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Oxysterol, Secosterols, and Cholesterol Intermediates Separation and Quantitation Using Triple Quadrupole Mass Spectrometry

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Novel Aspects

Using dual ion source with the benefit of APCI and ESI to achieve quantitation of oxysterol related compounds in QqQ-MS.

Introduction

Monitoring oxysterol levels has become increasingly popular, as potential links between oxysterol levels and certain neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis) as well as various types of cancers are being explored. To meet the increasing research demand, a LCMS oxysterol separation and quantitation method was developed using a Shimadzu LCMS-8060 liquid chromatography triple quadrupole mass spectrometer.

Methods

Oxysterol standards were obtained in methanol and diluted with 10:90 H2O:MeOH to working concentrations. Standards included the compounds indicated in Table 1. Human serum extract sample was obtained through alkaline hydrolysis of lipid esters followed by solid phase extraction.[1]

Table 1. Oxysterol Related Compounds

Compound name21	Abbreviation
24 (S)-hydroxycholesterol	24HC
(D7)22-hydroxycholesterol	22HC(d7)
25-hydroxycholesterol	25HC
27-hydroxycholesterol	27HC
(D7)7α-hydroxycholesterol	7αHC(d7)
7α-hydroxycholesterol	7αHC
7β-hydroxycholesterol	7βHC
7-Ketocholesterol	7KC
(D7) 7-Ketocholesterol	7KC(d7)
7α-hydroxycholestenone	7αHCn
(D3) Vitamin D3	VitD3(d3)
Zymosterol	Zymo
Desmosterol	Desmo
7α,27 dihydroxycholestenone	7α,27diHC,3one
Cholesterol	CH
7dehydrocholesterol	7DHC

Results

MRM Transitions of oxysterol related compounds

MRM transitions were developed and optimized on the triple quadrupole mass spectrometer using DUIS (Dual Ion Source), APCI, and ESI sources. In cases where two precursor ions showed a strong signal, MRM development and optimization were done on both precursor ions. This may provide an option for better sensitivity in different matrices for future applications.

Compound Name	Retention time (min)	DUIS		APCI		ESI	
		Target MRM	Reference MRM	Target MRM	Reference MRM	Target MRM	Reference MRM
24HC	4.0	385.45>367.30	385.45>324.30	367.35>91.05	367.35>104.85	385.10>367.25	385.10>109.00
24HC	4.0	367.30>281.10	367.30>104.90	385.40>367.30	385.40>104.90	367.45>95.15	367.45>105.25
22HC(d7)	4.2	374.40>91.10	374.40>255.25	374.40>104.80	374.40>133.15	374.20>104.90	374.20>132.95
25HC	4.1	385.30>367.40	385.30>324.25	367.35>95.00	367.35>135.10	367.35>105.15	367.35>147.15
25HC	4.1	367.35>81.15	367.35>105.10	385.35>367.30	385.35>133.10	385.20>367.30	385.20>324.45
27HC	4.5	385.05>67.15	385.05>93.10	385.10>135.10	385.10>149.10	385.15>95.05	385.15>324.35
7αHC(d7)	7.6	374.35>159.15	374.35>91.15	374.40>145.30	374.40>159.25	374.20>158.95	374.20>144.80
7αHC	7.7	367.35>117.10	367.35>66.90	367.45>144.95	367.45>95.00	367.15>145.40	367.15>159.35
7αHC	7.7	385.30>367.40	385.30>367.40	385.10>367.25	385.10>159.10	385.45>367.35	385.45>159.05
7βHC	7.9	385.30>367.30	385.30>324.35	367.15>159.10	367.15>145.30	367.10>95.15	367.10>158.95
7βHC	7.9	367.35>81.10	367.35>104.85	385.10>367.30	385.10>159.00	385.10>367.15	385.10>158.95
7KC	8.3	401.15>95.10	401.15>383.30	401.10>80.95	401.10>383.35	401.45>95.30	401.45>383.30
7KC(d7)	8.1	408.30>390.15	408.30>95.15	408.40>81.15	408.40>95.25	408.40>390.25	408.40>95.05
7αHCn	6.5	401.25>383.30	401.25>97.10	401.35>383.45	401.35>97.10	401.10>382.95	401.10>96.90
VitD3(d3)	3.0	386.25>232.20	386.25>368.45	386.40>368.35	386.40>92.90	386.40>368.30	386.40>95.20
Zymo	12.8	367.30>95.00	367.30>80.90	367.15>95.20	367.15>109.20	367.15>95.10	367.15>80.95
Desmo	13.3	367.25>95.05	367.25>95.05	367.35>135.25	367.35>104.90	367.15>95.00	367.15>104.95
7α,27diHC,3one	2.2	417.25>399.10	417.25>381.30	417.30>399.25	417.30>381.45	417.35>399.30	417.35>97.25
CH	17.4	369.30>161.10	369.30>94.90	369.40>95.20	369.40>146.95	369.40>147.25	369.40>135.25
7DHC	14.5	367.25>95.05	367.25>158.90	367.30>145.25	367.30>159.25	367.15>145.10	367.15>159.20

