



## Determination of musk compounds in sewage treatment plant sludge samples by solid-phase microextraction

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### Abstract

Headspace solid-phase microextraction, followed by GC–MS analysis is presented as a suitable technique for the determination of musk compounds in sewage treatment plant sludge. Five polycyclic musks (celestolide, phantolide, traseolide, galaxolide and tonalide) and four nitro musks (musk xylene, musk moskene, musk tibetene and musk ketone) were considered in the optimisation of the analytical method. The influence of extraction temperature, fibre coating, agitation, pH and salting out on the efficiency of the extraction along with the extraction kinetics were studied. An extraction temperature of 100 °C and sampling the headspace over the stirred sludge sample using polydimethylsiloxane -divinylbenzene as fibre coating lead to effective extraction. The method proposed is very simple and yields high sensitivity, good linearity and repeatability for all the analytes with limits of detection at the sub-ng/g level. The total analysis time, including extraction and GC analysis, was only 40 min, and no manipulation of the sample was required.

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### 1. Introduction

Personal care products (PCPs) are produced and used in large amounts around the world. Some examples are soaps, household detergents, perfumes, etc. Fragrances are common ingredients of PCPs and, in contrast to other chemicals, almost no knowledge exists on their long-term environmental effects [1]. Fragrances are mixtures of terpenes, esters and musk compounds. The last ones are ubiquitous, persistent, bioaccumulative pollutants, and some of them are and/or generate toxicologically active compounds [2–4]. Musk compounds are continuously introduced

into the environment mainly via urban wastewater effluents [1,5]. In contrast to other organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs) and others for which the European Community has regulated maximal concentrations in sewage sludge destined for agricultural use, musk compounds as well as other organics lack of any kind of regulation [2,6].

Polycyclic musks (substituted indans and tetralines) account for nearly 85% of the worldwide production of musks, while nitro musks account for the rest [7]. Both types of compounds are used in almost every fragrance containing PCP. Countries have no regulations for most of the marketed fragrances. The manufacturing industries have introduced some limitations on the kind and proportion of musks in PCP formulations. The consequence is the

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large amounts of musk compounds released to urban sewage treatment plants (STPs) and the bioconcentration of these musks in the sewage sludge. For galaxolide and tonalide, the two compounds most frequently detected in European water and sludge samples, concentration levels of about 10 mg/kg may be found in STP sludge [8].

Sludge is a very complex sample and the extraction of organic pollutants from this matrix usually implies solvent extraction of the dried sludge samples assisted by accelerated solvent extraction, sonication, microwave heating, solid-phase extraction or simple agitation. In all cases, several clean-up steps must be applied to the extracts before chromatographic analysis [2,6,9–16].

The objective of the present work was to present a solid-phase microextraction (SPME) combined with gas chromatography–mass spectrometry (GC–MS) method for the analysis of polycyclic and nitro musks in sewage sludge. SPME is a solventless technique that simplifies the long and tedious processes of sample preparation and analyte extraction in a single step [17]. There are very few papers dealing with the application of SPME to the analysis of organic pollutants in sludge samples [18] and according to our knowledge this is the first time that SPME is used to analyse musk compounds in sewage sludge. Full discussion of the factors (temperature, fibre type and pH) influencing the extraction of nine synthetic musk compounds, most of them used as ingredients of PCPs in Spain, is provided along with linearity, precision, and accuracy data.

## 2. Experimental section

### 2.1. Reagents and materials

The musk compound 4-acetyl-1,1-dimethyl-6-*tert.*-butylindan (celestolide, ADBI, [13171-00-1]) was kindly supplied by Ventós (Cornella de Llobregat, Barcelona, Spain); 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[*g*]-2-benzopyran (galaxolide, HHCB, [1222-05-5]), 7-acetyl-1,1,3,4,4,6-hexamethyltetralin (tonalide, AHTN, [1506-02-1]), 6-acetyl-1,1,2,3,3,5-hexamethylindan (phantolide,

