ThP411

Total cholesterol analysis in serum in 7 seconds per sample using LDTD-MS/MS

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OVERVIEW

Purpose

• High-throughput quantification of Total Cholesterol in serum.

Method

• A saponification reaction & Liquid-Liquid extraction was used for the Cholesterol analysis.

• Quantification:
- Linearity, $r^2 > 0.99$, over the calibration range (15.6 to 500 mg/dL)
- Accuracy ranging from 88.1 to 108.3 % using area ratio value
- Precision ranging from 6.2 to 11.8 % using area ratio value

• **Samples were analyzed with a run time of 7 seconds using LDTD-MS/MS system**

INTRODUCTION

Cholesterol is an essential component in blood. It plays different roles in intracellular transport, cell signaling and nerve conduction within the cell membrane. Cholesterol is also a precursor of different molecular biosynthesis: Vitamin D, steroid hormones like aldosterone and sex hormones. High concentration of Cholesterol in blood is associated to heart diseases. A report of National Cholesterol Education Program suggests a total blood cholesterol level lower than 200 mg/dL.

We developed a method using Laser Diode Thermal Desorption (LDTD) combined to MS/MS to quantify total cholesterol in serum in 7 seconds sample to sample. Sample pretreatment consists in a saponification reaction then a simple dilution with a mixture of solvents containing internal standard followed by a liquid-liquid extraction with hexane.

LDTD™ Ionization Source:

The LDTD uses a Laser Diode to produce and control heat on the sample support **(Figure 1)** which is a 96 wells plate**.** The energy is then transferred through the sample holder to the dry sample which vaporizes prior to being carried by a gas in a corona discharge region. High efficiency protonation and strong resistance to ionic suppression characterize this type of ionization, and is the result of the absence of solvent and mobile phase. This allows for very high throughput capabilities of 9 seconds sample-to-sample analysis time, without carry over.

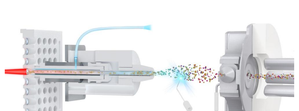

Figure 1 Schematic of the LDTD ionization source

METHOD

Saponification reaction and Liquid-liquid extraction

10 µL of serum (or water for a standard)
100 µL EtOH (or working solution for standard)
12 µL KOH (9N)
  Incubate 1h at 60°C
390 µL NaOH (1N)
400 µL Internal Standard (7 µg/mL Cholesterol-d7 in MeOH)
  Vortex
2 mL Hexane
  Vortex 30 seconds
Transfer 4 µL of the upper layer in Lazwell plate
Analyze after complete solvent evaporation

Instrumentation

• LDTD model: WX-960
• MS: Waters Xevo TQ MS

LDTD Parameters

• Laser power pattern :
  - ➢ Increase laser power to 45 % in 3.0 s
  - ➢ Maintained for 2 s.
  - ➢ Decrease laser power to 0 %
• Carrier gas flow : 3 L/min (Air)

MS Parameters

• APCI (+)
• MRM mode
• CE = 30
• Cone = 20
• Cholesterol: 369 → 161
• Cholesterol-d7: 376 → 161

Figure 2 LDTD system on Xevo TQ-S MS

RESULTS

Linearity results

The **Table 1** shows the calibration curve results of three different validation runs. A calibration curve has been prepared in water and analyzed in triplicate. It is necessary to prepare the calibration curve into water as cholesterol is endogenous in human plasma. **Figure 3** present a typical calibration curve for cholesterol. The desorption peak shape is shown in **Figure 4.**

Table 1 Inter-run curve parameters

	R	Slope (ratio area / concentration)	Y-intercept
Run 1	0.9981	0.0968	-0.2246
Run 2	0.9973	0.0906	0.6854
Run 3	0.9915	0.1077	0.6879

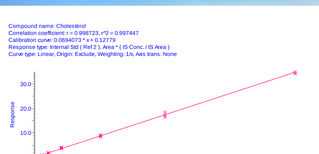

Figure 3 Typical Cholesterol standard curve

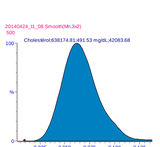

Figure 4 Typical Desorption peak

RESULTS

Precision/Accuracy results

Intra-run and inter-run precision/accuracy are reported in **Table 2** and **3**. Accuracy ranging from 88.1 to 108.3 % using area ratio value and precision ranging from 6.2 to 11.8 % using area ratio values were obtained.

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (mg/dL)	15.6	31.3	125	250	500
N	6	6	6	6	6
Mean (mg/dL)	13.74	31.13	132.66	267.04	511.70
%RSD	10.9	9.5	10.9	9.0	6.2
%Nom	88.1	99.5	106.1	106.8	102.3

Table 2 Intra-run precision and accuracy

	QC-Low	QC-Med	QC-High
Conc. (mg/dL)	31.3	125	250
N	18	18	18
Mean (mg/dL)	30.74	126.82	261.77
%RSD	11.8	11.7	9.4
%Nom	92.9	108.3	91.1

Table 3 Inter-run precision and accuracy

Carry over

Carry over was evaluated by analysis of 3 blanks after the high standard. Peak area blank were evaluated again the mean peak area value of lower standard to determinate interference percentage. **Table 4** shows the % Interference value of the 3 blanks.

	% Interference
BLK 1	0.59
BLK 2	0.53
BLK 3	0.49

Table 4 Blank interference value of carry over test

Stability verification

Following the extraction process, all samples were stored at 4°C to evaluate the wet stability of cholesterol. After 48h, all samples were re-spotted and analyzed. Linearity, precision and accuracy were evaluated to determine the stability. **Table 5** shows that a wet stability of 48h is obtained with good precision and accuracy of LOQ standard.

The stability of dry samples in LazWell plate was also determined. All standards and QCs are spotted, dried and kept at room temperature for 48h. Then, standards and QCs were analyzed and the linearity, precision and accuracy were verified. **Table 5** shows the dry stability results and the storage conditions of the LazWell.

	Wet Stability	Dry in LazWell
Time (h)	48	48
Temp. (°C)	4	RT
Conc. (mg/dL)	15.6	15.6
N	4	4
Mean (mg/dL)	13.43	14.25
%RSD	10.3	13.8
%Nom	86.1	91.3

Table 5 Stability results for cholesterol

Cross-validation

Serum samples were obtained from 6 different people. Samples were split in two (N=2). Samples were analyzed using LDTD-MS/MS technology and by an accredited clinical laboratory. This lab uses a clinical chemistry system as an in vitro diagnostic test. Less than 17% of difference on 6 different serum samples was obtained for the cross-validation.

Technique used by the clinical lab

An enzyme catalyzes the hydrolysis of cholesterol ester. The free cholesterol is oxidized and hydrogen peroxide is formed. Peroxide reacts with a specific reagent and forms a chromophore that absorbs at 540 nm. Results are showed in **Table 6**.

Serum	LDTD-MS/MS		Clinical Lab	% Difference
	Mean Conc.	CV	Mean Conc.	
	mg/dL	%	mg/dL	
Woman #1	180	11	158	13
Woman #2	167	3	185	-10
Woman #3	161	0.3	147	10
Man #1	160	3	170	-6
Man #2	236	5	220	7
Man #3	122	5	104	17

Table 6 Cross-validation results

CONCLUSIONS

- **Total cholesterol analysis in serum can be performed in 7 seconds by LDTD-MS/MS.**
- **Good precision and accuracy are obtained. No carryover was observed.**
- **Samples are stable at least 48 hours according to wet and dry stability tests.**
- **Cross-validation with an accredited clinical method shows less than 17% of difference on 6 different serum samples.**