Separation of sterols in various matrices using derivatization to carbamates

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Introduction

The advent of modern LC-MS platforms greatly widened the analytical possibilities even for the non-advanced users. Moreover, LC-MS methodology is nowadays being introduced into clinical practice and therefore, there is growing concern in using LC-MS for assessment of analytes previously measured with other approaches. However, the mass detection itself is in some cases not able to separate isobaric compounds and the analyst has to rely on the steps preceding the MS detection, most often the (U)HPLC. This the case of some sterol compounds, for example cholesterol-lathosterol pair, the latter being used as a surrogate marker of cholesterol biosynthesis in the clinical practice. Other sterols, phytosterols, are nowadays claimed to have cholesterol lowering effect and are sometimes also used as surrogate markers of cholesterol absorption.

The aim of the study was to develop method useful for separation of some noncholesterol sterols including isobaric pair lathosterol-cholesterol in plasma.

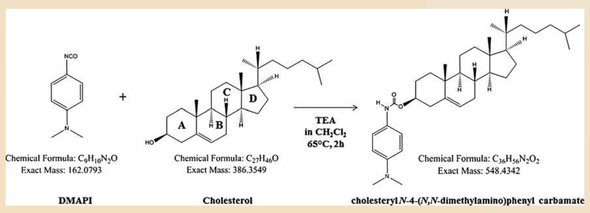
Materials and methods

Samples of nuts and seeds were extracted following the modified Folch procedure [1].

We adapted the method of Jonker [2] for acid hydrolysis with subsequent saponification of plasma samples or lipids extract (both with the internal standard epicoprostanol).

Dried sterols were resuspended with 200 µL of a solution containing DMAPI in dichloromethane (10 mg/mL) [3]. Thirty microlitre of triethylamine was added. The resulting mixture was vortexed and subsequently shaken for 2 h at 65 °C and 150 rpm in an incubator shaker. To quench the reaction, 150 µL of phosphate buffer (pH 8) was added, followed by 3 mL of hexane. The mixture was vortexed for 30 s and centrifuged. The upper layer containing the carbamate compounds was withdrawn and the organic solvent was evaporated under reduced pressure. The dry residues were reconstituted in 200 µL of acetonitrile/isopropanol (1:1, v/v) and 5 µL was injected into the UPLC-ESI-HRMS system.

Fig. 1: Reaction scheme for cholesterol derivatization with DMAPI



LC/MS	
Pump	Dionex Ultimate 3000
Analytical column	Hypersil GOLD column (150x2.1 mm, 3 µm), SecurityGuard column
Gradient	Binary mobile phase gradient consisted of
	A) acetonitrile/methanol/water (40/40/20 v/v/v) mixture with 0.1% acetic acid
	B) acetonitrile/methanol/water (45/45/10 v/v/v) mixture with 0.1% acetic acid
MS system	TSQ Quantum Access Max with H-ESI II probe
MRM transition	lathosterol 549.5 → 166.0 5α-cholestanol, epicoprostanol 551.5 → 166.0
	campesterol 563.6 → 166.0 β-sitosterol 577.5 → 166.0

Results and Discussion

We achieved complete separation of the lathosterol peak from cholesterol even in plasma, where cholesterol is present in high abundance (two to three orders of magnitude vs other sterols) thus preventing separation of the compounds only by SRM approach. Therefore, we included the derivatization step to increase the sensitivity of the MS detector thus enabling lower column load and more separation. The validation parameters showed good linearity with the range of 0,6-100 ng/ml and the results obtained were comparable with the commonly used GC methodology in our lab. The method was applied on the plasma samples of patients with end-stage renal disease, who showed higher concentrations of campesterol in comparison with matched control group. We also used the method for the analysis of sterols in nuts and seeds commonly available in the Czech market. The predominant sterols in nuts and seeds were β-sitosterol and campesterol with the exception of pumpkins and melons, where major sterols comprised of unusual D7 sterols.

Fig. 2: Example of sample analysis of human plasma

Tab.1: Sterols content in human plasma

	pacients on hemodialysis	controls
β-sitosterol	7,85 ± 3,87	6,93 ± 3,06
campesterol	11,60 ± 5,93*	9,20 ± 3,83
lathosterol	6,77 ± 3,91	6,79 ± 4,57

Data are in µmol/l (ave ± SD), n = 68 (both groups); control group age, sex, presence of DM matched;
* p < 0,05, ANCOVA (adjusted for BMI)

Tab. 2: Sterols content in nuts and seeds

	almond	apricot	brazil nut	cashew	coconut	flax seed	hazelnut	hempseed	chestnut	macadamia	peanut	pistachio	pumpkin*	sesame	sunflower	walnut
β-sitosterol	82,8 ± 2,0	91,5 ± 0,2	77,9 ± 3,1	83,7 ± 2,9	62,9 ± 0,8	55,0 ± 8,1	85,0 ± 0,7	79,4 ± 0,1	69,4 ± 0,1	82,2 ± 5,1	70,3 ± 3,5	85,8 ± 4,6	8,9 ± 2,5	72,8 ± 2,4	72,5 ± 6,9	81,0 ± 1,7
campesterol	1,5 ± 0,4	2,7 ± 0,0	0,8 ± 0,2	3,0 ± 1,3	5,1 ± 1,7	11,2 ± 3,4	2,5 ± 0,4	7,4 ± 0,6	5,9 ± 0,3	2,6 ± 0,8	7,6 ± 0,2	1,8 ± 0,5	0,9 ± 0,5	8,3 ± 0,8	4,2 ± 1,0	2,1 ± 0,9
stigmasterol	5,8 ± 1,0	0,2 ± 0,1	1,4 ± 0,3	3,7 ± 0,9	11,6 ± 0,9	6,8 ± 1,5	1,8 ± 0,4	3,4 ± 0,9	16,0 ± 0,0	5,4 ± 2,9	5,4 ± 0,8	4,0 ± 1,1	5,6 ± 2,5	8,0 ± 1,6	3,1 ± 1,0	4,4 ± 1,6

Data are in mol% (ave ± SD) of total sterol content in analyzed samples, further minor sterols comprised sitostanols, fucosterols, avenasterols and their isomers; average of 2-6 measurements; *major sterols comprised of unusual D7 sterols

Conclusion

The presented method is useful for the analysis of sterols including isobaric pair cholesterol-lathosterol in the various matrices including human plasma or sterol profiling of lipid extracts of seeds and nuts.

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