Evaluation of Matrix Effect when Matrix Factor is not Enough for LCMSMS Bioanalytical Method

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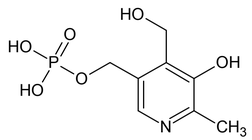
Introduction

Matrix effect must be evaluated for LCMSMS bioanalytical methods. EMA suggests to evaluate the matrix effect with the matrix factor procedure. Herein, two procedures are described using pyridoxine-5-phosphate (Pn5P) showing that matrix effect cannot be associated exclusively to ionic suppression or enhancement.



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Method

The matrix factor evaluation assesses the possible suppression/enhancement of the ionization of analytes by the presence of matrix components during MSMS analysis by comparing the response of several blank extracts reconstituted with a reference standard solution to the neat reference standard solution itself. The complementary matrix effect evaluation is done by analyzing low quality controls prepared in ten different lots of matrix. Our candidate, Pn5P, is extracted by acidic protein precipitation and analyzed by positive TurboIonSpray ionization using API5000. The matrix effect was evaluated using both procedures.

Extraction Procedure

- Internal Standard: Pyridoxal-5-phosphate-d_3
- Sample Volume: 75 µL of NaF/$K_2C_2O_4$ Human Plasma
- Extraction Type: Proteins precipitation
- Concentration factor: 0.5

LC-MS/MS Analysis

Chromatographic mode: Reversed Phase
Analytical Column: Atlantis dC18 75 x 4.6 mm
Column Temperature: Room Temperature
Elution mode: Isocratic
Mobile Phase A: Milli-Q Type Water / Acetonitrile (98/2), Ammonium formate 5mM, TFA 0.05%
Flow Rate: 1.0 mL/minute
Retention Times: 1.60 minutes
Acquisition time: 4.00 minutes
Detector: AB Sciex API 5000
Source: TurboIonSpray, Positive mode
Masses: 250→134 amu

Matrix Factor Evaluation Procedure

Extract Blank Samples
↓
Reconstitute with Neat Solution of Analyte and Internal Standard
↓
Compare Analyte Area and IS Area vs. Neat Solutions
↓
Calculate Analyte and IS Matrix Factor
↓
Calculate IS-Normalized Matrix Factor
↓
Calculate %CV (<15%)

Matrix Effect Evaluation Procedure

Spike 10 Lots of Matrix with Low Analyte Concentration
↓
Extract the Matrix Effect QCs in Triplicate
↓
Determine Analyte Concentration
↓
Calculate Accuracy
↓
Must be within 15% of the Nominal Concentration

Results

When the matrix effect was evaluated using the matrix factor procedure, the %CV of the IS-normalized matrix factor was 5.0% **(Table 1)**. No difference in analyte or IS responses was observed between the different lots. When the matrix effect was evaluated with matrix lots spiked at low quality control level and analyzed in triplicate, the mean accuracy for each lot ranged from 5.7 to 142.8% of the nominal concentration **(Table 2)**. This demonstrates that the matrix effect was not due to ionic suppression/enhancement but to sample extraction recovery variability depending on the matrix used.

Table 1. Matrix Factor Evaluation for Pyridoxine-5-Phosphate

Matrix Type	Untreated Standard (MFULOQ)		Reference Solution (RSULOQ)		Calculated Matrix Factor (Analyte)	Calculated Matrix Factor (IS)	IS-Normalized Matrix Factor
	Analyte Responses	Internal Standard Responses	Analyte Responses	Internal Standard Responses			
ME02	7767	110755	7339	106504	1.0771	1.0399	1.0357
ME03	7944	102612	7438	113498	1.1016	0.9635	1.1434
ME04	7671	111723	7078	111262	1.0638	1.0490	1.0141
ME05	7237	106453	7502	105326	1.0036	0.9995	1.0041
ME08	7975	110998	6956	102160	1.1059	1.0422	1.0612
ME10	7727	104112	6954	101560	1.0715	0.9775	1.0962
Mean			7211.1	106728.3			1.0591
SD(±)							0.0531
CV(%)							5.01

