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Oxysterols in cosmetics—Determination by planar solid phase extraction and gas chromatography–mass spectrometry

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ABSTRACT

Sterol oxidation products (SOPs) are linked to several toxicological effects. Therefore, investigation of potential dietary uptake sources particularly food of animal origin has been a key issue for these compounds. For the simultaneous determination of oxysterols from cholesterol, phytosterols, dihydrolanosterol and lanosterol in complex cosmetic matrices, planar solid phase extraction (pSPE) was applied as clean-up tool. SOPs were first separated from more non-polar and polar matrix constituents by normal phase thin-layer chromatography and then focussed into one target zone. Zone extraction was performed with the TLC–MS interface, followed by gas chromatography-mass spectrometry analysis. pSPE showed to be effective for cleaning up cosmetic samples as sample extracts were free of interferences, and gas chromatographic columns did not show any signs of overloading. Recoveries were between 86 and 113% with relative standard deviations of below 10% (n = 6). Results of our market survey in 2016 showed that some cosmetics with ingredients of plant origin contained phytosterol oxidation products (POPs) in the low ppm range and therefore in line with levels reported for food. In lanolin containing products, total SOPs levels (cholesterol oxidation products (COPs), lanosterol oxidation products (LOPs), dihydrolanosterol oxidation products (DOPs)) being in the low percent range exceeded reported levels for food by several orders of magnitudes.

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1. Introduction

Sterols are a class of non-polar lipids which are found in fat of plant and animal origin. Cholesterol is the major sterol of animal fat, whereas lanosterol and dihydrolanosterol are components of sheep wool fat and its refined product lanolin. Phytosterols are present in plant oils, with β-sitosterol, campesterol and stigmasterol being the most common congeners. Containing at least one carbon–carbon double bond, sterols are prone to reactions with oxygen resulting in the formation of sterol oxidation products (SOPs). [1–4]

SOPs, particularly cholesterol oxidation products (COPs) and phytosterol oxidation products (POPs) have repeatedly been reported to possess adverse biological properties [2,5–11]. For the assessment of countermeasures against high SOPs intake, COPs and POPs contents have been determined in a wide variety of foods, with the focus on food processing procedures suspected of increasing SOPs levels [5,10,12]. Besides food, cosmetic products may also play a role as exogenous SOPs sources as they often contain lanolin, phytosterols, the unsaponifiable extract of oils and pure vegetable oils. [5,6]. In the case of lip care products or nursing ointments, the way of absorption might even be oral [6]. Especially lanolin is known to contain high cholesterol (∼15%), lanosterol (∼7%) and dihydrolanosterol (∼5%) levels [4,13,14]. Furthermore, as far as we know, no data have been published on the occurrence of lanosterol oxidation products (LOPs) and dihydrolanosterol oxidation products (DOPs) in consumer products. These aspects render lanolin an issue of special concern.

In order to estimate human SOPs intake, sophisticated analytical methods capable of determining low levels in complex matrices e.g. cosmetics are required. Most published methods for the determination of SOPs in food include a clean-up step of the saponified or transesterified lipid extract prior to gas chromatography, preferably coupled to a mass spectrometer [15,16]. The removal of matrix compounds is mostly performed with solid phase extraction (SPE) [16–19] or preparative liquid chromatography (LC) [20,21]. Both clean-up techniques, however, have a low capacity for sample extracts and overloading may lead to insufficient removal of interfering components or even to clogged SPE cartridges or LC columns. These methods also involve large volumes of organic solvents, are

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time consuming and costly and do not allow for visual monitoring during the clean-up step. Visual control is especially important when samples of varying composition involving complex matrices are to be analysed as it enables minor adjustments to be made in time, if necessary.

Thin-layer chromatography (TLC) is a fast and inexpensive technique for separating, detecting and quantifying target compounds. In addition, TLC can also be applied for sample clean-up. This clean-up technique is called planar solid phase extraction (pSPE) [22]. TLC plates are single use items and therefore, reconditioning of the stationary phase is no issue. In addition, increasing the area of the application zone as well as simultaneous multiple applications is a simple way to increase sample size. Our decision to test pSPE for our study came with paraffin containing samples leading to the mentioned problems with our former SPE clean-up method [6].

Our aim was to develop a pSPE-GC–MS method for the simultaneous determination of COPs, POPs and for the first time lanosterol oxidation products (LOPs) and dihydrolanosterol oxidation products (DOPs) in cosmetics where sterols from both plant and animal origin often occur together.

2. Experimental

2.1. Chemicals and materials

7-Ketocholesterol (7-KC), 7β-hydroxycholesterol (7β-HC), cholesterol-5β,6β epoxide (β-CE), cholesterol-5α,6α epoxide (α-CE), cholestan-3ß,5,6ß-triol (CT), 5α-cholestane, β-sitosterol (>70%), 25-hydroxycholesterol (25-HC), C.I. 11005, 4-(4-nitrobenzyl)pyridine (NBP), and polytetrafluoroethylene (PTFE) membrane filters (0.2 and 0.45 μm) were purchased from Sigma-Aldrich (Buchs, Switzerland); 7α-hydroxycholesterol (7α-HC) from Chemos (Regensburg, Germany). Dichloromethane, diethyl ether (both analytical grade), n −hexane, acetone, methanol (all GC grade), sodium methylate (30% solution in methanol, for synthesis), tetraethylenepentamine and citric acid monohydrate were from Merck (Darmstadt, Germany) and Sylon HTP (1,1,1,3,3,3-hexamethyldisilazane: trimethylchlorosilane: pyridine; v:v:v; 3:1:9) from Supelco (Gland, Switzerland). All TLC and HPTLC plates were from Merck and obtained from VWR (Dietikon, Switzerland). They were prewashed with methanol and dried in an oven at 100 °C for 10 min before use. Propylene centrifuge tubes (15 mL; 50 mL) were from SPL Lifescience (Gyeonggi-do, Korea), polypropylene pipettes from alpha laboratories (Eastleigh, UK). Lanolin was obtained from a local pharmacy.

