ELSEVIER

Journal of Chromatography A, 857 (1999) 231–238

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

# Determination of tocopherols and sterols in vegetable oils by solid-phase extraction and subsequent capillary gas chromatographic analysis

Marion Lechner*, Birgit Reiter, Eberhard Lorbeer

*Institute of Organic Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Vienna, Austria*

Received 6 April 1999; received in revised form 16 June 1999; accepted 18 June 1999

**Abstract**

In general, analyses of tocopherols and sterols are performed separately in vegetable oils. By applying solid-phase extraction (SPE) prior to capillary gas chromatography, a simple and reliable procedure for the quantification of both tocopherols and sterols in a single analytical run has been developed. SPE was used as sample clean up procedure for the separation of these minor components from the triacylglycerol matrix, replacing time consuming saponification or on-line LC–GC. The analysis of tocopherols and free sterols in five different vegetable oils illustrates robustness and reliability of this method outlined. Quantification of the analytes was performed by external calibration with reference substances and internal standardization. The recovery of the procedure as well as the repeatability of the quantitative results have been evaluated. 

*Keywords:* Vegetable oils; Tocopherols; Sterols

## 1. Introduction

In seed oils, triacylglycerols are the main constituents making up about 98% of the oils. The remaining nonglyceridic fraction consists of different compound classes such as hydrocarbons, tocopherols, sterols and sterol esters. The analyses of these components provide rich information about the identity and the quality of vegetable oils, since their contents and compositions are more characteristic than the fatty acid profiles [1,2]. Especially the analysis of free and esterified sterols is of great value for the quality control of edible oils [3–6] and for the detection of oil admixtures [7–9]. Furthermore, sterols and tocopherols belong to the group of nutritionally significant lipids which are determined routinely in foods and edible oils [10–13].

The tocopherol content of oils and fats can be determined by a wide range of analytical techniques such as thin-layer chromatography (TLC) [14], capillary gas chromatography (cGC) [15,16], supercritical fluid chromatography (SFC) [15] or high-performance liquid chromatography (HPLC). The most common used technique is normal-phase HPLC with ultraviolet or fluorescence detection [12,13,17–20], although reproducibility of the data is often poor. Conventional methods for the analysis of the

*Corresponding author. Fax: +43-1-31367-2280.

0021-9673/99/$ – see front matter 
PII: S0021-9673(99)00751-7

sterol fraction in oils and fats involve saponification of the sample with the unsaponifiable matter being extracted and analyzed directly [21,22]. Alternatively, the saponificated sterols are isolated by preparative TLC and subsequently analyzed by cGC [23–26].

Although tocopherols and sterols are mostly determined separately in oils and fats, it is possible to analyze them simultaneously. In 1983, Slover et al. saponificated vegetable oils, isolated and derivatized the unsaponifiable matter and analyzed both tocopherols and sterols by cGC in a single analytical run [21]. Ballesteros et al. published a cGC method for the determination of cholesterol, $\alpha$-tocopherol and $\alpha$-tocopherol acetate in edible oils with automatic removal of the interfering triacylglycerols by continuous on-line transesterification [27]. Mariani et al. determined free and esterified sterols together with tocopherols, squalene and wax esters in oils by applying column chromatography prior to cGC analysis [28]. By on-line LC–GC, Grob et al. circumvented laborious sample preparations and analyzed free sterols, sterol esters and waxes in edible oils and fats in a single analytical run [4,29,30]. By modifying the derivatization and enlarging the transferred LC fraction, the range of compounds were expanded to squalene, tocopherols and epoxy-squalenes [3].

Solid-phase extraction (SPE) is a well established method for the concentration and extraction of lipids in biological samples [31]. In this work, SPE was applied to isolate the nonglyceridic components of vegetable oils. Prior to SPE, the free hydroxyl groups of sterols and tocopherols have been silylated to reduce their polarity. Ensuing, hydrocarbons, tocopherols, sterols and sterol esters can be separated from the slightly more polar triacylglycerol matrix by SPE. The eluted fraction was analyzed by cGC without further sample preparation. Quantification of the analytes was performed by internal standardization and external calibration with reference substances. Betulin was applied as internal standard for the analysis of both free and esterified sterols, since it is eluted between these two component classes. As a second internal standard the synthetic tocopherol isomer 5,7-dimethyltocol was used. The recovery of the whole procedure at different concentration levels and the repeatability of the quantitative results have been evaluated.

