Journal of Chromatography A, 1217 (2010) 4682–4687

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Improving analysis of apolar organic compounds by the use of a capillary titania-based column: Application to the direct determination of faecal sterols cholesterol and coprostanol in wastewater samples

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ARTICLE INFO

Article history:
Received 28 January 2010
Received in revised form 23 April 2010
Accepted 3 May 2010
Available online 6 May 2010

Keywords:
In-tube solid-phase microextraction (IT-SPME)
Reversed-phase TiO_2 capillary column (RP-TiO_2 capillary column)
Sterols
Wastewater samples

ABSTRACT

This article reports a new procedure for the direct determination of faecal sterols coprostanol and cholesterol in wastewater samples as tracers of human sewage contamination. The method combines in-tube solid-phase microextraction (IT-SPME) for analyte enrichment and capillary liquid chromatography (LC) for separation with diode array detection for identification and quantification. A titania-based polymeric capillary column and a conventional octadecyl silica (ODS) capillary column were evaluated and compared for their ability to separate the analytes. The titania-based column allowed the separation of the analytes in much shorter chromatographic times and with better chromatographic profiles, which in turn resulted in better detectability. In addition, IT-SPME allowed the direct injection into the chromatographic system of sample volumes as large as 200 μL, thus making unnecessary off-line clean-up and concentration steps. In such a way, the tested compounds could be directly analysed in less than 10 min, the limits of detection (LODs) being 10 and 1.2 μg/L for coprostanol and cholesterol, respectively. The reliability of the proposed method was tested by processing several wastewater samples.

1. Introduction

Recent research efforts are oriented towards the development of sensitive and simplified analytical methodologies which reduce the time of analysis, the use of chemicals and the generation of wastes. This is especially important in environmental analysis as a great number of samples must be processed in order to assure the environmental quality. The study of the applicability of new materials is also an important research field for the development of new procedures or for the improvement of existing ones. An example is the study of new stationary phases for liquid chromatography such as those based on metal oxides like titania [1,2]. Titania-based materials are attractive phases because they offer better pH and thermal stability than silica-based stationary phases [2–5]. In addition, differences in the chromatographic retention and selectivity arising from the surface chemistry may facilitate the separation of compounds which cannot be satisfactorily resolved on silica-based phases. In particular, the lower hydrophobicity of reversed-phase (RP) TiO_2 phases compared to RP-SiO_2 materials may be useful to reduce the retention times of very apolar compounds [6].

Several methods for the analysis of faecal sterols in waters have been published in the last years owing to their utility as indicator of faecal human contamination. Coprostanol is the major human sterol found in water, and it is produced by microbial reduction of cholesterol [7]. The concentration ratio of coprostanol to cholesterol has been proposed to estimate the degree of faecal matter in water samples. Waters having coprostanol to cholesterol concentration ratios greater than 0.2 are considered contaminated by faecal material [8]. The most used technique for the analysis of faecal sterols in environmental waters is gas chromatography (GC). To achieve the required sensitivity and selectivity the analytes must be extracted from the samples and then concentrated and derivatized before the chromatographic step. For extraction different strategies have been proposed which involve supercritical fluid extraction [9], sonication assisted extraction [10], Soxhlet extraction [11] and liquid–liquid extraction [12]. Although good extraction efficiencies and sensitivities are generally achieved, the resulting procedures are very laborious and very time consuming (total analysis times longer than 24 h have been reported [10]). Although LC is well-suited for the analysis of organic compounds in water matrices, only a few procedures using this technique have been proposed for the analysis of sterols, probably because these compounds are less sensitive towards common LC detectors than other classes of pollutants. The reported procedures allow the measurement of cholesterol at mg/mL concentration levels. Such sensitivity may be

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doi:10.1016/j.chroma.2010.05.001