2.2. Gas chromatography–mass spectrometry (GC–MS) and liquid chromatography-high resolution mass spectrometry (LC-HRMS)

Compounds were separated and detected with a Thermo Finnigan Trace GC (Thermo Scientific, Brechbühler, Schlieren, Switzerland) coupled to a PolarisQ MS (Thermo Scientific) using two capillary columns, a DB17 ms (10 m × 0.25 mm ID; 0.25 μm film) and a DB5 ms (30 m × 0.25 mm ID; 0.25 μm film) (both Agilent Technologies, Santa Clara, USA) connected with a pressfit. A diphenyltetramethyldisiloxane (DPTMDS) deactivated precolumn and postcolumn (both 1 m × 0.25 mm ID) were used. Injections were performed with a PAL autosampler (CTC analytics, Zwingen, Switzerland) into a programmable temperature vaporizer (PTV) injector used in the PTV large volume mode and equipped with a silcost liner (2 mm ID). Injection speed was 50 $\mu L\,s^{-1}$ and pre/post inject delay was 3000 ms. The injector was set at 100 °C (1 min, 50 kPa) with a split ratio of 10:1 during evaporation time. For the transfer of the analytes to the column, the temperature was raised at 14.5 $°C\,s^{-1}$ to 280 °C (1 min, 140 kPa) and the split valve was shut. For cleaning the injector, the injector temperature was raised to 310 °C at 14.5 $°C\,s^{-1}$ (10 min, 140 kPa) with a split ratio of 10:1. Helium was used as a carrier gas at a pressure of 50 kPa for 1 min then at a pressure of 140 kPa for the remaining of the GC runtime. Initial oven temperature was set at 60 °C for 2.5 min, then raised to 320 °C at a rate of 20 $°C\,min^{-1}$ and held constant for 15 min. Full-scan mass spectra (*m*/*z* 50–650) were recorded with a 17-min delay in the electron impact ionization (EI) mode at 30 eV (trap offset 10, AGC target 50, high mass adjust 50%, wave form off). The ion source temperature was set at 220 °C, and the transfer line temperature at 280 °C. Processing and interpretation of mass spectra were carried out with Xcalibur 2.1 (Thermo Scientific). Quantification was performed with an internal standard (5α-cholestane) using the extracted ion chromatograms of main mass signals (Table 1).

The LC-high resolution mass spectrometry (HRMS) system consisted of a LTQ Orbitrap XL equipped with a heated ESI II source (Thermo Scientific), a HTS-PAL autosampler (CTC analytics). Separation was performed on a Waters Atlantis T3 column (150 mm × 3 mm; 5 μm) using a gradient elution (A: nanopure water and B: methanol). The flow rate was 200 $\mu L\,min^{-1}$ and gradient elution started with 95% of A and 5% of B (0–2 min). The eluent was then first steadily modified to 50% of A and 50% of B (5 min-17 min) and then modified to 5% of A and 95% of B (17–25 min). After, the eluent was reset to the initial composition for 7 min (25 min–32 min). Ionisation was performed with electrospray ionisation (ESI) in the positive mode (capillary temperature 300 °C, sheath gas flow 50 arbitrary units, auxillary gas flow 5 arbitrary units, source voltage 4 kV) and full scan spectra were recorded in *m*/*z* range of 115–1000. Processing of data was performed with Xcalibur 2.1

2.3. Isolation of lanosterol and dihydrolanosterol

Lanosterol and dihydrolanosterol were isolated from a transesterified lanolin solution (2.7.2) by preparative liquid chromatography on a Spectraystem including UV6000, AS3000, D4000 (Thermo Scientific, Reinach, Switzerland) coupled to a fraction collector from Gilson (Mettmenstetten, Switzerland). The separation was performed on a Lichrosorb-100 RP18 column (5 μm, 250 cm × 4 mm) (Knauer, Berlin, Germany) with methanol at 1 $mL\,min^{-1}$. Detection was performed with a diode array detector at 210 nm. Eluate fractions containing lanosterol or dihydrolanosterol were collected and methanol vaporised with a nitrogen evaporator. Several milligrams of each sterol were obtained by repeating the procedure as often as needed (25 μg lanolin per run). Purities were checked with gas chromatography (GC) − flame ionisation detection (FID) and were found to be >88%.

2.4. Generation of phytosterol oxidation products (POPs) and dihydrolanosterol oxidation products (DOPs) by thermooxidation

Oxidised phytosterol and dihydrolanosterol congeners were obtained by thermooxidation. Solutions (10 mg in 10 mL acetone) of sitosterol and dihydrolanosterol, respectively, were pipetted into a 200-mL erlenmeyer flask and kept at 120 °C for 15 h using a heating plate. After cooling the flask under cold running water, the residue containing unreacted precursor and sterol oxides was dissolved in 10 mL of acetone under sonication. The POPs and DOPs solutions were then ready for identification (2.6) and quantification. For the latter, levels of congeners were determined with GC-FID using the corresponding congeners of COPs as standards. As the response factors of FID for substances having the same chemical group are often similar, this approach was deemed to be suitable. The sitosterol stock solution also contained campesterol. We therefore also expected to find campesterol oxidation products (CaOPs).

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