## 2. Experimental

### 2.1. Sample material and chemicals

Rapeseed, sunflower, soybean, castor, poppy and cuphea oil have been obtained by extracting crushed seeds with *n*-hexane according to German Standard Methods [27] in 1995. Protected from light, the oils have been stored at room temperature.

The reference substances squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene), stigmasterol (3$\beta$-hydroxy-24-ethyl-5,22-cholestadiene), cholesteryl stearate (5-cholesten-3$\beta$-yl-octadecanoate) and betulin (lup-20 [29]-ene-3$\beta$,28-diol) were purchased from Sigma (Deisenhofen, Germany) and at least 96% pure. A tocopherol kit consisting of $\alpha$-, $\beta$-, $\delta$- and $\gamma$-tocopherol (>95% each) was obtained from Merck (Darmstadt, Germany); 5,7-dimethyltocol (97%) was purchased from Matreya (Pleasant Gap, PA, USA). MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide) was obtained from Fluka (Buchs, Switzerland). Analytical grade pyridine, methyl *tert.*-butyl ether and *n*-hexane were supplied by Loba Feinchemie (Fischamend, Austria).

### 2.2. Equipment

Six milliliters Bakerbond spe columns packed with 1000 mg silica gel were utilized for SPE together with a vacuum manifold column processor (all from J.T. Baker, Deventer, The Netherlands). A HRGC 5300 Mega Series gas chromatograph (Carlo Erba, Milan, Italy) equipped with an on-column injector (OCI) and a flame ionization detection (FID) system was used for all GC analyses. Data acquisition and evaluation were achieved with the Chromcard for Windows 1.12 software (Fisons Instruments, Milan, Italy).

### 2.3. Preparation of samples and standard solutions

The following stock solutions in pyridine were prepared: stigmasterol (1.19 mg/ml), cholesteryl stearate (1.14 mg/ml), betulin (5.06 mg/ml), $\alpha$-tocopherol (2.0 mg/ml), $\beta$-tocopherol (2.0 mg/ml), $\gamma$-tocopherol (2.0 mg/ml), $\delta$-tocopherol (2.0 mg/ml) and 5,7-dimethyltocol (5.02 mg/ml).

The concentration of the reference substances in the solutions for calibration varied between 0.60 μg/ml and 11.9 μg/ml for stigmasterol, 0.57 μg/ml and 11.4 μg/ml for cholesteryl stearate and 0.4 μg/ml and 8.0 μg/ml for $\alpha$-, $\beta$- and $\delta$-tocopherols. The concentrations of the internal standards were 5.06 μg/ml for betulin and 5.02 μg/ml for 5,7-dimethyltocol in all standard solutions. For calibration, five standard solutions were prepared by transferring appropriate amounts of the stock solutions of stigmasterol, cholesteryl stearate, $\alpha$-tocopherol, $\beta$-tocopherol and $\delta$-tocopherol together with 10 μl of betulin and 10 μl of 5,7-dimethyltocol stock solutions in 10 ml screw cap vials. After adding 40 μl of MSTFA, the solutions were heated at 70°C for 15 min and diluted with *n*-hexane–methyl *tert.*-butyl ether (99:1, v/v) to 0.5 ml.

For preparing the samples 20–30 mg of vegetable oil, 10 μl of betulin and 10 μl of 5,7-dimethyltocol stock solutions were added to 50 μl pyridine and 40 μl of MSTFA into 10 ml screw cap vials.

