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Nitro musks, nitro musk amino metabolites and polycyclic musks in sewage sludges
Quantitative determination by HRGC-ion-trap-MS/MS and mass spectral characterization of the amino metabolites

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Abstract

Nitro- and polycyclic musks were quantified in sewage sludge samples from different catchment areas using high-resolution gas chromatography (HRGC) and ion-trap MS/MS. Collision induced dissociation (CID) turned out to be a useful tool for quantification of the analytes. Negative chemical ionization (NCI) quadrupole MS in the selected ion mode (SIM) showed similar sensitivities compared to ion trap MS/MS. Among the nitro musks, musk ketone (MK) and musk xylene (MX) were the main compounds in predominantly domestic sewage sludges, found at low μg/kg dry matter (d.m.) whereas polycyclic musks were present in domestic as well as in industrial sludges up to 12 mg/kg d.m. Galaxolide (HHCB) and Tonalide (AHTN) were the major polycyclic musks found in the sludges. Amino metabolites of the nitro musks, amino musk xylene (AMA), amino musk moskene (AMM) and amino musk ketone (AMK) were detected for the first time in sewage sludges, and reached partly higher concentrations compared to the parent compounds. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Nitro musks; Polycyclic musks; Amino metabolites; Sewage sludge; HRGC-ion-trap MS/MS; HRGC-EI/PCI- and NCI quadrupole MS

1. Introduction

The amendment of sewage sludge as fertilizer to agricultural land represents an economical way to use the high amounts of sludge produced by the wastewater treatment plants. Currently, approximately 2.1 × 10^8 kg (dry matter) of sewage sludge are produced annually in Switzerland, of which 1.07 × 10^8 kg (51%) are applied in agriculture (Kettler, 1998). In the last couple of years agriculture has moved towards more and more environmental friendly techniques with the goal of producing high quality food products. This also requires the use of fertilizers of high quality. For sewage sludge and compost limit values for heavy metals have been established (Verordnung über umweltgefährdende Stoffe, 1996). These inorganic compounds are analyzed on a routine basis. However, the characterization and long-term observation of organic contaminants in sludge has received little attention so far due to the absence of limit values. It is well known that sewage sludge contains many xenobiotic (anthropogenic)

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organic chemicals which might have a negative impact on soil organisms and fertility (Tarradellas and Diercxsens, 1983; Drescher et al., 1989; Demirjian et al., 1987; Klöpffer, 1996; Lega et al., 1997; Halling-Sorensen et al., 1998). Among these, non-polar and highly lipophilic compounds like polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dioxins (PCDD) and dibenzofurans (PCDF) are important contaminants as they persist for a long time in the different environmental compartments. Quite recently, two other groups of compounds exhibiting similar chemical and physical properties like those mentioned above, have been described to occur as ubiquitous contaminants: nitro musk and polycyclic musk substances. The nitro musks include a group of five synthetic alkylated nitro benzenes with a typical musk odor: musk moskene (MM), musk tibetene (MT), musk xylene (MX), musk ketone (MK) and musk ambrette (MA). The polycyclic musks are chemically alkylated tetralin or indane systems. Main representatives of the polycyclic musk are HHCB (Galaxolide), AHTN (Tonalide), ADBI (Celestolide), AHMI (Phantolide) and AITI (Traseolide). These compounds are widely used as fragrance ingredients in washing and cleaning agents, air fresheners, shampoos, perfumes and other cosmetic products, as food additives in fish baits, and in cigarettes. They have been detected in fish, surface and sewage waters (Eschke et al., 1994a,b, 1995; Rimkus and Wolf, 1993a), human milk and adipose tissue (Rimkus and Wolf, 1996, 1993b; Ott et al., 1999), sediment samples (Lach and Steffen, 1997; Winkler et al., 1998) as well as in the North Sea (Gatermann et al., 1995). The lack of biological and chemical degradation of these compounds as well as their bioaccumulation potential has raised considerable attention in the field of environmental chemistry (Suter-Eichenberger et al., 1998; Käfferlein et al., 1998). Very recently, metabolites of nitro musks have been described in the aquatic environment (Gatermann et al., 1998). These amino transformation products have been shown to occur also in mammals during metabolism (Käfferlein et al., 1998). From a toxicological point of view these metabolites might well be more toxic than their parent compounds (Gatermann et al., 1998).

To our knowledge very little is known about the contamination of nitro- and polycyclic musk compounds in sewage sludges. A study from the Netherlands has dealt with the analysis of musk fragrances in a few sewage sludge samples (Blok, 1997). Due to the importance of sludge use in Swiss agriculture, the aim of this study was to determine nitro and polycyclic musks in sludges from different catchment areas and also to look for the presence of amino metabolites of the nitro musk compounds. Moreover mass spectral data informations are provided for sensitive detection of these metabolites.

2. Experimental section

2.1. Laboratory contamination with musk compounds

Due to the widespread use of musks in house hold products it is important to remove all possible sources from the laboratory (soaps, hand cream, towels, etc.) which might contain these compounds in order to eliminate contamination. Also, blank samples should be run in parallel to every series of samples extracted.

