

Solid phase extraction of β -sitosterol and α -tocopherol from sunflower oil deodorizer distillate using desilicated zeolite

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SUMMARY: In this study, the efficiency of using zeolite-based adsorbents in a solid phase extraction (SPE) procedure of α -tocopherol and β -sitosterol isolation from Sunflower Oil Deodorizer Distillate (SuDOD) without pre-treatment was investigated. The results showed that 99.2% α -tocopherol and 97.3% β -sitosterol were successfully isolated as pure fractions from SuDOD, when desilicated ZSM-5-type zeolite (DSiZSM-5) was used as adsorbent on a SPE. A simple and rapid HPLC method for simultaneous α -tocopherol and β -sitosterol analysis was developed and validated according to AOAC guidelines. It was found that the inclusion of a DSiZSM-5 SPE step increased the precision of the α -tocopherol and β -sitosterol analysis. In conclusion, DSiZSM-5 zeolite was proven to be an efficient adsorbent which can be used not only for the recovery of α -tocopherol and β -sitosterol from SuDOD in industrial scale, but also in a laboratory scale clean-up method prior to the analysis of α -tocopherol and β -sitosterol.

KEYWORDS: SPE; Sterol; Sunflower oil deodorizer distillate; Tocopherol; Zeolite

RESUMEN: *Extracción en fase sólida de β -sitosterol y α -tocoferol de destilados de aceite de girasol desodorizado utilizando zeolita desilicada.* En este estudio, se investigó la eficacia del uso de adsorbentes a base de zeolita en el procedimiento de extracción en fase sólida (EFS) para el aislamiento de α -tocoferol y β -sitosterol a partir de destilados de aceites de girasol desodorizados (SuDOD) sin ningún tratamiento previo. Los resultados mostraron que el 99,2% de α -tocoferol y el 97,3% de β -sitosterol se aislaron con éxito como fracciones puras de SuDOD, cuando se usó zeolita de tipo ZSM-5 desilicada (DSiZSM-5) como adsorbente en una EFS. Se desarrolló y validó un método HPLC simple y rápido para el análisis simultáneo de α -tocoferol y β -sitosterol de acuerdo con las directrices de la AOAC. Se encontró que la inclusión del paso DSiZSM-5 EFS aumentó la precisión del análisis de α -tocoferol y β -sitosterol. En conclusión, se demostró que la zeolita DSiZSM-5 es un adsorbente eficiente que puede usarse, no solo para la recuperación de α -tocoferol y β -sitosterol de SuDOD a escala industrial, sino también en un método de limpieza a escala de laboratorio antes del análisis de α -tocoferol y β -sitosterol.

PALABRAS CLAVE: Destilado de aceite de girasol desodorizado; EFS; Esteroles; Tocoferol; Zeolita

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1. INTRODUCTION

The recovery of valuable bioactive compounds from the by-products and wastes generated during food processing has become an extremely important strategy for the valorization of food wastes (Martins *et al.*, 2017). The wastes generated during the vegetable oil refining processes has attracted considerable attention as they contain significant amounts of such biomolecules, for instance, sterols, tocopherols, squalene and unsaturated fatty acids which have many applications in the food and pharmaceutical industries (Socaci *et al.*, 2017). Deodorization distillate is regarded as the most significant source of valuable compounds among the various wastes produced during the oil refining process (Yan *et al.*, 2012).

Different methods have been used in the literature to recover and test precious substances from the deodorizer distillate during the refining of vegetable oil. These methods include solid phase extraction (SPE) (Li *et al.*, 2008; Alghamdi *et al.*, 2018), liquid-liquid extraction (Ghosh *et al.*, 1996), esterification/transesterification (Gunawan *et al.*, 2009), molecular distillation (Vazquez *et al.*, 2006) and crystallization (Jiang *et al.*, 2006) for extraction, separation and purification (Moreira *et al.*, 2004). The most important considerations for determining the industrial applicability of the methods mentioned above are efficiency, simplicity and economic cost. In this regard, SPE is the most frequently used method among other separation techniques, in both micro and large scale because of its low cost and effectiveness (Reyes-Garcés *et al.*, 2018).

