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LC-MS/MS quantitation of Abbott compound in cerebrospinal fluid through use of synthetic cerebrospinal fluid

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Abstract

Drug concentrations in cerebrospinal fluid is used as a surrogate for drug concentrations in the brain. Development and validation activities using human cerebrospinal fluid (CSF) can be expensive due to the limited availability and high cost of the matrix. The use of synthetic cerebrospinal fluid (sCSF) during development and validation allows for a multitude of experiments to be performed at low cost. A dilute and shoot method was developed and validated for Abbott compound. Calibration standards were prepared in sCSF while LLOQ, ULOQ and QC samples were prepared in CSF. Two hundred microliters of sample were mixed with 50 µL of internal standard (D_2, $^{13}C_2$-Abbott compound), the mixture was centrifuged and injected. Detection was performed by electrospray ionization (ESI) tandem mass spectrometry in the positive ionization mode. The method was shown to be linear from 0.0500 ng/mL to 25.0 ng/mL. The lower limit of quantitation was 0.0500 ng/mL. The inter-run CVs were ≤ 10.3%. The bias was between 3.9% and 12.4%. The method was shown to be selective and free of matrix effects. Suitable stability evaluations have been performed.

Keywords: Cerebrospinal fluid, CSF, synthetic CSF, LC-MS/MS

1. Introduction

Cerebrospinal fluid (CSF) concentrations are often used as a surrogate for concentrations of drug in the brain.[1-4] Human CSF is a rare matrix and has sometimes been substituted with distilled water [5] or a solution created to mimic CSF [6-9].

2. Materials and Methods

2.1. Chemicals and Reagents

Abbott compound and its stable label internal standard, D_2, $^{13}C_2$-Abbott compound, were manufactured by Abbott Laboratories (North Chicago, IL). Acetonitrile was purchased from Sigma-Aldrich (St. Louis, MO), methanol was purchased from EMD (Gibbstown, NJ), ethanol was purchased from Decon Labs (King of Prussia, PA) and purified water was obtained from a Millipore (Billerica, MA) system. Ammonium acetate was purchased from J.T. Baker (Phillipsburg, NJ). Sodium chloride, magnesium chloride hexahydrate, sodium phosphate dibasic heptahydrate, and sodium phosphate monobasic monohydrate were purchased from EMD (Gibbstown, NJ). Potassium chloride and calcium chloride monohydrate were purchased from J.T. Baker (Phillipsburg, NJ). Human cerebrospinal fluid was purchased from Biological Specialty (Colmar, PA).

2.2. Sample Preparation

Synthetic cerebrospinal fluid (sCSF) was prepared using the Alzet recipe [10]. The sCSF was used to prepare the calibration curve samples. Real CSF was used to prepare LLOQ, ULOQ and QC samples. Sample preparation was performed in a 96-well deep well plate. 50 µL of internal standard solution at approximately 15 ng/mL in 50/50 (v/v) methanol/water was added to the plate followed by 200 µL of sample. The plate was vortexed to ensure complete mixing and was then placed in the autosampler at a set point of 7 ºC until injection. The injection volume was 25 µL.

2.3. LC-MS/MS Analysis

The HPLC system consisted of a Shimadzu (Columbia, MD) LC-10 AD pump and a Shimadzu SIL-HTc autosampler and system controller. A Security Guard C12 (4 x 2.0 mm) and Synergi Max-RP analytical column with 2.5 µm particle size (100 x 3.00 mm, both from Phenomenex, Torrance, CA) were used to separate the compound of interest from the endogenous matrix components. The mobile phase consisted of 1 mM ammonium acetate in 60/40 (v/v) acetonitrile/water. During the first 1.5 minutes of the

run, column effluent was directed to waste. From 1.5 minutes to 4.0 minutes the flow was directed to the mass spectrometer and data was acquired. At 4.0 minutes the flow was again directed to waste.