2.2. Sample characterization

Sewage sludges from different catchment areas were collected: seven samples containing mainly domestic sewage obtained from separate sewer systems (A-type); three samples containing a mixture of domestic sewage, storm water runoff, and low amounts of industrial waste water obtained from combined sewer systems (B-type) and two samples as described for the B-type but containing higher amounts of industrial waste water (C-type). Fresh sewage sludge was sampled into precleaned (hexane and acetone) glass bottles, stored over night at 4°C and extracted the next day.

2.3. Standards, chemicals and solvents

Nitro musks (musk tibetene, musk ketone, musk xylene, musk moskene, musk ambrette) were obtained from Essentia, Ätherische Öle, Winterthur, Switzerland, polycyclic musk compounds (Tonalide, Celestolide, Phantolide, Cashmeran, Traseolide, Galaxolide) were purchased from Promochem, Wesel, Germany.

Cyclohexane, chloroform, dichloromethane (DCM), ethylacetate, hexane, methanol, isopropanol, toluene and acetone, residue grade, were obtained from Merck, Dietikon, Switzerland and Promochem, Wesel. Silica gel 40 for column chromatography, anhydrous sodium sulfate (Na_2SO_4), sodium chloride (NaCl) and sodium hydrogen carbonate ($HNaCO_3$) was from Merck, Dietikon, palladium on activated alumina, celite 545 AW and ammonium formate was from Fluka, Buchs, Switzerland. Waters Sep-Pak Silica cartridges (0.5 g) were bought from Waters, Rupperswil, Switzerland.

2.4. Synthesis of the amino metabolites of the nitro musks

Amino metabolites were synthesized according to Ram and Ehrenkaufer (1984). Briefly, the nitro musk (100 mg) was diluted in 3.5 ml of dry methanol. To the solution 20 mg of palladium on activated alumina and 106 mg of ammonium formate were added and the reaction stirred at room temperature for 2–18 h depending on the reactivity of the nitro group. After completion of the reaction the mixture was filtered over celite, concentrated, redissolved in chloroform, washed with $NaHCO_3$ and deionized water, dried over Na_2SO_4 and concentrated to dryness. Purification of the product was performed using silica gel column chromatography (100 × 10 mm) and hexane/ethylacetate (2:1) as elution solvent. After reconcentration, the pure product was redissolved in hot hexane from which crystallization occurred upon leaving at 4°C.

2.5. Sample extraction

Wet sewage sludge (1 l, $n = 1$) was extracted with 600 ml of hexane at ambient temperature for 2 h by agitating vigorously. Phase separation was improved by adding NaCl and small amounts of isopropanol to the two-phase system. The organic phase was decanted, dried over Na_2SO_4 and concentrated at 35°C under a slight vacuum to a volume of 5 ml. Ethylacetate (5 ml) was added and the solution further used for the gel permeation chromatography (GPC) clean-up.

2.6. Clean-up: gel permeation chromatography (GPC) and solid phase extraction (SPE)

Gel permeation chromatography was carried out on a Autoprep GPC Model 1002B from Gerber Instruments, Effretikon, Switzerland. Cyclohexane/ethylacetate (1:1) was used as eluent with a flow rate of 5 ml/min. The column (300 mm × 20 mm) was filled with 30 g of Bio-Beads SX3 (Bio Rad Laboratories, Hercules, CA, USA). The optimum sampling window was between 15 and 27 min (75 and 135 ml). The GPC eluates were evaporated at 40°C to approximately 5 ml and concentrated to 1 ml by a gentle stream of nitrogen. After GPC, extracts were submitted to a final clean-up by use of a silica cartridge (SPE). The column was conditioned with 10 ml of DCM. After loading the cartridge with the extract, elution was carried out with 8 ml of DCM. The eluate was concentrated nearly to dryness by a gentle stream of nitrogen and the volume adjusted to exactly 1 ml with toluene. Careful evaporation was crucial and could easily be monitored by the recoveries of Cashmeran (DPMI).

2.7. HRGC and ion-trap mass spectrometry (MS) quantification using CID

HRGC was performed on a Varian 3400 GC equipped with a Varian 1078 injector, a Varian 8200 autosampler and a Varian Saturn 2000 ion-trap mass spectrometer with MS/MS option. Separation was achieved on a 60 m DB-1 capillary column, 0.25 mm ID., 0.25 μm film (J+W Scientific, supplied by MSP Friedli, Köniz, Switzerland). Helium was used as carrier gas. The temperature program was 100°C, 1 min hold, 15°C/min to 157°C, 0.6°C/min to 178°C, 5°C/min to 220°C, 10°C/min to 300°C, 6.8 min hold. The injector was set at 260°C. Injection (2 μl) was performed in the splitless mode using a splitless time of 0.7 min. Stock solutions (100 μg/ml) of the standard compounds were prepared in toluene. The standard mixture of 500 ng/ml was obtained by appropriate dilution of a mixture of the stock solutions in toluene. Standard mixtures of lower concentrations were prepared by suitably diluting the 500 ng/ml stock mixture with toluene. The mass range for spectrum acquisition was 100–300 m/z, CID-RF m/z 90 and mass width 3 m/z for all substances. Table 2 shows MS/MS parameters used for the external standard quantification.