Under vacuum, the silica gel SPE cartridges were conditioned with about 3 ml *n*-hexane–methyl *tert.*-butyl ether (99:1, v/v), followed by the application of the sample and the elution of the analytes with 4.5 ml *n*-hexane–methyl *tert.*-butyl ether (99:1, v/v). 2 μl of this solution were injected.

### 2.4. Gas chromatography

GC separation was performed using a 13 m×0.32 mm I.D., $d_f$=0.15 μm DB 17HT fused-silica capillary column (J&W Scientific, Folsom, CA, USA). Hydrogen was used as carrier gas at a flow-rate of 2.5 ml/min measured at 70°C. After injection of the sample at 70°C, the temperature of the GC oven was increased by 15°C/min to 230°C, then by 5°C/min to 250°C and by 25°C/min to 350°C, which was held for 3 min. Detector temperature was 360°C.

## 3. Results and discussion

### 3.1. Analyte recovery after SPE

To exclude incorrect quantification of the analytes due to adsorption on the silica, the analyte recovery after SPE was investigated. Two solutions were prepared by mixing equal concentrations of squalene, 5,7-dimethyltocol, stigmasterin, betulin and cholesteryl stearate. These substances vary widely in polarity with squalene being the most unpolar and betulin being the most polar component. After derivatization, one of these solutions (named S1) was subjected to SPE and subsequently analyzed by GC. As a reference, the second one was diluted to 5 ml with *n*-hexane–methyl *tert.*-butyl ether (99:1, v/v) and injected into the GC without further treatment. By referring the peak areas of the sample S1 to the peak areas obtained from the reference sample, the percent recoveries of the analytes in the sample S1 were calculated. To evaluate possible adsorption effects of the triacylglycerol matrix, an oil sample (named S2, poppy oil containing known amounts of stigmasterin and no detectable amounts of squalene or cholesteryl stearate) was spiked with squalene, 5,7-dimethyltocol, stigmasterin, betulin and cholesteryl stearate as reported for S1. After derivatization, this solution was subjected to SPE and subsequently analyzed by GC. Again, by referring the peak areas of this sample S2 to the peak areas obtained from the reference sample, the percent recoveries of the analytes in the sample S2 were calculated. As shown in Table 1, all substances were recovered quantitatively from the silica cartridge.

### 3.2. Qualitative analysis

A gas chromatogram of a mix containing squalene, all four tocopherol-homologues, 5,7-dimethyltocol, stigmasterin, betulin and cholesteryl stearate is shown in Fig. 1. On (50% phenyl)methylpolysiloxane (DB17HT), the analytes

Table 1
Recoveries of the analytes in two samples S1 (mix of reference substances) and S2 (mix of reference substances and poppy oil) obtained after SPE compared to a reference sample not subjected to SPE

| Analyte | Recovery (%) | |
|---|---|---|
| | S1 | S2 |
| Squalene | 101 | 95 |
| 5,7-Dimethyltocol | 98 | 98 |
| Stigmasterin | 100 | 99 |
| Betulin | 100 | 100 |
| Cholesteryl stearate | 97 | 109 |

