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Derivatisation and gas chromatography–chemical ionisation mass spectrometry of selected synthetic and natural endocrine disruptive chemicals

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Abstract

Methods for ultra trace detection of endocrine disruptive chemicals (EDCs) are needed because of their low levels of impact. Twenty-one EDCs were selected, including 17β-estradiol, 17α-ethinylestradiol, 17β-testosterone and bisphenol A. Derivatisation with eight different fluorine containing compounds was examined. All EDCs could be derivatised automatedly (autosampler) with heptafluorobutyric acid (HFB) anhydride and trifluoroacetic acid (TFA) anhydride, respectively. The detection of these HFB and TFA derivatives in different chemical ionisation modes was studied. Fourteen different reagent gases, including methane, ammonia, acetone and water, were tested with the HFB and TFA derivatives in the negative chemical ionisation mode. Furthermore both types of derivatives were measured in positive chemical ionisation mode. Methane or water provide a good detection of all 21 TFA derivatives and create mass spectra with few fragmentation and characteristic mass peaks. This could serve as a basis for tandem or multiple mass spectrometric measurements.

Keywords: Derivatisation, GC; Endocrine disruptive chemicals; Estrogens

1. Introduction

About a decade ago endocrine disruptive chemicals (EDCs) were discovered as possible hazard for men and fauna. The EDC class is very heterogeneous and comprises natural and synthetic hormones as well as industrial chemicals, herbicides and pesticides. Concerning men an increasing risk of cancerogenesis, disturbance in children's development and reduced fertility are attributed to EDCs in water and nutrition [1–3]. Stronger scientific evidence is given for the impairment of fauna [4–6]. Fast and easy methods with low limits of detection (LODs) for the quantitative analysis of EDCs are needed because more exposition data for risk assessment are necessary. Synergistic effects of different EDCs are not ruled out. Therefore low limits of detection are important as well.

A number of methods for analysis of synthetic and natural hormones in water have been developed, amongst them many methods employing gas chromatography–mass spectrometry (GC–MS) [7–15] and high-performance liquid chromatography (HPLC–

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MS) [16–18] or HPLC–radio immunoassay (RIA) [19]. Mainly natural and synthetic estrogenic compounds have been analysed. De Alda and Barcelo [16] and Kuch and Ballschmitter [11] additionally examined gestagens like levonorgestrel. Wegener et al. [10] and Lagana et al. [18] additionally included gestagens like progesterone and androgens like 17β-testosterone. Different types of water like ground water, drinking water, river water, sewage plant effluents and influents have been analysed. Solid-phase extraction (SPE) has always been used for enrichment and in some works HPLC and other chromatographic clean-ups have been employed. A derivatisation of compounds was not necessary for HPLC analysis. However analytes have been derivatised before GC measurements except in one work [7]. Often trimethylsilyl (TMS) derivatives have been synthesized and measured in the single ion monitoring (SIM) [10,11] or MS–MS [8,9] modes with electron impact (EI) ionisation. Measurements of fluorine containing derivatives with negative chemical ionisation (NCI) and detection in the SIM mode were carried out. Therefore pentafluorobenzyl (PFB) [12] and pentafluorobenzoyl (PFBO) derivatives [13,15] have been synthesized. One group employed mixed PFB–TMS derivatives [14]. All derivatisation procedures are very time consuming and require much manual work.

LODs vary between 0.05 and 1 ng/l for steroidal hormones in surface water and between 0.1 and 1 ng/l for wastewater. Generally HPLC methods are not superior to GC methods.

Since most hormones and their derivatives, respectively show huge fragmentation in mass spectra obtained from EI ionisation, many ions are lost for detection when employing MS–MS or SIM techniques. Our approach for a more efficient analysis is the use of chemical ionisation (CI) in order to reduce fragmentation enabling MS–MS or multiple MS detection (MS^n) with low LODs. Twenty-one natural and synthetic hormones, mainly estrogenic substances, were selected for the examinations (Fig. 1). Eight fluorine containing derivatisation reagents were tested with these analytes. All these reagents react with hydroxyl groups of the analytes (Fig. 2). It was our aim to find a simple and fast derivatisation reaction which is applicable to many natural and synthetic hormones. The reaction should result in derivatives with large molecular masses which yield ions with large masses in chemical ionisation. These ions should emerge clearly from the chemical background and the matrix. So preferably all hydroxyl groups of the analytes should be derivatised. This is essential for good chromatographic properties (stability, elution profile, etc.) as well. In order to achieve a high throughput in later methods of analysis the derivatisation reaction should ideally be automated.

Two reagents [heptafluorobutyric acid (HFB) anhydride and trifluoroacetic acid (TFA) anhydride] which readily formed derivatives with all analytes were chosen. Derivatisation reactions with these reagents were automated by an autosampler programme. NCI and PCI measurements of the derivatives were carried out with 14 different reagent gases including methane, ammonia, acetone and water.

In other works HFB derivatives of anabolic steroids extracted from urine have already been measured in the NCI mode with methane [20]. HFB derivatives of some estrogens and TFA derivatives of some anabolic steroid esters have been synthesized before but were not examined with different CI modes [21,22].

Mostly methane is used for CI in the negative and positive modes. But there are some measurements with other appropriate CI gases mentioned in the literature, e.g., octylphenol, bisphenol A and some steroid hormones with argon [23], free volatile fatty acids with ammonia [24], *cis*- and *trans*-indanediols with dimethylether [25], amphetamines with methanol [26] and benzene, naphthalene and ferrocene with water [27]. A number of reaction gases, e.g., argon, methane, nitrogen, hydrogen, dichloromethane, and their properties especially for NCI are described in Ref. [28].

2. Experimental

2.1. Reagents and chemicals

Estrone, estriol, 17α-estradiol, dienestrol, bisphenol A, 17β-testosterone, dihydrotestosterone, pregnenolone and dehydroepiandrosterone (purity at least >98%) were from Sigma–Aldrich (Taufkirchen, Germany). 17α-Ethinylestradiol, 17β-estradiol,

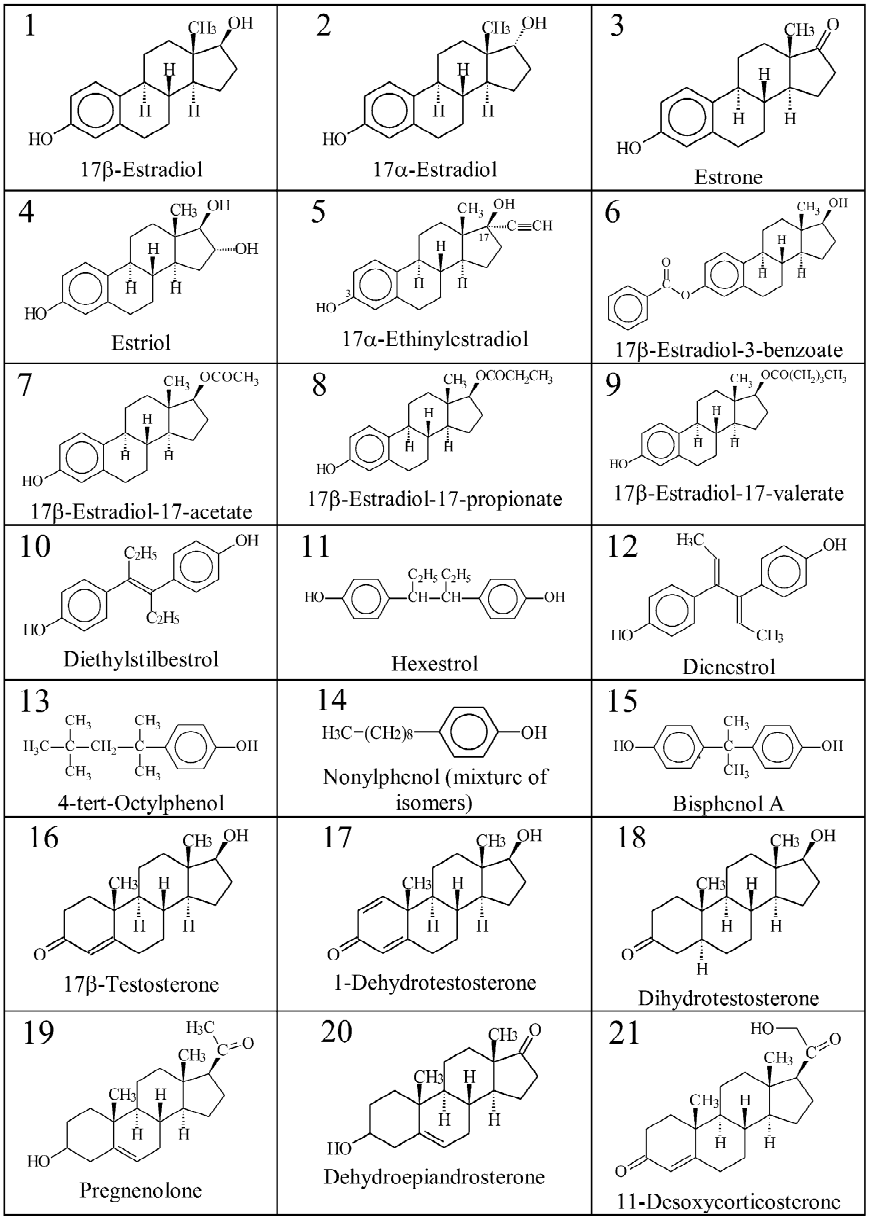


Fig. 1. Analysed EDCs.