2.8. HRGC and electron impact (EI), positive chemical ionization (PCI) and negative chemical ionization (NCI) quadrupole MS of the amino metabolites of the nitro musk compounds

HRGC was performed on a HP 6890 GC equipped with a HP 6890 Series automatic injector (enhanced parameters) and a HP 5973 mass spectrometer with PCI and NCI option. Separation was reached on a 30 m HP 35 MS capillary column, 0.25 mm ID and 0.25 μm film (Hewlett-Packard, Wilmington, Delaware, USA) using the following temperature program: 90°C, 1 min hold, 10°C/min to 300°C, 10 min hold. Helium was used as carrier gas at a linear velocity of 37 cm/s (temperature 90°C). Standards were injected (2 μl) in the on-column mode. In the CI mode (PCI and NCI) methane was used as a reagent gas. A chemical ion gas purifier for methane was installed just before methane was entering the MSD. The methane flow was set at 20% for PCI and 40% for NCI of a flow of 5 ml/min. MS temperature parameters: transfer line 290°C, quadrupole 106°C (PCI), 110°C (NCI), ion source 250°C (PCI), 110°C (NCI).

2.9. Recovery experiments and detection limits

Due to the high loads of mainly the polycyclic musk compounds in the investigated sludges, two different recovery experiments were carried out: (i) water was spiked with 500 ng/l of the analytes, (ii) samples of sewage sludge were spiked with 10 μg/l of the polycyclic musk compounds (except Galaxolide and Tonalide) and 1 μg/l of the nitro musks and the amino metabolites of the nitro musk compounds, respectively. Recovery rates were satisfactory for most of the compounds, which allowed for uncorrected calculation of the amounts found (Table 1). The gas chromatographic method allowed the detection of as little as 2 pg for most of the compounds on the column, corresponding to 2 ng/l in the sewage sludge. However, due to the high

Table 1
Recovery data of the different musk compounds from water and sewage sludge[a]

	Compound class	Name	Mean recovery rate (%)	
			Water	Sewage sludge
1	Polycyclic musk	DPMI	113	73
2	Polycyclic musk	ADBI	113	108
3	Polycyclic musk	AHMI	116	106
4	Nitro musk	MA	101	112
5	Nitro musk	MX	110	118
6	Polycyclic musk	HHCB	114	n.s.
7	Polycyclic musk	AITI	105	n.q.
8	Polycyclic musk	AHTN	113	n.s.
9	Nitro musk	MM	93	102
10	Amino metabolite	AMA	69	82
11	Nitro musk	MT	105	89
12	Amino metabolite	AMM	78	98
13	Nitro musk	MK	102	107
14	Amino metabolite	AMT	108	90
15	Amino metabolite	AMK	97	50
16	Amino metabolite	AMX	91	76
a.r.	Nitro musks		102	106
a.r.	Metabolites		89	80
a.r.	Polycyclic musks		112	n.c.

[a] $n = 2$, n.s.: not spiked, n.q.: not quantitated, a.r.: average recovery, n.c.: not calculated; compound identification see also Table 2.

background in the matrix, the real detection limit was estimated to be 100 ng/l, depending on the sludge under investigation.

2.10. Compound abbreviations and trade names

MT: 1-tert-Butyl-3,4,5-trimethyl-2,6-dinitrobenzene (musk tibetene), MK: 4-tert-Butyl-2,6-dimethyl-3,5-dinitroacetophenone (musk ketone), MX: 1-tert-Butyl-3,5-dimethyl-2,4,6-trinitrobenzene (musk xylene), MM: 1,1,3,3,5-Pentamethyl-4,6-dinitroindane (musk moskene), MA: 6-Methoxy-1-tert-butyl-2,4-dimethyl-3,5-dinitrobenzene (musk ambrette), HHCB (1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta(g)-2-benzopyran (Galaxolide), AHTN: 6-Acetyl-1,1,2,4,4,7-hexamethyl tetralin (Tonalide), ADBI: 4-Acetyl-6-tert-butyl-1,1-dimethylindane (Celestolide), AHMI: 6-Acetyl-1,1,2,3,3,5-hexamethylindane (Phantolide), AITI: 5-Acetyl-3-isopropyl,1,1,2,6-tetramethylindane (Traseolide), DPMI: 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4H-inden-4-one (Cashmeran). Amino metabolites of the nitro musks: AMA: amino musk ambrette, AMM: amino musk moskene, AMT: amino musk tibetene, AMK: amino musk ketone, AMX: amino musk xylene.

3. Results and discussion

3.1. HRGC and ion-trap MS quantification; mass spectral characterization of the amino metabolites of the nitro musks

Due to the non-polar character of the musk compounds efficient extraction was obtained using hexane. Recovery rates of the different compounds are summarized in Table 1. Nitro-and polycyclic musks were recovered quantitatively whereas recoveries of the amino metabolites were somewhat lower.