AHMI, [15323-35-0]), 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindan (traseolide, ATII, [68140-48-7]), were supplied by Promochem Iberia (Barcelona, Spain); 1-*tert.*-butyl-3,5-dimethyl-2,4,6-trinitrobenzene (musk xylene, [81-15-2]), 4,6-dinitro-1,1,3,3,5-pentamethylindane (musk moskene, [116-66-5]), 1-*tert.*-butyl-2,6-dinitro-2,4,5-trimethylbenzene (musk tibetene, [145-39-1]), 1-*tert.*-butyl-3,5-dimethyl-2,6-dinitro-4-acetylbenzene (musk ketone, [81-14-1]) were supplied by LGC Desealers (Barcelona, Spain). Isooctane, acetone, methanol, NaCl, were all purchased from Merck (Mollet del Vallés, Barcelona, Spain). All solvents and reagents were analytical grade. SPME holders and fibres were obtained from Supelco (Bellefonte, PA, USA). Four fibres were used: 85- $\mu$ m polyacrylate (PA), 100- $\mu$ m polydimethylsiloxane (PDMS), 75- $\mu$ m Carboxen-polydimethylsiloxane (CAR-PDMS), 65- $\mu$ m polydimethylsiloxane–divinylbenzene (PDMS–DVB) and 65- $\mu$ m Carbowax–divinylbenzene (CW–DVB). The fibres were conditioned as recommended by the manufacturer.

The real sludge samples were collected from an urban wastewater treatment plant in Galicia (NW Spain). Previous analysis of the water from the various units of this plant had shown the presence of some of the target compounds, mainly galaxolide and tonalide [19–21]. Thus, it was highly probable that these analytes would also be present in the sludge samples.

### 2.2. Experimental set-up

A 3-g amount of fresh sludge was placed in 22-ml headspace vials. Vials were sealed with a headspace aluminium cap furnished with a Teflon-faced septum and immersed in a thermostated water bath. Samples were equilibrated for 5 min before SPME. Afterwards, the fibre was exposed to the headspace over the magnetically stirred (600 r.p.m.) sludge sample for different times, depending on the experiment. Then, the fibre was immediately inserted into the GC injector and the chromatographic analysis was carried out. Desorption time was set at 3 min although an extra period of 5 min for desorption was consid-

ered to avoid carryover effects after the analysis of highly concentrated samples.

### 2.3. Gas chromatography–mass spectrometry

GC–MS analyses were performed in a Varian 3400 CX chromatograph equipped with a Saturn 3 ion trap mass detector and a split/splitless injector, operated by Saturn version 5.4 software. Musk compounds were separated on a Varian VA-5MS column (5% phenyl-polysilphenylene-siloxane, 25 m×0.25 mm I.D. coated with a 0.25- $\mu$ m film). The GC oven temperature program was: 60 °C hold 2 min, rate 10 °C/min to 250 °C, rate 20 °C/min to a final temperature of 280 °C, hold for 2.5 min (total analysis time, 25 min). Helium was used as carrier gas at a flow-rate of 1 ml/min. The injector was operated in splitless mode for 2 min and its temperature was set at 250 or 290 °C (depending on the type of fibre). Transfer line temperature was maintained at 280 °C. The ion-trap mass spectrometer was operated in the electron ionisation mode (70 eV), and the trap temperature was set at 200 °C. In order to increase detection sensitivity and selectivity, the total analysis time was divided into six mass acquisition segments, each one of 5 amu centred on the selected ion for each musk compound, with the exception of the third segment, with a larger mass range (213–284 mu) due to the lack of enough resolution for the peaks of traseolide, galaxolide, tonalide and musk xylene. Other experimental parameters were as follows: scan rate, 0.4 s/scan; multiplier voltage, 2100 V; axial modulation voltage, 4 V; filament emission current,

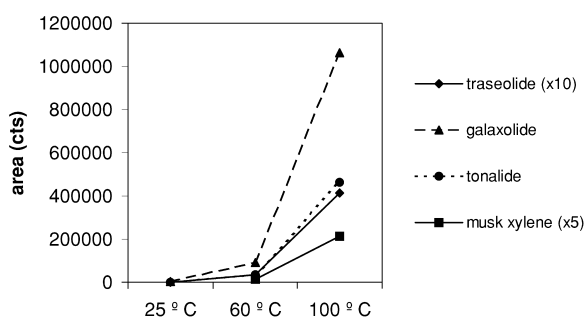


Fig. 1. Influence of temperature on the efficiency of extraction (results are expressed as area counts).

10  $\mu$ A; ionisation control performed in the automatic mode; filament/multiplier delay, 12 min.

