Biochimie 95 (2013) 489–495

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Application of gas chromatography–triple quadrupole mass spectrometry to the determination of sterol components in biological samples in consideration of the ionization mode

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ARTICLE INFO

Article history:
Received 20 June 2012
Accepted 5 September 2012
Available online 4 October 2012

Keywords:
GC–triple quad MS
Oxysterols
Bile acids
Chemical ionization

ABSTRACT

The hyphenation of gas chromatography (GC) and triple quadrupole mass spectrometry is a promising approach to increase sensitivity and selectivity as compared to single quad mass spectrometry. We present in this paper the application of GC–triple quadrupole mass spectrometry for determination of sterol components in biological samples. Due to the fact that sterols are quite small molecules an appropriate ionization mode has to be found for advantageous exploitation of the triple quad function. Electron ionization (EI), positive and negative chemical ionization (PCI, NCI) have been tested regarding sensitivity improvement in oxysterol and bile acid analysis in plasma samples. Target analytes were 24-, 25- and 27-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol, 3β,5α,6β-cholestanetriol, cholic acid, chenodeoxycholic acid, deoxycholic acid and lithocholic acid. In contrast to bile acids, oxysterols could be analyzed with the highest degree of sensitivity by application of PCI in multiple reaction monitoring mode whereas 7β-hydroxycholesterol and 7-ketocholesterol showed even better results with NCI.

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1. Introduction

The determination of oxysterols plays an important role for the understanding of the pathogenesis of neurodegenerative diseases, atherosclerosis, genetic disorders of lipid metabolism, and inflammation [1–5]. Oxysterols are directly involved in the regulation of cholesterol homeostasis as raft membrane microdomain constituents and activators of liver X receptor (LXR) [6–8].

Beside their function in solubilization and digestion of lipid-soluble nutrients, bile acids are known as signaling molecules with endocrine functions. Bile acids are involved in lipid metabolism and energy homeostasis, as modulators of nuclear hormone receptors and are ligands for G-protein coupled receptors like TGR5 [9–11].

For several years a variety of approaches for the analysis of oxysterols in plasma, cell lysates, or tissues have been proposed. The most established techniques are based on gas or liquid chromatography coupled with mass spectrometry. Gas chromatography–single quad mass spectrometry (GC–single quad MS) is characterized by excellent separation of different species, but lacks time efficiency since GC runs take 20–30 min and need at least one derivatization step. Successful applications have been shown in many fields of oxysterol research and have been extensively reviewed, for details see Refs. [12–16]. Also, bile acids are targets in GC based techniques. Many protocols were described for sterol and bile acid analyses based on GC methods starting at the very first in the 1960s [17]. For example, Kimura and co-workers developed a GC–single quad MS based method for the analysis of 28 urinary bile acid species as methyl ester-dimethylethylsilylether methoxime derivatives [18]. Recently, Tsai et al. determined bile acids in tissues by GC coupled to ion trap MS with total ion chromatograms (TIC) and extracted ion chromatograms (EIC) with high sensitivity [19]. They also focused on the ionization mode to reduce the background. A combined analysis panel of cholesterol, oxysterols, and bile acids would offer detailed sterol profiling in clinical and epidemiologic studies. A very successful attempt toward a sterol panel was recently proposed by Kumar et al. for seven bile acid species and seven oxysterols in urine of rats [20]. They presented a fully validated method based on GC–single quad MS.

A drawback regarding the bile acid analysis with GC–MS is the fact that the analysis is often restricted to unconjugated bile acids. However, direct GC determination of free, glycine- and glucuronide-conjugated bile acids without deconjugation has been reported [21]. In contrast, liquid-chromatography coupled with

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http://dx.doi.org/10.1016/j.biochi.2012.09.015

