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Sensitive Liquid Chromatography/mass Spectrometry Methods for Quantification of Pomalidomide in Mouse Plasma and Brain Tissue

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Abstract

Pomalidomide was recently approved by the United States Food and Drug Administration for the treatment of patients with relapsed or refractory multiple myeloma who have received at least two prior therapies. As pomalidomide is increasingly evaluated in other diseases and animal disease models, this manuscript presents development and validation of a sensitive liquid chromatography tandem mass spectrometry assay for quantification of pomalidomide in mouse plasma and brain tissue to fill a gap in published preclinical pharmacokinetic and analytical data with this agent. After acetonitrile protein precipitation, pomalidomide and internal standard, hesperitin, were separated with reverse phase chromatography on a C-18 column with a gradient mobile phase of water and acetonitrile with 0.1% formic acid. Positive atmospheric pressure chemical ionization mass spectrometry with selected reaction monitoring mode was applied to achieve 0.3–3000 nM (0.082–819.73 ng/mL) linear range in mouse plasma and 0.6–6000 pmol/g in brain tissue. The within- and between-batch accuracy and precision were less than 15% for both plasma and brain tissue. The method was applied to measure pomalidomide concentrations in plasma and brain tissue in a pilot mouse pharmacokinetic study with an intravenous dose of 0.5 mg/kg. This assay can be applied for thorough characterization of pomalidomide pharmacokinetics and tissue distribution in mice.

Keywords

Pomalidomide; Liquid Chromatography-Mass Spectrometry; Mouse; Plasma; Brain; Pharmacokinetics

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1. Introduction

Pomalidomide (CC-4047), a derivative of thalidomide, is a member of the immunomodulatory drugs (IMiDs) family. Pomalidomide is 5000 fold more potent than thalidomide and 10 fold more potent than lenalidomide in anti-TNF α effects [1, 2]. Its other immunomodulatory properties, e.g., enhancing T-cell response, increasing natural killer cells activity and augmenting endothelial progenitor cells differentiation, make it a potential reagent for sickle cell disease, hematologic neoplasms, like multiple myeloma (MM), as well as some solid tumors, such as metastatic melanoma, prostate and colorectal cancers [3–6]. In February 2013, the U.S. Food and Drug Administration (FDA) gave accelerated approval for pomalidomide for the treatment of patients with relapsed or refractory MM who have received at least two prior therapies. Pomalidomide is also under review by the European Medicines Agency for approval in treating MM.

To date, published studies of pomalidomide have included effectiveness and activity in different neoplasms [7–10], molecular mechanisms [6, 11–14], a pharmacokinetics study of [14C] pomalidomide in humans [15], clinical trials [16–24] and reviews [3, 18, 25–27]. Most published clinical trials are phase I studies aimed at determining the maximum tolerated dose, dose limiting toxicity and response with pomalidomide alone or in combination with other therapeutic drugs [12, 14, 16]. Only one quantitative assay of pomalidomide has been published, which measured the chiral inversion of pomalidomide in phosphate-buffered saline and human plasma in the $\mu\text{g/mL}$ range using high-performance liquid chromatography with ultraviolet absorbance detection [28]. In the supplementary material of a separate study that focused on pomalidomide augmentation of fetal hemoglobin production in transgenic sickle cell mice, the authors presented limited mouse plasma pharmacokinetic (PK) data generated with a mass spectrometry method with a reported linear range of 0.5–500 ng/mL [29]. A LC-MS method was also used in the absorption, metabolism and excretion study of [14C] pomalidomide in humans [15]; however, as in the previous fetal hemoglobin study, no method validation details were presented.

In this study, we developed and validated a sensitive LC-MS/MS assays for pomalidomide quantification in both mouse plasma and brain tissue based on FDA guidance criteria [30]. The assay requires a small sample quantity (100 μL for plasma and 50 mg for brain tissue) but achieves a lower limit of quantitation (LLOQ) of 0.3 nM (0.082 ng/mL) in mouse plasma and 0.6 pmol/g in brain tissue.

