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New ionisation techniques for mass spectrometric techniques for environmental analysis

KWR Watercycle Research Institute

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New mass spectrometric techniques for environmental analysis

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Inzet massaspectrometrie met DART of SAWN biedt mogelijk grotere snelheid en minder voorbewerking bij monitoring nieuwe stoffen

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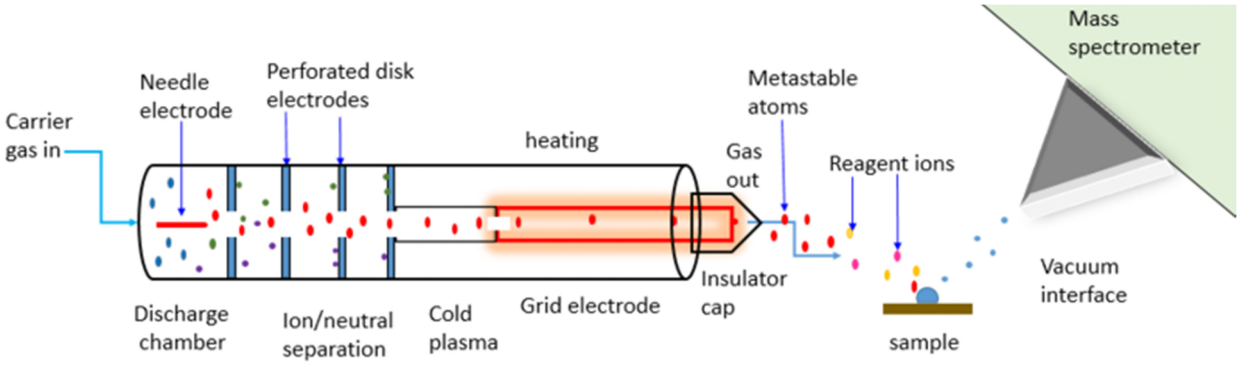
Twee veelbelovende technieken voor monitoring van nieuwe stoffen en analyse van onbekende verbindingen in watermonsters blijken geschikt om verder te testen op inzetbaarheid. Het gaat om DART (Direct Analysis in Real Time) en SAWN (Surface Assisted Wave Nebulisation), beide gebaseerd op massaspectrometrie. In het BTO project *Scouten Nieuwe Technieken* (BTO 2015.044) werd op grond van een literatuurstudie en een workshop met vertegenwoordigers van de waterlaboratoria geconcludeerd dat dergelijk *direct analysis mass spectrometry techniques* waardevol zouden kunnen zijn voor waterlaboratoria. Daarom zijn nu DART en SAWN onder de loep genomen, in samenwerking met UvA en WUR. De conclusie is dat beide technieken veel potentie hebben voor analyse van milieurelevante stoffen. Ze bieden een belangrijk voordeel ten opzichte van de huidige praktijk: bij deze nieuwe technieken is monstervoorbewerking nagenoeg overbodig. Ook leveren ze een significante inkorting van de meettijd. Na deze eerste verkenningen luidt de aanbeveling de technieken DART en SAWN uitgebreid te testen met watermonsters die voor waterbedrijven relevant zijn.



Schematische weergave van een DART ion source, één van de hier genoemde technieken om nieuwe stoffen in watermonsters aan te tonen

Belang: nieuwe technieken testen op bruikbaarheid en effectiviteit

Met de doorlopende introductie van nieuwe stoffen in het milieu komen steeds nieuwe technologieën op de markt om deze ontwikkeling te volgen. Daarom is het belangrijk de innovatieve technieken te testen op hun mate van geschiktheid voor monitoring van de waterkwaliteit en identificatie van nog onbekende verontreinigen. Met nieuwe technieken verbeteren de analyses in kwaliteit (nauwkeurigheid, gevoeligheid) en kunnen de kosten omlaag.

Aanpak: relevante technieken aangetoond en in het laboratorium getest

Eerder uitgevoerd literatuuronderzoek en gesprekken met deskundigen (BTO 2015.044) hebben twee mogelijk interessante technieken voor de watersector aangetoond. Dit betreft DART en SAWN, beide technieken die gebruikmaken van

massaspectrometrie. Vervolgens zijn deze twee technieken bij de UvA (SAWN) en bij de WUR (DART) op het laboratorium getest met milieurelevante stoffen. Het gaat om de geneesmiddelen diclofenac, metformin en carbamazepine.

Resultaten: monstervoorbewerking nagenoeg overbodig en significant kortere meettijd

Beide technieken hebben veel potentie om milieurelevante stoffen te analyseren. Belangrijk voordeel ten opzichte van de huidige praktijk is dat nieuwe technieken monstervoorbewerking nagenoeg overbodig maken. Ook betekenen ze een significante inkorting van de meettijd. Daarnaast kwamen mogelijke beperkingen van DART en SAWN in dit onderzoek naar voren, evenals problemen die bij de analyse van watermonsters zijn te verwachten, zoals soms moeilijk reproduceerbare data, lage gevoeligheid en technische problemen. In deze studie gaat het om verkennende experimenten. Daarom is het denkbaar dat huidige problemen in de toekomst oplosbaar zijn.

Implementatie: uitgebreid testen van DART en SAWN voor bepaling meest geschikte toepassing

Na deze eerste verkenningen van DART en SAWN luidt de aanbeveling deze technieken uitgebreid te testen met watermonsters die voor waterbedrijven relevant zijn. Vervolgens is het mogelijk te bepalen voor welke stoffen en watermatrices de technieken het meest geschikt zijn om de huidige aanpak te vervangen. Dit levert kostenbesparing op, evenals een kwalitatieve verbetering en vereenvoudiging van de analyses.

Rapport

Dit onderzoek is beschreven in het rapport *New ionisation techniques for mass spectrometric techniques for environmental analysis* (BTO 2018.040).

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1 Introduction

1.1 Reason for this project

This project is an offspring of the project “Scouten Nieuwe technieken” (BTO 2015.044). A literature review and a workshop led to the conclusion that direct analysis mass spectrometry techniques could be a valuable to the measure the water quality. Therefore, it has been decided to start a project, in which two analytical techniques (DART and SAWN) will be tested and evaluated. Additionally one other technique (Paper spray) will be introduced briefly as this might be a technique that could become interesting in the near future. The aim of this project is to decide whether these techniques are useful or not and to decide if a thorough assessment of them should be done.

2 DART-MS

2.1 Introduction

Direct Analysis in Real Time (DART) was first introduced by Cody et al. in 2005 and has since then grown from a self-built pilot to a commercialised product. [1] The two main advantages are the absence of tedious and time consuming sample preparation and the fact that it operates at ambient pressure. Therefore, it belongs to the ambient ionization techniques. To be more precise this ionization technique is an Atmospheric Pressure Chemical Ionisation (APCI). [2] The DART source offers the possibility to analyse most liquids and soil samples containing polar and non-polar compounds by high-throughput sample analysis. Due to the ionisation of merely exclusively low-molecular weight compounds (< 1kDa), the technique can tolerate samples with matrices containing high-molecular compounds. Another advantage of this technique is that it allows to generate fingerprints of samples within seconds and therefore allows to monitor the quality of a sample or sudden changes in the sample composition. DART can be used to verify the quality of single samples in seconds, making timely assessment of larger numbers of samples possible, thus increasing capability for threat detection. Both target and non-target analysis is possible in various aqueous matrices. Therefore, this method is suitable for the analysis of samples in case of an emergency (quick analysis) and for large amounts of samples. So far, DART is being applied in e.g. drugs analysis [3,4], food quality/safety [5], detection of explosives and clinical studies [2].

2.2 Principle

To analyse a sample it has to be presented in open atmosphere. [2,6] Therefore, the sample is placed between the outlet of the DART source and the orifice of the mass spectrometer. The analytes in the sample are subsequently ionised by heated gas leaving the DART source and transferred into the mass spectrometer. Important is that the gas can graze the sample and that the sample must not block the path to orifice of the mass spectrometer. There are multiple ways to present a sample in such a way. The most promising versions will be described below.

The first option is the simplest and fastest approach (1-2 min per sample). A glass rod (so called Dip-its) is dipped into the sample, and is subsequently analysed by placing it manually between the DART ion source and the MS orifice. The volatile compounds will evaporate, be ionised and transported by the gas flow from the DART to the MS. This method allows for a quick scan of a sample. However, the size of the droplet attached to the rod varies each time in size. Therefore, the reproducibility of the measurement is low. This is enhanced by the fact that the sample is held by hand between the source and the MS. Furthermore, the sample can spatter and consequently each time different amounts of sample can enter the MS. This means this method comes at the expense of reproducibility and is also prone to errors. Alternatively the glass rod can be replaced by a SPE-tip. These tips are solid phase microextraction devices designed to permit isolation of analytes from aqueous solutions and then direct analysis without further sample processing. The fibre tips are SPME fibres embedded in a micropipette tip for easy handling. The fibre coating is composed of either C18 or PDMS-DVB bonded silica particles embedded in a biocompatible polymer. The advantage of this method is that compounds get enriched in the SPE material and as a consequence lower concentrations in the sample are acceptable. However, the disadvantage is that the SPE material first needs to be equilibrated with the sample which takes about 1h.

This means the first sample will take about 1h before it can be analysed. Measurement of each sample will also only take 1-2 min.

Both the Dip-it and the SPE-tip can be placed in a holder that is mounted on a linear rail that move automatically (see Figure 1). Analysis of all the samples in the holder as shown in this figure does take about 3-5 min. This increases the reproducibility and provides the means for rapid quantitative and qualitative analysis.

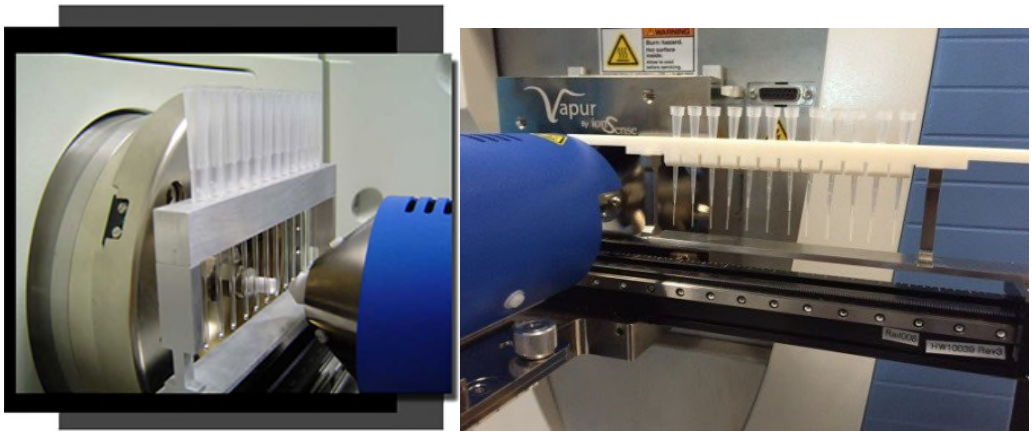


FIGURE 1: PICTURE OF THE ION SOURCE AND A HOLDER CONTAINING DIP-ITS (LEFT). PICTURE OF THE ION SOURCE AND A HOLDER CONTAINING THE SPE-TIPS (RIGHT).

Another means of presenting the sample is the use of a mesh. This is being placed on a rail (Figure 2). The sample is dripped on the mesh, the mesh is then allowed to dry and the DART is moving along the parts which carry the sample. This ensures that the samples are always are optimally positioned. Another advantage is that several samples can be analysed quickly. Also, in contrast to the Dip-it method, it is possible to add a specific amount of sample onto the mesh. Therefore, reproducibility is greatly enhanced and even quantification is possible. It takes about 30 min to dry the mesh and 1-2 min to analyse each sample.

Table 1 shows all introduced methods with their corresponding quality parameters.

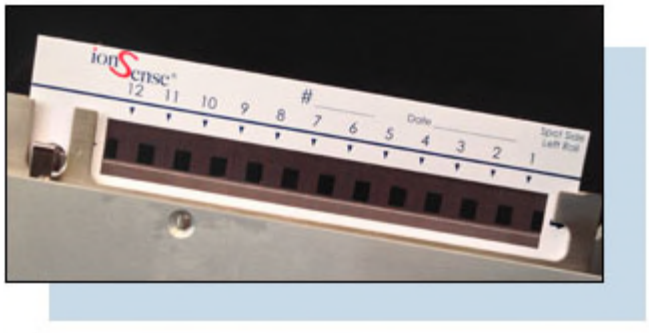


FIGURE 2: METAL MESH WITH TWELVE SAMPLE POSITIONS.

In all cases it is preferable, however not vital, that the samples are dry. This can increase the reproducibility of the experiment as wet samples tend to spatter. This is especially the case when employing Dip-its. All these methods have one advantage in common. Predominantly, merely volatile compounds will enter the MS and contamination of the same is greatly

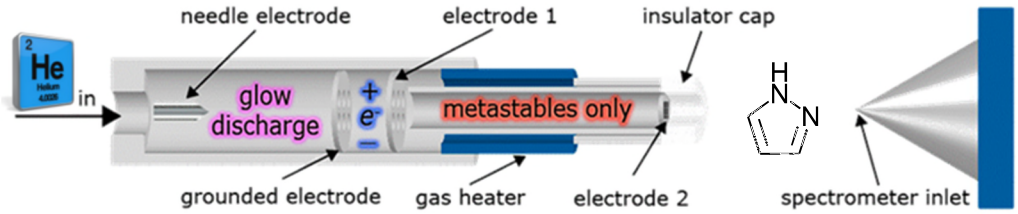
reduced compared to a direct injection. Furthermore, also solid materials (dry or soaked) can be analysed.

