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Combined gas chromatographic/mass spectrometric analysis of cholesterol precursors and plant sterols in cultured cells

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ABSTRACT

We developed a powerful gas chromatographic/mass spectrometric method allowing quantitative analysis of 11 structurally similar cholesterol precursors and plant sterols (squalene, desmosterol, 7-dehydrocholesterol, lathosterol, zymosterol, dihydro-lanosterol, lanosterol, FF-MAS, T-MAS, campesterol, sitosterol) from cultured human hepatocytes in a single chromatographic run. Deuterium labelled cholesterol, sitosterol and lathosterol were used as internal standards. Care was taken to select ions for the detection that gave the most appropriate discrimination in the assay. Replicate analyses gave a coefficient of variation less than 6%. Recovery experiments were satisfactory for 7-dehydrocholesterol, campesterol, desmosterol, lathosterol, zymosterol and cholesterol with less than 7% difference between expected and found levels. For other sterols, the difference between expected and found levels varied between 10 and 16%. It is concluded that this method is suitable for studies on the effect of different inhibitors and stimulators of cholesterol synthesis in cultured cells. Additionally, the method is relevant also for clinical applications since abnormally increased late cholesterol intermediates in patients are representations of the inherited disorders linked to different enzyme defects in the post-squalene cholesterol biosynthesis.

1. Introduction

Biosynthesis of cholesterol from acetate involves more than 30 different enzymes and a similar number of intermediates [1,2]. The rate-limiting enzyme in the overall conversion is HMG CoA reductase, catalyzing HMG CoA conversion into mevalonate [3]. Changes in the rate of cholesterol synthesis are associated with the alterations in the steady-state levels of the various intermediates. These changes can be expected to occur earlier and to be more marked than the corresponding changes in cholesterol levels. As a consequence, measurements of cholesterol precursors in cells, tissues or plasma are often used for the evaluation of cholesterol synthesis under different conditions. In a previous work from this laboratory, we found that plasma levels of lathosterol are a particularly sensitive marker for hepatic HMG CoA reductase activity in humans [4]. The determination of plasma or tissue levels of lathosterol is thus suitable for the evaluation of the effect of inhibitors of HMG CoA reductase. Other intermediates in cholesterol biosynthesis may, however, be more suitable as markers in connection with other types of inhibitors [5]. As an example, the inhibition of CYP51, catalyzing demethylation of 14-methylated sterols, can be expected to result in the accumulation of lanosterol and a reduction of lathosterol [6]. Up to this time six inherited disorders have been identified that can be linked to different enzyme defects in the post-squalene cholesterol biosynthetic pathway. Desmosterolosis, Smith-Lemli-Opitz syndrome (SLOS), lathosterolosis, Conradi-Huenermann-Happle syndrome (CDPX2), Greenberg skeletal dysplasia (also known as HEM skeletal dysplasia) and CHILD (Congenital Hemidysplasia with Ichthyosiform erythroderma and Limb Defects) syndrome are connected with abnormally increased levels of desmosterol, 7-dehydrocholesterol, lathosterol, zymosterol, FF-MAS and T-MAS, respectively. Disease-causing mutations in genes encoding the implicated enzymes (DHCR24, DHCR7, SC5DL, EBP, DHCR14 and EBP and/or NSDHL) were shown to be responsible for the metabolic defects [2]. A number of methods based on combined gas chromatography/mass spectrometry (GC/MS) have been described for assays of different cholesterol precursors in various biological systems [7–11]. Many of these methods do not have sufficient sensitivity for quantitative

Abbreviations: FF-MAS, follicular fluid meiosis activating sterol; T-MAS, testis meiosis activating sterol; HMG CoA, hydroxymethyl-glutaryl coenzyme A; GC/MS, gas chromatography/mass spectrometry.

