PO-CON1531E

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Integration of amino acid, acylcarnitine and steroids analysis in single FIA/LC-MS/MS platform

ASMS 2015 ThP-251

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Introduction

Analysis of amino acids (AA) and acylcarnitines (AC) in dried blood spot (DBS) sample collection method by flow injection analysis (FIA) is now widely used. On the other hand, traditionally, analysis of steroid such as 17-hydroxyprogesterone is done by immunoassays but LC/MS/MS will be an attractive analytical alternative because it can also screen for other related steroids.

The use of LC/MS/MS results in a reduction of false positives and a more accurate quantitative performance. The requirements against steroid analysis by LC/MS/MS are getting more stringent issues. In this study, we present a strategy for performing both AA/AC and steroids analysis within a single LC/MS/MS platform.

Methods and Materials

The experimental setup designed to combine a FIA measurement covers 8 AAs and 17 ACs and a LCMS measurement for 5 steroids includes cortisol, 21-deoxycortisol (21-DOF), 11-deoxycortisol (11-DOF), androstenedione (4-AD) and 17-hydroxyprogesterone (17-OHP) with 2 position 6 port high pressure valve.

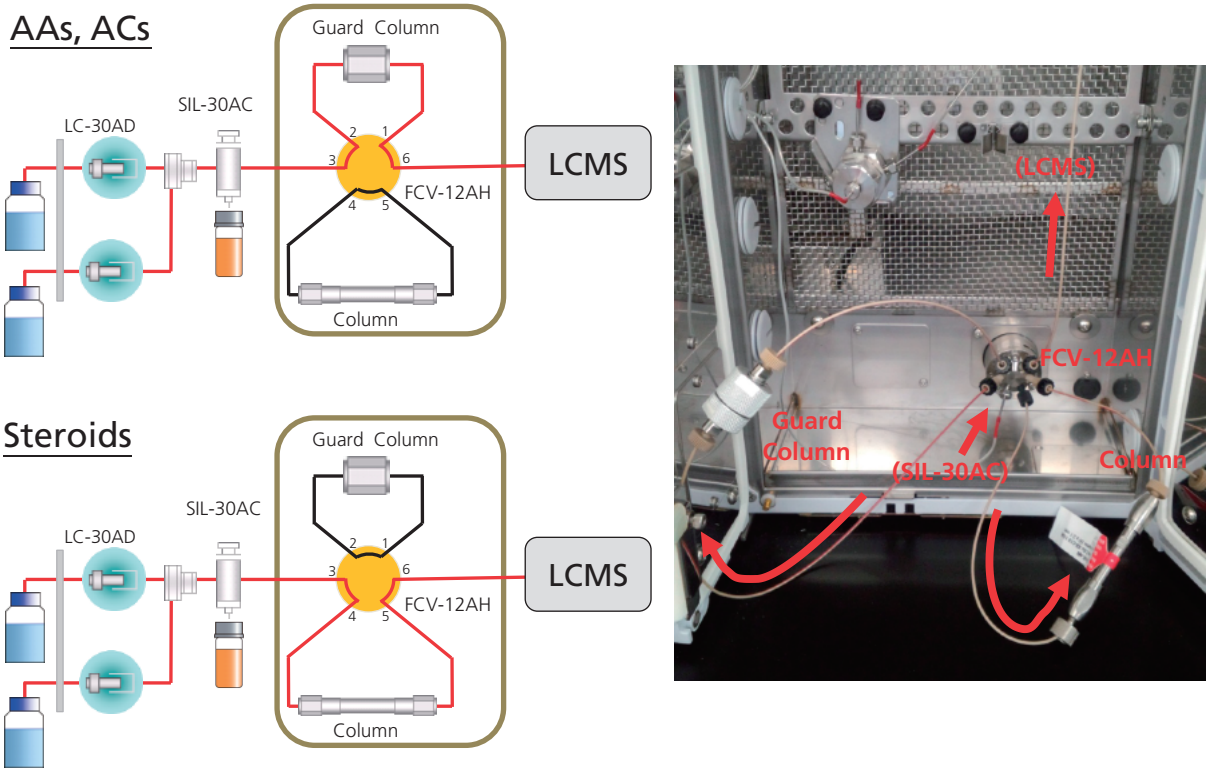

Figure 1 Flow Diagram of FIA/LC-MS/MS system

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The isotopically labeled internal standards for amino acids, acylcarnitines, and steroids were purchased from Cambridge Isotope Laboratories, Inc. Quality control materials were obtained from the Newborn Screening Quality Assurance Program at the Centers for Disease Control and Prevention (CDC).