TABLE 1: ANALYSIS AND PREPARATION TIME, REPRODUCIBILITY AND THE POSSIBILITY TO CONCENTRATE THE SAMPLES ARE SHOWN FOR FIVE METHODS. REPRODUCIBILIY IS A RELATIVE VALUE.

Method	Analysis time per sample	Preparation time	Reproducibility	Sample concentration
Dip-it (by hand)	1-2 min	negligible	-	No
Dip-it Autosampler	1-2 min	5 min	+	no
SPE-it (by hand)	1-2 min	1 h	++	Yes
SPE-it Autosampler	1-2 min	1 h	+++	Yes
Mesh	1-2 min	30 min	+++	yes

2.3 Ionisation mechanism

DART is based on the ionisation of target molecules in the gas-phase through long-lived electronic excited neutral (noble) gases [2]. Figure 1 shows the principle of the DART ion source. Glow discharge creates ions, electrons, excited state noble gases, usually Ar or He. These gases move forward toward a second electrode, which removes most of the charged particles and gases. This ensures that only the metastable electronic excited neutral gases will leave the ion source. Immediately after leaving the DART, the excited gases will ionise atmospheric gases (N_2, O_2) and eventually the analytes via the so-called Penning ionisation.



$He^* + N_2 > He + N_2^+ + e^-$

$He^* + A > He + A^+ + e^-$

Direct ionisation of the analyte molecules (primary ionisation) happens relatively rarely. This is attributed to the great excess of N_2 and O_2 in the air. The ionisation of atmospheric gases is the first step of a cascade of reactions, which will eventually lead to the formation of positively and negatively charged ions. Table 2 shows the most common positively and negatively charged analyte molecules. It is important to realise that the DART source can only ionise molecules that are in the gas phase. For this reason it is paramount that the gas leaving the DART is heated (usually between 350 and 500 C). The heated gas will vaporise the analytes that are placed between the outlet of the ion source and the orifice of the mass spectrometer.

TABLE 2: ANALYTE IONS FREQUENTLY OBSERVED IN DART-MS.

Analyte polarity	Positive ions	Negative ions
Non-polar	$M^{+\bullet}$, $[M + H]^+$, $[M + O + H]^+$	$M^{-\bullet}$, $[M - H]^-$, $[M - H + O_2]^-$, $[M + O_2]^{-\bullet}$, $[M + Cl]^-$
Medium polar to polar	$[M + H]^+$, $[M + O + H]^+$, $[M + NH_4]^+$ (other adducts are possible when the counter ions are present)	$[M - H]^-$, $[M + OH]^-$, $[M + CN]^-$, $[M + Cl]^-$ (other adducts are possible when the counter ions are present)

An extensive description of ionisation mechanism can be found elsewhere. [2]

2.4 Assessment

2.4.1 General comments

It is important to realise that in contrast to HPLC-hrMS, in case of DART-hrMS, there are no chromatograms. The sample that is being analysed enters the orifice of the MS directly and hence there is no chromatographic separation of the compounds. DART-hrMS is in this sense a direct injection. Instead of a chromatogram, a ion chronogram is received, that can contain several samples. Furthermore, it is important to mention that the DART ioniser can be installed on any given mass spectrometer as it works independent from the mass spectrometers' software. Also, the DART is very forgiving regarding the matrix. Whereas in case of a HPLC method often a laborious sample work-up is necessary or in case of a direct injection the MS requires cleaning from time to time, the user of a DART is not confronted with these problems.

2.4.2 Test analytes

The test analytes are diclofenac, carbamazepine, metformin and naphthalene. These compounds are all environmentally relevant and representatives for negatively charged compounds (diclofenac), positively charged compounds (metformin), (a)polar) neutral compounds (naphthalene and carbamazepine).

2.4.3 LOQ

A compound was accepted as positive when the signal to noise ratio was higher than 10 and both the ^{12}C monoisotopic mass and the ^{13}C monoisotopic mass of the compounds where detectable. Diclofenac was the exception. For this compound the isotopic ration of the chlorine isotopes was used.

2.4.4 Sample preparation

SPE-it samples were prepared as follows. The SPE-it tip was left in H_2O:MeOH (50:50) for 15 min and afterwards it was placed in the sample for 45 min prior to analysis.

Dip-it's were dipped into the sample and analysed immediately without drying.

Mesh samples were prepared by dripping the desired amount (5-20 µL) of sample onto the mesh. Afterwards the mesh was allowed to dry on a hotplate set to 50 ºC until dry.

2.4.5 DART-Orbitrap MS Settings

A DART-SVP (simplified voltage and pressure) ion source (IonSense, Saugus, MA) was coupled to an Exactive orbitrap high-resolution mass spectrometer (Thermo Fisher Scientific, San Jose, CA). All full-scan measurements were performed with a scan range of m/z 100.0–1000.0 in

positive mode, a mass resolution of 100 000 (full width at half-maximum, fwhm m/z 400), and no maximum injection time. The DART-SVP source was operated in either positive or negative-ion mode and with a temperature setting of 350 °C unless indicated otherwise. All samples were analyzed in transmission mode with an X-Z transmission module (IonSense) at a scan speed of 0.2 mm/s. As ionization gas, helium was used at a flow rate of ~3.7 L/min.

2.4.6 Direct injection Settings

A tribrid Orbitrap-Fusion mass spectrometer (ThermoFisher Scientific, Bremen, Germany) provided with an electrospray ionisation source was interfaced to a Vanquish HPLC system (ThermoFisher Scientific). For the chromatographic separation an XBridge BEH C18 XP column (150 mm × 2.1 mm I.D., particle size 2.5 µm) (Waters, Etten-Leur, The Netherlands) preceded by a 2.0 mm × 2.1 mm I.D. Phenomenex SecurityGuard Ultra column. Phenomenex, Torrance, USA) maintained at a temperature of 25 °C was used. The gradient started with 5% acetonitrile, 95% water and 0.05% formic acid (v/v/v), increased to 100% acetonitrile with 0.05% formic acid in 25 min, and was held constant for 4 min. The flow rate was 0.25 mL/min and 100 µL of sample was used for injection. With every batch run mass calibration was performed using Pierce ESI positive ion calibration solution. The vaporizer and capillary temperature were maintained at 300 and 300 °C, respectively. Sheath, auxiliary and sweep gas was set to arbitrary units of 40, 10 and 5.

The source voltage was set to 3.0 kV in the positive mode. The RF lens was set to 50 %. Full scan high accuracy mass spectra was acquired in the range of 65–1300 m/z with the resolution set at 120,000 fwhm in the positive ionization mode and quadruple isolation was used for acquisition with a 5 ppm mass window. The maximum injection times was set to 100 ms.

2.4.7 Spiked samples

Five (environmentally) relevant compounds were chosen to be tested in the DART-hrMS setup. The analytes were chosen to cover a broad range of attributes. The compounds selected are diclofenac, carbamazepine, metformin, naphthalene and cocaine. As first step it was tested if these compounds can be ionised using the DART source. Therefore, stock solution of these compounds in MilliQ water were analysed with the Dip-it method. Table 3 shows the results for these experiments. Four of the compounds were clearly visible. Signal to noise ratio was higher than 10 and all relevant isotopes could be seen. Naphthalene could not be detected, despite a concentration of 2 mg/L. This experiment should be repeated as visual inspection of the stock solution raise suspicion that naphthalene did not dissolve completely for unknown reasons. Furthermore, the signal intensities for the different isotopic masses were calculated. In case of diclofenac the signal intensity and area were used to calculate the isotopic pattern for chlorine ($^{35}Cl/^{37}Cl$). A molecule containing 2 chlorine atoms such as diclofenac will give an isotopic pattern of 9:6:1. For the other compounds the amount of carbon atoms per molecule is calculated from the carbon isotopic ration ($^{12}C/^{13}C$) using the following formula.

$$Carbon\ atoms = \frac{100 * {}^{13}C}{1.1\,{}^{12}C}$$

TABLE 3: DIP-IT METHOD: SIGNAL INTENSITIES (SI) AND CARBON ATOM NUMBER CALCULATED BASED ON THE SI AND AREA. ISOTOPIC RATIO OF CHLORINE IN DICLOFENAC BASED ON SI AND AREA.

Compound	Int. ($M+H^+$)	Int. ($^{13}C\ M+H^+$)		SI-Carbon number	Area-Carbon number
Carbamazepine (2 mg/L)	1.61E7	2.67E6		15.07 (+0.07)	13.1 (-1.9)

$C_{15}H_{12}N_2O$					
Metformin (10 mg/L)	3.61E6	1.18E5		2.97 (-0.03)	2.78 (-0.22)
$C_4H_{11}N_5$					
Cocaine (unknown con.)	3.88E7	7.37E6		17.3 (+0.3)	16.5 (-0.5)
$C_{17}H_{21}NO_4$					
	Int. (M^-)	**Int. ($^{35}Cl^{37}Cl\ M^-$)**	**Int. ($^{37}Cl^{37}Cl\ M^-$)**	**SI-Ratio**	**Area-Ratio**
Diclofenac (10 mg/L)	4.27E6	2.64E6	**4.25E5**	**9:5.6:0.9**	**9:5.6:0.9**
Expected Cl isotope ratio: 9:6:1					

Signal intensities for carbamazepine are significantly better than for the other compounds despite its lower concentration. For three of the four compounds the calculated isotopic ratios are in accordance with the expected ratio. The ratio calculated for carbamazepine and cocaine reflects the amount of carbon atoms in the molecule when it is calculated based on signal intensity. When using the peak area to calculate the amount of carbon atoms the number is off by two carbon atoms. The amount of carbon atoms for metformin cannot be calculated correctly in both cases. In case of diclofenac both calculation methods give the expected Cl isotopic ratio (9:6:1). The error caused when using the area can be explained by the signal. As there is no smooth signal but a rather "spikey" course of the signal, the area is greatly affected by these spikes. Also smoothing the signal will further contribute to this problem.

Next the detection limit for three compounds in MilliQ was determined (metformin, diclofenac and carbamazepine). The following tables Table 4Table 5Table 6 show the spectra and data, respectively. For these experiments the sample was dripped on a metal mesh to add exactly defined amounts of the sample. The samples were subsequently allowed to dry on a heated stirring plate. Three different volumes were tested (5 µL, 10 µL and 20 µL). The tables show that at least 20 µL should be used. Using only 5 µL results in no signal at all for neither of the compounds.

Using 20 µL results in a LOQ for diclofenac between 5 and 2 mg/L, carbamazepine 0.01 mg/L and metformin 2.5 mg/L. These results show clearly that not every compound is ionised equally well. For carbamazepine consequently also the LOQ in surface water was determined. The lowest possible concentration still detectable was 5 µg/L for a 20 µL sample. The surface water used for the dilution was void of carbamazepine. This result could imply that the presence of a complex matrix does improve the ionisability, at least it does not affect the LOQ negatively. For carbamazepine also the chronograms are shown as an example (Figure 3).

TABLE 4: CARBAMAZEPINE: DIFFERENT CONCENTRATIONS AND VOUMINA OF CARBAMAZPEINE IN MQ-WATER DRIPPED ON A MESH PLATE. N.D. = NOT DETERMINED

Concentration	Volume	Amount	Intensity Ratio	Area Ratio	S/N
MilliQ water					
1 mg/L	5 µL	5 ng	n.d.	n.d.	n.d.
0.5 mg/L	5 µL	2.5 ng	n.d.	n.d.	n.d.
0.2 mg/L	5 µL	1 ng	n.d.	n.d.	n.d.
0.1 mg/L	5 µL	0.5 ng	n.d.	n.d.	n.d.
0.01 mg/L	5 µL	0.05 ng	n.d.	n.d.	n.d.
0.001 mg/L	5 µL	0.005 ng	n.d.	n.d.	n.d.
1 mg/L	10 µL	10 ng	14.13	13.29	1403
0.5 mg/L	10 µL	5 ng	12.98	13.10	76
0.2 mg/L	10 µL	2 ng	14.36	12.93	171
0.1 mg/L	10 µL	1 ng	14.78	11.11	110
0.01 mg/L	10 µL	0.1 ng	n.d.	n.d.	n.d.
0.001 mg/L	10 µL	0.01 ng	n.d.	n.d.	n.d.
1 mg/L	20 µL	20 ng	14.78	12.36	5461
0.5 mg/L	20 µL	10 ng	15.95	11.45	3615
0.2 mg/L	20 µL	4 ng	13.04	11.93	1405
0.1 mg/L	20 µL	2 ng	14.06	10.36	1087
0.01 mg/L	20 µL	0.2 ng	10.88	7.04	100
0.001 mg/L	20 µL	0.02 ng	n.d.	n.d.	n.d.
Surface water					
0.5 mg/L	20 µL	10 ng	14.55	11.09	4583
0.05 mg/L	20 µL	1 ng	13.35	10.07	1206
0.01 mg/L	20 µL	0.2 ng	10.33	11.54	259
0.005 mg/L	20 µL	0.1 ng	8.56	9.46	136
0.001 mg/L	20 µL	0.02 ng	17.10	13.67	20
0.0005 mg/L	20 µL	0.01 ng	35.19	n.d.	n.d.

