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High-performance liquid chromatography coupled to direct analysis in real time mass spectrometry: Investigations on gradient elution and influence of complex matrices on signal intensities

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ABSTRACT

Direct analysis in real time (DART) time-of-flight mass spectrometry (TOF-MS) has been tested for its suitability as a detector for gradient elution HPLC. Thereby a strong dependency of signal intensity on the amount of organic solvent present in the eluent could be observed. Adding a make-up liquid (iso-propanol) post-column to the HPLC effluent greatly enhanced detection limits for early eluting compounds. Limits of detection achieved employing this approach were in the range of 7–27 $\mu g\,L^{-1}$ for the parabene test mixture and 15–87 $\mu g\,L^{-1}$ for the pharmaceuticals. In further investigations DART ionization was compared to several other widely used atmospheric pressure ionization methods with respect to signal suppression phenomena occurring in when samples with problematic matrices are analyzed. For this purpose extracts from environmental and waste water samples were selected as model matrices which were subsequently spiked with a set of six substances commonly present in personal care products as well as six pharmaceuticals at concentration levels between 100 $\mu g\,L^{-1}$ and 500 $\mu g\,L^{-1}$ corresponding to 100 $ng\,L^{-1}$ and 500 $ng\,L^{-1}$ respectively in the original sample. With ionization suppression of less than 11% for most analytes investigated, DART ionization showed similar to even somewhat superior behavior compared to atmospheric pressure chemical ionization (APCI) and atmospheric pressure photo ionization (APPI) for the Danube river water extract; for the more challenging matrix of the sewage plant effluent extract DART provided better results with ion suppression being less than 11% for 9 out of 12 analytes while values for APCI were lying between 20% and >90%. Electrospray ionization (ESI) was much more affected by suppression effects than DART with values between 26% and 80% for Danube river water; in combination with the sewage plant effluent matrix suppression >50% was observed for all analytes.

1. Introduction

Mass spectrometry (MS) has evolved into one of the most successful detection techniques in combination with liquid phase separations during the last decades [1,2]. It provides increased selectivity (in many cases also increased sensitivity) and a substantial gain in information due to the possibility to record MS or even MS^n spectra from the eluted compounds. Focusing on the ionization techniques employed for the coupling of liquid-phase separation methods to MS, electrospray ionization (ESI) still plays the predominant role, followed by atmospheric pressure chemical ionization (APCI) and the more recently developed atmospheric pressure photo ionization (APPI). All these ionization techniques have their strong points but also certain deficits. Whereas ESI covers a wide range of molecular weights with respect to the solutes (from small molecules to large bio-molecules) its best suited for relatively polar compounds. APCI and APPI show limitations regarding molecular weight (they are not suited for large molecules) but show an improved ability (compared to ESI) to provide good ion yields also for less polar compounds [2].

Despite the fact that MS detection in principle also allows the evaluation of peaks that are either not (fully) resolved chromatographically or co-eluting with matrix components, biasing of results due to effects related to ion suppression has to be taken into account [3]. Particularly ESI [4] but to a minor degree also APCI [5,6] and APPI [5,7,8] are affected by changes in signal intensities (mostly reduction) due to ion suppression effects. These effects

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play an important role especially when real samples with complex matrices such as environmental samples [9–13] or biological samples [14,15] have to be analyzed. For this reason a series of studies investigating such effects, including also comparisons between different ionization techniques with respect to suppression issues have been published so far [9,16–21]. The most common approach to minimize matrix effects with ESI or APCI is dilution of the samples, whereby the limiting factor for this strategy is MS sensitivity. An alternative to these ionization methods helping to overcome matrix effects, namely the combination of liquid chromatography (at very flow rates in the $\mu L\,min^{-1}$ range at most) with direct electron ionization has been proposed by the group of Cappiello [22,23].

Direct analysis in real time (DART)-MS, introduced 2005 by Cody et al. is a relatively new ionization technique [24,25]. Its primary field of application lies in the direct analysis of solids or liquids with applications ranging from the analysis of polymers [26], cosmetics [27] to fungicides on wheat [28], counterfeit drugs [29,30], detection of warfare agents [31], analysis of environmental samples after stir bar sorptive extraction [32], or counterterrorism applications such as explosives on clothes or shoes [33]. Recently our group reported the possibility to use DART-TOF-MS as a detector for HPLC [34]. Here DART ionization allowed the use of normally MS incompatible buffer systems like phosphate due to the fact that there is no contact between the eluate and parts of the ion source or the MS respectively and its relatively low tendency towards ion suppression, a fact that was already indicated in connection with applications in the fields of drug discovery [35].

In the present work the performance of DART-MS as a detector in gradient elution chromatography is investigated. The major focus within this manuscript is set on the issue of changes in signal intensities due to matrix effects whereby a comparison of DART with other common MS-ionization techniques was performed. For this purpose extracts from environmental and waste water samples have been selected as model matrices, because these types of samples are often associated with substantial ion suppression effects particularly when ESI is employed [9].

2. Experimental

2.1. Instrumentation

All measurements were performed with a DART ion source from IonSense Inc. (Saugus, MA, USA) coupled to a JMC-100-TLC (AccuTOF) time-of-flight mass spectrometer (JEOL, Peabody, MA, USA). The DART ion source was operated with helium gas for analysis and nitrogen gas in the standby mode. Helium (4.6) was from Linde Gas GmbH (Stadl-Paura, Austria). In the negative ion mode, mass calibration for the TOF-MS was performed employing a set of 5 substances, namely benzoic acid, 4-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, veratric acid and 1,3,5-benzotri-carboxylic acid with m/z (all $[M-H]^-$) of 121.0295, 137.0244, 153.0193, 181.0506 and 209.0092 respectively. In the positive ion mode PEG 600 was employed for mass calibration. The following MS-related parameters were employed: peaks voltage + 600 V (negative ion mode)/ +500 V (positive ion mode), needle voltage + 3300 V, discharge electrode voltage ± 100 V (positive/negative mode respectively). Grid electrode voltages were set to −75 V (negative ion mode) and +150 V (positive ion mode) respectively.