17β-estradiol-3-benzoate, 17β-estradiol-17-acetate, 17β-estradiol-17-propionate, 17β-estradiol-17-valerate, hexestrol, diethylstilbestrol, 1-dehydrotestosterone, 11-desoxycorticosterone (all analytical standards) were purchased from Riedel-de Haën (Seelze, Germany). Nonylphenol and 4-*tert*.-octylphenol were purchased as technical mixtures from Riedel-de Haën and Fluka (Buchs, Switzerland), respectively.

N-heptafluorobutyrilimidazole and flophemesylamine were purchased from Lancaster (Mühlheim, Germany), pentafluorobenzyl bromide (99%), pentafluoropyridine (99%) and octafluorotoluene (98%)

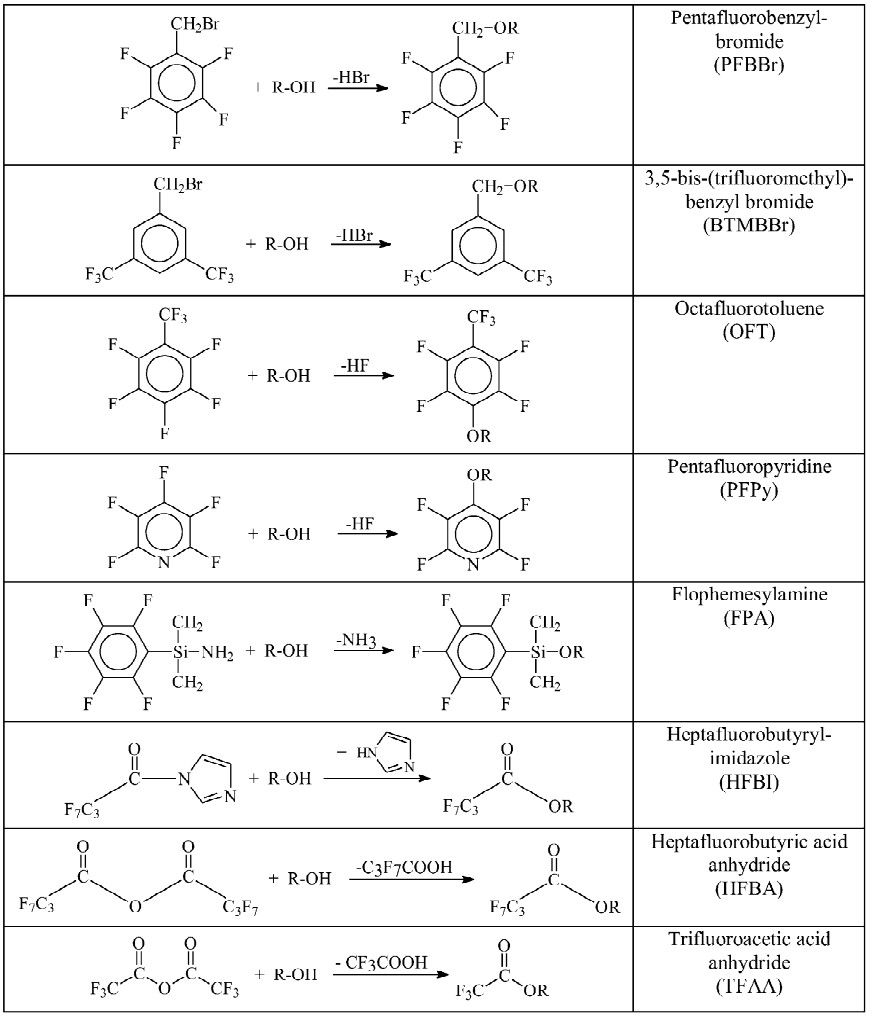


Fig. 2. Derivatisation reagents and the respective reactions.

from ABCR (Karlsruhe, Germany), trifluoroacetic acid anhydride (99+%), heptafluorobutyric acid anhydride (98+%) and 3,5-bis-(trifluoromethyl)-benzyl bromide (97%) from Acros (Geel, Belgium).

The gases methane (2.5), ammonia (3.8), helium (5.0), argon (4.8), nitrogen (4.0) and hydrogen (5.0) were purchased from Messer-Griesheim (Krefeld, Germany). *n*-Pentane, *n*-hexane, cyclohexane, toluene, ethyl acetate, acetone, acetonitrile, tetrahydrofuran, diethylether, diisopropylether, methanol, methylenechloride, chloroform, dimethylsulfoxide and dimethylformamide were of analytical grade from J.T. Baker (Deventer, The Netherlands). HPLC-grade water was from J.T. Baker as well. *M*-Xylene, 2-ethylbenzene, 3-ethylbenzene, 4-ethylbenzene, *n*-butylbenzene and *tert.*-butylbenzene were of analytical grade from Sigma–Aldrich. 1,2,3-Trimethylbenzene and 1,3,5-trimethylbenzene of analytical grade were from Riedel-de Haën and Fluka, respectively. NaOH, K_2CO_3, pyridine and triethylamine of analytical grade were from J.T. Baker. CsF and tetrabutylammoniumhydrogenesulfate of analytical grade were from Sigma–Aldrich and Acros, respectively.

2.2. Equipment

The GC–MS system was a GCQ instrument from Finnigan MAT (San Jose, CA, USA).

For MS parameters see Figs. 4 and 5.

The GC column was a DB-XLB (Agilent/J&W Scientific, Palo Alto, CA, USA), of 30 m×0.25 mm I.D., and 0.25 μm film thickness. For GC parameters see Fig. 5.

The injection system was an Optic 2-200 from ATAS (Veldhoven, The Netherlands).

The PTV (programmable temperature vaporiser) injector had a large-volume injection facility (separate solvent split).

The autosampler was a Combi PAL from CTC Analytics (Zwingen, Switzerland).

Software used was Xcalibur, version 1.1 from Finnigan (San Jose, CA, USA), CycleComposer, version 1.3.1 from CTC Analytics (Zwingen, Switzerland).

The mixers used were a Thermomixer Comfort 2 ml, from Eppendorf (Hamburg, Germany) and a Vortex Genie 2 from Scientific Industries (New York, NY, USA).

2.3. Experiments

2.3.1. Derivatisation

Stock solutions of 0.4 g/l of each analyte were prepared in acetone. These stock solutions were mixed and diluted to give another stock solution of all analytes with a concentration of 0.019 g/l in acetone.

Analytes 1 and 5 which include phenolic as well as aliphatic hydroxyl groups were employed as model substances in order to optimise derivatisations. 17α-Ethinylestradiol (compound 5), which is of huge importance for environmental analysis, was unstable under certain conditions. By derivatising compound 5 the reaction conditions could be tested for their smoothness against labile compounds.

A range of solvents was tested with the derivatisation reagents. Different polarities and abilities of interaction (e.g., van der Waals, polar) should be covered by the selection. No solvents with acidic X–H groups as methanol or acetic acid could be chosen, for they react with the derivatisation reagent itself. Different Brönstedt (K_2CO_3, NaOH and CsF) and Lewis bases (pyridine, triethylamine) were employed to promote the derivatisation reactions. Salts were added as solids to the reaction solution and did not dissolve. Bases can at least serve in two manners: (a) they activate the hydroxyl groups and (b) they form insoluble precipitations with one reaction product and therefore drive the chemical equilibrium towards the product side. Part (b) is, e.g., possible for the reaction of hydroxyl groups with PFBBr and K_2CO_3 in which the PFB derivative and insoluble KBr are formed.

The general strategy for optimisation of the derivatisation was as follows:

(i) Find an appropriate solvent for the reaction. Use a base if it is referred to in the literature.

(ii) If the use of a base is mentioned in the literature or it seems suitable, use the best solvent determined in step (i) and test different bases with this solvent.

(iii) If steps (i) and (ii) give promising results, derivatise all compounds separately.

(iv) If step (iii) gives good results again, optimise the derivatisation concerning volumes, reaction times, etc.

Tables 1–7 contain the experimental conditions concerning points (i) and (ii) whereas Table 8 describes the final conditions concerning points (iii) and (iv).

The different derivatisation reactions were evaluated by taking into account these facts:

(i) Completeness of derivatisation, only one main product.

(ii) Conversion of all hydroxyl groups.

(iii) Few byproducts.

Furthermore the requirements of a derivatisation reaction described in the introduction were considered.

Before reaction the acetone of the stock solutions was evaporated in a stream of argon at 55 °C and the analytes were redissolved in the respective solvent. Reactions were carried out in 2-ml safe seal micro tubes (PP). If the reaction should take place at room temperature the Vortex mixer was used. For reactions at a specific higher temperature the Thermomixer was employed at 1200 rpm. After mixing the reaction mixture was evaporated in some cases in a stream of argon at an appropriate temperature. If a precipitation was formed the solution was cen-

Table 1
Derivatisation parameters for testing solvents and bases with PFBBr

Derivatisation reagent	PFBBr	
Tested solvents	Acetone, acetonitrile, tetrahydrofuran, ethyl acetate, chloroform, *n*-hexane, cyclohexane, pyridine, dimethylsulfoxide	
Tested bases	K_2CO_3 (0.01 g), NaOH (0.003 g), CsF (0.01 g), triethylamine (10 μl), pyridine (6 μl)	
	Test of solvents:	Test of bases:
	40 μg compound 1	40 μg compound 1
	500 μl solvent	500 μl acetone
	0.01 g K_2CO_3	Amount of base (see above)
	5 μl PFBBr	5 μl PFBBr
	1 h, 50 °C	1 h, 50 °C
	Evaporation (except DMSO) redissolving in 200 μl acetone	Evaporation
	Injection 1 μl	Redissolving in 200 μl acetone
		Injection 1 μl