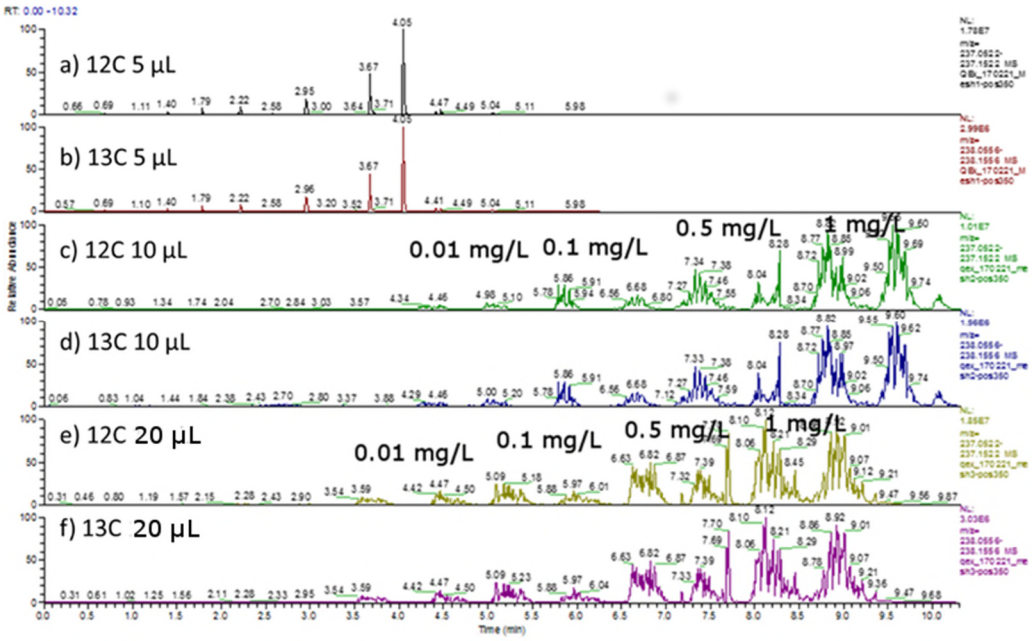


FIGURE 3 ION CHRONOGRAM OF A CARBAMAZEPINE DILUTION DRIPPED ON A MESH. THE ION CHRONOGRAMS FOR THE ^{12}C- AND ^{13}C-ISOTOPES ARE SHOWN. A) AND B): 5 µL OF SAMPLE ANALYSED, C) AND D) 10 µL OF SAMPLE ANALYSED, E) AND F) 20 µL OF SAMPLE ANALYSED. SEE ALSO TABLE 4.SIGNALS SEEN IN A) AND B) ARE MASSA SPIKES AND ARE TYPICAL FOR LOW CONCENTRATIONS AND ARE NOT A POSITIVE SIGNALS FOR THE COMPOUNDS. NOTE THAT THE VALUES ON THE Y-AXIS ARE RELATIVE VALUES.

TABLE 5: METFORMIN: DIFFERENT CONCENTRATIONS AND VOUMINA OF CARBAMAZPEINE IN MQ-WATER DRIPPED ON A MESH PLATE. N.D. = NOT DETERMINED

Concentration	Volume	Amount	Intensity Ratio	Area Ratio	S/N
5 mg/L	5 µL	25 ng	n.d.	n.d.	n.d.
2.5 mg/L	5 µL	12.5 ng	n.d.	n.d.	n.d.
1.0 mg/L	5 µL	0.5 ng	n.d.	n.d.	n.d.
0.5 mg/L	5 µL	0.25 ng	n.d.	n.d.	n.d.
0.05 mg/L	5 µL	0.025 ng	n.d.	n.d.	n.d.
0.005 mg/L	5 µL	0.0025 ng	n.d.	n.d.	n.d.
5 mg/L	10 µL	50 ng	1.17	3.03	587
2.5 mg/L	10 µL	25 ng	9.57	n.d.	112
1.0 mg/L	10 µL	1 ng	n.d.	n.d.	15
0.5 mg/L	10 µL	0.5 ng	n.d.	n.d.	n.d.
0.05 mg/L	10 µL	0.05 ng	n.d.	n.d.	n.d.
0.005 mg/L	10 µL	0.005 ng	n.d.	n.d.	n.d.
5 mg/L	20 µL	100 ng	3.88	3.02	2963
2.5 mg/L	20 µL	50 ng	3.27	3.30	1180
1.0 mg/L	20 µL	2 ng	15.27	n.d.	177
0.5 mg/L	20 µL	1 ng	n.d.	n.d.	67
0.05 mg/L	20 µL	0.1 ng	n.d.	n.d.	31
0.005 mg/L	20 µL	0.01 ng	n.d.	n.d.	n.d.

TABLE 6: DICLOFENAC: DIFFERENT CONCENTRATIONS AND VOUMINA IN MQ-WATER DRIPPED ON A MESH PLATE. N.D. = NOT DETERMINED

Concentration	Volume	Amount	Intensity Ratio	Area Ratio	S/N
10 mg/L	20 µL	200 ng	9:6:0.5	9:5.5:0.5	91
5 mg/L	20 µL	100 ng	9:2.5:n.d.	9:4.6:n.d.	17
2 mg/L	20 µL	40 ng	9:7.8:n.d.	9:2.2:n.d.	6
1 mg/L	20 µL	20 ng	n.d.	n.d.	n.d.
0.1 mg/L	20 µL	0.2 ng	n.d.	n.d.	n.d.
0.01 mg/L	20 µL	0.02 ng	n.d.	n.d.	n.d.

Furthermore, for carbamazepine a calibration curve was created by diluting a stock solution of 1.8 mg/L (MilliQ) to 20 µg/L. To each dilution an internal standard (benzotriazole-d4, 9.6 mg/L) was added. These solutions were measured using a mesh and SPE-it tips. In each case the calibration line was determined using the ratio of the areas of carbamazepine and the internal standard and the signal intensities of carbamazepine and the internal standard. For the mesh experiments the solutions (20 uL) were dripped on a mesh, aowed to evaporate and subsequently scanned. Figure 4 shows the resulting data. The detection limit for carbamazepine is about 20 µg/L. At this concentration both the 12C and 13C signal are still visible and the S/N is higher than 10 (not shown). For the calibration line based on the areas a R^2 of 0.98 is calculated. The calibration line calculated from the intensities has a R^2 of 0.89.

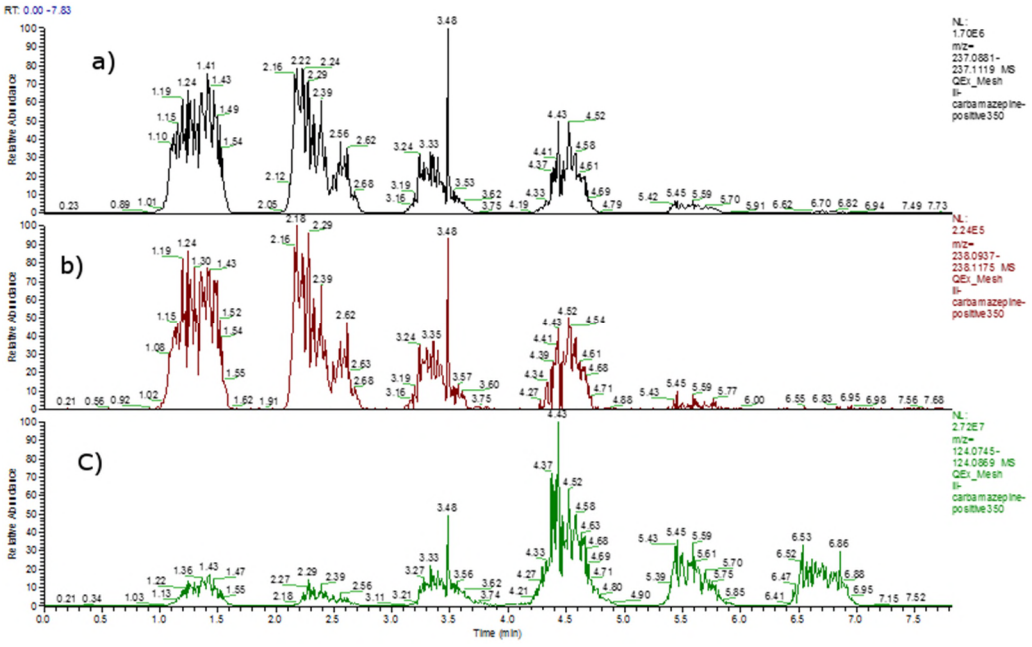


FIGURE 4: SIGNALS OF THE CARBAMAZEPINE DILUTIONS. A) ^{12}C SIGNAL CARBAMAZEPINE, B) ^{13}C SIGNAL CARBAMAZEPINE, C) ^{12}C SIGNAL INTERNAL STANDARD (BENZOTRIAZOLE-D4). SEE ALSO TABLE 7

TABLE 7: CARBAMAZEPINE SERIAL DILUTION ON A MESH. (MG/L). C = CARBAMAZEPINE. IS = INTERNAL STANDARD(BENZOTRIAZOLE-D4). SEE ALSO FIGURE 4 AND FIGURE 5.

Conc.	Area Carbam.	Area IS	Area C / Area IS	Signal C/IS
1.8	22528345	1.13E+08	0.198662	0.362
0.9	19981015	2.42E+08	0.082627	0.316
0.45	8135712	2.82E+08	0.028869	0.112
0.2	8410731	3.35E+08	0.025128	0.037
0.1	1539247	2.6E+08	0.005924	0.013
0.02	250441.4	2.88E+08	0.00087	0.002

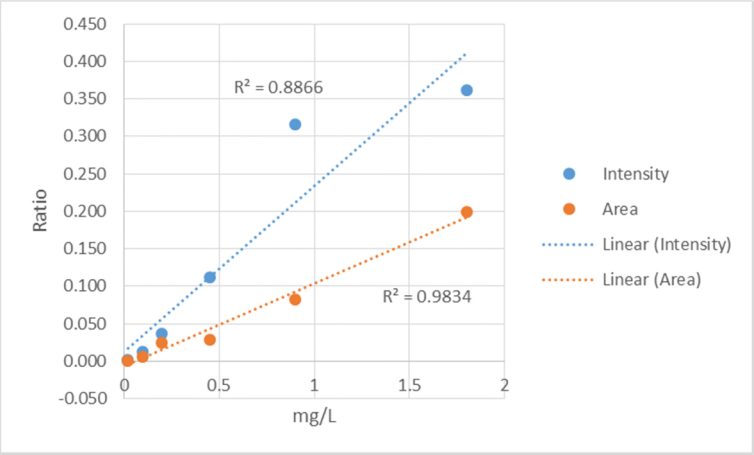


FIGURE 5: CARBAMAZEPINE IN MILLIQ DILTUED FROM 1.8 MG/L TO 0.02 MG/L ON A MESH. RATIO IS THE SIGNAL INTENSITY OR AREA OF THE CARBAMAZEPINE DIVIDED BY THE INTERNAL STANDARD (BENZOTRIAZOLE-D4). SEE ALSO TABLE 7: CARBAMAZEPINE SERIAL DILUTION ON A MESH. (MG/L).

For the SPE-it experiments the SPE tips where placed in a mixture of water and methanol (50:50) for 15 min. Next they were placed in the solutions for 45 min and subsequently measured. A calibration line can be received for both calculation methods with R^2 values of about 0.99. However, for the lowest concentration of 0.02 mg/L the mass for the ^{13}C-carbamzepine isotope cannot be detected anymore. This shows that at least for carbamazepine there is no added value in using the SPE-it method with regard to sensitivity.

TABLE 8: CARBAMAZEPINE SERIAL DILUTION WITH SPE-IT. (MG/L). C = CARBAMAZEPINE. IS = INTERNAL STANDARD. A) SIGNA TO NOISE RATION < 10.

Conc.	Area Carbam.	Area IS	Area C / Area IS	Signal C/IS
1.8	12303130	9.85E+06	1.25E+00	1.883929
0.9	10428669	1.50E+07	6.95E-01	0.836983
0.45	6698446	3.10E+07	2.16E-01	0.495667
0.2	3511977	2.85E+07	1.23E-01	0.204965
0.1	1146571	3.89E+07	2.95E-02	0.035331

0.02[a)]	141002	4.14E+07	3.40E-03	0.00494

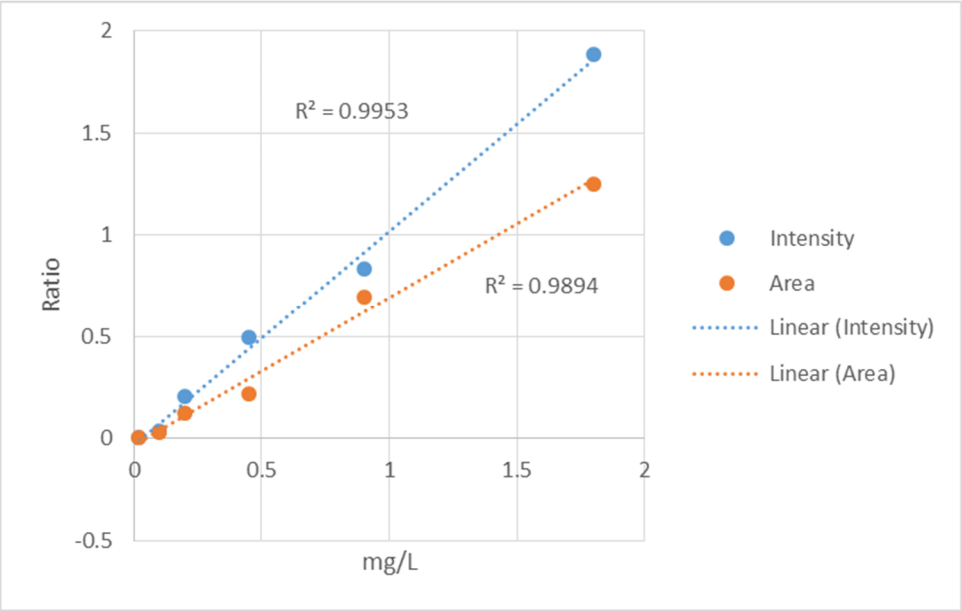


FIGURE 6: CARBAMAZEPINE IN MILLIQ DILTUED FROM 1.8 MG/L TO 0.02 MG/L WITH SPE-IT. RATIO IS THE SIGNAL INTENSITY OR AREA OF THE CARBAMAZEPINE DEVIDED BY THE INTERNAL STANDARD.

