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A Convenient Strategy for Quantitative Determination of Drug Concentrations in Tissue Homogenates Using a Liquid Chromatography/Tandem Mass Spectrometry Assay for Plasma Samples

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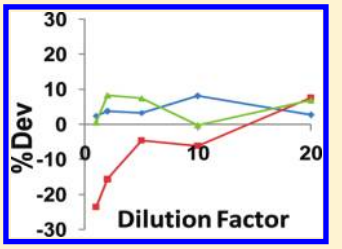
ABSTRACT: Quantitative determination of drug concentrations in tissue homogenates via liquid chromatography−tandem mass spectrometry (LC-MS/MS) is commonly conducted using the standards and analytical quality controls (QCs) prepared in the same matrix (tissue homogenates), to keep the matrix and its effects consistent on the analytes during sample extraction and analysis. In this manuscript, we proposed to analyze tissue homogenate samples using an LC-MS/MS assay with the standards and analytical QCs prepared in plasma after tissue homogenate samples were appropriately diluted with plasma. BMS-650032 was used as a model compound, and its validated dog plasma assay was used for dog liver sample analyses. The tissue matrix effect was evaluated by diluting liver homogenate QCs with drug-free plasma at different dilution factors to determine the minimum required dilution factor (MRDF) at which tissue matrix has insignificant impact to the plasma assay. The percentage deviation of the measured concentration from the nominal concentration was used as an indicator of the tissue matrix effect. The results suggested that the tissue matrix effect was decreased as the plasma dilution factor increased. Based on the results of the tissue matrix effect evaluation, liver homogenate samples were analyzed after appropriate dilutions with plasma at the MRDF or greater dilution factors. The results confirmed that this approach generates accurate data, and the process is very convenient and economic. This approach has been used on the analyses of different tissues (liver and brain) and biofluid (bile) to support several drug development programs.



INTRODUCTION

In tissue sample analyses performed via liquid chromatography−tandem mass spectrometry (LC-MS/MS), standards and analytical quality controls (QCs) are ideally prepared in drug-free tissue homogenates to maintain the matrix consistency between the standards, analytical QCs, and tissue homogenate samples. In these cases,[1−9] analyte working solutions were either spiked onto drug-free solid tissue before homogenization or directly spiked into tissue homogenates to prepare standards and analytical QCs, with tissue samples homogenized and extracted in the same way as the standards and analytical QCs. However, using a substitute matrix such as 4% bovine albumin,[10] water,[11] or blood[12,13] to prepare standards and analytical QCs for tissue sample analysis was also reported when a drug-free tissue was not available. It was observed that analyte recoveries were different between the standards and tissue homogenate samples, and they caused significant data biases. In addition, the impact on data accuracy was even more significant when an analogue internal standard was used.[12] Therefore, a correction factor, the recovery difference between the standards and tissue samples, was used to correct reported study sample drug concentrations. In this study, the data bias resulting from the recovery difference between standards and tissue homogenate samples was defined as the tissue matrix effect. In occasional cases, the tissue matrix effect was minimized when standards were prepared with the homogenization solution, even when a stable isotopically labeled internal standard was not used.[13]

In this manuscript, we successfully used a validated LC-MS/MS method for the quantitative determination of BMS-650032 (an inhibitor of hepatitis C virus NS3 protein; see Figure 1) in dog plasma to determine BMS-650032 in dog liver homogenate samples. The standards and analytical QCs were prepared in plasma, whereas the tissue samples were homogenized in acetonitrile/Hank's buffered salt solution (20:80, v/v). The matrix differences were minimized by diluting the tissue homogenate samples with dog plasma before sample extraction. The tissue matrix effect was evaluated by calculating the percentage deviation (%Dev) of the measured concentrations of homogenate QCs (after dilution with plasma at different dilution factors) from their nominal concentrations in order to determine a minimal required dilution factor (MRDF), at which the tissue matrix effect was not significant (i.e., the %Dev value of the measured from the nominal concentration was minimal). The tissue samples from several drug safety evaluation studies have

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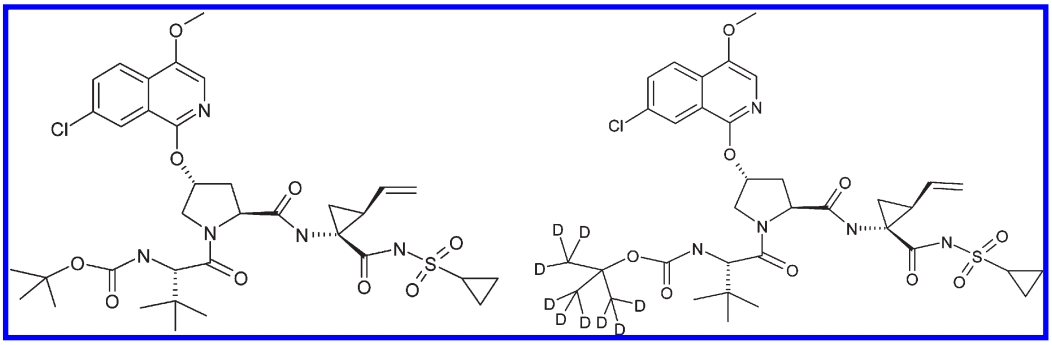

Figure 1. Chemical structures of BMS-650032 and the internal standard, D_9-BMS-650032.

been successfully analyzed after dilution with plasma at the MRDF or larger dilution factors.