2. Materials and methods

2.1. Chemicals and reagents

Pomalidomide was synthesized using a modified method similar to the previously reported one [31], and the purity of the drug was confirmed >95% by nuclear magnetic resonance and HPLC-UV analysis. The internal standard (IS), hesperetin was purchased from Sigma (St. Louis, MO). Methanol, acetonitrile and water were HPLC grade and purchased from Thermo Fisher Scientific (Waltham, MA). All other chemicals were analytical grade and used without further purification. Blank mouse plasma was purchased from Innovative Research (Novi, MI), and blank mouse brain tissue was obtained directly from FVB mice.

2.2. Standard and quality control solutions

High concentration solutions (10 mM and 10 mg/mL) were obtained by dissolving pomalidomide and hesperetin powder in DMSO, respectively. Stock solutions of pomalidomide (1 mM) and hesperetin (1 mg/mL) were prepared in 50% methanol (methanol:

water =50:50, v/v) and aliquotted for storage. Serial pomalidomide standard neat solutions (10x) and IS working solution (10 µg/mL) were freshly prepared immediately before each run by diluting stock solutions of pomalidomide and IS with 50% methanol, respectively.

Plasma standard curve samples were produced by spiking 10 µL of standard neat solutions (10x) of pomalidomide into 100 µL of blank mouse plasma. The linear range was 0.3 to 3000 nM with concentrations at 0.3, 1, 3, 10, 30, 100, 300, 1000 and 3000 nM. Quality control (QC) samples at 1, 3, 100, 800 and 2400 nM were prepared in a similar way from an independently prepared stock solution. Brain standard curve samples were prepared by adding 10 µL of pomalidomide standard neat solutions (10x) into 50 mg of blank, homogenized mouse brain tissue followed by adding 1 mL of acetonitrile and further shearing with a homogenizer (PYREX, Corning) to produce final concentrations from 0.6 to 6000 pmol/g. QC samples (2, 60 and 2000 pmol/g) were prepared in a similar way at low, medium and high concentration levels.

2.3. LC-MS/MS

The LC-MS/MS system comprised a Thermo Scientific Accela U-HPLC pump, autosampler and TSQ Quantum Discovery triple quadrupole mass spectrometer (Thermo Fisher Scientific Corporation, San Jose, CA) with an atmospheric pressure chemical ionization (APCI) source. Sample separation was performed on a reverse-phase Zorbax (Agilent) Extend-C18 column (3.5 µm, 2.1 × 50mm) with a C18 guard column. Mobile phases were water (A) and acetonitrile (B), each with 0.1% formic acid. The 10 min gradient elution (flow rate: 0.4 mL/min) included the following linear components: 0–2 min, 10% B; 2–3 min, 10–50% B; 3–6 min, 50–90% B; 6–7 min, 90% B, 7–7.10 min, 90–10% B and 7.10–10 min, 10% B. Xcalibur software was employed for system control and data processing.

Single reaction monitoring was used for detection. Transition channels of the protonated molecular ions were 274.02→201.00 for pomalidomide and 303.06→153.01 for hesperetin, respectively. Parameters optimized under a direct infusion of analyte and IS using a syringe pump were as follows: spray voltage, 4900V; sheath gas pressure, 30 mTorr; aux gas pressure, 25 mTorr; capillary temperature, 320 °C; collision gas pressure, 25 mTorr and skimmer offset, 8 V. Tube lense offsets were 90 and 85 for pomalidomide and hesperetin, respectively. Scan time was 0.03s. Both Q1 and Q3 peak widths were 0.7 full widths at half-maximum m/z.