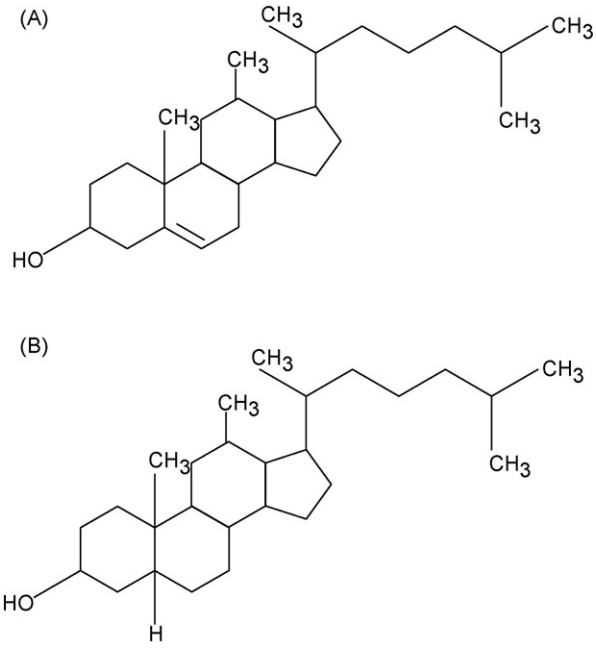

Fig. 1. Chemical structure of (A) cholesterol and (B) coprostanol.

adequate for analysing pharmaceuticals [13], food and biological samples [14], but it is not sufficient for monitoring faecal sterols in water samples. To our knowledge, only one LC procedure has been reported for measuring coprostanol in water, which combined solid-phase extraction and post-column derivatization [15]. Therefore, the development of simple and sensitive methods for determining faecal sterols continues to be of great interest for monitoring water samples.

There are different approaches for increasing the sensitivity in LC. The combination of a reduction of the column diameter (micro-, capillary or nano-LC) and the injection of a large sample volume via an in-tube solid-phase microextraction (IT-SPME) device is an effective alternative for the analysis of organic pollutants in waters, as demonstrated for pesticides and polycyclic aromatic hydrocarbons [16–18].

The aim of this work was to develop a direct and sensitive procedure for the analysis of faecal sterols in water samples using IT-SPME and capillary LC. Cholesterol and coprostanol were selected as model compounds because they are the most frequently detected sterols in wastewaters [19], and also because of their significance to establish sewage contamination [7,8]. Firstly, and since sterols are very apolar substances, we studied whether the employment of a titania-based stationary phase could be an alternative to conventional ODS columns. After optimizing the chromatographic conditions, the main parameters affecting the efficiency of the IT-SPME were optimized. On the basis of the results obtained, possible conditions for the direct analysis of coprostanol and cholesterol (Fig. 1) in wastewater samples were proposed. The reliability of proposed methodology was tested by analysing several wastewater samples.

2. Experimental

2.1. Chemicals

All reagents were of analytical grade. Coprostanol and cholesterol were obtained from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile and methanol were of HPLC grade (Scharlau, Barcelona, Spain).

The stock solutions of sterols (1.0 g/L) were prepared in methanol. Working solutions containing up to 5 μg/mL of coprostanol and 400 μg/mL of cholesterol were prepared by dilution of the stock solutions with water, whereas working solutions containing higher concentrations of the analytes were prepared by dilution of the stock solutions with methanol.

2.2. Equipment and chromatographic conditions

The capillary chromatographic system used consisted of a LC capillary pump (Agilent 1100 series, Waldbronn, Germany) and a UV–Vis diode array detector (Agilent, 1200 series) equipped with a 80-nL flow cell. The wavelength was set at 210 nm and the spectra were recorded between 190 and 400 nm. An injection valve with an internal loop of 2 μL was used for direct injection of the analytes into the chromatographic column. In the IT-SPME–capillary LC configuration samples were injected via a six-port injection valve equipped with a GC capillary column which acted the injection loop. GC TRB-5 capillary columns of 30 and 70 cm lengths, with 0.32 mm i.d. coated with 5% diphenyl–95% polydimethylsiloxane (PDMS) (3-μm coating thickness) were used for the IT-SPME (Teknokroma, Barcelona, Spain).

