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Technical Report

Sugar and Sterol Analysis in Human Body Fluids on a True Dual Column GCMS System

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1. Summary

Being flexible is one of the most important services in an analytical supporting laboratories. This especially the case in hospitals where fast accurate respons is essential.

2. Introduction

Galactosaemia is an autosomal recessively inherited disorder in galactose metabolism (OMIM #230400). The disease is most frequently caused by a deficiency of galactose-1-phosphate uridyltransferase (GALT). Patients are unable to metabolize galactose to glucose. Ingestion of lactose, which is a disaccharide of galactose and glucose, causes a prolonged rise of blood galactose and the appearance of galactose and galactitol in urine.

Smith-Lemli-Opitz syndrome (SLOS, OMIM #270400) is a malformation syndrome caused by deficiency of 7-dehydrocholesterol reductase (DHCR) which catalyzes the last step of cholesterol biosynthesis. A deficiency of the enzyme results in an accumulation of 7- and 8-dehydrocholesterol (7+8-DHC) and, in most patients, in a decreased concentration of cholesterol.

Cerebrotendinous xanthomatosis (CTX, OMIM #213700) is an autosomal recessively inherited disorder of bile acid synthesis. Due to a deficiency of the mitochondrial enzyme sterol 27-hydroxylase, production of chenodeoxycholic acid (CDCA) is impaired. This results in an excessive production of cholestanol and cholesterol and accumulation of these sterols in several tissues and in plasma. Furthermore, these patients excrete bile alcohols in urine.

Gas chromatography is an important method in the screening and follow up of patients with the inborn errors of metabolism mentioned above and is the method of choice in the analysis of monosaccharides, polyols and sterols. GC separates complex mixtures with high resolution, and allows a reliable qualitative and quantitative interpretation. A GCMS is necessary for identification of the compound and permits also quantification of monosaccharides and polyols (e.g. glucose, galactose, galactitol) and sterols such as cholesterol, cholestanol, 7- and 8-dehydrocholesterol.

For both analyses sensitive and validated GCMS methods are necessary, that previously required two dedicated GCMS systems for either monosaccharides/polyols or sterols or, as an alternative, frequent changing of GC-columns. The latter has the major disadvantage as it requires a complete shut-down of the GCMS system and, after changing the column, a restart. It takes subsequently approximately 3 hours before the GCMS is operational. The down-time for changing columns is therefore 4-5 hours, a situation that is not acceptable in a clinical laboratory setting with relatively high sample throughput.

The Shimadzu GCMS-QP2010 is a dual-column system in which one ion source accommodates both analytical columns. (Leijs et.al.[3]) Due to its large pumping capacity, it is possible to install two GC capillary columns simultaneously in the Shimadzu GCMS-QP2010. This makes column change with significant down-time of the instrument unnecessary. Both analytical assays described above were successfully validated on the dual-column system (with both columns installed).

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3. Instrumentation

Our laboratory is equipped with a Shimadzu GCMS-QP2010 system with an AOC-20i auto sampler. The GCMS-QP2010 has a high pressure electronic flow control, high split ratios as well as fast heating and cooling of the column oven (250°C/min).

The mass spectrometer has a fast scan speed (10,000 amu/sec and 50 scans/sec) and a very short inter-scan delay time. The high-energy ion source, large-capacity dual turbo-molecular pump differential pumping system (260 L/s + 65 L/s), and low-noise detector unit makes this system very sensitive. For highest sensitivity of the target compounds and easy identification of all unknowns in the same run, SCAN and SIM modes can be programmed in one method file. For the determination of monosaccharides and polyols we used a Varian VF-01 ms WCOT column (20 m × 0.15 mm × 0.15 µm film thickness). Column linear velocity (helium) 35.0 cm/sec, split ratio 1:20, Injector temperature 280°C, Ion Source temperature 200°C, Interface temperature 250°C (Table 1).

For the determination of sterols we used a Varian VF-1701 ms WCOT column (30 m × 0.25 mm × 0.15 µm film thickness). Column linear velocity (helium) 35.0 cm/sec, split ratio 1:80, Injector temperature 280°C, Ion Source temperature 200°C, Interface temperature 250°C (Table 1).