2.4.8 Repeatability:

For the repeatability experiments two compounds (atrazine-d5 and benzotriazole-d4) that are commonly employed as internal standards are chosen. The repeatability experiments were conducted for with the mesh and SPE-it method. Table 9 shows the repeatability for the mesh method. Same amounts of these compounds were measured six times. The compounds were dripped on a mesh, left to dry and subsequently measured. The table shows for atrazine a standard deviation between 21 and 29% for the peak area and the peak intensity, respectively. In case of benzotriazole the standard deviations are higher (62 and 55%). Furthermore, nearly for all measurements the ratio between the area's or the peak intensity of the two compounds differs. This should not be the case as the ratio of these two compounds in each sample is the same. These result show that I) that samples values show a great variance, II) that the variance is compound-dependent and that III) the compounds are not always equally well ionised. Plotting the area against the signal intensity for each compound shows a good correlation of these two values ($R^2 > 0.95$) (see Figure 7).

TABLE 9: REPEATABILITY EXPERIMENTS FOR TWO COMPOUNDS. 20 UL DROPS ON MESH.

	Atrazine-d5 (9.6 mg/L)			**Benzotriazole-d4 (9.6 mg/L)**			**Ratio A/B**	
Measurement	Area	Signal		Area	Signal		Area	Signal
1	1.18E+08	2.54E+06		6.54E+07	1.76E+06		1.8	1.4
2	2.12E+08	4.67E+06		3.91E+07	9.52E+05		5.4	4.9
3	2.15E+08	7.24E+06		8.41E+07	2.04E+06		2.6	3.5
4	2.48E+08	6.80E+06		2.62E+08	5.72E+06		0.9	1.2
5	2.34E+08	5.40E+06		1.23E+08	3.18E+06		1.9	1.7

6	2.56E+08	6.06E+06		1.25E+08	3.01E+06		2.0	2.0
Average	2.14E+08	5.45E+06		1.16E+08	2.78E+06			
STD (%)	21	29		62	55			

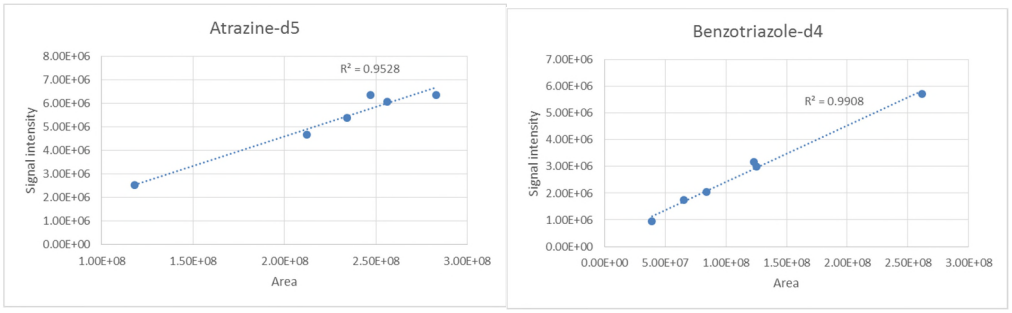


FIGURE 7: MESH METHOD: AREA PLOTTED AGAINST SIGNAL INTENSITY FOR ATRAZINE AND BENZOTRIAZOLE.

The same experiment was repeated using SPE-it instead of a mesh (Figure 8 and Table 10). The same effects as mentioned above can be seen. In this case however for atrazine the correlation between area and signal intensity is worse (R^2 = 0.73).

TABLE 10: REPEATABILITY EXPERIMENTS FOR TWO COMPOUNDS. SPE-IT METHOD.

	Atrazine-d5 (9.6 mg/L)			**Benzotriazole-d4 (9.6 mg/L)**			**Ratio A/B**	
Measurement	Area	Signal		Area	Signal		Area	Signal
1	1.16E+08	1.14E+07		9.49E+06	6.88E+05		12.2	16.6
2	1.97E+08	1.81E+07		1.51E+07	1.25E+06		13.0	14.5
3	3.46E+08	1.99E+07		3.08E+07	2.36E+06		11.2	8.4
4	3.88E+08	2.77E+07		2.84E+07	1.86E+06		13.7	14.9
5	3.15E+08	2.21E+07		3.89E+07	2.38E+06		8.1	9.3
6	3.21E+08	2.85E+07		4.05E+07	2.56E+06		7.9	11.1
Average	2.80E+08	2.13E+07		2.72E+07	1.85E+06			
STD (%)	34	27		42	37			

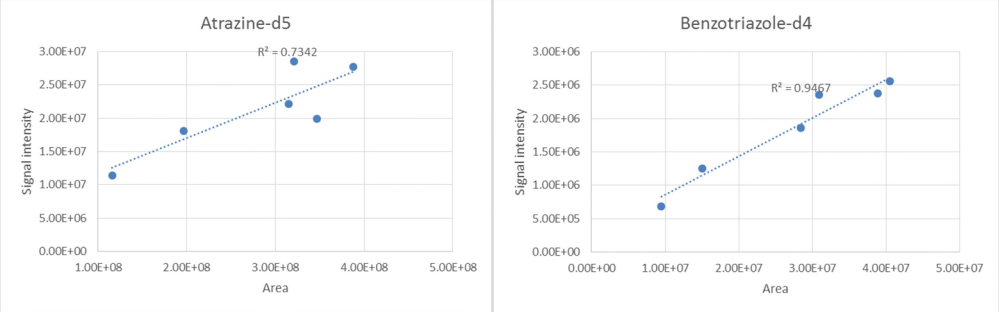


FIGURE 8: SPE-IT METHOD: AREA PLOTTED AGAINST SIGNAL INTENSITY FOR ATRAZINE AND BENZOTRIAZOLE.

The main conclusions from this experiments are:

1) It is imperative to use an internal standard for quantitative analysis and if samples need to be compared. Otherwise, the signal intensities from one sample are not comparable to the signal intensity of the next nor will it be possible to compare the same sample measured several times.
2) LOQs are highly depended on the compound. Whereas, for carbamazepine concentrations between 1 - 10 µg/L where still detectable, the LOD for metformin was 1 mg/L
3) It is important to decide before hand if the signal intensity or signal area should be used for the evaluation.

2.4.9 Matrix effect

In another experiment the relative signal intensity of these two standards in MilliQ and surface water with the MESH method was determined. The results showed that the two compounds can be detected in MilliQ water, but how any response when the spiked surface water was analysed. The reason for this is still unclear.

2.4.10 Comparison Direct injection and DART

For determining the overall time effort needed to analyse an x-amount of samples, certain assumptions have been made regarding the time to process samples. The first one is that merely 12 samples will be analysed because this is the maximum number of samples that can be analysed in one run using either the mesh or the SPE-it method. The direct injection involves spiking a certain amount of liquid sample with internal standards and subsequent filtration to a vial. For the DART analyses two ways of introducing the sample have been examined. (i) Using a mesh which requires depositing a sample on a metal mesh and allow time to evaporate the water. (ii) The SPE method in which the tip of a small pipette containing SPE material is immersed for 12 minutes to allow an equilibrium.

In total it becomes evident (Figure 9Figure 10) that DART has an advantage over direct injection when using a mesh but only when there are more than 17 samples. For using the DART-SPE tip approach there is no advantage. The DART-SPE-it approach is too time-consuming and laborious to outperform the direct injection.

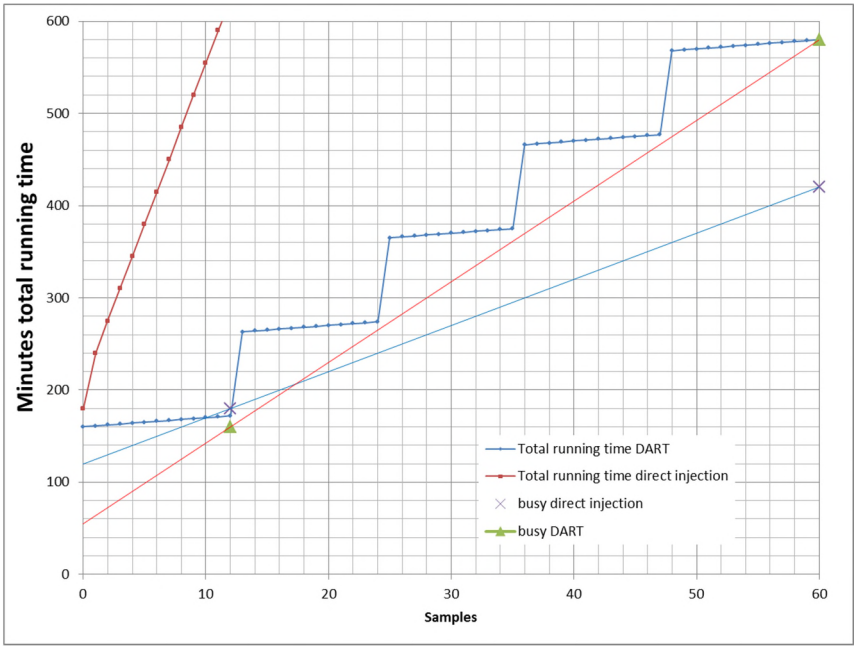


FIGURE 9: DART-MESH AGAINST DIRECT INJECTION: EXPECTED METHOD RUNNING TIMES FOR EACH TECHNIQUE FOR 1-60 SAMPLES.

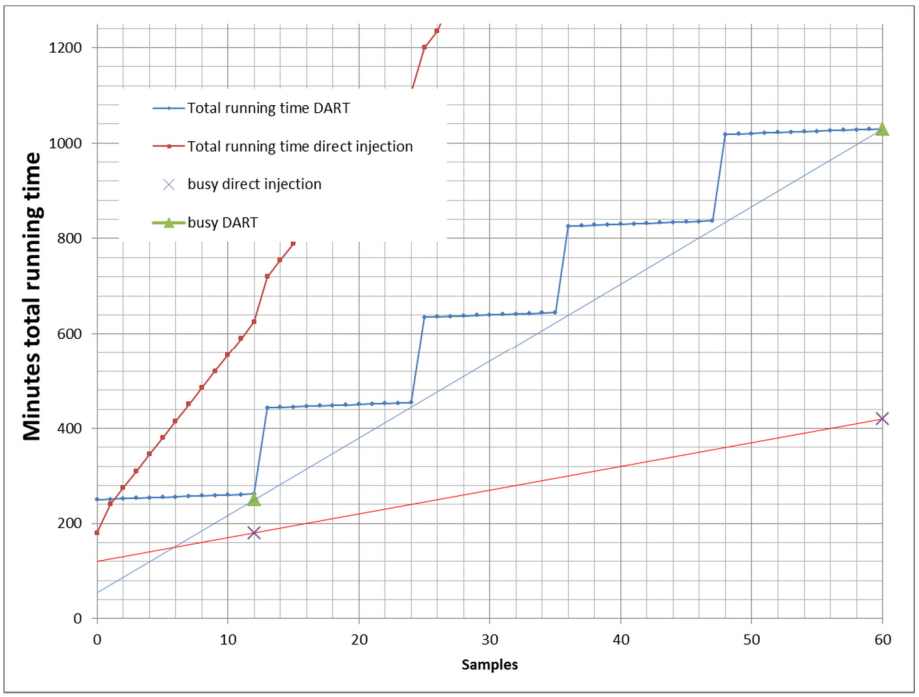


FIGURE 10: DART-SPE AGAINST DIRECT INJECTION: EXPECTED METHOD RUNNING TIMES FOR EACH TECHNIQUE FOR 1-60 SAMPLES.

A direct injection with a chromatographic separation does not need extensive evaluation. The processing method automatic integrates and quantitates according to a calibration sequence or via an internal standard. It will only require validating the results. Deep knowledge of the mass spectrum is not needed. The DART does not produce a chromatographic peak but a chronogram or an all ion high resolution mass spectrum. When

handling difficult matrices, multiple adduct forming can take place. Hence only suspect screening for compounds which do not have a background will be easy to interpret. For these compound it will take minutes to produce results.

3 Surface assisted wave nebulization

3.1 Introduction

Surface acoustic wave nebulization (SAWN) is a novel atmospheric ionisation method for MS, first introduced by Heron et. al. in 2010. [7-9] This technique excels for the absence of pressure pumps, capillaries that can clog, or a direct voltage applied to the sample. SAWN is a chip-based technology (see Figure 11) which directly nebulises and ionises various dissolved compounds via acoustic waves that travel along the surface of a piezoelectric[1] chip with two opposite facing inter digital transducers (IDTs). The ionization mechanism, however, is not yet fully understood. The surface acoustic waves are Rayleigh waves generated on the surface of a piezoelectric crystal (LiNbO3) by applying a radio frequency through the ITDs. Due to the piezoelectric nature of chip, an electric gradient on the surface of chip is also produced known as the piezoelectric effect.