MATERIALS AND REAGENTS

Drug-free dog livers and K_2EDTA plasma were obtained from Bioreclamation, Inc. (Hicksville, NY, USA). High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Burdick & Jackson (Muskegon, MI, USA). Analytical-grade ammonium formate, isopropanol, hexane, and ethyl acetate were obtained from J.T. Baker (Phillipsburg, NJ, USA). Analytical-grade formic acid and ammonium bicarbonate were from EMD Chemical, Inc. (Gibbstown, NJ, USA). Hank's buffered salt solution (HBSS) was obtained from Cellgro (Herndon, VA, USA). Deionized water was prepared from a Barnstead Nanopure Diamond system (Dubuque, IA, USA).

The reference materials of BMS-650032 and deuterium labeled internal standard (Figure 1) were obtained from Bristol–Myers Squibb Company (New Brunswick, NJ, USA).

INSTRUMENTS

A Sciex API4000 triple quadrupole tandem mass spectrometer (Applied Biosciences, Forster City, CA, USA) was coupled with a Shimadzu (Kyoto, Japan) LC system that consisted of two LC-10AD VP pumps and an SIL-HTC autosampler. Waters Atlantis dC18 columns (3 μm, 2.1 mm $\times$ 50 mm) were purchased from Waters Co. (Milford, MA, USA). The Omni TH tissue homogenizer was from Omni International, Inc. (Kennesaw, GA, USA).

EXPERIMENTAL SECTION

The Validated Method for Quantitative Determination of BMS-650032 in Dog EDTA Plasma. An LC-MS/MS method has been previously developed and validated for quantitative determination of BMS-650032 in dog K_2EDTA plasma, according to the FDA Guidance for Bioanalytical Method Validation[14] and internal SOPs. In this study, this method was used to determine the concentration of BMS-650032 in dog liver homogenates. The procedure and performance of the validated plasma method were described below. Two milliliters (2 mL) of each calibration standard (5.00, 10.0, 20.0, 50.0, 100, 500, 1000, or 2000 ng/mL) and 6 mL of each quality control sample (5.00, 15.0, 125, 1000, 1600, or 50000 ng/mL) were prepared in drug-free dog plasma by spiking from separate BMS-650032 stock solutions (1.0 mg/mL in methanol from separate weighings) and serial dilutions with dog plasma. After the addition of 50 μL of the internal standard (D_9-BMS-650032 at 100 ng/mL in methanol/water [50:50, v/v]) to 50 μL of each standard and QC followed by a brief vortexing, 100 μL of 1.0 M ammonium formate buffer (pH ~3) and 600 μL of ethyl acetate/hexane (10:90, v/v) were then added. The mixture was vortexed for 1 min, followed by centrifugation for 4 min at ~2000*g*. The organic layer was removed and evaporated under nitrogen for 30 min at 40 °C. The residue was reconstituted in 100 μL of 5 mM ammonium bicarbonate in acetonitrile/water (50:50, v/v) and then 5 μL were injected into the LC-MS/MS system. Chromatographic separation was achieved in 4 min on a Waters Atlantis dC18 analytical column (2.1 $\times$ 50 mm, 3 μm) with mobile phases A (10 mM ammonium bicarbonate) and B (acetonitrile) under a gradient program (30% B for 0.1 min, linear increase to 70% B in 2.4 min, keep at 70% B for 0.5 min, decrease to 30% B in 0.1 min, keep at 30% B for 0.9 min; flowrate of 0.4 mL/min). Detection was accomplished using a Sciex API4000 tandem mass spectrometer in positive-ion electrospray and multiple reaction monitoring (MRM) mode (BMS-650032, m/z 748.3 > 535.3; D_9-BMS-650032, m/z 757.3 > 536.3) (see Figure 2). The optimal mass spectrometer parameters were as follows:

collision gas (CAD), 6 arbitrary units (6 au);
curtain gas (CUR), 30 au;
ion source gas 1 (GS1), 30 au;
ion source gas 2 (GS2), 60 au;
turbo ion spray voltage (IS), 4500 V;
turbo probe temperature (TEM), 500 °C;
dwell time, 200 ms;
declustering potential (DP), 60 V;
entrance potential (EP), 12 V;
collision energy (CE), 39 eV; and
collision cell exit potential (CXP), 14 V.

The standard curves, which ranged from 5.00 ng/mL to 2000 ng/mL for BMS-650032, were fitted to a $1/x^2$ weighted linear regression model. The intra-assay precision, based on four analytical QCs at 15.0, 125, 1000, and 1600 ng/mL, was within 1.8% CV for the analyte. The inter-assay precision was within 1.7% CV, which was previously established in the full validation for rat plasma assay. The assay accuracy, expressed as %Dev, was within ±7.5% of the nominal values for the analyte. At the lower limit of quantitation (LLOQ) of 5.00 ng/mL for BMS-650032, the deviations of the predicted concentrations from the nominal values were within ±11.0% for the six LLOQ samples from different matrix lots. The LLOQ response ratio (analyte-to-internal standard peak area ratio), when compared to the QC0 response ratio, was ≥16.4 in the six matrix lots for the analyte. The analyte was stable in dog plasma for at least 24 h at room temperature, and for at least 63 days at approximately −20 °C.

