Determination of Betaine, Choline, Carnitine, Dimethylethanolamine and Acetyl-choline in Finished Goods by UPLC/MS

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PURPOSE

Betaine/Choline/Carnitine/Dimethylethanolamine (DMEA)/Acetyl-choline (Fig. 1) are transporters, which are involved in many functions including memory and muscle control. To quantitate these transporters, an evaporative light scattering detector (ELSD) instead of traditional diode-array detector is commonly used since transporters have no double bonds and UV-Vis absorption. However, ELSD has limitations, such as low sensitivity, poor selectivity and non-linear response. In this presentation, we outline a sensitive, specific and robust quantitative method for transporters using UPLC-MS.

METHOD

Sample Preparation and Extraction:
About 2 g of sample was extracted by acetic acid, water and internal standard (IS). The extracted sample was cleaned by 0.20 µM filtration before analysis.

UPLC-MS Conditions
UPLC system: Waters ACQUITY UPLC H-Class core system including a quaternary solvent manager, sample management and column heater
Column: ACQUITY UPLC BEH C_{18} (Waters)
Mobile Phase A: HPBA and H_2O
Mobile Phase B: Acetonitrile
Flow rate: 0.40 mL/min
Pump Gradient Cycle time: 7.0 minutes
MS detector: QDA (Waters)
MS Parameters: Table 1

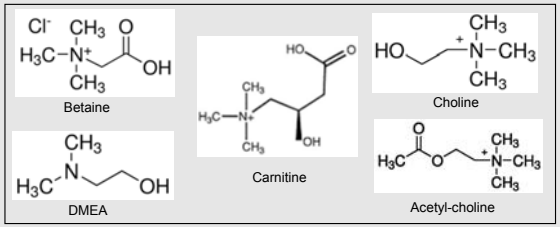

Figure 1: The Chemical Structure of Five Transporters.

MS CONDITIONS					
Mass Range (Da)	Capillary Voltage (V)		Probe Temperature (°C)		Gain
80.0-210.0	1.5		600		1.0
Compounds Parameters					
Analyte	SIM Mass (Da)	Retention Time (min)	IS	Cone Voltage (V)	Polarity
Betaine	118	1.2	Betaine-D9	15	Positive
Choline	104	1.5	Choline-D9	15	Positive
Carnitine	162	1.5	Choline-D9	15	Positive
DMAE	90	1.6	Choline-D9	15	Positive
Acetyl Choline	146	2.4	Choline-D9	15	Positive
Choline-D9 (IS)	113	1.5	N/A	15	Positive
Betaine-D9 (IS)	127	1.2	N/A	15	Positive

Table 1: UPLC/MS Conditions

RESULTS and DISCUSSION

During the method development stage, different columns and mobile phases were investigated. The results indicated that BEH C18 and HFBA/water/acetonitrile revealed ideal peak shape and sensitivity. Different potential IS were screened, and two stable labeled IS were selected for all five analytes. In order to eliminate the matrix effect, an extra 3 minutes of forward flush for each injection with high organic solvent was applied in LC gradient program. The method was successfully validated over the range of 1.00-10.0 µg/mL with the target concentration of sample preparation at 5.00 µg/mL. The specificity experiment showed that there was no contribution between analytes/IS and no visible interference peak showed in blank diluent at expected analyte retention times (Fig. 2). The LLOQ has sufficient sensitivity (S/N>10) (Fig. 3). System suitability consisted of six replicate injections of the middle standard solution and was injected before sample analysis, and RSD was ≤10% (Table 2). Quadratic regression with $1/X^2$ weighing factor provided the best fit, and the correlation coefficient r^2 is ≥0.995 (Fig. 4). The accuracy experiment showed that the spiking recovery is within ±20% (Table 3). The %RSD of precision and repeatability experiment was <10% (Table 4). Four days stability was established for both extracted sample and standard solution in ambient conditions protected from light.