When a small droplet is dripped onto the chip, between the two opposing ITDs, the generated progressive surface acoustic waves (pSAWs) will alter the surface tension of the liquid generating capillary waves inside the droplet on the chip and charged aerosols with a diameter between 1 to 1000 µm are formed which will enter the orifice of the MS. The ionised droplets further evaporate after entering the heated inlet of the mass spectrometer. This type ionisation mechanism is fundamentally different from other existing ionization techniques such as ESI, MALDI, APCI, APPI.

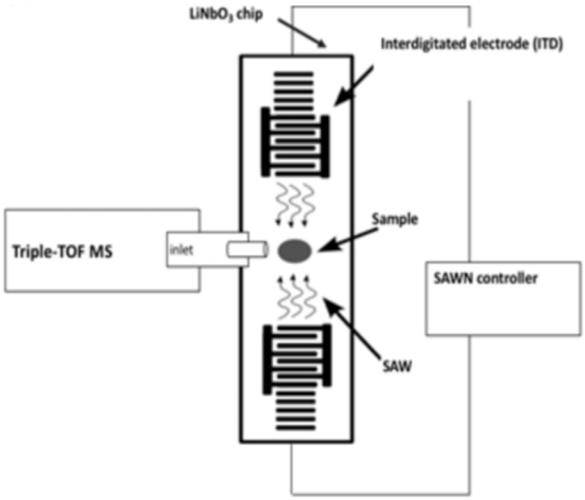


FIGURE 11: GRAPHICAL REPRESENTATION OF A SAWN CHIP COUPLED TO A TRIPLE-TOF MS (NOT TRUE TO SCALE)

The ionisation together with the aerosol formation function as an ion delivery system for the MS. For a successful SAWN analysis, it is necessary to utilise a SAWN chip equipped with two IDTs. The two opposing pSAWs combined, form a standing SAW (sSAW)

[1] Piezoelectricity is the appearance of positive electric charge on one side of certain non-conducting crystals and negative charge on the opposite side when the crystals are subjected to mechanical pressure. In the opposite case, namely applying an electrical charge to a crystal, results in physical changes.

which generates a higher percentage of smaller droplets in the 1µm to 10 µm range as well as an increase of S/N ratio ranging up to a factor of 100. The two IDTs are controlled by an external controller applying a RF of 9.6 MHz to the IDTs. The amount of power applied to the chip by the controller can be manually set to any given percentage of the maximum output power of 7.5 Watts. The resulting output power and corresponds to the amplitude of the RF applied to the IDTs.

The first successful SAWN-MS analysis was reported by Heron et. al. in 2010 [8]. Heron et. al. introduced SAWN as a new ionization method after successfully sequencing peptides via SAWN-MS/MS. Huang et. al. subsequently showed that SAWN produces ions with a higher survival yield compared to ESI, wherefore the ions must have had a lower internal energy distribution, concluding that SAWN is a softer ionisation method than ESI. Yoon et. al. observed in 2014 a difference in fragmentation patterns with very labile phospholipids between SAWN-MS and ESI-MS. With SAWN-MS, there was no in-source fragmentation observed of the phospholipid, whilst there was with ESI-MS. A difference in fragmentation pattern was not confirmed by van der Heiden et.al. in the analysis of several explosives via SAWN-MS and ESI-MS (*Anal. Chem.* 2018 under review). However, significant differences in peak ratios were observed in the spectra of explosives, with the SAWN spectra preserving valuable information about the explosives.

Furthermore, SAWN has proven to be a successful ionisation method for bacterial phenotyping with MS, as well as for dye analysis from textile samples [10] (*J. Mass. Spectrom.* 2018 under review). The sample size for the textiles was reduced by a factor 10 up to 1000. SAWN has also proven to be compatible with online LC-MS analysis of protein digests. Low flow rates of 5 µL/min for the LC system were identified as the optimum condition for SAWN-MS. Higher flow rates had a negative impact on the signal to noise (S/N) ratio of SAWN-MS spectra. The compatibility of SAWN with online LC separation shows that this technique can be applied in wide variety of fields where precise separation is necessary.

This wide application field and rapid improvements of SAWN truly shows this technique holds the potential to compete with ESI for MS ionization, and can significantly reduce analysis time for pharmaceutical analysis in environmental analysis.

In this project ESI-MS was compared with a SAWN-MS set up testing the following analytes: diclofenac and carbamazepine dissolved in different types of water

3.2 Instrument

The ESI-MS experiments were conducted with a Bruker Daltonics micrOTOF mass spectrometer (Bremen, Germany) and acquisition of the data was achieved with the software from Bruker Daltonics, micrOTOFcontrol version 2.3. A syringe pump from New Era Pump Systems, Inc. (Farmingdale, USA) was used. The SAWN-MS experiments were conducted on a Sciex Triple TOF +5600 mass spectrometer (Concord, USA) and a SAWN device from Deurion (Seattle, USA). Piekview 2.0 software was used for data processing.

3.3 Chemicals

Formic acid (99.9%) and ammonium acetate (99.9%) were purchased from Sigma-Aldrich (Schnelldorf, Germany). Methanol (MeOH), acetonitrile (ACN) and water (H2O), all of MS grade, were purchased from Biosolve BV (Valkenswaard, the Netherlands). DCF and CBZ were purchased from Sigma-Aldrich (Schnelldorf, Germany) and supplied as standard solutions by KWR (Nieuwegein, the Netherlands), together with the surface water samples and the internal standard stock solutions 10 mg/mL in water (atrazine-d5, benzotriazole-d4 and bentazone-

d6). The internal standards, stock solutions of DCF and CBZ and surface water samples were stored at 4 °C.

The stock and working solutions of DCF and CBZ were prepared in same manner for both ESI and SAWN. The standard solutions of DCF and CBZ were diluted to form stock solutions of 1ppm. Working solutions were prepared by dilution of the stock solutions of DCF and CBZ. Serial dilutions were performed to obtain the appropriate working solutions for the calibration curves, and the internal standards were added to obtain final concentrations of 10ppb in each working solution. All the samples were vortexed for 30 seconds before acquisition. All the experiments were carried out in triplets.

3.4 Methods

The ESI-MS mass spectra were acquired in the mass range 100 to 400 m/z in positive and negative ion mode. The ESI source gas temperature was set to 200 °C with a flow rate of 4.0 µL/min. The source voltage was set to +2500 V with an end plate off set of -1000 V for CBZ. For DCF opposite parameters were used but in opposite polarity. The hexapole RF was set to 130pp. The flow rate of the syringe was set to 10 µL/min.

SAWN-MS spectra were acquired in the mass range 100 to 400 m/z in positive and negative ion mode. The source temperature was set to 150 ºC and the inlet and outlet gas pressure were set to 0 psi. The curtain was set to 10 psi (minimum). The SAWN-chip was placed approximately 2.0 centimetres right below the inlet of the mass spectrometer. The chip was controlled with software through an android tablet connected to the chip. The power ranged from 45% to 65%, corresponding to 3.375 to 4.875 Watts applied to the chip, depended on the solvent mixture. A higher power was used for more aqueous solvent mixtures

A droplet of approximately 1 µL was pipetted on the chip whilst continuous power was applied to the chip. The droplet nebulized after few seconds and entered the inlet of the mass spectrometer. This procedure of pipetting the sample on the chip was repeated until about 6 µL of the sample was infused in the MS and the mass spectra acquired (approximately 1 to 1.5 minutes). The data was accumulated into one data file for processing

3.5

The ESI-MS experiments were successfully conducted on the Bruker micrOTOF mass spectrometer. However, this MS instrument was not compatible with the SAWN.

The problem is probably caused by inefficient evaporation of the droplets of the sample before they reached the time-of-flight (TOF) mass analyser of the Bruker micrOTOF (Figure 12). This lead to an instable vacuum inside the mass analyser which causes to system to switch automatically to safety mode to restore the vacuum. The safety mode blocks the acquisition of the sample immediately as a result of which no samples spectra could be acquired. A stable pseudo vacuum of approximately 5×10^{-7} mbar inside the TOF mass analyser is required for the system to operate successfully. Every time nebulized aerosols of sample entered the MS, the monitored pressure inside the TOF jumped up to approximately 10^{-5} mbar, switching the system automatically to safety mode and blocking the acquisition of sample.

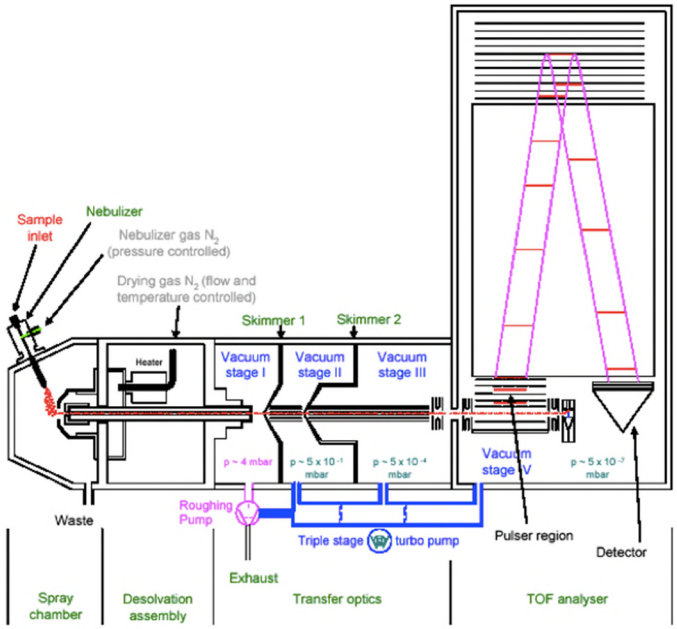


FIGURE 12: OVERVIEW OF DESIGN OF THE BRUKER MICROTOF MASS SPECTROMETER.

The inefficient evaporation of the sample can probably be traced back to the set-up of the Bruker micrOTOF (Figure 12). One reason could be the relative simple design of the Bruker micrOTOF compared to other more sophisticated instruments. The aerosols travel a relative short path to the TOF mass analyser of the Bruker. The pathway to the analyser consists of two skimmers, a hexapole and pressure system building down to approximately 5 x 10^{-7} mbar in the mass analyser (Figure 12). Other mass spectrometers have more sophisticated pathways with multiple vacuum stages, where the ions are filtered and guided by multiple electrically charged skimmers, quadrupoles and hexapoles. Most other mass spectrometers also have lower pressure systems than 10^{-7} mbar. All these factors ensure that only gaseous ions can survive in the mass spectrometer and reach the detector. It could be due to this simple design of the Bruker micrOTOF that the aerosols produced by the SAWN-chip did not evaporate completely, as a result of which nongaseous molecules reached the TOF mass analyser and forcing the system to switch to safety mode.

Additionally, the Bruker micrOTOF is the only instrument equipped with a 30 cm long glass capillary just behind the inlet of the mass spectrometer (Desolvation assembly) and this could also be the cause or contribute to the incomplete evaporation of the sample. The other mass spectrometers reported in literature involved in SAWN-MS research do not have any glass capillaries in their design. The instruments reported in the literature were from Waters, Thermo and Sciex.

Nevertheless, the exact cause for the incompatibility of SAWN and the Bruker micrOTOF is not fully understood. Further research on droplet size, ionization and pathway to the mass analyser on Bruker mass spectrometers should be conducted to understand the incompatibility of SAWN with this type of mass spectrometer.

As a consequence, the SAWN experiments were conducted on the Sciex Triple TOF +5600 mass spectrometer. This is a more comprehensive mass spectrometer compared to the

Bruker micrOTOF. It has three quadrupoles, three skimmers and a pressure system down to 10^{-9} mbar (Figure 13). This more complex pathway of the Sciex Triple TOF can improve the evaporation of the sample. The issues experienced with the Bruker micrOTOF did not emerge with the Sciex Triple TOF. This observation supports the hypothesis proposed earlier.

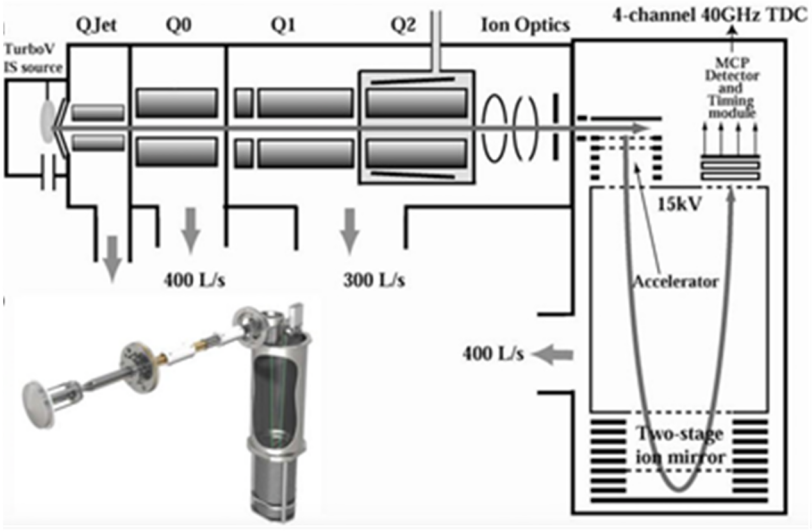


FIGURE 13: OVERVIEW OF DESIGN OF THE SCIEX TRIPLE TOF 5600+ MASS SPECTROMETER.

3.6 Solvent compatibility

This section reports a difference in solvent compatibility for SAWN and ESI. The optimal solvent composition mixtures of MeOH-H_2O were evaluated for SAWN and ESI.

The experimental conditions for ESI-MS and SAWN-MS were first optimized for CBZ in positive ion mode, and for DCF in negative ion mode. The optimized experimental settings were derived by monitoring the signal to noise (S/N) ratio of the signals specific for DCF and CBZ. For CBZ, this included the molecular ion $[M+H]^+$ at m/z = 237.1, and molecular sodium adduct $[M+Na]^+$ at m/z = 259.1. For DCF the molecular ion signal $[M-H]^-$ at m/z = 294.0 and the isotopic peaks at m/z = 296.0 and m/z = 298 were monitored.