Analytical Conditions

HPLC

Mobile Phase A : 0.1% Formic acid - water
Mobile Phase B : Methanol
Column temperature : 40 °C

[for Amino Acids and Acylcarnitines]

Guard Column : GL Sciences Cartridge Guard Column E (10 mmL x 1.5 mm I.D.)
Gradient Program :

Time	B conc. (%)	Flow rate (mL min)
0	90	0.13
0.65	90	0.13
0.66	90	0.7
1.00	90	0.7

Injection Volume : 1 µL

[for Steroids]

Column : Phenomenex Kinetex 2.6u XB-C18 (50 mmL x 2.1 mm I.D., 2.6µm)
Flow Rate : 0.3 mL/min
Gradient Program :

Time	B conc. (%)
0	50
0.5	55
1.5	55
3.0	90
5.0	90

Injection Volume : 10 µL

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Mass (LCMS-8050 triple quadrupole mass spectrometry)

Ionization	: heated ESI		
Nebulizing Gas Flow	: 3 L / min	Drying Gas Pressure	: 10 L / min
Heating gas flow	: 10 L/min	DL Temperature	: 250 °C
BH Temperature	: 500 °C	Interface Temperature	: 400 °C
MRM parameter	:		

Target	Q1>Q3		IS	Q1>Q3
Phe	166.10>120.10	-	Phe IS	172.10>126.10
Leu	132.10>86.10	-	Leu IS	135.10>89.10
Met	150.10>104.10	-	Met IS	153.10>107.10
Tyr	182.10>136.10	-	Tyr IS	188.10>142.10
Val	118.10>72.10	-	Val IS	126.10>80.10
Cit	176.10>113.10	-	Cit IS	178.10>115.10
Arg	175.10>70.10	-	Arg IS	180.10>75.10
Ala	90.00>44.00	-	Ala IS	94.00>48.00
C0	162.10>103.00	-	C0 IS	171.10>103.00
C2	204.10>85.00	-	C2 IS	207.10>85.00
C3	218.10>85.00	-	C3 IS	221.10>85.00
C4	232.20>85.00	-	C4 IS	235.20>85.00
C4OH	248.20>85.00			

Target	Q1>Q3		IS	Q1>Q3
C5	246.20>85.00	-	C5 IS	255.20>85.00
C5DC	276.10>85.00	-	C5DC IS	279.10>85.00
C5OH	262.20>85.00	-	C5OH IS	265.20>85.00
C6	260.20>85.00	-	C8 IS	291.20>85.00
C8	288.20>85.00			
C10	316.20>85.00	-	C12 IS	347.30>85.00
C12	344.30>85.00			
C14	372.30>85.00	-	C14 IS	381.30>85.00
C16	400.30>85.00	-	C16 IS	403.30>85.00
C16OH	416.30>85.00			
C18	428.40>85.00	-	C18 IS	431.40>85.00
C18OH	444.40>85.00			

	R.T(min)	Q1>Q3	CE (V)
Cortisol	1.50	363.15>121.10	-31
		363.15>327.10	-17
21-DOF	1.85	347.15>311.15	-15
		347.15>121.10	-28
11-DOF	2.20	347.15>109.10	-31
		347.15>97.05	-27
4-AD	2.65	287.15>97.05	-24
		287.15>109.10	-24
17-OHP	3.02	331.15>109.05	-29
		331.15>97.10	-30

	R.T(min)	Q1>Q3	CE (V)
Cortisol-D2	1.50	365.15>122.10	-27
		365.15>329.10	-18
21-DOF-D8	1.82	355.20>319.25	-18
		355.20>46.10	-45
11-DOF-D2	2.23	349.20>109.05	-33
		349.20>97.10	-29
4-AD-13C3	2.65	290.15>100.00	-22
		290.15>112.00	-28
17-OHP-D8	3.00	339.20>100.10	-27
		339.20>113.10	-31

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DBS samples (d = 5mm) were placed in 96-well plates, and AAs, ACs and steroids were extracted with 180 µL of 80% acetonitrile-water solution consists of the known concentrations of stable isotope labeled standards of each compounds. The extraction were performed in an ultrasonic bath for 30 min. Samples were measured using a Nexera UHPLC system coupled to LCMS-8050 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan).