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evaluation of cholesterol homeostasis in cultured cells. They do also not allow separation of all major post-squalene cholesterol precursors in a single run, which is particularly important when dealing with precious clinical samples. In the present work, the method of Tamboli et al. [10] and Lindenthal et al. [11], has been optimized and modified, to allow separation/quantification of nine structurally related cholesterol intermediates (squalene, desmosterol, 7-dehydrocholesterol, lathosterol, zymosterol, dihydro-lanosterol, lanosterol, FF-MAS, T-MAS) and two plant sterols (campesterol and sitosterol). With 2 deuterated internal standards (d4-lathosterol and d6-sitosterol), this combines to 13 sterols that are quantitatively determined after a single chromatographic run. Cholesterol as the most abundant mammalian sterol is analyzed in a separate chromatographic run. Our method has sufficient accuracy to allow quantitative studies of cholesterol homeostasis in cultured mammalian cells as well as to quantify cholesterol precursors in clinical samples.

2. Experimental

2.1. Chemicals and reagents

All chemicals and solvents were of the highest purity available. Commercial sterol standards desmosterol, 7-dehydrocholesterol, lathosterol, zymosterol, campesterol, 24,25-dihydro-lanosterol, lanosterol, sitosterol were purchased from Steraloids (Newport, RI, USA). Cholesterol was purchased from Sigma Chemie GmbH (Deisenhofen, Germany). Hexadeuterium labelled cholesterol as well as tetradeuterium labelled lathosterol were obtained from Medical Isotopes Inc. (Pelham, NH, USA). Unlabelled squalene was obtained from Sigma–Aldrich GmbH (Steinheim, Germany). FF-MAS and T-MAS were purchased from Laboratory of Reproductive Biology (The Juliane Marie Center for Children, Women and Reproduction, University Hospital of Copenhagen, Copenhagen, Denmark). Chemicals for hepatocyte isolation, cell culture media and supplements were purchased from Sigma Chemie GmbH (Deisenhofen, Germany) and Merck (Darmstadt, Germany).

2.2. Synthesis of deuterium labelled campesterol/sitosterol

Oppenauer-oxidation of a mixture of sitosterol/campesterol (47:53, w:w) led to the corresponding 3-oxo-delta-4 compound (24-methyl or ethyl-cholest-4-en-3-one). Addition of $NaOCH_3$, CH_3OD and D_2O led to H/D-exchange and intermediate formation of the 3-oxo-derivatives of 2,2,4,6,6-D_5-campesterol/sitosterol. Protection of the 3β-OH-group using AcOH led to the corresponding 3β-OAC group and isomerisation to the corresponding enolate sterol with double bonds at C3 and C5. Reduction with $NaBD_4$ gave the corresponding 3β-OAC-compound with deuteration at position 2,2,3,4,4,6 and subsequent hydrolysis using $NaHCO_3$ gave a mixture of 2,2,3,4,4,6-campesterol/sitosterol. The mixture obtained was purified by silica gel chromatography (60–230 mesh). The labelled sterols were eluted with n-hexane/acetone (1:1, v/v) and recrystallized from acetone, yielding a white powder with m.p. 139 °C. The yield was 25% from the original mixture.

2.3. Isolation and culture of human hepatocytes

Human liver tissue was obtained from a 44-year-old male cadaver donor (HH-172) at the Transplantation and Surgical Clinic (Semmelweis University, Budapest, Hungary). Permission of the Hungarian Regional Committee of Science and Research Ethics was obtained to use human tissues. Liver cells were isolated by the method of Bayliss and Skett [12]. One of the branches of portal vein in the liver tissue was cannulated, and the tissue was perfused with Ca^{2+}-free Earl's balanced salt solution (EBSS) containing EGTA (0.5 mM) and then with Ca^{2+}-free EBSS, finally with the perfusate containing collagenase (Type IV, 25 mg/100 ml) and Ca^{2+} (2 mM). Perfusion was carried out at pH 7.4 and at 37 °C. Softened tissue was gently minced and suspended in ice-cold hepatocyte dispersal buffer. The cells were filtered and isolated by low-speed centrifugation ($50 \times g$), and washed three times in hepatocyte dispersal buffer and once in culture medium. Hepatocytes having viability better than 90% as determined by trypan blue exclusion were used in the experiments. The cells were plated at a density of 1.5×10^5 cells/cm^2 in plastic dishes precoated with collagen in serum-containing medium (5%) described by Ferrini et al. [13]. After an overnight culture, the medium was replaced by serum-free medium. Culture medium has been renewed every 24 h. Forty-eight hours after serum deprivation, cells were cultured for 48 h.