For the separation of the analytes a Zirchom Sachtopore RP-TiO_2 100 mm × 0.5 mm i.d., 5-μm column (Sugelabor, Madrid, Spain) or a Zorbax SB C18 (SiO_2-based) 150 mm × 0.5 mm i.d., 5-μm column (Agilent) was used. Unless otherwise stated, the mobile-phase used with the TiO_2-based column was a mixture of acetonitrile:water 90:10 (v/v) at a flow-rate of 15 μL/min, and in assays with the SiO_2-based column the mobile-phase was 100% acetonitrile and the flow-rate was 20 μL/min. All solvent were filtered thorough 0.45-mm nylon membranes (Teknokroma) before use.

2.3. IT-SPME procedure

The TRB-5 capillary column was connected to a conventional six-port injection valve and used as the injection loop. Capillary connections to valve were facilitated by the use of 2.5-cm sleeve of 1/16-in. polyether ether ketone (PEEK) tubing; 1/16-in. PEEK nuts and ferrules were used to complete the connections. Samples were manually processed into the system by means of a 1.0-mL precision syringe. After sample loading the valve was manually rotated, so the analytes were desorbed from the extractive phase of the GC capillary with the mobile-phase, and transferred to the analytical column for separation and detection [16–18].

All the experiments were carried out in triplicate and at room temperature.

2.4. Water samples

Several wastewater samples collected at different water treatment plants were directly analysed (without filtration). A water sample collected in a septic tank was also processed; this sample was filtered through a 0.45-mm nylon membrane (Teknokroma) before injection into the IT-SPME device. After the arrival to the laboratory water samples were stored in dark in brown glass flasks at 4 °C until analysis. Each sample was analysed in triplicate and at room temperature.

3. Results and discussion

3.1. RP-TiO_2 capillary chromatographic column for separation of sterols

In the present study, two different capillary LC columns were evaluated and compared for the separation of coprostanol and cholesterol, a conventional SiO_2-based column and a TiO_2-based

Table 1
Comparison of the resolution, retention factors and selectivity obtained with the SiO_2- and the TiO_2-based columns using conditions A and B, respectively.

	Resolution, *Rs*	Retention factor, *k*	Selectivity, α
C18-based column	4.0	Coprostanol 9.6 Cholesterol 18	1.87
RP-TiO_2-based column	2.6	Coprostanol 3.6 Cholesterol 6.7	1.86

column. Standard solutions of coprostanol and cholesterol at concentrations of 200 and 5 μg/mL, respectively, were used. These solutions were directly injected using an injection valve equipped with a 2-μL internal loop. For each column several acetonitrile:water mixtures and flow-rates were assayed.

When using the SiO_2-based column coprostanol and cholesterol were satisfactorily resolved but, as expected, both compounds were highly retained. As an example, Fig. 2A depicts the chromatogram obtained with a mobile-phase containing 100% acetonitrile at the highest flow-rate assayed, 20 μL/min (conditions A). Even under such conditions the time required to complete the chromatographic separation was higher than 20 min. When using the RP-TiO_2 column satisfactory resolution was also obtained under a variety of conditions, but the time required to complete the chromatographic separation was drastically shortened. Best results were observed when using a mobile-phase consisting of acetonitrile:water 90:10 (v/v), and a flow-rate 15 μL/min (conditions B). Under such conditions the analytes were eluted in less than 10 min (Fig. 2B). In addition, the employment of the TiO_2-based column resulted in lower peak widths. The peak widths were 0.8 min for coprostanol and 1.2 min for cholesterol, whereas in the SiO_2-based column the peak widths were 1.6 min for coprostanol and 3.2 min for cholesterol. Consequently, the chromatographic peaks obtained with the RP-TiO_2 were also higher (see Fig. 2).

Table 1 lists the resolution, retention factors and selectivity obtained with the SiO_2 and TiO_2-based columns, and the elution conditions A and B, respectively. Good resolution (*Rs*) values were obtained in both cases with similar selectivity (α). The retention factor (*k*) was more favourable with the RP-TiO_2 column than with the SiO_2-based column. Thus, the RP-TiO_2 column was clearly advantageous for the separation of coprostanol and cholesterol as satisfactory chromatographic separation was reached in shorter times. Further studies were carried out with the TiO_2-based column using the optimized mobile-phase composition and flow-rate.