Result

DBS provides a number of advantages, for examples, a less invasive and much simpler sample collection method rather than venipuncture technique. Furthermore, it provides you simpler storage and transportation as well as it can lower the infection risk of various pathogens, and requires a smaller blood volume. To date, DBS-LC-MS/MS has emerged as an important method for quantitative analysis of small molecules.
Previously we developed an innovative AAs and ACs screening method makes it possible to inject just 1 µl of sample and successfully reduce analytical run time as fast as 74 seconds (conventional method >120 sec.) using the combination of Nexera MP and LCMS-8040 (Shimadzu Corporation, Japan). In addition to that, we independently developed a method for steroids in DBS. Steroids were separated on a Phenomenex kinetex XB-C18 (50x2mm, 2.6µm) at a column temperature of 40 °C for 5 min.
In this study, we present a strategy for performing AAs, ACs and steroids analysis within a single LC/MS/MS platform. AAs, ACs and steroids were extracted from only one dried blood spot. This system enables to automatically analyze 7 min in all target analytes in 2 injections.

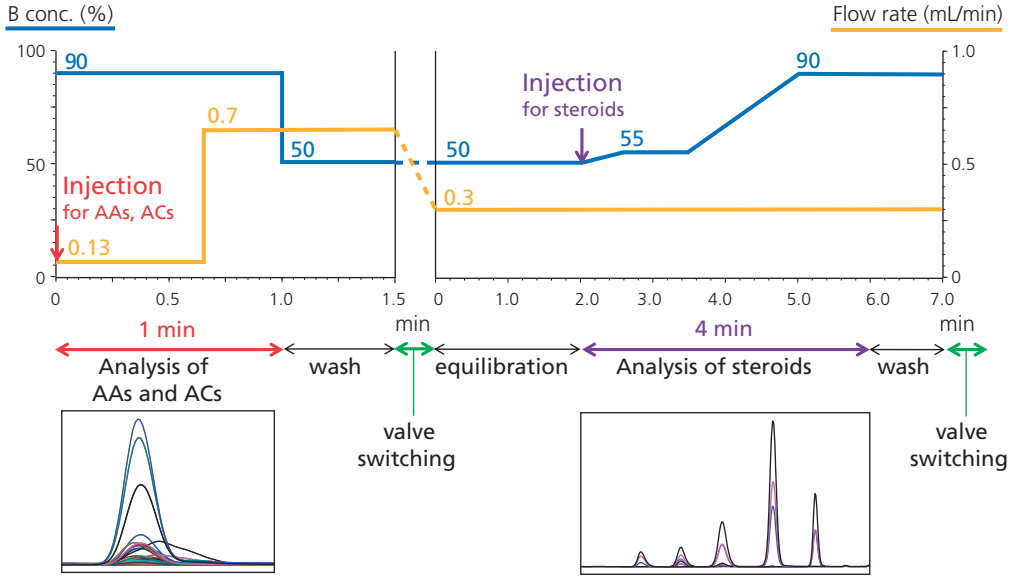

Figure 2 Time Program of amino acids, acylcarnitines and steroids analysis

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Table 1 Data Summary of 8 amino acids, 17 acylcarnitines and 5 steroids

	Phe	Leu	Met	Tyr	Val	Cit	Arg	Ala		C0	C2	C3	C4
No.1321	65.3	172.91	17.57	54.12	132.99	32.19	14.33	192.19	No.1361	22.25	14.65	1.43	0.1
CV	1%	5%	9%	1%	4%	4%	9%	3%	CV	4%	9%	2%	11%
Target	66.1	131.6	15.9	49.1	127.2	26.2	15	179.1	Target	17	12.4	1.2	0.1
No.1322	166.28	291.55	58.11	236.31	315.48	55.37	100.26	295.09	No.1362	32.23	25.4	4.75	0.97
CV	1%	4%	8%	2%	1%	3%	2%	3%	CV	1%	9%	4%	10%
Target	157	221.2	52.2	211	262.2	52.5	97.4	261	Target	29	21.5	4	0.9
No.1323	253.12	418.99	141.41	415.87	438.18	127.41	192.38	391.76	No.1363	47.27	32.72	9.78	2.28
CV	1%	1%	9%	5%	1%	4%	1%	5%	CV	4%	4%	6%	8%
Target	245.1	345.3	123.8	375.5	360.2	118.7	184.2	348.6	Target	40.1	30.5	8	2.2
No.1324	319.28	598.09	221.83	554.31	500.46	254.09	269.5	441.5	No.1364	62.3	48.82	16.36	5.05
CV	0%	3%	5%	2%	1%	1%	0%	5%	CV	4%	5%	5%	7%
Target	330.5	552.8	192.5	516.9	464.4	237.2	260.5	422.2	target	55.6	40.6	13.2	4.4

