Application Note
Food & Beverages

Phytosterols

Introduction

Plant sterols and plant stanols, known commonly as phytosterols, are plant-derived compounds that are structurally related to cholesterol [1]. It is well established that high intakes of plant sterols or stanols can lower serum total and low-density lipoprotein (LDL)-cholesterol concentrations in humans [2]. Important food sources of phytosterols include unrefined vegetable oils, whole grains, nuts, seeds, and legumes. Furthermore, phytosterols enriched foods and beverages are available in many countries nowadays. However, there are no clinical data available indicating that such foods reduce cardiovascular events [3]. Sterols can be electrochemically detected without derivatisation, on boron-doped diamond (BDD) electrodes at high potentials (see figure 3). This note shows the proof of principle for the fast and sensitive analysis of Phytosterols using the ALEXYS LC-EC analyzer.

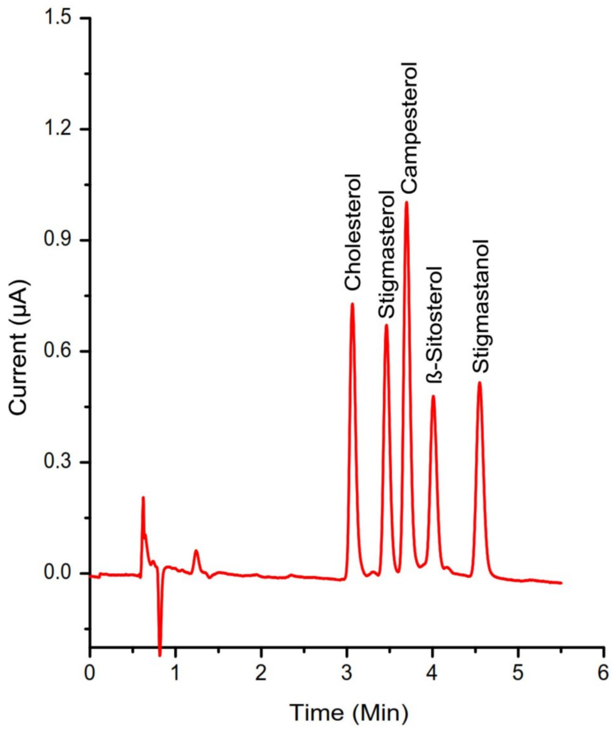

Fig. 2. *Example chromatogram of a 1 µL injection of 10 µM standard mix of Cholesterol, Stigmasterol, Campesterol, ß-Sitosterol and Stigmastanol in acetonitrile.*

Fig. 1. *ALEXYS LC-EC analyzer, UHPLC (1 channel).*

Method

The ALEXYS Neurotransmitter Analyzer (figure 1) consists of a P6.1L pump with integrated degasser, DECADE Elite electrochemical detector, AS 110 autosampler and Clarity data acquisition software. The LC-EC conditions are listed in table 1. Separation was achieved using an Acquity UPLC BEH C8 column at 45°C, in combination with an acidic mobile phase with $LiClO_4$ as electrolyte and 75% Acetonitrile. After separation, the sterols are detected in DC mode at a potential of 2.2 Volt versus Pd/H_2 on a BDD electrode using a DECADE Elite detector in combination with a FlexCell.

Table 1. LC-EC conditions

HPLC	ALEXYS LC-EC Analyzer, UHPLC, 1 channel
Column	Acquity UPLC BEH C8, 1x100 mm 1.7 µm
Mobile phase	0.1% trifluoroacetic acid (TFA), 50mM $LiClO_4$ in Acetonitrile/H_2O 75:25%
AS wash solution	Acetonitrile
Flow rate	100 µL/min
Backpressure	About 190 bar
Injection	1 µL (partial loop fill)
Temperature	45°C for separation & detection
Flow cell	FlexCell with Boron-doped Diamond WE HyREF^{TM} REF , 50 µm spacer
E-cell	2.2 V
I-cell	about 250 nA
ADF	0.5 Hz
Range	10 µA/V