The mass spectrometer used was a TSQ Quantum Ultra (Thermo Fisher Scientific, Waltham, MA). Analytes were ionized by electrospray ionization (ESI) in the positive mode. The spray voltage was set to 4500, the capillary temperature was 325 ºC, the sheath gas was set at 75 AU, the auxiliary gas was set at 22 AU and the source collision energy was set at 17eV. Highly selective reaction monitoring (H-SRM) with the Q1 peak widths set at 0.2 amu was selected and the $[M + H]^+$ ions were selected as the precursor ions. Scan width was set to 0.250 *m/z* with a scan time of 0.20 seconds. The collision gas pressure was 1.5 mTorr and the collision energy was 32eV. The H-SRM transitions monitored were *m/*z 411.18 → 144.10 (Abbott compound) and *m/*z 415.18 → 148.10 (D_2, $^{13}C_2$-Abbott compound).

2.4 Method Validation

2.4.1. Linearity

A stock solution of Abbott compound in ethanol was prepared. Spiking solutions were prepared in 50/50 (v/v) methanol/water by serial dilution of the stock solution. Calibration curve standards were prepared in synthetic cerebrospinal fluid (sCSF) using individual spiking solutions for each concentration level. Standards ranged from 0.0500 ng/mL to 25.0 ng/mL. Standard curves were plotted as peak area ratio (analyte/internal standard) versus theoretical concentration. A least squares regression was performed to evaluate the linearity of the calibration curve.

2.4.2. Precision, Accuracy and Limits of Quantitation

A stock solution of Abbott compound in ethanol was prepared. Spiking solutions were prepared in 50/50 (v/v) methanol water by serial dilution of the stock solution. Quality control (QC) samples at three concentrations, lower limit of quantitation (LLOQ) samples and upper limit of quantitation (ULOQ) samples were prepared in cerebrospinal fluid (CSF) using individual spiking solutions for each concentration level. QC, LLOQ and ULOQ samples were evaluated in replicates of six on three different days and intra-run and inter-run statistics were calculated.

2.4.3. Selectivity and Matrix Effects

To demonstrate the selectivity of the method, six lots of CSF without internal standard were screened for interference from endogenous matrix components. An LLOQ without internal standard was used as the comparator for these samples. No interference was observed for Abbott compound. To demonstrate that the assay performance is independent of the sample matrix, six lots of CSF were used to prepare samples at the same concentration as the low QC. The samples were evaluated and demonstrated that there were no matrix effects.

2.4.4. Stability

The stability of samples undergoing multiple freeze/thaw cycles, stability at room temperature, frozen storage stability, and prepared sample stability were evaluated.

2.4.5. Incurred Sample Reproducibility

The reproducibility of the method was evaluated using samples collected as part of a phase I clinical study.

3. Results

3.1. LC-MS/MS analysis

Representative chromatograms of Abbott compound and D_2, $^{13}C_2$-Abbott compound are shown in Figure 1.

3.2. Method Validation

3.2.1. Linearity

The standard curve was found to be linear over the range 0.0500 ng/mL to 25.0 ng/mL and showed good reproducibility and coefficient of determination ≥ 0.996 (n = 4).

3.2.2. Precision, Accuracy and Limits of Quantitation

QC samples at three concentrations, along with LLOQ and ULOQ samples, were assayed in replicates of 6 on three different days. The intra-run CV was $\leq 20.6\%$ for the LLOQ and was $\leq 14.9\%$ for concentrations higher than the LLOQ. The intra-run bias was between -5.9% and 10.7% for the LLOQ and was between 0.2% and 22.1% for concentrations higher than the LLOQ. The inter-run CV was 14.6% for the LLOQ and was $\leq 10.3\%$ for concentrations higher than the LLOQ, the inter-run bias was 1.7% for the LLOQ and was between 3.9% and 15.5% for concentrations higher than the LLOQ. The results of the precision, accuracy and limits of quantitation experiments are presented in Table 1.

3.2.3. Selectivity and Matrix Effects

The six lots of CSF without internal standard showed no significant peaks at the retention time of the analyte or internal standard. The low QC samples prepared in six different lots of CSF showed mean biases (n=6) between -7.7% and -1.7% which demonstrated the method is independent of the matrix (Table 2).