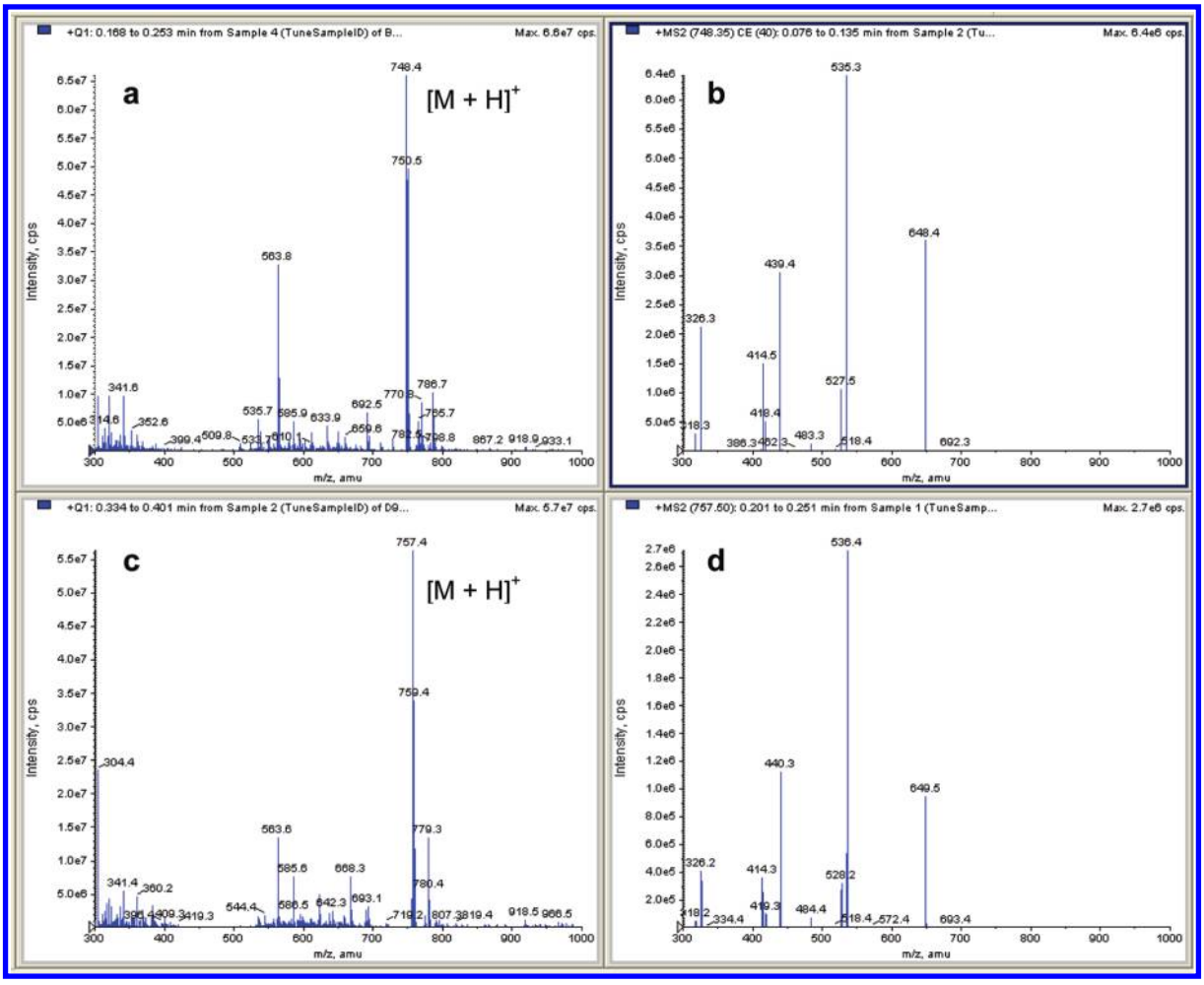

Figure 2. Representative Q1 mass spectra and MS/MS product ion spectra of $[M+H]^+$ for BMS-650032 ((a,b) m/z 748.4 > 535.3) and D_9-BMS-650032 ((c,d) m/z 757.5 > 536.4).

The LC-MS/MS matrix effects were 1.01 and 1.06 for BMS-650032 at concentrations of 15.0 and 1600 ng/mL, and 0.99 and 1.01 for D_9-BMS-650032 at a concentration of 100 ng/mL in the presence of BMS-650032 at concentrations of 15.0 and 1600 ng/mL. Recoveries were 52.4% and 64.2% for BMS-650032, and 59.7% and 71.0% for the internal standard (100 ng/mL) at analyte concentrations of 15.0 and 1600 ng/mL. The average autosampler injection carryover from high QCs (1600 ng/mL) to the subsequent blanks was 0.03% for BMS-650032.

