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# Fully automated determination of the sterol composition and total content in edible oils and fats by online liquid chromatography–gas chromatography–flame ionization detection

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ABSTRACT

Sterol analysis of edible oils and fats is important in authenticity control. The gas chromatographic determination of the sterol distribution and total content is described by ISO norm 12228. Extraction, purification, and detection of the sterols are time-consuming and error-prone. Collaborative trials prove this regularly. Purification by thin-layer chromatography (TLC) and robust GC determination of all mentioned sterols is not straightforward. Therefore, a fully automated LC-GC-FID method was developed to facilitate the determination of sterols. The only manual step left was to weigh the sample into an autosampler vial. Saponification and extraction were performed by an autosampler while purification, separation, and detection were accomplished by online coupled normal-phase LC-GC-FID. Interlacing of sample preparation and analysis allowed an average sample throughput of one sample per hour. The obtained quantitative results were fully comparable with the ISO method with one apparent exception. In the case of sunflower oils, an additional unknown sterol was detected generally missed by ISO 12228. The reason was found in the omission of sterol silylation before subjection to GC-FID. The derivatization reaction changed the retention time and hid this compound behind a major sterol. The compound could be identified as 14-methyl fecosterol. Its structure was elucidated by GC–MS and ensured by HPLC and GC retention times. Finally, validation of the designed method confirmed its suitability for routine environments.

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## 1. Introduction

Sterols are naturally occurring compounds in animals and plants. The best-known sterol is cholesterol, which is found in animals, and stabilizes cell membranes. Similar functions can be attributed to sitosterol in plants. Among other compounds, this sterol is counted to the plant sterols (phytosterols) [1].

Sterols, in particular phytosterols, are important compounds for the nutrition and health industry. They are known to have a variety of biological effects [1]. It was reported in the past that compounds derived from phytosterols and their saturated analogues (phytostanols) have beneficial effects on the cardiologic system [2]. Foodstuffs, such as margarines, are enriched with phytosteryl or phytostanyl esters to lower the cholesterol level in the human body. This effect is based on the structural similarities between cholesterol and phytosterols. They compete for the same absorption sites in the human organism [2]. Phytosterols are also used as emulsifiers in the cosmetic industry and are important steroidal precursors for hormone pharmaceuticals [3].

Sterols are found in the unsaponifiable matter and belong to the class of triterpenes. The structure is derived from sterane hydroxylated at C-3 (see Fig. 1). The sterol skeleton carries a double bond at varying position, mainly found at C-5(6) (Δ5) or C-7(8) (Δ7). C-17 contains a variable branched alkyl sidechain with possible additional double bonds. Sterols can be divided into three main classes based on the number of methyl groups at C-4, two (4,4-dimethyl), one (4-methyl) and none (4-desmethyl). The term sterol is often used as a synonym for 4-desmethyl sterols and stanols. 4,4-Dimethyl and 4-methyl sterols are metabolic intermediates transformed into 4-desmethyl sterols at the end of the pathway.

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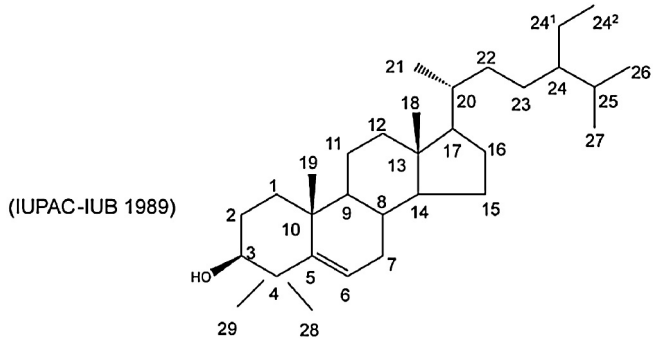


**Fig. 1.** Structure and nomenclature of sterols (from [4] with permission).

**Table 1**
Phytosterols and stanols covered by ISO 12228 [8].

| Peak no. | Compound name |
|---|---|
| 1 | Cholesterol |
| 2 | Cholestanol (ISTD, not shown) |
| 3 | Brassicasterol |
| 4 | 24-Methylene cholesterol |
| 5 | Campesterol |
| 6 | Campestanol |
| 7 | Stigmasterol |
| 8 | Δ7-Campesterol |
| 9 | Δ5,23-Stigmastadienol |
| 10 | Clerosterol |
| 11 | Sitosterol |
| 12 | Sitostanol |
| 13 | Δ5-Avenasterol |
| 14 | Δ5,24-Stigmastadienol |
| 15 | Δ7-Stigmastenol |
| 16 | Δ7-Avenasterol |

Sterols can occur either as free alcohols or bound to other molecules, e.g., fatty acids, ferulates, or glycosides [4]. In animals, cholesterol is the most abundant sterol while in plants the most encountered phytosterols are sitosterol, campesterol, and stigmasterol [5]. In addition, numerous minor sterols with percentages less than 5% of the total sterol content can be found. The total sterol amount in edible oils generally varies between approximately 300 and 10,000 mg/kg [6].

