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Normal-phase liquid chromatography–atmospheric-pressure photoionization–mass spectrometry analysis of cholesterol and phytosterol oxidation products

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ABSTRACT

During thermal processing of sterols, complex mixtures of sterol oxidation products may be formed. Here, a new method for the separation and detection of such products is described. The method is based on normal-phase liquid chromatography (NPLC) for separation and atmospheric-pressure photoionization–mass spectrometry (APPI–MS) for detection. The method was optimized using commercial cholesterol oxidation products and tested on an experimentally derived mixture of phytosterol oxidation products. The investigated parameters include solvent and dopant selection, dopant concentration, polar modifiers, the type of stationary phase, and flow rate.

Best chromatographic separation and highest sensitivity were achieved using a diol-bonded silica column, employing a solvent system consisting of hexane and isopropanol. The dopant of choice was chlorobenzene, added post-column to the solvent stream at 10% of the flow rate.

The developed NPLC–APPI–MS method proved to be a valuable tool for the separation and detection of sterol oxidation products.

1. Introduction

Sterols are a class of lipids present in virtually all living organisms. Cholesterol is the major sterol from animal origin, whereas phytosterols are found in plant species such as nuts and seeds. During processing of (vegetable) oils and fats, e.g. bleaching and heating, sterols may undergo auto-oxidation. Several oxidation products can be formed, most notably dienes, epoxides, hydroxides, and ketones, and combinations of those (Fig. 1) [1–7].

Sterol oxidation products are commonly analyzed by gas chromatography, preferably coupled to a mass spectrometer [3,8–16]. Gas chromatography is preferred by many because of its unprecedented resolving power, allowing the separation of very complex mixtures. However, gas chromatography requires derivatization of mid-polar to polar sterol oxides to increase their volatility. Alternatively, separation techniques based on liquid chromatography are used for the separation of sterol oxides. Examples include the analysis of phytosterols in edible oils [17] and cholesterol oxides in mouse brain [18–20] and human plasma [21–23] by reversed-phase HPLC coupled to mass spectrometry. However, reversed-phase chromatography is generally based on polar, aqueous mobile phases, which are unsuitable to dissolve rather non-polar lipids such as sterols and sterol oxidation products. Alternatively, normal-phase liquid chromatography (NPLC) has been proven an excellent technique to separate compounds of limited polarity [24], including triglycerides [25], complex crude oil mixtures [26], and apolar vitamins [27,28]. NPLC is also regularly employed for the separation of sterols and sterol oxides [29–35]. In normal-phase chromatography, the stationary phase typically consists of silica that may be modified with polar groups such as cyanide or hydroxide [24]. By choosing a mobile phase less polar than the analyte, retention of the analyte occurs via polar interaction. Importantly, the elution order reflexes the degree of polarity of the analytes, with the least polar compounds eluting first [24]. When separating sterol oxides, this means that the non-polar steradienes and ketones will elute first, followed by the mid-polar sterols and their oxidated derivatives and finally by the polar hydroxysterols. As a result, the identification of different sterol oxides is facilitated by the specificity of normal-phase chromatography towards polarity.

As mode for detection and identification, mass spectrometry (MS) has been evolved as the most potent technique due to its sensitivity, its selectivity, and its flexibility to analyze a broad range of compounds. A number of different ionization techniques are