2.4. Sterol extraction

The human primary hepatocytes (2–4 million cells) were washed in phosphate buffer pH 7.4, harvested and stored at −80 °C. After thawing, the cells were dried in a vacuum centrifuge for about 4 h at 40 °C. The cells were then weighed (3–7 mg), put into glass vials with 2 ml of Folch (chloroform/MeOH, 2:1; v/v) with argon on top and sealed with a teflon cap. After 24 h at room temperature, 100 μl and 1800 μl of the extract were transferred into new vials for the assay of cholesterol and other sterols, respectively. Internal standards were added; 2 μg d6-cholesterol for cholesterol analysis and 150 ng d6-sitosterol + 150 ng d4-lathosterol for the other sterols. The extracts were evaporated under a stream of argon, 1 ml of hydrolysis solution (8 g NaOH dissolved in 20 ml distilled water + 180 ml EtOH (99.5%)) was added and shaken for 2 h in a water bath at 65 °C. Distilled water (0.5 ml), and cyclohexane (3 ml) were added, vortexed for 30 s and centrifuged at 3500 rpm for 10 min. The upper organic phase was transferred into a new vial, and the extraction was repeated once more with 3 ml of cyclohexane. Extracts were pooled and evaporated under a stream of argon in heating blocks at 60 °C for derivatization.

2.5. Derivatization

TMS reagent (pyridine/hexamethyldisilazane/chloromethylsilane, 3:2:1, v/v/v), 100 μl, was added to the dried extracts and the sealed tube was treated at 60 °C for 30 min. The solvent and reagents were removed under a stream of argon at 60 °C until complete dryness. The residue was dissolved in hexane, in 80 μl for sterols and in 200 μl for cholesterol. The clear hexane phase was transferred into glass vial, suitable for GC/MS injection.

2.6. Gas chromatography/mass spectrometry (GC/MS)

The samples for cholesterol analysis were run on an Agilent HP 6890N gas chromatograph–Agilent HP 5973 MSD quadropole mass spectrometer using electron impact ionisation mode (Stockholm, Sweden). One microliter of each sample was injected into the gas chromatograph inlet via autosampler. The injector temperature was held at 280 °C throughout the analysis. Injection was performed in a pulsed splitless mode. Separation was performed on a HP-ultra1 (Scantec Lab AB, Gothenburg, Sweden) 25 m capillary column (0.20 mm i.d., 0.33 μm phase thickness), using helium as carrier gas at a flow rate of 1 ml min^{-1}. The initial column temperature of 180 °C was held for 1 min, then programmed at a rate of 20 °C min^{-1} until reaching 250 °C, then raised to 300 °C at a rate of 5 °C min^{-1} and maintained at this temperature for further 8.5 min, giving a total runtime of 23.0 min.

In preliminary experiments attempts were made to achieve a better separation between the different cholesterol precursors by the use of a 50 m fused silica column (CS-FS-OV 101, Fa.Chrmopack) 0.32 mm i.d. and 0.25 μm phase thickness as well as a 25 m SE 30 column with similar content.