Separation of most of the compounds could be achieved by the chromatographic conditions described. Coelution was observed for Galaxolide (compound 6) and Traseolide (7) as well as for MM (9) and AMA (10) as shown in Fig. 1. Solution to this problem was offered by the multiple-reaction-monitoring tool (MRM) of Saturn 2000, which allows to change the precursor ion from scan to scan. MRM worked well for the standard mixtures and for musk moskene and amino musk ambrette in the samples, but as the concentrations of Galaxolide and Traseolide in the sludges differed orders of magnitude and the precursor ion of Traseolide was also present in the mass spectrum of Galaxolide, a reliable quantification of Traseolide was not possible.

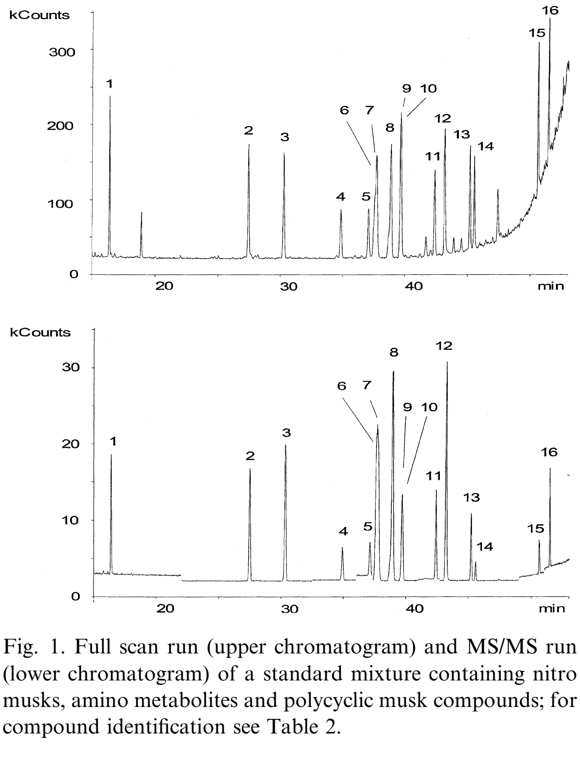

Fig. 1. Full scan run (upper chromatogram) and MS/MS run (lower chromatogram) of a standard mixture containing nitro musks, amino metabolites and polycyclic musk compounds; for compound identification see Table 2.

Quantification of the analytes was based on successor ions after collision induced dissociation (CID) in the ion-trap (Table 1). Full scan and MS/MS spectra of the amino metabolites of the nitro musks and chemical structures are shown in Figs. 2 and 3. All spectra exhibit a molecular weight peak of 30 mass units lower compared to the parent compounds indicating that one nitro group had been reduced. Basically two groups of mass spectra can be distinguished: one having the molecular ion (M^+) as base peak as for AMA, AMT and AMK, the other group having the $M^+ - CH_3$ fragment ion as base peak as for AMM and AMX. CID experiments on the precursor ion (see Table 2) resulted in mass spectra with characteristic successor ions. Their presence can be rationalized as follows: The MS/MS spectra of AMA, AMT and AMK show a strong, common $M^+ - 73$ fragment ion (AMA: m/z 165, AMT: m/z 163 and AMK: m/z 191) presumably due to the cleavage of the tertiary butyl group and the amino function ($M^+ - 57 - 16$). This hypothesis was supported by a second set of CID experiments performed on the $M^+ - 73$ fragment ions of AMA, AMT and AMK (figure not shown). For AMA for example, loss of m/z 30 (NO) and m/z 58 (NO + CO) clearly demonstrate that a nitro group is involved and therefore the 16 mass units as part from the $M^+ - 73$ fragment observed originate from the NH_2 group and not of a cleavage of O from a nitro group. In no case losses of m/z 57 or 16 were observed in these CID experiments showing that cleavage of these groups occurred initially.

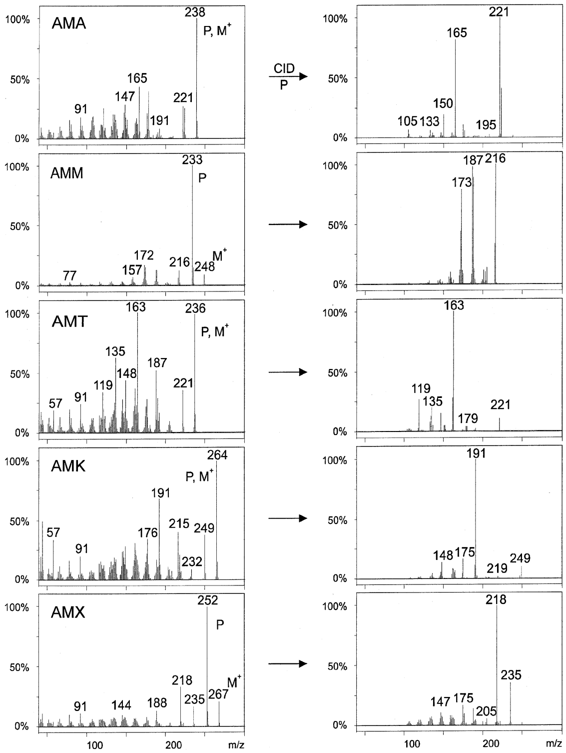

Fig. 2. Full scan ion-trap mass spectra of the amino metabolites of the nitro musks (left column) and MS/MS spectra (right column) after collision induced dissociation (CID); P: precursor ion, M^+: molecular ion.