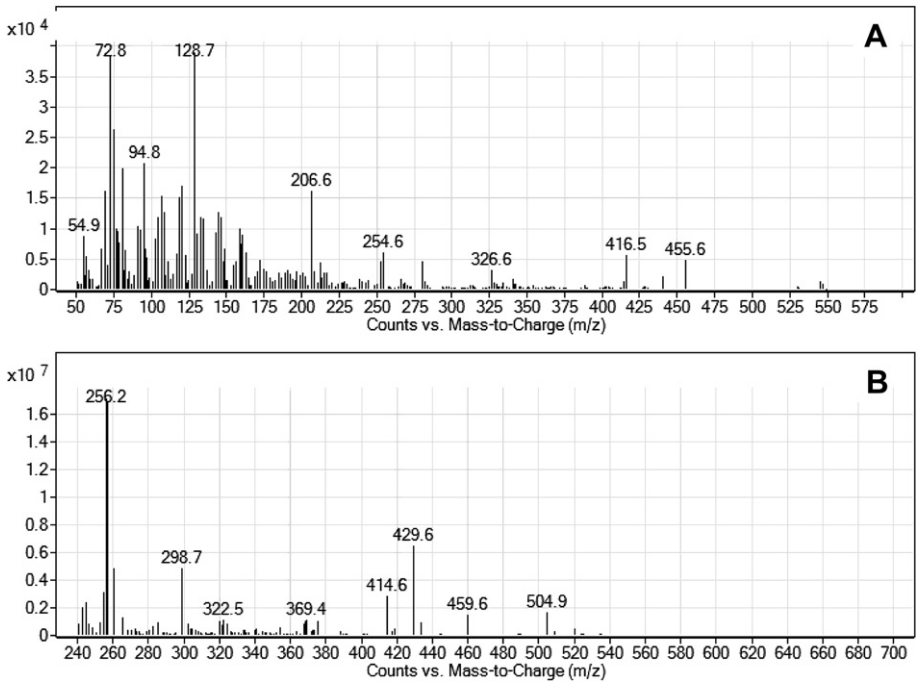

Fig. 1. Mass spectra of Q1 scan after electron ionization (EI) of the trimethylsilyl derivatives of A) 27-hydroxycholesterol and B) lithocholic acid.

triple quad spectrometry (LC–triple quad MS) allows the rapid and straightforward analysis of free and conjugated bile acids [22,23].

The LC–triple quad MS methodology provides also advantages for oxysterol analyses. Free sterols can be analyzed immediately after extraction without derivatization [24–26]. In order to improve the ionization efficiency and thus to enhance the sensitivity derivatization has been proposed when using LC–triple quad MS methods. The most promising approaches target the conversion to picolinyl esters [27] and Girard-P tagging after oxidation of the hydroxyl group [28]. Especially the latter derivatization procedure has been extensively applied in oxysterol analysis on an LTQ-Orbitrap with excellent potential for molecular structure determination [29–31].

The newest instrument developments enable the hyphenation of GC and triple quad MS. Using the first quadrupole for precursor ion selection, the second as collision cell and the third quadrupole for product ion selection a high discrimination against background signals should be achieved. Henceforward, the well-known advantages of precursor-product ion specificity known from LC–MS systems are transferred to a GC based system. Defining high abundant precursor ions and specific product ions makes the choice of ionization particularly important. Electron ionization (EI) with 70 eV, which is the widely used mode in GC, might be too harsh to provide precursor ions for small molecules such as sterols. In contrast, chemical ionization (CI) suffers from high background signals resulting from the high pressure of reagent gas. In this article, the authors compare the ionization modes EI, positive CI, and negative CI regarding the overall sensitivity for oxysterol and bile acid analysis with the MS in single quad and triple quad function.

Table 1
Characteristic ions and MRMs of oxysterols and bile acids after electron ionization (EI) obtained with standard solutions (TMS derivatives). Most intense ions are represented in bold.

Compound	t_r [min]	Ion 1	Ion 2	Ion 3	MRM
7β-Hydroxycholesterol	14.77	454.6	457.6	**545.8**	545.8/455.4
3β,5α,6β-Cholestanetriol	16.29	473.7	545.6	455	545.6/545.6
7-Ketocholesterol	17.27	455.8	**471.5**		472/382
24-Hydroxycholesterol	16.89	322.4	**412.6**		412.6/159.0
25-Hydroxycholesterol	17.13	455.2			455/376
27-Hydroxycholesterol	18.05	206.6	**416.2**	**455.6**	416.2/214.8
LCA	14.98	**256**	429	504.8	**429**/**215**
DCA	15.28	**254**	428.0	592.5	593/593[a]
CDCA	15.39	**254**	**427.7**		427.9/255[a]
CA	15.53	**253.5**	425.8	515.8	515.8/253.2;[a] 425.8/253[a]
UDCA	15.78	254	**427.5**	517.6	427.9/255.0;[a] **517.6**/**517.6**

[a] Very low intensity.

2. Materials and methods

2.1. Chemicals and solutions

Hexane, methanol, sodium chloride, and potassium hydroxide were purchased from VWR GmbH (Darmstadt, Germany). N-Methyl-N-trimethylsilyl-trifluoracetamide (MSTFA) was obtained from Macherey-Nagel GmbH & Co. KG (Düren, Germany). 7β-, 24-, 25-, 27-hydroxycholesterol, 3β,5α,6β-cholestanetriol, and 7-ketocholesterol were purchased from Avanti Polar Lipids (Alabaster, Alabama, USA). 24-Hydroxycholesterol-D_{10} and 27-hydroxycholesterol-D_6 were obtained from Sugaris GmbH (Münster, Germany). Cholic acid (CA), chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA), deoxycholic acid (DCA), lithocholic acid (LCA) and butylated hydroxytoluene were obtained from Sigma–Aldrich Chemie GmbH (Taufkirchen, Germany). 7-Ketocholesterol D_7 was purchased from Toronto Research Chemicals (North York, Ontario, Canada).

The standard substances were dissolved in chloroform/methanol (50/50) and stock solutions were prepared.

2.2. Samples and sample preparation

EDTA-plasma and serum were obtained from healthy volunteers by standard venipuncture techniques. Samples were immediately centrifuged at 2000 × g for 10 min. Butylated hydroxytoluene (BHT)

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