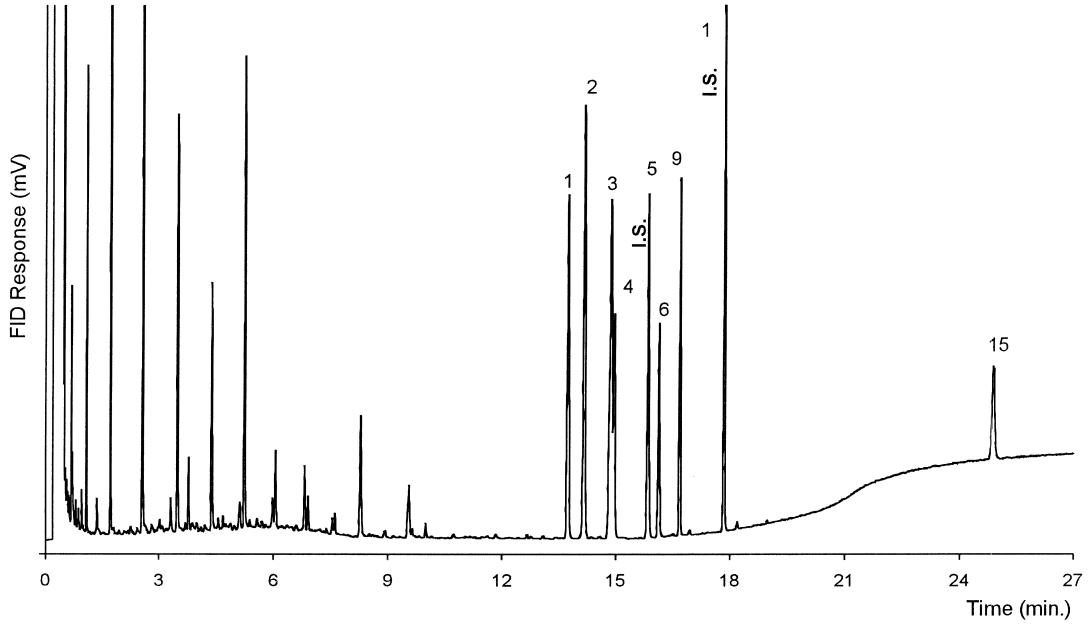


Fig. 1. SPE–GC–FID chromatogram of a silylated reference sample analyzed on a DB 17HT fused-silica column. Peak assignment: 1=squalene, 2=$\delta$-tocopherol, 3=$\beta$-tocopherol, 4=$\gamma$-tocopherol, 5=5,7-dimethyltocol (I.S.), 6=$\alpha$-tocopherol, 9=stigmasterol, 14=betulin (I.S.), 15=cholesteryl stearate.

are separated in compound classes according to their carbon numbers. Squalene is eluted first, followed by the tocopherol isomers with increasing numbers of methyl groups bound to the chromanol ring: $\delta$-tocopherol followed by the three dimethyltocols isomers $\beta$-, $\gamma$- and 5,7-dimethyltocol and finally $\alpha$-tocopherol. Subsequently stigmasterin is eluted, followed by the signals of betulin and of cholesteryl stearate.

A typical gas chromatogram of rape seed oil spiked with 5,7-dimethyltocol and betulin as internal standards is shown in Fig. 2. Under the GC conditions applied, the sterol esters undergo nearly complete thermal degradation, indicated by the unusual multiplet of signals at the end of the gas chromatogram. The data given in this paper have been obtained by analyzing the oils with the DB 17HT column, since special interest was paid to the complete separation of all four tocopherol homologues. Nevertheless, the insert in Fig. 2 shows the same rape seed sample analyzed on a 9 m DB-5 fused-silica column ((5%phenyl)polydimethylsiloxane). Under these GC conditions, the sterol esters are eluted nearly undecomposed and could be quantified. On the other hand, the signals of $\beta$- and $\gamma$-tocopherol would superimpose and these tocopherols could not be quantified separately.

### *3.3. Calibration*

For quantification of free and esterified sterols, a calibration with the reference substances stigmasterol and cholesteryl stearate and with betulin as internal standard was accomplished. Calibration functions were determined for $\alpha$-tocopherol, $\beta$-tocopherol and $\delta$-tocopherol with 5,7-dimethyltocol as internal standard. The response factor obtained for $\beta$-tocopherol was applied to the isomeric $\gamma$-tocopherol, therefore no individual calibration was performed for $\gamma$-tocopherol. Standard solutions containing five different concentration levels of these reference substances and constant amounts of the internal standards were subjected to SPE and analyzed on the DB 17HT column five times each. The calibration functions