AMA reveals a $M^+ - 17$ fragment as base peak (m/z 221) which corresponds to the cleavage of an OH radical ion ($OH^\cdot$). This reaction is frequently observed for ortho-nitro toluenes. The same type of cleavage can be deduced from the MS/MS spectrum of AMX which shows fragment ions at m/z 235 and 218 corresponding to $M^+ - 15 - 17$ ($M^+ - CH_3 - OH^\cdot$ and $M^+ - 15 - 2 \cdot 17$ ($M^+ - CH_3 - (OH^\cdot)_2$). In AMX this $OH^\cdot$ cleavage is very pronounced as there are two nitro groups ortho to a methyl group. Finally the MS/MS spectrum of AMM shows strong fragmentation ions at m/z 216, 187 and 173 which can be explained as follows: m/z 216 ($M^+ - 32$) corresponds to the cleavage of a CH_3 group and an $OH^\cdot$ ($M^+ - 15 - 17$) similarly to AMX, m/z 187 and 173 are successor ions by additional cleavage of a C_2H_5 ($M^+ - 15 - 17 - 29$) and C_3H_7 ($M^+ - 15 - 17 - 43$) originating from ring-opening and cleavage of the tetramethylated cyclopentane ring.

With mass spectrometrical methods the location of the reduction of the nitro group in the molecule cannot be determined. In AMT and AMK the nitro groups are chemically aequivalent whereas in AMA, AMM and AMX the nitro groups have a different chemical environment. For the latter compounds ^1H-NMR, ^{13}C-NMR, NOE and long-range coupling experiments should resolve this problem. This aspect will be treated elsewhere (Berset et al., 1999).

3.2. EI, PCI and NCI quadrupole MS of the amino metabolites

Performing a comparison study applying EI, PCI and NCI–MS useful information was further gathered regarding fragmentation and response sensitivity of the amino metabolites (Table 3). Besides the M^+ peak numerous fragment ions can be observed in EI–MS, whereas $M^+ - CH_3$ and m/z 57 (C_4H_9) are dominant ones. PCI–MS shows less fragmentation, typical a molecular ion MH^+ due to proton transfer reactions and adduct ions M^++29 and M^++41 can be identified, well known from PCI with methane. NCI–MS results in only few fragmentation ions. In most cases the molecular ion (M^-) turned out to be the base peak besides m/z 46 characteristic for the presence of a nitro group in the molecule. Regarding the sensitivity of the different ionization methods NCI was the most sensitive one, allowing the detection of femtograms, followed by EI and finally PCI. Therefore, quadrupole NCI–MS might be a valuable alternative to ion trap MS/MS for low level quantification of these metabolites.

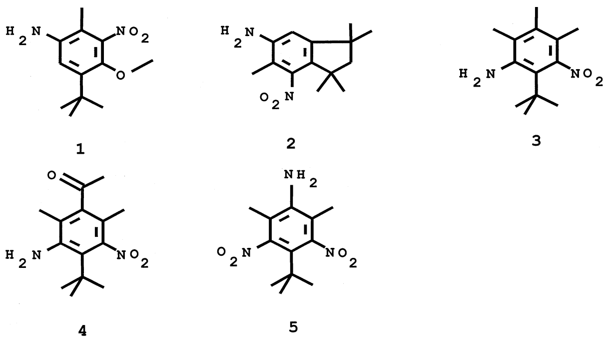

Fig. 3. Structures of the amino metabolites of the nitro musks: 1: AMA, 2: AMM, 3: AMT, 4: AMK, 5: AMX; see also Table 2.

Table 2
Conditions for resonant CID MS/MS fragmentation

	Musk compound	Precursor ion (m/z)	Successor ions for quantification (m/z)	CID res. (V)	t_R (min)
1	Cashmeran	191	135, 163, 173	0.35	16.3
2	Celestolide	229	173, 187	0.35	27.4
3	Phantolide	229	173, 187	0.35	30.3
4	Musk ambrette	253	219, 251	0.30	34.9
5	Musk xylene	282	265, 280	0.35	37.1
6	Galaxolide[a]	243	187, 213	0.35	37.6
7	Traseolide[a]	215	171, 173	0.40	37.6
8	Tonalide	243	173, 187	0.35	38.9
9	Musk moskene[a]	263	229, 245, 246	0.30	39.7
10	Amino musk ambrette[a]	238	165, 221, 223	0.60	39.7
11	Musk tibetene	251	204, 234, 249	0.30	42.4
12	Amino musk moskene	233	188, 216, 233	0.35	43.2
13	Musk ketone	279	191, 262	0.35	45.2
14	Amino musk tibetene	236	162, 163	0.35	45.6
15	Amino musk ketone	264	148, 191	0.30	50.6
16	Amino musk xylene	252	218, 235	0.35	51.4

[a] These substances were separated in the mass spectrometer by multiple-reaction-monitoring (MRM).