Table 2
Derivatisation parameters for testing solvents and bases with BTMBBr

Derivatisation reagent	BTMBBr	
Tested solvents	Acetone, acetonitrile, tetrahydrofuran, ethyl acetate, diisopropylether, chloroform, *n*-hexane, cyclohexane, toluene, pyridine, dimethylsulfoxide	
Tested bases	K_2CO_3 (0.01 g), NaOH (0.003 g), CsF (0.01 g), triethylamine (10 μl), pyridine (6 μl)	
Experimental conditions	Test of solvents:	Test of bases:
	Each 80 μg compound 1, 5, 15 in one solution	80 μg compound 1
	300 μl solvent	300 μl acetonitrile
	0.01 g K_2CO_3	Amount of base (see above)
	10 μl BTMBBr	10 μl BTMBBr
	1 h, 55 °C	1 h, 80 °C
	Evaporation (except DMSO)	Evaporation
	Redissolving in 300 μl acetone	Redissolving in 300 μl *n*-hexane
	Injection 1 μl	Injection 1 μl

Table 3
Derivatisation parameters for testing solvents and bases with OFT and PFPy

Derivatisation reagent	OFT/PFPy	
Tested solvents	Acetone, acetonitrile, tetrahydrofuran, ethyl acetate, diisopropylether, chloroform, methylenechloride, *n*-hexane, cyclohexane, toluene, pyridine, dimethylsulfoxide, dimethylformamide	
Tested bases	K_2CO_3 (0.01 g), NaOH (0.003 g), CsF (0.01 g), triethylamine (10 μl), pyridine (6 μl)	
Experimental conditions	Test of solvents:	Test of bases:
	160 μg compound 1	80 μg compound 1
	300 μl solvent	300 μl DMSO or acetone
	0.01 g K_2CO_3	Amount of base (see above)
	10 μl OFT or PFPy	10 μl OFT or PFPy
	1 h, 55 °C (except methylenechloride at room temperature)	1 h, 55 °C
	Evaporation (except DMSO, DMF)	Evaporation
	Redissolving in 300 μl acetone	Redissolving in 300 μl acetone
	Injection 1 μl	Injection 1 μl

Table 4
Derivatisation parameters for testing solvents with FPA

Derivatisation reagent	FPA
Tested solvents	Acetone, acetonitrile, tetrahydrofuran, ethyl acetate, diisopropylether, chloroform, *n*-hexane, cyclohexane, toluene, pyridine, dimethylsulfoxide
Tested bases	No bases tested
Experimental conditions	Test of solvents:
	160 μg compound 1, 5, 15 in one solution 300 μl solvent 5 μl FPA 1 h, 50 °C Evaporation (except DMSO) Redissolving in 300 μl *n*-hexane Injection 1 μl

Table 5
Derivatisation parameters for testing solvents with HFBI

Derivatisation reagent	HFBI
Tested solvents	Acetone, acetonitrile, tetrahydrofuran, ethyl acetate, diisopropylether, chloroform, *n*-hexane, cyclohexane, toluene, pyridine, dimethylsulfoxide, dimethylformamide
Tested bases	No bases tested (see Refs. [36,37])
Experimental conditions	Test of solvents:
	Each 10 μg compound 1 and 6 in one solution 250 μl solvent 20 μl HFBI 45 min, 55 °C Injection 1 μl

Table 6
Derivatisation parameters for testing solvents with HFBA

Derivatisation reagent	HFBA	
Tested solvents	Acetone, acetonitrile, tetrahydrofuran, ethyl acetate, diisopropylether, chloroform, *n*-hexane, cyclohexane, toluene, *n*-butylbenzene, *tert*.-butylbenzene, *m*-xylene, 2-ethyltoluene, 3-ethyltoluene, 4-ethyltoluene, 1,2,3-trimethylbenzene, 1,3,5-trimethylbenzene,dimethylsulfoxide, dimethylformamide	
Tested bases	K_2CO_3 (0.01 g), CsF (0.01 g), triethylamine (10 μl), pyridine (6 μl)	
Experimental conditions	Test of solvents:	Test of bases:
	10 μg compound 1 500 μl solvent 10 μl HFBA 30 min, 55 °C Evaporation Redissolving in 200 μl acetone Injection 1 μl	10 μg compound 1 300 μl toluene Amount of base (see above) 10 μl HFBA 1 h, 55 °C Evaporation Redissolving in 200 μl acetone Injection 1 μl

Table 7
Derivatisation parameters for testing solvents with TFAA

Derivatisation reagent	TFAA
Tested solvents	Acetone, acetonitrile, tetrahydrofuran, ethyl acetate, diisopropylether, chloroform, methylenechloride, *n*-hexane, cyclohexane, toluene, dimethylsulfoxide, dimethylformamide
Tested bases	No bases tested because of the results with HFBA
Experimental conditions	Test of solvents: 120 μg compound 1 300 μl solvent 10 μl TFAA 1 h, room temperature Injection 1 μl

trifuged. For measurements the solutions were filled in a 2 ml glass autosampler vial.

2.3.1.1. Derivatisation with PFBBr

For determination of the most suitable solvent K_2CO_3 was employed as mentioned in Refs. [29–31].

Besides the general experiments explained in Tables 1 and 8 the reaction was carried out phase transfer catalyzed, principally described in Ref. [32]. The conditions were: 160 μg compound 1, redissolved in 300 μl ethyl acetate+300 μl 1 *M* NaOH+0.005 g tetrabutylammoniumhydrogenesulfate as phase transfer catalyst+10 μl PFBBr, 1 h at 70 °C, direct injection of 1 μl organic phase.

2.3.1.2. Derivatisation with BTMBBr

For determination of the most suitable solvent K_2CO_3 was employed as mentioned in Ref. [33].

Besides the general experiments explained in Table 2 the reaction was carried out phase transfer catalyzed [33] under similar conditions as in the PFBBr reaction: 160 μg compound 1, redissolved in 300 μl ethyl acetate+300 μl 1 *M* NaOH+0.005 g tetrabutylammoniumhydrogenesulfate as phase transfer catalyst+10 μl BTMBBr, 1 h at 70 °C, direct injection of 1 μl organic phase.

2.3.1.3. Derivatisation with OFT or PFPy

For determination of the most suitable solvent K_2CO_3 was employed (Table 3) as in the reactions with PFBBr and BTMBBr (Tables 1 and 2).

A phase transfer catalyzed reaction was tested as well. Therefore conditions similar to those described in Ref. [34] were employed: 160 μg compound 1, redissolved in 300 μl CH_2Cl_2+300 μl 1 *M* NaOH+0.005 g tetrabutylammoniumhydrogenesulfate as phase transfer catalyst+5 μl OFT or PFPy, reaction

Table 8
Final parameters for the derivatisation of EDCs

Derivatisation reagent	PFBBr	OFT/PFPy	HFBI	HFBA	TFAA
Derivatised analytes	1–15, 40 μg each in separate solutions	1–15, 120 μg each in separate solutions	1–15, 40 μg each in separate solutions	1–21, 3.8 μg each in one solution	1–21, 3.8 μg each in one solution
Solvent	400 μl acetone	300 μl DMSO	250 μl *n*-hexane–diisopropylether (1:1, v/v)	150 μl toluene	150 μl toluene
Base	0.01 g K_2CO_3	0.01 g CsF	–	–	–
Volume derivatisation reagent	5 μl	10 μl OFT/20 μl PFPy	20 μl	6 μl	6 μl
Reaction conditions	1 h, 50 °C	45 min, 55 °C	45 min, 55 °C, centrifugation	5 min, room temperature	5 min, room temperature
Evaporation/redissolving	50 °C/200 μl acetone	–	–	–	–
Use of an autosampler	–	–	–	Yes	Yes
Injection volume	1 μl	1 μl	1 μl	1 μl	1 μl

1 h at room temperature and direct injection of 1 μl organic phase.

2.3.1.4. Derivatisation with FPA

According to the literature [35] the reaction of FPA with the analytes was carried out without any auxiliary chemicals. The conditions can be seen in Table 4.

2.3.1.5. Derivatisation with HFBI

According to the literature [36,37] the reaction of HFBI with the analytes was carried out without any auxiliary chemicals.

Besides the experiments presented in Tables 5 and 8, compounds 1 and 5 were derivatised directly in HFBI without another solvent [36,37]. The following conditions were applied: 10 μg each of compound 1 and 5 redissolved in 20 μl HFBI, reaction 45 min at 55 °C, +250 μl *n*-hexane, injection of 1 μl solution.

2.3.1.6. Derivatisation with HFBA

According to the literature [38] the reactions for determination of the most suitable solvent were carried out without any auxiliary chemicals (Table 6). Afterwards different bases have been tested which is mentioned in the literature as well [38].

Because the results of the derivatisation of compounds 1–21 (Table 8) were positive other parameters have been tested. A reaction at room temperature has been tested versus one at 55 °C. The parameters were as follows: 120 μg compound 5 redissolved in 300 μl toluene+10 μl HFBA, 1 h reaction time, injection of 1 μl solution. Furthermore it has been tested how the evaporation of the toluene after the reaction affects the yields of derivatives. Therefore the following experiments were carried out: 3.8 μg each of compounds 1–15 in one solution redissolved in 150 μl toluene+5 μl HFBA, 20 min at room temperature, direct injection of 1 μl or evaporation at 75 °C and redissolving in 150 μl toluene.

Since the reaction needed no solid auxiliary chemicals an autosampler could be employed for adding HFBA, shaking and injection of the solution. Reaction time and volume of HFBA were optimised. Therefore 3.8 μg each of compounds 1–15 in one solution redissolved in 150 μl toluene was employed. In the first experiment 5, 6, 7 or 9 μl of HFBA were added, the reaction time was 5 min and 1 μl was directly injected. In the second experiment 6 μl of HFBA was added and the reaction times were 5, 10, 20, 30 and 60 min. Each 1 μl volume of the solutions was injected into the GC system.