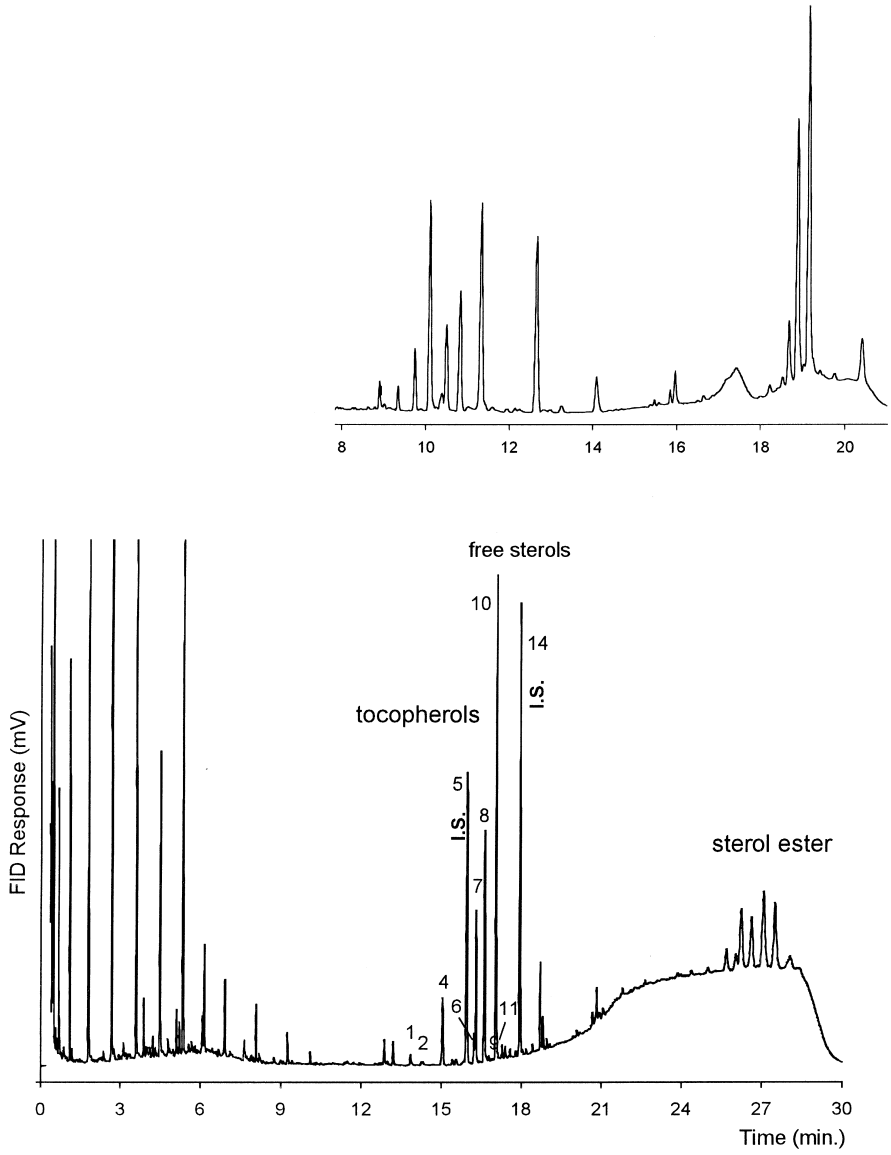


Fig. 2. SPE–GC–FID chromatogram of rapeseed (*Brassica napus*) oil with 5,7-dimethyltocol and betulin as internal standards analyzed on a DB 17HT fused-silica column. Peak assignment: 1=squalene, 2=$\delta$-tocopherol, 4=$\gamma$-tocopherol, 5=5,7-dimethyltocol (I.S.), 6=$\alpha$-tocopherol, 7=brassicasterol, 8=campesterol, 9=stigmasterol, 10=$\beta$-sitosterol, 11=$\Delta^5$-avenosterol, 14=betulin (I.S.). The sterol esters are nearly quantitatively degraded. The insert shows the same sample analyzed on a 9 m DB-5 fused-silica column, on which the sterol esters are degraded on a less lower amount.

obtained are shown in Table 2. All calibration functions with the exception of cholesteryl stearate showed excellent linearity, which is indicated by low values of standard errors and high correlation coefficients within the concentration range of interest. The response factor for sterol esters was 1.86 indicating strong degradation of these compounds on the GC column applied.