The most commonly used solvent composition reported in the literature for the analysis of CBZ and DCF by ESI-MS is a mixture of MeOH: H_2O (9:1, v/v). To study the effect of different solvent compositions of H_2O and MeOH on the S/N ratio of DCF and CBZ, MeOH: H_2O compositions of (0.9:0.1), (0.5:0.5), and (0.1:0.9) (v/v) were evaluated. The S/N results of DCF and CBZ are shown in Table 11.

TABLE 11: S/N RATIOS OF CBZ (1PPM) AND DCF (1PPM) IN DIFFERENT SOLVENT COMPOSITION MIXTURES OF MEOH: H2O BY ESI-MS AND SAWN-MS.

Solvent mixture composition	CBZ - S/N ratios		DCF - S/N ratios	
	ESI-MS	*SAWN-MS*	*ESI-MS*	*SAWN-MS*
MeOH: H_2O (9:1, *v/v*)	≈ $1 * 10^4$	≈ $1.5 * 10^3$	≈ $1.6 * 10^4$	≈ $5.1 * 10^3$

MeOH: H_2O (1:1, *v/v*)	≈ 2 * 10^2	≈ 2 * 10^3	≈ 9 * 10^3	≈ 5.5 * 10^3
MeOH: H_2O (1:9, *v/v*)	≈ 10	≈ 2.4 * 10^3	≈ 1 * 10^2	≈ 7 * 10^3

Table 11 shows a better performance for ESI-MS with regard to the S/N ratio of DCF and CBZ for higher organic MeOH fractions. The S/N decreases in case of ESI-MS with an increasing amount of water. ESI relies on organic solvent. This behaviour is reported in the literature. For SAWN-MS, however, no such behaviour is observed. No significant difference is reported between the different solvent compositions. This is beneficial for the analysis of CBZ and DCF in surface water samples. SAWN is compatible with 100% pure aqueous solvents, which makes it possible to analyse CBZ and DCF directly in their original matrix. No need for an organic solvent component is a big advantage of SAWN over ESI. The transfer of a target analyte to an organic solvent before analysis involves extraction and dilution procedures, which are tedious and can be avoided when using SAWN. Also, addition of an organic solvent to the water sample would dilute the said.

However, it is important to bear in mind that different mass spectrometers were used for ESI and SAWN for above mentioned reasons. The absolute values of the S/N ratios in Table 11 from ESI and SAWN should therefore not be directly compared to each other.

3.7 Interpretation of ESI-MS and SAWN-MS spectra of carbamazepine

Experiments show that there is no differences in ion formation between SAWN-MS and ESI-MS for CBZ. The optimal experimental conditions and settings for ESI-MS (MeOH: H_2O (9:1, v/v)) and SAWN-MS (MeOH: H_2O (1:9, v/v)), as described before (see Table 11), were used for the acquisition of the spectra of CBZ. Figure 14 shows the spectra obtained by ESI-MS and SAWN-MS

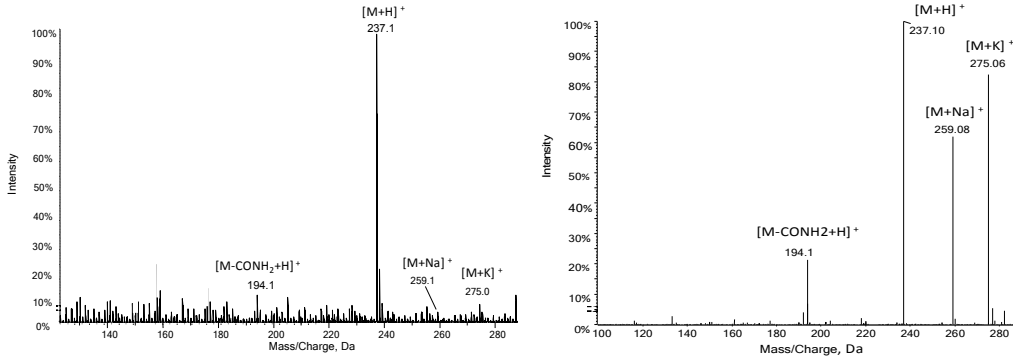


FIGURE 14: MASS SPECTRA OF CARBAMAZEPINE (1PPM) BY ESI-MS IN MEOH (LEFT): H_2O (9:1, V/V) AND SAWN-MS IN MEOH: H_2O (1:9, V/V) (RIGHT).

The ions shown in Figure 14 are consistent in all the evaluated experimental conditions for CBZ, concluding SAWN and ESI produce the same kind of ions for CBZ. The ions present are the molecular ion, the molecular sodium adduct and the molecular potassium adduct, respectively $[M+H]^+$ at m/z = 237.1, $[M+Na]^+$ at m/z = 259.1 and $[M+K]^+$ at m/z = 275.1. Also the fragment $[M+H\text{-}CONH_2]^+$ at m/z = 194.1, which is normally detected when analysing CBZ with tandem MS, showed up in both spectra. This ion is either formed by insource fragmentation or by decomposition of CBZ over time. The other spectra (not shown)

obtained by ESI-MS and SAWN-MS with the solvent compositions reported in Table 112 showed the same ions, but with lower S/N ratios.

A difference which is observed between the spectra in Figure 14 is a higher percentage of sodium and potassium adducts formed relative to the molecular ion with SAWN-MS compared to ESI-MS. One possible reason for this is the higher water content of the SAWN samples.

The ESI-MS experiments with samples consisting of high aqueous fractions (MeOH: H_2O (1:1, 1:9, v/v)) lacked signal intensities for CBZ (Figure 13). The addition of the additives ammonium acetate (AmAc) and formic acid were studied to evaluate their effect on the S/N ratios of the signals of CBZ. The additives are commonly reported to support ionization if S/N ratios are poor. AmAc was successful in improving the S/N ratios of CBZ samples with ESI-MS, intensities were increased with a factor 50. Formic acid did not show significant improvements (not shown).

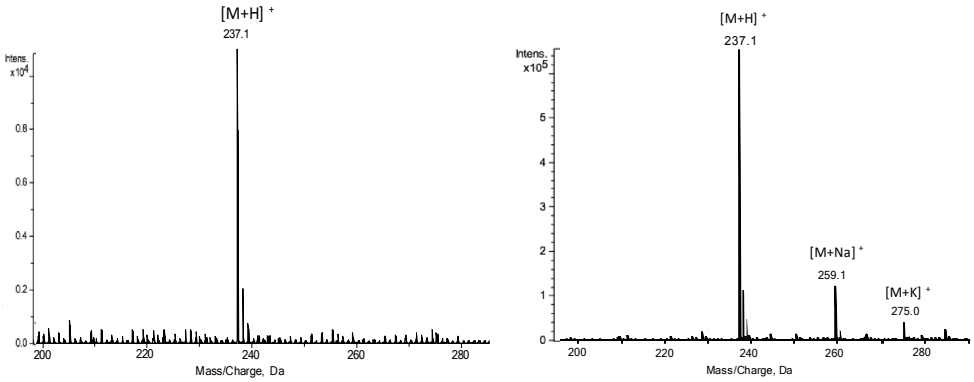


FIGURE 15: MASS SPECTRA OF CARBAMAZEPINE BY ESI-MS IN MEOH: H_2O (1:9, V/V) (A) WITHOUT AMMONIUM ACETATE (B) WITH 50MM AMMONIUM ACETATE.

Consecutively, the optimal conditions for SAWN-MS were used for the analysis of CBZ in MilliQ water to see if the aqueous solvent with a higher ionic strength has any significant effect on the spectrum. The results are shown in Figure 16. This figure shows no differences between the SAWN-MS spectrum of CBZ in LC/MS grade water (Figure 12) compared to the MilliQ matrix (Figure 16). The S/N ratio decreases with a factor of 10 in the MilliQ solvent but it can be concluded that SAWN-MS successfully detects CBZ in MilliQ and LC/MS grade water.

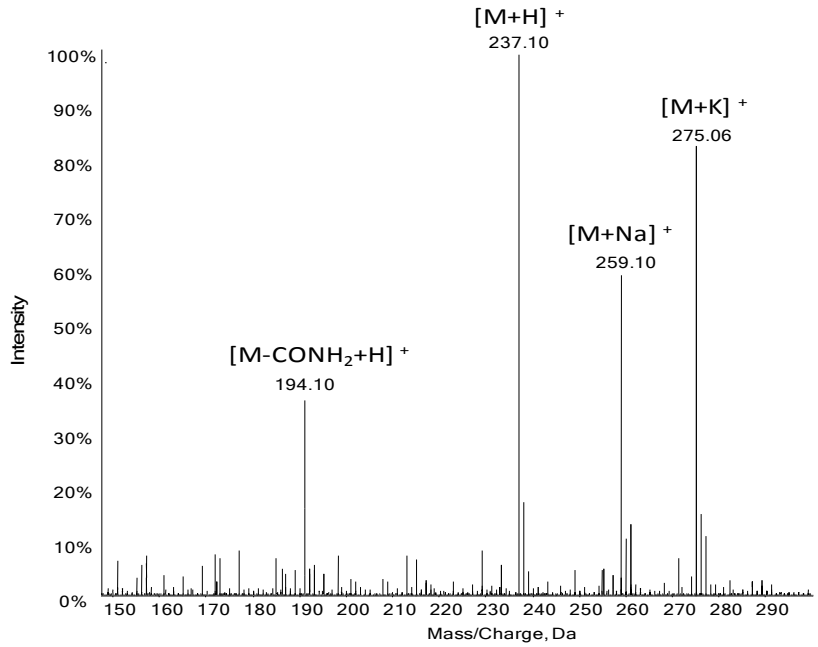


FIGURE 16: MASS SPECTRUM OF CARBAMAZEPINE (1PPM) IN MILLI-Q WATER OBTAINED BY SAWN-MS.

3.8 Interpretation of ESI-MS and SAWN-MS spectra of sodium diclofenac

The optimal experimental conditions for ESI-MS (MeOH: H_2O (9:1, *v/v*)) and SAWN-MS (MeOH: H_2O (1:9, *v/v*)) as described before (see Table 11) were used for the acquisition of the spectra shown in Figure 17. The results showed no difference in ion formation between SAWN and ESI.

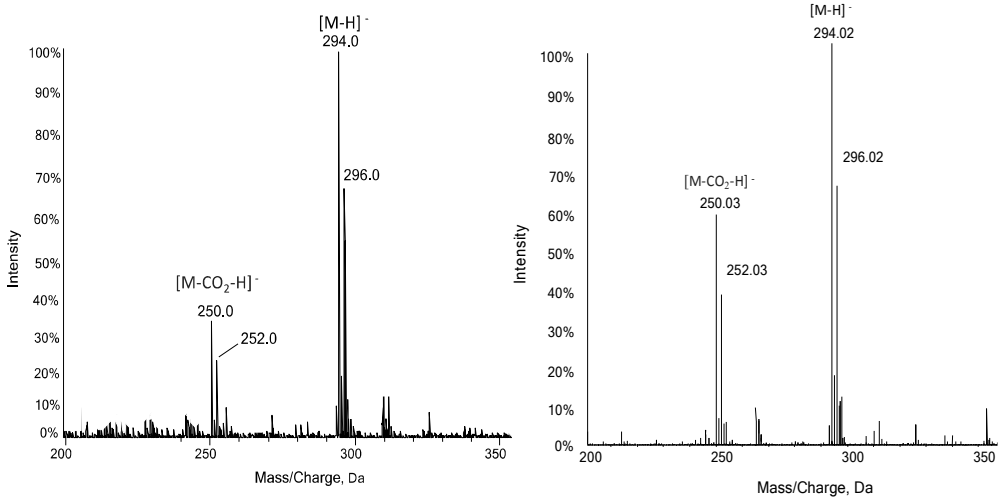


FIGURE 17: MASS SPECTRA OBTAINED FOR SODIUM DICLOFENAC (1PPM) BY (LEFT) ESI-MS IN MEOH: H_2O (9:1, *V/V*) (RIGHT) SAWN-MS IN MEOH: H_2O (1:9, *V/V*).

The ions observed in Figure 17 are consistent in all the evaluated experimental conditions for DCF, concluding SAWN and ESI produce the same kind of ions for DCF. The molecular ion $[M-H]^-$ is detected at m/z = 294.0, m/z = 296.0 and m/z = 298, with corresponding ratios of

9:6:1 between the peaks. This isotope pattern is produced by the chlorine isotope $Cl^{35}Cl^{37}$ pair of DCF, confirming this is the actual spectrum of DCF. The other major peak present in both the ESI and SAWN spectra, is the peak produced by the loss of a COO^- group of the molecular ion $[M- CO_2-H]^-$, at respectively m/z = 250.0, m/z = 252.0 and m/z 254.0. Again, the isotope pattern of chlorine can be seen. For the rest no significant differences are observed between the spectra. The ratios between the two different peaks are the same as well.

Consecutively, the optimal conditions for SAWN-MS were used to for the analysis of DCF in MilliQ water to see if a solvent with a higher ionic conductivity has any significant effect on the spectrum. The result is shown below in Figure 18 and no major differences between the SAWN-MS spectrum of DCF in LC/MS grade water (Figure 17) compared to a MilliQ solvent (Figure 18) is observed. SAWN-MS successfully detects DCF in MilliQ and LC/MS grade water.