ESI-MS, APCI-MS, and APPI-MS measurements were performed on an Agilent MSD SL ion trap mass spectrometer (Agilent Technologies, Waldbronn, Germany) equipped with an ESI, APCI or APPI source respectively (Agilent Technologies). Optimization of parameters for ESI, APCI and APPI and the ion trap MS was performed by infusion of a sum-standard containing the test analytes

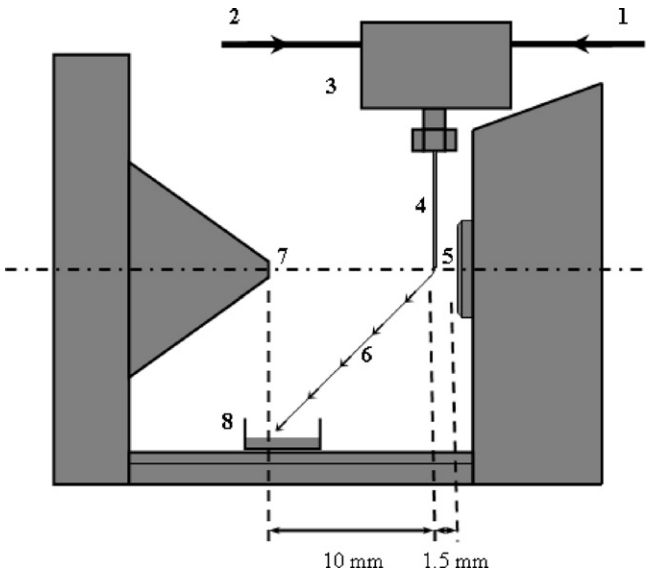

Fig. 1. Schematic drawing of the setup used for the coupling of gradient elution HPLC to DART-TOF-MS. (1) PEEK capillary from HPLC, (2) PEEK capillary from make-up liquid pump, (3) T-piece, (4) fused silica capillary (100 μm ID), (5) insulator cap with grid electrode, He outlet, (6) liquid jet, (7) MS inlet, and (8) groove for fraction collection.

for positive (pharmaceuticals) or negative ionization (personal care products) respectively. The following optimized parameters were employed (parameters listed in the order positive ion mode/negative ion mode): ESI, nebulizer gas pressure 50 psi, drying gas flow rate $10\,L\,min^{-1}$, drying gas temperature 350 °C, capillary voltage −3500 V/+3500 V; APCI, vaporizer temperature 400 °C, nebulizer gas pressure 60 psi, drying gas flow rate $7\,L\,min^{-1}$, drying gas temperature 350 °C, capillary voltage −1500 V/+1000 V; corona needle current 4500 nA/35000 nA; APPI, vaporizer temperature 400 °C, nebulizer gas pressure 60 psi, drying gas flow rate $7\,L\,min^{-1}$, drying gas temperature 350 °C; dopand-assisted APPI, capillary voltage −1000V/−; 5% acetone added post-column via T-piece. The trap scan range was set from 100 *m/z* to 250 *m/z*.

For HPLC an Agilent 1100 modular HPLC system with a 30 mm × 4.6 mm I.D. Agilent Eclipse XDB-C18 column packed with 1.8 μm particles (Agilent Technologies) was used. Injection volume was 75 μL throughout this work. The coupling of HPLC to DART-TOF-MS was accomplished by using a setup depicted in Fig. 1. As an end-piece, a fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 100 μm was employed. Further modifications were the use of a stainless steel T-piece (instead of a zero dead-volume junction) allowing the introduction of a make-up flow. The make-up flow was supplied by an HP 1050 HPLC pump (Agilent Technologies). Elution was performed using a ternary gradient with 0.05 M NaH_2PO_4 in H_2O/MeOH (1:1) as eluent A, H_2O as eluent B and MeOH as eluent C. The following gradient profiles were applied: for the personal care products test mixture: 0 min 20A/30B/50C to 20A/20B/60C in 1 min and finally to 20A/0B/80C in 0.3 min at a flow rate of $1\,mL\,min^{-1}$. For the pharmaceuticals test mixture: 0 min 20A/75B/5C to 20A/10B/70C in 2 min and finally to 20A/0B/80C in 1 min at a flow rate of $1\,mL\,min^{-1}$ or at a flow rate of $0.5\,mL\,min^{-1}$ in combination with $0.5\,mL\,min^{-1}$ iso-propanol as a make-up flow.

2.2. Materials and reagents

The following chemicals were employed in this study: set of personal care products: methyl-, ethyl-, propyl- and butyl-parabene, 2,4 dihydroxybenzophenone (all from Sigma–Aldrich, St.

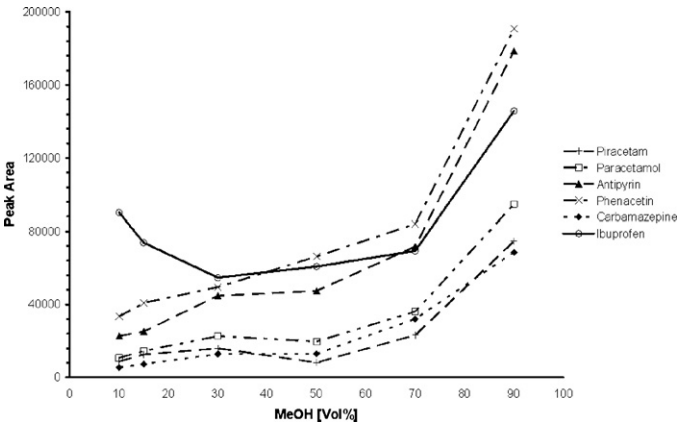

Fig. 2. Influence of the methanol content on peak areas obtained for six pharmaceuticals. Data points are mean values of 3 determinations.