2.3.1.7. Derivatisation with TFAA

According to the literature [38] the reactions for determination of the most suitable solvent were carried out without any auxiliary chemicals (Table 7). Because of the results with HFBA no bases were tested with TFAA.

An autosampler was employed as well, reaction time and volume of TFAA were optimised. Therefore 3.8 μg each of compounds 1–21 in one solution redissolved in 150 μl toluene was employed. In the first experiment 2, 4, 6, 8 or 10 μl of TFAA was added, the reaction time was 5 min and 1 μl was directly injected. In the second experiment 5 μl of TFAA was added and the reaction times were 2, 5, 10, 20 and 40 min. Each 1-μl volume of the solutions was injected into the GC system.

2.3.2. Mass spectrometric detection

During testing and optimisation of the derivatisation all mass spectra were recorded in the EI mode to monitor the yields of derivatives.

The employed GCQ mass spectrometer is equipped with an ion trap analyzer with external ionisation. This feature allows one to produce and detect positive as well as negative ions in the chemical ionisation mode (so-called ‘‘high pressure CI’’ with about 100 Pa in the ionisation volume).

HFB derivatives were measured in the NCI mode with following reagent gases: methane, ammonia, nitrogen, helium, argon, hydrogen, methanol, *n*-pentane, toluene, ethyl acetate, acetone, diethylether, methylenechloride and water. A measurement in the PCI mode with methane as reagent gas was carried out as well. TFA derivatives were measured in the PCI mode with methane, ammmonia, argon, hydrogen, methanol, *n*-pentane, diethylether and water as reagent gases. Again measurements in the NCI mode were carried out with methane, ammonia, acetone and water as reagent gases.

Liquid reagent ‘‘gases’’ were filled in a little steel tube which was connected to the mass spectrometer via a needle valve. They were transported to the ionisation volume solely due to their vapour pres-

sure. In general following parameters were chosen for PCI and NCI modes: 240 °C ion source temperature, 100 eV electron energy, 350 μA emission current and 8 V (PCI)/3 V (NCI) trap offset. The reagent gas was adjusted by the needle valve on the front side of the GCQ mass spectrometer resulting in fore pressures of 8, 10.7 and 13.3 Pa, respectively, in order to optimise the yield of ions. This fore pressure is the only possibility to control and reproduce the reagent gas pressure in the GCQ mass spectrometer.

2.3.2.1. NCI detection

After having found adequate reagent gases, the mass spectra of HFB derivatives should be improved without decrease of ion yield. That means fragmentation was tried to be controlled to result in ions specific for the individual compound. Therefore the ideal foreline pressure of the respective reagent gas was set. Electron energies were altered to 70 and 30 eV and the source temperatures to 200 and 180 °C. Furthermore the emission current was set to 500 μA, with an electron energy of 70 eV and 200 °C source temperature.

NCI measurements of the TFA derivatives have been carried out with methane, ammonia, acetone and water (all adequate for NCI detection of the HFB derivatives). Conditions for methane: general parameters (see Section 2.3.2), but 200 °C ion source temperature and 13.3 Pa fore pressure. Conditions for acetone: general parameters, but 200 °C ion source temperature and 8 Pa fore pressure. Conditions for water: general parameters, but 200 °C ion source temperature and 12 Pa fore pressure. Conditions for ammonia: fore pressure 10.7 and 13.3 Pa, 200 and 240 °C source temperature, 30, 70 and 100 eV electron energy, 350 and 600 μA emission current, 3 V trap offset.

2.3.2.2. PCI detection

For PCI measurements of the TFA derivatives the general parameters (see Section 2.3.2) for testing the different reagent gases were employed (except source temperature 200 °C). Different fore pressures were evaluated (8, 10.7 and 13.3 Pa). For further optimisation firstly the electron energy was lowered to 70 eV, secondly the emission current was increased to 500 μA and thirdly the source temperature was increased to 240 °C all at a constant fore pressure of 13.3 Pa.

A PCI measurement of the HFB derivatives was carried out with methane at following conditions: 13.3 Pa fore pressure, 200 °C ion source temperature, 100 eV electron energy, 500 μA emission current and 8 V trap offset.

3. Results and discussion

3.1. Derivatisation

General reaction schemes of the derivatisation reactions can be seen in Fig. 2.

3.1.1. Derivatisation with PFBBr

Best derivatisation yields were achieved in acetone with K_2CO_3 as base. The use of acetone as solvent in a reaction with PFBBr is rather seldom reported in the literature [39]. K_2CO_3 is mostly employed in derivatisations with PFBBr [29–31]. Obviously amine bases or NaOH activate hydroxyl groups to a lesser extent than K_2CO_3. With amine bases in acetone no precipitation of the respective ammoniumbromide is formed to force the reaction to the product side. The mild basic conditions created by K_2CO_3 should be ideal for derivatisation of compounds containing labile functional groups like ester groups. It was found that only phenolic hydroxyl groups reacted with the PFBBr, aliphatic ones did not react. Again this is in compliance with literature, where PFBBr is often used for derivatising reactive hydroxyl groups like phenolic ones and hydroxyl groups of acids [30,31]. So analytes with one phenolic and one aliphatic hydroxyl group (e.g., compound 1) formed a mono PFB derivative and analytes with only one aliphatic hydroxyl group (e.g., compound 6) did not form derivatives at all. Derivatisation of the phenolic hydroxyl group of 17β-estradiol (compound 1) is already reported in literature, the aliphatic hydroxyl group has been silylated before [40].

Generally many byproducts were formed. In the phase transfer catalysed reaction only aliphatic hydroxyl groups reacted as well.

3.1.2. Derivatisation with BTMBBr

Best derivatisation yields were achieved in acetone or acetonitrile with NaOH as base. Again only phenolic hydroxyl groups reacted, aliphatic ones did not. There are many parallels between the derivatisation with PFBBr and BTMBBr like: same solvent, same compounds to be derivatised and same leaving group (bromide). In contrast to the derivatisation with PFBBr NaOH is the most suitable base which yielded almost a 200-fold amount of mono derivative than K_2CO_3. Even amine bases worked better than K_2CO_3. This could mean that the reaction mechanisms of the derivatisation with PFBBr and BTMBBr are different although they are similar reagents.

The phase transfer catalysis had no advantages again. Generally many byproducts were formed.

3.1.3. Derivatisation with OFT or PFPy

Best derivatisation yields were achieved in DMSO with CsF as base and as well phenolic as aliphatic hydroxyl groups reacted. This is in compliance with the literature, but in contrast to our experiments dimethylformamide (DMF) was used as solvent [41]. The reaction may be understood as a nucleophilic aromatic substitution ($S_{N,Ar}$) of a fluoride at the OFT or PFPy, respectively [42]. Problems appeared with compounds 4 (estriol), 5 (17α-ethinylestradiol) and 6 (17β-estradiol-3-benzoate). Compound 4 decomposed during the reaction and only a small yield of triple OFT or PFPy derivative resulted. Compound 5 lost C_2H_2 (completely with OFT and partly with PFPy) forming compound 3 (estrone). Therefore the mono OFT derivative of compound 3 could be detected as main product of the reaction of compound 5 with OFT. The mono PFPy derivatives of compounds 5 and 3 were the main products of the reaction of compound 5 with PFPy. Compound 6 hydrolysed forming compound 1 (17β-estradiol) which could be detected as di OFT or PFPy derivative. To prevent, e.g., C_2H_2-loss (compound 5) or hydrolysis (compound 6) the reaction was carried out at room temperature which resulted in little yields of derivatives. Phase transfer catalysed reaction did not form any derivatives at all.

OFT and PFPy mainly react in the *para* position [34], but with all compounds little amounts of *ortho* OFT derivatives occurred. Only small amounts of the other byproducts were formed.

3.1.4. Derivatisation with FPA

Compounds 1 (17β-estradiol), 5 (17α-ethinylestradiol) and 15 (bisphenol A) were tested with this derivatisation reagent (Table 4). Cyclohexane, toluene and *n*-hexane turned out to be the most suitable solvents which is in contrast to the literature where pyridine is used [35]. Generally many byproducts were formed and the peaks of the derivatives were broad on a DB-5-MS column (Agilent/J&W Scientific). Compounds 1 and 15 yielded di FP derivatives, compound 5 only the mono FP derivative. Moreover compound 5 decomposed to compound 3 (estrone) and the respective mono FP derivative could be detected as a byproduct.

3.1.5. Derivatisation with HFBI

In the experiments with different solvents (Table 5) *n*-hexane turned out to be the most suitable solvent for derivatisation of compound 1. Diisopropylether turned out to be most appropriate for compound 6. Therefore a 1:1 (v/v) mixture of both was employed and as well phenolic as aliphatic hydroxyl groups reacted. With some analytes the derivatisation was not complete so that mono HFB derivatives or underivatised compounds remained. Compound 5 only formed a mono derivative as already observed with OFT, PFPy and FPA.

The yields of the derivatisation in pure HFBI were much lower than the yields with *n*-hexane–diisopropylether (1:1) as solvent.

3.1.6. Derivatisation with HFBA

The best derivatisation yields were achieved in toluene without any base. As well phenolic as aliphatic hydroxyl groups reacted. The use of toluene as solvent is unusual. Literature mostly reports on the use of acetonitrile with HFBA [20,21,38]. Derivatives of some anabolic steroids and estrogens have already been synthesized before [20,21]. The procedures were rather time consuming with heating for 30–60 min at temperatures from 50 to 80 °C and subsequent evaporation and redissolving.

