Analysis of sterol lipids as carbamates in metabolic syndrome

Marek Vecka, Barbora Staňková, Petra Tomášová, Magdalena Dušejovská, Aleš Žák

IVth Department of Medicine and Institute of Medical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles Univeristy and General University Hospital in Prague, Prague, Czech Republic

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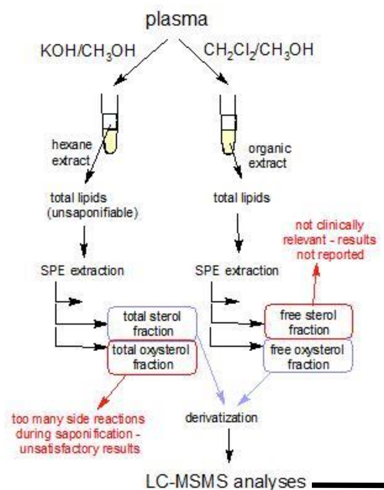
Aim: One advantage of targeted lipidomics versus untargeted approach is higher sensitivity. This is useful for human plasma samples, in which the presence of highly abundant isobaric sterol compounds (e.g. cholesterol) complicates the separation. Moreover, the issue can be solved by derivatization further lowering detection limits and column load. The aim of this study was method development for analysis of noncholesterol sterols and oxysterols including isobaric species in human plasma.

Methods: Lipids in human plasma were directly saponified or extracted [1], and the isolated SPE fractions [2] were derivatized to carbamates [3] and sterol lipids analysed with LC/MSMS platform. The method was applied on comparison of clinical samples of individuals with metabolic syndrome to control group.



HPLC

column: Hypersil GOLD column type C18 (150 × 2.1 mm, 3 μm); flow 0.4 ml/min, 50°C

mobile phase: sterols A H_2O (0.1% v/v HCOOH)
B ACN/CH_3OH (7/3 v/v)
A/B 25/75 (0 min) - 25/75 (30 min) - 15/85 (59 min) - 25/75 (60 min), equil. 4 min
oxysterols A H_2O (0.1% v/v HCOOH)
B ACN/CH_3OH/*i*-PrOH (400/100/5 v/v/v)
A/B 35/65 (0 min) - 28/72 (10 min) - 15/85 (31 min) - 35/65 (33 min), equil. 3 min

MS

TSQ Quantum Access Max with HESI-II probe

spray voltage	+4300 V	vaporizer	350 °C
sheath gas	34 a.u.	auxilliary flow	15 a.u.
sweep gas	1.2 a.u.	capillary	320 °C
skimmer offset	not used	collision gas	Ar, 1.0 mTorr

SRM parameters: parent ion → product ions (collision E); tube lens voltage

desmosterol	547.5 →	166(40) /181(29); 117
lathosterol	549.5 →	166(40) /181(30); 123
cholesterol	549.5 →	166(42) /181(31); 124
epicoprostanol(IS)	551.5 →	166(43) /181(32); 128
5α-cholestanol	551.5 →	166(43) /181(22); 123
campesterol	563.5 →	166(42) /181(33); 119
β-sitosterol	577.5 →	166(44) /181(34); 122
lanosterol	589.5 →	166(46) /181(31); 127

oxycholesterols with keto group (C4,3b7O)	563.5 →	181(18) /383(21); 89
C4-d7 (IS)	570.5 →	166(18) /390.5(21); 89
oxycholesterols with 3OH (TRIOL)	585.5 →	166(42) /181(35); 113
oxycholesterols with 2OH	565.5 →	166(42) /181(24); 111
oxycholesterols-d4 (IS)	569.5 →	166(42) /181(24); 111
oxycholesterols-d7 (IS)	572.5 →	166(42) /181(24); 111

