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A quantitative approach for pesticide analysis in grape juice by direct interfacing of a matrix compatible SPME phase to dielectric barrier discharge ionization-mass spectrometry

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Abstract

We evaluated the performance of a dielectric barrier discharge ionization (DBDI) source for pesticide analysis in grape juice, a fairly complex matrix due to the high content of sugars ($\approx 20\%$ w/w) and pigments. A fast sample preparation method based on direct immersion solid-phase microextraction

(SPME) was developed, and novel matrix compatible SPME fibers were used to reduce in-source matrix suppression effects. A high resolution LTQ Orbitrap mass spectrometer allowed for rapid quantification in full scan mode. This direct SPME-DBDI-MS approach was proven to be effective for the rapid and direct analysis of complex sample matrices, with limits of detection in the parts-per-trillion (ppt) range and inter- and intra-day precision below 30% relative standard deviation (RSD) for samples spiked at 1, 10 and 10 ng/ml, with overall performance comparable or even superior to existing chromatographic approaches.

Keywords: DBDI, active capillary plasma ionization, ambient mass spectrometry, SPME, pesticides, complex matrices, matrix effects.

Introduction

In recent years, a large number of ambient ionization techniques have been reported, with desorption electrospray ionization (DESI) and direct analysis in real time (DART) among the most widespread^{1,2}. In addition, dielectric barrier discharge ionization (DBDI) sources have attracted a lot of attention in the scientific community, after the introduction of the low temperature plasma (LTP) source³.

1 A dielectric barrier discharge is achieved when an alternating current is delivered to two
2 electrodes that are separated by a dielectric material. Several DBD configurations with
3 different electrode and dielectric dimensions and shapes have been reported ⁴⁻⁶. The applied
4 current induces a polarization of the dielectric and a mild discharge, which generates the low-
5 temperature plasma. In general, a DBD generates a non-equilibrium plasma, meaning that the
6 charged species generated have a higher kinetic energy than the neutral molecules. The fairly
7 low overall energy involved in the discharge leads to the cold nature of the resulting plasma.
8 When a DBD is used to ionize gas-phase molecules, the amount of energy that is transferred
9 to them is generally relatively low. Fragmentation of the analytes can be observed, to some
10 extent, by tuning specific source parameters like electrode dimensions, operating voltage and
11 frequency⁷, type of gas, as well as with a longer residence time of the analytes inside the
12 plasma. In our setup, the molecules mainly interact with the reactive species generated by the
13 plasma, causing little or no fragmentation during the ionization process. The great success
14 and increasingly widespread use of DBDI sources for ambient mass spectrometry in the last
15 few years is also due to the sensitivity of this technique, since the ionization efficiency can be
16 very high.

17 In this work, we employ an “active capillary” embodiment of a DBDI source developed in
18 our research group ^{8,9}, and interface it directly to a novel solid-phase microextraction (SPME)
19 device. The first direct SPME-DBDI-MS and the SPME-GC-DBDI-MS hyphenations were
20 recently reported by Mirabelli et al. ^{10,11}, successfully using a low temperature plasma source
21 for pesticide and drug quantification. Compared to existing direct couplings of SPME to MS
22 ¹², using DART ¹³, nano-ESI ¹⁴ or DESI ¹⁵, the approach proposed here is based on a thermal
23 desorption of the analytes prior to ionization. These two steps remain separate, and can be
24 tuned independently, allowing for a more flexible method development. In addition, this
25 setup does not require a precise tuning of the position of the SPME device in the ionization
26 source or in front of the mass spectrometer inlet, parameters which are critical in the
27 aforementioned SPME direct coupling approaches to obtain reproducible results.

