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RenataDX Screening System: Analytical Performance for Butyl Esters of Amino Acids, Free Carnitine, 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 butyl esters of amino acids, free carnitine, and acylcarnitines in dried blood spots.

RenataDX Screening System.

Experimental

Dried blood spot (DBS) control samples were extracted. Derivatized extracts from 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 to a new 96-well microtitre plate for derivatization with n-butanol HCl. Sample residue was reconstituted in mobile phase.

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	187	4.1	57.5	435	10.4	5070	824	5.8	864
Alanine	195	6.2	411	695	9.3	10700	856	6.5	1800
Proline	N/D	N/A	N/A	269	9.7	1880	614	6.4	756
Valine	70.8	6.5	113	279	9.5	672	523	6.5	1232
Leucine	67.4	6.6	479	299	10.0	7260	534	6.8	2000
Phenylalanine	30.0	7.2	384	152	9.1	6400	508	8.0	1860
Citrulline	10.8	6.3	609	57.5	8.5	30.2	272	6.0	3300
Tyrosine	145	2.6	116	291	5.6	1140	626	4.7	3290
Methionine	10.9	7.2	68.6	58.3	12.0	165	187	7.8	1170
Arginine	4.30	13.0	34.8	24.7	4.1	557	44.1	1.2	852

Table 1. Performance characteristics of the amino acid 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/D=not detected; 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)	11.8	6.4	281	39.5	10.1	862	117	8.5	1150
Acetylcarntine (C2)	9.5	5.7	197	24.1	9.3	2410	70.1	5.5	1870
Propionylcarnitine (C3)	0.77	7.2	78.7	4.43	9.9	796	14.3	7.2	674
Hydroxyvalerylcarnitine (C4OH)	0.04	16.4	2.49	N/S	N/A	N/A	N/S	N/A	N/A
Butyrylcarnitine (C4)	0.07	13.9	20.3	0.77	7.3	116	4.01	5.3	207
Isovalerylcarnitine (C5)	0.06	9.3	8.61	0.40	8.9	55.3	2.01	7.1	637
Glutarylcarnitine (C5DC)	N/D	N/A	N/A	0.43	10.5	17.4	2.30	6.5	143
Hydroxyisovalerylcarnitine (C5OH)	0.32	7.7	38.7	N/S	N/A	N/A	N/S	N/A	N/A
Hexanoylcarnitine (C6)	0.09	9.8	7.67	0.44	7.5	52.7	2.09	6.4	91.6
Octanoylcarnitine (C8)	N/D	N/A	N/A	0.39	12.8	80.4	2.28	8.5	428
Decanoylcarnitine (C10)	N/D	N/A	N/A	0.26	14.8	106	1.46	12.1	217
Dodecanoylcarnitine (C12)	N/D	N/A	N/A	0.39	13.7	52.2	2.29	13.5	668
Tetradecanoylcarnitine (C14)	N/D	N/A	N/A	0.41	11.7	164	2.23	11.4	342
Palmitoylcarnitine (C16)	0.70	9.3	160	4.17	11.3	394	14.1	8.8	2510
Octadecanoylcarnitine (C18)	0.55	8.6	79.4	2.46	9.0	617	10.4	8.1	516

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 butyl esters of

amino acids, free carnitine, and acylcarnitines. The endogenous concentration of arginine was near the limit of detection of the RenataDX Screening 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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