Figure 1 Total sterol fraction analysis

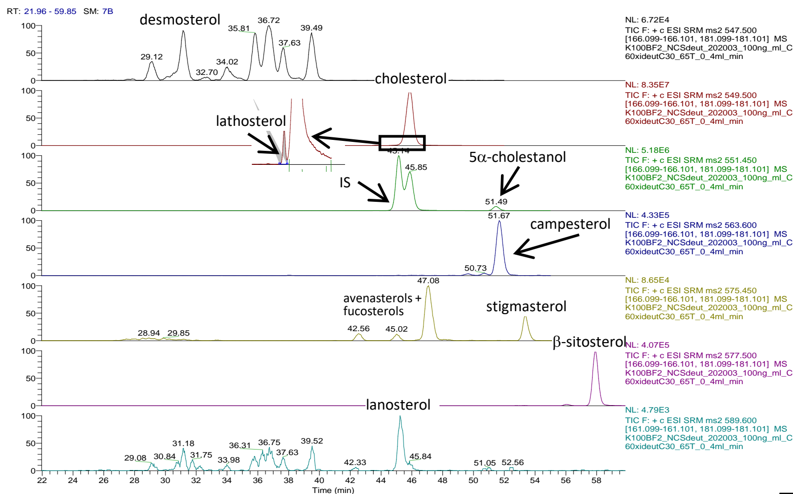
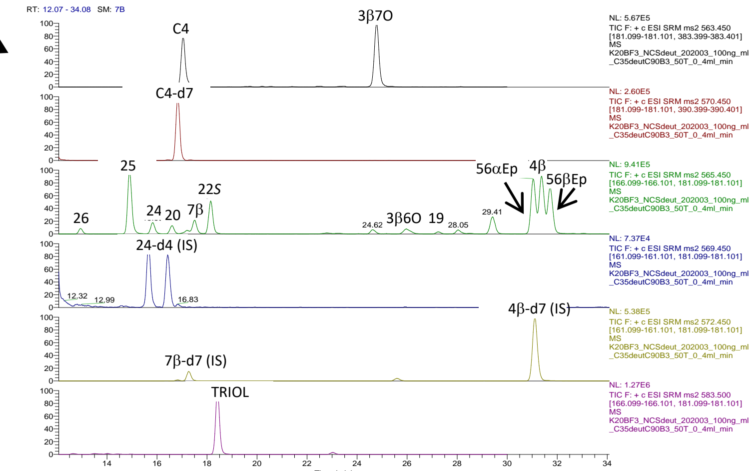


Figure 2 Free oxysterol fraction analysis



IS – internal standard, numbers indicate the location of hydroxyl group in oxysterol molecule, TRIOL – 3β,5α,6β-trihydroxycholesterol

Table 1 Basic characteristics of groups

parameter	control	MetSy
gender (M/F)	7/7	7/7
age (yrs)	33.5 ± 7.1[a]	37.2 ± 4.9
BMI (kg.m^{-2})	23.4 ± 2.0	31.4 ± 2.9***
TAG (mmol/L)	0.88 ± 0.30	2.81 ± 2.39***[b]
TC (mmol/L)	4.65 ± 0.66	5.19 ± 1.29***

*[a] – mean ± SD; BMI – body mass index, TAG – triacylglycerols, TC – total cholesterol; ***-p < 0.001 (t-test); [b] – transformed*

Results: We were able to analyze several noncholesterol sterols including lathosterol, campesterol, and β-sitosterol. From oxygenated derivatives of cholesterol, various mono-, di-, oxo- and epoxy- derivatives of cholesterol were analysed including C4 (metabolic precursor of bile acids). The method was applied for analysis of human plasma.

Table 2 Concentrations of selected plasma sterol lipids in groups

parameter	control	MetSy
total lathosterol (μmol/L)	5.6 ± 1.9[a]	8.7 ± 4.3*
total campesterol (μmol/L)	8.8 ± 3.7	9.1 ± 3.1
total β-sitosterol (μmol/L)	6.3 ± 2.4	5.7 ± 1.6
free C4 (ng/mL)	23.7 ± 9.1	25.4 ± 14.4
free 24*R*/*S*OH cholesterol (ng/mL)[b]	4.3 ± 2.7	5.2 ± 4.3
free 26OH cholesterol (ng/mL)[b]	9.4 ± 9.7	6.4 ± 6.5
free 7αOH cholesterol (ng/mL)[b]	10.1 ± 4.3	15.0 ± 14.7

[a] – mean ± SD; **-p < 0.05 (t-test), transformed for analysis*

Conclusions: With the presented method, it is possible to gain deeper insight into metabolism of sterols. The implementation of saponification step enables to analyze total concentrations of sterol lipids. Assessment of oxygenated cholesterol derivatives, including C4, is recommended without saponification step.

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Contact: marek.vecka@lf1.cuni.cz

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