Analytes 1–21 readily formed derivatives with HFBA. Again only the mono derivative of compound 5 (17α-ethinylestradiol) was formed. Probably

the ethinyl group sterically hinders the hydroxyl group at C-17 so that it is not accessible by the tested derivatisation reagents. Every hydroxyl group of all the other compounds reacted with HFBA.

Derivatisation of compound 5 at room temperature yielded the double amount of mono derivative than the derivatisation at 55 °C.

During evaporation of toluene derivatives were evaporated as well and in some cases bondages were cleaved so that the underivatised compounds were partly reobtained (e.g., compounds 7, 8, 9).

Changing the HFBA volume between 5 and 10 μl did not affect the derivatisation yields markably. Derivatisation was completed after 5 min mixing at room temperature and extremely few byproducts were formed.

3.1.7. Derivatisation with TFAA

The best derivatisation yields were achieved in toluene without any base. As well phenolic as aliphatic hydroxyl groups reacted. Again the use of toluene as solvent is unusual. The literature mostly reports on the use of acetonitrile with TFAA [38].

Analytes 1–21 readily formed derivatives with TFAA. Again only the mono derivative of compound 5 (17α-ethinylestradiol) occured. The other compounds formed derivatives according to their number of hydroxyl groups.

Changing the TFAA volume between 2 and 10 μl did not affect the yield of derivatives markably for most compounds. Some compounds (e.g., 4, 10, 11, 12, 13, 15) showed an increasing yield in the range from 2 to 6 μl TFAA. Derivatives of compound 5 (17α-ethinylestradiol) slightly decreased with increasing TFAA volume. This decrease could be due to a dehydratisation at C-17 by TFAA. In preceding measurements it has been found out, that a derivative with a lack of 18 mass units (loss of H_2O) in relation to the mono derivative of compound 5 can result in little yields. The H_2O can only be eliminated at C-17 because the phenolic hydroxyl group has been acylated by TFAA.

Generally the reaction was completed after 5 min at room temperature (see Fig. 3a–c). Only some compounds show slightly increasing yields of derivatives after that (e.g., 13, 14). A reaction time of 2 min resulted in less derivatives of all compounds. The yields of compound 5 (17α-ethinylestradiol), 15 (bisphenol A), 16 (testosterone), 21 (11-desoxycorticosterone) decreased with increasing reaction time. The explanations for these observations are, that the derivative of compound 5 was dehydratised (see above) and compound 16 and 21 formed di TFA derivatives (formation possible due to keto-enol tautomerie) which increased with time.

3.1.8. Conclusions concerning derivatisation

PFBBr and BTMBBr are not suitable for the derivatisation of the chosen analytes because they only reacted with phenolic hydroxyl groups. Furthermore much byproducts were formed. Multi step derivatisation methods with, e.g., preceding silylation of aliphatic hydroxyl groups [40] were ruled out, because of their complexity and incapability to be automated.

OFT and PFPy are generally applicable but problems with some compounds appeared. The employed CsF may clog the syringe and so disturb derivatisation by an autosampler. CsF has to be weighed manually and therefore prolongs the sample preparation time in comparison to HFBA and TFAA.

FPA is not appropriate as derivatisation reagent because it formed much byproducts and the derivatives showed poor peak shape.

HFBI could be employed but the formed imidazole may clog the autosampler syringe. Moreover it is not as stable as HFBA or TFAA and shows no other advantages compared to the anhydrides.

The anhydrides HFBA and TFAA are excellent for (automated) derivatisation of the chosen analytes. The reaction (5 min at room temperature) ran smoothly without auxiliary reagents. The automation of the reaction provides precision in reagent volumes and most notably in reaction time. For most compounds the yield of derivatives is dependent on the reaction time, e.g., the mono derivative of compound 5 decreases with time (Fig. 3a). So a defined period of time between derivatisation and measurement is of great importance for reproducibility. By employment of an autosampler faults caused by manual sample preparation can be minimised, moreover the throughput of samples can be increased.

All analytes could be derivatised without forming much byproducts. The aliphatic hydroxyl group of compound 5 (17α-ethinylestradiol) did not react. So other sterically hindered hydroxyl groups should not

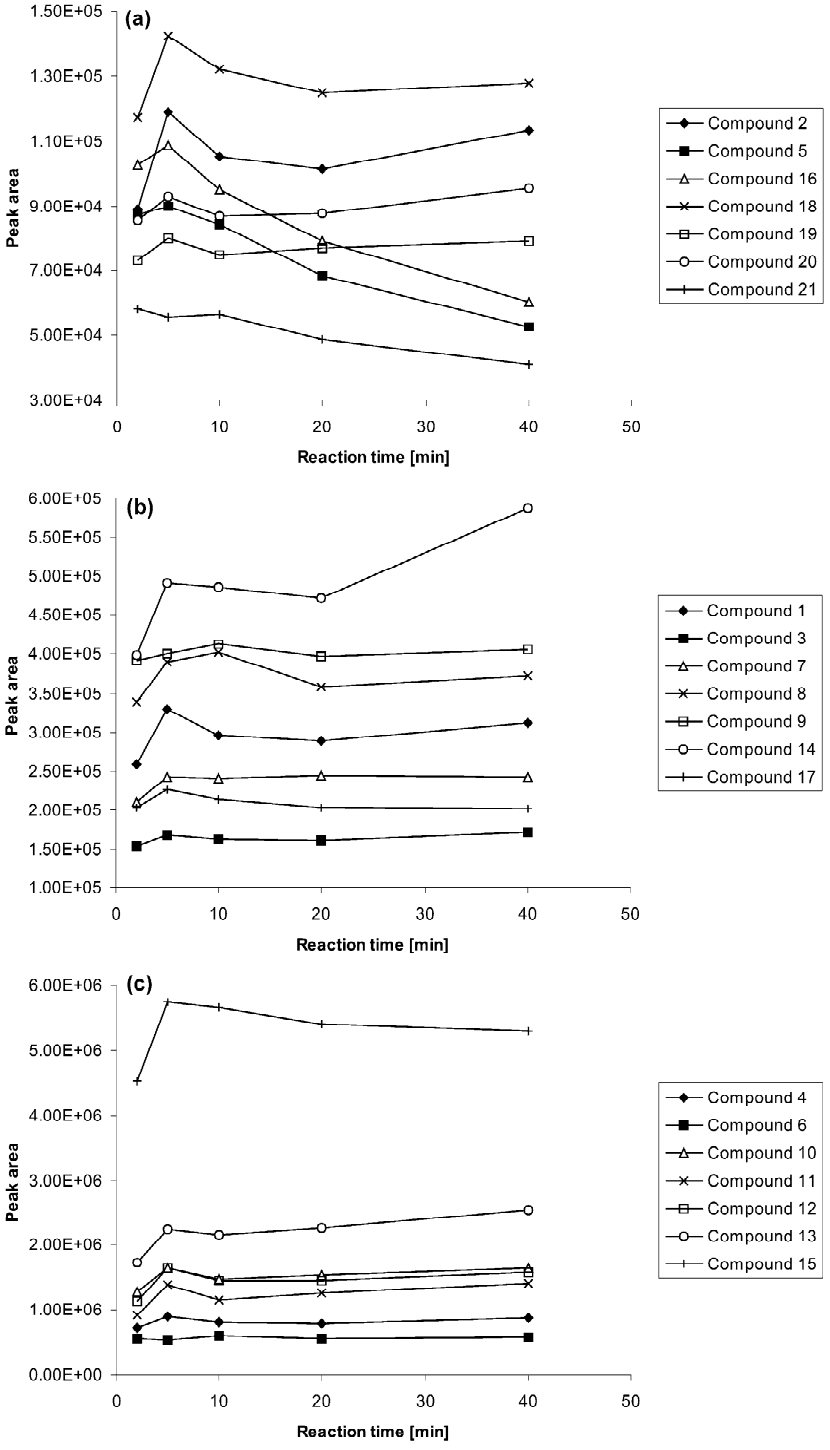


Fig. 3. Yields of derivatives for the derivatisation with TFAA in dependence on time (for parameters see Section 2.3.1.7). (a) Compounds 2, 5, 16, 18, 19, 20, 21; (b) compounds 1, 3, 7, 8, 9, 14, 17; (c) compounds 4, 6, 10, 11, 12, 13, 15.

react as well. This was verified with mestranol, another important synthetic estrogen differing from compound 5 by an ester group at C-3, which could not be derivatised at all. Generally it could be concluded that TFAA is more stable than HFBA. It was possible to derivatise the analytes completely with TFAA which was stored at room temperature and sunlight several days. The septum of the vial used for storage had already been penetrated several times by the autosampler syringe. Doing the same with a vial filled with HFBA resulted in incomplete derivatisation. Therefore derivatisation with TFAA is more rugged than with HFBA. HFB and TFA derivatives were chosen for further experiments concerning CI-MS detection.

3.2. Mass spectrometric detection

Generally HFB and TFA derivatives showed much fragmentation in the EI-MS detection mode. This is disadvantageous for a low limit of detection with MS–MS. A detection method had to be found which produces compound specific ions in sufficient yields without much fragmentation. Therefore NCI-MS and PCI-MS were employed.

Generally the type of reagent gas and its fore pressure were the most important parameters for yield of ions in CI detection. The electron energy was ideal at a value of 100 eV (maximum for the GCQ mass spectrometer) due to achieve much primary ionisation of the reagent gas. The emission current was set to 350 μA, a far higher value lets the filament burn out more rapidly.

