Analysis of lathosterol and plant sterols on GC-MS and LC-MS/MS

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Introduction

Cholesterol is found in every cell of the mammalian body and is important for diverse functions. Several inherited disorders have been identified that can be linked to different enzyme defects in the post-squalene cholesterol biosynthesis which result in severe malformations [1]. Lathosterol, campesterol and sitosterol can be helpful parameters in case of errors in the metabolism due to a gene mutation. So far, gas-chromatography mass spectrometry (GC-MS) has been the golden standard to measure sterols, but in recent years combined liquid-chromatography mass spectrometry (LC-MS) is becoming more prevalent as well [1, 2]. The aim of this study is to develop a simple LC-MS/MS method that allows separation and quantification of the three sterols and to compare it with the established GC-MS method.

Materials and methods

Sample preparation was performed by adding deuterium labelled internal standards and hydrolysis to free esterified sterols. Thereafter the organic phase was extracted and evaporated under a stream of nitrogen. Then, samples were derivatized with TMS, evaporated and dissolved in hexane to run on the GC-MS. For LC-MS/MS analysis, the derivatization step was omitted and the samples were dissolved in methanol.

A previously validated method including a temperature gradient was used for GC-MS analysis.

To develop a LC-MS/MS method, three different columns were tested for separation. Other parameters that were experimented with are the composition of the mobile phase, gradient of the mobile phase, flow rate, column temperature and type of ionization.

For validation of the methods, following parameters were determined: linearity, intraday and interday precision, accuracy, recovery, lower limit of quantification (LLOQ), upper limit of quantification (ULOQ) and carry-over.

Results

The best separation was obtained with the ACQUITY UPLC HSS PFP 1.8 µm, 2.1 mm x 100 mm column using a combination of water and methanol as mobile phase with the gradient shown in Figure 1A. The flow rate was 0.4 mL/min, the column temperature was 15 °C and positive UniSpray Ionization was used.

There was no peak detected in the solvent blank, which indicates that there was no carry-over. Other validation results are shown in Table 1.

Some sample runs on the LC-MS/MS could not be included in the statistics due to no detection of peaks.