In SPE, the selection of the material used as adsorbing solid phase is the most critical factor which affects the separation performance for the target compound in the most cost-effective way (Maya *et al.*, 2018). Therefore, although they are cheap and highly abundant materials, no research has been carried out yet concerning the usage of zeolites for the recovery of valuable biomolecules from vegetable oil deodorizer distillates.

Zeolites are widely used for the shape-selective separation of molecules, a property which is mainly attributed to its porous structure and interaction with target molecules (Gounaris *et al.*, 2008). It has also been reported that restricted access to, and/or diffusional constraints within their micropores might limit the adsorption capacity of the zeolites (Verboekend *et al.*, 2011). Therefore, the modification of the microporous structure in a way to buildup the mesoporous frameworks has been proposed to facilitate the access to the micropores which possess active sites. Among the different methods applied, the desilication of zeolites with alkaline treatment has been proposed as one the most efficient and easy ways to create a mesoporous framework within zeolites. (Le Van Mao *et al.*, 1995) reported that

alkaline treatment caused an increase in the Al content of ZSM-5 and enhanced ion exchange capacity.

Therefore, in this study, we investigated the potential of using zeolites (raw and desilicated) in the solid phase extraction cartridge (SPE) for the isolation of β -sitosterol and α -tocopherol from sunflower oil deodorizer distillate (SuDOD) without any pretreatment such as esterification, crystallization, etc. An in-house HPLC method was also developed for the simultaneous determination of β -sitosterol and α -tocopherol by using a UV Vis. detector at 210 nm. The method was validated according to AOAC (Association of Official Analytical Chemists) (2002) guidelines and compared to the reference standards for the determination of α -tocopherol and β -sitosterol.

2. MATERIALS AND METHODS

2.1. Reagents and chemicals

ZSM-5-type Zeolite with a high silicon content ($\text{SiO}_2/\text{Al}_2\text{O}_3$ ratio of 469 (Si/Al 938)) and β -sitosterol (75%) were purchased from Acros Organics (Geel, Belgium). DL- α -tocopherol was donation from Supelco (Bellefonte, U.S.A). Hexane (HPLC-Isocratic grade), heptane and sodium sulphate were purchased from Carlo Erba Reagents (Milano, Italy); 2-propanol for HPLC and dichloromethane (DCM) were provided by VWR Chemicals (Radnor, Pennsylvania, USA). Potassium hydroxide pellets (KOH), N,O-Bis(trimethylsilyl)trifluoroacetamide, trimethyl chlorosilane, 5- α -cholestan-3- β -ol, tetrahydrofuran (THF) and pyridine were purchased from Merck (Darmstadt Germany). Purified water was obtained using a Milli-Q Plus system (Millipore, Billerica, MA).

In order to separate α -tocopherol and β -sitosterol, standard stock solutions were prepared in hexane at a concentration of $1000 \text{ mg}\cdot\text{L}^{-1}$ and stored in amber bottles at a temperature of -20°C . Working standard solutions were made by diluting the stock solutions with hexane in volumetric flasks. The following concentrations were obtained: 1, 2, 5, 10, 25, 50 and $100 \text{ mg}\cdot\text{L}^{-1}$.

2.2. SuDOD sample

The SuDOD samples were obtained from the vegetable oil plant production line of Beta Ziraat A.Ş. (Konya/Turkey). Approximately 1 kg SuDOD sample was homogenized with a pilot scale mixer at 500 rpm for 15 minutes prior to use in the subsequent studies.

2.3. Instrumentation

The HPLC (Agilent 1100 series, USA) system and Gas Chromatography (GC) (Perkin Elmer, Autosystem XL, USA) system were used

throughout this study. The HPLC 1100 system used for chromatographic analyses consisted of a quaternary pump (Agilent, G1311A/USA, serial number DE43630657), an automatic injector (Agilent G1367A WPALS/USA, serial number DE50404041), a column oven (Agilent, G1316A COLCOM/USA, serial number DE43644342), a degasser (Agilent, G1379A/USA, serial number JP40720677) and an UV detector (Agilent 1260 Infinity, G1314F/USA, serial number DEABB00154). Evaluations and quantifications were made on HP ChemStation software B.03.02-SR2 for a liquid chromatographic system. Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectra of all the Si/Al ratio experiments were measured on a Thermo Nicolet 6700 spectrometer FTIR (Nicolet Analytical Instruments, USA), vacuum oven (Nüve EV018, Turkey), solid phase extraction vacuum manifold system (GL Sciences, Japan), Turbovap system (Biotage, Sweden), pilot scale mixer with reactor (Heidolph, Germany) and ultrasound (Bandelin Sonorex, Germany) were used in this study.

