

# Analysis of plant stanyl fatty acid esters in enriched margarine using an on-line coupled Agilent 1220 Infinity LC-7890 GC system

## Application Note

Food

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### Abstract

This Application Note describes the investigation of intact plant stanyl fatty acid esters in an enriched commercial margarine using an on-line coupled Agilent 1220 Infinity LC-7890A GC system. The lipid extract was directly analyzed without prior purification steps. The LC fraction of plant stanyl esters was transferred on-line into the GC system using the solvent vent mode of the multimode inlet for solvent evaporation. The on-line LC-GC combination showed very good linearity and repeatability.



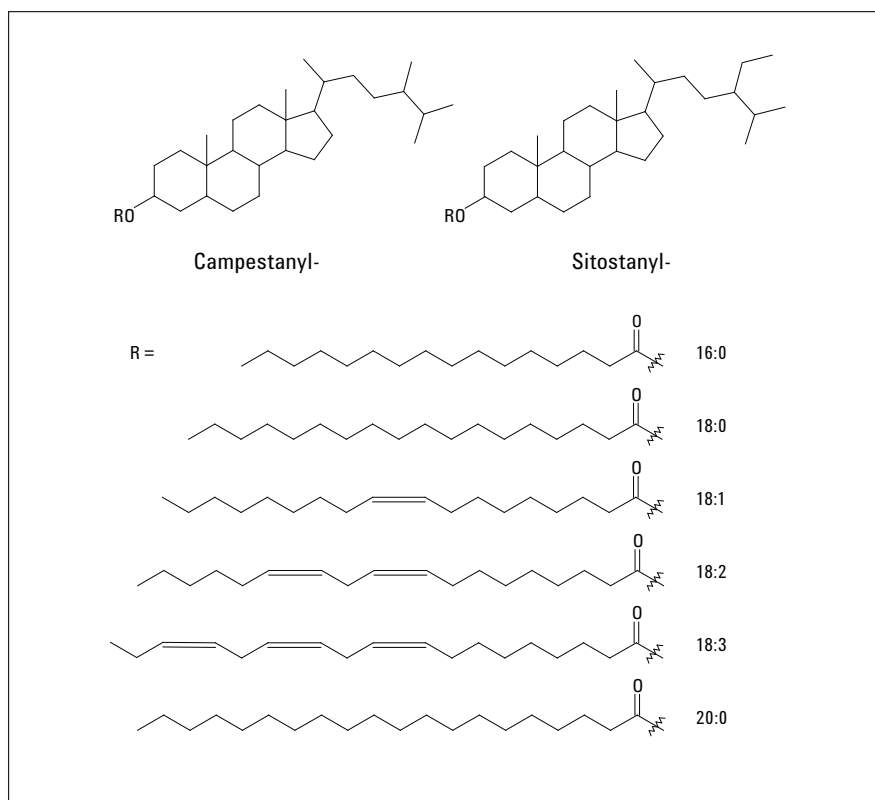
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## Introduction

Plant steryl and stanyl esters (Figure. 1) are added to food products like skimmed milk-drinking yogurts or margarines because of their cholesterol-lowering properties. The capillary gas chromatographic investigation of plant stanyl fatty acid esters from skimmed milk products can be performed directly after the lipid extraction<sup>1</sup>. However, the presence of di- and triglycerides may hamper the direct GC quantification. Therefore, the analysis in lipid extracts from foods with high fat contents like margarine requires a fractionation prior to the GC separation by laborious off-line techniques, such as TLC or SPE. The on-line coupling of LC and GC offers an efficient and elegant alternative. The plant stanyl esters can be fractionated by liquid chromatography and transferred on-line into the GC system. In this way, the pre-fractionation step and the capillary gas chromatographic analysis of the transferred LC fraction are performed in a closed system in one run. Hence, the risk of sample loss and contamination is reduced and the approach results in better repeatability<sup>2,3</sup>.

In a recently published paper<sup>1</sup>, the analysis of plant stanyl esters in enriched margarines using an on-line LC-GC system equipped with a loop-type interface was reported. Using the loop-type interface, the solvent evaporation was performed in the GC capillary columns by means of a pre-column system in combination with an early solvent vapor exit. Due to the high solvent amounts which were loaded on the pre-column system with each transfer, a loss of resolution was observed after a few runs.

The on-line coupling of an Agilent 1220 Infinity LC system and an Agilent 7890A GC system, with a 2-position/6-port switching valve using the solvent vent mode of the multimode



**Figure 1**  
Structures of plant stanyl fatty acid esters.

inlet of the GC, allowed for the evaporation of the solvent prior to the capillary column<sup>4</sup>. A pre-column system and/or a solvent vapor exit were not necessary. This combination was already suitable for the analysis of cholesteryl esters<sup>4</sup>.

In this Application Note, the use of an Agilent on-line coupled LC-GC combination for the quantification of plant stanyl esters in enriched margarine is presented.

## Experimental

### Chemicals and materials

The plant stanyl ester mixture “plant stanol ester, STAEST-115” was provided by Raisio Group (Raisio, Finland). The internal standard cholesteryl palmitate ( $\geq 98\%$ ) was obtained from Sigma Aldrich (Taufkirchen, Germany).