Table 2
Calibration functions for free sterols, sterol esters and tocopherols. For the quantification of $\gamma$-tocopherol the response factor obtained for $\beta$-tocopherol was applied. $M_c/M_{st} = bA_c/A_{st}$ $(n=5)$[a]

| | $b$ | Standard error | $r^2$ |
|---|---|---|---|
| Stigmasterin | 1.03 | 0.015 | 0.999 |
| Cholesteryl stearate | 1.86 | 0.035 | 0.989 |
| $\alpha$-Tocopherol | 0.64 | 0.019 | 0.998 |
| $\beta$-Tocopherol | 0.56 | 0.020 | 0.998 |
| $\delta$-Tocopherol | 0.60 | 0.015 | 0.999 |

[a] Abbreviations: $M_c$, mass of component; $M_{st}$, mass of internal standard; $A_c$, peak area of component; $A_{st}$, peak area of internal standard; $r$, correlation factor.

### 3.4. Analyte recovery of the SPE–GC method

For the evaluation of the recovery, poppy oil was spiked with known amounts of $\alpha$-, $\beta$-, $\gamma$- and $\delta$-tocopherol and stigmasterin at three different concentration levels and constant amounts of the standard substances. By comparing the quantitative results obtained by SPE–GC analysis and the actual concentrations of the spiked components, the per cent recovery was calculated. The results are shown in Table 3 and demonstrate the good recovery for the compounds investigated. With the exception of tocopherols added at very low concentrations level, the recoveries of all analytes were excellent reaching from 93 to 111%. For tocopherols spiked at 0.02% to the sample, recoveries showed lower values reaching from 75 to 90%.

Table 3
Average recovery for $\alpha$-, $\beta$-, $\gamma$- and $\delta$-tocopherol and stigmasterol at three concentration levels in spiked poppy oil $(n=3)$

| | Concentration (% in oil) | Recovery (%) |
|---|---|---|
| $\alpha$-Tocopherol | 0.02 | 75 |
| | 0.04 | 102 |
| | 0.08 | 96 |
| $\beta$-Tocopherol | 0.02 | 88 |
| | 0.04 | 96 |
| | 0.08 | 107 |
| $\gamma$-Tocopherol | 0.02 | 80 |
| | 0.04 | 93 |
| | 0.08 | 103 |
| $\delta$-Tocopherol | 0.02 | 90 |
| | 0.04 | 104 |
| | 0.08 | 111 |
| Stigmasterin | 0.05 | 102 |
| | 0.1 | 103 |
| | 0.2 | 96 |

### 3.5. Quantitative analysis

The quantitative and qualitative composition of the tocopherols and the free sterols were determined in five different vegetable oils in duplicate by SPE–GC. The concentrations of the individual tocopherols and free sterols were calculated based on the response factors given in Table 2 with $\gamma$-tocopherol quantified with the response factor obtained for $\beta$-tocopherol. The sterol esters were not quantified due to their strong thermal degradation on the GC column applied. The results of these analyses are given in Table 4. The qualitative composition of tocopherols is in accordance with data from the literature [3,17,20,21]. The quantitative results obtained are reasonable considering the progressive degradation of these components in the oil samples due to their long storage period. The qualitative and quantitative results obtained for free sterols are consistent with data obtained by on-line LC–GC analyses [3,5,30], but are not comparable with data obtained by conventional saponification, since this method produces the total sterol comprising of free sterols, sterol esters and sterols bound to other compounds.

### 3.6. Repeatability of the SPE–GC method

Repeatability of the quantitative results was checked by consecutive injection of the same rape seed sample and by repeated complete analysis including sample preparation. The data given in Table 5 indicate the excellent repeatability of the outlined method for the analysis of tocopherols and free sterols.

