Gas Chromatography Mass Spectrometry Targets of Cholic Acid, Chenodeoxycholic Acid and Deoxycholic Acid for the Comparison of Total Bile Acid Assays in Serum.

Ducroq DH, Shadi N, Fraser H, Thomas MA

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

Routine methods used to measure total bile acids in serum are very non-specific and measure all of the major bile acids present in serum. The most common methods are enzymatic, using 3-α hydroxysteroid dehydrogenase to convert bile acids to 3-ketosteroids, with monitoring of the formation of NADH. Comparison of returned EQA results with the consensus mean may not fully highlight any bias due to non-specificity of the methods used. Use of GCMS targeted material would therefore assist in standardising results from these assays.

REFERENCE METHOD

An Isotope Dilution Gas Chromatography – Mass Spectrometry (ID-GCMS) method has been adapted from the method of Setchell[1]. The analysis was undertaken by the Weqas Reference Laboratory. Samples were processed as detailed in the method flow diagram (fig. 1), forming the methyl trimethylsilyl ether (Me TMS) derivatives for GCMS analysis (fig. 2). GCMS conditions are detailed in table 1. Gravimetric analysis was used throughout, allowing uncertainty measurements to be estimated according to GUM, with traceability by use of gravimetrically prepared QC material (table 2, certified material not available). All sample volumes were adjusted such that the ratio of analyte to internal standard was approximately 1 (50ng). Measured results were calculated using a bracketed standard curve as illustrated in figure 3.

Figure 1
Method Flow Diagram

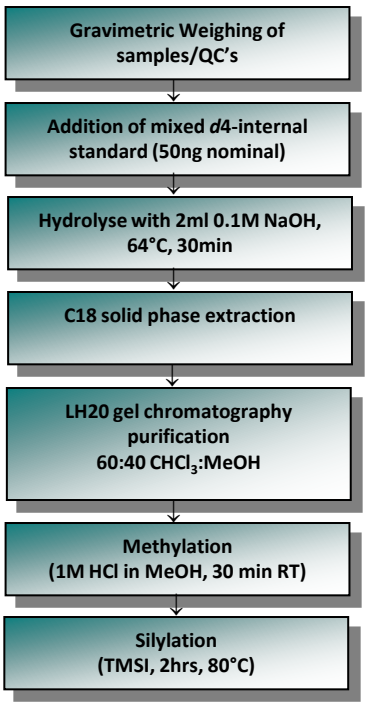


Figure 3 Typical standard curve (Deoxycholic acid)

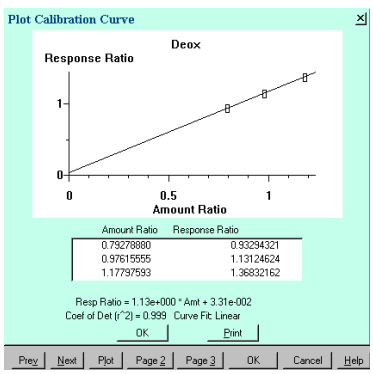


Figure 4 Typical TIC

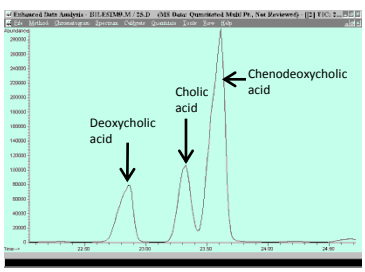


Table 1 *GCMS Conditions (Agilent 5973 MSD)*

Column	DB5-MS (J&W 25m, 0.2mm diameter x 0.33µm film)
Injector	Gerstel ptv, 75°C initial for 2min, ramp 270°C, 0.2µl injection
Oven	75°C initial, ramp 25°C/min to 295°C, hold 16min
Gas flow	0.5ml/min

Table 2 *Traceability*

Analyte	Standard (purity)	Control material
Chenodeoxycholic Acid	Sigma (98%)	"In house" (none available commercially – see Table 3)
Deoxycholic Acid	Sigma (99%)	
Cholic Acid	Sigma (99%)	

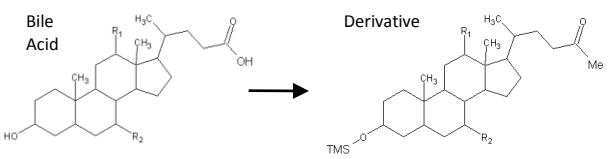
RESULTS

From figure 4 the total ion count shows good separation of each of the major bile acids analysed. Further specificity for each is achieved by single ion monitoring of the respective ions as detailed in Figure 2. Each bile acid forms a fragment ion of m/z 370 therefore the long retention time was necessary to give base peak separation between cholic acid and chenodeoxycholic acid (fig. 4). Studies have shown no interference from other major bile acids (ursodeoxycholic acid and hyodeoxycholic acid acid).

Reproducibility was assessed using quality control material consisting of charcoal stripped serum spiked with each bile acid (table 3). The %cv for each was less than 3.5% for each level tested.

For EQA purposes, target values were provided as the sum of each of the bile acids measured by ID-GCMS.

Figure 2 Methyl Trimethylsilyl Ether Derivatives (Me TMS)



			Monitored Mass (m/z)
Cholic Acid:	R1 = OH, R2 = OH	R1 = O-TMS, R2 = O-TMS	623/627 (M-15)
Chenodeoxycholic Acid:	R1 = H, R2 = OH	R1 = H, R2 = O-TMS	370/372 (fragment ion)
Deoxycholic Acid:	R1 = OH, R2 = H	R1 = O-TMS, R2 = H	535/539 (M-15)

Table 3 *Reproducibility (Internal QC material)*

	Mean (nmol/l) {cv} High	Medium	Low
Chenodeoxycholic Acid	48.5 {1.42}	25.74 {2.70}	10.03 {0.36}
Deoxycolic Acid	47.85 {2.48}	27.15 {3.14}	9.93 {2.94}
Cholic Acid	47.48 {3.31}	26.08 {3.38}	10.19 {0.97}

CONCLUSION

ID GC-MS values will be assigned to all bile acid 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. Similar studies are currently being undertaken for steroids, and creatinine. 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, cholesterol, triglyceride, HDL cholesterol, steroids, creatinine , uric acid, glucose and enzymes.

REFERENCES

1. Setchell KDR. Identification of a New Inborn Error in Bile Acid Synthesis: Mutation of the Oxysterol 7α-Hydroxylase Gene Causes Severe Neonatal Liver Disease. J Clin Invest 1998;102/9:1690-1703.