28 Although several geometries of SPME were reported, the most widespread one to date is
29 certainly the fiber geometry, in which a thin layer of extracting phase material (coating) is
30 used to coat a fused silica fiber or a metal wire. Recently, thermal desorption based SPME
31 approaches have moved a step ahead toward the analysis of complex matrices, especially
32 after the introduction of new, matrix-compatible SPME coating ¹⁶. The anti-fouling properties
33 of the matrix compatible coating are due to a thin (10-20 μm) and smooth outer layer of pure

polydimethylsiloxane (PDMS) that overcoats the inner SPME extractive phase, preventing irreversible attachment of matrix components¹⁶. The use of this matrix compatible coating enabled the analysis of pesticides in various complex matrices, with very satisfactory results in terms of durability of the SPME coating, reproducibility and limit of quantitation obtained, often achieving better or comparable performance than other conventional sample preparation approaches used for food contamination assessment^{17–20}. One of the main features of the matrix-compatible coating is its ability to endure direct immersion in complex food matrices and avoid the attachment of matrix constituents, which would undergo degradation during thermal desorption, and thus degrade the surface of the SPME phase, compromising its extraction performance. The inertness of the SPME coating towards matrix components results not only in better performance and longer coating lifetime (over 100 consecutive extractions), but also in reduced instrumental contamination¹⁸. The latter is especially important when a direct coupling of SPME to mass spectrometry is carried out²¹. In this work, a novel matrix compatible PDMS/DVB/PDMS fiber was used to quantitatively determine pesticides in grape juice. The matrix compatibility observed for this method allowed the simultaneous analysis of several pesticides, avoiding the need for additional sample extraction, pre-treatment and derivatization. In this way, conventional and time-consuming chromatographic separation can be circumvented, and quantitative determination of the analytes by DBDI-HRMS was achieved with superior results compared to the conventional approaches based on GC-EI-ToF/MS.

Experimental section

Materials. HPLC-grade water and acetonitrile and dimethyldichlorosilane (DMDCS, 5% in toluene) were obtained from Sigma-Aldrich (Buchs, Switzerland). PESTANAL© analytical-grade standards were purchased from Sigma-Aldrich: famphur, parathion, parathion-met, sulfotep, thionazin, ametryn, atrazine, atrazine-D₅, metolachlor, prometon, prometryn, terbutryn, propazine, simazine, pyrimethanil, cyproconazole, quinoxifen, flusilazole, trifloxystrobin, azoxystrobin, pyridaben, pyraclostrobin, pyriproxyfen, tebuconazole, cyprodinil, difenoconazole and malathion. Organic grape juice was purchased at local grocery stores in Zurich (Switzerland) and in Waterloo (ON, Canada). PDMS/DVB/PDMS coatings were prepared in-house following the procedure described by Souza-Silva et al.¹⁶

Sample preparation. Individual pesticide standard stock solutions were prepared at a concentration of 5 mg/mL in acetonitrile, and stored at -20 °C. A mix of the pesticides at

100 µg/mL in acetonitrile was prepared from the stock solutions. Diluted solutions used for SPME analysis were prepared in ACN from the mix and were stored at a temperature of 4 °C. The grape juice samples were spiked with an aliquot of the pesticides standards mixtures, always keeping the total amount of solvent spiked in the matrix below 1% (v:v).

SPME extraction. The extractions from grape juice were done using PDMS/DVB/PDMS fibers. For the SPME-DBDI-MS experiments, a pre-equilibrium extraction time of 20 min was chosen, performed at room temperature (25 °C) with a magnetic stirring of 700 rpm. The extraction volume was 9 mL. For SPME-GC-EI-ToF/MS experiments, an extraction time of 30 min was chosen, since for this approach the extraction time is less important, because one needs to wait for the chromatographic run to terminate anyway before performing the next injection.

Post-extraction SPME cleanup. After each extraction from grape juice, the fibers were rinsed by immersion into HPLC-grade water for 3 seconds. This cleanup step was also performed at room temperature (25 °C), with a magnetic stirring of 700 rpm and a volume of 9 mL of water. At the end of the rinsing step, the fiber was gently dried by wiping it with a Kimwipe® tissue.