2.4. Sample preparation

For plasma samples, 10 µL of IS working solution (10 µg/mL) was spiked into 100 µL plasma sample and followed by 1 mL acetonitrile for protein precipitation. The mixture was vortex-mixed for 30 seconds and centrifuged at 18,000 g for 10 min at 4°C. Then the supernatant was transferred to a glass tube and evaporated under a gentle nitrogen gas stream. The residue was reconstituted with 120 µL 5% acetonitrile. After centrifugation at 18,000 g for 10 min at 4°C, 20 µL of supernatant was injected into the LC–MS/MS system.

For brain tissue samples, 50 mg of brain tissue was homogenized after 1mL of -acetonitrile was added. 10 µL of IS working solution (10 µg/mL) was spiked into the homogenate. The mixture was vortex-mixed for 30 seconds and centrifuged at 18,000 g for 10 min at 4°C. Subsequent steps were identical to those for plasma sample preparation.

2.5. Assay validation

Method validation for this assay was performed according to FDA guidelines [30]. Six different sources of blank mouse plasma and pooled blank mouse brain from 15 FVB mice were used in method evaluation. The linear range for plasma was 0.3–3000 nM with low,

medium and high level QCs at 1, 3, 100, 800 and 2400 nM. For brain, the linear range of standard curve was 0.6–6000 pmol/g with QC samples at 2, 60 and 2000 pmol/g. Within- and between-batch accuracy and precision were determined with the QC samples. The absolute recoveries of pomalidomide were calculated by comparing peak areas of extracted QC samples with corresponding areas of pomalidomide from standard neat solutions. Matrix effects were evaluated by comparing the peak areas of analyte in post-spiked samples versus neat solutions at three QC concentration levels.

Short-term and long-term plasma stability samples were analyzed after 1 week, 4 weeks, 2 and 3 months storage at -80°C , separately. Freeze-thaw stability samples were obtained by freezing–thawing QC samples three times. Stability of pomalidomide stock solutions stored at -80°C for one month were evaluated by comparing plasma QC samples (1, 100 and 2400 nM, triplicates at each concentration) prepared from stored stock solutions and freshly made stock solutions. Hesperitin stock solution stability was also evaluated. IS working solutions (10 $\mu\text{g/mL}$) were prepared separately with freshly-made stock solution and stock solution already stored at -80°C for one month. 10 μL of IS working solutions (10 $\mu\text{g/mL}$) were then spiked into 100 μL plasma sample and followed by the sample preparation method described above. The stability of IS stock solution was calculated by comparing peak areas of triplicate IS samples prepared from stored and newly prepared stock solutions, respectively. Stability of QC samples at 4°C in the autosampler tray for 12 hr after reconstitution was also evaluated for both brain and plasma.

2.6. Pharmacokinetic study

The method was successfully applied to a pilot pharmacokinetic study of pomalidomide in FVB mice. Pomalidomide was given by bolus injection through tail vein at a dose of 0.5 mg/kg. Plasma and brain tissue were collected at 5, 10, 20, 30, 60, 90, 150, 240, 360 and 540 minutes after drug administration (one mouse per time point), and samples were stored at -80°C until analysis.

3. Result and Discussions

3.1. Mass Spectrometry

The response of pomalidomide was evaluated under both electrospray ionization and APCI positive ion modes. APCI was selected due to the superior signal intensity obtained compared to electrospray ionization. Mass spectrometer parameters were optimized for pomalidomide response. Chemical structures, full scan product ion spectra of $[\text{M}+\text{H}]^{+}$ and potential fragment structures [15, 32] for pomalidomide and hesperetin are shown in Fig. 1. Transitions with maximum signal to noise ratios were chosen for the quantifications of pomalidomide and hesperetin; these were m/z 274.02 $\rightarrow$ 201.00 and 303.06 $\rightarrow$ 153.01, respectively.