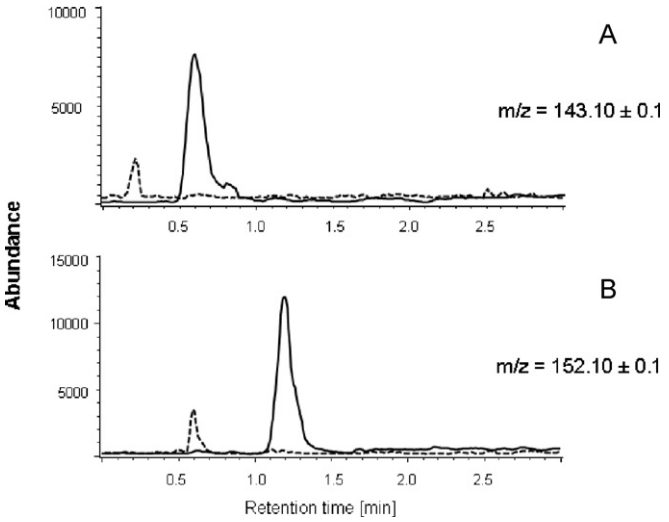

Fig. 3. Chromatograms for piracetam (A) and paracetamol (B) at a concentration level of 500 μg L^{-1} with (continuous line) and without (dashed line) make up flow (iso-propanol). Chromatographic and MS conditions see Section 2.

Louis, MO, USA) and 2-hydroxy-4-methoxybenzophenone (Merck, Darmstadt, Germany). Set of pharmaceuticals: piracetam, paracetamol, antipyrin, carbamazepine, ibuprofen (Sigma–Aldrich,) and phenacetin (Merck). Sodium dihydrogen phosphate hydrate, ammonium acetate, acetic acid, benzoic acid, 4-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, veratric acid, 1,3,5-benzo-tricarboxylic acid, acetonitrile, iso-propanol and methanol were from Merck. All chemicals had a purity of >98% and were used with 18 MΩ Milli-Q purified water (Millipore, Bedford, MA, USA).

2.3. Environmental and waste water samples and extracts

Water samples were collected from the Danube river in Linz or from a local sewage plant effluent. 1 L of the water sample was acidified using sulfuric acid, filtered through a 0.45 μm filter and passed through an Oasis HLB 6 cc cartridge (Waters, Milford, USA). Subsequently the cartridge was eluted with 10 mL of methanol and the eluate evaporated to dryness using N_2 and reconstituted with 1 mL of 10% MeOH. The resulting extract solution was used without any further treatment.

3. Results and discussion

3.1. Choice of ionization mode in DART-TOF-MS

Two different sets of test substances were employed for this purpose; a set of six common ingredients from personal care products and a set of six pharmaceuticals. For the first set the negative ion mode provided substantially better sensitivity than the detection of positive ions; a fact that is in accordance with our previous observations [34]. Focusing on differences in ionization between DART and the other ionization techniques included in this study, only 2-hydroxy-4-methoxybenzophenone showed substantially different behavior. In the negative ion mode, no evaluable signal for 2-hydroxy-4-methoxybenzophenone was obtained with ESI, APCI and APPI. This is in accordance with reports in the literature, where contrary to other benzophenones the positive ion mode is employed for this substance [9,36,37]. In the present work we selected the negative ion mode for this set of analytes as it provided better results for all six compounds with DART and for five out of six compounds using the other ionization techniques.

The pharmaceuticals antipyrin, paracetamol, piracetam, and carbamazepine could be detected only in the positive ion mode as protonated species. For phenacetin both the negative and the positive ion mode were suitable with better results achieved in the latter mode (again protonated species). The carboxylic acid ibuprofen is usually detected in the negative ion mode after deprotonation. In the case of DART ionization, the negative ion mode only provided moderate sensitivity. An almost 100% enhancement in signal intensity was achieved when a fragment (m/z = 161.1525) was detected in the positive ion mode. This fragment is also observed in electron impact ionization of ibuprofen and formed via the loss of HCOO with subsequent detection of a positive radical ion. So, for the detection of the pharmaceutical test mixture the positive ion mode was chosen.

Table 1
Limits of detection, linear ranges, equations for the calibration curves and regression coefficient for HPLC DART-TOF-MS of test mixtures.

	LOD[a] (μg L^{-1})	Linear range (μg L^{-1})	Equation	R^2
Methyl parabene	9	50–2000	$y = 792018x - 173$	1.000
Ethyl parabene	27	50–1000	$y = 792787x - 11231$	0.9999
Propyl parabene	13	50–1000	$y = 757135x - 3555$	0.9999
Butyl parabene	7	10–2000	$y = 747962x - 5187$	0.9998
2,4-Dihydroxybenzophenone	7	10–2000	$y = 768844x - 6138$	0.9998
2-Hydroxy-4-methoxybenzophenone	7	10–2000	$y = 689847x - 3004$	0.9997
Piracetam	310 (65)[b]	500–2000	$y = 123390x - 41165$	0.9811
Paracetamol	104 (87)[b]	500–2000	$y = 56466x - 5561$	0.9978
Antipyrin	22	50–1000	$y = 270046x - 35230$	0.9991
Phenacetin	15	50–2000	$y = 309837x - 5055$	0.9991
Carbamzepine	25	50–2000	$y = 134973x - 1617$	0.9986
Ibuprofen	25	50–2000	$y = 209252x - 543$	0.9999

[a] 3 × baseline noise, mean value from 4 measurements.
[b] LOD with make up liquid in parentheses.