## 3. Results and discussion

### 3.1. Preliminary experiments

In Table 1, the retention times at the optimised chromatographic conditions are shown, as well as the quantification and identification ions for all target musks. Initial experiments were performed to test the ability of SPME to extract musk compounds from real sludge. Based on our previous SPME studies on musk compounds in water samples, PDMS–DVB coated fibres were initially used [19]. At 60 and 100 °C and 15 min of exposition of the fibre to the sample headspace (HS-SPME, headspace solid-phase microextraction), four of the target musk compounds, traseolide, galaxolide, tonalide and musk xylene were detected as shown in Fig. 1. At 100 °C the

Table 1  
Retention times and selected ions for the analysis of the target musk compounds

	Retention time (min)	Identification ions	Quantification ion
Celestolide	16.37	244, 173	229
Phantolide	16.80	187, 244	229
Traseolide	17.85	173, 258	215
Galaxolide	17.95	213, 258	243
Tonalide	18.02	187, 159	243
Musk xylene	17.97	297, 283	282
Musk moskene	18.26	278, 264, 221	263
Musk tibetene	18.81	266, 252, 115	251
Musk ketone	19.24	294, 128, 280	279

responses obtained were approximately 10 times higher than those obtained at 60 °C. In order not to exceed the glass vial pressure limits, temperatures above 100 °C were not tested.

In addition to fresh sludge, the purpose of the present work was also to develop a general method for the extraction of musks from dry sludge. Thus, some experiments using dry sludge samples were also carried out at early stages. Water has proven to be a very effective additive to facilitate the release of analytes from matrices such as soils and sediments, and a very significant increase in sensitivity is achieved [17,22]. For this reason the amount of water that must be added to dry sludge to obtain the highest response for musk compounds was studied. The amount of dry sludge used was about 0.16 g corresponding to 3 g of fresh primary sludge. The sample used in these experiments was also primary sludge from a sewage treatment plant in Asturias (Spain). The analyses were performed at 100 °C with a sampling time of 15 min. In this sample, the presence of galaxolide, tonalide and traseolide was confirmed, and the results obtained are shown in Fig. 2. When 0.5–6 ml of water was added to the dry sludge, the responses were very similar. However, when the amount of water was lower (0.25 ml) the responses were also lower. Therefore, 3 ml of water was chosen for further experiments.

### 3.2. Influence of pH

A fresh primary sludge sample with a pH of 6.4 was used. Except for those compounds found natu-

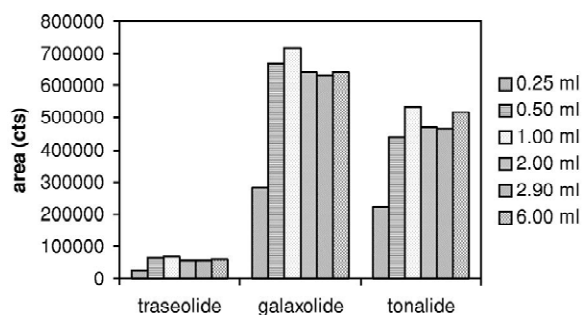


Fig. 2. Influence of the amount of water added to dried sludge (0.25 g) on the efficiency of extraction (results are expressed as area counts).

rally in the sludge, the sample was spiked with the remaining target compounds. The pH effects were studied at three levels—acidic (pH 2), neutral (pH 6.4) and basic (pH 12)—using HCl or concentrated NaOH aqueous solutions. All experiments were run in duplicate and the results are shown in Fig. 3. The responses obtained at pH 2 were lower for all compounds. At alkaline pH, the responses were similar or slightly higher than at neutral pH. Another pair of experiments was performed by adding 2 ml of the NaOH solution (pH 14). This strong alkaline treatment has proven to be very efficient for certain samples [23,24], but led to the decomposition of some organic compounds such as DDT, BHC and dieldrin [23]. The responses obtained for the polycyclic musks were similar to the ones obtained at neutral pH and at pH 12. On the contrary, the responses for the nitro musks were significantly lower. In addition, the chromatographic background in these last experiments was similar to that obtained at neutral pH. Considering all these results the pH of the sample was not modified for further experiments.

### 3.3. Influence of agitation of the sample

Samples were magnetically stirred at 600 r.p.m.