Dog Liver Sample Collection and Homogenization. Beagle dogs (~9−11 months of age, body weights from 6−10 kg) were dosed daily with BMS-650032 at 15, 50, or 100 mg/kg for 9 months. Liver samples were obtained from all cohorts of designated drug-treated study animals at necropsy after the last dose of BMS-650032. A section of the liver (~1 g from the left lateral lobe) was collected into a 5-mL polypropylene tube, weighed, and snap frozen in liquid nitrogen, and stored frozen at −70 °C. The frozen liver samples were thawed at room temperature and homogenized in acetonitrile/HBSS (20:80, v/v) at a tissue/buffer ratio of 1:4 (w/v). Homogenization was paused 10−20 s after every 30 s of homogenization at a medium speed. The homogenization was repeated 3−4 times until a uniform homogenate was obtained. The homogenizer probe was washed sequentially with water, methanol, and water after each sample homogenization. The homogenates were then stored at approximately −70 °C prior to analyses.

Preparation of Plasma Standards and Analytical QCs, and Liver Homogenate QCs. Both stock solutions of BMS-650032 and its internal standard at a concentration of 1.0 mg/mL were prepared by dissolving 3−4 mg corresponding reference materials in methanol. The BMS-650032 stock solutions from separate weighings were used for preparation of dog plasma standards, and the analytical QCs and liver homogenate QCs (25.0, 1600, and 50000 ng/mL). The homogenate QCs were only used to evaluate the tissue matrix effect. After preparation, the homogenate QCs were kept at room temperature for ~30 min prior to storage at −70 °C. The plasma standards were freshly prepared and used on the day of preparation; the analytical QCs were stored at −20 °C, a temperature at which BMS-650032 was demonstrated to be stable for 63 days during the validation.

Tissue Matrix Effect Evaluation. The liver homogenate QCs (25.0, 1600, and 50 000 ng/mL) were diluted with drug-free dog plasma. Homogenate QC-1 (25.0 ng/mL) was diluted at dilution factors of 1 and 2; homogenate QC-2 (1600 ng/mL) was diluted at dilution factors of 1, 2, 5, 10, and 20; and homogenate QC-3 (50000 ng/mL) was diluted at a dilution factor of 100. All the dilutions were applied in three replicates. Higher dilutions of QC-1 were not performed because the results would have been at