### 1.1. Phytosterols as identifiers for edible oils and fats

Because of unique plant-specific compositions, phytosterols are used as identifiers for natural products such as edible oils and fats. The control of the composition and total amount of phytosterols is an important tool for ensuring the purity of high quality oils, e.g., extra virgin olive oils. ISO 12228 describes a gas chromatographic method for the determination of fifteen individual 4-desmethyl phytosterols and stanols in edible oils and fats [7,8]. In Fig. 2 and Table 1, an overview of all covered compounds is given.

Admixtures of low-grade oils, e.g., rapeseed or sunflower oil, can be traced down to a few percent due to their prominent sterol compositions [9]. However, heating or bleaching of inexpensive oils can be used for desterolization [10]. Hence, the unique sterol composition is destroyed. Admixtures cannot be safely detected anymore by sole analysis of the sterol profile. Because of this, numerous purity and quality criteria are used.

For instance, EU regulation 1348/2013 specifies markers to ensure the quality of olive oils. Among other parameters, it regulates the allowed compositions and upper limits of 4-desmethyl sterols. Depending on the olive oil quality level, e.g., extra virgin, virgin, pomace, etc., differing limits were defined. For example, extra virgin olive oils must contain more than 1000 mg/kg phytosterols with a sitosterol content exceeding 93% (with regard to the total sterol content).

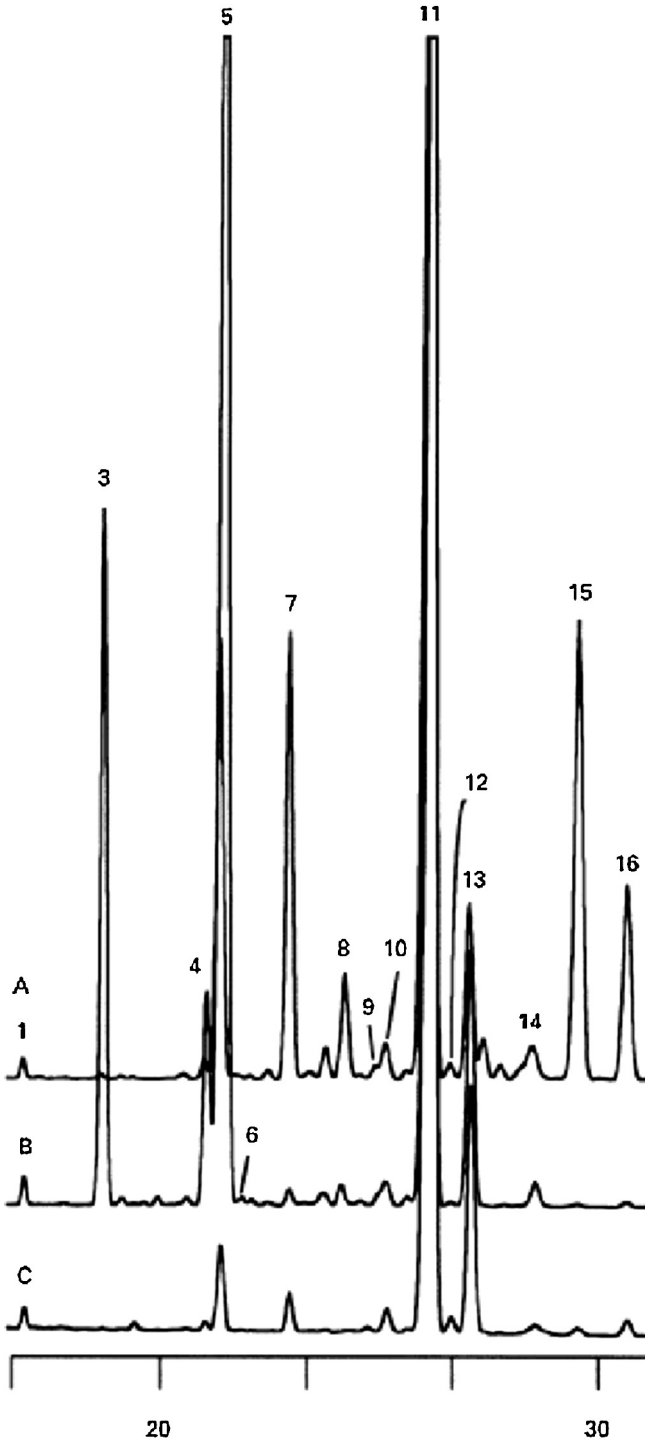


**Fig. 2.** GC-FID chromatogram of phytosterols and stanols covered by ISO 12228 (adapted from [8] – A: Sunflower, B: Rapeseed, C: Olive oil – Peak allocations found in Table 1).

### 1.2. Analytics of phytosterols in edible oils and fats

Numerous publications dealing with the determination of phytosterols in edible oils can be found [11–14]. ISO 12228 is most widespread in routine environments in Europe. In general, the first step involves liberation of all bound phytosterols into their free

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