Table 1 *Analytical conditions*

Instruments

GC/MS:	GCMS-QP2010 Ultra
Auto-injector:	AOC-20i + s
Column :	VF-01 ms (20 m × 0.15 mm I.D. df=0.15 µm, Varian)
	VF-1701 ms (30 m × 0.25 mm I.D. df=0.15 µm, Varian)

Analytical Conditions
Monosaccharides and polyols

GC

Injection Temp.:	280°C
Injection Mode:	Split
Column Temp.:	120°C (5 min) – (10°C/min) - 130°C (0 min)
	– (3°C/min) - 210°C (0 min) – (15°C/min) - 240°C (0 min)
	– (3°C/min) - 265°C (0 min) – (15°C/min) - 295°C (5 min)
Carrier Gas:	He (Constant Linear Velocity)
Linear Velocity:	35.0 cm/sec
Split ratio:	20
Injection volume:	1 µL

MS

Ion Source Temp.:	200°C
Interface Temp.:	250°C
Scan Range:	*m/z* 50 – 400
Event Time:	0.36 sec

Sterols

GC

Injection Temp.:	280°C
Injection Mode:	Split
Column Temp.:	120°C (1 min) – (75°C/min) - 270°C (0 min)– (3°C/min)
	- 300°C (3 min)
Carrier Gas:	He (Constant Linear Velocity)
Linear Velocity:	35.0 cm/sec
Split ratio:	80.0
Injection volume:	1 µL

MS

Ion Source Temp.:	200°C
Interface Temp.:	250°C
Scan Range:	*m/z* 50 – 500
Event Time:	0.4 sec

4. Results

Monosaccharides and polyols:

The sample preparation of urine samples for an analysis of monosaccharides and polyols was performed as described earlier by Jansen *et al*.[1] A typical TIC chromatogram is displayed in Fig. 1.

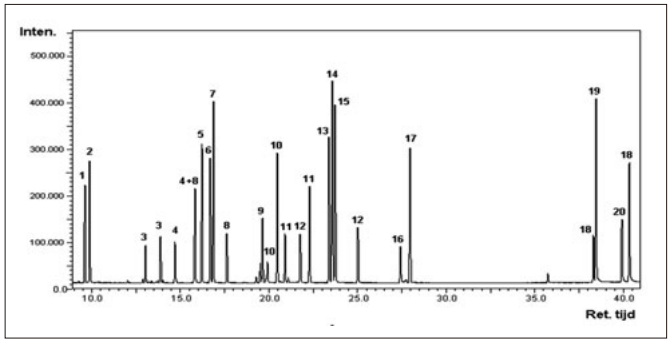


Fig. 1 *TIC-Chromatogram of monosaccharides and polyols*
1=Treitol; 2=Erytritol; 3=Arabinose; 4=Fucose;
5=Xylitol; 6=Arabitol; 7=Ribitol; 8=Xylose; 9=Fructose;
10=Allose; 11=Galactose; 12=Glucose; 13=Mannitol;
14=Sorbitol; 15=Galactitol; 16=Mannoheptulose (IS 1);
17=Myoinositol; 18=Lactose; 19=Sucrose; 20=Trehalose
(IS 2); IS: Internal Standard.

Quantification of urinary monosaccharides and polyols was performed by comparing the peak area ratio of a fragment ion that is characteristic for each analyte and its corresponding internal standard to that of the standards which were spiked into a pool urine.

As some analytes had more than one peak the sum of both peaks areas was taken for quantification. For example the calibration curve of galactitol (*m/z* 217) in pool urine (Fig. 2).

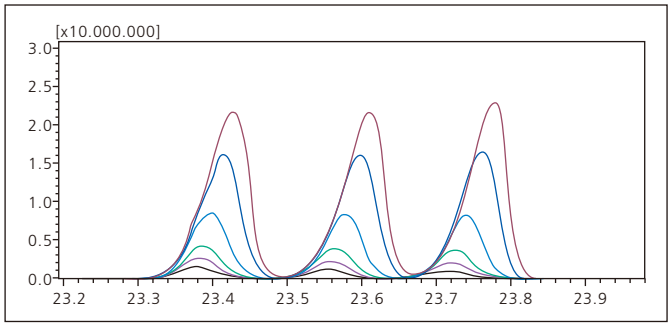


Fig. 2 *Calibration standards of mannitol,*
sorbitol and galactitol (25, 50, 100, 250, 500, 750 nmol)

The calibration curve with area ratios (area compound/area IS) was generated by linear regression within the limits of the calibration range. The calibration range was determined by statistical analysis of concentration data from real samples. The calibration curve was linear over a range of 1 µmol/mmol creatinine to 750 µmol/mmol creatinine for all monosaccharides and polyols, with a linear correlation coefficient (r^2) of > 0.999 for all compounds. The detection limits (LOD) of the monosaccharides and polyols was ~ 1 µmol/mmol creatinine (s/n > 10). The use of an internal standard method compensates the interday changes in GCMS stability and the calibration errors caused by possible influence of the matrices.