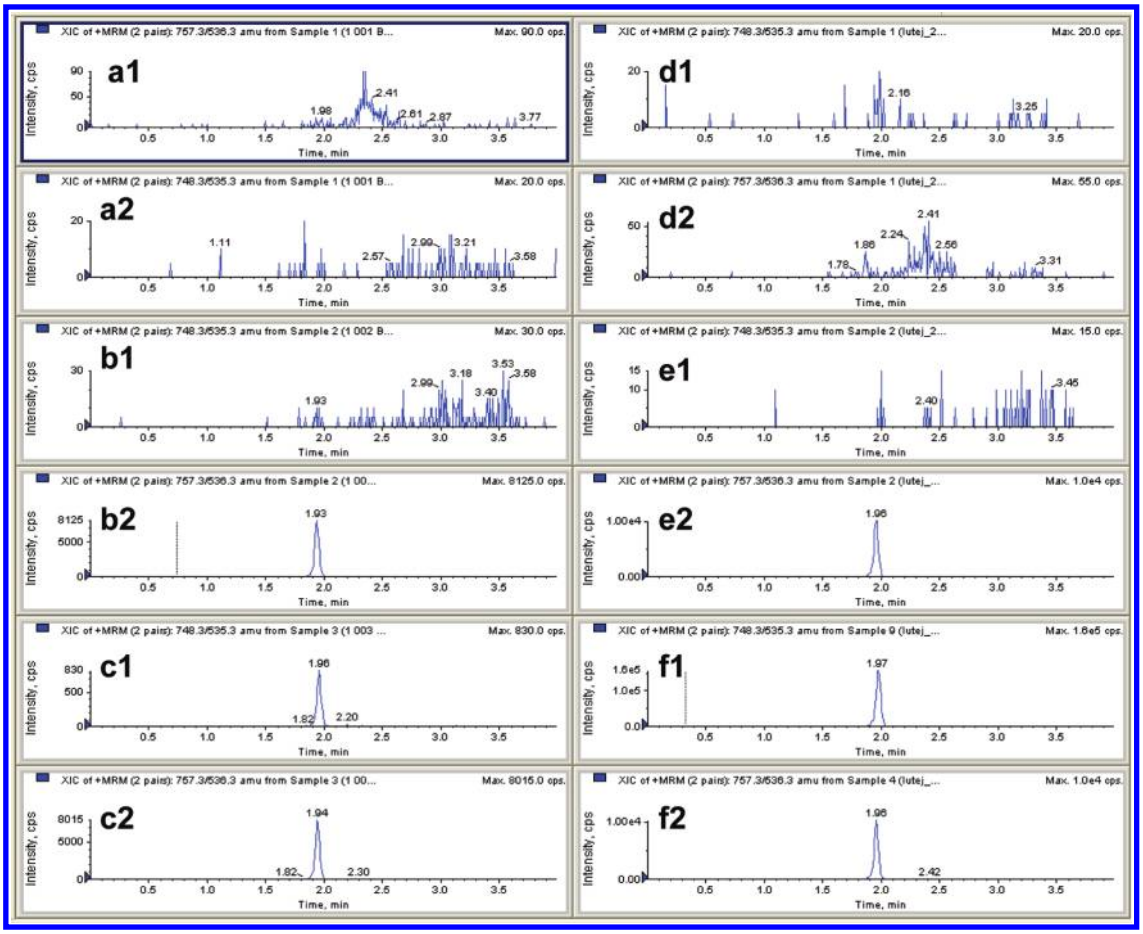

Figure 3. Representative chromatograms of BMS-650032 and its internal standard (D_9-BMS-650032) in plasma blank (panels a1 and a2), plasma QC0 (panels b1 and b2), plasma LLOQ (panels c1 and c2), liver homogenate blank (panels d1 and d2), liver homogenate QC0 (panels e1 and e2), and liver homogenate QC without plasma dilution (panels f1 and f2).

Table 1. Calibration Curves for Quantitative Determination of BMS-650032 in Dog Liver Homogenate

regression equation	correlation coefficient (r)	weighting factor	range (ng/mL)
response[a] = 0.0207 × Conc. + 0.0139	0.9987	$1/x^2$	5.00−2000
response[a] = 0.0232 × Conc. + 0.0080	0.9996	$1/x^2$	5.00−2000

[a] Response is defined as the analyte/internal standard ratio.

Table 2. Accuracy and Precision

parameter	Value			
	low QC	GM QC	mid QC	high QC
nominal conc. (ng/mL)	15.0	125	1000	1600
mean observed conc. (ng/mL)	14.6	129	1045	1693
%Dev	−2.6	2.9	4.5	5.8
within-run precision (%CV)	3.7	3.1	2.5	4.4
between-run precision (%CV)	1.8	1.8	0.1	0.0
total variation (%CV)	4.1	3.6	2.5	36.5
n	6	6	6	6
number of runs	2	2	2	2

or below the LLOQ. To ensure pipetting accuracy, a minimum of 50 μL of the liver homogenate QC was pipetted with wide bore pipet tips. The diluted homogenate QC samples were processed and analyzed along with plasma standards and analytical QCs using the validated LC-MS/MS plasma assay. The measured concentration of the diluted homogenate QC at each dilution factor was corrected by the corresponding dilution factor. The %Dev from nominal values and %CV of the replicates for the diluted homogenate QC were calculated and used to evaluate the tissue matrix effect at each dilution factor. The matrix effect was considered to be significant when the %Dev value was greater than ±15% at a given dilution factor.

RESULTS AND DISCUSSION

Assay Performance. Two analytical runs were conducted for evaluation of the tissue matrix effect, which include plasma standards, analytical QCs, and liver homogenate QCs diluted with plasma at different dilution factors. The representative chromatograms of BMS-650032 and the internal standard are shown in Figure 3: in a plasma blank (panels a1 and a2), a QC0 (panels b1 and b2), a plasma LLOQ (panels c1 and c2), a tissue homogenate blank (panels d1 and d2), a tissue homogenate QC0 (panels e1 and e2), and a liver homogenate QC without plasma dilution (panels f1 and f2). No interference with BMS-650032 and the internal standard from plasma or liver homogenate was observed. The retention time of chromatographic peaks was ~2 min. The

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Table 3. Tissue Matrix Effect Evaluation

dilution factor	nominal conc. (ng/mL)	measured conc. (ng/mL)	%Dev	%CV
2	25.0	19.9	−20.4	
		20.3	−18.8	
		18.6	−25.6	
			−21.6 (mean)	4.5
1	25.0	13.6	−45.6	
		13.3	−46.8	
		11.6	−53.6	
			−48.7 (mean)	8.4
20	1600	1830	14.4	
		1870	16.9	
		1760	10.0	
			13.8 (mean)	3.1
10	1600	1750	9.4	
		1810	13.1	
		1720	7.5	
			10.0 (mean)	2.6
5	1600	1750	9.4	
		1700	6.3	
		1680	5.0	
			6.9 (mean)	2.1
2	1600	1380	−13.8	
		1350	−15.6	
		1020	−36.3	
			−21.9 (mean)	16.0
1	1600	905	−43.4	
		1010	−36.9	
		790	−50.6	
			−43.6 (mean)	12.2
100	50000	54300	8.6	
		54600	9.2	
		50600	1.2	
			6.3 (mean)	4.2

plasma standard curves were obtained from a linear regression with a weighting factor of $1/x^2$ (see Table 1). The accuracy and precision data for the analytical QCs is shown in Table 2. The intra-assay precision was within 4.4% CV for the analyte, and the inter-assay precision was within 1.8%. The plasma assay accuracy (%Dev) was within ±5.8% of the nominal values for the analyte.

Determination of Minimum Required Dilution Factor (MRDF). The final concentration of each diluted homogenate QC was obtained by back-calculating from the plasma standard curves and multiplying by the corresponding dilution factor. If more than two-thirds of the diluted homogenate QCs at a given dilution factor have a %Dev value of less than ±15.0% and a %CV value of <15.0%, the tissue matrix effect is considered to be insignificant at this dilution factor; if the %Dev and %CV values at any higher dilution factors also met the same criteria, this dilution factor is considered minimum required dilution factor (MRDF). All tissue homogenate samples will be diluted with plasma at the MRDF or larger dilution factors prior to extraction and analyses.

