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アプリケーションノート

Renatadx Screening System: Analytical Performance for Amino Acids, Free Carnitines and Acylcarnitines in Dried Blood Spots

Waters Corporation

For *in vitro* diagnostic use. Not available in all countries.

Introduction

The Waters RenataDX Screening System enables flowinjection analysis and quantification of organic compounds in biological matrices.

This document describes a test of the analytical performance of the RenataDX Screening System for the analysis of amino acids, free carnitines, and acylcarnitines in dried blood spots.

RenataDX Screening System.

Experimental

Extracted dried blood spot (DBS) control samples were analyzed with the RenataDX Screening System, under the control of MassLynx IVD Software (v4.2), with data processed using IonLynx Application Manager.

Sample Description

A single 3-mm diameter DBS punch was incubated in a methanol-based internal standard solution. After the incubation period, the samples were transferred from the extraction plate to a clean 96-well microtitre plate.

Flow-Injection Analysis Conditions

System tubing:	~1 meter PEEK (0.005" ID) with post injection valve

	inline filter (2 µm pore size)
Mobile phase A:	80% Acetonitrile$_{(aq)}$ with 0.05% (v/v) formic acid
3777C wash 1:	20% Methanol$_{(aq)}$
3777C wash 2:	80% Acetonitrile$_{(aq)}$ with 0.05% (v/v) formic acid
Flow rate:	Variable flow rate from 150 µL/min to 15 µL/min, with 500 µL/min flush

MS Conditions

Resolution:	MS1 (0.70 FWHM), MS2 (0.70 FWHM)
Acquisition mode:	MRM
Polarity:	ESI+

Results and Discussion

The imprecision of extraction and analysis of amino acids and acylcarnitines is illustrated in Tables 1 and 2. The Peak-to-Peak (PtP) Signal-to-Noise ratio (S/N) is shown, as an indication of the analytical sensitivity of the system.

Compound	Endogenous			QC1			QC2		
	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)
Glycine	218	13.7	18.5	605	11.3	5.19	1030	10.0	56.7
Alanine	229	10.2	101	851	9.72	652	928	7.08	156
Proline	81.1	11.7	71.1	314	9.07	178	669	7.24	200
Valine	54.2	9.92	157	239	9.36	593	405	7.60	468
Leucine	88.2	9.52	179	384	10.1	618	564	6.98	1232
Phenylalanine	33.4	9.95	106	179	9.47	798	506	6.54	1789
Citrulline	N/D	N/A	N/A	58.0	17.0	11.3	264	10.9	44.6
Tyrosine	34.5	9.40	19.9	191	9.00	269	513	7.70	671
Methionine	10.5	10.2	28.0	77.9	8.53	94.4	244	7.57	151
Arginine	N/D	N/A	N/A	34.9	10.4	21.6	123	7.24	103

Table 1. Performance characteristics of the amino acid analytes. Between-batch imprecision experiments were performed on five occasions (n=25); µM in whole blood, accounting for the dilution of the DBS material into the extraction solution; endogenous=DBS control from a single donor; QC1 and 2 of commercial origin; N/D=not detected i.e. imprecision ≥20%CV ± S/N (PtP)≤3; N/A=not applicable.

Compound	Endogenous			QC1			QC2		
	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)	Conc (µM)	%CV	S/N (PtP)
Free carnitine (C0)	10.9	10.9	79.8	38.9	10.7	199	99.9	7.91	756
Acetylcarntine (C2)	6.93	9.63	98.1	17.7	9.42	343	52.4	6.94	890
Propionylcarnitine (C3)	0.66	9.50	70.2	3.99	10.5	296	11.5	6.77	2376
Malonyl / Hydroxyvalerylcarnitine (C3DC/C4OH)	0.04	16.6	15.7	N/S	N/A	N/A	N/S	N/A	N/A
Butyrylcarnitine (C4)	0.07	11.8	14.3	0.72	8.91	106	3.32	6.22	867
Isovalerylcarnitine (C5)	0.04	11.6	8.44	0.40	11.5	63.9	1.81	8.31	106
Glutarylcarnitine (C5DC)	N/D	N/A	N/A	0.48	13.4	12.3	2.12	14.6	34.8
Methylmalonyl / Hydroxyisovalerylcarnitine (C4DC/C5OH)	0.37	9.72	106	0.15	10.1	21.7	0.22	8.51	49.4
Hexanoylcarnitine (C6)	N/D	N/A	N/A	0.41	11.7	176	2.07	7.52	407
Octanoylcarnitine (C8)	N/D	N/A	N/A	0.44	12.8	86.7	2.23	9.48	268
Decanoylcarnitine (C10)	N/D	N/A	N/A	0.40	15.7	386	2.00	11.9	654
Dodecanoylcarnitine (C12)	N/D	N/A	N/A	0.40	15.5	249	2.04	11.0	504
Tetradecanoylcarnitine (C14)	0.03	18.6	9.40	0.45	13.1	413	2.13	9.63	1025
Palmitoylcarnitine (C16)	0.68	13.3	387	4.08	11.3	248	12.3	8.30	816
Octadecenoylcarnitine (C18)	0.49	12.1	78.3	2.21	9.24	131	8.15	6.98	427

Table 2. Performance characteristics of the free carnitine and acylcarnitine analytes. Between-batch imprecision experiments were performed over five occasions (n=25); µM in whole blood, accounting for the dilution of the DBS material into the extraction solution; endogenous=DBS from a single donor; QC1 and 2 of commercial origin; N/S=not supplemented; N/A=not applicable; N/D=not detected, i.e. imprecision ≥20%CV ± S/N (PtP)≤3.

Conclusion

The Waters RenataDX Screening System has demonstrated the capability to measure a subset of amino acids, free carnitines, and acylcarnitines. The endogenous concentration of some analytes was at the limit of

detection of the RenataDX System.

Disclaimer

The analytical performance data presented here is for illustrative purposes only. Waters does not recommend or suggest analysis of the analytes described herein. These data are intended solely to demonstrate the performance capabilities of the system for analytes representative of those commonly analyzed using flow-injection analysis and tandem mass spectrometry. Performance in an individual laboratory may differ due to a number of factors, including laboratory methods, materials used, intra-operator technique, and system conditions. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the analytes in this analysis.

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