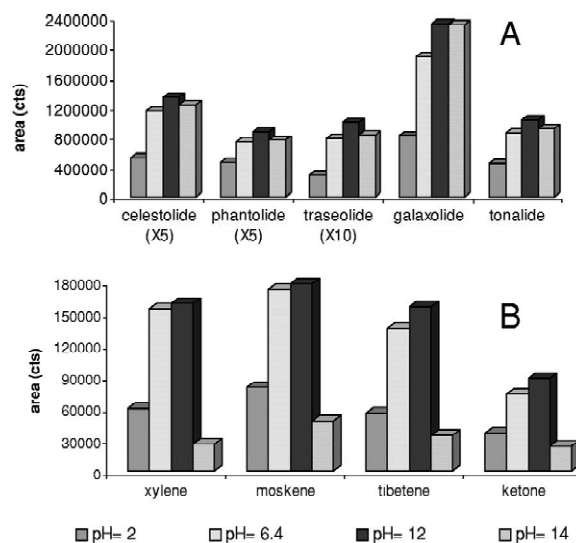


Fig. 3. Influence of pH on the efficiency of extraction. (A) Polycyclic musks. (B) Nitro musks. Results are expressed as area counts.

and the results obtained were compared with those obtained without agitation. The responses with stirring were about 30% higher. Consequently, in later experiments, the samples were stirred.

### 3.4. Influence of the fibre coating

In a previous study for the extraction of polycyclic musks from water [19], the PDMS–DVB was selected as the best fibre coating. However, the kinetics of the SPME process are very different for water and sludge samples, and the most efficient extraction fibre may not be the same. Various fibres were tested: 85- $\mu\text{m}$  PA, 100- $\mu\text{m}$  PDMS, 75- $\mu\text{m}$  CAR-PDMS, 65- $\mu\text{m}$  PDMS–DVB and 65- $\mu\text{m}$  CW-DVB. The extraction time and temperature were 15 min and 100 °C, respectively. The results for the nine musk compounds are shown in Fig. 4. PDMS–DVB was

the most efficient extraction fibre for all compounds, followed by CAR-PDMS. The responses obtained with the other fibres were between two and six times lower than the PDMS–DVB responses.

### 3.5. Extraction time profile

In order to investigate the kinetics of the HS-SPME process for the extraction of musks from sludge, the extraction time profiles for the compounds were established by plotting the detector response against the extraction time. Using fresh primary sludge as well as dry primary sludge, different extraction times (0, 15, 30, 60 and 90 min) were tested at 100 °C using the PDMS–DVB fibre. The extraction time profiles are shown in Fig. 5. Polycyclic musks as well as the most volatile nitro musk (the musk xylene) reached equilibrium in

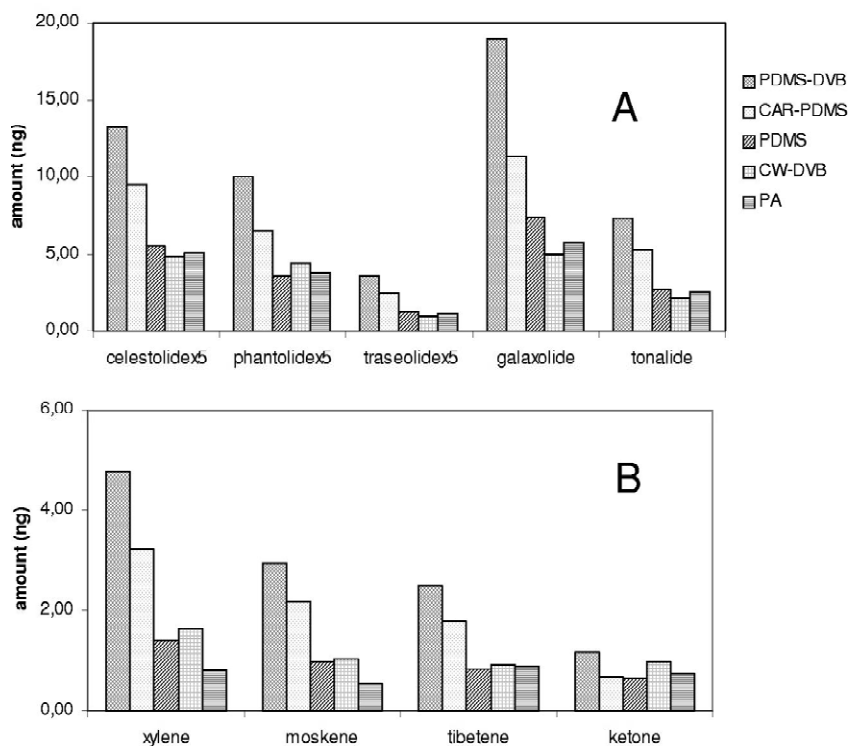


Fig. 4. Comparison of five commercial fibres for the extraction of musk compounds from sludge samples. (A) Polycyclic musks. (B) Nitro musks. Results are expressed as amount of analyte (ng).