2.4. Solid phase extraction (SPE) of α -tocopherol and β -sitosterol

For the SPE assays, both ZSM-5 zeolite and its desilicated (DSiZSM-5) form were used as adsorbents. DSiZSM-5 zeolite was synthesized according to the typical method described by Silaghi *et al.*, (2014) which involves treatment of ZSM-5 with 2 mol·L⁻¹ KOH as a base catalyzer at 110 °C for 6 hours. The Si/Al ratio was determined by using a ATR-FTIR spectrometer in order to verify the desilication process. The calculations were carried out by dividing the characteristic pentasil Si-O-Si peak at 540 cm⁻¹ by the total Si-O-Si and Si-O-Al band at 440 cm⁻¹ in the corresponding FTIR spectrum (Gaag *et al.*, 1984). Then, either ZSM-5 or DSiZSM-5 was applied into the SPE cartridge to recover α -tocopherol and β -sitosterol from SuDOD.

For the SPE assays, 1 g of either ZSM-5 or DSiZSM-5 was added to the 12 mL SPE cartridge, which was conditioned by passing 30 mL of hexane through the adsorbent, and 0.25 mL SuDOD were loaded into the cartridge. The elution of the α -tocopherol and β -sitosterol was achieved by applying a 3 step elution procedure which successively passed 40 mL of 3 different elution solvents, namely hexane: DCM, 10:1, 5:1 and 1:1 (v:v), through the cartridge at a flow rate of 1.2 mL·min⁻¹. After each step, elution solvents were collected and concentrated under a gentle stream of nitrogen at 50 °C. Finally, 1 mL of hexane was added to the residues and they were analyzed for α -tocopherol and β -sitosterol contents. All procedures were performed in triplicate and the samples were injected in duplicate. The amount determined as a percentage of the amount spiked was regarded as the recovery.

2.5. β -sitosterol content determination with the reference method

ISO 12228:1999 was used as the reference standard method for the sterol composition analysis. In this method, 1 mL 5 α -cholestan-3 β -ol (1000 mg·L⁻¹) was added to 0.5 g of sample as an internal standard. The saponification was carried out with 5.0 mL saturated methanolic KOH at 80 °C for 1 hour. Three successive extractions were carried out with 5 mL of hexane under nitrogen until reducing the volume of hexane to 10 mL, which was further dried with anhydrous sodium sulphate. 0.5 mL dried hexane extract was silylated with a solution of 250 μ L bis(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane (4:1) and 250 μ L dry pure pyridine at 60 °C for 15 min. A Gas Chromatograph equipped with a flame ionization detector (FID) was used for the sterol composition analysis of the SuDOD samples. Separation of the sterols was performed using a SE-54 (5%-phenyl-1%vinylmethylpolysiloxane, 30 m x 0.32 mm x 0.25 μ m) column; carrier gas, helium; flow rate, 0.8 mL/min; injector temperature, 280 °C; detector temperature, 300 °C; oven temperature program, initial temperature was 60 °C for 2 min, increased at 40 °C·min⁻¹ to 220 °C, held for 1 min and then increased at 5 °C·min⁻¹ to 310 °C, and held for 30 min. β -sitosterol was identified using the relative retention time (RRT) of 5 α -cholestan-3 β -ol. Data were collected and quantified with the TotalChrom Navigator software and the results of β -sitosterol were expressed as mg·kg⁻¹ of SuDOD.