3.2. Chromatography

A Zorbax Extend-C18 column (3.5 μm , $2.1 \times 50\text{mm}$) was observed to provide adequate separation of analytes after a simple protein precipitation sample prep, and formic acid was added to the mobile phases to improve peak shape. Hesperetin was chosen as internal standard due to its relatively high recovery, similar polarity and retention time to pomalidomide. A mobile phase gradient was developed to separate pomalidomide from other potentially interfering peaks. The gradient allowed analytes to enter the mass spectrometer after at least 1.5 column dead volumes thus enabling diversion of hydrophilic matrix components to waste prior to directing flow back to the mass spectrometer for analyte detection. Ultimately, these conditions enabled good separation, acceptable matrix effect and recovery to yield a highly sensitive and sufficiently robust assay.

3.3. Assay validation

3.3.1 Selectivity and sensitivity—The selectivity and sensitivity of the method was evaluated by comparing blank matrix, blank matrix spiked with pomalidomide at the LLOQ, and a study sample containing a low concentration of the drug (Fig. 2 and Fig. 3). No interfering peaks were observed at the retention times of pomalidomide and IS in both plasma and brain. LLOQ is the lowest concentration in the standard curve with accuracy within 20%, and the limits of detection, determined as the lowest concentration with signal-to-noise ratio ≥ 3 , were 0.1 nM for pomalidomide in plasma and 0.2 pmol/g in brain.

3.3.2 Linearity, accuracy and precision—Peaks were integrated using the Interactive Chemical Integration System algorithm, and linearity was calculated by weighted least squares regression of analyte/IS peak area ratios versus analyte concentration. Linearity ranges of standard curves were 0.3–3000 nM for plasma ($R^2 > 0.9915$) and 0.6–6000 pmol/g in brain ($R^2 > 0.9935$). Representative standard curves were $y = 0.000805065x + 0.000480823$ ($R^2 = 0.9984$) for plasma and $y = 0.0120067x + 0.0210464$ ($R^2 = 0.9968$) for brain, respectively. Given the broad five orders of magnitude concentration ranges in the standard curves, we compared the strategies of breaking calibration curves into two overlapping sections versus using a single calibration curve for quantification. Results of this evaluation demonstrated both strategies yielded results that were acceptable based on FDA criteria. Notably, the single standard curve approach yielded slightly better results relative to splitting the curve in two when a $1/X^2$ weighting scheme was employed with the single curve. Given the five orders of magnitude range, a $1/X^2$ weighting scheme is a reasonable and appropriate approach. Therefore, the single calibration curve with $1/X^2$ weighting was adopted for assay validation.

Accuracies and precisions were calculated based on three batches of plasma and brain QC samples, respectively. Within-batch and between-batch precisions were below 11.6% for both plasma and brain, and accuracies were 92.1–99.3% and 90.5–112.8% for plasma and brain tissue, respectively (Table 1). The QC levels differed between plasma and brain because the plasma method was developed first, then QC levels were slightly adjusted to optimally cover the slightly different range of concentrations within the brain standard curve. In addition, to conserve brain tissue, in each batch we used 3 replicate QC samples for brain as compared to 6 replicate QC samples for plasma at each QC level.

3.3.3 Recovery, Matrix effect and stabilities—Absolute recoveries were obtained by comparing QC samples and corresponding standard neat solution. Absolute recoveries of pomalidomide at three QC concentrations were 114.9 ± 8.8 , 91.1 ± 7.0 and $103.3 \pm 6.5\%$ for plasma (3, 100 and 800 nM) and 101.7 ± 9.9 , 90.1 ± 4.2 and $94.4 \pm 9.7\%$ for brain tissue (2, 60 and 2000 pmol/g), respectively. Matrix effect on mass spectrometry signal (suppression or enhancement) was calculated by comparing post-spiked samples with neat solutions at different QC concentration levels. The matrix effects of pomalidomide in mouse plasma and brain were 102.7–113.7% and 98.4–116.5% of the nominal concentrations, respectively. Stabilities of pomalidomide stock solution stored at -80°C for one month was calculated as 96.3 ± 12.5 , 91.9 ± 4.0 and $92.1 \pm 1.2\%$, respectively, at dilutions of 1, 100 and 2400 nM. Stability of the internal standard stock solution was $93.2 \pm 4.6\%$. The stability of pomalidomide brain QC samples in the autosampler tray at 4°C for 12 hrs was 87.5–113.5%. Pomalidomide was stable in plasma under all the storage conditions evaluated with mean recoveries of 85.2–113.7%.

