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Application News

SSI-LCMS-075

Liquid Chromatography Mass Spectrometry

Quantitative Analysis of Cholesterol using LCMS-2020

LCMS-2020
Single Quadropule Mass Spectrometer

Summary

Cholesterol was analyzed on the LCMS-2020 and a lower limit of quantitation (LLOQ) of 0.01 ppm was achieved.

Background

Cholesterol is a naturally produced substance found in all cells of the body. There are two forms of lipoproteins that carry cholesterol through the bloodstream, low-density lipoproteins (LDL) and high-density lipoproteins (HDL). These lipoproteins are good for the body when they are at a healthy level, but can be harmful if LDL is too high or HDL is too low.

It is important to test for cholesterol to determine the levels of LDL and HDL in the body. Understanding the levels of LDL and HDL in the body helps a person to maintain good cardiac health. The goal is to keep arteries clear and reduce the risk of build up that could cause a stroke or heart attack.

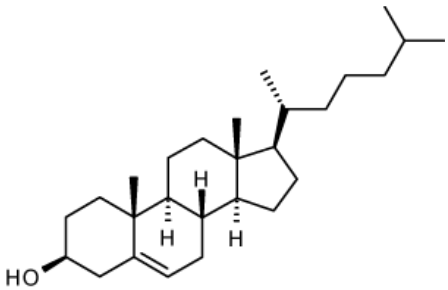

Figure 1 Chemical Structure for Cholesterol

Method

Cholesterol powder was dissolved in acetonitrile in order to make a 50 ppm stock solution. The stock solution was then used to construct a six point calibration curve ranging from 0.01 ppm to 10 ppm which was made up in acetonitrile with 0.1% formic acid.

Using APCI positive ionization, Cholesterol was acquired in SIM mode with a m/z of 369.5. System Parameters for the mass spectrometer (MS) and the liquid chromatography (LC) system are described in **Table 1 and 2.**

A Phenomenex Kinetex C18 (2.6 µm x 2.1 mm x 50 mm) column was used for isocratic elution utilizing 95% Mobile Phase B for 3 minutes at a flow rate of 0.5 mL/min. Cholesterol eluted off the column with a retention time of 2.25 min. The column temperature was 40°C and an injection volume of 5 µL was used.

Mobile Phase A consisted of 0.1% formic acid in DiH_2O while Mobile Phase B contained 0.1% formic acid in acetonitrile.

During data processing the peaks were integrated and standard smoothing parameters of 3 counts and a 1 second width were applied.

MS Parameters	
SIM m/z	369.5
Acquisition Time	0-3 min
Acquisition Mode	SIM
Polarity	Positive
Event Time	0.5 sec
Scan Speed	307

Table 1 MS parameters

LCMS-2020 System Parameters	
Drying Gas	5 L/min
Nebulizing Gas	3 L/min
Interface Temperature	350 °C
Heat Block Temperature	200 °C
DL Temperature	200 °C
Injection Volume	1 µL

Table 2 Interface parameters (APCI)

Results and Discussion

Following the development of chromatographic conditions, a 6 point calibration curve for Cholesterol was generated. **Table 3** shows the calibration levels injected and the corresponding accuracies for each level. The curve has a linearity coefficient of R^2=0.9998 as shown in **Figure 2. Figure 3A** shows the LLOQ of Cholesterol to be 0.01 ppm. **Figure 3B** also shows Cholesterol but at a concentration of 10 ppm.

The level of detection was determined to be 0.005 ppm with a signal to noise ratio of 15 RMS while the LLOQ was determined to be 0.01 ppm with a signal to noise of 29 RMS.

Blank samples were injected periodically throughout the batch and showed no detectable levels of carryover for Cholesterol. **Figure 3C** shows the chromatogram of a representative blank sample.

Cholesterol						
Level #	Sample Type	Std. Conc.	Conc. (ppm)	Ret. Time	Area	Accuracy[%]
1	Standard(Calc.Point)	10	10.045	2.216	124,532	100.4
2	Standard(Calc.Point)	1	0.984	2.214	12,226	98.4
3	Standard(Calc.Point)	0.5	0.468	2.216	5,834	93.6
4	Standard(Calc.Point)	0.1	0.097	2.216	1,234	97.1
5	Standard(Calc.Point)	0.05	0.056	2.216	728	112.3
6	Standard(Calc.Point)	0.01	0.01	2.215	153	98.1