3.2.4. Stability

Low QC and High QC samples were exposed to 4 freeze/thaw cycles and 14 hours of room temperature storage. The samples were compared to samples that underwent their first thaw on the day of analysis. The control samples (n=5) showed biases of 6.6% and 5.6% for the low and high QC, respectively. The stability samples (n=5) showed biases of 5.5% and 4.4% for the low and high QC, respectively (Table 3). All biases were from the theoretical concentration. Samples at all three QC levels were stored in a -20 ºC for 35 days prior to analysis and were analyzed using a calibration curve that had not been frozen. The stability samples (n=3) showed biases between 5.9% and 11.5% (Table 4). A plate consisting of standards and QCs was prepared, injected, stored in the autosampler at approximately 7 ºC, and the QCs re-injected following 88 hours of storage. The re-injected QC concentrations (n=18) were compared to the original observed concentrations for each sample. The differences ranged from -12.5% to 15.4% (Table 5). A plate consisting of standards and QCs was prepared and placed in the autosampler for storage at approximately 7 ºC for 91 hours prior to starting injections. The QC samples (n=4) showed mean biases between 0.5% and 13.2% (Table 6).

3.2.5. Incurred Sample Reproducibility

Samples from the T_{max} and the terminal portion of the CSF concentration time profile of samples were evaluated for reproducibility. A total of 16 samples were selected and each sample showed a repeat concentration within 20% of the mean of the original and repeat concentrations.

4. Discussion

The method described allows for accurate, precise and reproducible quantitation of Abbott compound in cerebrospinal fluid samples using a calibration curve prepared in synthetic cerebrospinal fluid. The sample preparation is short, simple and easily performed by a robotic liquid handler. The use of synthetic cerebrospinal fluid for the

preparation of the calibration curve allows for a significant cost savings with no loss of quality for the data. This has led to an adoption of sCSF as the preferred matrix during development of methods intended for analysis of human cerebrospinal fluid.

5. References

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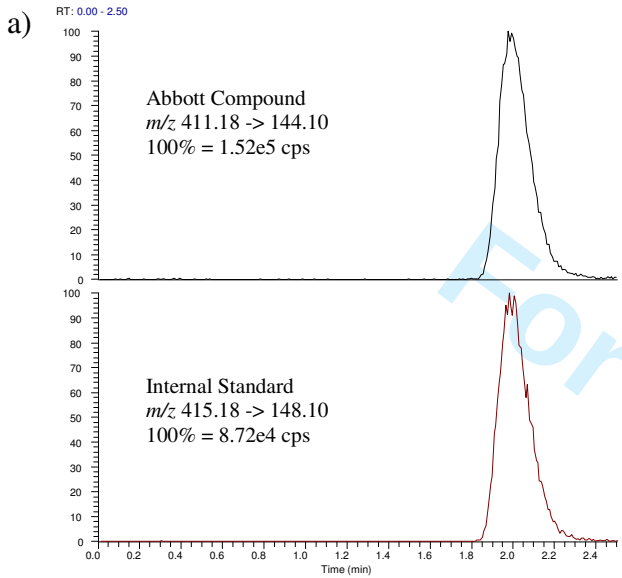
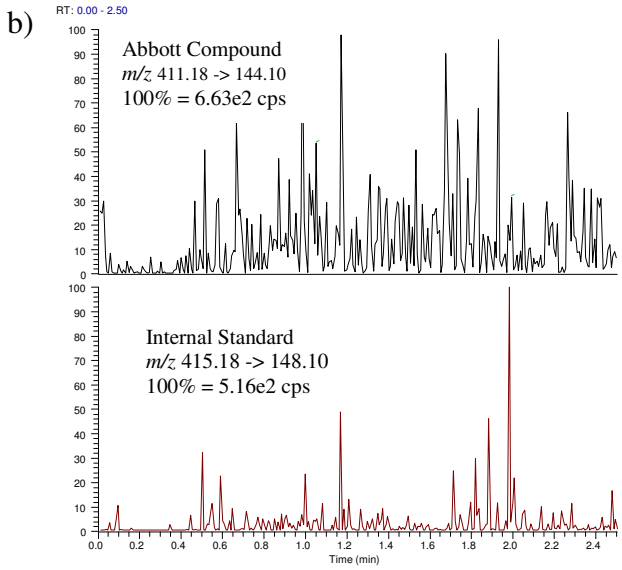
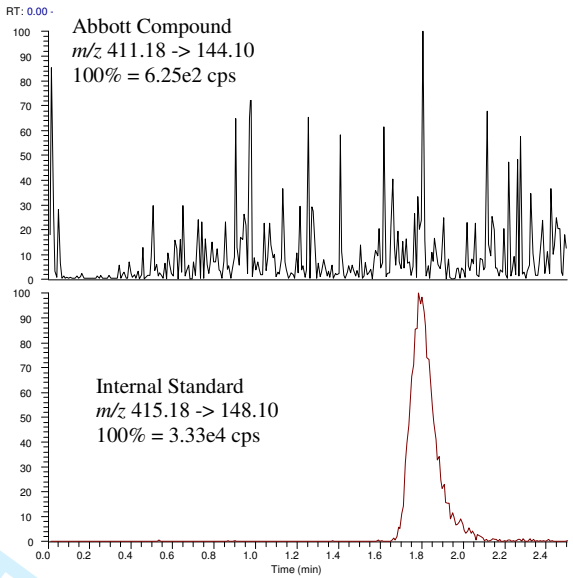
6. Figure Captions

