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Application News
SSI-LCMS-082

Liquid Chromatography Mass Spectrometry

Separation and Quantitation of Oxysterol Compounds Using LC-MS/MS

Liquid Chromatograph Mass Spectrometer

LCMS-8060

Summary: A mixture of 16 oxysterols and cholesterol related compounds were separated using a Shimadzu Nexera UHPLC and analyzed by a Shimadzu LCMS-8060 system. Detection and quantitation limits were obtained using APCI, ESI, and Dual Ion (DUIS) sources.

Background: There is high demand for oxysterol quantitation due to their correlation with neurodegenerative diseases. The ratios of various oxysterols in biological fluids are used by researchers to study disease states. An LCMS oxysterol quantitation method was developed using a Shimadzu LCMS-8060. Detection and quantitation limits were determined using multiple reaction monitoring (MRM) mode for each analyte.

Method: Oxysterol standards were obtained in methanol and diluted with 10:90 H_2O:MeOH. Standards included the compounds indicated in Table 1. An MRM method was determined and optimized using LabSolutions 5.82 on a Shimadzu triple quadrupole mass spectrometer (LCMS-8060). A Shim-pack XR-ODS III column was used for the separation. Injection volume was 2 µL with autosampler temperature being set at 10°C. Flow rate was initially at 0.4 mL/min and adjusted to 0.7 mL/min toward the end of the run to speed the elution of highly retained compounds.

Table 1. Oxysterol Related Compounds

Compound name	Abbreviation
24 (S)-hydroxycholesterol	24HC
(D7)22-hydroscycholesterol	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

MRM transitions were developed and optimized on the triple quadrupole mass spectrometer using DUIS, 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.

The DUIS method established nebulizing, heating, and drying gas flows at 3, 17, and 3 L/min. Interface, desolvation line, and heat block temperatures were set at 300°C, 250°C, and 400°C, respectively. With the APCI source, temperatures for the interface, desolvation line, and heat block were set at 350°C, 200°C, and 200°C, respectively. Drying gas flow rate was 5 L/min. For ESI, nebulizing, heating, and drying gas flows were set at 3, 10, and 10 L/min. Interface, desolvation line, and heat block temperatures were 300°C, 250°C, and 400°C.

Table 2. MRM transitions of oxysterol related compounds

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

Multiple MRM transitions were determined from each precursor ion, the two most intense are shown.

Chromatographic separation was accomplished using a Shimadzu Nexera UHPLC system with a Shim-pack XR-ODS III column. Challenging separations of 24HC and 25HC as well as 7αHC and 7βHC were achieved to allow individual quantitation of these analytes. Maximum operating pressure for a typical run was about 11,000 psi.

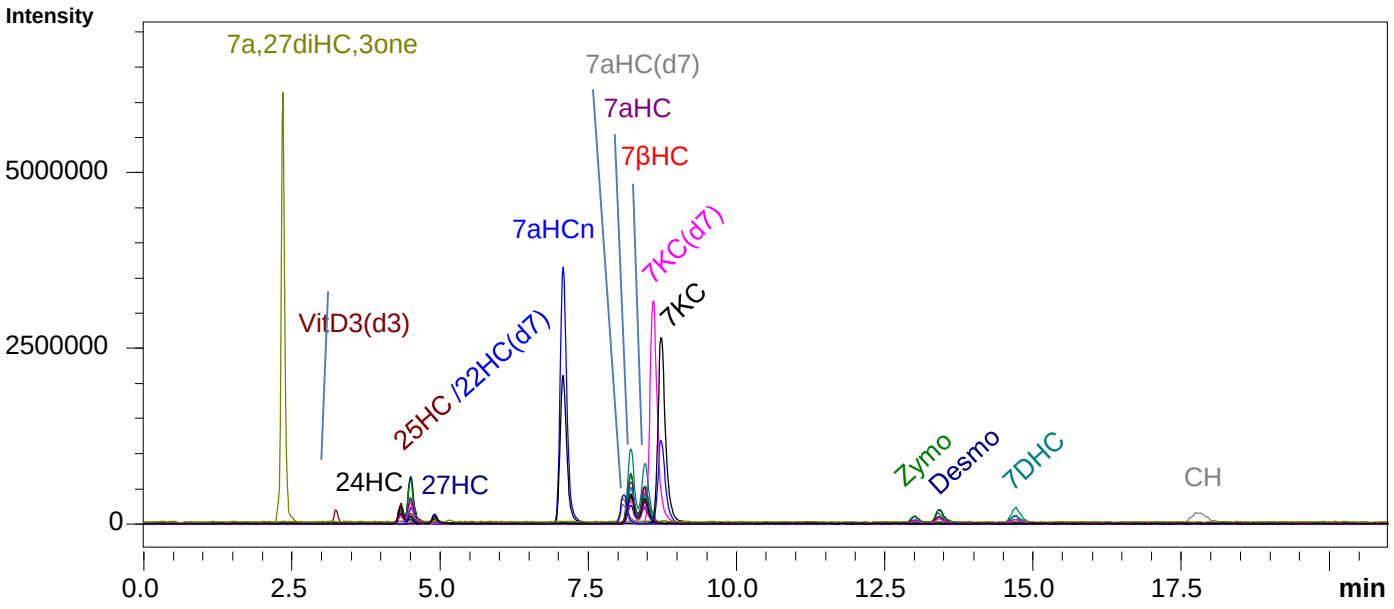

Figure 1. Chromatogram of the 16 oxysterol related compounds.