Desorption system for SPME coupling to DBDI. SPME fibers were desorbed in a lab-built stainless-steel desorption chamber (Fig.1). A constant gas flow was maintained in this chamber when connected to the MS through a leak-proof connection with the DBDI source. The desorption chamber used in a previous study¹⁰ was modified, to reduce adsorption of the analytes desorbed from the fiber to the metal walls of the desorption chamber. This was necessary because of the complex nature of the matrix analyzed. The internal diameter of the chamber was adapted to accommodate a glass capillary, whose walls were passivated by silanization using DMDCS (5% in toluene). The glassware was rinsed with DMDCS for 15 seconds, rinsed three times with toluene and methanol, and dried with nitrogen. A gas overflow was maintained behind the fiber location in the chamber, to avoid any dilution of the analytes or backflush. The gas flow inside the glass capillary was generated from the underpressure inside the MS and not from a backpressure. The ionization source could be kept at ambient pressure, to ensure its correct operation.

Mass spectrometry. The mass spectrometer used for the direct analyses was a Thermo LTQ Orbitrap (Thermo Fischer Scientific). The total gas flow entering the source was constant and dictated by the inlet of the mass spectrometer, i.e., the metal transfer capillary dimensions, and measured to be 0.7 L/min. The optimization of the ionization source parameters has been described elsewhere^{10,22}. Quantification was performed in positive ionization mode, with full

scan centroid acquisition and a mass resolution of 30,000 FWHM at 400 m/z . The MS interface parameters were as follows: capillary voltage, 4 V; tube lens voltage, 65 V; capillary temperature, 275 °C. Data acquisition was performed with a mass window of 200 to 500 m/z , with 1 microscan, and with a maximum injection time of 200 ms. AGC was on. With our version of the Orbitrap and with the resolving power used, the scan speed was up to 1.5 scans per second.

Fig. 1. Schematic of the “in-line geometry” DBDI source used in this study. The analytes are thermally desorbed from the SPME fibers and introduced in neutral form into the ionization source together with humidified nitrogen. The reactive species responsible for the ionization are generated inside the source, resulting in a very high ion transmission to the MS, and a more consistent sampling. The inner (I.E.) and outer (O.E.) electrodes of the DBDI source are shown.

Ionization source. The details of the active capillary plasma source have already been described elsewhere^{10,22}: an AC voltage is applied to two concentric tube-shaped electrodes that are separated by a glass capillary connected to the MS-inlet. The DBDI voltage was 1.6 kV_{p-p}, applied between the inner and outer electrode. The operating frequency was 5.75 kHz. The resulting low-temperature plasma generates mostly protonated ions, with little to no fragmentation. This is mainly due to the very short residence time of the compounds inside the reactive plasma, which is generated between the electrodes and has a tube shape. This particular plasma shape results in the gas phase neutral analytes to come in contact with the plasma itself only for a very short time, while mostly interacting with plasma-generated reactive species during transfer into the vacuum.

Although air and nitrogen can be used as reagent gasses, with similar performance, the latter was used to avoid interferences from room air contaminants (e.g., plasticizers). The high temperatures required for SPME fibers during the desorption also required the use of nitrogen to avoid oxidation of the extracting polymers. The nitrogen was humidified to 90% (R.H. at

25 °C) to increase sensitivity: an increased concentration of water and hydronium clusters inside the source allows more efficient transfer of protons to the neutral analytes.

Quantification. A matrix-matched calibration approach was used as quantitation strategy. For DBDI analysis, samples were spiked with pesticide concentrations between 1 pg/mL and 100 ng/mL, and analyzed in triplicate. Atrazine-D₅ and metolachlor were used as internal standards at a concentration of 1 ng/mL. The mass accuracy achieved was very satisfactory, with a relative error below 1.5 ppm when using lock masses. A mass tolerance of 2 ppm was used to generate extracted ion chromatograms (EICs), integrated over a time period of 2 minutes from the fiber exposure inside the desorption chamber. This time interval ensured that most of the ion signal was integrated. In all cases, a 1/x weighting was used for the calibration. The limits of quantification (LOQs) were calculated according to FDA guidelines (lowest calibration point having RSD < 20% and accuracy between 80 and 120%).

Moreover, the LODs were verified experimentally, by analyzing samples with decreasing concentrations of analytes. No background noise was observed in the EIC within a 2 ppm mass tolerance window around the exact mass of each compound, due to the use of the HR-LTQ Orbitrap/MS. Therefore, the classical approach based on the evaluation of signal-to-noise ratio could not be applied.