Benecol (taste-type Kevyt kasvira-valevite 32%, with added plant stanyl esters) margarine was purchased in a supermarket in Finland; the plant stanol content was labeled as 8 wt-%, total lipids as 32 wt-%.

### Sample preparation of margarine<sup>1</sup>

The margarine sample (20–40 mg, accuracy of  $\pm 0.1$  mg) was weighed into a vessel; internal standard (cholesteryl palmitate, 750  $\mu\text{g}$ ), 5 mL of *n*-hexane/MTBE (3:2) and sodium sulfate (anhydrous) were added and sonicated for 1 minute. The solution was filtered through a 0.45  $\mu\text{m}$  membrane filter assembled with a 5 mL syringe. The vessel and the filter were washed twice with 5 mL *n*-hexane/MTBE (3:2). After dilution (1:5) of the combined extracts, the solution was used for on-line LC-GC analysis.

## Quantification

The five-point calibration functions of nine individual stanyl esters were generated in a range of 0.2 – 1.0 µg of total stanyl ester ("plant stanol ester, STAEST-115") per 2 µL i.v. Each calibration point was done in triplicate. Linear regression analysis was performed in coordinate ratios of areas (individual stanyl ester/IS) and amounts (individual stanyl ester/IS).

## Equipment

The coupling of the Agilent 1220 Infinity LC system to the Agilent 7890A GC system was accomplished using an Agilent 2-position/6-port switching valve equipped with a 200 µL sample loop (Table 1). The evaporation of the eluent was performed using the temperature programmable MM Inlet in the PTV solvent vent mode<sup>4</sup>.

## Results and discussion

The chromatograms obtained by on-line LC-GC analysis of a margarine enriched with plant stanyl esters are presented in Figure 2. The LC-fractionation (Figure 2a) was performed isocratically on a silica gel column with *n*-hexane/MTBE (96+4; v+v) as mobile phase. The plant stanyl esters eluted after approximately 4 minutes. The transfer was performed 4.25 minutes after injection. The transfer conditions for the analysis of cholesteryl esters<sup>4</sup> were also suitable for plant stanyl esters. The GC separation of the transferred fraction was similar to that reported for the on-line LC-GC analysis via a loop type interface<sup>1</sup>. The intact plant stanyl fatty acid esters were distinguishable according to their carbon number and, in the case of unsaturated fatty acid moieties, to the number of double bonds; only the esters of saturated and monounsaturated fatty acids of the same chain length eluted at the same time.

### Chromatographic conditions

#### LC conditions

Injection volume:	2 µL
Eluent:	<i>n</i> -hexane/ <i>tert</i> -butylmethyl ether (96:4, v/v)
Column temperature:	27 °C
Column flow:	0.200 mL/min
Column type:	Eurospher-100Si (250 x 2 mm I.D., 5 µm)
Wavelength:	205 nm

#### LC controlled interface

Transfer valve:	4.25 min: Position 1 → 2 7.50 min: Position 2 → 1
GC start:	4.20 min: Change contacts switch contact A to closed 4.25 min: Change contacts switch contact A to open

#### GC conditions

Front MM inlet:	Mode: Solvent vent Carrier: H <sub>2</sub> Pressure: 7.8 psi Septum purge flow: 3 mL/min Vent pressure: 4 psi until 0.5 min Vent flow: 1000 mL/min Temperature program: Initial: 50 °C for 0.5 min Rate 1: 900 °C/min to 350 °C for 2 min Purge flow to split vent: 2.5 mL/min at 0.5 min Gas saver: 20 mL/min after 5 min
Column number 1:	Column type: Restek Rtx-200MS: 30 m x 250 µm; 0.1 µm df; Constant flow: 1.5 mL/min
Column number 2:	Transfer line, controlled by PCM C-1 Pressure program: Initial: 5 psi for 0.3 min Rate 1: 10 psi/min to 20 psi
Oven:	Temperature program: Initial: 40 °C for 2 min Rate 1: 100 °C/min to 100 °C for 0 min Rate 2: 15 °C/min to 310 °C for 2 min Rate 3: 1.5 °C/min to 340 °C for 3 min
Detector:	FID: 360 °C (H <sub>2</sub> : 30 mL/min, Air: 400 mL/min; Makeup: 25 mL/min)

Table 1  
Liquid and gas chromatographic conditions.