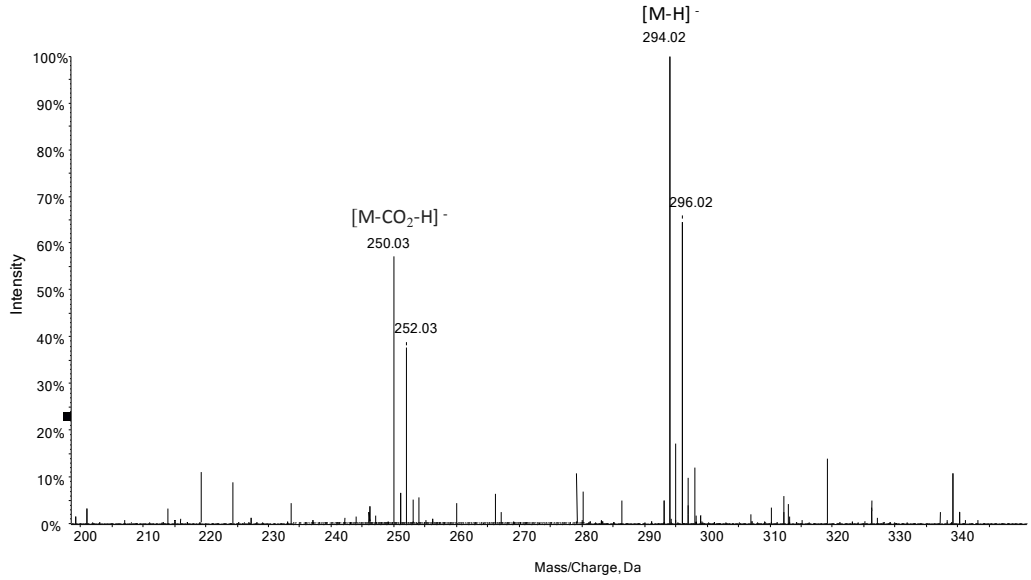


FIGURE 18: MASS SPECTRUM OBTAINED FOR SODIUM DICLOFENAC (1PPM) BY SAWN-MS IN MILLIQ WATER.

3.9 Calibration curves, LODs and LOQs for DCF and CBZ by SAWN-MS

SAWN-MS results have shown that DCF can be successfully analysed in negative ion mode and CBZ in positive ion mode using both LC/MS-grade and MilliQ water. The optimum experimental conditions found for DCF and CBZ in aqueous solvents were used to study the relation between the response of SAWN-MS and the concentrations of DCF and CBZ in H_2O (LC/MS-grade). For this purpose, calibration curves were constructed using various concentrations ranging from 500 ppt (ng/L) to 500 ppb (µg/L). The internal standards (I.S.) used for the calibration curves with the corresponding m/z signals and abbreviations are shown below in Table 12. Atrazine-d5 (ATZ5) was used for CBZ in positive mode, and benzotriazole-d4 (BZZ4) for DCF in negative mode. The concentrations of the two I.S. were set to 10 ppb, since this was the lowest accurate detectable concentration of I.S. close to the range of the analyte, to avoid big concentration difference between the I.S. and the analyte.

TABLE 12: I.S. USED FOR CALIBRATION CURVES OF DCF AND CBZ BY SAWN-MS.

I.S.	Abbreviation	Molecular weight	*m/z* signal positive (+) mode	*m/z* signal negative (-) mode
Atrazine-d5	ATZ5	221.13243 Da	221.13243	-
Benzotriazole-d4	BZZ4	124.08073 Da	124.08073	122.08

The ordinary least squares (OLS) regression model was used to fit the data points of the calibration curve and to study the linearity between the response and the concentration. Each concentration of DCF and CBZ was measured in triplets. Outliers were removed from the data set. Table 13 shows the data obtained for the calibration curves of CBZ, together with the average response and relative standard deviations (RSTD) of the analyte. Table 14 shows the same data for DCF. Figure 19, Figure 20 and Figure 21 show the calibration curves constructed from the data in Table 13 and Table 14 for DCF and CBZ.

TABLE 13: CALIBRATION POINTS AND RSDTS FOR CARBAMAZEPINE IN H_2O (LC/MS GRADE) BY SAWN-MS.

Conc. CBZ (ppb)	Average response of: $[M+H]^+$/I.S.	Average response of: $[M+Na]^+$/I.S.	RSDT for: $[M+H]^+$/I.S.	RSTD for: $[M+Na]^+$/I.S.
0	0,0027	0,0055	5.86%	4.76%
0.5	0.076	0.056	13.88%	6.34%
1	0.466	0.228	20.14%	30.34%
50	2.831	1.263	3.76%	25.38%
500	57.80	39.35	31.44%	3.56%

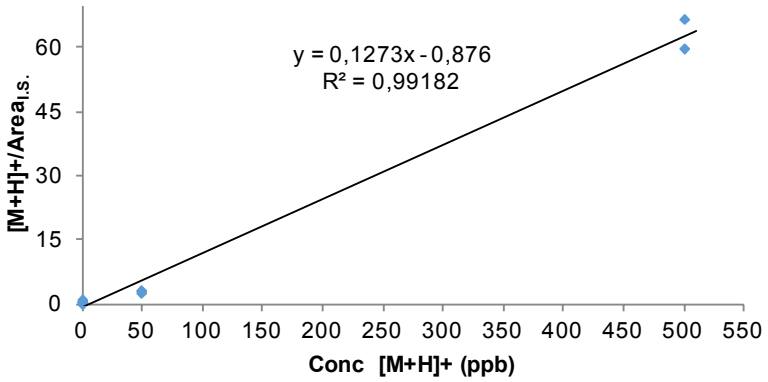


FIGURE 19: CALIBRATION CURVE FOR MOLECULAR ION OF CBZ IN H2O (LC/MS GRADE) BY SAWN-MS

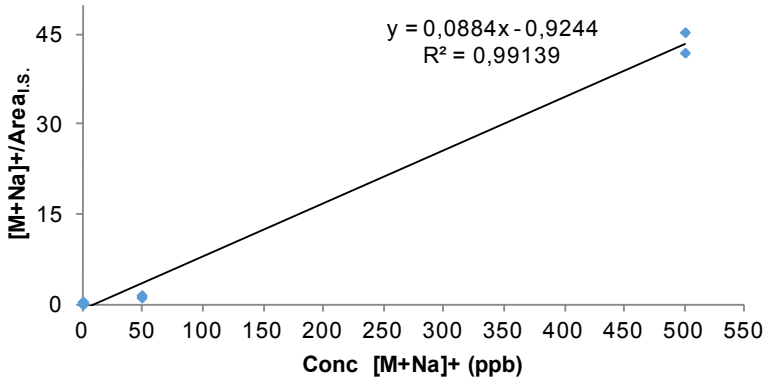


FIGURE 20: CALIBRATION CURVE FOR CBZ SODIUM ADDUCT IN H_2O (LC/MS GRADE) BY SAWN-MS.

TABLE 14: CALIBRATION POINTS AND RSDTS FOR DICLOFENAC IN H_2O (LC/MS GRADE) BY SAWN-MS.

Conc. DCF (ppb)	Average response of: $[M-H]^-$/I.S.	RSTD for: $[M-H]^-$/I.S.
0	0,007	4.34%
1	0.008	17.07%
5	0.039	14.11%
10	0.080	1.53%
100	0.795	2.14%
500	4.535	10.91%

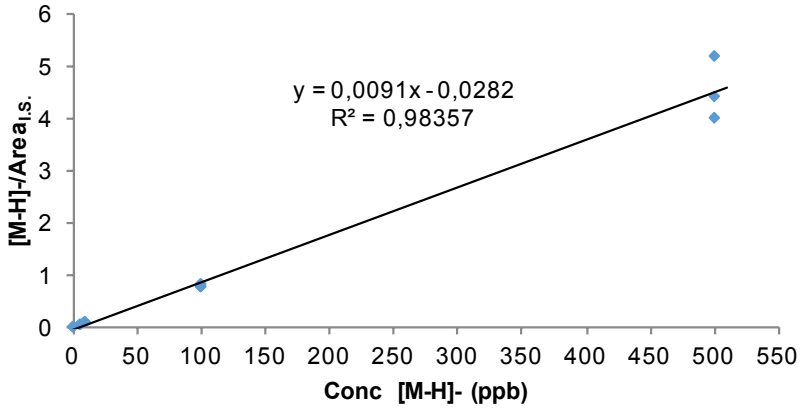


FIGURE 21: CALIBRATION CURVE OF THE MOLECULAR ION OF DCF IN H2O (LC/MS GRADE) BY SAWN-MS

The three calibration curves of CBZ and DCF show all excellent linearity correlation (R^2 > 0.98) between response (Y-axis) and the concentration of the analyte (X-axis) over the range of 500 ppt to 500 ppb (Figure 19 and Figure 20) for CBZ and in the range of 1ppb to 500 ppb for DCF.

The calibration curves have both negative Y-intercepts for DCF and CBZ, whilst a positive or zero Y-intercepts is expected with calibration curves. The negative Y-intercepts are not significantly large, -0.876, -0.9244 and -0.0282, respectively for DCF and CBZ. The negative Y-intercepts for DCF and CBZ indicate that either the fit of the OLS regression model needs improvement, or that the data provided for the model needs to be improved.

In some cases the RSTDs reflects a significant scattering of the MS signal for the samples of the same concentration (Table 13 and Table 14). This emphasises the necessity to measure each sample several times.

More points for DCF and CBZ in the lower ppt range were acquired, with the intention to include these points in the curve. However, at lower concentration no linear correlation was seen and therefore these measurements were not included. These factors all contribute to a less precise calibration curve and negative Y-intercepts, but overall the linearity of the curves shows an acceptable correlation in range from 500 ppt to 500 ppb.

One should bear in mind that calibration curves require an precise method with minimised manual error. With the current procedure of manually pipetting small dilutions of volumes in the µL range on the SAWN-chip, and nebulization of these small µL droplets in open air conditions, it is very hard to exclude all the manual errors in this procedure. At the moment, SAWN is less reproducible compared to other well established conventional ionization techniques (e.g. ESI, MALDI etc.). Especially the creation of vapour in open space makes this technique receptive for air movement caused by e.g. ventilation units.

The LODs and LOQs for DCF and CBZ in H_2O (LC/MS grade) were determined according to two procedures; the calibration curve slope method and by visual approximation. One can determine the LOD and LOQ from a calibration curve according to the following formulas; LOD = 3 * (STD of Y-intercept)/slope and LOQ = 10 * (STD of Y-intercept)/slope. The other method is more conventional; analyse a series of dilutions and find the spectrum of the analyte were the S/N ratio ≈ 5. The concentration of this sample times 3 equals the LOD, and

times 10 equals the LOQ. The results of the two methods for DCF and CBZ in MilliQ are shown below in Table 15.

TABLE 15: ESTIMATED LODS AND LOQS FOR DCF AND CBZ IN H_2O (LC/MS GRADE) ACCORDING TO THE CALIBRATION CURVE SLOPE METHOD AND VISUAL DETERMINATION.

Compound:	Calibration Curve Slope method:		Visual determination:	
	LOD (conc.)	LOQ (conc.)	LOD (conc.)	LOQ (conc.)
DCF	1.71 ppb	5.72 ppb	1.5 ppb	5.0 ppb
CBZ	0.55 ppb	1.48 ppb	0.3 ppb	1.0 ppb

As Table 15 shows, the LODs and LOQs predicted by the calibration curve slope method are close to the visual approximation method. The LODs and LOQs of the visual approximation are of the same magnitude, but have slightly lower values. This also suggest that the calibration curves can be improved to obtain the same results via the two different methods. It also suggests that the calibration curves are not really that far off, from the true LODs and LOQs of DCF and CBZ since the two methods give both almost the same values.

3.10 DCF and CBZ in surface water analysis by SAWN-MS

For the proof of principle of SAWN being compatible with MS without any invasive pre-sample treatment, surface water samples were spiked with 1 ppm DCF and CBZ and filtered once through a 0.45 μm filter and analyzed by SAWN-MS under the optimal conditions.

At first, the chip stopped nebulizing the sample, even when the power was increased to 100%. Consecutively, the chip started working again but the sample was not nebulized as observed previously with the LC/MS grade solvent and MilliQ water. The chip nebulized irregularly producing bursts of aerosols, however, no ions were detected.

After further inspection of the chip a coating was observed on the region where the sample was applied on the chip, impeding the nebulization process of the liquid sample. The surface of the chip on that spot was not smooth any more but felt rather rough. This section is marked in Figure 22.

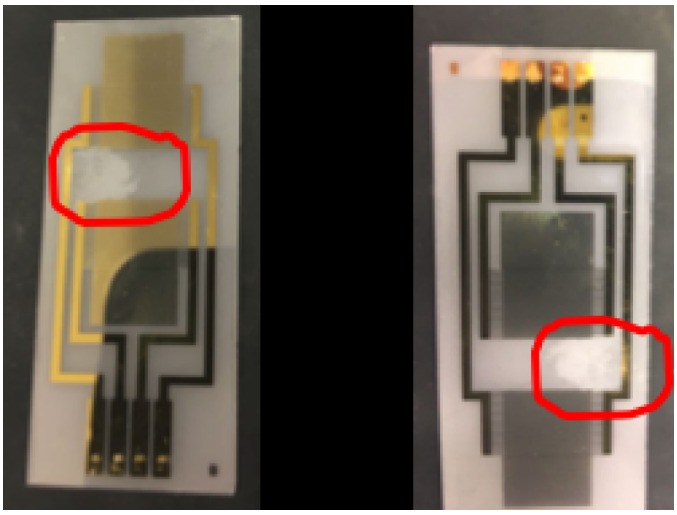


FIGURE 22: DAMAGED SURFACE OF THE SAWN CHIP AFTER SURFACE WATER ANALYSIS.