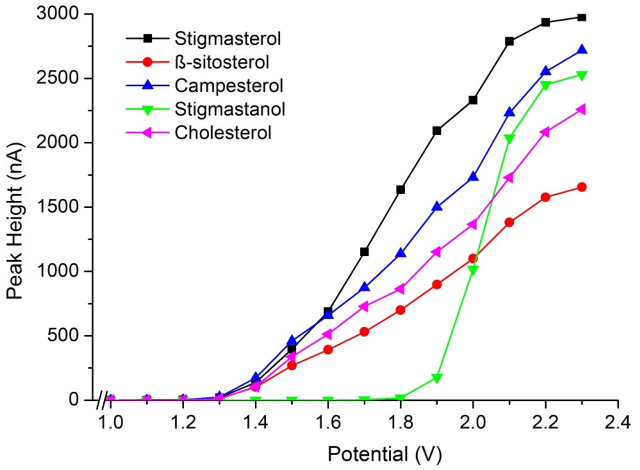

Fig. 3. *Hydrodynamic voltammogram (I-V curve) of Cholesterol, Stigmasterol, Campesterol, ß-Sitosterol and Stigmastanol in acetonitrile.*

An example chromatogram of an 1 µL injection of an 10 µM standard mix of the sterols in acetonitrile is shown in figure 2. All sterols are resolved within 5 minutes. The Limit of Detection (LOD) for the sterols calculated from the response of the standard mix are in the range of 100 – 300 nM (0.1 – 0.3 pmol, on-column). The repeatability was assessed using Cholesterol standards. RSD's of 1.1 and 0.5% (n=10) were obtained for a 1 and 10 µM standard, respectively. Note that the sterols are practically insoluble in water, also acetonitrile was not optimal as solvent for all sterols at high concentrations. Slight precipitation of ß-Sitosterol was observed in our 10 mM stock standard, after storage for weeks in the fridge at 4°C. In reference [4] directions are given about suitable solvents for sterols. In a 10 mM ß-Sitosterol stock solution in iso-propanol no precipitation was observed over time.

For the measurement of real samples appropriate sample preparation methods and internal standards might be required. In literature reference [5] and [6] some directions are given for different type of samples. Ref [5] describes the sample prep and analysis of phytosterols in whole blood, ref [6] about phytosterols in dairy products and vegetable fats.

References

1. Wiki, Phytosterols, https://en.wikipedia.org/wiki/Phytosterol
2. C.E. Cabral et al., Phytosterols in the Treatment of Hypercholesterolemia and Prevention of Cardiovascular Diseases, Arq Bras Cardiol. 109 (2017), 475–482.
3. O. Weingartner et al., Controversial role of plant sterol esters in the management of hypercholesterolaemia, European Heart Journal, 30(4) (2009), Pages 404–409
4. Phytosterols, phytostanols and their esters, FAO JECFA Monographs 5 (2008), http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/monograph5/additive-509-m5.pdf
5. Thermo, Rapid and Direct Analysis of Free Phytosterols by Reversed Phase HPLC with Electrochemical Detection, application note, PN-70986-EN
6. I. Borkovcova et al., Determination of Sterols in Dairy Products and Vegetable Fats by HPLC and GC Methods, Czech J. Food Sci., 27 (2009), S217-S219

Ordering information

180.0091W	ALEXYS LC-EC Analyzer, UHPLC, 1 channel
191.0055UL	AS 110 autosampler UHPLC cool 6p
102.4305M	FlexCell with BDD WE and HyREF REF
250.1164*	Acquity UPLC BEH C8 column, 1x100 mm ID, 1.7 µm (186002876)
250.1165*	Acquity UHPLC in-line filter kit + 6 frits (205000343)
250.1702	In-line filter, solvent

*) Columns are products of Waters Corporation (Milford, USA), between parenthesis the Waters pn's are shown for reference.

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