For quantification by GC-EI-ToF/MS, Atrazine-D₅ was used as internal standard, spiked at 10 ng/mL. Calibration curves were obtained in a concentration range within 0.5 and 100 ng/mL. Chromatograms were acquired in full scan mode and analyte specific quantitation ions were extracted for peak integration. A library match threshold of 700 was selected as criterion for peak rejection. LOQs were determined from the lowest calibration point of the calibration curves providing accuracy within 20% of the nominal concentration and RSD < 20%, as per Food and Drug Administration guidelines.

GC-EI-ToF/MS analysis. Automated SPME extraction/desorption, separation and detection of targeted analytes were performed by a GC-EI-ToF/MS Pegasus 4D (LECO Corp., St Joseph, MI, USA) equipped with a GERSTEL MPS2 MultiPurpose Autosampler (Gerstel Inc., USA). An Agilent 7890 GC oven was used as a chromatographic system, equipped with a commercial capillary column (Rtx®-5SilMS, 30 m x 0.25 mm x 0.25 µm, Restek Corp., Bellefonte, PA, USA). Ultra-high purity helium (99.999%) was used as carrier gas with a constant flow rate of 1.2 mL/min. Desorption of analytes from the SPME coating was performed in splitless mode at 270 °C for 1.5 min. Evaluation of carryover of analytes onto the fiber coating was performed by re-desorbing the same coating immediately after analysis. Chromatographic separation of analytes was achieved using the following temperature

program: an initial temperature of 35 °C was held for 5 min; a ramp of 6 °C/min was then used to reach a temperature of 250 °C, which was then held for 10 min, for a total run time of 50.83 min. The transfer line and ion source temperatures were 250 °C and 200 °C, respectively. A solvent delay of 60 s was used. Electron impact ionization was performed at 70 eV; ions ranging from 35-600 m/z were acquired.

Results and discussion

In this work, we tested the performances of the matrix-compatible SPME coatings, namely PDMS/DVB/PDMS, for the first time directly coupled to DBDI-mass spectrometry, using the analysis of pesticides in grape juice as an example. The selection of this matrix represents a challenging case, due to its high pigment and sugar content. The composition of the grape juice matrix can easily induce side reactions during thermal desorption, resulting in artifacts in gas chromatography²³. Among the advantages of matrix compatible SPME coatings are a greatly reduced risk of instrument contamination and less matrix effects and chromatographic interferences. Both are very useful for direct coupling of the technique to mass spectrometry^{18,19,24}. Even if the use of matrix compatible coatings drastically reduces these problems, it is critical to assess the efficiency of the SPME-DBDI coupling, where chromatography is not performed, because matrix effects and contamination could in principle be even more pronounced.

Firstly, the extraction time profile of the analytes in grape juice was investigated, using durations between 5 and 120 min, by using the traditional GC-EI-ToF/MS setup. Considering that most of the analytes under investigation did not reach equilibrium even at 120 min, a 30 min of extraction time was selected as a good compromise between sensitivity and method throughput. To increase throughput, the extraction time was reduced to 20 min for the direct SPME-DBDI-MS approach.

When chromatography is bypassed, all the compounds desorbed from the SPME fibers are ionized and detected simultaneously. Therefore, the total ion current (TIC) obtained consisted of only one peak that included characteristic $[M+H]^+$ ions of all pesticides tested (Fig.2). Pesticides belonging to different chemical classes and with a broad range of physico-chemical properties were extracted and quantified by the direct coupling approach. In Figure 3 calibration curves of representative target analytes are shown, and in Table 1 detailed information regarding the validation parameters are shown.

Fig. 2. a) TIC-MS and b) high-resolution mass spectrum of the direct SPME-DBDI-MS (positive ionization mode) of a mix of pesticides (see table 1) at 10 ng/mL, analyzed with a LTQ Orbitrap mass spectrometer. Some of the pesticide peaks are labelled (see Table 1 for their exact masses).