Figure 1. Representative chromatograms of a) a reference mixture of 10 ng/mL Abbott compound and 10 ng/mL D_2, $^{13}C_2$-Abbott compound in mobile phase; b) blank CSF; c) blank CSF with internal standard; d) LLOQ sample with internal standard







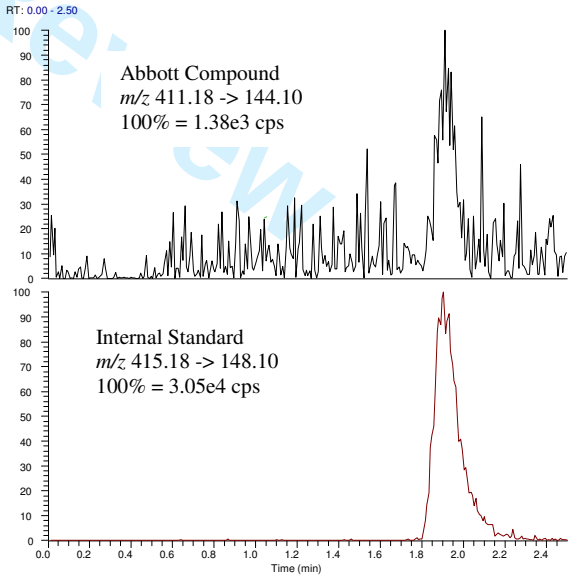


Table 1. Accuracy and Precision data

Run	Level Theo. Conc.	LLOQ 0.0500 ng/mL	Low QC 0.136 ng/mL	Mid QC 1.70 ng/mL	High QC 21.2 ng/mL	ULOQ 25.0 ng/mL
1	Intra-run Mean	0.0471	0.147	1.80	21.2	27.6
	Intra-run SD	0.00969	0.0181	0.0841	0.845	0.559
	Intra-run %CV	20.6	12.3	4.7	4.0	2.0
	Intra-run %Bias	-5.9	7.7	5.9	0.2	10.4
	n	6	6	6	6	6
2	Intra-run Mean	0.0501	0.154	1.83	22.5	28.5
	Intra-run SD	0.00349	0.0161	0.0737	1.12	1.49
	Intra-run %CV	7.0	10.5	4.0	5.0	5.2
	Intra-run %Bias	0.3	13.5	7.4	6.2	13.9
	n	6	6	6	6	6
3	Intra-run Mean	0.0554	0.158	1.88	22.4	30.5
	Intra-run SD	0.00622	0.0132	0.0598	0.866	4.55
	Intra-run %CV	11.2	8.4	3.2	3.9	14.9
	Intra-run %Bias	10.7	16.1	10.5	5.5	22.1
	n	6	6	6	6	6
	Inter-run Mean	0.0508	0.153	1.84	22.0	28.9
	Inter-run SD	0.00742	0.0157	0.0765	1.07	2.90
	Inter-run %CV	14.6	10.3	4.2	4.9	10.0
	Inter-run %Bias	1.7	12.4	7.9	3.9	15.5
	n	18	18	18	18	18