Results can be seen in Fig. 4a–d and Table 9. They are pointed out and discussed in the following subchapters.

3.2.1. NCI detection

3.2.1.1. NCI detection of HFB derivatives

Best ionisation yields measured by peak areas of the compounds were achieved with methane (13.3 Pa), ammonia (10.7 Pa), acetone (8 Pa) and diethylether (8 Pa). They were up to two-times greater than the ionisation yields in the EI mode.

Spectra of all derivatives looked similar and were therefore very unspecific. The ion m/z 197 was continuously the only peak in the mass spectrum or at least the base peak (exceptions: compounds 6, 16, 18, 21) (see, also Ref. [43]). It is the heptafluorobutyryl fragment of the derivatives. Spectra were not influenced much by the type of reagent gas or its fore pressure. With all tested gases similar unspecific spectra occured. Lowering the ion source temperature to 180 °C or the electron energy to 70 eV slightly influenced fragmentation so that other peaks than m/z 197 appeared. Still m/z 197 was base peak for all compounds (exceptions: compounds 6, 16, 18, 21). Least energy was transferred by acetone and diethylether and therefore least fragmentation of larger ions occured (rather ''soft'' NCI gases). Ammonia transferred most energy onto the derivatives and therefore induced most fragmentation of larger ions (rather ''hard'' NCI gas). Nevertheless these differences are marginal so that spectra looked very similar as mentioned before.

3.2.1.2. NCI detection of TFA derivatives

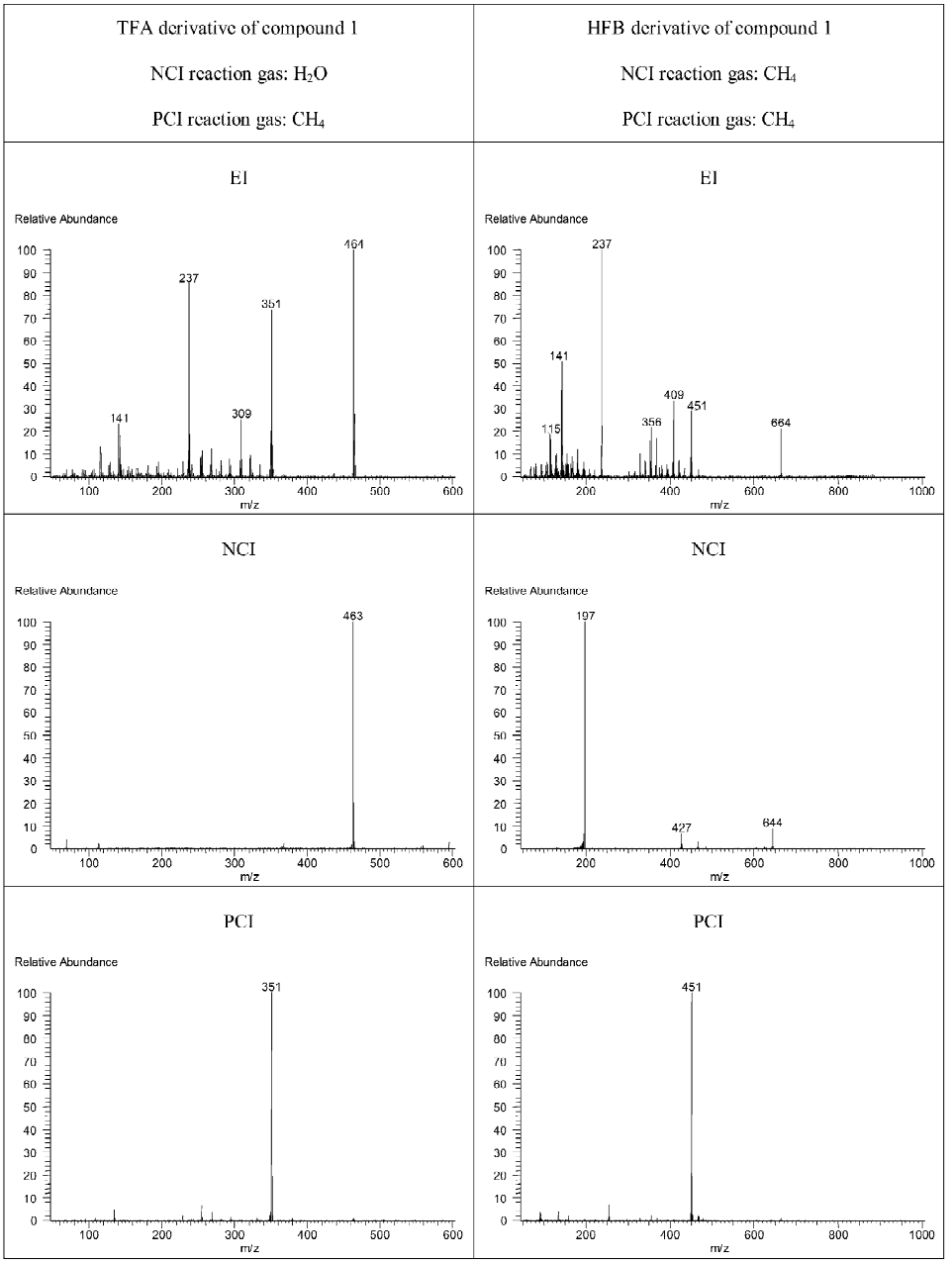
Most derivatives could not be detected with methane and acetone as reagent gases. Ammonia and water proved to be applicable although they only yielded down to 1/10 of the ionisation of the EI mode. Compound 20 (dehydroepiandrosterone) and compound 19 (pregnenolone) were detected weakly with ammonia. The same occured with compound 20 and water as reagent gas.

Spectra contained derivative specific ions where $[M–H]^-$ was oftentimes the base peak. Few fragmentation occured resulting in 1–3 major ions. Derivatives of compounds 4 (estriol) and 21 (11-desoxycorticosterone) fragmented to m/z 69 (CF_3^-) and m/z 113 (CF_3COO^-) as major ions which are unspecific. Again ammonia induced more fragmentation of larger ions and is therefore a ''harder'' NCI reagent gas than water.

3.2.2. PCI detection

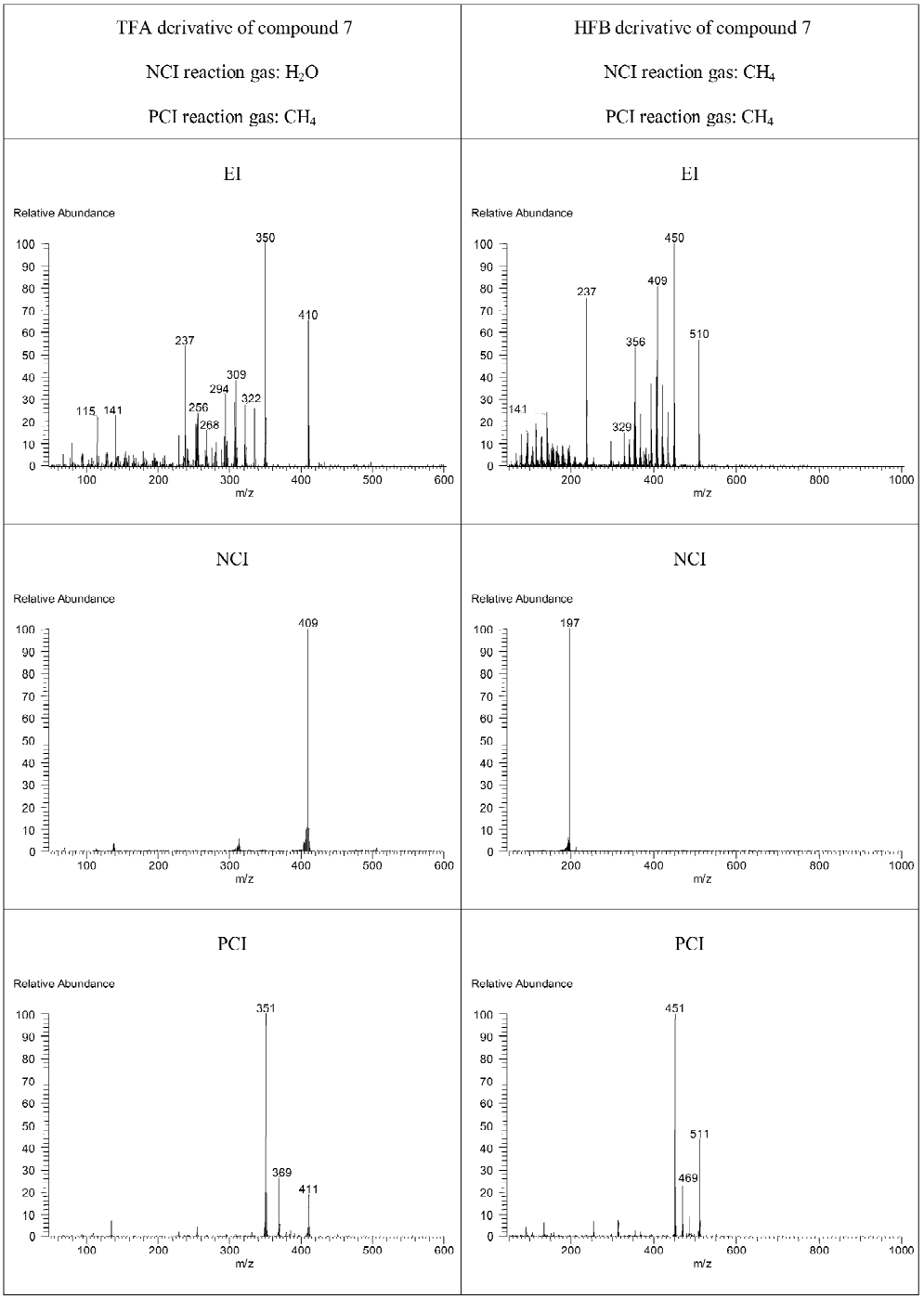
3.2.2.1. PCI detection of TFA derivatives

