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Determination of sterols using liquid chromatography with off-line surface-assisted laser desorption/ionization mass spectrometry

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ABSTRACT

A new method, reversed phase liquid chromatography with off-line surface-assisted laser desorption/ionization mass spectrometry (RPLC–SALDI MS) for the determination of brassicasterol (BR), cholesterol (CH), stigmasterol (ST), campesterol (CA) and β-sitosterol (SI) in oil samples has been developed. The sample preparation consisted of alkaline saponification followed by extraction of the unsaponificable fraction with diethyl ether. The recovery of the sterols ranged from 91 to 95% with RSD less than 4%. Separation of the five major sterols on a C18 column using methanol-water gradient was achieved in about 10 min. An on-line UV detector was employed for the initial sterol detection prior to effluent deposition using a laboratory-built spotter with 1:73 splitter. Off-line SALDI MS was then applied for mass determination/identification and quantification of the separated sterols. Ionization of the nonpolar analytes was achieved by silver ion cationization with silver nanoparticles used as the SALDI matrix providing limits of detection 12, 6 and 11 fmol for CH, ST and SI, respectively. Because of the incorporated splitter, the effective limits of detection of the RPLC–SALDI MS analysis were 4, 3 and 4 pmol (or 0.08, 0.06 and 0.08 μg/mL) for CH, ST and SI, respectively. For quantification, 6-ketocholestanol (KE) was used as the internal standard. The method has been applied for the identification and quantification of sterols in olive, linseed and sunflower oil samples. The described off-line coupling of RPLC to SALDI MS represents an alternative to GC–MS for analysis of nonpolar compounds.

1. Introduction

Phytosterols are compounds occurring ubiquitously in plants and are routinely determined in foods and diets due to their wide range of biological activities and physical properties [1]. These triterpenes, see Fig. 1, can be found in all plant cell membranes [2]. Vegetable oils are composed mainly of triacylglycerols and a complex mixture of minor compounds (2–5%) with a wide range of chemical structures. The predominant phytosterols in vegetable oils, such as olive oils, are β-sitosterol (SI) and stigmasterol (ST). Campesterol (CA) and brassicasterol (BR) can be found in sunflower and linseed oils [3–5]. Cholesterol (CH) is typically produced by the plants in negligible amounts [1,3]. The total amount of sterols depends on many factors [6,7] and their distribution characterizes the quality of the oils. It can reveal possible adulteration of oils, e.g. BR indicating the presence of rapeseed oil in olive oil or CH pointing to animal fat in vegetable oils. The sterols in food are usually analyzed by GC with a flame ionization detector (FID), while GC with mass spectrometry (MS) using electron ionization is used to confirm peak identities [8–11]. The major limitations of the GC methods are the time-consuming derivatization step, which might be a source of artifacts, and thermal stability of columns.

More recently, great attention has been paid to LC with ultraviolet detection (UV) [12,13] as well as to LC–MS employing atmospheric pressure chemical ionization (APCI) for sterol characterization in food materials [5,7,14–17]. Due to the growing interest in the physiological properties of plant sterols and related high quality monitoring of food products containing them, the analytical techniques undergo continuous development; analysis was downscaled using nano-LC [16] and employment of ultrahigh-performance liquid chromatography (UHPLC) led to a significant gain in efficiency and reduced analysis time [7,15,17]. Because of the highly lipophilic nature of sterols, APCI is the most widely used ionization technique for their LC–MS analysis. A variety of mass

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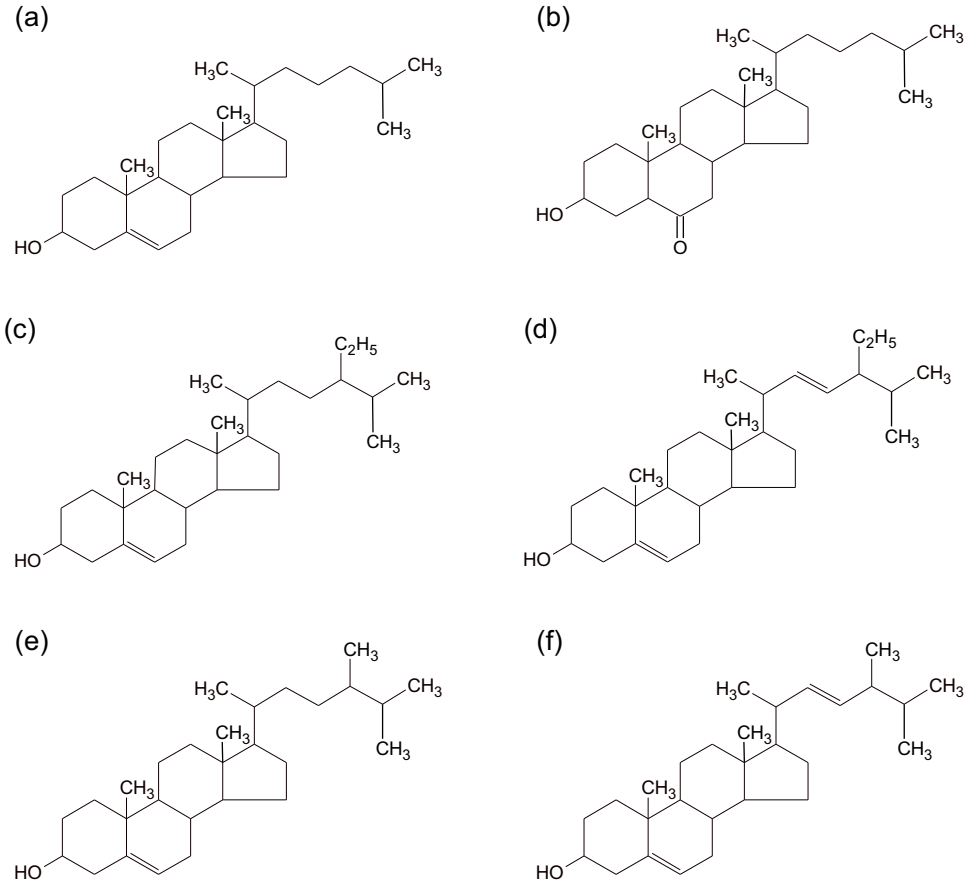