3.3. Concentrations of musk compounds in the sewage sludges of different catchment areas

Levels of nitro musks, polycyclic musks and metabolites of the nitro musks were determined in some selected sewage sludges containing no (type A), low (type B) or higher (type C) amounts of industrial waste water. A typical chromatogram of an extract is shown in Fig. 4 and quantitative results are presented in Table 4. Among the nitro musks MK was identified in 7 out of 12 sludges whereas MX was detected in just one sludge. Not surprisingly these nitro musks were found mainly in A-type sludges containing only domestic sewage water. Concentrations were rather low with an average concentration of 5 μg/kg d.m. for MK and 30 μg/kg d.m. for MX. These results are similar to recent studies which found values of 10–60 μg/kg d.m. (mean 30 μg/kg d.m.) for MK and 5 μg/kg d.m. for MX (Sauer et al., 1997; Blok, 1998). The concentrations found in our study reflect the industrial production volume of these compounds, MX and MK being the major nitro musks still used in different products. Also efforts undertaken by the fragrance industry in Switzerland since 1994 to reduce the amount of MX in detergents and cleaning agents are well documented by these results.

Considerable research is focused on transformation products of the nitro musks which have been described to occur in the environment and biota. These metabolites are formed by reduction of a nitro group to the corresponding amine. Very recently, published data have shown that the 4-amino-musk-xylene was much more toxic for Daphnia magna than the parent compound (Behechti et al., 1998) emphasizing the importance of these compounds for risk assessment

Table 3
Main mass ions of the amino metabolites obtained from full scan spectra using EI, PCI and NCI quadrupole MS[a]

	EI–MS	PCI–MS	NCI–MS	Tentative interpretation of some important mass ions
AMA	53 (22), 57 (29), 65 (25), 67 (23), 74 (27), 91 (23), 107 (40), 120 (39), 132 (21), 134 (21), 136 (21), 146 (20), 147 (26), 165 (33), 177 (35), 191 (8), 208 (4), 221 (13), 223 (23), **238** (100)	41 (88), 183 (28), 193 (13), 209 (7), 222 (14), **239** (100), 267 (10), 279 (4)	46 (2), **238** (100), 239 (14)	*EI–MS*: 238 (M^+), 223 ($M^+ - 15$, $M^+ - CH_3$), 221 ($M^+ - 17$, $M^+ - OH$), 165 ($M^+ - 73$, $M^+ - C_4H_9 - NH_2$), 57 ($C_4H_9^+$); *PCI–MS*: 239 (MH^+), 267 ($M^+ + 29$, $(M + C_2H_5)^+$), 279 ($M^+ + 41$, $(M + C_3H_5)^+$) 222 ($MH^+ - 17$, $MH^+ - OH$), 209 ($MH^+ - 30$, $MH^+ - NO$), 193 ($MH^+ - 46$, $MH^+ - NO_2$); *NCI–MS*: 238 (M^-), 46 (NO_2^-)
AMM	51 (15), 65 (6), 71 (7), 77 (7), 91 (5), 115 (5), 130 (6), 144 (5), 157 (11), 172 (17), 187 (10), 216 (9), **233** (100), 248 (20)	41 (40), 193 (56), 202 (27), 233 (17), **249** (100), 277 (17), 289 (9)	**248** (100), 249 (16)	*EI–MS*: 248 (M^+), 233 ($M^+ - 15$, $M^+ - CH_3$), 216 ($M^+ - 32$, $M^+ - CH_3$–OH); *PCI–MS*: 249 (MH^+), 277 ($M^+ + 29$, $(M + C_2H_5)^+$), 289 ($M^+ + 41$, $(M + C_3H_5)^+$), 233 ($MH^+ - 16$, $MH^+ - NH_2$ or O), 219 ($MH^+ - 30$, $MH^+ - NO$); *NCI–MS*: 248 (M^-)
AMT	**57** (100), 65 (27), 77 (36), 91 (26), 107 (15), 119 (29), 135 (68), 148 (48), 163 (93), 175 (29), 187 (49), 204 (7), 221 (40), 236 (91)	57 (8), **181** (100), 190 (24), 221 (6) 236 (24), 237 (16), 265 (2), 277 (2)	46 (6), 180 (3), 220 (2), **236** (100), 237 (15)	*EI–MS*: 236 (M^+), 221 ($M^+ - 15$, $M^+ - CH_3$), 163 ($M^+ - 73$, $M^+ - C_4H_9 - NH_2$), 57 ($C_4H_9^+$); *PCI–MS*: 236 ($((M - H) + H^+)$), 237 (MH^+), 265 ($M^+ + 29$, $(M + C_2H_5)^+$), 277 ($M^+ + 41$, $(M + C_3H_5)^+$), 190 ($(M - H) + H^+$–46, $((M - H) + H)^+ - NO_2$); *NCI–MS*: 236 (M^-), 46 (NO_2^-),
AMK	**57** (100), 65 (15), 77 (18), 91 (15), 103 (6), 115 (10), 134 (9), 144 (17), 160 (15), 176 (17), 191 (27), 202 (6), 215 (18), 249 (20), 264 (53)	57 (9), 164 (9), 178 (7), 209 (43), 218 (11), 219 (8) 233 (3), 249 (4), 265 (22), 293 (5), 305 (2)	46 (16), **264** (100), 265 (16)	*EI–MS*: 264 (M^+), 249 ($M^+ - 15$, $M^+ - CH_3$), 232 ($M^+ - 32$, $M^+ - CH_3$–OH), 191 ($M^+ - 73$, $M^+ - C_4H_9 - NH_2$), 57 ($C_4H_9^+$); *PCI–MS*: 265 (MH^+), 293 ($M^+ + 29$, $(M + C_2H_5)^+$), 305 ($M^+ + 41$, $(M + C_3H_5)^+$), 249 ($MH^+ - 16$, $MH^+ - NH_2$ or O), 219 ($MH^+ - 46$, $MH^+ - NO_2$); *NCI–MS*: 264 (M^-), 46 (NO_2^-),
AMX	57 (33), 65 (20), 77 (22), 91 (19), 106 (10), 118 (10), 130 (11), 144 (17), 159 (9), 175 (6), 187 (8), 218 (21), 235 (12), **252** (100), 267 (42)	57 (55), 177 (5), 189 (5), 212 (12), 252 (3), 268 (3), 296 (9), 308 (2)	**46** (100), 204 (3), 211 (3), 251 (2), 267 (77), 268 (11)	*EI–MS*: 267 (M^+), 252 ($M^+ - 15$, $M^+ - CH_3$), 235 ($M^+ - 32$, $M^+ - CH_3$–OH), 218 ($M^+ - 49$, $M^+ - CH_3 - 2 \cdot (OH)$); *PCI–MS*: 267 ($((M\text{–}H) + H^+)$), 268 ($MH^+$), 296 ($M^+ + 29$, $(M^+ + C_2H_5)^+$), 308 ($M^+ + 41$, $(M + C_3H_5)^+$); *NCI–MS*: 267 (M^-), 46 (NO_2^-)