The samples for sterol analysis were run on an Agilent HP 5890 gas chromatograph–Agilent HP 5972 MSD quadropole mass spectrometer using electron impact ionisation mode (Stockholm, Sweden). Two microliters of each sample were injected into the gas chromatograph inlet via autosampler. The injector temperature was held at 280 °C throughout the analysis. Injection was performed in a splitless mode. Separation was performed on a HP-5MS (Scantec Lab AB, Gothenburg, Sweden) 30 m capillary column (0.25 mm i.d., 0.25 μm phase thickness), using helium as carrier gas at a flow rate of 0.8 ml min^{-1}. The initial column temperature of 180 °C was held for 1 min, then programmed at a rate of 20 °C min^{-1} until reaching 250 °C, then raised to 300 °C at a rate of 4 °C min^{-1} and maintained at this temperature for further 7 min, giving a total runtime of 24.0 min.

The concentrations of each sterol were estimated on the basis of an adequate standard curve using its respective sterol standard. Cholesterol was quantified using d6-cholesterol as the internal standard; campesterol and sitosterol by using d6-sitosterol and the other sterols by using d4-lathosterol.

2.7. Recovery calculation

Replicas of samples ($n = 6$) with and without the addition of the authentic compound were analyzed by GC/MS. Mean, standard deviation and coefficient of variation were calculated (Table 2). The recovery (R) was calculated as the mean of measured sterol concentration with sterol standard addition (M_+) divided by the mean of expected sterol concentration (E), whilst E was calculated as the mean of measured sterol concentration without sterol standard addition (M_-) plus sterol standard amount added (AA), as shown in Eq. (1):

$$R = \frac{\bar{M}_+}{\bar{E}}; \quad \bar{E} = \bar{M}_- + AA \tag{1}$$

3. Results and discussion

3.1. Selection of the normalization parameters for sterol quantification

Normalization is an extremely important issue when dealing with biological samples since it can contribute to a big variation in the final quantification of biological molecules. In the literature, the concentration of sterols is usually expressed as ng/mg of wet/dry tissue weight [14–16]. We have performed a number of replicate pilot experiments to decide whether the concentration of the sterols should be expressed in relation to wet weight or dry weight of cells, ng/mg protein or ng/mg cell DNA. Under the conditions employed, the most reproducible results were always obtained expressing the results in relation to weight of the dried samples using the procedure described in Section 2.4.

3.2. Quantification of cholesterol

The concentration of cholesterol is two to four orders of magnitude higher than that of all other sterols (Table 2) and it is difficult to get a linear response of the instrument in such a broad range. Thus we assayed cholesterol under separate chromatographic conditions using 1/20 of the extract compared to 18/20 used in the assay of the precursors.

3.3. Selection of optimal ions for sterol identification

In order to get maximal stability in the measurements, 13 different ions were followed through the entire chromatographic analysis without interruption. In view of the fact, that the mass spectra of some of precursors are very similar (Fig. 1) and they have a similar or identical retention time, selection of optimal ions for detection is not trivial. Table 1 gives an overview of the retention times and ions used in the assay.

Attempts to find chromatographic conditions giving baseline separation among all the different sterols has failed. Under the applied chromatographic conditions, desmosterol and 7-dehydrocholesterol could thus not be separated (only 0.03 min difference in retention time). Attempts to obtain a better separation by the use of a 50-m fused silica column (CS-FS-OV 101) or a 25-m SE 30 column or use of different temperature gradients did not yield a better separation.