The exact cause for this behaviour of the nebulisation process and the damaged chip cannot be concluded directly from the obtained spectra. The main reason could be the need of several sample treatment steps since this behaviour was not observed with the MilliQ water and LC/MS grade water. Potentially, adjusting the pH of the sample and/or the removal of several organic (acidic) molecules present in the surface water, or the presence of several metals could solve this problem. For instance, a similar behaviour was observed in previous research carried out in the group of HIMS at UvA directed to the analysis of compounds that contain chromium and platinum. The surface of the chip should be studied to understand what might have caused the coating on the chip. A working online LC method for the separation of DCF and CBZ in surface water samples could be coupled to the SAWN chip to see if this procedure gives rise to the same issues as with the direct analysis described earlier.

3.11 Conclusions and future recommendations

In this report, the compatibility of SAWN with two different types of mass spectrometers was investigated, It was concluded that the SAWN is not compatible with the Bruker micrOTOF mass spectrometer. Two potential causes for this incompatibility can be named: The relative simple pathway to the mass analyzer and the glass capillary present in this instrument were suspected to prevent complete evaporation of the sample, and therefore preventing to maintain stable vacuum of 10^{-7} mbar inside the mass analyzer of the mass spectrometer. Hence, to obtain successful SAWN analysis, the design of the mass spectrometer should be considered before analysis, since SAWN might be incompatible with some specific types of mass spectrometers.

The potential of SAWN-MS for the analysis of DCF and CBZ in water samples was studied.

The results obtained by analyzing standards in water showed that SAWN-MS allows the identification of low ppb-levels within an analysis time of about 1 min.

The effect of different solvent mixture compositions of MeOH: H_2O (LC/MS grade) on the S/N ratios of DCF and CBZ were studied and compared by SAWN-MS and ESI-MS. SAWN showed to be compatible with 100% pure aqueous solvents which was not the case for ESI-MS. A higher solvent compatibility is suggested for SAWN, which is particularly interesting for surface water analysis due to the fact that the analyte can be analyzed in its original matrix.

The mass spectra obtained by ESI and SAWN for DCF and CBZ were compared with each other. No difference in ions were found which suggest that SAWN and ESI produce the same kind of ions for DCF and CBZ. More salt adduct formation, however, was observed when analyzing the pharmaceuticals in a more aqueous solvent. This could be the result of better adduct formation in more aqueous solvents. The calibrations curves, LODs and LOQs in H_2O were derived for DCF and CBZ by SAWN-MS. All the curves showed excellent linearity (R^2 > 0.98) in the range up to 500ppb. The LODs and LOQs were derived according to the calibration curve slope method and by visual approximation. The LOD and LOQ in Milli-Q water for the molecular ion of DCF were estimated between 1.5 – 1.71 ppb and 5.0 – 5.72 ppb respectively, which is in the range of concentrations frequently detected in the environment. The LOD and LOQ for the molecular ion of CBZ were estimated between 0.3 – 0.55 ppb and 1.0 – 1.48 ppb respectively, which are also in the range of concentrations in which this pharmaceutical is detected in in the environment.

It was also concluded that there is still room for improvement of the calibration curves, since the two methods for LOD and LOQ determination gave not yet the exact same results and because negative Y-intercepts of the calibration curves were observed. This could be corrected in future experiments by either including more calibration points that are more evenly spread out in the range of the curve, or by using a different model (e.g. weighted least square) to the current points.

The direct analysis of spiked surface water samples with DCF and CBZ by SAWN-MS was not successful. The surface of the SAWN-chip where the sample was applied showed a coating and the liquid was not nebulized. The cause of this behavior remains to be further investigated and future research should be conducted to establish the type of sample pre-treatment required for the successful analysis of DCF and CBZ in surface water samples by SAWN-MS.

4 Paper spray

4.1 Introduction

PaperSpray belongs to the ambient atmospheric pressure ionisation (AAPI). [11]All AAPI techniques have in common that they aim at direct sampling of analytes in untreated samples in their ambient state. PaperSpray is a simple technique for introducing unprocessed samples of fluids to the mass spectrometer. PaperSpray combines paper chromatography substrates and electrospray ionisation to enable direct analysis of dried fluids without off-line reconstitution or liquid chromatography. This method is suitable for fast qualitative and quantitative analysis of complex matrices.

4.2 Principle

For this method no sample preparation is necessary if a liquid sample is to be analysed. This means the range of application spans from tap water to sewage influent. Figure 1 shows the general principle of this methods. [11]

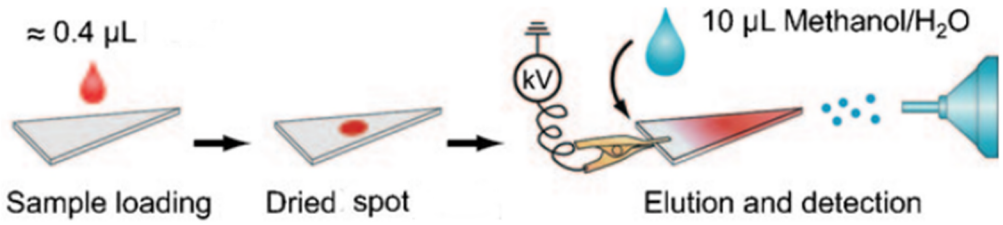


FIGURE 23: PRINCIPLE OF PAPERSPRAY [11]

A droplet of about 0.4 µL will be added to a triangular piece of paper. The droplet is allowed to evaporate and the compounds remain on the paper. It is crucial at this point not to add too much liquid because this would lead to leaking through the paper and inevitably a quantitative analysis of the sample would not be possible anymore. In the next step about 10 µL of a methanol/H_20 mixture are added to the paper at the wide end and a voltage is applied to the paper. Under the influence of the voltage the compounds will be ionised and move toward the sharp point of the paper. From there they will enter the MS inlet. The sharp point of the paper is placed in front of the MS inlet in such a way that quantitative analysis is possible. Alternatively, any other conducting paper or material with a sharp end can be used.

At the tip of the paper the charge accumulates and a Taylor cone is formed, which releases an aerosol (fine liquid droplets in a gas). Each of these droplets carries several like-charged ions (positive or negative). When due to heat these droplets have shrunk to a certain size (Rayleigh limit) the ions in the droplet start to repel each other (coulombic repulsion) which leads the formation of even smaller droplets and eventually bare ions. These ions will then enter the mass analyser.

4.3 Detection of Drugs of Abuse

Two papers have demonstrated the applicability of paper spray as a method to detect drugs of abuse in various bodily (fluids) [12,13]. A solution of heroin in methanol/water was added onto paper and the compounds were analysed in positive mode. The protonated heroin *m/z* 370 was detected and its identify was confirmed by MS/MS (Figure 24: Analysis of Heroin soaked paper with paperspray-MS in positive mode [13]. In another experiment a solution of cocaine was analysed. The concentrations used for the experiments are in the order of several µg/L.

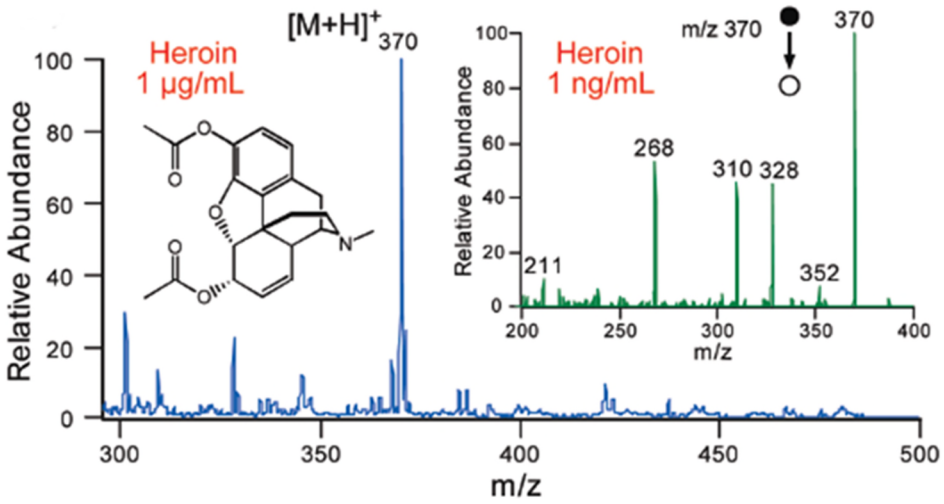


FIGURE 24: ANALYSIS OF HEROIN SOAKED PAPER WITH PAPERSPRAY-MS IN POSITIVE MODE [13].

4.4 Combination of Chromatography and PaperSpray

The fact that any conductive paper can be used for PaperSpray MS allowed to explore the possibly to employ chromatographic paper. For the experiment two different dyes were separated chromatographically using methanol. After the separated was completed the part with the dyes were cut from paper as shown in Figure 25. Both paper triangles were analysed separately. The data from the MS confirmed what can be seen with the naked eye. On the triangle with the green dye the dominant mass was *m/z* 284 and on the triangle with the purple dye the mass *m/z* 358 significantly outnumbers the mass of the green dye.

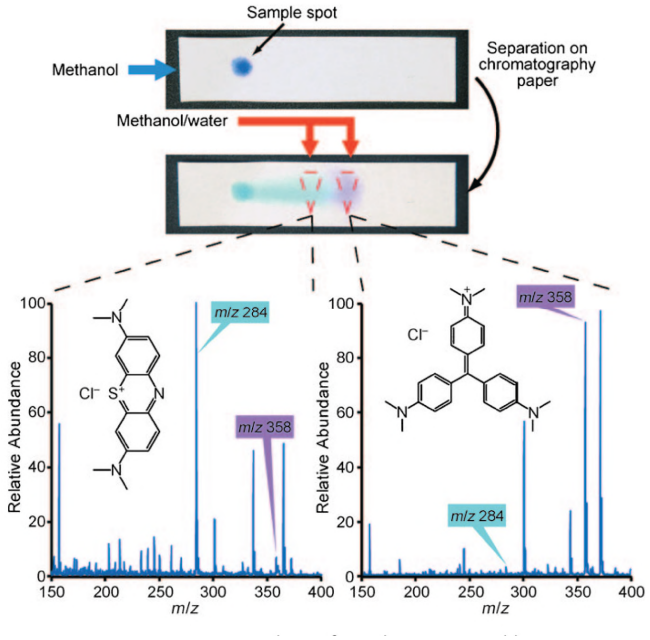


FIGURE 25: CHROMATOGRPAHIC SEPARATION OF TWO DYES WITH SUBSEQUENT PAPERSPRAY ANALYSIS.

5 Conclusion

Two ionisation techniques for mass spectrometry were evaluated and their applicability for employment in the water sector was assessed. The two techniques are the surfaced assisted wave nebulisation (SAWN), a method that is still under development and direct analysis in real time (DART), a commercially available technique. Both techniques work at ambient atmosphere and no chromatographic separation of the samples is needed. The sample is ionised and directly introduced into the mass spectrometer. This means that both techniques are comparable to direct infusion with a few important differences. In both cases (I) only volatile compounds can enter the mass spectrometer. This means that without risk of contamination the MS, samples containing high amount of inorganic salts or large organic compounds (e.g. plastic, wood, leaves) can be analysed. Both techniques (II) also share the advantage that measurements in general are very fast and (III) can be done without any sample pre-treatment. A sample can therefore be analysed within a few minutes. Using an autosampler for DART even enables measurement of about 50 - 60 samples per hour. As for the SAWN no autosampler exists yet, realistically no more than 20 samples can be measured. Both techniques are fast an can compete with the direct injection method in that regard. In case of practicability both techniques outperform the direct injection. Both techniques however suffer from the disadvantage that the amount of sample entering the orifice of the MS highly depends on the thoroughness of the user and therefore reproducibility will not be as good as in case of a direct injection. This is a consequence of the fact that the sample is ionised in front of the MS inlet and moves without any protection towards the inlet. Therefore sample can get lost on the way to the inlet. This problem was clearly visible in case of the SAWN technique as the sample is vaporised. The formed plume is visible to the naked eye. However, there are possibilities to minimise this effect.

The next question that needed to be answered is if SAWN and DART can be used for environmental samples with concentrations typically in the µg/L (ppb) rang. Therefore, aqueous samples with environmentally relevant compounds were tested. Two compounds were investigated. Carbamazepine, a compound that is measured in positive mode and diclofenac, a compound that is measured in negative mode. The results of the experiments show that SAWN is capable to reach ppb levels for both compounds, whereas the DART ionisation method only succeeded in reaching ppb levels for carbamazepine. The lowest possible limit for diclofenac was in the ppm range, which is not a realistic concentration in an environment sample, except in case of a calamity. However, it must be emphasised that these experiment were done mainly in MilliQ water or MilliQ water with additives. So far, environmental sample were not tested. This would be the next step.

The overall verdict for these ionisation techniques is positive. Both techniques have the great advantage that they are very fast techniques, allow for large numbers of samples to be measured in relatively short a time and tolerate “dirty” samples. The current shortfalls, such as the way the sample is presented and the detection limits, can be overcome in time. These are problems that can be expected when techniques are used for the first time in a new employment field. The next step for both techniques would be to analyse environmental samples, spiked and non-spiked.

One thing that need to be considered is that the large amount of data that is acquired also needs to be interpreted. Therefore, for these techniques to be become practical, suitable

software needs to be developed as well. Otherwise more data will be generated than ever can be evaluated.

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