2.6. α -tocopherol content determination with the reference method

ISO 9936:2016 was used as the reference standard method for the α -tocopherol analysis. According to this method, 1 g of sample was vortexed for 2 min with 10 mL of n-heptane. After filtering through a 0.45 μ m syringe-filter, the extract was injected into the HPLC-FLD system equipped with a normal-phase Luna (Silica 250 mm x 4.6 mm 5 μ m) column. The isocratic mobile phase consisted of 3.5% (volume fraction) THF in n-heptane at a flow rate of 1 mL·min⁻¹. The wavelengths for excitation and emission were 295 nm and 330 nm, respectively. Data were collected and quantified with Chemstation software and the results were expressed as mg·kg⁻¹ of SuDOD.

2.7. HPLC method for the simultaneous determination of β -sitosterol and α -tocopherol contents

A simultaneous chromatographic analysis of the β -sitosterol and α -tocopherol contents was carried out with the HPLC system. TotalChrom Navigator software was used for the system management and

data acquisition. Separation was achieved with a normal-phase Luna Silica 250 mm x 4.6 mm 5 μm i.d.. Three different elution solvents, namely, *i)* hexane/2-propanol (97/3, v/v), *ii)* heptane/2-propanol (97/3, v/v) and *iii)* hexane/2-propanol (99/1, v/v) were tested at a flow rate of $1.0\text{ mL}\cdot\text{min}^{-1}$ for the best peak separation and geometry. Detection of the β -sitosterol and α -tocopherol was performed with a UV detector set at 210 nm, in accordance with the literature (Czauderna *et al.*, 2009). The retention times of β -sitosterol and α -tocopherol were determined by injecting 25 μL of standard solutions containing $50\text{ mg}\cdot\text{L}^{-1}$ β -sitosterol, $50\text{ mg}\cdot\text{L}^{-1}$ α -tocopherol and a mixture solution containing both the β -sitosterol and α -tocopherol at a concentration of $50\text{ mg}\cdot\text{L}^{-1}$ in hexane. The analytical method developed for the determination of α -tocopherol and β -sitosterol in SuDOD was validated according to AOAC Guidelines (AOAC, 2002). Specificity and selectivity, linearity, intraday and interday precision, accuracy, the limits of detection (LOD), and the limit of quantification (LOQ) were used as method validation parameters. Calibration plots were constructed for the two analytes using peak area as a function of the standard concentration of each compound. All analysis and LOD and LOQ of the esterified SuDOD sample were determined in triplicate with successive dilution and considering the signal to noise ratio relative to the background signal as 3 and 10, respectively. The accuracy was verified by the analysis of the SuDOD sample with the reference methods (ISO, 1999; ISO, 2016).

3. RESULTS AND DISCUSSION

3.1. SPE studies with ZSM-5 and DSiZSM-5

In order to test the efficiency of zeolites for the isolation of α -tocopherol and β -sitosterol from SuDOD, ZSM-5 zeolite was first used in its ordinary form. The results of the analysis showed that ordinary ZSM-5 zeolite was not efficient in adsorbing and separating α -tocopherol and β -sitosterol in SuDOD samples. The results of the α -tocopherol and β -sitosterol analysis carried out according to reference ISO methods showed that there was no significant difference between the α -tocopherol and β -sitosterol contents of the elutions and almost all the α -tocopherol and β -sitosterol were collected from the first elution.

In order to increase the selectivity of ZSM-5 towards different lipophilic constituents found in SuDOD, we modified the porous structure of the ZSM-5 according to the simple desilication method described by (Silaghi *et al.*, 2014). After the desilication process the Si/Al ratio was quantified by comparing the transmittance values of the pentasil Si-O-Si peak at 540 cm^{-1} to the total Si-O-Si and Si-O-Al band at 440 cm^{-1} in the corresponding

FTIR spectrum in order to verify the desilication (Figure 1). The results showed that the Si/Al ratio of ZSM-5 decreased from its initial value of 938 to 520, indicating an efficient desilication. The desilicated ZSM-5-type zeolite (DSiZSM-5) was tested under the same conditions used for the ordinary ZSM-5 zeolite as described in the materials and methods section. It was found that α -tocopherol and β -sitosterol could only be eluted in second and third elution steps with 5:1 and 1:1 Hexane:DCM (v:v), respectively. The results showed that 99.2% and 97.3% of the α -tocopherol and β -sitosterol found in SuDOD could be isolated as pure fractions with DSiZSM-5. It was postulated that the metal hydroxyl (Si-OH, Al-OH) groups and the mesoporous framework formed after the desilication of the ZSM-5 (Silaghi *et al.*, 2014) might have increased the molecular interactions and size exclusion capacity of ZSM-5, which enabled the effective separation of the different lipophilic constituents found in SuDOD.