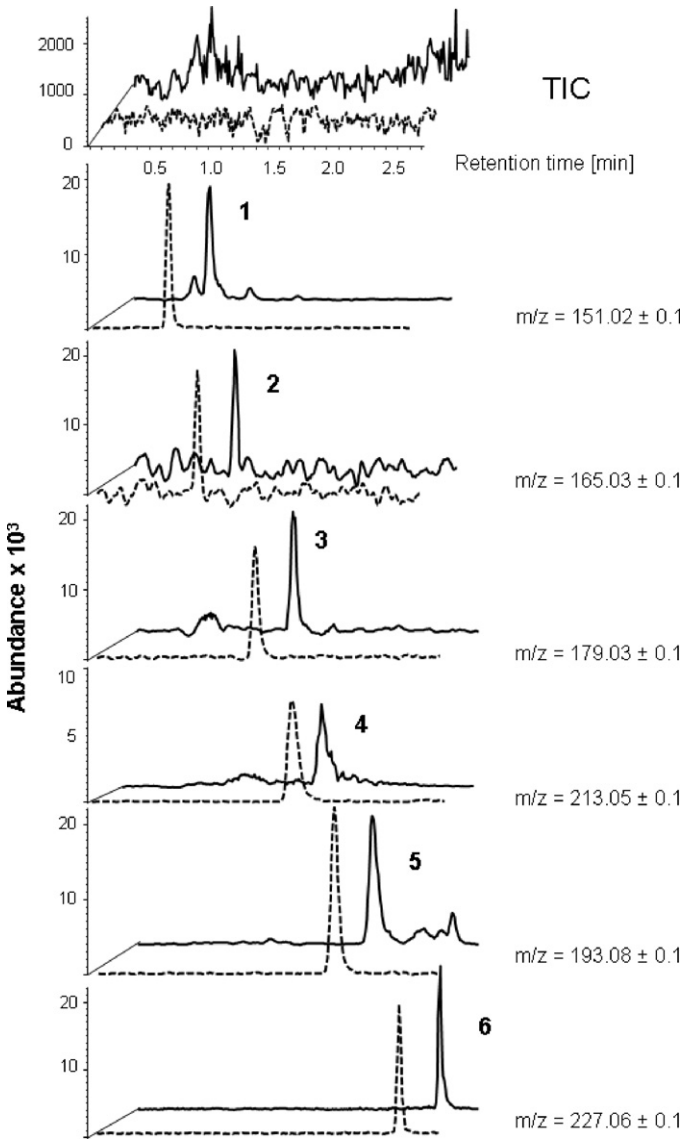

Fig. 4. TIC and extracted ion chromatograms for a test mixture of six compounds commonly found in personal care products. Sewage plant effluent extract spiked at a concentration level of 100 μg L^{-1} (continuous line) and standard mixture at a concentration level of 100 μg L^{-1} (dashed line). HPLC and DART-TOF-MS conditions: see text. Peak assignment: (1) methyl parabene, (2) ethyl parabene, (3) propyl parabene, (4) 2,4-dihydroxybenzophenone, (5) butyl parabene, and (6) 2-hydroxy-4-methoxybenzophenone.

3.2. Gradient elution HPLC with DART-TOF-MS detection

As the applicability of DART-TOF as a detector for isocratic elution HPLC has already been investigated in our previous paper [34], in a next step the compatibility of DART-TOF-MS detection with gradient elution HPLC was tested. As DART allows the use of phosphate-based eluents (without any loss in sensitivity compared to ESI-compatible buffers like ammonium-acetate [34]), for both test mixtures gradients based on such buffers with an increasing concentration of methanol or acetonitrile respectively were used. As both solvents led to very similar results, further work was performed using methanol. Thereby we observed that sensitivity was worse for the earlier eluting substances compared to those occurring later in the chromatogram. As this behavior was more pronounced for the pharmaceuticals, where a gradient starting with only 15% of organic solvent was used, a correlation between DART-TOF-MS sensitivity and the amount of organic solvent in the effluent seemed obvious (a dependency that can also be observed in ESI, APCI and APPI [38]). In order to substantiate this assumption, a standard mixture including all six analytes under investigation was introduced by flow injection using 20 mM phosphate buffer with 0–90% organic solvent. As can be seen from Fig. 2, a strong dependence between peak intensities and % methanol exists, with methanol contents above 65% leading to a substantial increase in peak heights. In order to exploit these findings for enhancing the detectability of early eluting substances during HPLC analysis, a make-up flow was added post-column to the HPLC effluent through a T-piece (see Fig. 1). Because in the coupling of HPLC to DART-TOF-MS the formation of a stable liquid jet (depending on the capillary diameter and the flow rate) is of substantial importance, the overall flow rate of 1 mL min^{-1} was kept constant [34]. This was achieved by using a reduced HPLC flow rate together with a make-up flow. Investigations using different make-up solvents revealed that the most substantial enhancement in peak intensities was obtained using iso-propanol (followed by methanol and finally acetonitrile which only showed little effect) at a flow rate of 0.5 mL min^{-1}, corresponding to a total of around 60% organic solvent (10% methanol + 50% iso-propanol) for the two early eluting compounds. In Fig. 3 chromatograms obtained for the first two peaks in the chromatogram with and without make-up flow are compared, revealing the substantial enhancement in peak intensity achieved through the addition of iso-propanol as the make-up liquid. Differences in time scale are due to the use of different HPLC flow rates. For the later eluting peaks the addition of a make-up liquid did not provide any advantages. A similar behavior was observed for the parabenes. Nevertheless, due to the fact that for the HPLC separation of parabenes a gradient starting with 50% of methanol was employed, the benefits from the addition of a make-up liquid were only minor and did not justify the additional effort.

