = 0.00600x + 1.48 \times 10^{-5}$	0.998
Spleen[a] 0.0025–1 μg/mL	$y = 0.00867x - 4.95 \times 10^{-6}$	0.999
	$y = 0.00799x + 1.38 \times 10^{-5}$	0.999
	$y = 0.00923x + 1.88 \times 10^{-5}$	0.999
	$y = 0.00841x + 8.29 \times 10^{-5}$	0.999
	$y = 0.00899x + 1.53 \times 10^{-5}$	0.998
Kidney[a] 0.0025–1 μg/mL	$y = 0.00727x + 1.17 \times 10^{-5}$	0.998
	$y = 0.00538x + 1.35 \times 10^{-5}$	0.998
	$y = 0.00529x + 1.45 \times 10^{-5}$	0.998
	$y = 0.00761x + 5.44 \times 10^{-6}$	0.999
	$y = 0.00698x + 1.68 \times 10^{-5}$	0.998
Lung[a] 0.0025–1 μg/mL	$y = 0.00733x + 1.97 \times 10^{-5}$	0.998
	$y = 0.00648x + 1.21 \times 10^{-5}$	0.998
	$y = 0.00520x - 9.94 \times 10^{-7}$	0.998
	$y = 0.00601x + 8.90 \times 10^{-6}$	0.998
	$y = 0.00722x + 1.50 \times 10^{-5}$	0.998

[a] Concentrations of tissues expressed as μg/mL tissue homogenate.

3.4.3. Accuracy and precision

The accuracy and precision of between- and within-day data for LBH589 were determined at QC concentrations of 0.0025, 0.0075, 0.4 and 0.8 μg/mL in plasma and tissue homogenate. Table 2 shows the obtained results. The accuracy ranged from −2.41 to 2.62% and the precision ranged from 0.92 to 8.40% in all the QC assayed. In all cases, accuracy and precision were <15%, in agreement with FDA guidelines [15].

3.4.4. Matrix effect and extraction efficiency

Matrix effect results were 9.92 ± 2.19% for plasma, 16.96 ± 1.89% for liver, 10.79 ± 2.99% for spleen, 13.56 ± 2.73% for kidney and 16.92 ± 2.07% for lung. The extraction efficiency of LBH589 ranged from 61.80% to 85.83% in all samples. For tobramycin, the matrix effect ranged from 13.41% to 32.36% in plasma, liver, kidney and spleen, and from 41.25% to 55.4% in lung samples (Table 3). Matrix effects of LBH for each tissue presented moderate variability as the coefficients of variation were 22.1% for plasma, 11.2% for liver, 27.8% for spleen, 20.1% for kidney and 12.2% for lung. Tobramycin presented similar matrix effect values, which were 23.9% for plasma, 26.5% for liver, 7.52% for spleen, 24.0% for kidney and 14.0% for lung. Keeping in mind that we are dealing with *in vivo* data and the variability is usually rather higher than *in vitro*, these matrix effect values present acceptable coefficients of variation for each tissue, showing moderate reproducibility of the study. In this sense, there is no expected influence of the matrix effect on analytical results because it is constant for each type of matrix, as can be deduced from the coefficient of variation values. A possible reason for the observed matrix effect is the fact that this one is related to the amount of phospholipids, among other components, present in the matrix. This liquid–liquid extraction procedure has been shown not to be completely effective in removing all these components that

Table 2
Accuracy, precision and between- and within-day measured concentrations for analysis of LBH589 QC by the UHPLC method in plasma, liver, spleen, kidney and lung.