In another set of experiments, the precision (RSD) was calculated for both, retention times and analytical signals (peak areas), using a solution containing 200 μg/mL of coprostanol and 5 μg/mL of cholesterol. The RSD coefficients for retention times were lower than 1% ($n = 6$) for the two sterols tested. As regards the analytical signals, RSD values of 4 and 5% were obtained for coprostanol and cholesterol, respectively ($n = 6$). These results indicate that the performance of the RP-TiO_2 column was suitable and comparable to that reported when using conventional SiO_2-based stationary phases [13,14].

3.2. IT-SPME optimization

IT-SPME is an effective sample preparation technique which uses open-tubular capillary columns such as those typically used in GC for extraction of the target analytes. In this technique, also known as open-tubular trapping, the analytes are concentrated into the solid or liquid coating of the capillary column when the sample is passed through this column [20,21]. There are different options to effect IT-SPME. The replacement of the external loop of an injection valve by the capillary column is probably the most convenient configuration (in-valve IT-SPME), as it allows the introduction of large sample volumes (until sufficient analyte is extracted to reach the required detectability), and also because no extra instrumentations are required [16–18]. In the present study, in-valve IT-SPME was the configuration used for analysing coprostanol and cholesterol. As regards the column coating, a polydimethylsiloxane–polydiphenylsiloxane phase was selected taking into account the nonpolar nature of the analytes.

Initial assays were aimed to test whether the mobile-phase optimized for separation (see the above section) was adequate to desorb the analytes retained in the extractive capillary column. In this study, a 30-cm-length capillary column (internal volume of 20 μL) was used. Aliquots of 20 μL of a standard solution of containing 1 μg/mL of coprostanol and 0.25 μg/mL of cholesterol were introduced into the extractive capillary column, and then dynamically desorbed and transferred to the analytical capillary column for separation and detection. The resulting chromatograms were

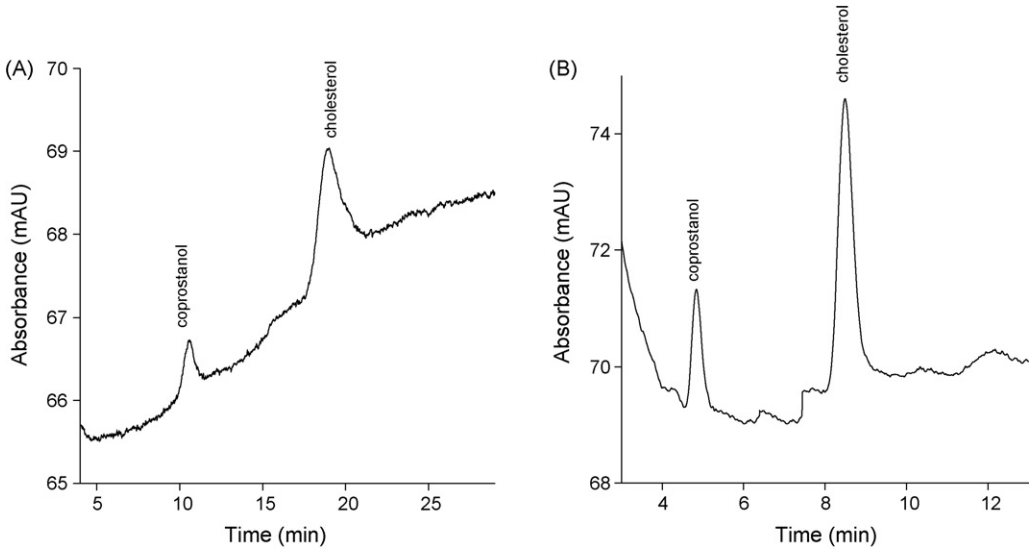

Fig. 2. Chromatograms obtained for a mixture of coprostanol and cholesterol (200 and 5 μg/mL, respectively) with (A) the RP-SiO_2 capillary column and (B) the RP-TiO_2 capillary column using direct sample injection. Conditions with the RP-SiO_2 column: volume of sample, 2 μL; mobile-phase composition, 100% acetonitrile; flow-rate, 20 μL/min. Conditions with the RP-TiO_2 column: volume of sample, 2 μL; mobile-phase composition, acetonitrile–water 90:10 (v/v); flow-rate, 15 μL/min. Separations were carried out at ambient temperature. For other experimental details, see text.