Subsequently calibration curves were constructed for both sets of test analytes. For the pharmaceuticals limits of detection (LOD) were between 310 μg L^{-1}/65 μg L^{-1} (without/with make-up flow) for piracetam and 15 μg L^{-1} for phenacetin. For parabenes somewhat lower LOD's ranging from 27 μg L^{-1} (ethylparabene) to 7 μg L^{-1} (butylparabene) were achieved. Each concentration level employed for the construction of calibration curves was measured at least four times. The highest concentration level included in the calibration was either determined by the linear range (antipyrin, ethyl- and propylparabene) or by the maximum concentrations expected in the real samples described later in this paper. The corresponding equations for calibration graphs, linear ranges as well as LOD values are summarized in Table 1.

3.3. Effect of surface and waste water matrices on signal intensities in HPLC-DART-TOF-MS

One additional benefit of DART over other ionization techniques is its relatively low tendency towards ion suppression [34,35]. This advantage can be utilized in different ways, either by using eluents that are commonly not compatible with other ionization techniques (e.g., phosphate buffer) or by the direct analysis of samples with problematic matrices. As the first point was a major aim in our previous work [34], this time the main focus was set on the evaluation of effects from matrix components on the signal intensities observed in DART-TOF-MS. Therefore the applicability of HPLC DART-TOF-MS for the determination of the two sets of test substances in samples commonly showing interferences due to complex matrix composition such as environmental and waste water samples (here chosen as a model for matrices well known for severe suppression phenomena e.g., in ESI-MS) was investigated. Danube river water was selected as an example for a matrix with a minor to medium degree of difficulty with respect to interferences expected, whereas the effluent from a sewage treatment plant

Table 2
Comparison of ion suppression for different ionization techniques for the personal care products test mixture at a concentration level of 100 μg L^{-1}.

	Danube river water suppression %/(RSD for peak areas (n = 4))			Sewage plant effluent suppression %/(RSD for peak areas (n = 4))		
	DART	ESI	APCI	DART	ESI	APCI
Methylparabene	7/(4)	42/(12)	14/(4)	2/(20)	56/(11)	>90[a]
Ethylparabene	–[b]/(10)	26/(11)	5/(4)	4/(10)	52/(7)	35/(7)
Propylparabene	11/(6)	44/(5)	–[b]/(1)	11/(6)	49/(7)	20/(5)
Butylparabene	3/(4)	48/(5)	–[b]/(4)	1/(9)	60/(3)	56/(12)
2,4-Dihydroxybenzophenone	10/(13)	40/(4)	–[b]/(6)	42/(14)	59/(8)	26/(9)
2-Hydroxy-4-methoxybenzophenone	–[b]/(8)	62 (9)–[c]	>75[a,c]	–[b]/(6)	–[a,c]	>75[a,c]

[a] Signal below LOD.
[b] No suppression observed.
[c] ESI and APCI measured in the positive ion mode.

was regarded as highly demanding regarding problems related to ion suppression caused by matrix components. In both cases, extracts were prepared by 1000-fold pre-concentration employing solid phase extraction. The extracts obtained were subsequently spiked with the two test mixtures at a level of 100 μg L^{-1} for the personal care product test mixture, corresponding to 100 ng L^{-1} in the original sample and 250 μg L^{-1} (except for piracetam and paracetamol where the spiking level was 500 μg L^{-1}) corresponding to 250 ng L^{-1} and 500 ng L^{-1} respectively in the original sample, for the pharamceuticals. The spiking level was selected according to the concentration range commonly found in real samples. A rather steep gradient was used for elution, providing fast analysis times but also only insufficient separation of matrix and analytes; although this would not be regarded as a desirable strategy in the actual analysis of real environmental samples, the situation encountered can be seen as very challenging with respect to suppression issues. Thereby a thorough comparison of the different ionization methods employed with respect to suppression phenomena was possible. For this purpose a standard mixture at an identical concentration level was analyzed directly after the spiked extract. Fig. 4 depicts chromatograms obtained for the set of six ingredients from personal care products spiked into the extract from the sewage plant effluent (continuous line) as well as the ion traces from the corresponding standard (dashed line). As can be seen from the total ion current (TIC), this extract is affected with a substantial amount of matrix components leading to an average abundance of 1.5×10^6 over the full time range. Focusing on ion suppression effects, almost no difference can be seen between signal intensities when comparing the extracted ion traces from the sewage plant effluent extract and the standard. In Table 2 ESI and APCI are compared with DART with respect to ion suppression observed for the personal care products in the two selected matrices. For comparison in all cases the negative ion mode was used and also identical HPLC conditions were employed, except for the ammonium acetate based buffer replacing the phosphate buffer used in combination with DART. Unfortunately no other ion sources were available for the TOF-MS equipped with the DART. So all other ion sources (ESI, APCI, APPI) had to be operated in connection with an ion trap MS instrument. Comparing the basic principles of operation for the investigated ionization techniques, differences in LOD's for standards but more likely a different behavior with respect to matrix affected samples is inherent. For standard mixtures, ESI and APCI showed comparable (or even better) LOD's than DART. For ESI they were in the range of 2 μg L^{-1} for butylparabene and 5 μg L^{-1} for methylparabene; for APCI LOD's between 3 μg L^{-1} for butylparabene and 7 μg L^{-1} for methylparabene were found. APPI without a dopand did not provide any usable results (LODĭs >100 μg L^{-1}). Employing 5% of acetone as a dopand (added post-column), APPI ionization delivered somewhat improved LOD's in the range of 14 μg L^{-1} for butylparabene and 40 μg L^{-1} for propylparabene.