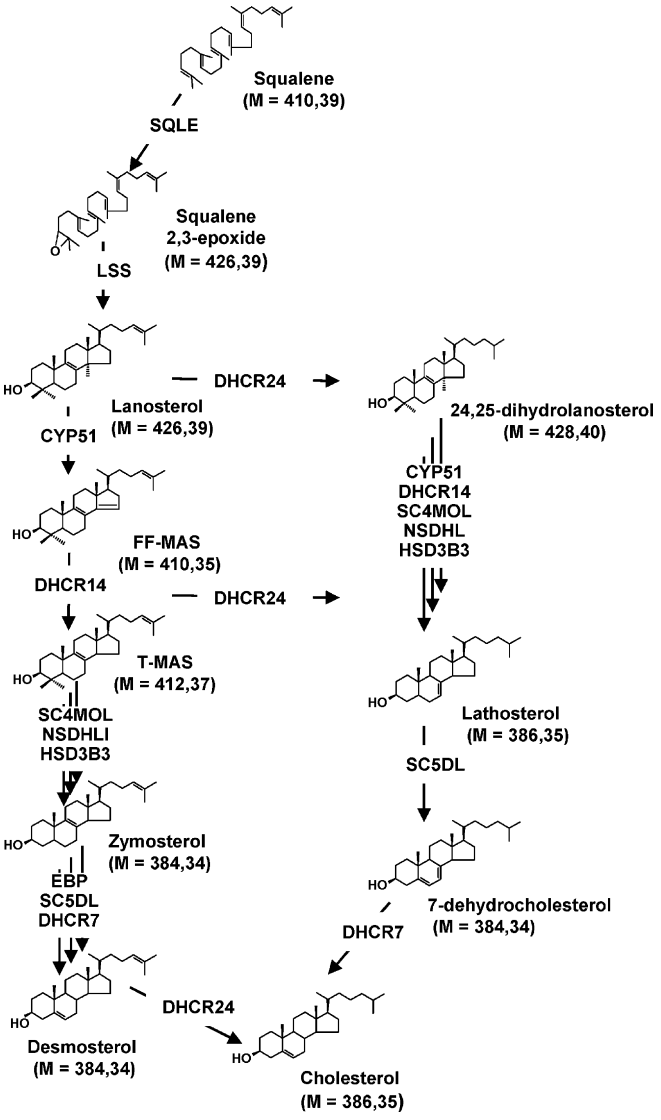

Fig. 1. Cholesterol biosynthesis. Chemical structures and molecular weights of measured sterol intermediates, with exception of squalene-2,3-epoxide that is not measured. Arrows represent enzymes catalyzing reactions, respectively. SQLE, squalene epoxidase; LSS, lanosterol synthase; CYP51, lanosterol 14α-demethylase; DHCR24, sterol Δ24-reductase; DHCR14, sterol Δ14-reductase; SC4MOL, sterol C4 methyl-oxidase; NSDHL, 3β-hydroxy Δ5-steroid dehydrogenase; HSD3B3, 3β-keto reductase; EBP, sterol 8,7-isomerase; SC5DL, sterol C5-desaturase; DHCR7, sterol Δ7-reductase.

Table 1
Detailed data for GC/MS sterol analyses.

Number	Trivial name	Chemical name	*m/z* ion measured	Retention time (min)[a]
1	Squalene	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene	410	11.48
2	Desmosterol	5,24-Cholestadien-3β-ol	343	16.33
3	7-Dehydro-cholesterol	5,7-Cholestadien-3β-ol	325	16.34
4	d4-Lathosterol	1,2,5α,6α-$^{2}H_4$-5α-cholest-7-en-3β-ol	462	16.48
5	Lathosterol	5α-Cholest-7-en-3β-ol	458	16.51
6	Zymosterol	5α-Cholesta-8,24-dien-3β-ol	456	16.55[a]
7	Campesterol	5-Cholesten-24α-methyl - 3β-ol	382	17.02
8	24,25-Dihydro-lanosterol	4,4,14α-Trimethylcholesta-8(9)en-3β-ol	395	17.50
9	Lanosterol	4,4,14α-Trimethylcholesta-8(9),24-dien-3β-ol	393	18.07
10	d6-Sitosterol	2,2,3,4,4,6-$^{2}H_6$-5-cholesten-24β-ethyl-3β-ol	402	18.10
11	Sitosterol	5-Cholesten-24β-ethyl-3β-ol	396	18.17
12	FF-MAS	4,4-Dimethylcholesta-8(9),14,24-trien-3β-ol	482	18.32
13	T-MAS	4,4-Dimethylcholesta-8(9),24-dien-3β-ol	484	18.41

[a] The retention times shown are those obtained in the chromatogram shown in Fig. 2, analysis of a sample that did not contain detectable levels of zymosterol. The retention time for zymosterol was calculated from the relative retention time of this sterol obtained in other chromatograms.