3.2. HPLC method development studies

Vegetable oil deodorizer distillates (VoDOD) are complex matrices, which contain various lipophilic constituents. Therefore, in order to eliminate the undesired signals arising from these lipophilic components which interfere with those of α -tocopherol and β -sitosterol, the utilization of different instrumental techniques is required for the accurate identification and quantification of α -tocopherol and β -sitosterol in VoDOD. In reference standard methods, in general, HPLC-FLD and GC-FID systems are suggested to be used for the determination of α -tocopherol and β -sitosterol in VoDOD, respectively.

As it was demonstrated in the previous section, it is possible to obtain high purity α -tocopherol and β -sitosterol fractions void of undesired lipophilic components using DSiZSM-5 SPE. By taking

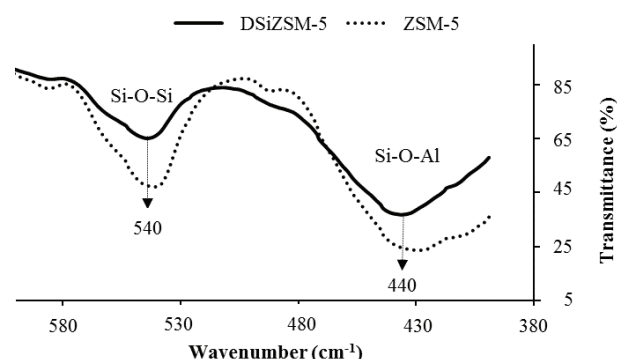


FIGURE 1. FT-IR spectra of pristine ZSM-5 (dotted line) and base ($2\text{ mol}\cdot\text{L}^{-1}$ KOH) treated ZSM-5 (DSiZSM-5; solid line). The absorbance peaks corresponding to Si-O-Si (540 cm^{-1}) and Si-O-Al (440 cm^{-1}) are marked with arrows on the figure.

advantage of this purification, we explored the possibility of developing a simple method, without any pre-treatment, which permits the analysis of α -tocopherol and β -sitosterol contents in SuDOD only by using a HPLC-UV system. In this way, it was possible to avoid the cumbersome GC method which includes saponification, extraction and derivatization steps for the sterol analysis.

In the literature, 210 and 294 nm were reported to be the wavelengths at which the β -sitosterol (Sheng *et al.*, 2009) and α -tocopherol (Renzi *et al.*, 2005) could be best detected, respectively. Therefore, in the present study, 2 different wavelengths, 210 and 295 nm, were investigated for their effectiveness in the simultaneous detection of β -sitosterol and α -tocopherol. The results showed that at 295 nm the peak of α -tocopherol was visible as expected but β -sitosterol could not be detected. At 210 nm, along with α -tocopherol, the β -sitosterol peak also became visible. The reason for selecting a single wavelength which enabled the simultaneous detection of α -tocopherol and β -sitosterol was to quantify any residual amount of each metabolite which may have existed in pure fractions.

As can be seen in Figure 2, the retention times for α -tocopherol and β -sitosterol were found as 6.3 and 19.8 min, using hexane/2-propanol (99/1, v/v) as the mobile phase, respectively, and each compound gave a specific and clear Gaussian peak shape with a good baseline resolution. In a further attempt to improve the peak separation, geometry and intensity, different elution solvents, namely, *i*) hexane/2-propanol (97/3, v/v), *ii*) heptane/2-propanol (97/3, v/v) and *iii*) hexane/2-propanol (99/1, v/v) were also tested. The results showed that the best compromise between the peak separation, geometry and intensity could be obtained with hexane/2-propanol (99/1, v/v) solvent system at 1.0 mL·min⁻¹ flow rate.