Fig. 3. Direct SPME-DBDI-MS calibration curves for prometryn, terbutryn, trifloxystrobin and sulfotep, belonging to different chemical classes (triazines, beta-methoxyacryl esters and organophosphates). Prometryn and terbutryn were quantified together since they are isomers, and could not be resolved by MS.

Compound	exact mass [M+H] ⁺	I _s	calibr range pg/mL	LogP	Curve equation	LOQ ^a	Exp. LOD ^b	R ²
						pg/mL	pg/mL	
Famphur	326.02803	A	100-10k	2.1	y=5.51E-04x-6.83E-02	300	100	0.995
Parathion	292.04031	M	3k-100k	3.8	y=9.67E-06x-3.63E-02	10000	3000	0.981
Parathion-met	264.00901	M	10k-100k	2.7	y=8.44E-06x-7.28E-02	30000	10000	0.997
Sulfotep	323.03001	A	30-30k	4.0	y=1.57E-03x-5.13E-02	100	30	0.998
Thionazin	249.04573	A	30-100k	1.6	y=1.37E-03x-5.65E-02	300	30	0.999
Ametryn	228.12774	M	10-10k	3.1	y=1.48E-04x-2.54E-03	100	10	0.987
Atrazine	216.10105	M	10-100k	2.6	y=1.64E-04x-7.96E-04	300	10	0.998
Prometon	226.16624	A	10-10k	2.9	y=2.82E-04x-2.51E-03	300	10	0.993
Prometr+Terbut	242.14339	A	10-10k	-	y=6.86E-03x-1.14E-02	30	10	0.995
Propazine	230.11670	M	10-100k	3.0	y=4.76E-04x-4.17E-03	30	10	0.998
Simazine	202.08540	M	10-100k	2.3	y=5.49E-05x+5.96E-04	300	10	0.998
Pyrimethanil	200.11822	A	10-3k	2.8	y=3.48E-03x+1.86E-01	30	-	0.990

Cyproconazole	292.12112	M	100-30k	2.7	$y=1.56E-04x-2.75E-02$	1000	100	0.988
Quinoxifen	308.00397	A	3-3k	6.3	$y=7.01E-03x-2.58E-03$	30	3	0.995
Flusilazole	316.10761	M	10-3k	3.9	$y=1.32E-03x-1.63E+02$	100	10	0.996
Trifloxystrobin	409.13697	M	3-3k	5.1	$y=2.84E-03x-8.50E-03$	10	3	0.999
Azoxystrobin	404.12410	A	100-3k	5.1	$y=5.23E-04x-6.35E-02$	300	100	0.986
Pyridaben	365.14489	A	30-10k	4.7	$y=1.53E-03x-4.66E-02$	100	10	0.998
Pyraclostrobin	388.10586	A	3-10k	4.3	$y=1.02E-02x-8.82E-02$	100	3	0.933
Pyriproxyfen	322.14377	A	3-3k	4.8	$y=1.30E-02x-3.15E-02$	10	3	0.998
Tebuconazole	308.15242	A	10-3k	3.6	$y=1.44E-03x-2.13E-02$	100	10	0.995
Cyprodinil	226.13387	A	3-3k	4.0	$y=1.08E-02x-6.6E-03$	100	-	0.984
Difenoconazole	406.07197	M	10-3k	4.9	$y=5.63E-04x-8.54E-03$	100	10	0.988
Malathion	331.04334	M	1k-30k	2.9	$y=2.50E-06x-6.69E-04$	1000	1000	0.999

Table 1. Linear regression parameters for the pesticides investigated, determined by SPME-DBDI-MS. ^acalculated based on FDA guidelines, ^bdetermined by injecting standards at decreasing concentrations. Two internal standards at 1 ng/mL were used, i.e., atrazine-D5 (A) and metolachlor (M).