3.4. Pharmacokinetic study

The LC-MS/MS assay for quantification of pomalidomide in mouse plasma and brain tissue was employed to quantify drug in samples obtained from mice injected intravenously with

pomalidomide solution. A total of 10 mice were injected in this pilot study, and plasma and tissue samples were collected at time points between 5 minutes and 9 hours. The resulting data is displayed in the concentration versus time profile displayed in Fig. 4. Notably, pomalidomide was quantifiable in all samples, thus demonstrating the capability of the assay for characterizing pomalidomide pharmacokinetics within the 9-hour span of sample times in this pilot study. Mouse plasma data appeared similar to the previous publication [18], which also displayed a secondary peak within the first couple of hours after dosing. Pomalidomide concentrations in brain were roughly 70% those in plasma at early time points and even higher at later time points. These data suggest pomalidomide had good penetration into brain and was potentially retained in brain tissue.

4. Conclusion

A sensitive LC-MS/MS method for pomalidomide quantification in mouse plasma and brain tissue was developed and validated. The LLOQ achieved for plasma and brain tissue measurement was 0.3nM and 0.6 pmol/g, respectively, with relatively small sample quantities (100 μ L plasma and 50 mg brain tissue). This assay was utilized to determine pomalidomide concentrations in mouse plasma and brain in a pilot pharmacokinetic study. The results demonstrated the utility of the broad calibration range of the assay, which can be applied to further characterize pomalidomide pharmacokinetics and tissue distribution in mouse disease models.

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References

1. Corral LG, Haslett PA, Muller GW, Chen R, Wong LM, Ocampo CJ, Patterson RT, Stirling DI, Kaplan G. *Journal of immunology*. 1999; 163:380–386.
2. Muller GW, Chen R, Huang SY, Corral LG, Wong LM, Patterson RT, Chen Y, Kaplan G, Stirling DI. *Bioorganic & medicinal chemistry letters*. 1999; 9:1625–1630. [PubMed: 10386948]
3. Crane E, List A. *Future oncology*. 2005; 1:575–583. [PubMed: 16556034]
4. Zhu D, Corral LG, Fleming YW, Stein B. *Cancer immunology, immunotherapy: CII*. 2008; 57:1849–1859.
5. Streetly MJ, Gyertson K, Daniel Y, Zeldis JB, Kazmi M, Schey SA. *British journal of haematology*. 2008; 141:41–51. [PubMed: 18324965]
6. Cooney MM, Nock C, Bokar J, Krishnamurthi S, Gibbons J, Rodal MB, Ness A, Remick SC, Dreicer R, Dowlati A. *Cancer chemotherapy and pharmacology*. 2012; 70:755–761. [PubMed: 22875080]
7. Gertz MA. *Leukemia & lymphoma*. 2013; 54:681–682. [PubMed: 22917017]
8. Weingärtner S, Zerr P, Tomcik M, Palumbo-Zerr K, Distler A, Dees C, Beyer C, Shankar SL, Cedzik D, Schafer PH, Distler O, Schett G, Distler JH. *Annals of the rheumatic diseases*. 2012; 71:1895–1899. [PubMed: 22904257]
9. Mussetti A, Dalto S, Montefusco V. *Leukemia & lymphoma*. 2013; 54:864–866. [PubMed: 22880953]
10. Dispenzieri A, Buadi F, Laumann K, LaPlant B, Hayman SR, Kumar SK, Dingli D, Zeldenrust SR, Mikhael JR, Hall R, Rajkumar SV, Reeder C, Fonseca R, Bergsagel PL, Stewart AK, Roy V, Witzig TE, Lust JA, Russell SJ, Gertz MA, Lacy MQ. *Blood*. 2012; 119:5397–5404. [PubMed: 22493299]
11. Zhu YX, Kortuem KM, Stewart AK. *Leukemia & lymphoma*. 2013; 54:683–687. [PubMed: 22966948]