Lastly, the effect of injection volume on the peak areas was determined by injecting 5, 10 and 25 μ L standard mixtures containing 50 mg·L⁻¹ of α -tocopherol and β -sitosterol. As can be seen from Figure 3, the ideal peak width and height for β -sitosterol and α -tocopherol was obtained with 25 μ L injection volume. According to these results, the optimal conditions for the method validation were defined as: injection volume: 25 μ L; mobile phase: hexane/2-propanol (99/1, v/v); flow rate: 1.0 mL·min⁻¹; column: 250 mm \times 4.6 mm, 5 μ m silica; detection wavelength: 210 nm.

For method validation, the linearity was first studied by using pure β -sitosterol and α -tocopherol standard solutions at five different concentration levels in the range of 5-100 mg·L⁻¹ covering their expected levels in SuDOD. In Table 1, concentration levels in SuDOD samples were expressed in mg·kg⁻¹ due to the fact that separation and pre-concentration steps were included in the procedure. Table 1 summarizes the characteristics of the calibration graphs obtained for β -sitosterol and α -tocopherol standards. The limit of detection (LOD) and limit of quantification (LOQ) values for α -tocopherol and β -sitosterol were 1.39, 1.19 and 4.62, 3.96, respectively.

The reliability of the method was verified by recovery and repeatability tests. The precision of the method was calculated as both injection (within-day) repeatability and between day reproducibility which was checked on 2 different days at the same concentration levels. The results revealed that the within-day and between-day precision figures are satisfactory at less than 2.14% and the method provides a precise analysis of the β -sitosterol and α -tocopherol contents of SuDOD. The accuracy of the method was tested upon performing recovery studies and the data for SuDOD samples spiked

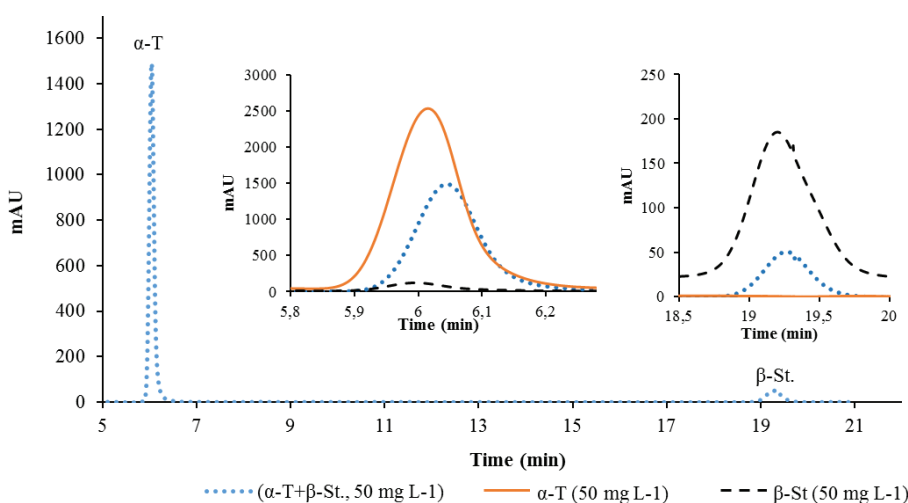


FIGURE 2. The HPLC-UV chromatograms of the standard solutions containing 50 mg·L⁻¹ α -tocopherol (α -T, orange solid line), 50 mg·L⁻¹ β -sitosterol (β -St., dashed black line) and 50 mg L⁻¹ α -tocopherol + β -sitosterol (blue dashed line) detected at 210 nm.