As can be seen from the data in Table 2, ESI ionization is affected by suppression effects already in the Danube river water matrix and even worse in the sewage plant effluent. This can be explained by the fact that the degree of ionization suppression is mainly determined by the droplet solution properties [4] and in this case particularly by the increased presence of non-volatiles in the sewage plant effluent matrix. APCI, in contrary to ESI a gas phase ionization method, shows only little suppression in the case of the Danube river water extract. Nevertheless in the case of samples that are more affected by matrix effect such as sewage plant effluent substantial suppression is observed under the conditions employed in this study. APPI was not included in this comparison as primarily due to the substantially higher LOD's (obtained for the parabens) but also some signal reduction caused by suppression effects no proper evaluation of signal intensities was possible for several compounds. DART, as a desorption method, presents a completely different picture. It might be assumed that ionized analyte

Table 3
Comparison of ion suppression for different ionization techniques for the pharmaceuticals test mixture in Danube river water at a concentration level of 250 μg L^{-1} (except 500 μg L^{-1} for paracetamol and piracetam).

	Danube river water suppression %/(RSD for peak areas (n = 4))				
	DART	DART(make up)	ESI	APCI	APPI(dopand)
Piracetam	n.d.[a]	10/(14)	35/(4)	37/(3)	17/(9)
Paracetamol	49/(15)	23/(5)	26/(4)	5/(6)	28/(1)
Antipyrin	1/(17)	–[b]/(13)	64/(6)	18/(7)	–[d]/(8)
Phenacetin	1/(14)	5/(10)	80/(4)	57/(6)	21/(2)
Carbamazepine	–[b]/(15)	n.d.[a]	63/(6)	6/(1)	–[d]/(4)
Ibuprofen	2/(10)	n.d.[a]	–[c]	–[c]	–[c]

[a] Not determined.
[b] No suppression observed.
[c] No sufficient response in ESI, APCI, and APPI.
[d] Signal enhancement.

Table 4
Comparison of ion suppression for different ionization techniques for the pharmaceuticals test mixture in the sewage plant effluent at a concentration level of 250 μg L^{-1} (except 500 μg L^{-1} for paracetamol and piracetam).

	Sewage plant effluent suppression %/(RSD for peak areas (n = 4))				
	DART	DART(make up)	ESI	APCI	APPI(dopand)
Piracetam	n.d.[a]	40/(9)	64/(7)	47/(9)	34/(3)
Paracetamol	n.d.[a]	22/(10)	65/(19)	20/(2)	43/(6)
Antipyrin	–[c]/(20)	–[c]/(5)	72/(6)	36/(1)	47/(8)
Phenacetin	–[c]/(9)	–[c]/(16)	>95[b]	92/(15)	>90[b]
Carbamazepine	–[c]/(19)	n.d.[a]	80/(14)	40/(9)	30/(7)
Ibuprofen	–[c]/(7)	n.d.[a]	–[d]	–[d]	–[d]

[a] Not determined.
[b] Signal below LOD.
[c] No suppression observed.
[d] No sufficient response in ESI, APCI, and APPI.

molecules are primarily desorbed from the surface of the liquid jet traversing the ionization region. For DART all investigated solutes from the personal care products test mixture are only affected by suppression to a minor degree (<11%). The only exception is 2,4-dihydroxybenzophenone which shows substantial suppression in the sewage plant effluent extract. To obtain suppression data for 2-hydroxy-4-methoxybenzophenone using ESI and APCI the positive ion mode had to be employed, due to reasons already stated in Section 3.1.

Focusing on the other test mixture consisting of six pharmaceuticals often found in the effluent of sewage plants, a representative chromatogram for a spiked sewage plant extract is shown in Fig. 5. As can be seen from this figure, no signal was obtained for paracetamol in the sewage plant effluent extract and no signal for piracetam in both extracts investigated, despite a spiking level of 500 μg L^{-1}. As already discussed in the previous section of this manuscript this behavior can be mainly attributed to the insufficient sensitivity of DART detection when eluents with a low organic solvent content are employed. Addition of a make up flow (0.5 mL min^{-1} of iso-propanol) greatly improved the situation and allowed the proper detection of both analytes. This procedure also reduces ion suppression to some degree due to dilution of the matrix.

Tables 3 and 4 provide a comparison of several ionization techniques (positive ion mode), namely ESI, APCI and APPI with dopand and DART ionization for the determination of the pharmaceuticals in Danube river (Table 3) and waste water extracts (Table 4) at a spiking level of 250 μg L^{-1} (except for piracetam and paracetamol where the spiking level was 500 μg L^{-1}). LOD's (for standards) for the other ion sources were comparable or even better than those achieved with DART. They were between 7 μg L^{-1} (antipyrin) and 52 μg L^{-1} (piracetam) for ESI, 3 μg L^{-1} (phenacetin) and 24 μg L^{-1} (piracetam) for APCI and 20 μg L^{-1} (antipyrin) and 62 μg L^{-1} (paracetamol) for APPI with 5% acetone as dopand. Ibuprofen can only be detected in the negative ion mode employing the ion sources mentioned before whereas with DART, as stated in the first paragraph of Section 3, best results are achieved when a fragment ion of this analyte is detected in the positive ion mode. Focusing on signal reduction due to matrix effects the following situation is encountered: Using the approach with a make-up liquid for the first two peaks and then switching off the make up flow, for DART only minor suppression effects are observed for most of the analytes within the Danube river water. For piracetam and paracetamol, despite the substantial improvement due to the addition of the make up liquid, suppression in the range of 20–40% still is encountered for the sewage plant effluent. Focusing on all six analytes a trend could be observed for DART ionization, namely that suppression effects decrease with an increasing percentage of

