Gas Chromatography Mass Spectrometry Reference Targets for the Comparison of Cholesterol Assays in Serum.

Morton MS, Fraser H, Strevens C, Ducroq DH

WEQAS
Unit 6, Parc Tŷ Glas,
Llanishen
Cardiff
CF14 5DU
Tel: +44 (0) 2920 314750
Fax: +44 (0) 2920 314760
Email: office@weqas.com

INTRODUCTION

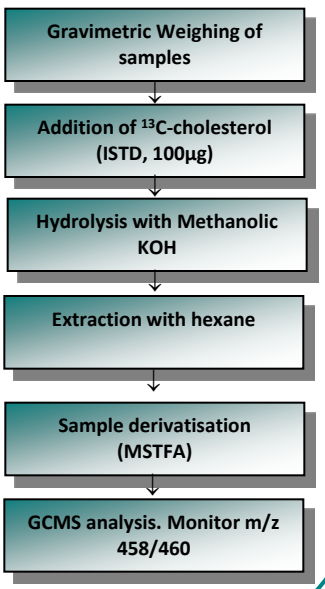
Metrological traceability of methods is a requirement of ISO15189 accreditation and labs are now becoming aware of the need for traceability of their routine methods. To meet this need analysis of traceable material in the chosen method is required. The preferred method of comparison of returned EQA results is to the SI unit utilising reference target, ensuring the transfer of accuracy from definitive methods to routine methods. Use of reference targeted material would therefore assist in standardising results from these assays.

Until recently, reference values for total cholesterol were assigned by a CDC laboratory using the Abel-Kendell method. A method has been developed for total cholesterol, assigning targeting values to EQA material (3.0 - 8.0mmol/l) utilising Isotope Dilution Gas Chromatography Mass Spectrometry (ID-GCMS). The methodology was based on a published reference method.

REFERENCE METHOD

An Isotope Dilution Gas Chromatography – Mass Spectrometry (ID-GCMS) method has been developed based on the method of Thienpont[1]. The analysis was undertaken by the Weqas Reference Laboratory. Samples were processed as detailed in the method flow diagram (fig. 1). The GCMS conditions were as indicated in table 1. Gravimetric analysis was used throughout, allowing uncertainty measurements to be estimated according to GUM, with traceability by use of certified standards and standard reference material (SRM, table 2). All sample/SRM volumes were adjusted such that the ratio of analyte to internal standard was approximately 1 (100µg). Measured results were calculated using a bracketed standard curve as illustrated in figure 2.

Figure 1
Method Flow Diagram



RESULTS

Incubation with methanolic KOH frees protein-bound cholesterol and hydrolyses sterol esters to produce total cholesterol. The hexane extracted cholesterol is converted to the mono TMS ether by MSTFA. During GCMS analysis a 250:1 split injection was necessary due to the level of analyte response observed (fig. 4). Reproducibility, repeatability and traceability were assessed using NIST 909c (tables 3 & 4). Linearity was assessed by analysing a high cholesterol serum pool mixed with a zero serum pool (stripped of cholesterol using cab-osil M-5) to produce a linear series (fig. 3). All results for the reference materials and analysed samples were within accepted specification (2). The reference method was used to analyse cholesterol in EQA samples to be distributed as part of the Weqas Lipid scheme

Table 1 *GCMS Conditions (Agilent 5973 MSD)*

Column	HP5 (25m, 0.25mm diameter x 0.25µm film)
Injector	Split 250:1, 270°C, 5µl injection
Oven	Isothermal 300°C
Gas flow	0.7ml/min

Table 2 *Traceability*

Analyte	Standard (certified purity)	Control material
Cholesterol	NIST 911c (99.2%)	SRM 909c

Figure 3 Cholesterol Linearity

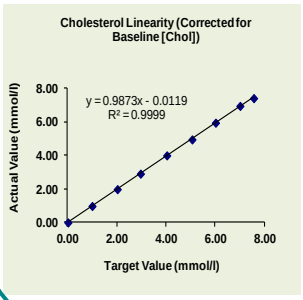


Figure 4 Typical GCMS Response

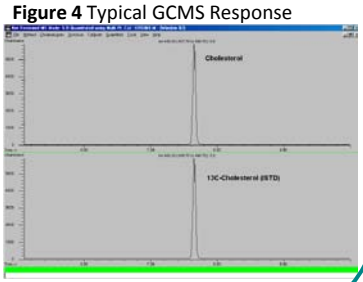


Figure 2 Typical standard curve

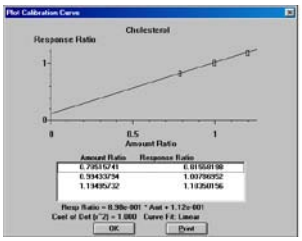


Table 3 *Reproducibility (within assay; SRM 909c)*

	SRM I
Target	**3.703mmol/L**
Mean	3.70mmol/L
SD	0.03
%CV	0.88
n	6
% Bias (from target)	-0.08

Table 4 *Repeatability (between assay; NIST 909c)*

	NIST909c
Target	**3.703mmol/L**
Mean	3.69mmol/L
SD	0.05
%CV	1.23
n	12
% Bias (from target)	-0.43

CONCLUSION

ID GC-MS reference values for cholesterol will be assigned to all lipid pools allowing a comparison of participant results to the Reference method for each Weqas distribution. The usefulness of such reference targeted data as an accuracy target in EQA schemes is obvious. The development of reference methods will ensure that Weqas can independently help manufacturers, users and competent authorities in the post –marketing vigilance of the EU Directive 98/79/EC. Weqas is committed to provide traceable reference method target values for the majority of analytes; currently the repertoire includes electrolytes, HbA1c, uric acid, triglyceride, HDL cholesterol, steroids, bile acids, glucose, creatinine and enzymes.

REFERENCES

1. Thienpont L.M. Candidate Reference Methods for Determining Target Values for Cholesterol, Creatinine, Uric Acid, and Glucose in External Quality Assessment and Internal Accuracy Control. II. Method Transfer. Clin Chem 1993;39/6:1001-1006 .

2. Stöckl D Analytical specifications of reference methods compilations and critical discussion. Eur J Clin Chem Biochem 1996;34: 319-337