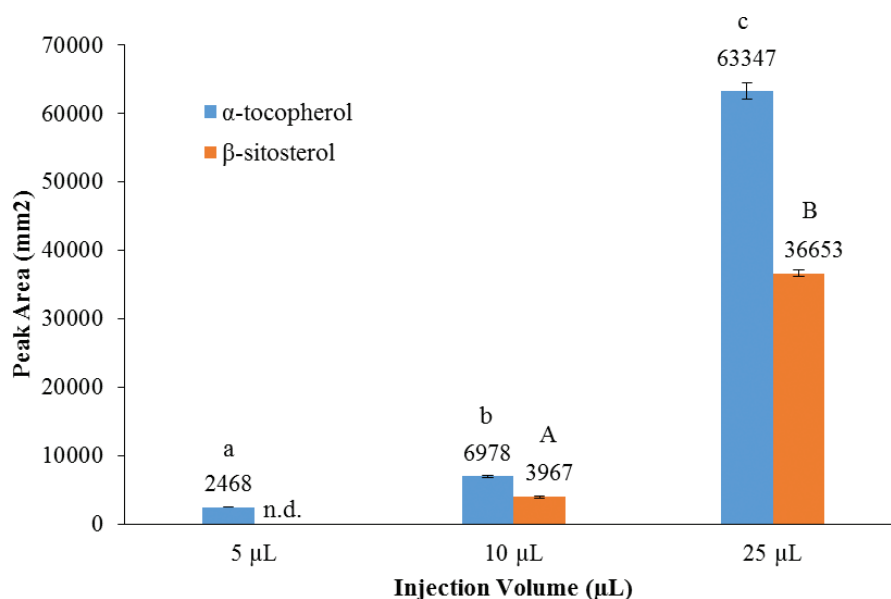


FIGURE 3. α -tocopherol and β -sitosterol peak areas corresponding to different injection volumes. Mean values and corresponding standard deviations ($n=3$) for each injection are given on the bars. The means with different letters are significantly different ($p < 0.05$) from each other. (n.d.: not detected).

TABLE 1. The data derived from calibration graphs of compounds obtained by HPLC-UV.

Results	α -tocopherol	β -sitosterol
Retention Time t_R (min)	6.8	19.8
Conc. in the vial (mg L^{-1})	5-100	5-100
Conc. in the sample (mg kg^{-1})	200-4000	125-2500
Linear Equation	$y = (72.35 \pm 1.13) * x + (29.83 \pm 49.21)$	$y = (7.97 \pm 0.14) * x + (1.44 \pm 6.19)$
Correlation coefficient (R^2)	0.9993	0.9995
LOD ^a (mg L^{-1})	1.39	1.19
LOQ ^b (mg L^{-1})	4.62	3.96
RSD ^c (% , $n=3$) within-day	0.91	0.79
RSD (% , $n=3$) between-day	2.14	2.12

^aLimit of Detection, ^bLimit of Quantification, ^cRelative Standard Deviation.

with different concentrations of standards, which are listed in Table 2.

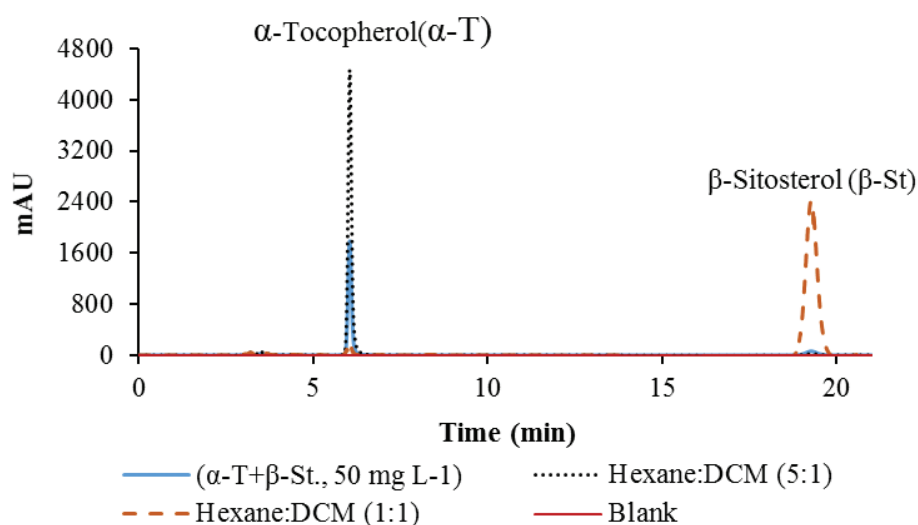
As shown in Table 2, the recovery values were above 95% for both analytes and in accordance with the AOAC Guidelines (AOAC, 2002), which dictate recovery values between 95 -102% for the samples containing a minimum of 10% of the target component.