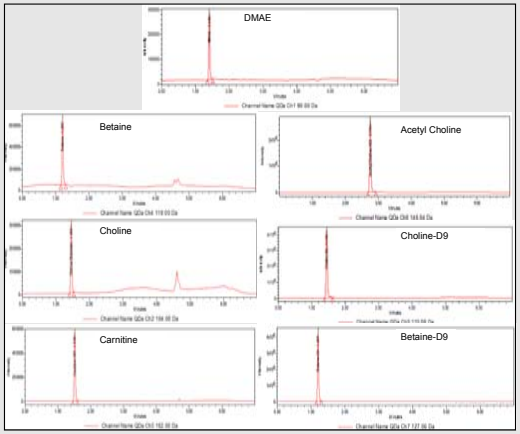

Figure 3: LLOQ Sample Chromatograms

Compound	Choline (Area)	Betaine (Area)	Carnitine (Area)	DMAE (Area)	Acetyl-choline (Area)
Replicate 1	4706363	8725271	6710747	2448160	6534087
Replicate 2	4863497	8858227	7154337	2625150	7058890
Replicate 3	5102429	9259073	7198337	2747349	7415177
Replicate 4	5043534	9098086	7228863	2677855	7528098
Replicate 5	5063835	9123960	7475731	2697444	7291131
Replicate 6	4676095	8821061	7187393	2530395	7315601
Average	4909292	8980946	7159235	2621059	7190497
Std Dev	188077	208577	248397	112375	357331
% RSD	**3.8**	**2.3**	**3.5**	**4.3**	**5.0**

Table 2: System suitability results

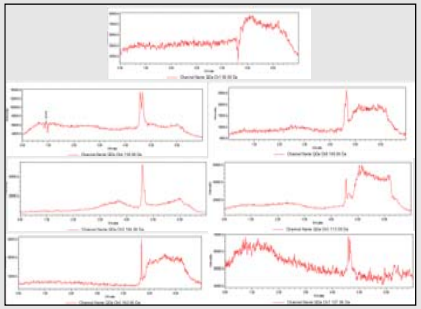

Figure 2: Blank diluent sample chromatograms

Analyte		QC Levels		
		Low QC (2.00 µg/mL)	Medium QC (5.00 µg/mL)	High QC (8.00 µg/mL)
Choline	Replicate 1	106	111	98.7
	Replicate 2	108	96.7	96.7
	Replicate 3	107	94.2	94.2
	Average	**107**	**101**	**96.5**
Betaine	Replicate 1	108	104	101
	Replicate 2	101	101	109
	Replicate 3	109	105	109
	Average	**106**	**103**	**106**
Carnitine	Replicate 1	128	115	93.3
	Replicate 2	132	107	79.9
	Replicate 3	126	117	83.5
	Average	**129**	**113**	**85.6**
DMAE	Replicate 1	79.3	91.8	98.7
	Replicate 2	102	84.9	96.7
	Replicate 3	87.6	98.8	94.2
	Average	89.8	91.8	96.5
Acetyl-Choline	Replicate 1	121	119	112
	Replicate 2	122	104	105
	Replicate 3	127	119	107
	Average	**123**	**114**	**108**

Table 3: Post Spiking Accuracy (n=3)

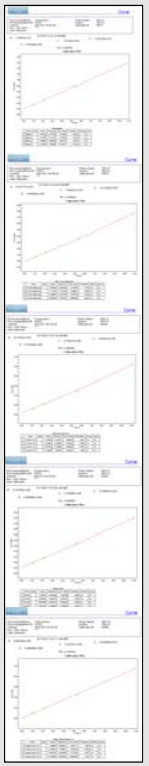

Figure 4: Calibration Curves

Analyte		Precision Medium QC (5.00 µg/mL)	Repeatability Medium QC (5.00 µg/mL)
Choline	Accuracy (%)	111	97.9
		96.7	106
		102	98.5
		101	98.9
		98.0	101
		99.8	105
	Average	101	101
	SD	5.0	4.1
	RSD%	**4.9**	**4.0**
	n	6	12
Betaine	Accuracy (%)	104	110
		101	106
		105	110
		107	107
		104	106
		106	106
	Average	104	106
	SD	1.9	2.5
	RSD%	**1.9**	**2.3**
	n	6	12
Carnitine	Accuracy (%)	115	112
		107	126
		117	116
		116	116
		108	117
		116	126
	Average	113	116
	SD	4.4	5.6
	RSD%	**3.9**	**4.8**
	n	6	12
DMAE	Accuracy (%)	91.8	78.3
		84.9	102
		98.8	85.1
		89.9	84.3
		79.4	88.9
		99.8	92.6
	Average	85.0	89.7
	SD	7.9	7.80
	RSD%	**9.3**	**8.70**
	n	6	12
Acetyl-Choline	Accuracy (%)	119	134
		104	146
		119	137
		122	134
		112	137
		120	150
	Average	116	128
	SD	6.70	14.1
	RSD%	5.80	11.0
	n	6	12

Table 4: Precision and Repeatability Results

CONCLUSIONS

This UPLC-MS assay for quantitation of transporters in finished goods has been successfully validated. The assay is sensitive, specific and robust for the analysis of transporters in finished goods.