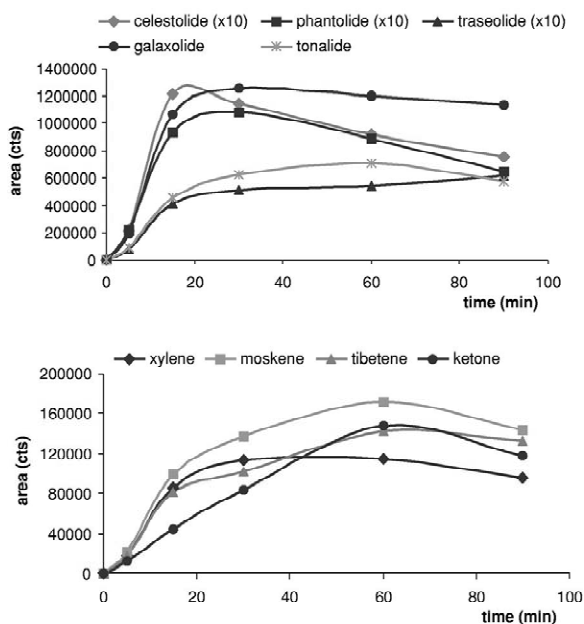


Fig. 5. Extraction time profiles for the target musk compounds obtained by HS-SPME at 100 °C using a PDMS–DVD fibre (results are expressed as area counts).

30 min. The SPME process is slower for the other nitro musks, especially for musk ketone. Equilibrium for all the compounds studied was achieved within 60 min. Considering that responses obtained in 30 min were quite close to those obtained in 15 min for the majority of compounds (with the exception of musk ketone) and that our aim was to develop a fast method, the sampling time was set at 15 min in later experiments.

### 3.6. Performance evaluation and validation of the proposed method. quantification of musks in real samples

The spiked concentration range was from 20 to 200 ng/g for all compounds with the exception of galaxolide and tonalide. These compounds were found in the sludge samples in high concentrations and the spike range for them was from 80 to 800 ng/g. Fig. 6 shows the ion chromatograms for a primary sludge spiked with 50 ng/g of musks (200

ng/g for galaxolide and tonalide). The compounds studied in the secondary sludge included those detected in the non-spiked sample. There was a directly proportional relationship between the extracted amount of musks and the initial concentration in the sample for both matrices. The correlation coefficients ( $R^2$ ) and the slopes of the calibration curves are shown in Table 2. The slopes obtained for primary sludge were three to four times lower than those obtained for secondary sludge, in inverse relationship to the solid content of the samples. Thus, as could be expected, the sensitivity of the method is related to the solid content in the sample.

The precision of the experimental procedure was also evaluated for both samples at different concentration levels. A series of five consecutive HS-SPMEs gave a relative standard deviation (RSD) ranging from 1.13 to 13.62% (Table 2).

Detection limits were also evaluated for both kinds of sludge (signal-to-noise ratio of 3) and are presented in Table 2. They were in the pg/g for all compounds in both samples, however, the limits were higher for primary sludge than for secondary sludge.

In these samples, some of the musk compounds were detected and their concentrations were evaluated using the standard addition protocol. Fig. 7 shows the ion chromatograms of two non-spiked samples obtained from the primary and secondary treatment units, respectively. Table 3 shows the results obtained. The predominant musks in all these samples were galaxolide and tonalide whose concentration was considerably higher than the ones for the other compounds. One of the nitro musks, the musk xylene was also found in both samples.