Matrix	Concent.	Measured concentration (μg/mL, mean ± S.D.)		Accuracy (bias %)	Precision (CV)	
		Between-day (5 batches, *n* = 15)	Within-day (1 batch, *n* = 5)		Between-day (5 batches, *n* = 15)	Within-day (1 batch, *n* = 5)
Plasma	0.0025	0.0025 ± 0.0001	0.0025 ± 0.0001	0.68	3.75	5.28
	0.0075	0.0074 ± 0.0002	0.0075 ± 0.0004	−0.52	3.80	5.55
	0.4	0.4406 ± 0.0236	0.3739 ± 0.0094	−2.41	5.80	2.53
	0.8	0.7862 ± 0.0365	0.7798 ± 0.0188	−2.11	4.65	2.41
Liver[a]	0.0025	0.0025 ± 0.0001	0.0024 ± 0.0001	0.97	5.41	3.80
	0.0075	0.0073 ± 0.0002	0.0076 ± 0.0002	0.51	3.85	2.83
	0.4	0.4016 ± 0.0167	0.4193 ± 0.0209	2.62	4.16	5.00
	0.8	0.8071 ± 0.0286	0.8049 ± 0.0244	0.75	3.55	3.04
Spleen[a]	0.0025	0.0025 ± 0.0002	0.0025 ± 0.0001	−0.89	8.40	4.33
	0.0075	0.0075 ± 0.0002	0.0077 ± 0.0002	1.62	2.06	3.27
	0.4	0.4056 ± 0.0205	0.4032 ± 0.0163	1.12	5.30	4.05
	0.8	0.7977 ± 0.0345	0.7780 ± 0.0445	−1.53	0.92	5.72
Kidney[a]	0.0025	0.0025 ± 0.0001	0.0025 ± 0.0001	1.57	3.24	4.75
	0.0075	0.0077 ± 0.0004	0.0075 ± 0.0004	1.47	5.39	5.25
	0.4	0.4102 ± 0.0150	0.4093 ± 0.0160	2.46	3.66	3.92
	0.8	0.7954 ± 0.0414	0.8029 ± 0.0225	−0.09	5.20	2.80
Lung[a]	0.0025	0.0025 ± 0.0001	0.0024 ± 0.0001	−1.08	5.83	6.18
	0.0075	0.0075 ± 0.0003	0.0073 ± 0.0004	−0.29	5.12	6.46
	0.4	0.4084 ± 0.0212	0.3945 ± 0.0125	0.37	5.19	3.19
	0.8	0.7779 ± 0.0332	0.8041 ± 0.0589	−1.13	4.27	7.33

[a] Concentrations of tissues expressed as μg/mL tissue homogenate.

Table 3
Matrix effect and extraction recovery of LBH589 and tobramycin in plasma and tissues.

Matrix	QC	Matrix effect (%)	Extraction recovery of LBH589 (%)	Matrix effect of tobramycin (%)
Plasma	QC1	12.36	61.80	13.65
	QC2	7.38	68.52	17.86
	QC3	8.96	70.81	20.90
	QC4	10.96	62.97	24.45
Liver	QC1	19.56	70.71	32.36
	QC2	15.96	69.71	30.99
	QC3	15.24	79.36	16.70
	QC4	17.08	74.70	26.68
Spleen	QC1	12.99	84.74	32.17
	QC2	8.39	79.31	31.08
	QC3	8.03	85.83	30.55
	QC4	13.75	77.37	26.93
Kidney	QC1	13.00	69.96	13.41
	QC2	16.71	79.17	17.57
	QC3	14.35	74.30	20.83
	QC4	10.18	64.27	24.04
Lung	QC1	16.60	61.99	41.25
	QC2	18.43	62.67	52.60
	QC3	14.11	63.96	55.40
	QC4	18.52	66.61	44.04

might have an influence in the matrix effect. Therefore, a possible solution for the reduction of the matrix effect would be a further purification of the sample by solid phase extraction, prior to the liquid–liquid extraction; however, a lower recovery of drug would be expected due to the presence of more purification steps.