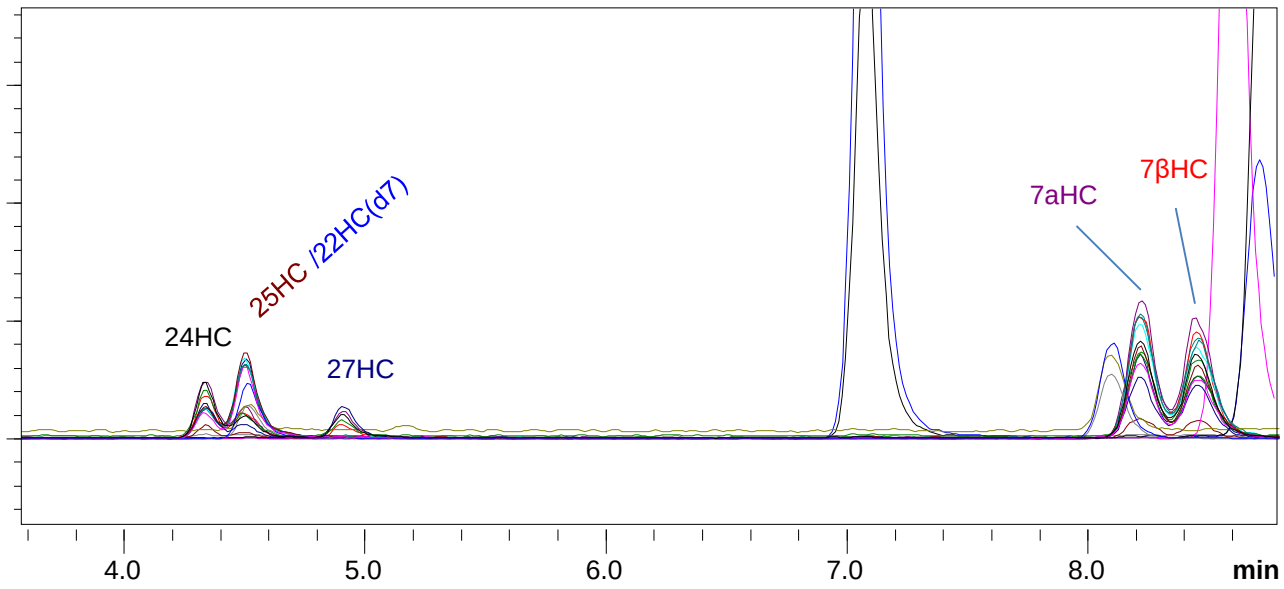

Figure 2. Chromatogram showing separation of key components.

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.

Table 3. LODs and LOQs determined by LCMS-8060 with ESI, APCI, and DUIS sources. Values shown are picograms on-column. Green highlighting indicates lower or lowest limits. Yellow highlighting indicates those limits which are somewhat higher than the lowest limits. Red highlighting indicates the highest limits.

	ESI		APCI		DUIS	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
24 (S)-hydroxycholesterol	20	30	30	50	20	50
(D7) 22-hydroscycholesterol	10	20	30	50	10	20
25-hydroxycholesterol	20	30	50	100	20	30
27-hydroxycholesterol	20	50	50	50	20	100
(D7) 7a-hydroxycholesterol	20	20	10	10	10	10
7a-hydroxycholesterol	4	10	10	10	10	20
7b-hydroxycholesterol	10	10	10	30	10	20
7-Ketocholesterol	1	4	4	10	1	4
(D7) 7-Ketocholesterol	0.5	2	2	4	0.5	2
7a-hydroxycholestenone	0.5	4	4	4	1	4
(D3) Vitamin D3	30	300	50	50	20	30
Zymosterol	100	>2000	30	300	300	300
Desmosterol	300	>2000	30	50	100	300
7a,27 dihydroxycholestenone	0.25	1	2	4	0.5	2
Cholesterol	100	1000	100	500	100	500
7dehydrocholesterol	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. DUIS offers the benefit of both APCI and ESI by allowing 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. The dual ion DUIS source is able to quantify all sixteen analytes in a single run instead of two runs using ESI and APCI sources separately.

In the case of (D3) Vitamin D3, detection and quantitation was even lower using DUIS vs. APCI and ESI. This is likely the result of probe position optimization, which is possible with DUIS (and ESI) but not with APCI.

Results and Discussion: MRM transitions for sixteen oxysterol related compounds 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.

Conclusion: A fast and sensitive method using a triple quadrupole mass spectrometer was developed to assist future applications for oxysterol related compound quantitation.

ULTRA FAST MASS SPECTROMETRY

LCMS-8030 LCMS-8040 LCMS-8050 LCMS-8060 LCMS-2020 LCMS-IT-TOF

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Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS, INC.
Applications Laboratory
7102 Riverwood Drive, Columbia, MD 21045
Phone: 800-477-1227 Fax: 410-381-1222
URL http://www.ssi.shimadzu.com

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