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Reversed Phase Liquid Chromatography

Chromatographic separation was accomplished using a Shimadzu Nexera UHPLC system with a Shim-pack XR-ODS III column (2.0 mm i.d. x 200 mm; 2.2 µm). Challenging separations of 24HC and 25HC as well as 7αHC and 7βHC were achieved to allow individual quantitation of these analytes.

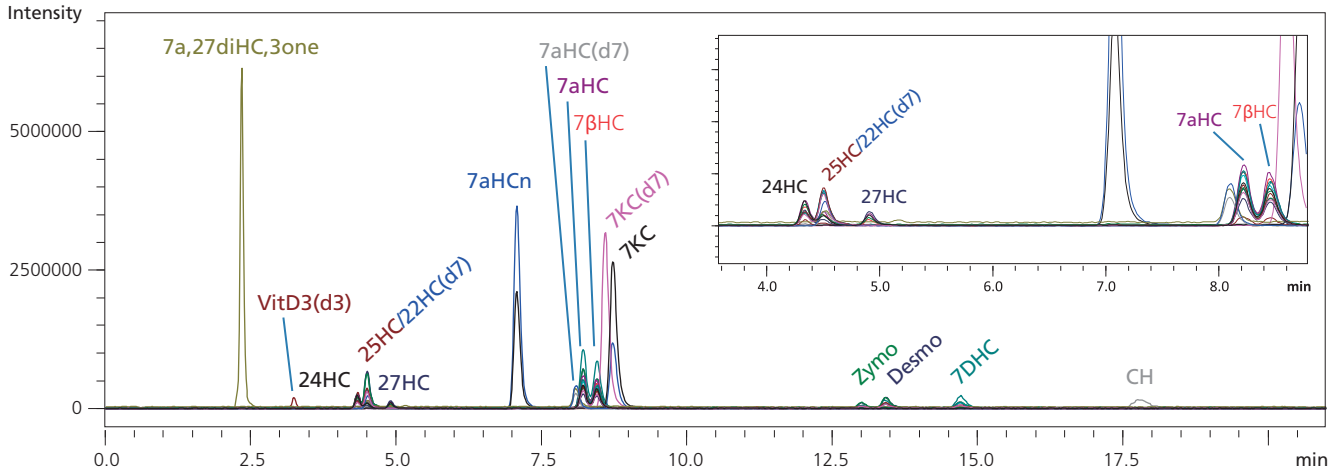

Figure 1. Chromatogram of the 16 of Oxysterol, Secosterols, and Cholesterol Intermediates.

Detection and Quantitation Limits

Detection limits determined using ESI, APCI, and DUIS sources are shown below. Limits of detection (LOD) and limits of quantitation (LOQ) were determined by a signal to noise ratio of 3:1 and 10:1, respectively. Values shown are picograms on-column.