3.4.5. LBH589 stability

Stability of plasma and liver homogenate samples was followed at −80 °C for 1 month. Also bench-top, autosampler and freeze-thaw stability were analyzed. Table 4 summarizes the results. Samples were stable for 6 h at room temperature in plasma and liver homogenate. LBH589 was also stable in plasma and liver homogenate at −80 °C for 30 days and for three cycles when stored at −80 °C and thawed to room temperature. The bench-top stability was also acceptable, ranging from −0.16 to 3.17%.

3.5. Method application

The applicability of this method was initially demonstrated *in vivo* by the determination of LBH589 concentrations in plasma and some tissue samples from mice treated with LBH589. Fig. 3 depicts the time–concentration course of LBH589 in mouse plasma after intraperitoneal administration of 20 mg/kg of LBH589 to BALB/c mice. LBH589 showed concentrations from 790 to 141 ng/mL, during the first 8 h after the administration. Fig. 4 shows the distribution of LBH589 to different organs in mice after 48 h of intraperitoneal administration of 20 mg/kg of LBH589. No drug was detectable in the liver. The LBH589 levels present in spleen and

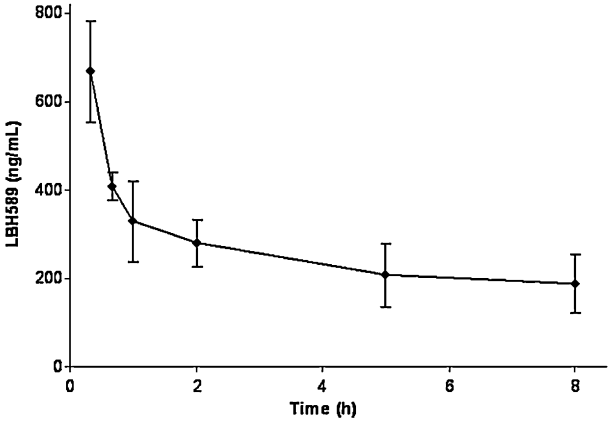

Fig. 3. Plasma concentration–time profile of LBH589 after the administration of an intraperitoneal dose of 20 mg/kg to mice (error bars represent SD, $n = 3$).

Table 4
Stability of LBH589 in plasma and liver under different conditions.

	QC2 (0.0075 μg/mL)			QC4 (0.8 μg/mL)		
	Measured concentration (μg/mL)	CV	Accuracy deviation (%)	Measured concentration (μg/mL)	CV	Accuracy deviation (%)
Stability in plasma						
Autosampler Stability (48 h)	0.0079 ± 0.0003	2.57	5.92	0.7747 ± 0.0419	5.58	3.16
Stability alter 3 freeze/thaw cycles	0.0077 ± 0.0003	4.87	3.37	0.8120 ± 0.0762	9.39	1.52
Short-term stability (6 h at room temperature)	0.0079 ± 0.0007	8.96	4.21	0.7746 ± 0.0752	9.81	3.17
Stability in storage (−80 °C, 1 month)	0.0077 ± 0.0002	3.58	3.74	0.8105 ± 0.0584	7.24	1.31
Stability in liver						
Autosampler Stability (48 h)	$0.0075 \pm 7.1 \times 10^{-5}$	0.92	0.70	0.7987 ± 0.0013	0.16	−0.16
Stability alter 3 freeze/thaw cycles	$0.0075 \pm 9.8 \times 10^{-5}$	1.13	1.29	0.8001 ± 0.0018	0.22	0.01
Short-term stability (6 h at room temperature)	$0.0075 \pm 4.2 \times 10^{-5}$	0.56	0.55	0.7997 ± 0.0009	0.11	−0.02
Stability in storage (−80 °C, 1 month)	$0.0075 \pm 3.5 \times 10^{-5}$	0.46	0.46	0.7999 ± 0.0010	0.12	−0.01

Concentrations of liver expressed as μg/mL tissue homogenate.