The direct SPME-DBDI approach showed the highest sensitivity for quinoxifen, trifloxystrobin, pyraclostrobin and pyriproxyfen, with experimental LODs of 3 pg/mL. All triazine pesticides showed LODs of 10 pg/mL, while the sensitivity for organophosphorous pesticides was variable. LODs from this class of compounds were from 30 pg/mL for sulfotep and thionazin, to 10 ng/mL for parathion-methyl, which exhibited the worst sensitivity. Two of the pesticides, pyrimethanil and cyprodinil, were also detected in the organic juice blank at concentrations below the LOQs (mass spectra in Fig. 4). Their identification was based on accurate mass, with mass errors of 0.38 and -0.77 ppm, respectively, when compared to their theoretical masses.

Fig. 4. Direct SPME-DBDI-MS high resolution mass spectra of an extraction from unspiked grape juice, showing the presence of pyrimethanil (a) and cyprodinil (b) identified based on accurate mass.

1 With the exception of parathion-methyl, all pesticides could be detected and quantified at
2 concentration levels well below 10 ng/mL. This is the maximum legally allowed
3 concentration level of pesticides in different food/feed matrices, i.e., the maximum residue
4 levels (MRLs), according to the current EU regulation (EC 396/2005). Our method could
5 achieve a sub-ng/mL sensitivity, which is desired to exclude the presence of even low
6 amounts of pesticides in organic food. In some cases, e.g., for surface and drinking water
7 analysis, where the regulation is generally stricter compared to food analysis, such a
8 sensitivity is even required.

9 The linear dynamic range (LDR) of the method was two to three orders of magnitude.
10 Compared to our previous work on water samples ¹⁰, in this work we noticed a narrower
11 LDR, due to the simultaneous ionization of all analytes in the DBD plasma, including matrix
12 components that can be either co-extracted or loosely attached to the coating surface. The
13 presence of matrix components is, in fact, detrimental both in terms of sensitivity and
14 reproducibility of the method. Generally, for fruit juices the most abundant interfering
15 compounds are phenolic compounds and sugars. The latter are especially undesirable during
16 the thermal desorption of the SPME, since the high temperatures reached in the desorption
17 chamber can cause their pyrolysis, leading to a significant reduction of the fiber lifetime and
18 production of artifacts. To minimize the above-mentioned phenomena, use of matrix-
19 compatible coating is highly beneficial. Moreover, a pre-desorption rinsing of the SPME
20 coating was carried out after each extraction step, following the procedure reported in the
21 experimental section. While the cleaning step helped removing loosely attached residues
22 from the outer coating surface, several matrix constituents adsorbed by the coating could be
23 detected (Fig. 5). The presence of matrix effects is also visible from the different linear
24 dynamic ranges achieved, with different compounds having different upper limits of
25 quantification. This suggests that the analytes and interfering molecules from the matrix are
26 competing inside the ionization source for the available protons (hydronium clusters) and
27 charges generated by the plasma. Although several matrix interfering compounds could be
28 observed, it is important to note that our DBDI source is generally less efficient in ionizing
29 phenolic compounds and sugars, which effectively reduces the problems associated with the
30 matrix effects. This is important especially when considering that many matrix components
31 can have a concentration significantly higher than the targeted analytes.

Fig. 5. Positive ionization mode high resolution mass spectra: a) DBD plasma background signal (no SPME desorption); b) desorption from a SPME fiber after a blank extraction from HPLC water; c) desorption from a SPME fiber after a blank extraction from grape juice. Signal intensity was normalized to the same scale for all three spectra (NL: 3.14E7, for the grape juice blank)

Hence, the direct coupling of matrix-compatible SPME and DBDI was successful in the analysis of grape juice, whose complex composition did not severely compromise the sensitivity and reproducibility of the method. In particular, reproducibility data are reported in Table 2, showing that intra-day RSDs usually below 10% were achieved for most pesticides at 1 ng/mL and 10 ng/mL.

compound	intra-day RSD%			inter-day RSD%	
	100 pg/mL	1 ng/mL	10 ng/mL	100 pg/mL	1 ng/mL
Famphur	28	12.3	14.2	27.8	21
Parathion	-	-	19.1	-	-
Parathion-met	-	-	-	-	-
Thionazin	19.4	15.4	10.5	25.7	29.1
Ametryn	25.9	10.9	12.6	26.0	12.0
Atrazine	10.9	7.4	8.3	14.6	7.0
Prometon	27.4	7.1	14.1	35.5	11.7