Table 2. Matrix Effect Evaluation for Pyridoxine-5-Phosphate

Sample	Nominal Concentration (ng/mL)	Concentration Found (ng/mL)	Accuracy %	Ratio	Analyte Area	IS Area
ME02	5.00	7.17	143.40	0.1462	13112	89678
ME02	5.00	7.27	145.40	0.1482	13520	91235
ME02	5.00	6.95	139.00	0.1419	12333	86909
MEAN		7.130	142.600	0.14543	12988.3	89274.0
SD		0.1637	3.2741	0.003219	603.09	2191.11
CV		2.30	2.30	2.21	4.64	2.45
ME03	5.00	3.22	64.40	0.0683	6657	97459
ME03	5.00	2.77	55.40	0.0594	5854	98487
ME03	5.00	2.80	56.00	0.0599	5624	93829
MEAN		2.930	58.600	0.06253	6045.0	96591.7
SD		0.2516	5.0319	0.005000	542.34	2447.13
CV		8.59	8.59	8.00	8.97	2.53
ME04	5.00	2.18	43.60	0.0478	4705	98399
ME04	5.00	1.99	39.80	0.0441	4327	98163
ME04	5.00	1.95	39.00	0.0432	3750	86837
MEAN		2.040	40.800	0.04503	4260.7	94466.3
SD		0.1229	2.4576	0.002438	480.94	6608.25
CV		6.02	6.02	5.41	11.29	7.00
ME05	5.00	2.75	55.00	0.0590	5580	94510
ME05	5.00	2.96	59.20	0.0632	5990	94764
ME05	5.00	2.85	57.00	0.0610	5526	90640
MEAN		2.853	57.067	0.06107	5698.7	93304.7
SD		0.1050	2.1008	0.002101	253.74	2311.16
CV		3.68	3.68	3.44	4.45	2.48
ME08	5.00	0.30	6.00	0.0107	1021	95664
ME08	5.00	0.44	8.80	0.0134	1299	97262
ME08	5.00	0.12	2.40	0.0071	670	93779
MEAN		0.287	5.733	0.01040	996.7	95568.3
SD		0.1604	3.2083	0.003161	315.21	1743.47
CV		55.89	55.96	30.39	31.63	1.82
ME10	5.00	1.73	34.60	0.0389	3631	93339
ME10	5.00	1.41	28.20	0.0327	3085	94443
ME10	5.00	1.38	27.60	0.0320	2945	92080
MEAN		1.507	30.133	0.03453	3220.3	93287.3
SD		0.1940	3.8799	0.003798	362.47	1182.35
CV		12.87	12.88	11.00	11.26	1.27

Chromatography

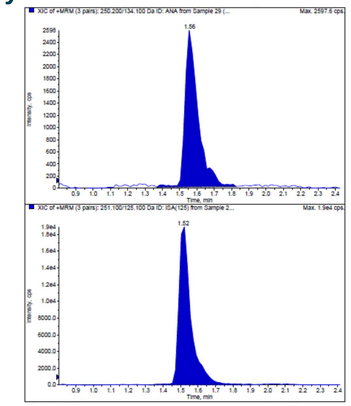

Figure 1. Representative Chromatogram of an Extracted Low Quality Control (QC1)

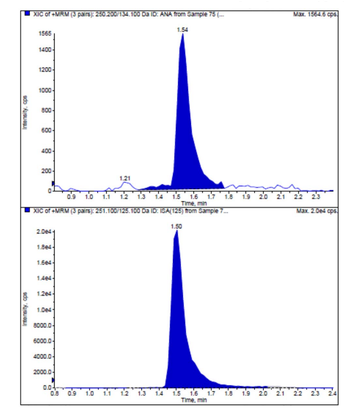

Figure 2. Representative Chromatogram of a Blank Extract reconstituted with Low Quality Control Reference Solution (MFQC1)

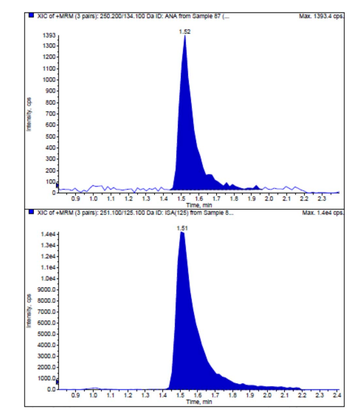

Figure 3. Representative Chromatogram of Low Quality Control Reference Solution (RSQC1)

Conclusion

Although the matrix factor test is suggested by the regulatory agencies, it would be strongly advisable to at least verify spiking different matrix lots during method development. In this case study, it was observed that quantitation might be affected by the matrix lot, the variability was not due to ionic suppression. This issue could not have been seen with the matrix factor procedure, but the simple spiking procedure in different matrix lots allowed for the development of a robust assay. The matrix effect issue was solved by changing the anticoagulant to EDTA K2.

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