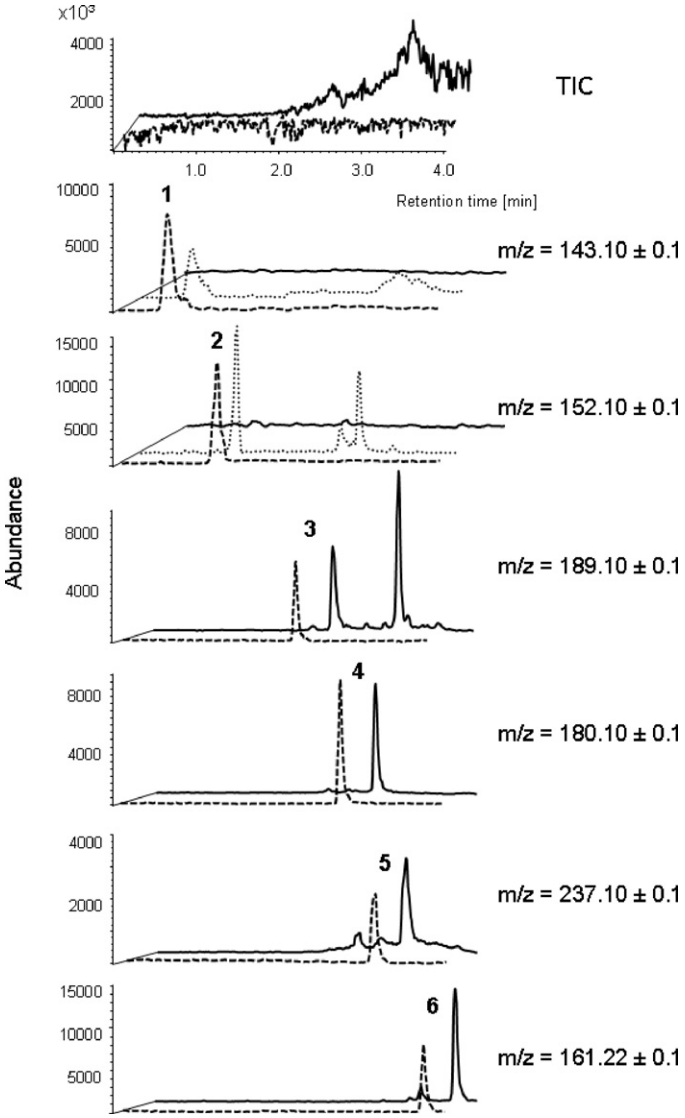

Fig. 5. TIC and extracted ion chromatograms for a test mixture of six pharamceuticals. Sewage plant effluent extract spiked at a concentration level of 250 μg L^{-1} (except 500 μg L^{-1} for paracetamol and piracetam) without addition of a make up flow (continuous line) and with make up flow (dotted line). Standard mixture at a concentration level of 250 μg L^{-1} (except 500 μg L^{-1} for paracetamol and piracetam), dashed line. HPLC and DART-TOF-MS conditions: see text. Peak assignment: (1) piracetam, (2) paracetamol, (3) antipyrin, (4) phenacetin, (5) carbamazepine, and (6) ibuprofen.

organic solvent in the eluent. As already observed in the case of the personal care product test mixture ESI suffered from substantial suppression effects in combination with both matrices investigated. APCI showed somewhat better results, but signal reduction was definitely more pronounced than with DART ionization. Focusing on the sewage plant effluent sample, the performance of APPI is comparable to that of APCI. This behavior is not really unexpected as the final step in ionization is identical for both techniques, if APPI is applied in the dopand assisted mode. APPI without dopand suffers from insufficient LOD's for several compounds (primarily piracetam/paracetamol) substantially obstructing the evaluation of analyte signals in the more complex matrix (sewage plant effluent).

4. Conclusion

In the present work the applicability of DART-TOF-MS as a detector for gradient elution HPLC could be demonstrated, whereby a strong dependency of the signal intensity on the amount of organic solvent present in the eluent was observed. Post column addition of a make-up liquid (in our case iso-propanol) can help to overcome this problem providing acceptable sensitivities also for early eluting substances. In a second part, the suitability of HPLC-DART-TOF-MS for the analysis of real samples was examined on the example of two model matrices, namely a Danube river water extract and an extract from a sewage treatment plant effluent. Thereby DART ionization showed a reduced tendency towards ion suppression effects compared to other widely employed ionization techniques like ESI, APCI and APPI. Ionization mechanisms for DART and even matrix effects have been discussed in the literature before but these investigation mainly dealt with samples that were statically placed in the gas stream of the DART source [39–41]. In our case ionization occurs directly from the surface of a fast moving liquid stream. Differences in the performance of DART and ESI in the analysis of solutes within complex matrices can be explained with the substantially different mechanism of ionization. Regarding APCI and APPI, similarities between these two ionization techniques and DART exist [39]. In order to fully understand the mechanistic aspects behind differences in the performance of DART-, APCI- and APPI-MS as detector in the HPLC analysis of the samples investigated in this work, additional studies are needed and will be the subject of further work.