After dilution with plasma at different dilution factors, dog liver homogenate QCs (25.0, 1600, and 50000 ng/mL) were analyzed, along with plasma standards and analytical QCs. The %Dev and %CV values for the homogenate QCs were calculated, as shown in Table 3. For the homogenate QC-2 (1600 ng/mL), the concentrations measured were significantly lower than the nominal value with the %Dev varying from −13.8% to −50.6% at dilution factors of 1 and 2. When the dilution factor was increased to 5 or higher, the %Dev values of the homogenate QCs were within ±15%, with only one individual %Dev value being above 15% (i.e., 16.9%), and the %CV values were within 3.1%. The results indicate that the tissue matrix effect impacted data accuracy (%Dev) at dilution factors of 1 and 2. Similar impacts were observed on the %Dev for homogenate QC-1 (25.0 ng/mL) at dilution factors of 1 and 2. Therefore, BMS-650032 liver homogenate samples can be analyzed along with the plasma standards after a 5-fold or greater dilution with plasma to generate accurate data. This conclusion was further confirmed by the results for homogenate QC-3 (50000 ng/mL) at a dilution factor of 100, which demonstrated good data accuracy and precision.

The biases observed at dilution factors of 1 and 2 indicate the difference of the analyte extraction recoveries in the plasma matrix and liver homogenate matrix (i.e., the tissue matrix effect). The stable isotopically labeled internal standard in the assay did not overcome the tissue matrix effect, as demonstrated in Figures 4a and 4b (large-dashed lines). Extending the time (additional 1 min) for vortexing after adding the internal standard into the homogenate QC-2 significantly decreased the biases (see the small-dashed line in Figure 4a), suggesting that the labeled internal standard overcame the tissue matrix effect after extensive mixing (see the small-dashed line in Figure 4b). The low recovery of the internal standard corrected the low analyte recovery at dilution factors of 1 and 2. As a control, the plasma high-QC (1600 mg/mL) was diluted and analyzed in a manner similar to that for the homogenate QC-2 (see solid lines in Figures 4a and 4b).

When acetonitrile/HBSS (20:80, v/v) was replaced with dog plasma as homogenization solution, it was observed that the tissue matrix effect was significantly reduced, because of the similarity of the matrices between plasma and the homogenates prepared in plasma. The %Dev value of the homogenate QCs prepared in plasma at a dilution factor of 1 (−10%; see the large-dashed line in Figure 4c) was significantly lower than that of the homogenate QCs prepared in acetonitrile/HBSS (20:80, v/v) at the same dilution factor (−25%; see the large-dashed line in Figure 4a). These results suggested that the assay accuracy (%Dev) was significantly improved by using plasma as a homogenization solution.

Quantitative Determination of BMS-650032 in Dog Liver Homogenates from Dosed Animals. Based on the results (MRDF = 5) from the tissue matrix effect evaluation, the liver samples homogenized in acetonitrile/HBSS (20:80, v/v) were prediluted with dog plasma at least 5-fold prior to sample extraction. The final reportable concentrations of BMS-650032 in liver (in terms of ng/g liver) were obtained from the measured concentrations by multiplying the homogenization dilution factor