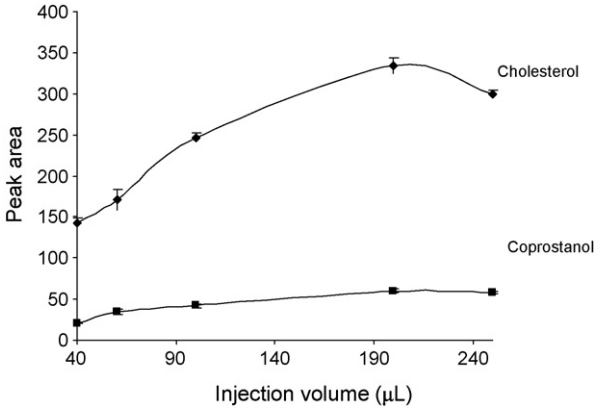

Fig. 3. Analytical signal obtained as function of the injection volume with the IT-SPME–capillary LC method for a solution containing 1 μg/mL of coprostanol and 0.5 μg/mL of cholesterol. For other experimental details, see text.

similar to those obtained by direct injection of the samples, and neither peak distortion nor loss in resolution was observed. Thus, it was concluded that the mobile-phase composition and flow-rate selected for the chromatographic separation (conditions B) was also adequate to desorb and transfer the analytes from the extractive to the analytical column.

The effect of the extractive capillary column length was evaluated by processing different sample volumes of the working solution in two columns of 30- and 60-cm-column lengths (internal volumes of 20 and 40 μL, respectively). Similar chromatographic profiles were observed with the two column lengths tested, although peak areas for the two analytes were higher when using the 60-cm capillary column. Consequently, the 60-cm capillary column was selected for further work.

Finally, in order to optimize the sample volume, different sample volumes from 40 to 250 μL were assayed using a standard solution containing a mixture of coprostanol (1 μg/mL) and cholesterol (0.5 μg/mL). The results of this study are depicted in Fig. 3. As observed, the peak areas for cholesterol increased as the volume of sample injected into the capillary increased within the interval 40–200 μL. A subsequent increase of the sample volume to 250 μL resulted in a decrement of the signal, which suggested that saturation for the capillary coating occurred. In contrast, the peak area of coprostanol increased within the tested volume interval. This indicates that the affinity of cholesterol by the column coating is higher than the affinity of coprostanol, which is in agreement with their respective polarities (the octanol/water partition coefficient of cholesterol is about 10 times higher than that of coprostanol). As it can be deduced, injecting a sample volume of 200 μL is the best option for the two analytes tested, and consequently, this was the sample volume selected in the final procedure.

The absolute recoveries obtained with the proposed IT-SPME procedure were established by comparing the amounts of the analytes that reached the analytical column with the amounts passed through the enrichment capillary column. The amount of each analyte that reached the analytical column was calculated from its peak area in the chromatogram and from a calibration equation constructed by injecting the analyte directly into the analytical column (2 μL of each working solution). The absolute recoveries for coprostanol (1 μg/mL) and cholesterol (0.5 μg/mL) were 20 ± 1 and 15 ± 1% (n = 3), respectively, which are values typically encountered when using in-valve IT-SPME. It should be remembered that, under the in-valve IT-SPME approach, the volume of sample passed through the extractive column is usually much larger than the inner volume of the capillary, and thus, the extraction is not exhaustive. Instead, sample is passed through the capillary until sufficient analyte is extracted or until the extractive phase is saturated.

As an illustrative example, Fig. 4A shows the chromatogram obtained for a solution containing coprostanol (0.2 μg/mL) and cholesterol (0.5 μg/mL) under the proposed conditions.