Prom+Terb	9.6	4.7	9.3	22.4	7.4
Propazine	12.6	9.7	7.6	15.2	8.3
Simazine	15.4	6.7	9.2	38.2	6.8
Pyrimethanil	18.0	7.9	20.9	9.4	9.7
Cyproconazole	48.1	6.7	7.0	84.3	22.0
Quinoxifen	11.9	6.7	7.5	12.0	8.3
Flusilazole	3.6	5.8	5.2	3.6	5.2
Trifloxystrobin	12.3	4.0	6.8	12.4	4.5
Azoxystrobin	37.8	3.2	5.1	29.7	5.8
Pyridaben	10.9	9.9	7.9	16.4	8.2
Pyraclostrobin	8.5	3.9	7.5	24.3	7.8
Pyriproxyfen	22.5	6.1	9.1	23.5	7.7
Tebuconazole	21.8	5.6	5.2	35.2	5.2
Cyprodinil	15.9	6.1	20.6	10.2	8.8
Difenoconazole	12.8	5.8	4.8	12.9	22.5

Table 2. Intra-day RSD% (n=5, evaluated at 100 pg/mL, 1 ng/mL and 10 ng/mL) and inter-day RSD (n=5, 3 days, evaluated at 100 pg/mL, 1 ng/mL) for the considered pesticides and drugs.

In order to compare the performance of the new SPME-DBDI-(HR)MS set up with a conventional analytical apparatus, the same analyses were carried out by SPME-GC-EI-ToF/MS. The results pertaining to linearity, linear dynamic range and achieved LOQs are shown in Table 3.

	Curve equation	LDR (ng/mL)	Weight	LOQ ^a (ng/mL)	R ²	RSD% ^b	RSD% ^c
Atrazine	y=0.394x-0.030	0.5-50	-	0.5	0.997	4.2	1.5
Pyrimethanil	y=3.703-0.014	0.5-50	1/X	0.5	0.999	1.6	1.7
Parathion-methyl	y=2.920-0.076	0.5-50	1/x	0.5	0.997	3.2	7.2
Metalaxyl	y=0.040-0.001	0.5-50	1/x	0.5	0.986	7.7	4.6
Malathion	y=7.531+27.89	1.5-15	-	1.5	0.991	1.7	2.5
Chlorpyrifos	y=1.034-0.045	0.5-50	1/x	0.5	0.988	4.7	10.4
Parathion	y=3.094-0.107	0.5-50	1/x	0.5	0.992	3.2	7.7
Cyprodinil	y=6.913-0.087	0.5-15	1/x	0.5	0.997	1.5	-
Flusilazole	y=6.352-0.255	0.5-50	1/x	0.5	0.991	1.4	8.6
Cyproconazol	y=0.399-0.022	1.5-100	1/x	1.5	0.998	2.6	6.6
Trifloxystrobin	y=13.98-0.273	0.5-50	1/x	0.5	0.996	5.5	2.3
Quinoxifen	y=9.042-0.265	0.5-15	1/x	0.5	0.993	6.1	1.4
Tebuconazole	y=1.506-0.176	0.5-100	-	0.5	0.994	3.3	7.4
Fenpropathrin	y=34.60-14.99	0.5-15	-	0.5	0.997	9.7	-
Pyriproxyfen	y=26.96-1.376	0.5-15	1/x	0.5	0.994	6.4	-
Pyridaben	y=5.937-0.232	1.5-50	1/x	1.5	0.991	6.2	11.4
Pyraclostrobin	y=2.291-0.083	0.5-100	1/x	0.5	0.994	5.4	10.8
Difenoconazole	y=0.419-0.065	0.5-50	1/x	0.5	0.988	1.8	1.3
Azoxystrobin	y=0.015-0.005	5-100	1/x	5	0.990	15.0	4.6

^a calculated from the calibration curves based on FDA guidelines
^b RSD calculated at spiking level of 5 ng/ml
^c RSD calculated at spiking level of 50 ng/ml