Methane at 13.3 Pa fore pressure yielded most ionisation which was comparable to the EI mode. Water at 13.3 Pa was a little more ineffective concerning ionisation. Lowering the electron energy to 70 eV resulted in a decrease of ionisation. Increasing the emission current to 500 μA did not have



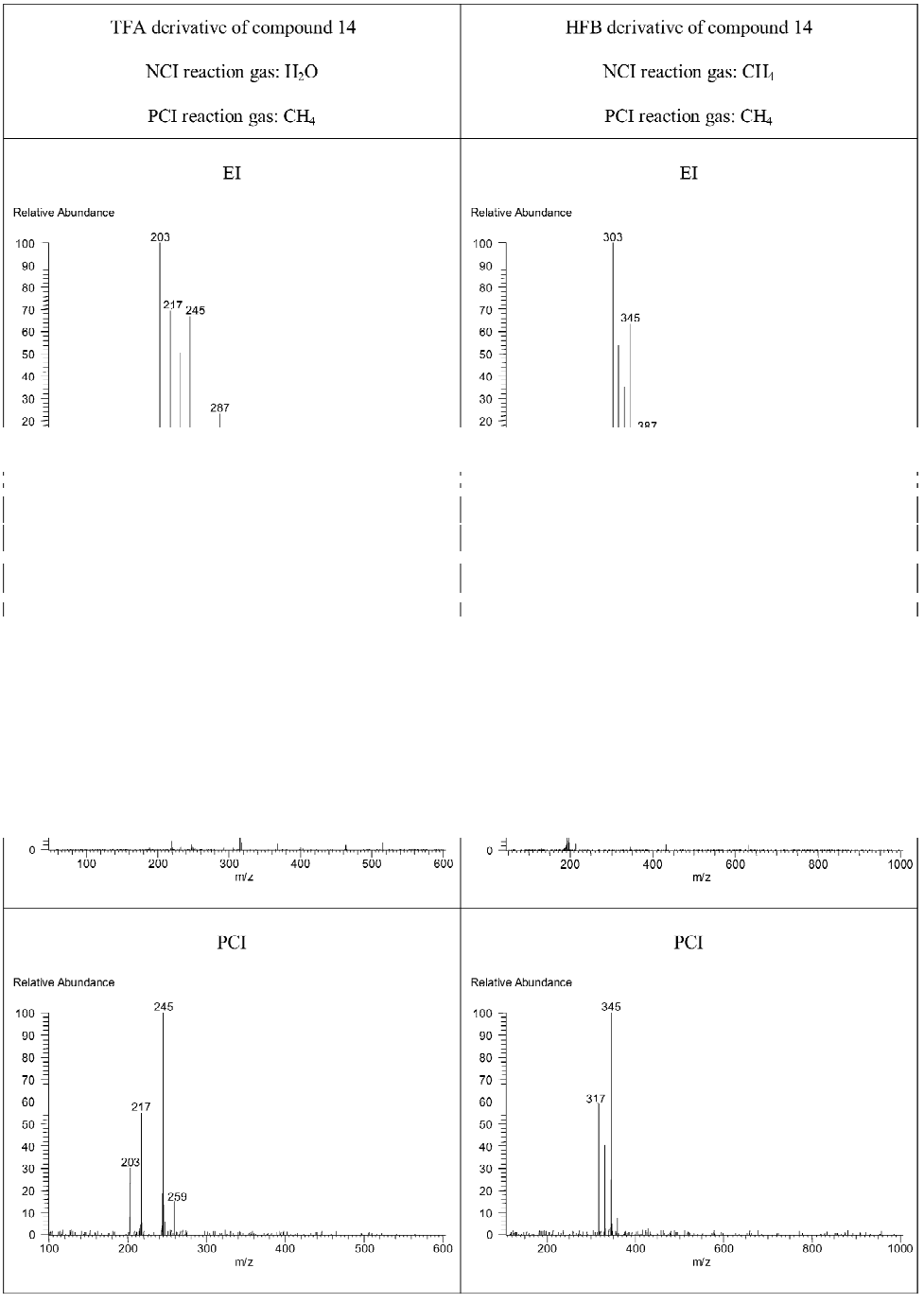
(a)

Fig. 4. MS spectra of derivatives of analytes (a) 1, (b) 7, (c) 14 and (d) 16 exemplifying characteristics of different detection modes. Parameters for the different detection modes: EI TFAA: full scan 50–600 u, source 240 °C, transfer line 350 °C, electron energy 70 eV, emission current 250 μA, trap offset 10 V. EI HFBA: full scan 50–1000 u, source 240 °C, transfer line 350 °C, electron energy 70 eV, emission current 250 μA, trap offset 10 V. NCI TFAA: water 12 Pa, full scan 50–600 u, source 200 °C, transfer line 350 °C, electron energy 100 eV, emission current 350 μA, trap offset 3 V. NCI HFBA: methane 13.3 Pa, full scan 50–1000 u, source 240 °C, transfer line 350 °C, electron energy 100 eV, emission current 350 μA, trap offset 3 V. PCI TFAA: methane 13.3 Pa, full scan 50–600 u, source 200 °C, transfer line 350 °C, electron energy 100 eV, emission current 350 μA, trap offset 8 V. PCI HFBA: methane 13.3 Pa, full scan 50–1000 u, source 200 °C, transfer line 350 °C, electron energy 100 eV, emission current 500 μA, trap offset 8 V.



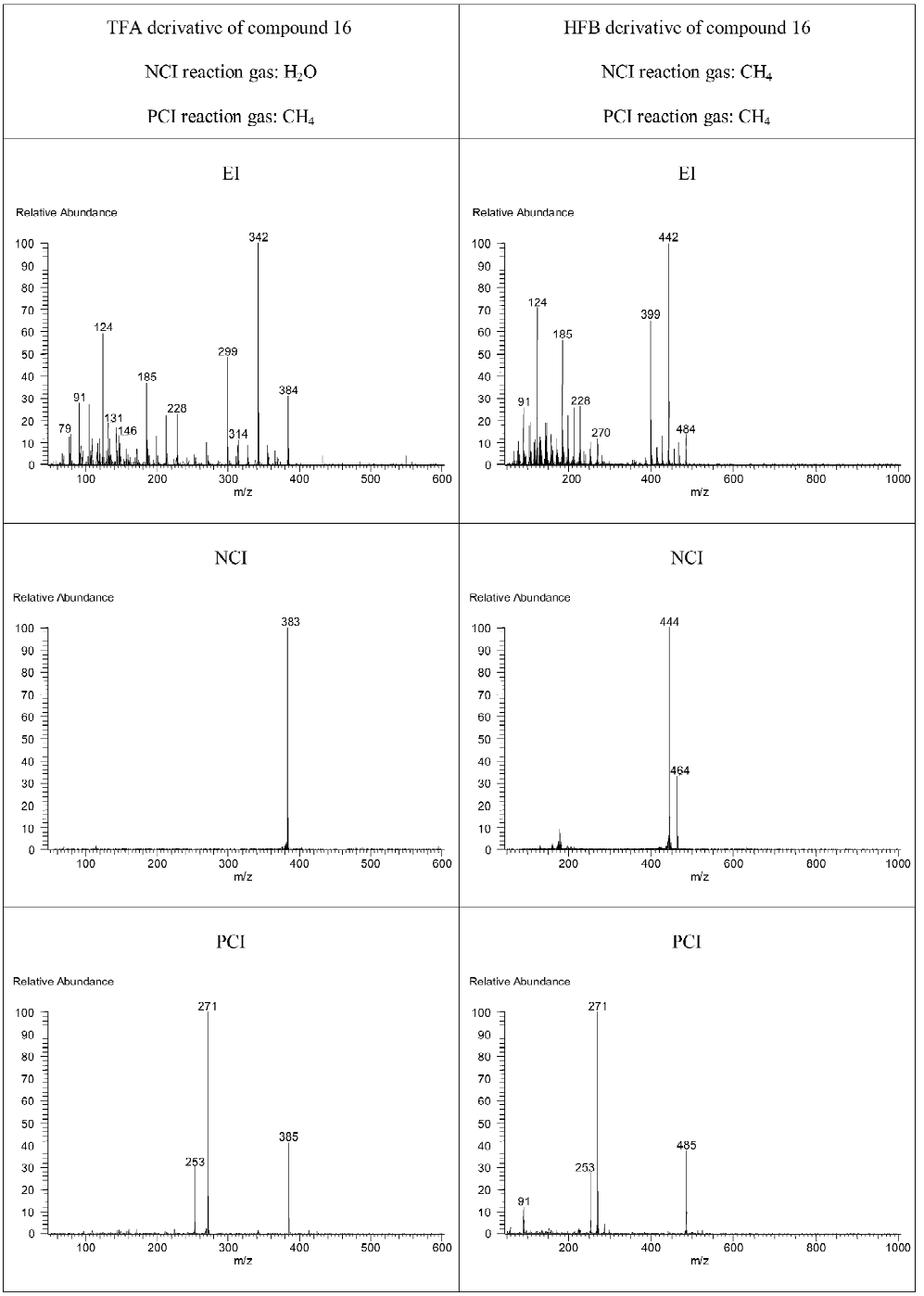
(b)

Fig. 4. (*continued*)



(c)

Fig. 4. (*continued*)



(d)

Fig. 4. (*continued*)