3.3. Analytical performance

Different assays were carried out to evaluate the analytical performance of the proposed IT-SPME–capillary LC method. The linearity was evaluated by processing solutions containing

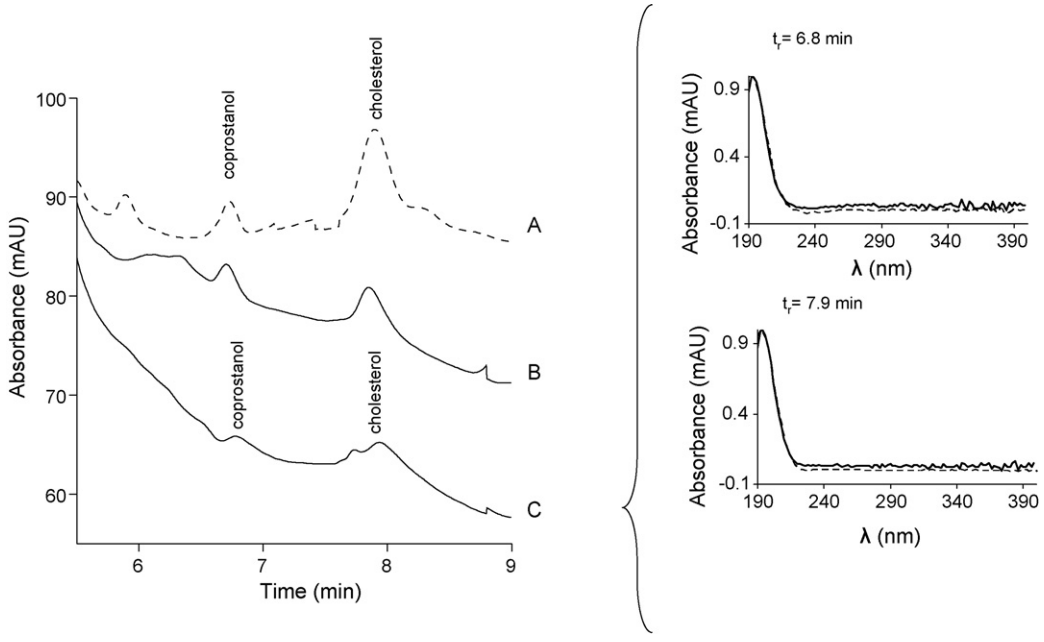

Fig. 4. Chromatograms obtained for (A) standard solution of coprostanol (0.2 μg/mL) and cholesterol (0.5 μg/mL), (B) wastewater S1 spiked with 0.2 μg/mL coprostanol and 0.05 μg/mL cholesterol, and (C) wastewater sample S1. Right: spectra of the sample obtained at retention times (t_r) 6.8 and 7.9 min. Doted line spectra at t_r 6.8 and 7.9 min correspond to coprostanol and cholesterol spectra in standard solutions. Conditions: volume of sample, 200 μL; mobile-phase composition, acetonitrile–water 90:10 (v/v); flow-rate, 15 μL/min; separations were carried out at ambient temperature. For other experimental details, see text.

Table 2
Analytical parameters for coprostanol and cholesterol obtained with the IT-SPME–capillary LC method.

	$y = b_0 + b_1 x$ (μg/L)		R^2	S_{xy}	Working range (μg/L)	LOD (μg/L)	LOQ (μg/L)
	$b_0 \pm s_{b0}$	$b_1 \pm s_{b1}$					
Coprostanol	1 ± 6	0.058 ± 0.002	0.998	7.711	40–5000	10.0	30.0
Cholesterol	2 ± 5	2.60 ± 0.07	0.998	7.529	5–125	1.3	4.0

coprostanol and cholesterol within the concentration intervals 40–5000 and 5–125 μg/L, respectively. The results obtained (Table 2) showed that the responses (peak areas) were linear within the tested concentration intervals. Thus, no saturation of the column capillary coating occurred within those concentration intervals.

The precision was evaluated through the consecutive injection of a solution containing coprostanol (500 μg/L) and cholesterol (62.5 μg/L). The RSD (%) values calculated for the analytical signals were 6% ($n = 6$) for coprostanol and 5% ($n = 6$) for cholesterol. The RSD values for the retention times were lower than 1% ($n = 6$) for both analytes. Therefore, the precision of the method was considered satisfactory.