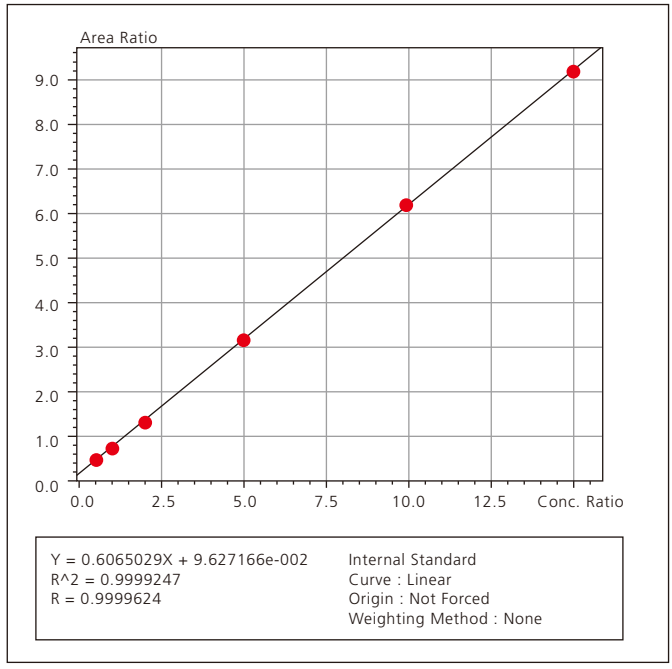


Fig. 3 *Calibration curve of galactitol*

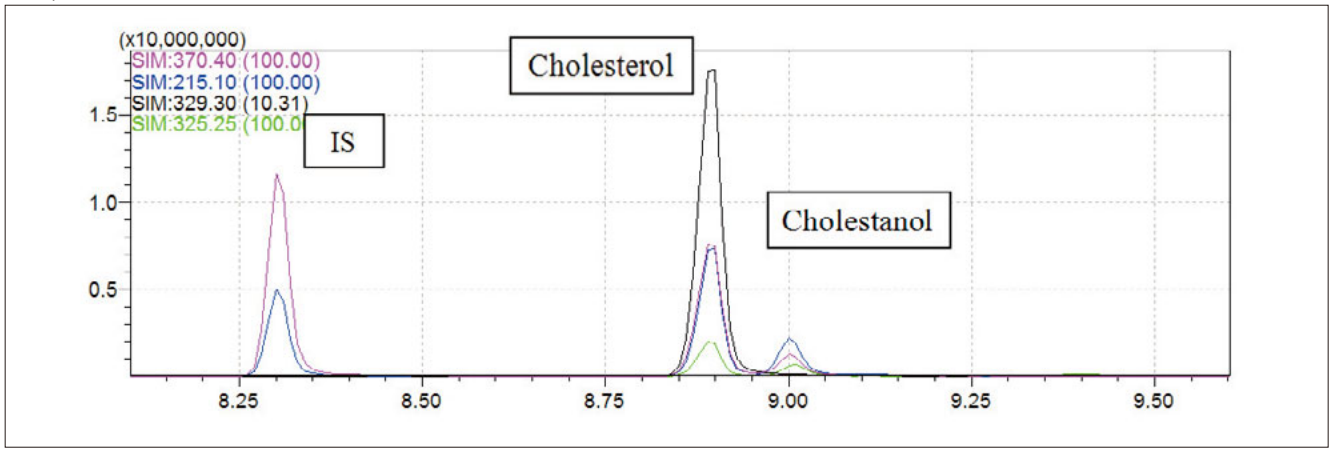
Table 2 *Validation results of 3 monosaccharides*

	Intra-day (n=10)		Inter-day (n=10)	
	Average (µmol/mmol creatinine)	VC (%)	Average (µmol/mmol creatinine)	VC (%)
fructose	107.2	4.3	104.2	9.2
glucose	64.3	2.3	70.7	5.6
galactitol	8.3	5.8	9.6	16.4

Sterols

The preparation of plasma/serum samples for an analysis of sterols was performed as described by Koopman *et al*.[2]

CTX-patient:



SLO-patient:

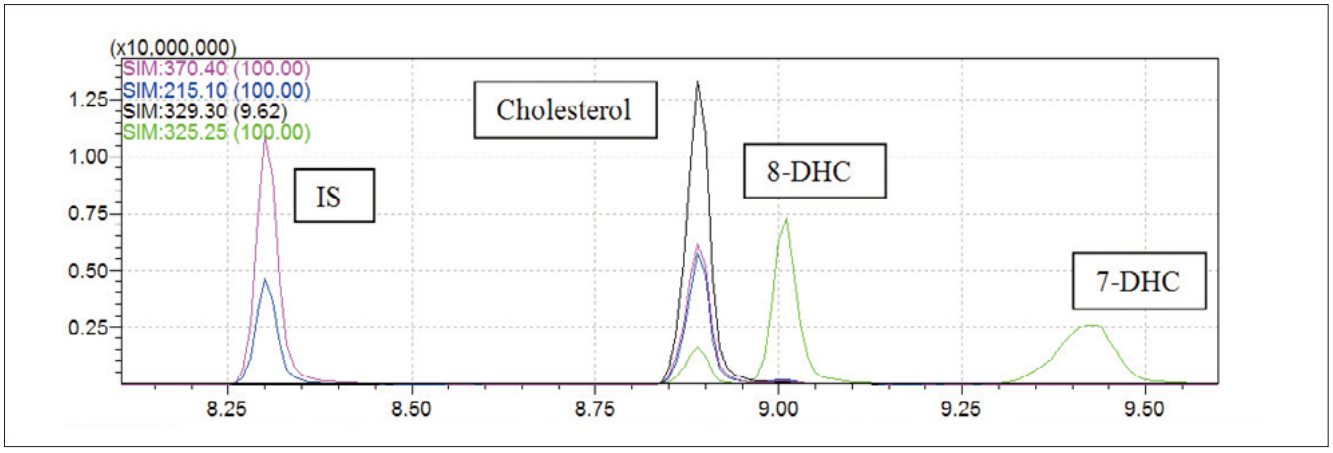


Fig. 4 *Typical plasma sterol chromatogram of a CTX patient (top panel) and SLO patient (bottom panel)*
Note the (near) identical retention times of cholestanol and 8-DHC. Both compounds can be discriminated and identified by unique ion fragment.

The sterols were identified in the scanning mode on the basis of their characteristic retention times and mass spectra. They were subsequently quantified by selective monitoring of a specific ion fragment after normalization with the internal standard and calibration with weighed standards.

The retention time of cholestanol and 8-DHC is nearly identical. The use of unique ion fragments (cholestanol *m/z* 215 and 8-DHC *m/z* 325) permits unequivocal identification.

Calibration curves with area ratios (area compound/area IS) were generated by linear regression within the limits of the calibration range. The calibration range was determined by statistical analysis of concentration data from real samples. The calibration curve was linear over a range of 1 µmol/L to 8500 µmol/L for cholesterol; 1-85 µmol/L for cholestanol and 1-250 µmol/L for 7- and 8-dehydrocholesterol, with a linear correlation coefficient (r^2) of > 0.990 for all compounds. The detection limits (LOD) of the sterols was ~ 2 µmol/L (s/n > 5).

The use of an internal standard method compensates the interday changes in GCMS stability and the calibration errors caused by possible matrix influence.

Table 3 *Validation results of sterols*

	Intra-day (n=10)		Inter-day (n=8)	
	Average (µmol/l)	VC (%)	Average (µmol/l)	VC (%)
Cholesterol	3100	1.5	3000	5.7
Cholestanol	31	4.2	28	6.5
7-DHC	208	8.0	208	9.2

5. Conclusion

The trimethylsilyl derivatives of many sugars such as glucose and fructose, have very similar EI mass spectra. In order to distinguish and quantify them, chromatographic parameters are of particular importance. Chromatographic retention times vary with the column length. The switching of columns that would be necessary to analyse different types of metabolites on the same GC(-MS) system always needs cutting the column with repercussions for retention times. This renders automatic identification more difficult (unless relative retention indices are used). A dual column set up, as in our Shimadzu GCMS-QP2010, solves this problem. The concentration of the sterols cholestanol, 7- and 8-DHC is very low. For the highest sensitivity of target compounds and easy identification of unknown compounds in the same run it is necessary to run SCAN and SIM-modes simultaneously in one method file. The GCMS-QP2010 mass spectrometer offers fast scan speed which permits such a dual-mode analysis.

The analysis of sugars and sterols demands two different columns to obtain data of the highest quality necessary in the metabolic diagnostics of abnormalities in monosaccharides/polyols and cholesterol metabolism. It takes normally a long time to cool down a GCMS system, change of columns and restart again. Since we work in our laboratory with the GCMS-QP2010, equipped with dual columns, both ending in one shared ion source we lose no time in changing columns. Both methods have been validated successfully, with both columns installed in the QP2010.