[a] ( ): % abundance of the base peak (bold marked).

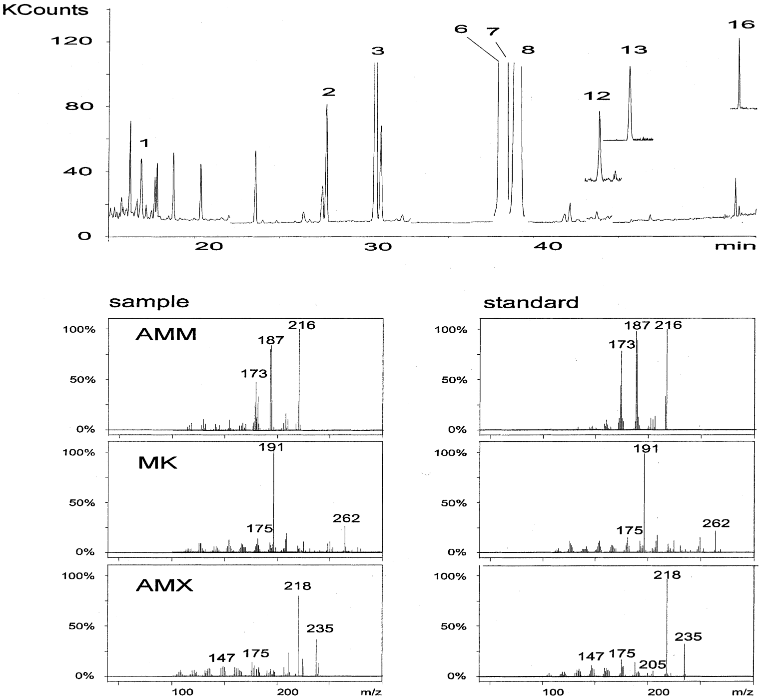

Fig. 4. Total ion chromatogram (TIC) of a sewage sludge extract and extracted ion chromatograms (EIC) of 12 (AMM), 13 (MK) and 16 (AMX); selected MS/MS spectra obtained from the sample and the corresponding standard.

considerations. In our sludge study three amino metabolites could be identified and quantified: amino musk xylene (AMX), amino musk moskene (AMM) and amino musk ketone (AMK). Concentrations were between 1 and 50 μg/kg d.m. and therefore reached levels in the same order as the parent compounds or even higher. AMX turned out to be the major metabolite, followed by AMM and AMK. Clearly, the two nitro musks MX and MM are found mainly as their amino metabolites in the sewage sludges. The decrease of the concentrations of the parent compounds and the increase of the amino metabolites strongly suggest, that besides adsorption to sludge particles transformation of nitro musks under anaerobic conditions is a relevant process in the sewage treatment plant (STP).