## 4. Conclusions

A method based on SPME coupled to GC–MS has been developed for the analysis of polycyclic and nitro musk compounds in sludge samples. Analytes were extracted by sampling the headspace over the sludge sample for only 15 min. This method does not require any pretreatment of the sample and may be applied to fresh or dried sludge samples. The pro-

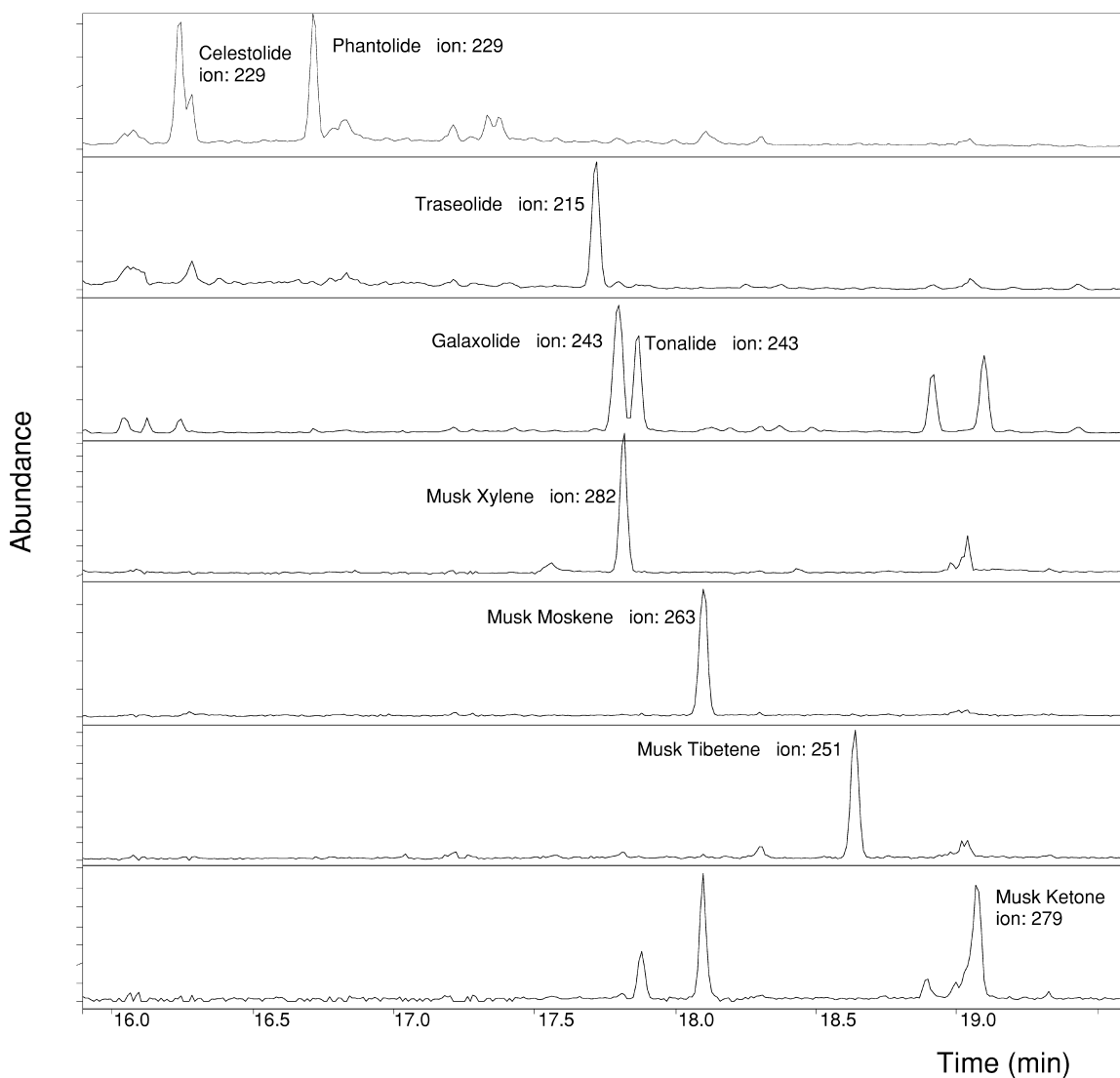


Fig. 6. GC–MS selected ion chromatograms for a primary sludge sample spiked with 200 ng/g of galaxolide and tonalide, and 50 ng/g of the other musk compounds.

posed method is simple and rapid. The performance of the method has been evaluated using two kinds of urban wastewater plant sludges, a primary sludge and a secondary sludge. The method provided good linearity and precision, and the detection limits were in the pg/g level. The analysis of sludge samples from an urban wastewater treatment plant showed the presence of six of the target analytes (mainly

galaxolide and tonalide) in concentrations ranging from 0.27 to 162 ng/g.