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References

[1] W.M.A. Niessen in, W.M.A. Niessen (Eds.), Encyclopedia of Mass Spectrometry, 8, 1st ed., Elsevier, Amsterdam, 2006, p. 1.
[2] E. Gelpi, J. Mass Spectrom. 37 (2002) 24.
[3] F. Gosetti, E. Mazzucco, D. Zampieri, M.C. Gennaro, J. Chromatogr. A 1217 (2010) 3929.
[4] R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, T. Olah, J. Am. Soc. Mass Spectrom. 11 (2000) 942.
[5] T. Sengster, M. Spence, P. Sinclair, R. Payne, C. Smith, Rapid Comunn. Mass Spectrom. 18 (2004) 1361.
[6] I. Marchi, S. Rudaz, J.-L. Veuthey, Talanta 78 (2009) 1.
[7] K.A. Hanold, S.M. Fischer, P.H. Cormia, C.E. Miller, J.A. Syage, Anal. Chem. 76 (2004) 2842.
[8] D.B. Robb, M.W. Blades, Anal. Chim. Acta 627 (2008) 34.
[9] A. Wick, G. Fink, T.A. Ternes, J. Chromatogr. A 1217 (2010) 2088.
[10] A. Kruve, A. Künnapas, K. Herodes, I. Leito, J. Chromatogr. A 1187 (2008) 58.
[11] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Talanta 74 (2008) 1299.
[12] J.C. Van De Steene, K.A. Mortier, W.E. Lambert, J. Chromatogr. A 1123 (2006) 71.
[13] A. Kloepfer, J. Benito Quintana, T. Reemtsma, J. Chromatogr. A 1067 (2005) 153.
[14] A. Van Eeckhaut, K. Lanckmans, S. Sarre, I. Smolders, Y. Michotte, J. Chromatogr. B 877 (2009) 2198.
[15] O.A. Ismaiel, M.S. Halquist, M.Y. Elmamly, A. Shalaby, H.T. Karnes, J. Chromatogr. B 875 (2008) 333.
[16] M. Himmelsbach, W. Buchberger, E. Reingruber, Polym. Degrad. Stab. 94 (2009) 1213.
[17] M.E. Rybak, D.L. Parker, C.M. Pfeiffer, J. Chromatogr. B 861 (2008) 145.
[18] H.R. Liang, R.L. Foltz, M. Meng, P. Bennett, Rapid Commun. Mass Spectrom. 17 (2003) 2815.
[19] G.-W. Lien, C. -Y- Chen, G—S. Wang, J. Chromatogr. A 1216 (2009) 956.
[20] I.-T. Wang, Y.-T. Feng, C.-Y. Chen, J. Chromatogr. B 878 (2010) 3095.
[21] G. Mascolo, V. Locaputo, G. Mininni, J. Chromatogr. A 1217 (2010) 4601.
[22] A. Cappiello, G. Famiglini, P. Palma, E. Pierini, V. Termopoli, H. Trufelli, Anal. Chem. 80 (2008) 9343.
[23] A. Cappiello, G. Famiglini, P. Palma, H. Trufelli, J. Liq. Chromatogr. Relat. Technol. 33 (2010) 1067.
[24] R.B. Cody, J.A. Laramee, H.D. Durst, Anal. Chem. 77 (2005) 2297.
[25] D.J. Weston, Analyst 135 (2010) 661.
[26] M. Haunschmidt, C.W. Klampfl, W. Buchberger, R. Hertsens, Analyst 135 (2010) 80.
[27] M. Haunschmidt, C.W. Klampfl, R. Buchberger, Hertsens, Anal. Methods 3 (2011) 99.
[28] J. Schurek, L. Vaclavik, H. Hooijerink, O. Lacina, J. Poustka, M. Sharman, M. Caldow, M.W.F. Nielen, J. Hajslova, Anal. Chem. 80 (2008) 9567.
[29] F.M. Fernandez, R.B. Cody, M.D. Green, C.Y. Mampton, R. McGready, S. Sengaloundeth, N.J. White, P.N. Newton, ChemMedChem 1 (2006) 702.
[30] I. Nyadong, G.A. Harris, S. Balayssac, A.S. Galhena, M. Malet-Martino, M.R. Martino, R. Mitchell Parry, M.D. Wang, F.M. Fernandez, V. Gilard, Anal. Chem. 81 (2009) 4803.
[31] J.M. Nilles, T.R. Connell, H. Dupont Durst, Anal. Chem. 81 (2009) 6744.
[32] M. Haunschmidt, C.W. Klampfl, W. Buchberger, R. Hertsens, Anal. Bioanal. Chem. 379 (2010) 269.
[33] R.B. Cody, Anal. Chem. 81 (2009) 1101.
[34] W. Eberherr, W. Buchberger, R. Hertsens, C.W. Klampfl, Anal. Chem. 82 (2010) 5792.
[35] S. Yu, E. Crawford, J. Tice, B. Musselman, J.-T. Wu, Anal. Chem. 81 (2009) 193.
[36] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, J. Chromatogr. A 1216 (2009) 6994.
[37] A. Zenker, H. Schmutz, K. Fent, J. Chromatogr. A 1202 (2008) 64.
[38] R. Kostiainen, T.J. Kauppila, J. Chromatogr. A 1216 (2009) 685.
[39] L. Song, A.B. Dykstra, H. Yao, J.E. Bartmess, J. Am. Soc. Mass Spectrom. 20 (2009) 42.
[40] C.N. McEwen, B.S. Larsen, J. Am. Soc. Mass Spectrom. 20 (2009) 1518.
[41] L. Song, S.C. Gibson, D. Bhandari, K.D. Cook, J.E. Bartmess, Anal. Chem. 81 (2009) 10080.