Based on the production volume, polycyclic musks represent about 93% of the market in Europe for synthetic musk compounds (OSPAR, 1998). As expected, they could be found in all sewage sludges most often in much higher concentrations than the corresponding nitro compounds (Table 4). Galaxolide (HHCB) and Tonalide (AHTN) were the main representatives reaching concentrations of 0.7–12.1 mg/kg d.m., followed by Phantolide (AHMI), Celestolide (ADBI) and Cashmeran (DPMI) which were present in concentrations below 1 mg/kg d.m.

4. Conclusions

HRGC and ion-trap MS/MS was used to identify and quantify two groups of synthetic musks in sewage sludge samples, namely the nitro and polycyclic musks. Among the nitro musks mainly MK was detected whereas MX was determined in just one sludge. These nitro musks were measured in the low μg/kg d.m. in sludges originating from domestic waste water. Amino transformation products of MX, MM and MK were detected for the first time in some sewage sludge samples which partly exceeded the concentrations of the parent compounds. This is important, taking into account the toxicity of some of these metabolites. Therefore, besides adsorption to the sewage sludge particles, transformation processes in the STP seem to play an important role with respect to the elimination of the nitro musks. Polycyclic musks were the main representatives of these synthetic compounds, found in all types of sewage sludges up to 12 mg/kg d.m. The two most important products detected were Galaxolide and Tonalide which, at the same time, are the compounds produced in highest quantities by the fragrance industry.

Future work will focus on (i) ecotoxicological studies of the amino-metabolites of the nitro musks as well as the polycyclic musks, (ii) soil degradation experiments of

Table 4
Concentrations of nitro musks, amino metabolites of the nitro musks and polycyclic musks in sewage sludges of different catchment areas[a]

Musk compound	Type A sludge											
	µg/l	µg/kg d.m.	µg/l	µg/kg d.m.	µg/l	µg/kg d.m.	µg/l	µg/kg d.m.	µg/l	µg/kg d.m.	µg/l	µg /kg d.m.
Nitro musks												
Ambrette	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Xylene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Moskene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tibetene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ketone	0.2	6.1	0.2	6.8	0.2	6.9	0.1	1.5	n.d.	n.d.	0.2	5.0
Amino metabolites												
AMA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AMX	0.4	10.9	0.4	15.3	1.2	49.1	n.d.	n.d.	n.d.	n.d.	0.9	24.9
AMM	0.3	7.9	n.d.	n.d.	0.1	1.6	n.d.	n.d.	0.1	0.7	n.d.	n.d.
AMT	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AMK	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Polycyclic musks												
Cashmeran	1.8	55.6	1.7	72.3	1.8	74.7	1.7	47.2	2.7	51.4	2.2	60.9
Celestolide	3.7	113	3.3	140	1.5	61.6	4.7	131	6.2	116	2.4	65.4
Phantolide	14.5	441	19.8	843	5.1	210	9.2	256	7.3	139	3.7	103
Galaxolide	178	5416	190	8068	145	5922	158	4374	124	2347	132	3658
Traseolide	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
Tonalide	44.5	1352	34.9	1485	44.1	1800	71.4	1983	51.5	973	42.7	1183

Musk compound	Type A sludge		Type B sludge						Type C sludge			
	µg/l	µg/kg d.m.	µg/l	µg/kg d.m.	µg/l	µg/kg d.m.	µg/l	µg/kg d.m.	µg/l	µg/kg d.m.	µg/l	µg/kg d.m.
Nitro musks												
Ambrette	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Xylene	n.d.	n.d.	1.6	32.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Moskene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tibetene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ketone	n.d.	n.d.	0.4	7.0	n.d.	n.d.	0.2	3.1	n.d.	n.d.	n.d.	n.d.
Amino metabolites												
AMA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AMX	n.d.	n.d.	1.6	31.5	n.d.	n.d.	0.9	15.6	n.d.	n.d.	n.d.	n.d.
AMM	n.d.	n.d.	n.d.	n.d.	0.3	7.2	n.d.	n.d.	1.2	36.2	n.d.	n.d.
AMT	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AMK	n.d.	n.d.	0.7	13.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Polycyclic musks												
Cashmeran	4.3	332	3.7	73.9	3.8	93.3	2.2	38.4	2.1	64.1	10.3	147
Celestolide	3.2	245	16.3	330	4.3	108	2.4	41	3.1	96.6	7.9	113
Phantolide	10.7	819	6.1	122	7.9	198	3.7	64.9	5.8	180	18.6	266
Galaxolide	158	12157	202	4074	150	3718	132	2293	104	3229	208	2977
Traseolide	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
Tonalide	54.1	4161	62.8	1269	57.0	1418	42.7	741	41.7	1290	54.9	784

[a] n.d.: not detected; n.q.: not quantified.

the chiral polycyclic musk compounds and (iii) the identification and spectroscopical characterization of polar metabolites of these substances.

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