	C4OH	C5	C5DC	C5OH	C6	C8	C10	C12	C14	C16	C16OH	C18	C18OH
No.1361	0.05	0.1	0.12	0.72	0	0.02	0.02	0.01	0.05	0.78	0.01	0.57	0
CV	17%	19%	14%	2%	-	31%	35%	24%	15%	2%	-	1%	-
Target	0.1	0.1	0	0.6	0	0	0	0	0.1	0.8	0	0.6	0
No.1362	0.28	0.48	0.51	1.09	0.45	0.49	0.49	0.42	0.46	3.14	0.08	1.23	0.07
CV	11%	4%	7%	5%	7%	2%	4%	4%	1%	2%	9%	2%	3%
Target	0.4	0.5	0.5	1	0.4	0.5	0.5	0.4	0.5	3.5	0.1	1.5	0.1
No.1363	0.53	1.51	0.93	2.11	0.93	1.04	1	0.87	1.44	7.23	0.36	2.08	0.33
CV	10%	4%	8%	4%	6%	2%	1%	1%	2%	2%	6%	1%	2%
Target	0.7	1.3	1	1.8	0.8	1	1	0.9	1.4	7.2	0.4	2.2	0.3
No.1364	1.42	2.79	2.45	3.07	2.37	2.54	2.5	2.1	2.83	10.41	0.72	4.49	0.68
CV	8%	4%	3%	6%	2%	1%	2%	0%	2%	1%	1%	0%	4%
Target	1.6	2.7	2.4	2.7	1.9	2.4	2.4	2	2.6	10.5	0.7	4.8	0.7

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	Conc. (ng/mL)	Area	%RSD	S/N	LOD (ng/mL)	LOQ (ng/mL)
Cortisol	5	15,919	6.05	66.1	0.23	0.76
	25	54,064	2.11	246.3		
	75	186,970	0.85	681.0		
	125	322,782	1.68	971.9		
	150	345,172	0.26	994.9		
	500	1,307,317	0.16	1606.4		

	Conc. (ng/mL)	Area	%RSD	S/N	LOD (ng/mL)	LOQ (ng/mL)
4-AD	5	97,910	0.62	184.1	0.08	0.27
	25	387,155	0.92	946.5		
	75	1,247,236	0.08	2338.6		
	125	2,187,611	0.26	5318.0		
	150	2,406,566	0.36	4459.4		
	500	9,143,414	0.69	36564.1		

	Conc. (ng/mL)	Area	%RSD	S/N	LOD (ng/mL)	LOQ (ng/mL)
21-DOF	5	23,687	1.87	27.8	0.54	1.80
	25	98,627	3.64	103.0		
	75	388,221	2.04	473.1		
	125	701,690	1.16	181.9		
	150	758,860	0.43	537.1		
	500	3,000,289	0.70	214.5		

	Conc. (ng/mL)	Area	%RSD	S/N	LOD (ng/mL)	LOQ (ng/mL)
17-OHP	5	40,107	6.12	79.3	0.19	0.63
	25	157,773	2.85	366.5		
	75	504,027	1.03	830.9		
	125	874,209	1.56	3824.8		
	150	951,763	0.62	1635.5		
	500	3,694,638	0.20	1932.9		

	Conc. (ng/mL)	Area	%RSD	S/N	LOD (ng/mL)	LOQ (ng/mL)
11-DOF	5	39,161	1.53	60.5	0.25	0.83
	25	167,525	2.43	218.1		
	75	534,404	0.39	436.3		
	125	964,525	0.26	94.2		
	150	1,029,424	0.53	695.0		
	500	3,872,397	0.60	97.9		

Conclusions

This platform is an effective tool for an initial screening and able to minimize sample consumption down to 1 uL. However, the use of a high performance LC/MS/MS system is highly recommended in order to achieve the appropriate level of sensitivity for the steroids especially. Using high performance LC/MS/MS (LCMS-8050, Shimadzu Corporation, Japan), the observed limit of detection (LOD) for the analysis of 17-hydroxyprogesterone was 0.19 ng/mL.

First Edition: May, 2015

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