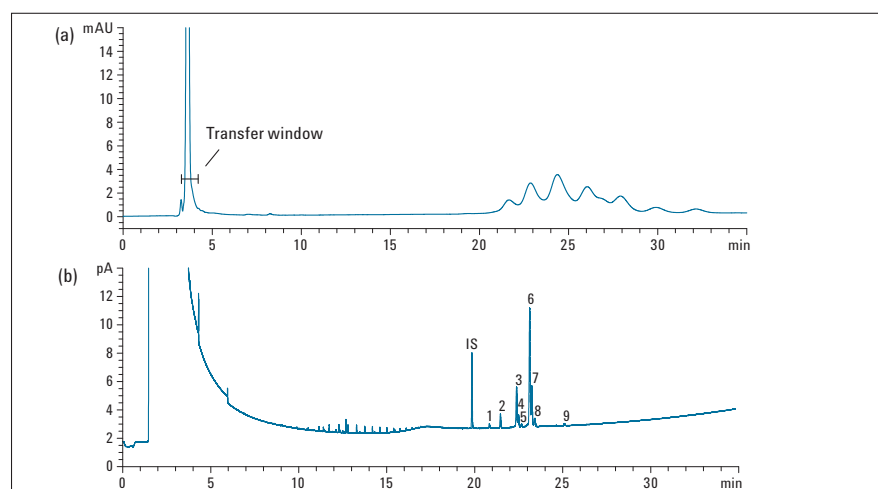


Figure 2  
Analysis of plant stanyl esters in enriched margarine by online LC-GC/FID; (a) LC-chromatogram and (b) GC-chromatogram of the transferred LC-fraction; peak numbering according to Table 2; (IS) internal standard cholesteryl palmitate.

Under the employed on-line LC-GC conditions, using the Agilent Multimode Inlet for the solvent evaporation<sup>4</sup>, the solvent load on the GC capillaries was low in comparison to the loop type coupled system. Even after 600 transfers, no loss of resolution was observed using the on-line LC-GC combination.

For the calibration, linear regression analysis was performed in the coordinate ratios of areas (individual stanyl ester/IS) and amounts (individual stanyl ester/IS). The correlation coefficients of the calculated calibration functions ( $R^2$ ) were in the range of 0.995 – 0.999, showing very good linearity of the on-line LC-GC/FID detector response.

The repeatability was determined by 10-fold injections of the same sample solution. The coefficients of variation were low (< 9%) for all plant stanyl esters (Table 2). The quantitative results were comparable to those obtained by means of the loop type interface coupled on-line LC-GC<sup>1</sup>.

## Conclusion

On-line coupling of an Agilent 1220 Infinity LC system and an Agilent 7890A GC system was shown to be suitable for the quantitative analysis of plant stanyl fatty acid esters in enriched margarine. The on-line LC-GC system was characterized by easy handling and a very robust separation performance for both dimensions. Therefore, the Agilent on-line LC-GC combination can be a valuable tool for the routine analysis of plant steryl and stanyl esters in functional foods.

No. <sup>a</sup>	Stanlyl ester	CV [%] <sup>b</sup>				Amount [g/100 g] <sup>c</sup>
		Extract 1	Extract 2	Extract 3		
1	Campestanlyl-16:0/16:1	7.7	2.0	5.1	0.18 ± 0.01 (8.1)	
2	Sitostanlyl-16:0/16:1	5.7	0.7	2.6	0.47 ± 0.02 (5.3)	
3	Campestanlyl-18:0/18:1	1.1	0.8	0.6	2.10 ± 0.06 (2.8)	
4	Campestanlyl-18:2	3.7	1.6	3.2	0.73 ± 0.04 (5.2)	
5	Campestanlyl-18:3	8.8	2.8	6.9	0.30 ± 0.02 (7.6)	
6	Sitostanlyl-18:0/18:1	1.1	0.9	1.5	6.36 ± 0.22 (3.5)	
7	Sitostanlyl-18:2	1.9	1.5	2.0	2.17 ± 0.11 (5.1)	
8	Sitostanlyl-18:3	6.5	2.2	7.2	0.81 ± 0.06 (8.0)	
9	Sitostanlyl-20:0/20:1	4.8	2.5	5.5	0.19 ± 0.01 (7.0)	
Total stanlyl esters		1.6	0.7	1.6	13.3 ± 0.5 (3.7)	
Esterified sterols		1.6	0.7	1.6	8.1 ± 0.3 (3.7)	

<sup>a</sup> Peak number correspond to Figure 2b

<sup>b</sup> Coefficient of variation [CV] determined by 10-fold injections of the same sample solution

<sup>c</sup> Values represent average ± standard deviations of 30 analyses (coefficient of variation [%])

**Table 2**

**Coefficients of variation of plant stanlyl esters.**

## References

1. A. Barnsteiner, T. Lubinus, A. di Gianvito, W. Schmid, K.-H. Engel, GC-Based Analysis of Plant Stanlyl Fatty Acid Esters in Enriched Foods. *Journal of Agricultural and Food Chemistry*, 2011, 59, (10), 5204-5214.
2. P. Dugo, G. Dugo, L. Mondello, On-line coupled LC-GC: theory and applications. *LC-GC Eur.* 2003, 16, (12a), 35-43.
3. T. Hyoetylaeinen, M.-L. Riekkola, On-line coupled liquid chromatography-gas chromatography. *J Chromatogr A* 2003, 1000, (1-2), 357-84.
4. R. Esche, A. Barnsteiner, K.-H. Engel, W. Kohlert, S. Fenzel, "Two-dimensional chromatography: LC-GC online coupling of an Agilent 1260 Infinity LC and an Agilent 7890A GC", Agilent Technical Overview, publication number 5990-8025EN; **2011**

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