	ESI		APCI		DUIS	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
24HC	20	30	30	50	20	50
22HC(d7)	10	20	30	50	10	20
25HC	20	30	50	100	20	30
27HC	20	50	50	50	20	100
7αHC(d7)	20	20	10	10	10	10
7αHC	4	10	10	10	10	20
7βHC	10	10	10	30	10	20
7KC	1	4	4	10	1	4
7KC(d7)	0.5	2	2	4	0.5	2
7αHCn	0.5	4	4	4	1	4
VitD3(d3)	30	300	50	50	20	30
Zymo	100	>2000	30	300	300	300
Desmo	300	>2000	30	50	100	300
7α,27diHC,3one	0.25	1	2	4	0.5	2
CH	100	1000	100	500	100	500
7DHC	100	300	100	300	100	500

Although ESI was able to obtain low detection limits for most oxysterol related compounds, it had difficulty reaching practical quantitation limits for zymosterol and desmosterol, even in neat standards. APCI allowed better quantitation for these two compounds, however, the LODs and LOQs for many other compounds were sacrificed. The Dual Ion Source, which adds simultaneous APCI voltage to standard ESI hardware, allows quantitation of zymosterol and desmosterol without significantly sacrificing the LODs of other compounds. Therefore, DUIS is the optimal source for analyzing this oxysterol related mixture. This combination source is able to quantify all sixteen analytes in a single run instead of two runs using ESI and APCI sources separately.

Human Serum Sample Analysis

Human serum samples (DC TROL) were analyzed using the LCMS method. %CV of all compounds from both samples were under 10% showing the stability of the system and the method.

	DC TROL Level 1			DC TROL Level 2		
	Average (ng/mL)	Std	%CV	Average (ng/mL)	Std	%CV
24HC	23.2	1.7	7.3	9.0	0.4	4.8
22HC(d7)	45.2	1.5	3.2	44.1	1.5	3.3
25HC	25.3	0.2	0.9	4.2	0.2	4.9
27HC	89.5	3.2	3.6	62.4	3.2	5.2
7αHC(d7)	174.5	10.4	5.9	164.7	3.8	2.3
7αHC	396.0	15.4	3.9	212.1	11.3	5.3
7βHC	838.3	36.4	4.3	249.4	8.7	3.5
7KC	295.7	11.0	3.7	69.3	0.9	1.4
7KC(d7)	113.3	3.8	3.4	116.7	4.2	3.6
7αHCn	3.0	0.1	5.0	2.7	0.2	6.0
VitD3(d3)	69.5	7.2	10.4	64.6	3.6	5.6
Zymo	337.5	11.1	3.3	103.3	5.1	5.0
Desmo	218.5	7.8	3.6	220.5	21.6	9.8
7α,27diHC,3one	7.9	0.2	2.4	7.7	0.1	1.2
7DHC	957.8	81.4	8.5	298.0	7.1	2.4

Reference

1. Narayanaswamy R, Iyer V, Khare P, Bodziak ML, Badgett D, Zivadinov R, Weinstock-Guttman B, Rideout TC, Ramanathan M, Browne RW. Simultaneous Determination of Oxysterols, Cholesterol and 25-Hydroxy-Vitamin D3 in Human Plasma by LC-UV-MS. *PloS one*. 2015. *10(4)*:e0123771

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Conclusion

MRM transitions for sixteen Oxysterol, Secosterols, and Cholesterol Intermediates were identified and optimized using the LCMS-8060. A UHPLC column was used to obtain separation. 24HC, 25HC, 7αHC, and 7βHC were chromatographically separated in a manner sufficient for individual quantitation. The total run time for this method was 21 minutes. Limits of detection and limits of quantitation for oxysterol related compounds were determined using APCI, ESI, and DUIS sources. Although LOD and LOQ vary per compound, LCMS-8060 was able to reach detection limits in the low picogram (mass on-column) range for oxysterol related compounds. DUIS was shown to be the optimal source for this oxysterol related compound mixture analysis. It demonstrates the advantages of both ESI and APCI and allows all sixteen compounds to be analyzed in a single run. The low %CV of each compound in human serum sample displays the precision of the method.

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