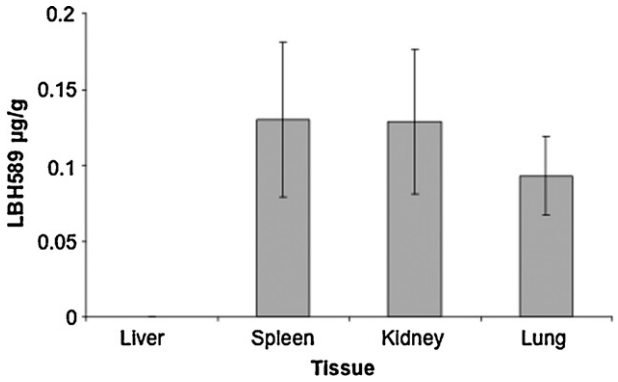

Fig. 4. LBH589 measured in different tissues 48 h after the administration of an intraperitoneal dose of 20 mg/kg of LBH589 to mice (error bars represent SD, $n = 10$).

kidney were quite similar, that is, 0.13 and 0.12 μg/g, respectively. In lung, the LBH589 level was 0.09 μg/g.

4. Conclusion

An UHPLC–MS/MS method for the bioanalysis of LBH589 was developed and validated. The method developed is specific, accurate, precise and reproducible for the analysis of LBH589 in mouse plasma and tissues. Moreover, this method is valuable for the determination of the pharmacokinetic behavior of LBH589 and its biodistribution in mice.

References

[1] W. Shao, J.D. Growney, Y. Feng, P. Wang, Y. Yan-Neale, G. O'Connor, 99th American Association of Cancer Research Annual Meeting, San Diego, CA, 2008.
[2] P. Atadja, Cancer Lett. 280 (2009) 233.
[3] D.Z. Qian, Y. Kato, S. Shabbeer, Y. Wei, H.M. Verheul, B. Salumbides, T. Sanni, P. Atadja, R. Pili, Clin. Cancer Res. 12 (2006) 634.
[4] L. Catley, E. Weisberg, Y.T. Tai, P. Atadja, S. Remiszewski, T. Hideshima, N. Mitsiades, R. Shringarpure, R. LeBlanc, D. Chauhan, N.C. Munshi, R. Schlossman, P. Richardson, J. Griffin, K.C. Anderson, Blood 102 (2003) 2615.
[5] E. Weisberg, L. Catley, J. Kujawa, P. Atadja, S. Remiszewski, P. Fuerst, C. Cavazza, K. Anderson, J.D. Griffin, Leukemia 18 (2004) 1951.
[6] P. George, P. Bali, S. Annavarapu, A. Scuto, W. Fiskus, F. Guo, C. Sigua, G. Sondarva, L. Moscinski, P. Atadja, K. Bhalla, Blood 105 (2005) 1768.
[7] P. Yeo, L. Xin, E. Goh, L.S. New, P. Zeng, X. Wu, P. Venkatesh, E. Kantharaj, Biomed. Chromatogr. 21 (2007) 184.
[8] M.I. Churchwell, N.C. Twaddle, L.R. Meeker, D.R. Doerge, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 825 (2005) 134.
[9] N. Stephanson, A. Helander, O. Beck, J. Mass Spectrom. 42 (2007) 940.
[10] J. Olsovska, M. Jelinkova, P. Man, M. Koberska, J. Janata, M. Flieger, J. Chromatogr. A 1139 (2007) 214.
[11] Y. Hsieh, C.J. Duncan, S. Lee, M. Liu, J. Pharm. Biomed. Anal. 44 (2007) 492.
[12] A. Estella-Hermoso de Mendoza, M.A. Campanero, F. Mollinedo, M.J. Blanco-Prieto, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 877 (2009) 4035.
[13] I.S. Lurie, J. Chromatogr. A 1100 (2005) 168.
[14] I. Citova, L. Havlikova, L. Urbanek, D. Solichova, L. Novakova, P. Solich, Anal. Bioanal. Chem. 388 (2007) 675.
[15] US Food and Drug Administration, Guidance for industry, Bioanalytical Method Validation, Centre for Drug Evaluation and Research, 2001.