Fig. 1. The structures of selected sterols: (a) cholesterol, CH; (b) 6-ketocholestanol, KE; (c) β-sitosterol, SI; (d) stigmasterol, ST; (e) campesterol, CA and (f) brassicasterol, BR.

spectrometer types are used in combination with LC; the simplest ones are single quadrupole (Q) instruments. Employment of a triple quadrupole (QqQ) system in combination with UHPLC results in a dramatic increase of sensitivity; the limits of detection (LOD) are 123–677 ng/mL for LC–QMS [5], about 30 ng/mL for UHPLC–QMS [17] and 6 ng/mL for UHPLC–QqQMS [15].

Another ionization technique that is popular in organic analysis is matrix-assisted laser desorption ionization (MALDI); it is widely used especially for the analysis of large molecules [18–22]. The matrix plays the key role in the ionization [23]. Applicability of common MALDI matrices based on organic aromatic acids is limited due to the production of background interference in the low *m*/*z* region complicating detection of small molecules. This is critical especially if diluted samples have to be analyzed. Furthermore, the most common MALDI matrices, such as 2,5-dihydroxybenzoic acid and α-cyano-4-hydroxycinnamic acid, are designed for analysis of polar compounds. Yet, a complete survey of MALDI matrices suitable for lipid analysis is available [24]. For analysis of mixtures MALDI MS may be coupled off-line to LC or capillary electrophoresis; this has been widely used in proteomics and synthetic polymer analysis [25]. After deposition of the effluent on the target, mass spectra from the discrete fractions are recorded with time-of-flight (TOF) mass analyzers, which are typically used in combination with MALDI. The off-line coupling offers certain advantages compared to the on-line mode, namely: decoupling of separation from mass analysis, sample archiving and the option of detailed re-analysis of analytes of interest. Due to the speed of recent TOF mass analyzers, the off-line reading of the plate can be much faster than the separation and the analysis time is not extended drastically [26,27]. For parallel separation arrangement and single mass analyzer, the analysis time per sample may be even shorter in comparison with the on-line coupling [28].

To date a number of non-conventional nanoparticle matrices and related ionization techniques has emerged taking advantage of their unique properties, such as a large specific surface area and high adsorption capacity [29]. Although the terminology in this field is somewhat inconsistent, the term surface-assisted laser desorption (SALDI) introduced by Sunner and Chen [30] describes the principle of the technique and remains the most commonly used. The main advantages of the SALDI approach are: reduced background in the low mass region due to the absence of a chemical matrix and improved sample homogeneity, which leads to a more reproducible and rapid analysis of small molecules. The typical nanomaterials in SALDI, carbon [31,32], silicon [33,34] and metals [35–38] are applied to both biomolecules and low molecular weight organic compounds. Silver nanoparticles derivatized with hydrophobic ligands have been employed for the extraction of peptides and proteins in biological samples by means of a liquid–liquid extraction procedure, a classic matrix was added prior to MALDI MS [39]. Negative mode SALDI MS with silver nanoparticles, prepared through sodium citrate mediated reduction of silver nitrate, was applied to determine estrogens in women's urine [40]. A readily available and stable source of silver nanoparticles is colloidal suspension of silver; it has been previously applied for in situ analysis of wax compounds on the surface of intact plant material [41], or fatty acids [42], glycosphingolipids [43] in animal parts.

In this work, we present a new method based on reversed phase liquid chromatography (RPLC) with off-line SALDI MS spectrometry with silver nanoparticles as the matrix for the analysis of sterols. To our knowledge, this off-line coupling and the class of matrix has