SIMULTANEOUS EXTRACTION, DERIVATIZATION AND CLEAN-UP OF STEROLS FROM SOIL USING ACCELERATED SOLVENT EXTRACTION

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Abstract

A one-step extraction, derivatization and clean-up technique for the determination of sterols in soil by accelerated solvent extraction (ASE) is presented. In this method, in situ derivatization of sterols using 99% N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) was carried out inside the extraction cell. The extracts were analyzed by gas chromatography with mass spectrometry detector (GC-MSD) and gas chromatography with flame ionization detector (GC-FID). The effects of solvent, sorbent and temperature on the in situ derivatization of sterols were investigated using Ottawa sand fortified at 50 ppm sterol mixture (cholesterol, stigmasterol and stigmastanol). The extraction performed using dichloromethane at extraction pressure of 1500 p.s.i, temperature 60ºC and static extraction time of 15 min gave recoveries from 87 to 96 %. The effect of several types of sorbents as on-line clean-up by inclusion of sorbents in the extraction cell on the extraction efficiencies were investigated using spiked soil sample. Increase in static extraction time significantly improved the recoveries of the analytes. The utilization of integrated extraction, derivatiation and clean-up strategy simplify and shorten the sample preparation step in an analysis.

Keywords: Accelerated solvent extraction, sterols, in situ derivatization

Introduction

Extraction of organic pollutants from soil is a critical step during soil analysis, because hydrophobic compounds are strongly sorbed to the soil material. The sorption occurs through a combination of surface adsorption and partitioning (or dissolution) into organic phases, the latter being generally regarded as the major mechanism. However, this partitioning is slow and the fraction of pollutants bound to the organic matter increases with time, a phenomenon referred to as 'aging'. Consequently, the reverse process desorption is also slow, limiting pollutant bioavailability and extractability. In an attempt to overcome these limitations, several other extraction techniques have been developed. Among them, microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE) have recently been under intensive study, with good efficiency and reliability being reported.

ASE is a sample preparation technique that combines elevated temperature and pressure with liquid solvents to achieve fast and efficient extraction of the analytes from the solid matrix [1]. ASE is recognized as a rather uncomplicated, exhaustive extraction technique, which is easy to learn and provides quantitative recoveries with little time spent on method development [2]. ASE has gained wide acceptance for the extraction of persistent organic pollutants (POPs) from various environmental and biological matrices. When POPs enter the human chain, as in a number of recent crises, thousands of samples have to be analyzed in a short time to protect humans from severe POPs exposure [2]. In such cases, ASE provides a good alternative to conventional extraction techniques.

In the extraction of sterols from soil using ASE, dirty extracts are usually obtained because humic organic matter (HOM) will also be extracted together with the analytes [3]. Therefore a clean-up step is required to remove interferences in the extract prior to gas chromatographic analysis [4]. In addition, sterols have to be derivatized to their corresponding ethers. Driven by the need for a faster, simpler and reliable analytical method, combining extraction, derivatization and clean-up steps is a promising strategy.

Experimental

Standards and reagents

Sterol standards (cholesterol, stigmasterol and stigmastanol) with purity greater than 95 % were purchased from Sigma-Aldrich (Chemie GmbH, Steinheim, Germany). Dichloromethane, acetone and toluene were purchased

from AJAX (LabChem, Malaysia). Florisil (60-100 mesh) (Fisher Scientific Loughborough, United Kingdom) and ultra pure silica gel (70-230 mesh) (Silicycle, Quebec, Canada) were activated for 24h at 130°C before used. Diatomaceous earth non-washed was purchased from Sigma-Aldrich (Steinheim, Germany). Sand, acid washed was obtained from Fisher Scientific (Hong Kong). Activated carbon was soaked with methanol for 24 hours and dried at 50ºC to activate it. The derivatization agent for silylation, Derivatization agent, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (99 BSTFA:1 TMCS) (Sigma-Aldrich (Chemie GmbH, Steinheim, Germany) and sterol standards were stored at ≈ 4°C to avoid a drop in activity.

Preparation of spiked sample

Soil sample (free of sterols) were air dried for about 48 hours and sieved (600µm mesh). The fine soil was transferred into air tight amber bottle and stored at about 4°C. The soil was spiked with sterol standard mixture and stored at about 4°C for 2 days before extraction.

ASE one step clean-up with in-situ derivatization procedure

Extractions were done using ASE 200 accelerated solvent extractor (Dionex, Sunnyvale, CA, USA) equipped with 22-mL stainless-steel cells. Cell loading was done in the following sequence: Cellulose filter was placed at the bottom of the extraction cell, then 2 g of sorbent, topped by cellulose filter followed by 5g of spiked soil blended with diatomaceous earth (1.5g). After sample loading, the cell cap was hand tightened and placed into the cell tray for extraction. Several extraction solvents were used and derivatizing agent, BSTFA with ratio of 99:1 was added to the selected solvent. The extract was then concentrated to 1 mL prior to gas chromatograph analysis.