## 4. Conclusion

The determination of minor components in oil matrices by cGC entails removing the bulk lipid material by applying an appropriate sample pretreatment. This sample clean up is commonly achieved by either chemical manipulations like saponification followed by column chromatography and thin-layer chromatography or by applying

Table 4
Content and composition of tocopherols and free sterols in the seed oils investigated

| | | Rape, *Brassica napus* | Sunflower, *Helianthus annuus* | Soybean, *Glycine max.* | Castor, *Ricinus communis* | Cuphea, *Cuphea wrigtii* |
|---|---|---|---|---|---|---|
| $\alpha$-Tocopherol | (μg/g) | 132 | 425 | n.d.[a] | 12 | 154 |
| $\beta$-Tocopherol | (μg/g) | n.d. | 21 | n.d. | n.d. | 19 |
| $\gamma$-Tocopherol | (μg/g) | 218 | n.d. | 14 | 260 | 122 |
| $\delta$-Tocopherol | (μg/g) | 19 | n.d. | 32 | 244 | 12 |
| Tocopherol content | (%) | 0.037 | 0.045 | 0.005 | 0.052 | 0.031 |
| RSD[a] | (%) | 5.8 | 1.0 | 5.3 | 5.2 | 0.6 |
| Brassicasterol | (μg/g) | 670 | n.d. | n.d. | n.d. | n.d. |
| Campesterol | (μg/g) | 1036 | 315 | 911 | 189 | 172 |
| Stigmasterol | (μg/g) | 29 | 198 | 824 | 543 | 83 |
| $\beta$-Sitosterol | (μg/g) | 1830 | 1860 | 1754 | 1171 | 2294 |
| $\Delta^5$-Avenasterol | (μg/g) | 33 | 131 | 52 | 435 | 241 |
| $\Delta^7$-Stigmastenol | (μg/g) | n.d. | 790 | 77 | 99 | 368 |
| $\Delta^7$-Avenasterol | (μg/g) | n.d. | 200 | n.d. | n.d. | n.d. |
| Content of free sterols | (%) | 0.360 | 0.339 | 0.362 | 0.244 | 0.316 |
| RSD | (%) | 2.0 | 4.0 | 1.4 | 3.1 | 3.8 |

[a] n.d.: not detected; RSD: relative standard deviation.

Table 5
Repeatability of the quantitative results for tocopherols and free sterols in rape seed oil obtained by consecutive injections and by repeated complete analysis including sample preparation ($n=5$)

| | Consecutive injection | | Complete analysis | |
|---|---|---|---|---|
| | Mean concentration (μg/g) | RSD[a] (%) | Mean concentration (μg/g) | RSD (%) |
| $\alpha$-Tocopherol | 134 | 1.9 | 132 | 4.6 |
| $\gamma$-Tocopherol | 240 | 1.9 | 216 | 7.1 |
| $\delta$-Tocopherol | 20 | 5.8 | 22 | 10.2 |
| Tocopherol content | 394 | 1.4 | 370 | 3.3 |
| Brassicasterol | 673 | 1.3 | 666 | 2.1 |
| Campesterol | 1059 | 0.6 | 1033 | 2.3 |
| Stigmasterol | 29 | 5.7 | 25 | 16.2 |
| $\beta$-Sitosterol | 1816 | 1.3 | 1775 | 6.4 |
| $\Delta^5$-Avenasterol | 36 | 6.9 | 36 | 10.0 |
| Content of free sterols | 3613 | 0.7 | 3535 | 2.2 |

[a] RSD: relative standard deviation.

sophisticated instruments like on-line LC–GC. The proposed method overcomes the problems posed by the triacylglycerol matrix in oils by applying SPE for the quantitative separation of the minor components. By subsequent injection to cGC, qualitative and quantitative information about tocopherols, free sterols and sterol esters in vegetable oils are provided in a single analytical run. The SPE–GC method presented in this work requires little analysis time and provides the possibility of complete automatization. It offers excellent recovery and good repeatability of the analytes and is therefore well suited to routine use.

## Acknowledgements

Financial support by the Austrian Federal Ministry of Agriculture and Forestry is gratefully acknowledged.

