New Opportunities for Wine Analysis through SPME Arrow and GC-MS/MS

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ABSTRACT

Purpose: this work illustrates the benefits of the SPME Arrow extraction method for wine aroma GC/MS investigation. It highlights the opportunities for improving quality assessment through highest sensitivity, robustness and versatility of quantification methods. In particular, the SPME Arrow has been evaluated for the determination, in wines, of molecules with minty aromas and of pesticide residues.

Methods: modern instrumentation allows to fully automate SPME extraction technique, facilitating method optimization. For this work the highest sensitivity of a Triple Quadrupole GC-MS/MS has been coupled to the new SPME Arrow, featuring highest sample capacity and robustness.

Results: the versatile automated solution illustrated in this paper provided several advantages for the quality assessment of wines, like the possibility to reduce the sample size and the sample preparation step, increase the selectivity and achieve lower detection limits.

INTRODUCTION

Gas chromatography is a major technique applied in wine aroma investigation. During the 90's, apparatus coupled to flame ionization detectors (FID) were used. Specific detectors (ECD, NPD, FPD…) also allowed new developments for wine analyzes. Nowadays, gas chromatography coupled to mass spectrometry (GC-MS) is preferred.

Initially analytical developments focused on wine volatile compounds, whether off-flavors or pleasant aromas. The analysis of wine odors is an essential step to study the impacts of various viticulture and winemaking processes on wine quality, but also to detect as early as possible defects in wine. Both helps to understand wine composition and, as a consequence, quality.

Chemical analysis is complementary to the tastings to determine the quality of the wine. Methods were also developed to detect pesticide residues in wine as this has become an important issue for winemakers, consumers as well as legislators.

When using a SPME extraction method, GC-MS has the additional advantages of requiring very small sample sizes, a minimum of sample preparation, and rapid analysis of target molecules. The size of sample is a key point for wine analysis. Considering aged wines, each bottle of wine is a single sample. A bottle is different from its "twin" because of small differences of corks or of ageing temperatures. The sample size must be as small as possible to allow a large amount of analyses of a single flask. On another hand, because of the complexity of the wine matrices and to optimize the chromatographic resolutions, the GC runs are quite longer, an automated technique with repeatable results for detecting these compounds is highly desirable.

Additionally, the highest selectivity provided by GC-MS/MS detection is offering several advantages in better handling co-elution and achieving better detection limits. Coupling the highest sensitivity of a Triple Quadrupole GC-MS/MS with the new SPME Arrow featuring highest sample capacity and robustness, a versatile automated solution is available for wine analysis, spanning from pesticides quantitation, aroma profiling and off-flavor detections.

MATERIALS AND METHODS

Instrumentation

The Thermo Scientific™ TSQ™ 8000 Evo GC-MS/MS has been equipped with a Thermo Scientific™ TriPlus™ RSH autosampler configured for SPME injection. The new SPME Arrow fibers have been used in place of the classic SPME, to leverage the highest sample capacity and lower the limit of detection (Figure 1). Thanks to the Automatic Tool Change (ATC), the TriPlus RSH is capable to automatically recognize the type of syringe and switch between the different injection techniques, like liquid injection or SPME, within the sequence.

Sample Preparation

The use of the SPME extraction technique reduces dramatically the sample preparation step. An aliquot of wine sample is simply put into 20 mL headspace vials for the analysis. Different type of wines (white, rosé, red) have been used to evaluate the impact of different matrices. A 2x and 5x dilutions of the sample prior SPME extraction have been also applied to evaluate the impact of ethanol on the extraction efficiency.

Test Method(s)

To optimize the extraction of target compounds, several conditions were tested such as the nature of SPME coating, time and temperature of extraction, needs of blank inclusion in the sequence, post-cleaning of the fiber. Classical parameters were also assessed (salt addition, stirring speed, desorption time, headspace (HS) or direct immersion (DI) of the fiber). Then, the performances of the method were evaluated.

Data Analysis

Thermo Scientific™ Chromeleon™ CDS software was used for instrument control and data analysis.