The ion at *m/z* 343 is dominant in the mass spectrum of desmosterol, but practically absent in the mass spectrum of 7-dehydrocholesterol. The situation is reverse with the ion at *m/z* 325.

The ratio between the ions *m/z* 343 and *m/z* 325 was thus 18 in the mass spectrum of the trimethylsilyl ether of desmosterol but less than 0.01 in the mass spectrum of the corresponding derivative of 7-dehydrocholesterol. This means that in the measurement of pure desmosterol falsely high levels of 7-dehydrocholesterol will be obtained, corresponding to 4% of the measured level of desmosterol. This was confirmed in several repeated measurements of pure desmosterol. In the measurement of pure 7-dehydrocholesterol less than 1% was measured as desmosterol. This was also confirmed in repeated measurements. Using the ions at *m/z* 343 for the assay of desmosterol and the ion at *m/z* 325 for 7-dehydrocholesterol, it was thus possible to get a sufficient discrimination between these two sterols. However, in measurements of very high levels of desmosterol (levels considerably higher than those of 7-dehydrocholesterol) a correction for the overlapping may be required (this situation never occurred in the primary hepatocyte samples studied here).

Lathosterol and zymosterol were eluted at similar retention times (0.05 min difference in retention time); however, the molecular ion of lathosterol at *m/z* 458 and the molecular ion of zymosterol at *m/z* 456 provided sufficient discrimination of these two sterols (cf. Fig. 2). The sufficient discrimination of the two precursors FF-MAS and T-MAS was achieved by the baseline separation and using the molecular ions at *m/z* 482 and *m/z* 484, respectively.

Fig. 2 shows a typical chromatogram obtained in the analysis of trimethylsilyl ether of a biological sample (extract of cultured human primary hepatocytes). This chromatogram does not include cholesterol, which appears just before the cholesterol precursors except squalene. Squalene appears before cholesterol (retention time 11.61) and there are no contaminating compounds in this part of the chromatogram (Fig. 2A). This specific sample did not contain significant levels of zymosterol.

3.4. Precision and recovery

In order to evaluate precision and recovery, replicate analyses of the different sterols were performed before and after the addition of known amounts of the authentic compounds. With exceptions (zymosterol, FF-MAS (550%) and sitosterol (900%)) the latter were added in amounts corresponding to 100–200% of the endogenous levels (Table 2). In the case of zymosterol cultured untreated primary human hepatocytes contain trace amount of zymosterol only; this cholesterol precursor may however accumulate under certain conditions. As shown in Table 2, a satisfactory precision was found in the analyses of all the sterols (CV less than 7%) with one exception (sitosterol at lower concentration). The results of the recovery experiments were satisfactory in case of 7-dehydrocholesterol, campesterol, desmosterol, lathosterol, zymosterol and cholesterol (difference between expected and found level < 7.5%). Less satisfactory results were obtained for sitosterol, squalene, lanosterol, FF-MAS, T-MAS, dihydro-lanosterol (difference between expected and found lev-

Table 2
Precisions and recovery of sterols measured expressed as ng/mg sterol weight per dry cell weight. *Expected* represents mean measured quantities of the sterol + the added quantity of the same sterol. *Measured* (*before addition*) represents the measured quantity of the sterol in the sample and *Measured* (*after addition*) the measured quantity of the sterol after addition of the same sterol. *Recovery* represents the ratio between measured and expected result (cf. Section 2.7).