Table 3. Linear regression parameters for the pesticides investigated, determined by SPME-GC-EI-ToF/MS. The internal standard was atrazine-D₅ at 10 ng/mL. ^acalculated from the calibration curves based on FDA guidelines (lowest calibration point having RSD < 20% and accuracy between 80 and 120%)

When comparing the direct coupling approach to the conventional GC-EI-ToF/MS approach, we found that the LDR is generally broader with DBD ionization. The sensitivity is also higher with the direct coupling approach for most of the compounds, even with a shorter extraction time. Parathion and parathion-methyl were found to be less easily ionized with DBDI compared to electron ionization (EI). This is most probably due to in-source suppression effects. The large difference in LODs compared to other organophosphorus compounds is indicative of suppression by matrix interferences. This is also supported by results recently reported for the SPME-GC-DBDI coupling ¹¹, where parathion and parathion-methyl were efficiently ionized and detected at low pg/mL concentration levels after SPME pre-concentration from water samples.

Generally speaking, EI produced on average similar LOQs for the different pesticides. In contrast, since the ionization in DBDI mainly relies on proton transfer in very mild conditions (room temperature, ambient pressure, low energy plasma) and ion-neutral interactions in the reactive plasma, a large difference in ionization efficiency can be observed for molecules with different structures and chemical properties, e.g., proton affinity and ionization energy.

When comparing the reported approach with literature values, a one-to-one comparison is in most cases not possible, because of the different extraction devices and the different mass spectrometers used. Compared to SPME-DART results reported in recent work by Gómez-Ríos et al.²¹, our SPME-DBDI approach allowed to obtain similar LOQs. For some of the analytes investigated, we were able to achieve even lower LOQs, e.g., for trifloxystrobin and pyriproxyfen (both with LOQs of 10 pg/mL for SPME-DBDI, compared to 100 pg/mL of the DART approach). In other cases, SPME-DART showed higher sensitivity, e.g., for cyproconazole and malathion (both with LOQs of 1000 pg/mL for SPME-DBDI, compared to 100 pg/mL of the DART approach). However, it is important to mention that the DART results were obtained using different extraction conditions, and a triple quadrupole instead of an Orbitrap used in this study. The sensitivity of the SPME-DBDI approach is expected to be even higher when combined to a triple quadrupole mass spectrometer.

Conclusions

In this work, we presented for the first time the coupling of a matrix compatible SPME fiber to a DBDI-MS system for rapid analysis of pesticides in grape juice. The use of a matrix compatible SPME coating is critical especially when direct-MS analysis is carried for complex food matrices, since the occurrence of matrix effects is drastically minimized or

avoided. Moreover, compared to other ambient ionization strategies, the SPME-DBDI-MS coupling reported here presents several advantages, such as the simplicity of the setup and its ease of use, that allow easy implementation in research as well as routine analytical laboratories. The throughput of the analytical procedure is extremely high, since no matrix pre-treatment is necessary due to the anti-fouling properties of the SPME coating used. Contrariwise to DART or DESI, the desorption of the analytes from the SPME device is completely separated from the ionization event in our setup. This enhances the reproducibility of the method and minimizes ion suppression, making the entire procedure more flexible and more reproducible at the same time. Moreover, the high sensitivity of the DBDI source used, in combination with the active capillary sample introduction system, allows pg/mL sensitivities to be reached for several classes of analytes, e.g., pesticides, even in complex matrices, with a shorter analysis time compared to classical chromatography-based approaches.

For all these reasons, this novel direct SPME-MS coupling represents a viable alternative for the analysis of complex samples without performing chromatography. Considering the high sensitivity of the proposed analytical set up, it is plausible to further reduce SPME extraction times, depending on the required quantitation level for a specific application. Therefore, multiple analytes can be accurately quantified within few minutes rather than hours, the time usually required when GC-MS or LC-MS are performed.

The ease of use of this analytical setup, in combination with the robustness of the ionization source, could allow the implementation of this technology into many analytical laboratories, also due to its compatibility with commercially available SPME devices and the possibility to automate the entire analytical workflow.

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