Table 9
Main ions of TFA and HFB derivatives with different ionisation methods

Compound	TFA		HFB	
	PCI (methane)	NCI (water)	PCI (methane)	NCI (methane)
1	351	463 $[M-H]^-$	451	197 $[C_3F_7CO]^-$
2	351	463 $[M-H]^-$	451	197 $[C_3F_7CO]^-$
3	349 $[M+H-H_2O]^+$, 367 $[M+H]^+$	365 $[M-H]^-$	467 $[M+H]^+$, 449 $[M+H-H_2O]^+$	197 $[C_3F_7CO]^-$
4	349	69 $[CF_3]^-$, 113 $[CF_3COO]^-$	449	197 $[C_3F_7CO]^-$, 213 $[C_3F_7COO]^-$, 639, 679 $[M-C_3F_7CO]^-$
5	375 $[M+H-H_2O]^+$, 393 $[M+H]^+$	391 $[M-H]^-$	475 $[M+H-H_2O]^+$, 493 $[M+H]^+$	197 $[C_3F_7CO]^-$
6	359 $[M-CF_3COO]^+$	471 $[M-H]^-$	359 $[M-C_3F_7COO]^+$	552, 178
7	351, 369, 411 $[M+H]^+$	409 $[M-H]^-$	451, 511 $[M+H]^+$, 469	197 $[C_3F_7CO]^-$
8	351, 425 $[M+H]^+$	423 $[M-H]^-$	451, 525 $[M+H]^+$	197 $[C_3F_7CO]^-$
9	351, 453 $[M+H]^+$	451 $[M-H]^-$	451, 553 $[M+H]^+$	197 $[C_3F_7CO]^-$
10	231, 461 $[M+H]^+$	459 $[M-H]^-$, 363 $[M-CF_3CO]^-$, 69 $[CF_3]^-$	331, 661 $[M+H]^+$, 464 $[M+H-C_3F_7CO]^+$, 465	266, 197 $[C_3F_7CO]^-$
11	231 $[M/2]^+$, 273, 217	461 $[M-H]^-$, 365 $[M-CF_3CO]^-$	373, 331 $[M/2]^+$, 317	197 $[C_3F_7CO]^-$, 465 $[M-C_3F_7CO]^-$
12	269, 459 $[M+H]^+$	457 $[M-H]^-$, 361 $[M-CF_3CO]^-$	659 $[M+H]^+$, 369, 462 $[M+H-C_3F_7CO]^+$, 463	197 $[C_3F_7CO]^-$, 264 $[M-2x(C_3F_7CO)]^-$
13	231 $[M-C_5H_{11}]^+$	301 $[M-H]^-$,	331 $[M-C_5H_{11}]^+$	197 $[C_3F_7CO]^-$
14	245, 217, 203 $[M-C_8H_{17}]^+$	315 $[M-H]^-$	345, 317, 331 $[M-C_6H_{13}]^+$	197 $[C_3F_7CO]^-$
15	231 $[M-(Ph-HFB)]^+$	419 $[M-H]^-$, 323 $[M-CF_3CO]^-$	331 $[M-(Ph-HFB)]^+$	197 $[C_3F_7CO]^-$, 226 $[M-2x(C_3F_7CO)]^-$
16	271 $[M-CF_3COO]^+$, 385 $[M+H]^+$, 253 $[M-CF_3COO-H_2O]^+$	383 $[M-H]^-$	271 $[M-C_3F_7COO]^+$, 485 $[M+H]^+$, 253 $[M-C_3F_7COO-H_2O]^+$	444, 464 $[M-HF]^-$
17	269, 383 $[M+H]^+$	381 $[M-H]^-$	270 $[M+H-C_3F_7COO]^+$, 483 $[M+H]^+$	462 $[M-HF]^-$
18	273 $[M-CF_3COO]^+$, 255 $[M-CF_3COO-H_2O]^+$, 387 $[M+H]^+$	386 $[M]^-$	273 $[M-C_3F_7COO]^+$, 255 $[M-C_3F_7COO-H_2O]^+$, 487 $[M+H]^+$	446, 466 $[M-HF]^-$
19	281, 299, 395 $[M+H-H_2O]^+$	411 $[M-H]^-$	299 $[M-C_3F_7COO]^+$, 281 $[M-C_3F_7COO-H_2O]^+$	492 $[M-HF]^-$
20	271, 253, 367 $[M+H-H_2O]^+$	383 $[M-H]^-$	271 $[M-C_3F_7COO]^+$, 467 $[M+H-H_2O]^+$	464 $[M-HF]^-$
21	427 $[M+H]^+$	69 $[CF_3]^-$, 113 $[CF_3COO]^-$	527 $[M+H]^+$	213 $[C_3F_7COO]^-$

much effect on ionisation as well as increasing the temperature to 240 °C.

Only 1–3 major specific fragments, mostly smaller than the molecular ion, resulted for all derivatives. Spectra with water as reagent gas were quite similar to the methane spectra. However with water as reagent gas ionisation was not as stable as with methane. Moreover the mass spectrometer sometimes produced error messages. Methane was the ideal reagent gas because the mass spectrometer worked more rugged with it than with water.

3.2.2.2. PCI detection of HFB derivatives

With methane at 13.3 Pa fore pressure the ionisation was similar to the measurement in the EI mode. Moreover it was comparable to the ionisation of the TFA derivatives in the PCI mode with methane at 13.3 Pa.

Again only 1–3 major specific fragments, mostly smaller than the molecular ion, resulted for all derivatives.

3.2.3. Conclusions

As it can be seen in Fig. 4a–d EI detection of TFA and HFB derivatives resulted in much fragmentation of the molecules. The molecular ion is abundant for compounds 1, 7 and 16 and is not abundant for compound 14. There are at least 3–5 major fragments which have a relative abundance greater than 50%. Moreover there are many fragments with relative abundances lesser than 50%. This is ideal for structural analysis, but is not appropriate for trace analysis. When isolating an ion for SIM or MS–MS detection modes all other fragments are discarded which results in a huge loss of detectable ions. It can be guessed that at least more than 70% of all ions generated are lost for detection.

NCI detection of HFB derivatives was efficient but very unspecific. This is visible in Fig. 4a–d where m/z 197 $[C_3F_7CO]^-$ is always the base peak (except compound 16, 17β-testosterone) in the NCI spectra of the HFBA derivatives. In real life samples interferences by matrix compounds which are derivatised as well and could have the same spectra will disturb the analysis.

NCI detection of TFA derivatives was specific but inefficient compared to the EI mode. The spectra look very suitable for trace analysis because only one specific fragment with a high mass larger than 300 is abundant. Nevertheless the good quality of spectra cannot compensate the poor yield of ions.

PCI detection of TFA and HFB derivatives were both efficient and specific. Ionisation yields were comparable to the EI mode. The spectra showed not much fragmentation only 1–2 fragments have a relative abundance of more than 50%. Compound specific ions mainly with m/z larger than 300 were formed. Therefore the PCI detection of TFA and HFB derivatives is the best compromise between suitable spectra quality and enough ionisation yields for trace analysis.

Separation of the derivatives could be achieved on a non polar column (Fig. 5). Some derivatives are not baseline resolved. This is no problem for a quantitative detection because the mass spectrometer software supports so-called ‘‘scan events’’. With these one can rapidly switch the detection mode (e.g., precursor ions for MS–MS, detection mode SIM/detection mode MS–MS) after every scan, which enables to detect even coeluting peaks separately.

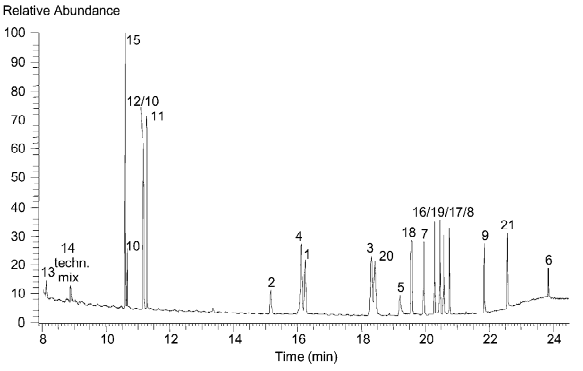


Fig. 5. Chromatogram of derivatives of all analytes as TFA derivatives (injection: 1 μl, 25 ng each) recorded in the PCI mode with methane Injector: helium 100–200 kPa during run, 40 °C, 5 °C/s, 300 °C, 1.5 min splitless. GC: DB-XLB (Agilent/J&W Scientific, Palo Alto, CA, USA), 30 m×0.25 mm I.D., 0.25 μm film, 40 °C, 1 min, 20 °C/min, 235 °C, 8 min, 30 °C/min, 360 °C, 2 min. MS: PCI, methane 13.3 Pa, full scan 50–600 u, source 200 °C, transfer line 350 °C, electron energy 100 eV, emission current 350 μA, trap offset 8 V.

4. General conclusions

A fast automated derivatisation method for 21 EDCs with TFAA and HFBA was developed. In contrast to derivatisation methods found in literature it only lasts 5 min and needs no evaporation or further manual work. Therefore a high throughput is possible.

Efficient and specific detection of both types of derivatives was possible in the PCI-MS mode with methane as reagent gas. Although ionisation yields and spectra of HFB and TFA derivatives were comparable, TFAA would be preferred. It is more stable than HFBA and therefore makes the automated derivatisation more rugged

In further experiments PCI-MS–MS or PCI-MS^n detection methods for the TFA derivatives shall be developed. Along with an effective sample concentration by SPE and large-volume injection of 100

μl extract into the GC system a powerful analysis of EDCs should be established in the future.

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