The limits of detection (LODs), established as the concentration of analyte required to generate a signal-to-noise ratio of 3, were obtained by injecting solutions of decreasing concentrations of the analytes. Before analysing each solution, water was processed to confirm the absence of contaminants and/or memory effects. The values obtained are listed in Table 2. In this table are also listed the limits of quantification (LOQs). According to the literature, the detectability of the method can be considered suitable to determine the content of coprostanol and cholesterol in wastewater [12,19]. Although not tested, improved detectability would be achieved by increasing the length of the extractive capillary.

The values in Table 2 indicate that the sensitivity of the method is better for cholesterol than for coprostanol. This can be explained by the fact that at the working wavelength and for the same molar concentrations of the analytes cholesterol presents higher absorbance, and also because the percentage of analyte recovered by the IT-SPME approach is higher for cholesterol than for coprostanol. On the other hand, considering that a coprostanol to cholesterol concentration ratio higher than 0.2 is indicative of the presence of faecal contamination, in waters containing a concentration of coprostanol equivalent to its LOQ the occurrence of faecal contamination could only be established for concentrations of cholesterol in the range 1.3–150 μg/L.

3.4. Analysis of real wastewater samples

Ten sewage-treatment work effluents collected from different water treatment plants were directly analysed with the proposed procedure. The samples were directly introduced into the IT-SPME device. Coprostanol and cholesterol were detected only in one of the samples analysed (S1). The chromatogram corresponding to such sample is shown in Fig. 4C. Fig. 4 (right) shows the spectra obtained for the sample at retention times of 6.8 and 7.9 min, respectively. The comparison of the spectra at these retention times confirmed the presence of coprostanol and cholesterol in this sample as they completely match with the spectra of standards of coprostanol and cholesterol (dotted lines). Although, the spectra of both compounds are quite similar, they can be distinguished by their retention times. No other peaks with spectra similar to those of the analytes were found at similar retention times. The concordance between retention times and UV spectra is generally accepted as a proof of the presence of a given compound in the tested sample [24]. Indeed, for studies including other sterols, LC–MS would be more reliable.

The concentration of coprostanol in sample S1 was 140 ± 10 μg/L ($n = 3$). Since the content of suspended particulate matter in these samples was low (<15 mg/L) it was assumed that this concentration was equivalent to the total concentration [22,23]. Cholesterol was also detected but at a concentration below its LOQ (4 μg/L). A chromatogram of S1 fortified with 200 μg/L of coprostanol and 50 μg/L of cholesterol is depicted in Fig. 4B. The percentages of the analytes recovered after IT-SPME were calculated from the differences of areas between the spiked and the unspiked sample. The increments were compared with the areas obtained after the direct injection of a solution containing an equivalent amount of the analytes. The mean recoveries obtained for coprostanol and cholesterol were 100 ± 7 and 92 ± 6%, respectively ($n = 6$).

Another sample (S2) was spiked with 400 μg/L of coprostanol and 40 μg/L of cholesterol and subjected to the described IT-SPME procedure. The mean recoveries obtained were 96 ± 9 and 95 ± 6% for coprostanol and cholesterol, respectively ($n = 3$).

The above results indicated that the recoveries in the spiked wastewater samples were statistically equivalent and independent on the concentration of the analytes. It was concluded that, as expected, the extraction efficiency of the method no affected by the particulate matter of the sample.

In order to investigate the applicability of the proposed procedure for samples with different origins, a sample collected in a septic tank impacted by human faecal contamination was also analysed. In this sample the content of suspended particulate matter was significantly higher in comparison with the other samples analysed. Thus, the sample was filtered before injection in order to prevent rapid pressure development in the IT-SPME device. The analysis of this sample shows the presence of 360 ± 20 μg/L of dissolved coprostanol ($n = 3$); cholesterol was not detected in this sample.

Table 3
Comparison of some relevant features of methods proposed for the analysis of sterols in environmental samples.

Type of sample	Sample pretreatment		Separation and detection	Total analysis time	LOD (μg/L)		Reference
	Extraction	Derivatization	Technique		Coprostanol	Cholesterol	
Wastewater	Supercritical fluid	Yes	GC–ECD	>1 h	<5	<5	[9]
River and coastal waters	Sonication assisted	Yes	GC–MS	>24 h	5×10^{-4}	–	[10]
River and creek waters	Soxhlet	Yes	GC–MS	>7 h	<0.027	<1	[11]
Wastewater and surface water	Liquid–liquid	Yes	GC–FID	>3 h	1–2	1–2	[12]
Wastewater	Solid-phase into C_{18} cartridges	Yes	Conventional LC–UV	>35 min[a]	Not reported	–	[15]
Wastewater	IT-SPME	No	Capillary LC–UV	<10 min	10	1.3	This work

[a] Time estimated only for the chromatographic run.