## References

[1] A. Seher, H. Vogel, Fette-Seifen-Anstrichm. 78 (1976) 301.
[2] E. Homberg, Fat Sci. Technol. 93 (1991) 516.
[3] A. Artho, K. Grob, C. Mariani, Fat Sci Technol. 95 (1993) 176.
[4] K. Grob, M. Lanfranchi, C. Mariani, J. Am. Oil Chem. Soc. 67 (1990) 626.
[5] K. Grob, A. Artho, C. Mariani, Fat Sci. Technol. 94 (1992) 394.
[6] O.J. de Blas, A. del Valle González, J. Am. Oil Chem. Soc. 73 (1996) 1685.
[7] P. Horstmann, A. Montag, Fat Sci. Technol. 89 (1987) 381.
[8] L. Alonso, J. Fontecha, L. Lozada, M. Juárez, J. Am. Oil Chem. Soc. 74 (1997) 131.
[9] M. Biedermann, K. Grob, C. Mariani, J.P. Schmidt, Z. Lebensm. -Unters. -Forsch. 202 (1996) 199.
[10] German Standard Methods for the Analysis of Fats and Other Lipids, F-III 1 (98), Fett/Lipid, 100 (1998) 422.
[11] German Standard Methods for the Analysis of Fats and Other Lipids, F-II 4 (84), Fette-Seifen-Anstrichm. 86 (1984) 148.
[12] IUPAC Method 2.432, International Union of Pure and Applied Chemistry, Standard Methods for the Analysis of Oils, Fats and Derivatives, Blackwell, Oxford, 7th ed., 1988.
[13] AOCS Official Method Ce 8-89, American Oil Chemists Society, Official Methods and Recommended Practices, Champaign, IL, 4th ed., 1990.
[14] S.R. Ames, J. Assoc. Off. Anal. Chem. 54 (1971) 1.
[15] J.M. Snyder, S.L. Taylor, J.W. King, J. Am. Oil Chem. Soc. 70 (1993) 349.
[16] W.F. de Greyt, V. Petrauskaite, M.J. Kellens, A.D. Huyghebaert, Fett/Lipid 100 (1998) 503.
[17] M. Balz, E. Schulte, H.-P. Thier, Fat Sci. Technol. 94 (1992) 209.
[18] S.L. Abidi, T.L. Mounts, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 509.
[19] G.W. Chase, C.C. Akoh, R.R. Eitenmiller, J. Am. Oil Chem. Soc. 71 (1994) 877.
[20] F. Zonta, B. Stancher, Riv. Ital. Sost. Grasse 60 (1983) 195.
[21] H.T. Slover, R.T. Thompson, G.V. Merola, J. Am. Oil Chem. Soc. 60 (1983) 1524.
[22] M. Arens, H.-J. Fiebig, E. Homberg, Fat Sci. Technol. 92 (1990) 189.
[23] G. Lercker, M.F. Caboni, Riv. Ital. Sostanze Grasse 62 (1985) 193.
[24] T. Itoh, T. Tamura, T. Matsumoto, J. Am. Oil Chem. Soc. 50 (1973) 122.
[25] E. Kovacheva, G. Ganchev, A. Neicheva, I. Ivanova, M. Konoushlieva, V. Andreev, J. Chromatogr. 509 (1990) 79.
[26] E. Homberg, B. Bielefeld, Fat Sci. Technol. 91 (1989) 23.
[27] E. Ballesteros, M. Gallego, M. Valcárcel, J. Chromatogr. A 719 (1996) 221.
[28] C. Mariani, E. Fedeli, K. Grob, Riv. Ital. Sostanze Grasse 68 (1991) 233.
[29] K. Grob, M. Lanfranchi, C. Mariani, J. Chromatogr. 471 (1989) 397.
[30] M. Biedermann, K. Grob, C. Mariani, Fat Sci. Technol. 95 (1993) 127.
[31] W.W. Christie, in: W.W. Christie (Ed.), Advances in Lipid Methology – One, Oily Press, Dundee, 1992, pp. 1–18.