MP 329

Total Cholesterol and HDL Cross Validation Between High Throughput LDTD-MS/MS Method and Reference Enzymatic Technique Used in Clinical Laboratory

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OVERVIEW

Purpose
- High-throughput quantification of HDL and Total Cholesterol in serum

Method
- Specific HDL separation
- 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
- Effective cross validation with accredited clinic quantitation
- **Samples analyzed with a run time of 7 seconds using LDTD-MS/MS system**

INTRODUCTION

High concentration of cholesterol in blood is associated with heart disease while HDL removes fats and cholesterol from cells and transports it back to the liver. Cholesterol level in blood is a frequently analyzed parameter that requires a high-throughput method due to the quantity of samples involved in routine analysis. HDL separation is achieved and all the samples are then treated in the same way for the extraction. A cross validation is made in an external clinical laboratory to compare with the LDTD results.

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 7 seconds sample-to-sample analysis time, without carry over.

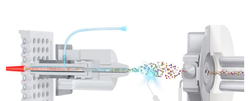

Figure 1 Schematic of the LDTD ionization source

METHOD

HDL Separation Procedure

Working solution 1: 1:1 ratio of Dextran Sulfate 20 g/L and Sodium Azide 0.5 g/L

Working solution 2: 1:1 ratio of $MgCl_2$ 0.7 mol/L and Sodium Azide 0.5 g/L

HDL Separation:

100 µL serum sample
10 µL working solution 1
10 µL working solution 2
Vortex and Incubate 30 min at room temperature
Centrifuge 30 min at 1500 g
Use supernatant as sample for cholesterol analysis

Extraction Procedure (cholesterol analysis)

10 µL sample (total cholesterol) or HDL supernatant (or water for a standard)
100 µL EtOH (or curve 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

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)

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

Figure 2 LDTD system on Xevo TQ-MS

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

RESULT

Linearity results

A calibration curve (15.6-500 mg/dL) has been prepared in water and analyzed in triplicate. Correlations were all over 0.9915. It is necessary to prepare the calibration curve in water as cholesterol is endogenous in human plasma. **Figure 3** presents a typical calibration curve for cholesterol.

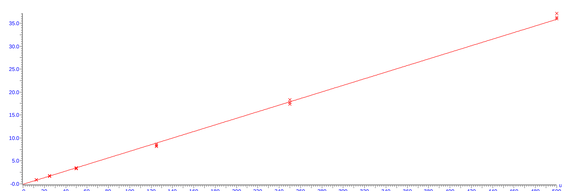

Figure 3 Typical Cholesterol standard curve

Precision/Accuracy results

Intra-run and inter-run precision/accuracy are calculated. Accuracy ranging from 88.1 to 108.3 % and precision ranging from 6.2 to 11.8 % using area ratio values were obtained. Results were reported in **Table 1** and **Table 2**.

Table 1
Intra-run precision and accuracy

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (mg/dL)	15.6	31.3	125.0	250.0	500.0
N	6	6	6	6	6
Mean (mg/dL)	13.7	31.1	132.7	267.0	511.7
%RSD	10.9	9.5	10.9	9.0	6.2
%Nom	88.1	99.5	106.1	106.8	102.3

Table 2
Inter-run precision and accuracy

	QC-Low	QC-Med	QC-High
Conc. (mg/dL)	31.3	125.0	250.0
N	18	18	18
Mean (mg/dL)	30.7	126.8	261.8
%RSD	11.8	11.7	9.4
%Nom	92.9	108.3	91.1

Carry over

Carry over was evaluated by analyzing 3 blanks after the highest standard. Blank peak areas were evaluated against the mean peak area value of the lower standard to determine the interference percentage. **Table 3** shows the % Interference value of the 3 blanks.

	% Interference
BLK 1	0.59
BLK 2	0.53
BLK 3	0.49

Table 3 Blank interference value of carry over test

Cross-validation with both HDL and Total Cholesterol

Serum samples were obtained from 6 different people. Samples were split in two fractions. One fraction set was analyzed using LDTD-MS/MS technology and other with an accredited clinical laboratory. Results were reported in **Table 4** and **Table 5**.

	LDTD-MS/MS mg/dL	Clinical lab mg/dL	% of difference
Woman #1	177.2	197.2	10.1
Woman #2	162.6	174.0	6.6
Woman #3	151.6	150.8	-0.5
Man #1	202.7	255.2	20.6
Man #2	227.3	224.2	-1.3
Man #3	155.2	162.4	4.4

Table 4- Cross-validation of serum samples for total Cholesterol

	LDTD-MS/MS mg/dL	Clinical lab mg/dL	% of difference
Woman #1	34.7	36.7	5.5
Woman #2	66.2	70.0	5.4
Woman #3	56.6	55.7	-1.6
Man #1	36.5	44.1	13.4
Man #2	38.1	37.5	-1.6
Man #3	42.4	42.2	3.9

Table 5- Cross-validation of serum samples for HDL Cholesterol

CONCLUSION

- **Total and HDL 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 20.6% of difference on 6 different serum samples.**