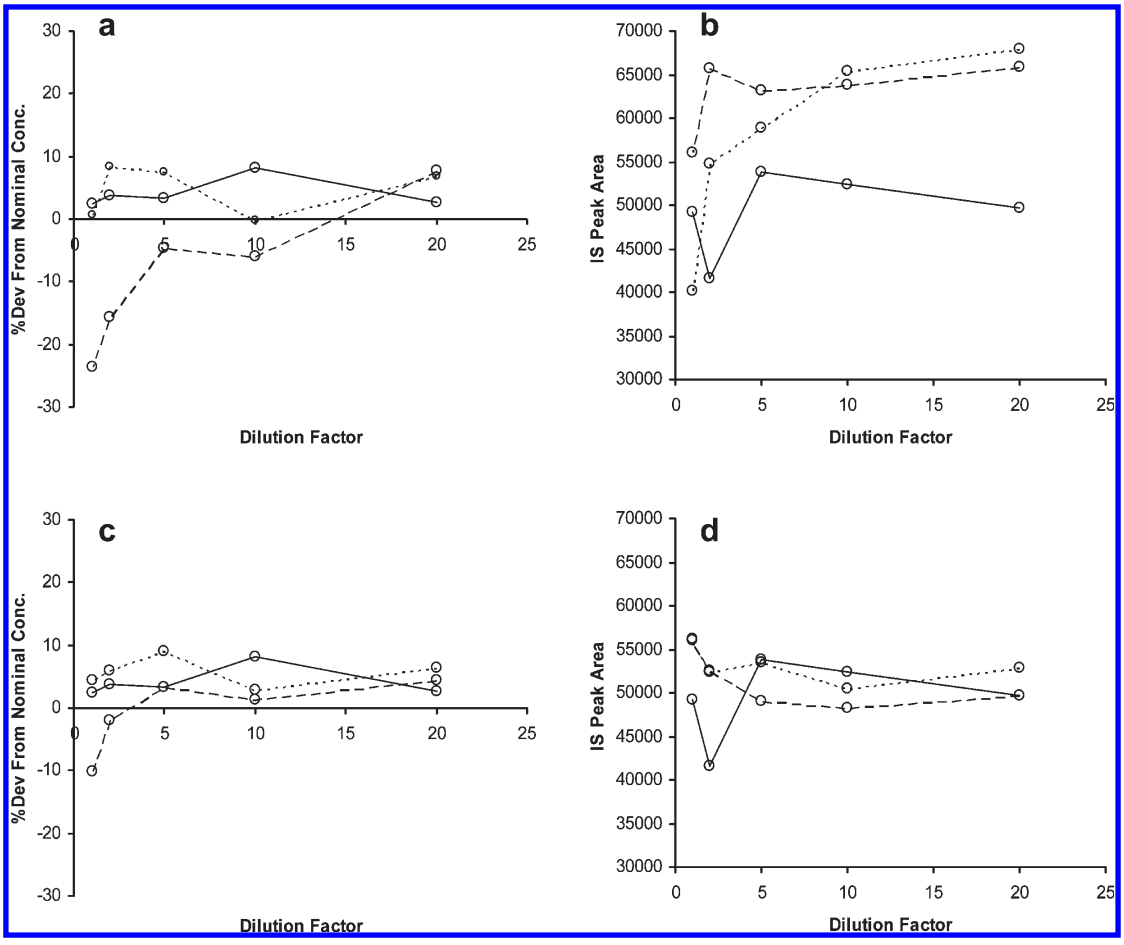

Figure 4. The accuracy (%Dev) of the liver homogenate QC in acetonitrile/HBSS (20:80, v/v) was improved by (a,b) extensively vortexing the mixture of the sample and the internal standard or (c,d) preparing the liver homogenate QC in the homogenates prepared with plasma. Legend: solid line (—), plasma QC; large-dashed line (– – –), liver homogenate QC; and small-dashed line (- - -), liver homogenate QC with extensive vortexing after the addition of the internal standard.

Table 4. Mean Liver and Plasma Concentrations, and Liver/Plasma Concentration Ratios at Necropsy (n = 4)

	BMS-650032					
	15 mg/kg/day		50 mg/kg/day		100 mg/kg/day	
parameter	male	female	male	female	male	female
liver (ng/g)	196 ± 147	336 ± 202	341 ± 396[a]	1650 ± 933	2427 ± 1958	5772 ± 2548[b]
plasma[c] (ng/mL)	11.0[d]	12.0[d]	13.5 ± 5.7[a]	70.2 ± 62.8	59.9 ± 64.2	123 ± 100[b]
liver/plasma ratio	36.2[d]	42.6[d]	30.1 ± 21.9[a]	30.4 ± 11.4	49.0 ± 16.0	62.4 ± 47.0[b]

[a] The number of values for the calculation was reduced from 4 to 3, because of misdosing. [b] The number of values for the calculation reduced from 4 to 3 due to emesis. [c] Plasma concentrations were measured using the validated plasma assay described. [d] Only one value is given; thus, a standard deviation value was not available.

of 4 mL/g (derived from homogenization process) and the corresponding analytical dilution factors. The representative results of BMS-650032 concentrations in the liver samples are shown in Table 4. The results indicated that BMS-650032 concentrations were higher in liver than plasma. However, mean BMS-650032 liver/plasma ratios were approximately similar across the dose ranges (30.07−62.41). Mean liver and plasma BMS-650032 concentrations were consistently lower in males than in females; however, mean BMS-650032 liver/plasma ratios were similar in males and females.

It is worth mentioning that the liver samples in this study were not homogenized with plasma, because, at the time of preparing liver homogenates, homogenization with plasma had yet to be investigated and acetonitrile/HBSS was considered a better matrix to maintain stability of the analyte since liver enzymes could be inhibited by acetonitrile during homogenization.

Strategy for Using Plasma Assay for Tissue Sample Analysis. In general, LC-MS/MS assays for plasma samples are fully developed and validated in early drug development. These available plasma assays can be directly applied to tissue sample

Table 5. Summary of Tissue Matrix Effect Evaluation Applied to Different Drugs in Various Tissues and Biofluids