Sterol	*n*	Measured (before addition) (ng/mg)			Amount added		Expected (ng/mg)	Measured (after addition) (ng/mg)			Recovery (%)	
		Mean	S.D.	CV(%)	(ng/mg)	%		Mean	S.D.	CV (%)	Mean	S.D.
Squalene	6	35.05	1.38	3.9	37.7	107	72.7	62.66	1.52	2.4	86.1	4.0
Desmosterol	6	2.30	0.08	3.3	3.4	148	5.7	5.59	0.20	3.5	97.9	4.7
7-Dehydro-cholesterol	6	1.48	0.09	6.0	3.3	228	4.8	5.09[a]	0.28	5.5	106.3[a]	8.6
Lathosterol	6	7.50	0.32	4.2	8.1	108	15.6	15.29	0.47	3.1	97.9	5.1
Zymosterol	6	0.00	0.00		3.2		3.2	3.04	0.09	3.1	94.3	2.3
Campesterol	6	8.22	0.56	6.9	19.7	227	27.9	29.97	1.10	3.7	107.3	8.3
Dihydro-lanosterol	6	1.21	0.06	4.9	2.0	164	3.2	2.81	0.08	2.8	87.9	5.0
Lanosterol	6	3.17	0.14	4.5	4.9	154	8.0	7.00	0.19	2.8	87.1	4.6
Sitosterol	6	3.45	0.59	17.1	28.2	904	31.6	34.63	1.71	4.9	109.6	19.6
FF-MAS	6	0.32	0.02	6.4	1.7	554	2.1	1.82	0.05	2.9	88.0	6.2
T-MAS	6	1.32	0.07	5.6	2.3	176	3.6	3.06	0.11	3.7	84.0	5.7
Cholesterol	6	8608	540	6.3	11194	130	19802	19289	634	3.3	97.4	6.9

[a] When corrected for the contribution from desmosterol due to the presence of *m/z* 343 in the mass spectrum of 7-dehydrocholesterol (cf. above) the mean was calculated to be 4.87 and the recovery 101%.