In order to evaluate the accuracy of the proposed method, the α -tocopherol and β -sitosterol contents of the real industrial SuDOD samples were determined both by reference methods (ISO 9936:2016 for tocopherol, ISO 12228:1999 for sterol) and the in-house HPLC-UV method for the α -tocopherol and β -sitosterol contents (Figure 4). For doing so, SuDOD samples were passed through the DSiZSM-5 SPE cartridge directly and fractions were collected and analyzed.

As can be seen from Table 3, SuDOD samples contain 4.1% α -tocopherol and 5.2% β -sitosterol when analyzed according to the conventional (ISO) protocols without SPE. Significantly ($p < 0.05$) lower values, which were checked by t-test, 3.9% of α -tocopherol and 4.9% of β -sitosterol, were obtained when the SuDOD samples were passed through the SPE cartridge prior to analyses with reference ISO methods. The SuDOD samples were found to contain even lower values, 3.8% of α -tocopherol and 4.7% of β -sitosterol, when analyzed with the in-house HPLC method after having passed through the SPE cartridge. These results indicated that the utilization of SPE clean-up and the in-house method resulted in lower recovery and sensitivity in analyzing α -tocopherol and β -sitosterol compared to the conventional procedure. However, this minute difference can be ignored in light of the simplicity

TABLE 2. Recovery data for sunflower oil deodorizer distillate (SuDOD) samples spiked with different concentrations of α -tocopherol and β -sitosterol standards.

Analyte	Concentration of spiked standard		Recovery \pm SD ^a (%, n=6)
	In the vial (mg·L ⁻¹)	In the SuDOD sample (mg·kg ⁻¹)	
α -tocopherol	5	200	97 \pm 3
	50	2000	102 \pm 3
	100	4000	99 \pm 3
β -sitosterol	5	125	96 \pm 3
	50	1250	97 \pm 2
	100	2500	99 \pm 3

^aStandard Deviation.FIGURE 4. Chromatograms of sunflower oil deodorizer distillate (SuDOD) samples Hexane:dichloromethane(DCM) (1:1) (orange dashed line) and Hexane:DCM (5:1) (black dashed line), standard solution containing 50 mg·L⁻¹ of α -tocopherol (α -T) and β -sitosterol (β -St.) (blue solid line) and blank (red dashed line) sample.TABLE 3. Comparison of α -tocopherol and β -sitosterol contents of sunflower oil deodorizer distillate (SuDOD) samples treated with or without desilicated ZSM-5 (DSiZSM-5) in solid phase extraction (SPE), determined by using reference and in-house methods.

Analyte	With SPE		Without SPE
	Reference method	In-house method	Reference method
α -tocopherol (g·100g ⁻¹)	3.91 ^a \pm 0.04	3.82 ^a \pm 0.14	4.13 ^b \pm 0.07
β -sitosterol (g·100g ⁻¹)	4.87 ^a \pm 0.03	4.73 ^a \pm 0.13	5.23 ^b \pm 0.06

^aThe results were given as mean \pm standard deviation (n= 3). ^bIn a row, means with the same superscript letters were not significantly different ($p > 0.05$) according to the Student's t-test.

and speed of the method developed in this study which can be considered as a reasonable screening method for laboratories with limited infrastructure and resources.

4. CONCLUSIONS

It can be concluded that desilicated ZSM-5 zeolite can be used as an efficient adsorbent in SPE

for the isolation of α -tocopherol and β -sitosterol from SuDOD with high purity. The exclusion of lipophilic impurities from the SuDOD in the SPE clean-up procedure also permitted the development of a simple HPLC-UV method for the analysis of α -tocopherol and β -sitosterol. The advantage of using zeolite base adsorbents is that they are more abundant and cheaper than silica and polymeric adsorbents commonly used in SPE. In the future,

it should be of interest to investigate in detail the effect of desilication and dealumination on the porous structure of the zeolites and their performances in the isolation of target molecules from complex matrices.

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