3.5. Utility

The results of the present study demonstrated that the IT-SPME–capillary LC approach is a valid alternative for the analysis of cholesterol and coprostanol in wastewater. The method combines large sample injection by IT-SPME and separation by capillary LC using a titania-based stationary column. In Table 3 are listed some relevant features of methods proposed for the analysis of sterols in waters. As it can be deduced from such table, the main advantage of the proposed method over other procedures proposed for the analysis of wastewaters is its simplicity, as coprostanol and cholesterol can be directly analysed in the whole samples, provided that the content of suspended particulate matter is not too high to clog the extractive capillary. In contrast, previously described GC and LC assays entail the extraction and derivatization of the analytes [9,12,13]. Therefore, such methods are much more time consuming, and several off-line operations are generally required. Total analysis times of several hours are generally required whereas with the proposed method the analysis can be completed in less than 10 min. The employment of a titania-based column has been found to be advantageous over a conventional RP silica-based column. The reason is that, since faecal sterols are highly apolar compounds they were strongly retained on the silica-based column. It should be noted that in the only LC procedure proposed for the determination of coprostanol in water (see Table 3), the time required for the chromatographic step was significantly longer than that of the proposed method. The use of the RP-TiO_2 column also resulted in better chromatographic profiles. On the other hand, the in-valve IT-SPME methodology allowed the injection of relatively large sample volumes (200 μL). As a result, the detectability reached was adequate for the direct determination of the tested analytes in wastewaters. As it can be deduced from Table 3, the LODs obtained are comparable to those previously reported for the determination of faecal sterols in wastewater samples [12]. Nevertheless, for other types of waters, more sensitive methodologies may be required.

On the other hand, previously described methods require organic solvents for extraction and chemical reagents for derivatization, and in some instances, extra instrumentation (extractors and evaporators). In contrast, with the proposed procedure the consumption of chemicals for sample conditioning is totally avoided. Moreover, this methodology only requires the replacement of the inner injection loop by a capillary column such as those used in GC, and the same capillary column can be reutilised for several times. In the present study more than 80 samples could be analysed with the same capillary without observing a deterioration of its extraction capability. In addition, the entire procedure could be easily automatised by replacing the injection loop of a conventional programmable autosampler by the extractive capillary column. This would render the procedure more simple and cost-effective.

4. Conclusions

In the present study it has been demonstrated that the IT-SPME–capillary LC methodology is an excellent alternative for the analysis of faecal sterols coprostanol and cholesterol in whole wastewater. The in-valve IT-SPME technique allowed the direct injection of up to 200 μL of the samples, thus providing excellent detectability. The detectability was further improved using a TiO_2-based column for the chromatographic separation. Thus, TiO_2-stationary phases are a good alternative to conventional ODS columns for the separation of coprostanol and cholesterol. In principle the methodology could be extended to other sterols, but a modification of the separation/detection conditions would most probably be required.

The proposed method is very simple and rapid, and enables the determination of coprostanol and cholesterol in wastewater samples without off-line sample manipulation in less than 10 min. The method can be also used to analyse the dissolved fraction of cholesterol and coprostanol in samples with high contents of suspended particulate matter, if the samples are previously filtered. In addition, the consumption of reagents and generation of wastes is reduced to a minimum (mobile-phase). Therefore, the proposed assay is a reliable, cost-effective and environmental-friendly alternative for monitoring analysis of faecal pollution in wastewaters.

Acknowledgements

The authors are grateful to the Spanish Ministerio de Educación y Ciencia (project CTQ 2008-01329/BQU) and to the Conselleria dïEducació, Generalitat Valenciana, for the project ACOMP09/306. Y.M.M. expresses her grateful for her JdC research contract (Ministerio de Ciencia e Innovación).

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