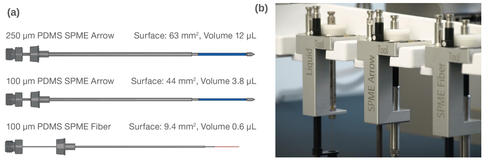

Figure 1. (a) Comparison between classical SPME fiber and SPME Arrow fibers featuring significantly higher surface and sample capacity. (b) The Automatic Tool Change (ATC) of the TriPlus RSH autosampler allows high level of automation and flexibility

RESULTS AND DISCUSSION

Mint flavor in wines

An important part of this study has been focused on minty aroma because of the description of aromatic expression of high quality red Bordeaux wines. The descriptions show the importance of fresh odors in the expression of wine quality[1].

Deciphering the sought-after freshness perceived in old red Bordeaux wines accounts among the current challenges in wine science. In that context, the contribution of monoterpene ketone piperitone to the minty nuances in red wines was recently investigated[2]. Piperitone is a secondary metabolite issued from the limonene biotransformation. The study of this metabolism permits to discover a large panel of molecules with powerful mint–like and fresh somesthetic properties[3].

A first quantification method has been set up and allowed to prove the importance of these compounds in the aromatic expression of aged wines. This method, based on a complex SPE/SBSE extraction step, was not adapted to routine analyses[4]. That is why, SPME Arrow combined to GC-MS/MS was tested for this application and this new method is now applied to wine composition studies and to understand vine management impact on aged wines quality.

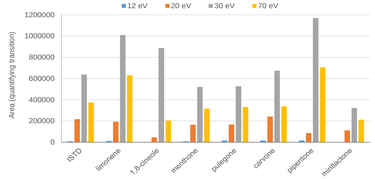

Figure 2. MS detection optimization. The ionization at 30 eV gives the highest peak area of the quantifying transition for all the minty aroma compounds (analysis performed in spiked red wines).

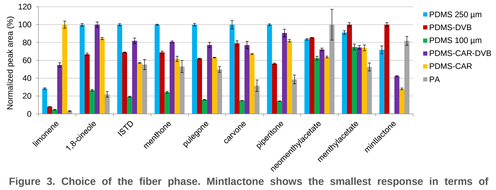

Figure 3. Choice of the fiber phase. Mintlactone shows the smallest response in terms of absolute peak area. Therefore, the PDMS-DVB coating was chosen. Limonene is not a limiting component as it is found in wine in greater amounts than all other compounds and reducing its response is even appreciable to improve peak shape.

Table 1. Method performances

Compound	Linearity		Detection and quantification limits		Intraday precision (repeatability)				Interday precision (reproducibility)
	Concentration range (ng/L)	R^2	LOD (ng/L)	LOQ (ng/L)	spiking 1 (ng/L)	RSD (%)	spiking 2 (ng/L)	RSD (%)	RSD (%)
limonene	695-2976	0.9517	36	120	864	19	945	11	23
1,8-cineole	35-2480	0.9971	20	70	60	6	291	2	10
menthone	6-3759	0.9978	3	7	46	4	391	3	10
pulegone	6-2989	0.9986	3	8	33	5	311	4	8
carvone	33-2931	0.9913	9	30	54	25	266	12	11
piperitone	14-3805	0.9993	5	17	51	6	386	5	7
neomenthylacetate	18-2540	0.9952	3	11	42	8	279	8	13
menthylacetate	14-3465	0.9914	4	15	50	6	370	8	13
mintlactone	103-2574	0.9858	60	200	617	12	1700	6	11

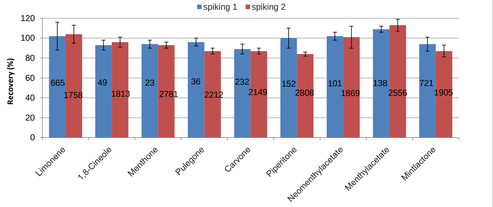

Figure 4. Recovery of the method for 2 spiking levels in red wine (added concentrations in ng/L are indicated on the bars). The recovery levels are very satisfying.