12. Gorgun G, Calabrese E, Soydan E, Hideshima T, Perrone G, Bandi M, Cirstea D, Santo L, Hu Y, Tai YT, Nahar S, Mimura N, Fabre C, Raje N, Munshi N, Richardson P, Anderson KC. *Blood*. 2010; 116:3227–3237. [PubMed: 20651070]
13. Escoubet-Lozach L, Lin IL, Jensen-Pergakes K, Brady HA, Gandhi AK, Schafer PH, Muller GW, Worland PJ, Chan KW, Verhelle D. *Cancer research*. 2009; 69:7347–7356. [PubMed: 19738071]
14. Moutouh-de Parseval LA, Verhelle D, Glezer E, Jensen-Pergakes K, Ferguson GD, Corral LG, Morris CL, Muller G, Brady H, Chan K. *The Journal of clinical investigation*. 2008; 118:248–258. [PubMed: 18064299]
15. Hoffmann M, Kasserra C, Reyes J, Schafer P, Kosek J, Capone L, Parton A, Kim-Kang H, Surapaneni S, Kumar G. *Cancer chemotherapy and pharmacology*. 2013; 71:489–501. [PubMed: 23203815]
16. Begna KH, Pardanani A, Mesa R, Litzow MR, Hogan WJ, Hanson CA, Tefferi A. *American journal of hematology*. 2012; 87:66–68. [PubMed: 22081489]
17. Lacy MQ, Allred JB, Gertz MA, Hayman SR, Short KD, Buadi F, Dispenzieri A, Kumar S, Greipp PR, Lust JA, Russell SJ, Dingli D, Zeldenrust S, Fonseca R, Bergsagel PL, Roy V, Stewart AK, Laumann K, Mandrekar SJ, Reeder C, Rajkumar SV, Mikhael JR. *Blood*. 2011; 118:2970–2975. [PubMed: 21690557]
18. Infante JR, Jones SF, Bendell JC, Spigel DR, Yardley DA, Weekes CD, Messersmith WA, Hainsworth JD, Burris HA 3rd. *European journal of cancer*. 2011; 47:199–205. [PubMed: 21051221]
19. Mesa RA, Pardanani AD, Hussein K, Wu W, Schwager S, Litzow MR, Hogan WJ, Tefferi A. *American journal of hematology*. 2010; 85:129–130. [PubMed: 20052748]
20. Begna KH, Mesa RA, Pardanani A, Hogan WJ, Litzow MR, McClure RF, Tefferi A. *Leukemia: official journal of the Leukemia Society of America, Leukemia Research Fund, UK*. 2011; 25:301–304.
21. Schey S, Ramasamy K. *Expert opinion on investigational drugs*. 2011; 20:691–700. [PubMed: 21413906]
22. Ellis PM, Jungnelius U, Zhang J, Fandi A, Beck R, Shepherd FA. *Journal of thoracic oncology: official publication of the International Association for the Study of Lung Cancer*. 2013; 8:423–428. [PubMed: 23370364]
23. Leleu X, Attal M, Arnulf B, Moreau P, Traulle C, Marit G, Mathiot C, Petillon MO, Macro M, Roussel M, Pegourie B, Kolb B, Stoppa AM, Hennache B, Brechignac S, Meuleman N, Thielemans B, Garderet L, Royer B, Hulin C, Benboubker L, Decaux O, Escoffre-Barbe M, Michallet M, Caillot D, Ferman JP, Avet-Loiseau H, Facon T, Intergroupe Francophone du M. *Blood*. 2013; 121:1968–1975. [PubMed: 23319574]
24. Richardson PG, Siegel D, Baz R, Kelley SL, Munshi NC, Laubach J, Sullivan D, Alsina M, Schlossman R, Ghobrial IM, Doss D, Loughney N, McBride L, Bilotti E, Anand P, Nardelli L, Wear S, Larkins G, Chen M, Zaki MH, Jacques C, Anderson KC. *Blood*. 2013; 121:1961–1967. [PubMed: 23243282]
25. Knight R. *Seminars in oncology*. 2005; 32:S24–30. [PubMed: 16085014]
26. Boder P, Stankiewicz W. *Recent patents on endocrine, metabolic & immune drug discovery*. 2011; 5:192–196.
27. Lacy MQ, Tefferi A. *Leukemia & lymphoma*. 2011; 52:560–566. [PubMed: 21338284]
28. Teo SK, Chen Y, Muller GW, Chen RS, Thomas SD, Stirling DI, Chandula RS. *Chirality*. 2003; 15:348–351. [PubMed: 12666243]
29. Meiler SE, Wade M, Kutlar F, Yerigenahally SD, Xue Y, Moutouh-de Parseval LA, Corral LG, Swerdlow PS, Kutlar A. *Blood*. 2011; 118:1109–1112. [PubMed: 21536862]
30. FDA guidance for Industry, Bioanalytical Method Validation. US Department of Health and Human Service, F.a.D. Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM); May. 2001
31. Mei Tang HW, Zhang Aiyang, Liu Zenglu, Mao Zhenmin. *Chinese Journal of Pharmaceuticals*. 2009; 40:721–723.
32. Kim SBHU, Kown Oh-S, Yoo HH. *Mass Spectrometry Letters*. 2011; 2:20–24.