sample for tissue matrix effect evaluation[a]	species	tissue sample	homogenization solution	established MRDF[b]	internal standard[c]
Compound 1					
incurred samples	rat	liver	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
incurred samples	monkey	liver	acetonitrile/HBSS (80:20, v/v)	10	SIL-IS
incurred samples	monkey	bile	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
incurred samples	dog	liver	acetonitrile/HBSS (80:20, v/v)	2	SIL-IS
homogenate QC	monkey	liver	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
bile QC	monkey	bile	acetonitrile/HBSS (80:20, v/v)	20	SIL-IS
homogenate QC	rat	liver	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
Compound 2					
incurred samples	rat	liver	acetonitrile/HBSS (80:20, v/v)	5	SIL-IS
incurred samples	dog	liver	acetonitrile/HBSS (80:20, v/v)	2	SIL-IS
incurred samples	monkey	liver	acetonitrile/HBSS (80:20, v/v)	10	SIL-IS
incurred samples	monkey	bile	acetonitrile/HBSS (80:20, v/v)	200[d]	SIL-IS
homogenate QC	monkey	liver	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
bile QC	monkey	bile	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
homogenate QC	rat	liver	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
Compound 3					
incurred samples	rat	liver	acetonitrile/HBSS (80:20, v/v)	10	SIL-IS
incurred samples	dog	liver	acetonitrile/HBSS (80:20, v/v)	2[e]	SIL-IS
homogenate QC	monkey	liver	ACN/HBSS (80:20, v/v)	1	SIL-IS
bile QC	monkey	bile	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
homogenate QC	rat	liver	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
Compound 4					
incurred samples	rat	liver	acetonitrile/HBSS (80:20, v/v)	2	SIL-IS
incurred samples	dog	liver	acetonitrile/HBSS (80:20, v/v)	1[e]	SIL-IS
homogenate QC	monkey	liver	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
bile QC	monkey	bile	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
homogenate QC	rat	liver	acetonitrile/HBSS (80:20, v/v)	1	SIL-IS
Compound 5					
homogenate QC	rat	brain	plasma	1	SIL-IS
homogenate QC	monkey	brain	plasma	1	SIL-IS
Compound 6					
homogenate QC	rat	brain	plasma	1	No IS
homogenate QC	monkey	brain	plasma	1	No IS

[a] Standards were prepared in plasma. [b] MRDF = Minimum required dilution factor. [c] SIL-IS = stable isotopic labeled internal standard. No IS = no internal standard is present. [d] Only dilution factors of 100, 200, and 500 were tested, because high drug concentrations in bile were expected. [e] An acceptance criterion of 30% was applied, because significant data variability was observed.

analyses after appropriate dilutions with plasma, as long as the tissue matrix effect has no impact on the data accuracy and precision. The results from this study have demonstrated that the dilution of tissue homogenate samples with plasma is a practical strategy to minimize the tissue matrix effect on the assay. The advantages of this strategy are that (a) there is no need to develop a unique and separate method for tissue samples when a plasma assay is already available, which saves time, resources, and effort; and (b) it is not necessary to collect or purchase large quantities of drug-free tissues for diluting homogenate samples (when drug concentrations in the homogenates are greater than the upper limit of quantitation, ULOQ) and prepare standards and analytical QCs, which also saves cost and effort. The strategy of using plasma standards and analytical QCs for the quantitative determination of drug concentrations in tissue samples has been used in several drug development programs. We have conducted ~30 tissue matrix effect evaluations for several drug candidates in the liver, bile, and the brain. In Table 5, some representative results for different tissue assays are summarized. The tissue matrix effect was observed mostly at dilution factors of <10. We also observed an insignificant tissue matrix effect without the need of plasma dilution (MRDF = 1) in several evaluations.

In the tissue matrix effect evaluation, incurred tissue samples from studies can also be used instead of using homogenate QCs. The incurred tissue samples are better representatives of all study samples. In addition, it is not necessary to purchase any drug-free tissue and prepare homogenate QCs for the tissue matrix effect evaluation. However, there are two pitfalls: (a) the tissue matrix effect evaluation could not be conducted before tissue samples are collected, and (b) nominal drug concentrations in the

incurred tissue samples are unknown. The measured concentration at the largest available dilution factor can be used as the nominal concentration, assuming that the tissue matrix effect is completely minimized by plasma dilution. Some representative results from incurred study samples are summarized in Table 5.

In case the matrix difference between the plasma standards and homogenate samples would not impact the extraction recovery, which is an alternative to the dilution with the MRDF before extraction, it can also be an option to reduce the injection volume with such a factor. This will be investigated further in the future. In addition, the MRDF will be largely dependent on the selectivity of the chromatographic separation. Further optimization of the chromatography may significantly minimize the matrix effect differences during method development.

There are also disadvantages in application of this strategy. First, the LLOQ will be increased due to plasma dilution for minimizing the tissue matrix effect, which brings limits to the ability of the assays to measure low concentrations of drugs and metabolites in tissue samples. Second, the assays will be more variable, when a stable isotopically labeled internal standard is not available, because an analogue internal standard may not overcome or compensate for the tissue matrix effect. Thus, significant biases may be expected. Several options may help overcome this issue: (a) preparing standards and analytical QCs in drug-free tissue homogenates (i.e., dog liver homogenate in acetonitrile/HBSS [20:80, v/v]); (b) preparing standards and analytical QCs in the homogenization solution (i.e., acetonitrile/HBSS [20:80, v/v]); (c) homogenizing tissue samples in plasma; (d) adding tissue homogenates (1:1, v/v) to plasma standards and analytical QCs, and adding plasma (1:1, v/v) to tissue homogenate samples to compensate the matrix difference between the plasma standards and homogenate samples.

CONCLUSIONS

In conclusion, the use of plasma standards and analytical quality controls (QCs) to quantitatively determine drug concentrations in tissue homogenate samples after plasma dilution at the MRDF or greater dilution factors has been proven to be practical, convenient, and accurate.

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