Table 2. Matrix Effects

	MEQC1 0.136 ng/mL	MEQC2 0.136 ng/mL	MEQC3 0.136 ng/mL	MEQC4 0.136 ng/mL	MEQC5 0.136 ng/mL	MEQC6 0.136 ng/mL
	0.164	0.147	0.142	0.132	0.123	0.128
	0.138	0.152	0.128	0.127	0.140	0.109
	0.117	0.113	0.129	0.139	0.137	0.125
	0.121	0.111	0.105	0.112	0.135	0.139
	0.129	0.120	0.128	0.148	0.129	0.129
	0.133	0.143	0.121	0.125	0.109	0.130
Mean	0.134	0.131	0.126	0.131	0.129	0.127
S.D.	0.0167	0.0184	0.0121	0.0124	0.0115	0.00985
%CV	12.5	14	9.6	9.5	8.9	7.8
%Bias	-1.7	-3.7	-7.7	-4.0	-5.3	-6.9
n	6	6	6	6	6	6

Table 3. Freeze/Thaw and Room Temperature Stability

	Observed Concentration (ng/mL)	Observed Concentration (ng/mL)
1 F/T Cycle	0.149	21.4
	0.130	22.4
	0.147	22.3
	0.159	22.2
	0.140	23.6
Mean	0.145	22.4
S.D.	0.0108	0.789
%CV	7.4	3.5
%Bias	6.6	5.6
n	5	5
4 F/T Cycles	0.146	21.3
	0.147	22.3
	0.147	23.3
	0.135	21.5
	0.142	22.3
Mean	0.143	22.1
S.D.	0.00513	0.792
%CV	3.6	3.6
%Bias	5.5	4.4
n	5	5

Table 4. Frozen Storage Stability

Stored 35 Days	Low QC 0.136 ng/mL	Mid QC 1.70 ng/mL	High QC 21.2 ng/mL
	0.136	1.81	22.2
	0.148	1.79	22.8
	0.171	1.80	23.3
Mean	0.152	1.8	22.8
S.D.	0.0178	0.01	0.551
%CV	11.7	0.6	2.4
%Bias	11.5	5.9	7.4
n	3	3	3

Table 5. Autosampler Storage Stability

Original injection concentration (ng/mL)	Re-injection concentration (ng/mL)	%Difference
0.154	0.145	-5.8
0.160	0.140	-12.5
0.145	0.145	0.0
0.138	0.147	6.5
0.135	0.137	1.5
0.136	0.157	15.4
1.86	1.86	0.0
2.13	1.89	-11.3
1.87	1.77	-5.3
1.80	1.84	2.2
1.79	1.77	-1.1
1.84	1.87	1.6
25.5	23.6	-7.5
23.9	22.7	-5.0
24.6	22.2	-9.8
23.7	23.1	-2.5
22.4	22.3	-0.4
22.2	21.5	-3.2

Table 6. Run Storage Stability

	Low QC 0.136 ng/mL	Mid QC 1.70 ng/mL	High QC 21.2 ng/mL
	0.157	1.81	21.3
	0.173	1.94	21.8
	0.153	1.82	20.0
	0.133	1.80	22.1
Mean	0.154	1.84	21.3
S.D.	0.0165	0.0655	0.927
%CV	10.7	3.6	4.4
%Bias	13.2	8.2	0.5
n	4	4	4