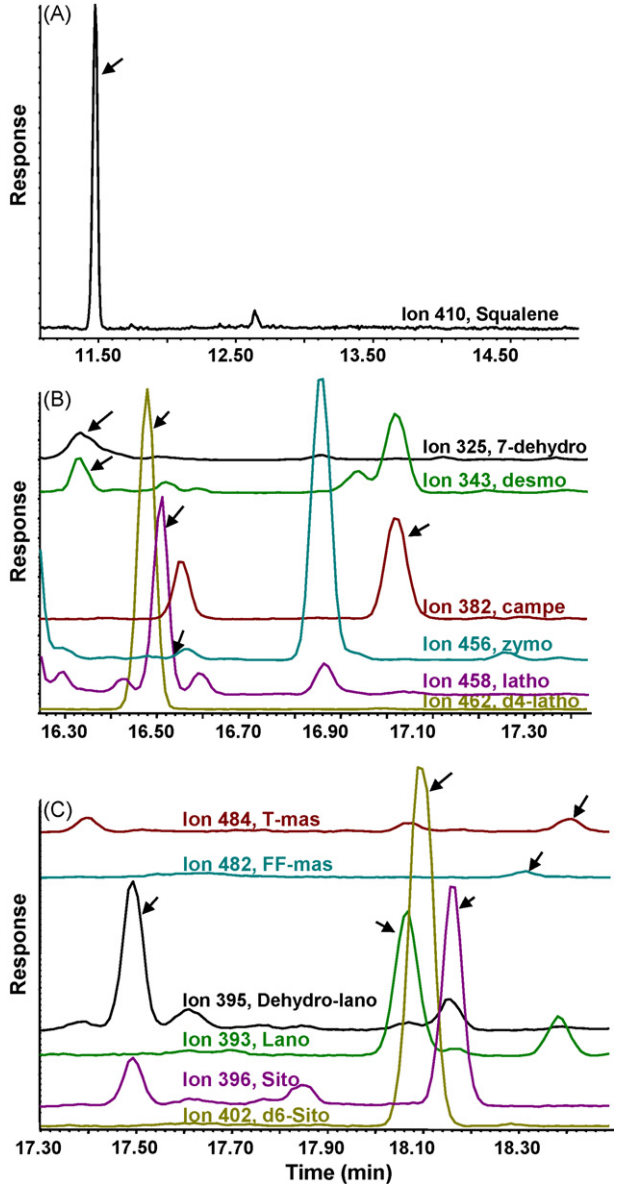

Fig. 2. Chromatogram obtained in analysis of trimethylsilyl ether of an extract of human primary hepatocytes. (A) Chromatogram between 11.46 and 14.55 min; (B) chromatogram between 16.24 and 17.30 min; (C) chromatogram between 17.30 and 18.50 min. The part of the chromatogram preceding 16.30 min containing cholesterol is not shown. The sample analyzed here did not contain zymosterol and the arrow shows the retention time of zymosterol as calculated from relative retention time obtained in analysis of a standard.

els between 10% and 16%). Repeated experiments gave similar results.

The possibility of sterol contamination from solvents or glassware was investigated by the analysis of blank samples that underwent the same extraction, hydrolysis and derivatization procedures as the authentic samples. With the exception of trace amounts of squalene and sitosterol found in some blank experiments, no significant amounts of the other sterols were detected in the blank samples (data not shown).

The less satisfactory recovery tests for lanosterol and dihydrolanosterol may be the consequence of the very low levels and/or presence of contaminants with similar mass spectrum and retention time. Due to the analytical variations, the changes detectable with this assay are of the magnitude 5–20% depending upon the specific precursor measured.

The analytical variations must, however, always be evaluated in relation to the magnitude of the changes expected in connection with experiments with different inhibitors and stimulators. In a typical experiment using human primary hepatocytes exposed to a statin, the reduction of the levels of cholesterol precursors was as follows: lathosterol, 91%; lanosterol, 76%; dihydro-lanosterol, 67%; zymosterol, 81%; T-MAS, 86%; 7-dehydrocholesterol, 81%; desmosterol, 92% and squalene, 78% (Björkhem et al., unpublished). In relation to these changes, the analytical variations are small and it is possible to draw firm conclusions with respect to the degree of inhibition.

3.5. Biological and clinical significance

Intermediates of the late post-squalene cholesterol synthesis were suggested to have tissue-specific signalling roles that are not dedicated to cholesterol only [17]. Elevated cholesterol precursors were recently determined as a hallmark for Cerebrotendinous xanthomatosis [18]. So far six inherited disorders are linked to different enzyme defects in the post-squalene cholesterol synthesis where abnormally increased levels of cholesterol intermediates in patients are representations of the diseases [2]. Our method that was developed for the human primary hepatocytes (primary cultures from the human liver), allows quantification of these important sterols with not yet fully explored biological roles in mammalian primary cell cultures or tissues, opening a possibility for application also in the clinics.

4. Conclusions

We describe a powerful method for separation and quantification of nine structurally similar cholesterol precursors and two plant sterols in cultured human cells. The number of analytes has been increased in relation to the previously published methods [7–11]. More specific ions were chosen in relation to previous publications. As an example the ion at m/z 456 was previously used in the quantification of desmosterol in spite of the fact that this ion is present also in the mass spectrum of 7-dehydrocholesterol which was probably not separated from desmosterol under the conditions employed [8]. In contrast to the previously published similar methods for the assay of several cholesterol precursors [10,11] we have evaluated the precision of the assay, made recovery tests, and defined the limits of the methodology. Together with 2 deuterated standards, the method allows for the first time the separation/quantification of 11 sterol related ions in a single non-interrupted chromatographic run.

Our method is suitable for studies of sterol uptake and cholesterol homeostasis in normal, transfected, drug-inhibited or silenced cell systems. Since the method allows quantification of the major post-squalene cholesterol intermediates in a single chromatographic analysis, it is ready to be applied also on the precious clinical samples.

Conflict of interest

There are no competing interests.

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