Highlights

- Pomalidomide is FDA approved for multiple myeloma and is under evaluation for use in other diseases
- First report of a fully validated LC-MS/MS assay for pomalidomide in mouse plasma and brain
- First report of pomalidomide plasma and brain pharmacokinetics in mice
- Sensitive assay with LLOQ of 0.3 nM (0.082 ng/mL) for plasma and 0.6 pmol/g for brain
- Small amounts of samples are needed for this sensitive method (100 μ L plasma or 50mg brain tissue)

Fig. 1.
Chemical structures, APCI full-scan product ion spectra and fragmentation pathways [15, 32] of [M+H]⁺ for: (A) pomalidomide and (B) hesperetin

Fig. 2. Representative chromatograms of: (A) blank plasma, (B) blank plasma spiked with pomalidomide at the lower limit of quantitation (0.3 nM) and (C) a representative mouse plasma sample 9h after drug administration. Peak I, pomalidomide; Peak II, hesperetin.

Fig. 3. Representative chromatograms of: (A) blank mouse brain, (B) blank mouse brain spiked with pomalidomide at the lower limit of quantitation (0.6 pmol/g) and (C) a representative brain sample 6h after drug administration. Peak I, pomalidomide; Peak II, hesperetin.

Fig. 4. A representative mouse plasma and brain concentration- time profile after pomalidomide IV injection at a dose of 0.5 mg/kg.

Table 1

Within-batch and between-batch accuracy and precision for three validation runs

Matrix	Nominal Conc.(nM)	Within-batch			Between-batch		
		n	Accuracy (%)	Precision (%CV)	n	Accuracy (%)	Precision (%CV)
Plasma	1	6	105.6	12.0	18	102.7	14.2
	3	6	99.3	11.6	18	92.1	10.4
	100	6	97.7	8.9	18	94.4	7.5
	800	6	96.3	6.3	18	93.8	6.0
	2400	6	101.4	2.5	18	103.3	4.7
Matrix	Nominal Conc.(pmol/g)	Within-batch			Between-batch		
		n	Accuracy (%)	Precision (%CV)	n	Accuracy (%)	Precision (%CV)
Brain tissue	2	3	112.8	5.3	9	107.5	8.5
	60	3	105.8	6.7	9	105.3	6.1
	2000	3	90.5	11.4	9	95.3	8.0