Table 3 Calibration Curve for Cholesterol

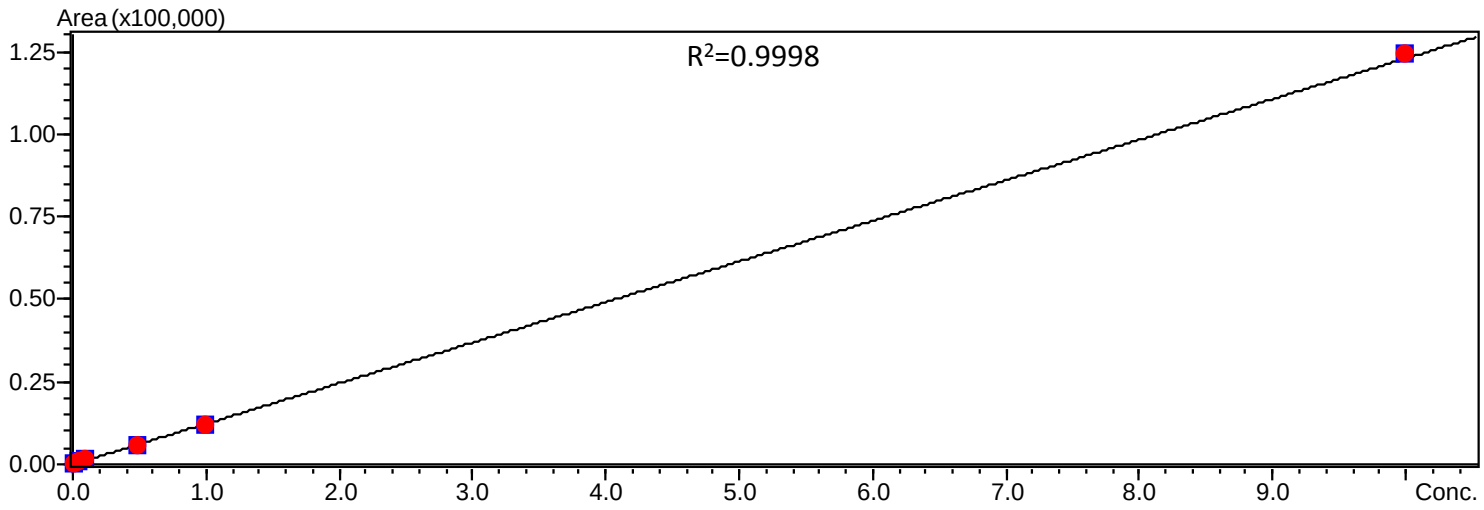

Figure 2 Calibration Curve for Cholesterol

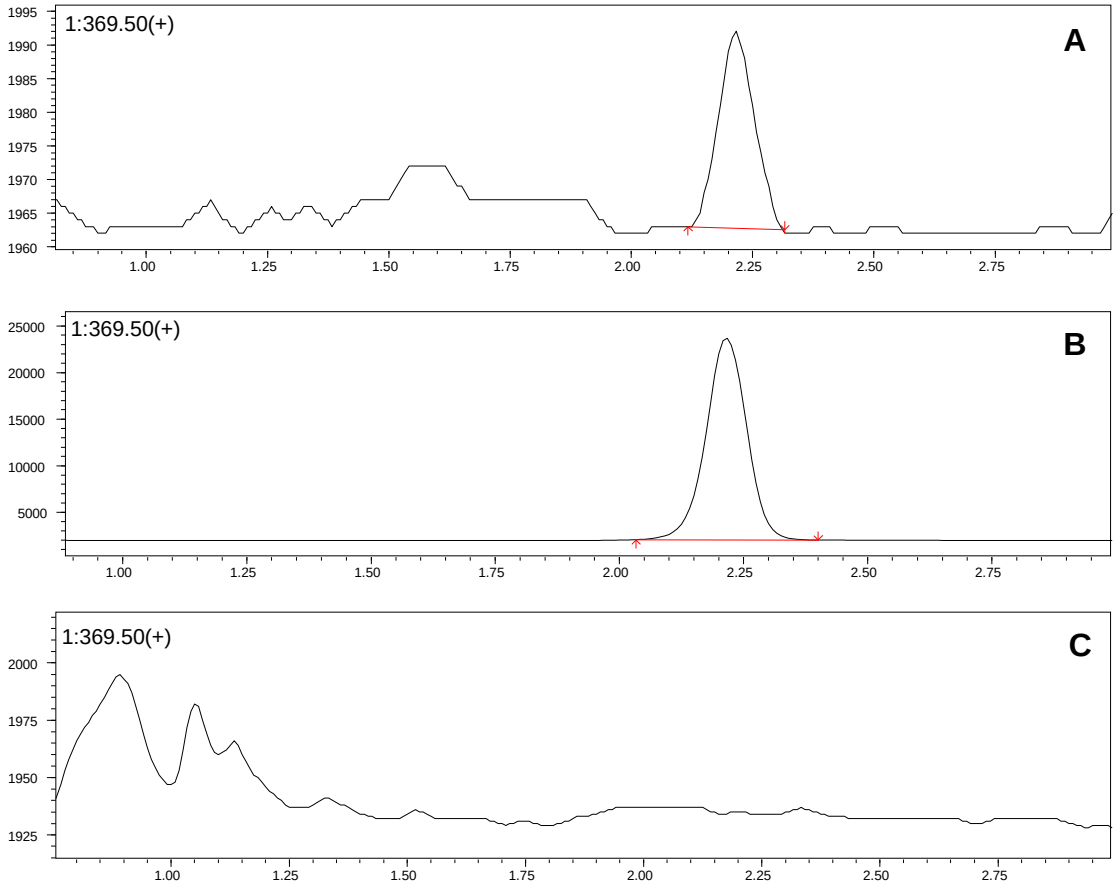

Figure 3 Chromatograms of Cholesterol at 0.01 ppm (A), 10 ppm (B) and a Blank Sample (C).

Conclusion

This work demonstrates a rapid method for the detection and quantification of Cholesterol down to a LLOQ of 0.01ppm using a Shimadzu LCMS-2020.

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