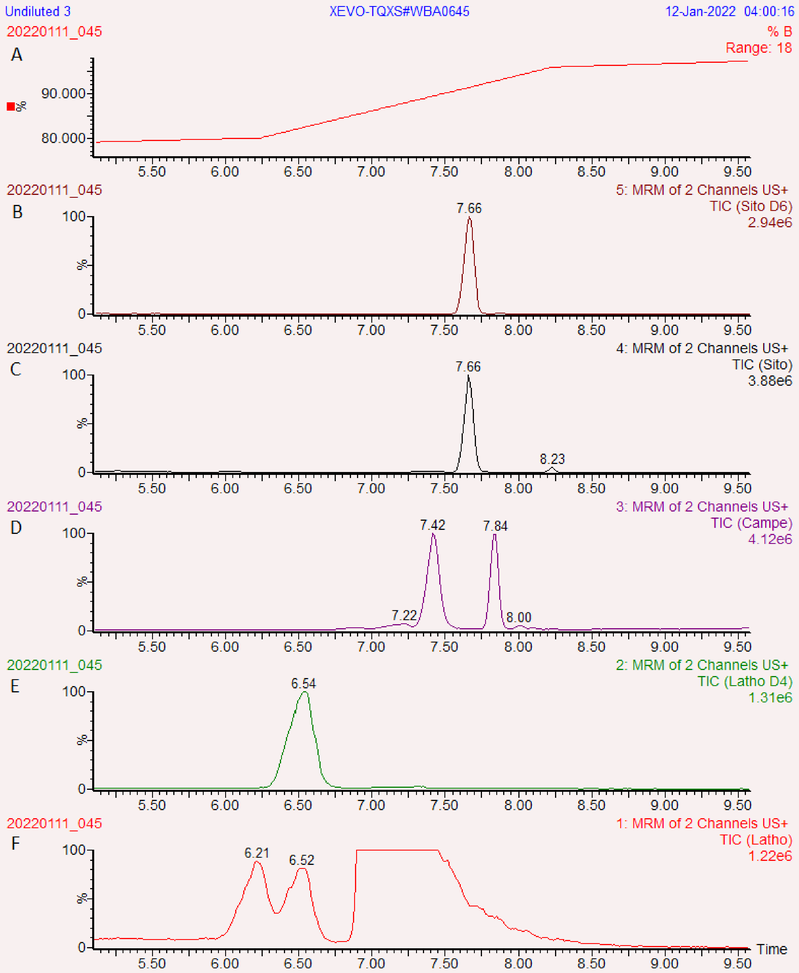

Figure 1. (A) LC-MS/MS gradient and (B-F) chromatograms of internal standards and serum samples.

Table 1. Validation results

		Intraday precision RSD (%)	Accuracy (%)	Recovery (%)	LLOQ (ng/mL)
GC-MS	Lathosterol	1.6[a]	90.5[c]	106[c]	35.7[e]
	Campesterol	3.5[a]	96.5[c]	104[c]	34.9[e]
	Sitosterol	2.4[a]	98.4[c]	91[c]	198[e]
LC-MS/MS	Lathosterol	5.2[b]	83.9[d]	109[d]	5.0[e]
	Campesterol	4.4[b]	86.6[d]	36.7[d]	0.49[e]
	Sitosterol	4.3[b]	92.0[d]	97.9[d]	0.74[e]

a) n = 10 runs; b) n = 11 runs; c) n = 12 runs; d) n = 15 runs; e) n = 3 runs

Discussion

Several aspects of LC-MS/MS needed to be optimized, including the choice of column. Lowering the column temperature from 60 °C to 15 °C had a positive impact on the separation of cholesterol and lathosterol. While a recovery higher than 100% is not reasonable, it was not unexpected at it has been reported in similar studies [2, 3]. However, the reason for this is not known. The recovery for campesterol in LC-MS/MS is significantly low and requires further investigation. Greater accuracy was found with GC-MS than with LC-MS/MS. This was not surprising, considering that the GC-MS method is a well-established and robust method that has been optimized over many years. LC-MS/MS is more sensitive than GC-MS, this is shown by the LLOQ. An advantage of this is that less sample needs to be used. A weakness of the LC-MS/MS is the stability, no peaks could be found in some sample runs. Presumably, this is due to air bubbles in the sample vials.

Conclusion

Preliminary results show that LC-MS/MS looks promising for the analysis of sterols. The developed method succeeds in separating and quantifying lathosterol, campesterol and sitosterol. However, further investigation and proper validation with more samples is required. The LC-MS/MS method is faster and more sensitive. But, the stability is lower and the machine is more difficult to operate. Also, sample preparation may need to be optimized. To avoid air bubbles hindering analysis, sonication of the sample could be done prior to loading on the machine. The need for additional steps in preparation makes LC-MS/MS less attractive as a better alternative to GC-MS.

Acknowledgements

We gratefully acknowledge the staff of Clinical Chemistry in Karolinska University Hospital Laboratory for granting access to their facilities and for sharing their knowledge about GC-MS.

References

1. Skubic C, Vovk I, Rozman D, Križman M. Simplified LC-MS method for analysis of sterols in biological samples. Molecules. 2002 Sep 9;25(18):4116.

2. Acimovic J, Lövgren-Sandblom A, Monostory K, Rozman D, Golicnik M, Lutjohann D, et al. Combined gas chromatographic/mass spectrometric analysis of cholesterol precursors and plant sterols in cultured cells. J Chromatogr B Analyt Technol Biomed Life Sci. 2009 Jul 15;877(22):2081-6.

3. McDonald JG, Smith DD, Stiles AR, Russell DW. A comprehensive method for extraction and quantitative analysis of sterols and secosteroids from human plasma. J Lipid Res. 2012 Jul;53(7):1399-409.