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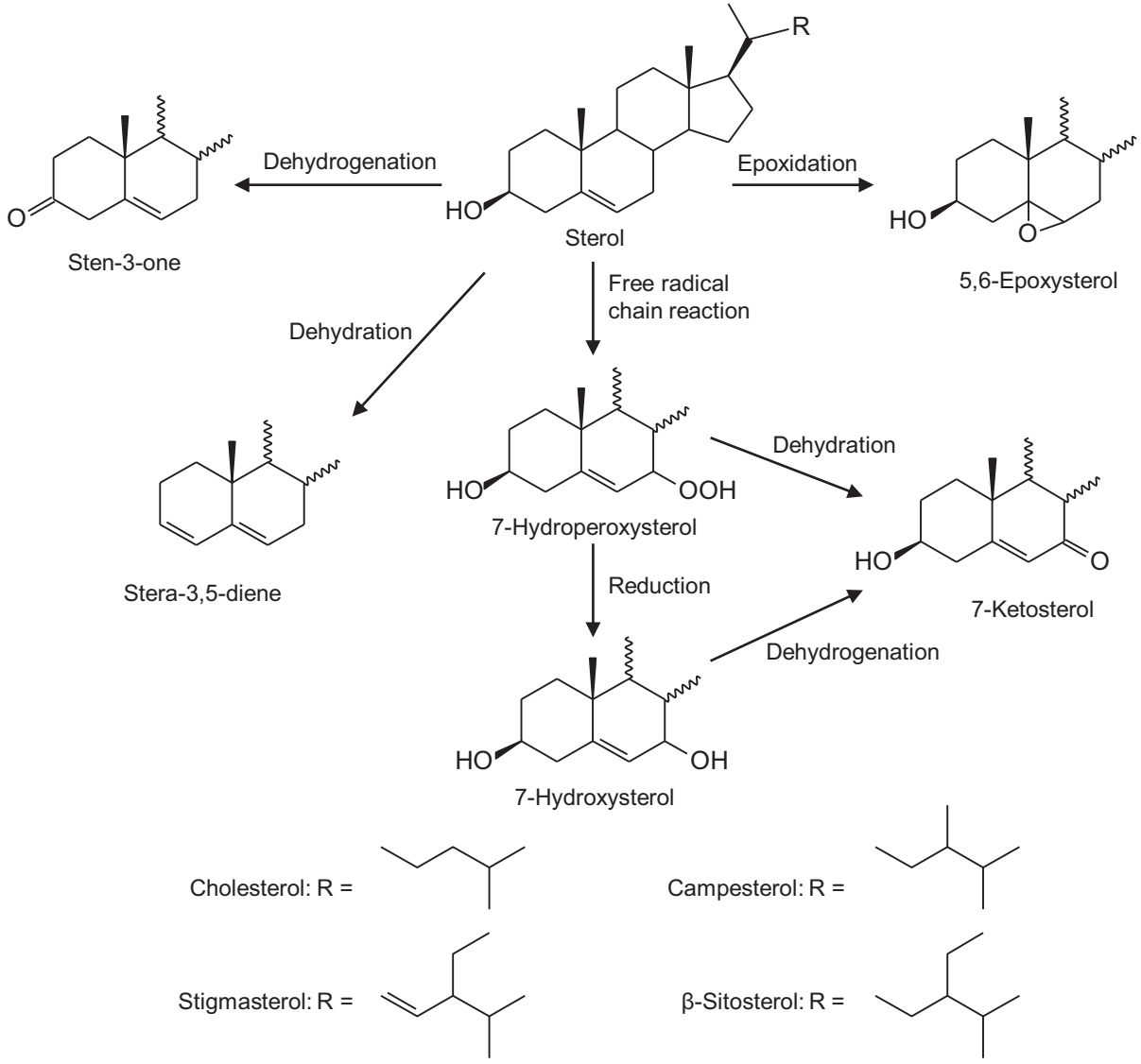

Fig. 1. Major sterol oxidation products formed during refining and processing of sterols.

available, of which electrospray ionization (ESI) is the most widely utilized. However, despite its broad applicability, ESI is less suited for the ionization of non-polar compounds. To be able to ionize such species, atmospheric-pressure chemical ionization (APCI) and, more recently, the closely related atmospheric-pressure photoionization (APPI) have been developed [36]. APPI was suggested to have advantages over both ESI and APCI because it is ideally suited for ionizing low- and non-polar analytes, whereas the other two ionization techniques work best with polar and ionic compounds [36–38]. Another advantage of APPI over specifically ESI is that it has been shown to be less susceptible to matrix effects and in addition, its linear dynamic range is generally higher than that of ESI [39,40]. Together, APPI would be the ideal choice for the ionization of sterol oxidation products, and indeed, other research groups have previously recognized its potential in studying sterols and their derivatives [18,37,40–44].

Although NPLC has been shown to be an excellent separation tool and APPI–MS is perfectly suited for detecting non-polar compounds, to our knowledge, a combination of these two techniques has not yet been described for the determination of sterol oxidation products. Furthermore, previous studies mainly focused on mid-polar and polar sterol oxides or separately analyzed non-polar and more polar compounds. Here, we report on the development of a method for the simultaneous analysis of non-polar, mid-polar, and polar cholesterol- and phytosterol oxidation products based on normal-phase liquid chromatography-atmospheric-pressure photoionization-mass spectrometry. Using cholesterol oxide standards and an experimentally obtained mixture of phytosterol oxidation products, the effect of dopant, solvent composition, and flow rate on the ionization efficiency was investigated and the NPLC method was optimized for separating oxidation products of varying polarity.

2. Experimental

2.1. Materials

Cholesterol, cholesta-3,5-diene, 5-cholesten-3-one, 22-ketocholesterol, 5-cholesten-3β-ol-7-one, cholesta-4,6-dien-3-one, 7β-hydroxycholesterol, 20α-hydroxycholesterol, 22(S)-hydroxycholesterol, 25-hydroxycholesterol, 5α,6β-dihydroxycholesterol, and 5α,6α-epoxycholesterol were from Sigma–Aldrich (Zwijndrecht, The Netherlands), 4-cholesten-3-one and stigmasterol were from Fluka (Sigma–Aldrich), and 19-