References

1. Jansen *et al*. (1986) Capillary gas chromatographic profiling of urinary, plasma and erythrocyte sugars and polyols as their trimethylsilyl derivatives, preceded by a simple and rapid repurification method. *Clinica Chimica Acta*, 157 277-294.
2. Koopman *et al*. (1994) Capillary gas chromatographic determination of cholestanol/cholesterol ratio in biological fluids. Its potential usefulness for the follow-up of some liver diseases and its lack of specificity in diagnosing CTX (cerebrotendinous xanthomatosis). *Clinica Chimica Acta* **137** 305-315.
3. Hans Leijs *et al*. (2005) Quantitative Analysis of the 26 Allergens for Cosmetic Labeling in Fragrance Raw Material and Perfume Oils, *J. Agric. Food. Chem.* 2005, **53**, 5487-5491.

Twin Line MS System Eliminates the Need to Swap Columns

The Twin Line MS system enables the installation of two capillary columns into the MS simultaneously. With this setup, users can smoothly switch between applications without venting the MS vacuum or physically modifying the column installation.

Since the outlets of the two columns are directly introduced into the MS interface without using press tight connectors and retention gap columns, existing methods and retention times for a single column can be used. In addition, no adsorption losses and a large-capacity differential evacuation system help achieve sensitivity equivalent to a single column.

Setup is simple using GCMSsolution Ver. 2.6 software, as shown in Fig. 2. First, set the two flow paths in the configuration settings. Next, select the column (analysis line) to use for the method. A batch schedule can be used to switch columns for serial analysis (Fig. 3), which can significantly enhance productivity in a clinical laboratory.

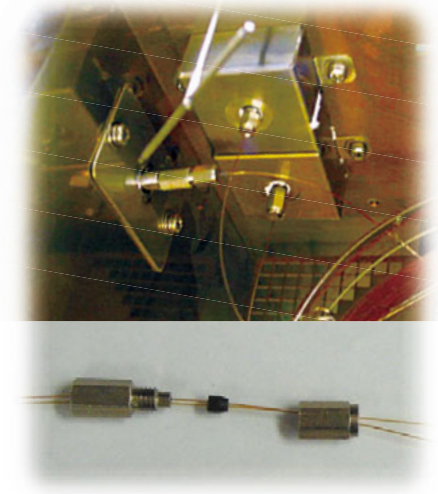


Fig. 1 Twin Line MS System

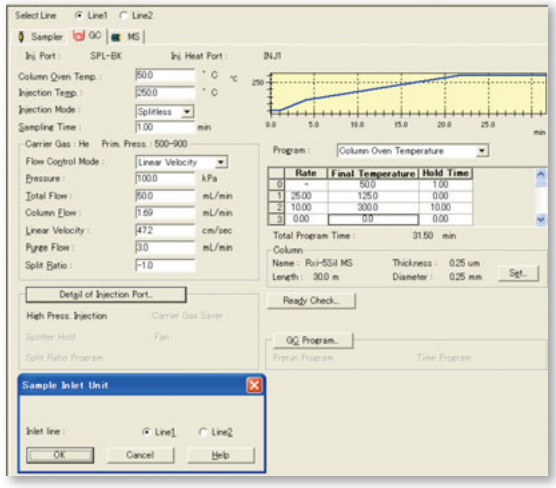


Fig. 2 Method Setting Select the column (analysis line) to use for the method

Folder: D:¥Data¥CS035_2010¥2010_11¥20101116

	Vial#	Sample Name	Sample ID	Sample Type	Analysis Type	Method File	Data File	Level#
1	1	Sample A		0:Unknown	IT QT	Scan_Line1.qgm	SampleA_Line1_001.qgd	1
2	1	Sample A		0:Unknown	IT QT	Scan_Line2.qgm	SampleA_Line2_001.qgd	1
3	2	Sample B		0:Unknown	IT QT	Scan_Line1.qgm	SampleB_Line1_001.qgd	1
4	2	Sample B		0:Unknown	IT QT	Scan_Line2.qgm	SampleB_Line2_001.qgd	1
5	3	Sample C		0:Unknown	IT QT	Scan_Line1.qgm	SampleC_Line1_001.qgd	1
6	3	Sample C		0:Unknown	IT QT	Scan_Line2.qgm	SampleC_Line2_001.qgd	1

Fig. 3 Batch Schedule Serial analysis of Samples A and B at inlet 1 and 2.

Note) The Twin Line MS System requires an optional installation kit and is limited to certain column dimensions.

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