Ex-situ derivatization

The extract was concentrated to 1 mL and derivatization agent (99 BSTFA:1 TMCS) was then added. Derivatization was carried out for 1 hour at 60ºC.

Gas chromatographic analysis

Gas chromatography with flame ionization detector (GC-FID). Gas chromatographic separation and identification of PAHs and sterols were performed using HP6890 series II (Agilent Technologies Inc., Palo Alto, CA, USA) with splitless injection and flame ionization detection. A 30 m x 0.25 mm id x 0.25µm film thickness HP5-MS capillary column (Agilent Technologies) was used to achieve separation of PAHs and sterols with the following temperature program: initial temperature, 230°C; increase at 8°C min^{-1} to 305°C. The injector and detector temperature was set at 310°C and 280°C respectively.

Gas chromatography with mass spectrometry detector (GC-MSD**).** An Agilent Technologies 6890N gas chromatograph coupled with a MD5973 Series mass selective detector and an HP 7673 GC auto-sampler was employed. Samples were separated on a 30m × 250µm x 0.25µm, HP-5 (Agilent technologies) fused silica capillary column. The column temperature was programmed as follows: initial temperature, 230°C and increased to 305°C at 8°C min^{-1}, increased to 310°C at 5°C min^{-1} and held for 1 min. Helium was used as carrier gas. Injector temperature was maintained at 310°C. The interface temperature was held at 280°C.

Results and Discussion

In-situ derivatization of sterols

In this study, extraction and derivatization of sterol was done simultaneously inside the extraction cell. Derivatization agent, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (99 BSTFA:1 TMCS) was added into the solvent used for extraction. The efficiency of in-situ derivatization was studied using several types of solvents. ASE was operated with extraction temperature of 60°C, extraction pressure of 1500 psi and static time of 15 min. The extraction temperature set was lower than the temperature suggested in the USEPA Method 3545 since 60°C was the temperature commonly used in derivatization procedure. Sand was spiked with 50 ppm standard sterols and extraction recoveries based on derivatized sterols were determined. The derivatized sterols were confirmed using gas chromatograph with mass spectrometry detector.

Effect of solvent on in-situ derivatization. A correct choice of solvent is important in obtaining an optimal extraction. To achieve fast and efficient extraction of the target compounds from a solid matrix, the polarity of the extraction solvent or mixture of solvents should closely match that of target compounds. Choosing the best solvent to extract sterols from sample matrix is crucial due to unique characteristic of sterols and the chosen extraction solvent should also be able to mix with the derivatization agent. Polar solvents with Hildebrand

solubility parameters that are close to those of HOM matrices are capable of dissolving the polymeric HOM. This will result in dirty extracts requiring additional clean-up steps. In this respect, preference should be given to non-polar solvents including benzene and toluene [3]. Solvents chosen in this work were toluene, dichloromethane (DCM) and DCM:acetone (1:1). Table 1 shows the recoveries of sterols based on derivatized compounds using these solvents. Based on the recoveries of sterols, DCM gave the highest recoveries. The derivatization agent added not only able to derivatize the sterols, but also able to weaken strong analyte matrix interactions. The BSTFA agent is capable of disrupting the strong analyte-matrix interactions enabling the organic solvent to diffuse into weak interaction and absorb analyte of interest. DCM is suitable as an extraction solvent and able to yield single derivative for in-situ derivatization.

Table 1. Recoveries (%) of target compounds using several solvents and solvent mixture.

Compound	DCM (n=3)	DCM:acetone(1:1) (n=3)	Toluene
Cholesterol	95.8	36.2	46.8
Stigmasterol	90.9	44.0	43.3
Stigmastanol	87.2	42.3	46.6

Effect of temperature on in-situ derivatization. Increase in extraction temperature led to improve mass transfer of the analytes thus will recoveries [5]. Moreover, surface tension and viscosity of the solvent are both reduced with increasing temperature leading to improved sample wetting and matrix penetration [5]. Since temperature is an important factor contributing to the increased recoveries in ASE, the effect of increasing the temperature to 100ºC (based on the USEPA Method 3545) on the derivatization of sterols was studied. As shown in Table 2, it was found that extraction at 100ºC yielded lower recoveries compared to those obtained at extraction temperature of 60ºC.

Table 2. Recoveries (%) of sterols at extraction temperatures of 60ºC and 100ºC

Compounds	Extraction temperature	
	60ºC	100ºC
Cholesterol	95.8	55.3
Stigmasterol	90.9	65.8
Stigmastanol	87.2	55.3

Effect of clean-up sorbent on the elution of derivatized sterols

Several types of sorbent ranging from polar to non polar were selected and placed inside the extraction cell. The ability of each sorbent to selectively elute derivatized sterols was analyzed using spiked sand. As shown in Table 3, silica gel and florisil, categorized as polar sorbents are ideal for the isolation of non polar analytes and have strong affinity to retain sterols. DCM was unable to elute sterols from silica gel sorbent. Activated carbon is a sorbent that can absorb compounds of a large range polarity. It was found that a significant amount of sterols was retained by activated carbon when DCM was used as the eluting solvent. For non polar sorbents such as C_8 and C_{18}, higher percentage of derivatized sterols were extracted and eluted from the sorbent by DCM. Therefore, C_{18} and C_8 were selected as the suitable sorbents for further method development.