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Table 2

Linearity, repeatability and limit of detection for the target musk compounds in primary and secondary sludge

	Primary sludge				Secondary sludge			
	Linearity		Precision (RSD%)	LOD ng/g	Linearity		Precision (RSD%)	LOD ng/g
	R <sup>2</sup>	Slope			R <sup>2</sup>	Slope		
Celestolide	0.9978	3130	8.0	0.049	0.9940	10 468	12.2	0.023
Phantolide	0.9969	3164	9.8	0.064	0.9979	11 174	13.6	0.028
Traseolide	0.9961	3540	1.7	0.154	0.9987	10 496	10.6	0.063
Galaxolide	0.9854	2861	4.4	0.088	0.9993	11 925	8.4	0.032
Tonalide	0.9972	2196	5.8	0.140	0.9989	6651	10.8	0.052
Musk xylene	0.9990	908	1.1	0.370	0.9990	3286	4.9	0.101
Musk moskene	0.9995	1711	12.6	0.311	–	–	–	–
Musk tibetene	0.9968	1152	8.5	0.448	–	–	–	–
Musk ketone	0.9997	515	4.0	0.611	–	–	–	–

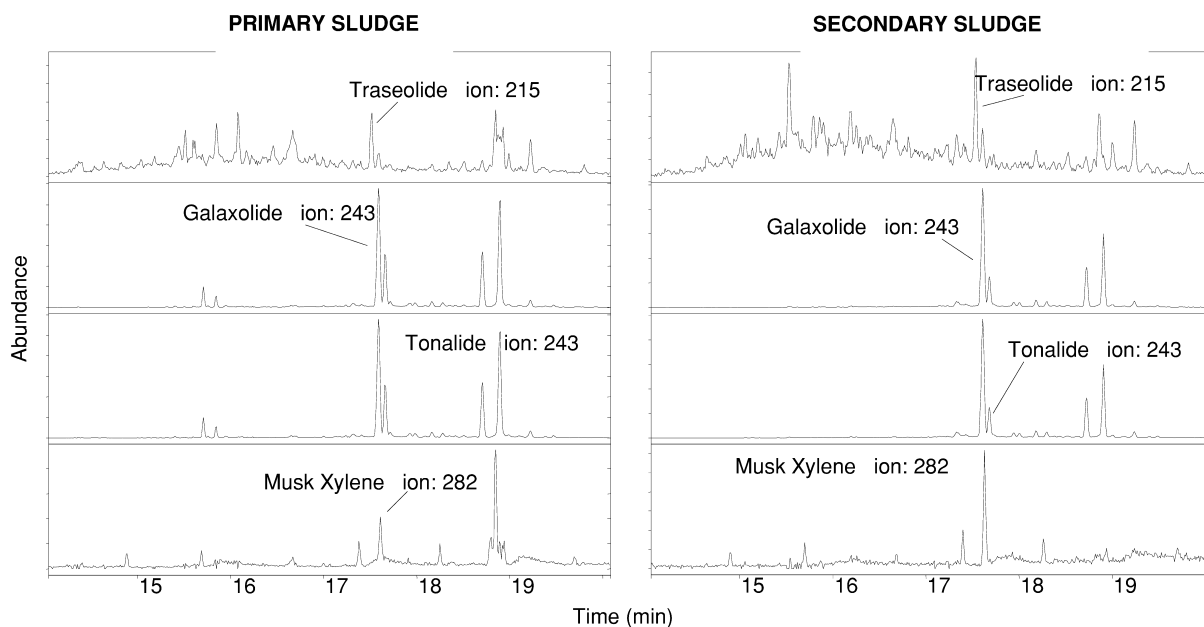


Fig. 7. Selected ion chromatograms of real sludge samples taken from the primary and secondary units of an urban sewage treatment plant.

Table 3

Concentration (expressed as ng/g) of the target musk compounds found in real contaminated sludge samples from an urban sewage treatment plant

	Primary sludge (ng/g)	Secondary sludge (ng/g)
Celestolide	1.5±0.2	1.4±0.2
Phantolide	0.73±0.07	0.27±0.03
Traseolide	4.8±0.5	7.0±0.7
Galaxolide	129±12	162±17
Tonalide	64±9	52±5
Musk xylene	12±2	16±2

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