Table 3. Recoveries (%) of sterols using several sorbents

Compound	Sorbents				
	Silica gel	Florisil	Activated carbon	C_{18}	C_8
Cholesterol	-	48.2	65.7	72.8	76.4
Stigmasterol	-	53.5	63.4	87.5	90.5
Stigmastanol	-	52.9	58.4	72.2	92.2

Efficiency of C_8 and C_{18} as sorbent for clean-up step

The efficiency of simultaneous extraction, derivatization and clean-up step was further analyzed using spiked soil sample. ASE was operated at extraction temperature of 60ºC, extraction pressure of 1000 psi and extraction time of 15 minutes. The recoveries of sterols are shown in Table 4.

Table 4. Recoveries (%) of sterols from spiked soil sample

Compound	C_{18} clean-up	C_8 clean-up
Cholesterol	75.7	86.4
Stigmasterol	79.0	87.9
Stigmastanol	88.5	88.7

The effectiveness of the clean-up step was analyzed by measuring the absorbance of humic organic matter (HOM) using a UV-VIS spectrophotometer (at range 300 - 400 nm). As shown in Figure 1, lower absorbance was observed for the extract with C_8 clean-up indicating that the sorbent was able to trap some of the HOM, producing cleaner extract that can be directly analyzed using GC.

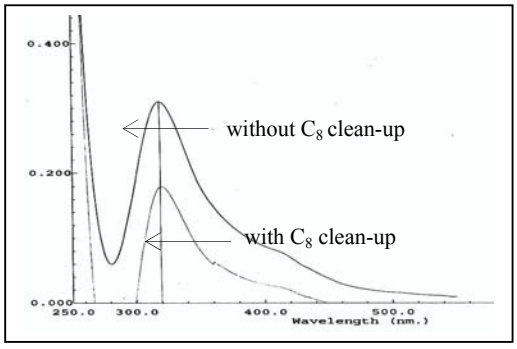

Fig. 1. Absorption spectrum of soil extracts with and without C_8 clean-up

Comparison of simultaneous extraction, derivatization and clean-up method with USEPA Method 3545

The efficiency of the developed method was compared to the USEPA Method 3545 using ASE operated at extraction pressure of 1500 psi, extraction temperature of 100ºC and 5 minutes static extraction time using spiked soil sample. The extract was then concentrated and derivatized using BSTFA:TMCS (99:1) at 60ºC for 1 hour prior to GC analysis. As shown in Table 4.5, the recoveries obtained form the two methods are comparable although a slightly lower recovery for stigmasterol was obtained using the developed method with extraction time of 15 minutes. The lower extraction temperature in the developed method (60ºC) compared to the USEPA method (100ºC) might resulted in the lower recovery. Since extraction time is an important

parameter in exhaustive extraction, the extraction time of the developed method was increased to 30 minutes to enhance extraction recoveries. As shown in Table 5, the increase in extraction time increases the recoveries of the sterols.

Table 5. Comparison of extraction techniques based on the recoveries (%) of sterols from spiked soil

Compound	USEPA 3545 ASE method with ex-situ derivatization 5 min static time	ASE with in-situ derivatization and C_8 clean-up	
		15 min static time	30 min static time
Cholesterol	88.3	86.4	83.4
Stigmasterol	98.2	87.9	99.9
Stigmastanol	89.2	88.7	92.3

Conclusion

A simple and rapid method for the determination of sterols in soil has been developed by combining extraction, derivatization and clean-up using ASE. From this study it can be concluded that single run extraction to extract sterols using ASE can be achieved with good extraction recoveries. Dichloromethane is the best solvent to extract sterols and C_8 is chosen as the effective sorbent to adsorb HOM. The ASE operating conditions proposed in the developed method were extraction temperature of 60ºC, extraction pressure of 1000 psi and extraction time of 30 minutes. Using the proposed method, comparable recoveries of sterols were obtained compared to those obtained using USEPA Method 3545.

Eventhough the extraction time for the developed method (30 minutes) is longer than the USEPA Method 3545 (5 minutes), the simultaneous extraction, derivatization and clean-up method offer many advantages. In-situ derivatization done inside the extraction cell reduces sample handling and decreases analysis time. Clean-up step achieved inside the extraction cell resulted in cleaner extract with reduced sample preparation steps. Therefore, the developed simultaneous extraction, derivatization and clean-up of sterols from soil leads to substantial time saving as compared to other conventional techniques.

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