Table 2. Wine analyses. Mean concentrations (± SD) determined in 14 red Bordeaux wines

	Mean concentrations (ng/L, n=3)							
	Limonene	1,8-Cineole	Menthone	Pulegone	Carvone	Piperitone	Neomenthyl acetate	Menthyl acetate	Mintlactone
1	467±2	70±3	7±1	< LOQ	35±1	36±1	19±1	22±2	n. d.
2	< LOQ	80±3	7±0	< LOQ	< LOQ	25±1	2234±184	24±3	n. d.
3	359±56	209±10	30±0	9±0	< LOQ	1638±49	830±46	20±2	n. d.
4	451±34	86±3	6±0	4±0	< LOQ	548±15	1443±124	32±1	n. d.
5	540±34	285±13	208±5	170±9	274±24	444±38	240±20	295±16	207±15
6	262±23	108±4	< LOQ	9±1	30±4	1637±94	1469± 96	32±1	n. d.
7	154±17	100±4	9±0	< LOQ	36±4	37±2	222±23	28±3	n. d.
8	445±30	144±5	53±3	47±5	53±4	3592±300	n. d.	26±2	n. d.
9	118±2	1221±71	13±1	< LOQ	58±5	55±4	993±72	31±1	n. d.
10	245±15	925±10	18±2	19±0	56±5	6773±131	1242±52	36±1	< LOQ
11	292±6	370±9	14±1	15±1	33±1	4258±158	1288±53	54±1	n. d.
12	506±55	110±6	8±0	< LOQ	< LOQ	44±2	246±5	175±14	n. d.
13	205±13	279±10	15±1	16±0	38±1	2312±30	1051±19	20±2	n. d.
14	183±9	164±2	22±0	19±1	31±1	2119±128	1030±73	29±0	n. d.

Pesticide residues in wine

SPME-Arrow is also being tested for pesticide residue analysis in wines.

A large number of active ingredients, with various physico-chemical properties, are authorized for vineyard protection and because of the diversity of their chemical structures, HPLC and GC are indispensable complementary tools for their analysis.

Moreover an unambiguous identification is essential and this is achieved with MS detection or better MS/MS or HRMS detection.

Extraction of pesticide residues from food products is generally performed using the QuEChERS extraction. However, since the wine is an aqueous liquid matrix, other extraction techniques can be used and, in particular, SPME has been found to be a simple and effective approach.

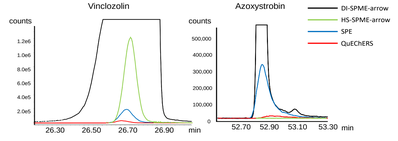

Figure 5. Comparison of QuEChERS / SPE / HS-SPME-arrow / DI-SPME-arrow extractions. For all compounds tested, SPME-arrow in immersion (DI-SPME-Arrow) gives the highest peak area.

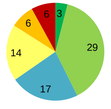

Figure 6. Linearity. The coefficient of determination (r^2) is above 0.90 for all 75 pesticide residues tested and even better than 0.99 for most of them in white wine.

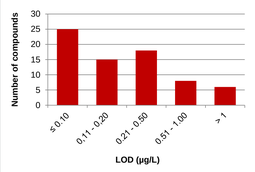

Figure 7. Limits of detection (LOD). LOD are very low for all compounds tested and below 6 µg/L, which is highly acceptable.

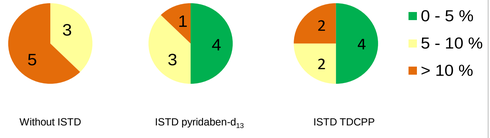

Figure 8. Repeatability. Evaluation is in progress but the repeatability improves significantly when an internal standard (ISTD) is used. RSDs are between 9 and 19% without ISTD and fall between 2 and 13% when an ISTD is used with most of them below 10%.

Table 4. Memory effect. Some compounds with high affinity for the fiber coating may produce false-positives when a sample is analyzed just after a heavily loaded one. As shown in the table the residual concentration is generally below 1 µg/L. This effect must be taken into account when defining the LODs in order to avoid false-positives.

µg/L	Difenoconazole	Difenoconazole	Chlorobenzilate	Chlorfenson	Fenarimol
[sample]	45	224	773	269	204
[residual]	0.1	1	0.1	0.2	0.2

CONCLUSIONS

- A SPME-Arrow GC-MS/MS method has been optimized and validated for the quantification of a series of minty aromas in red wines. The small size of the sample (5 mL) and the fully automated quantitation method permit to progress in the knowledge of high quality red wines and, as a result, to anticipate the consequences of climatic changes on this quality.
- The SPME-Arrow coupled to GC-MS/MS looks promising for pesticide residue analysis. It offers an alternative to QuEChERS extraction of aqueous liquid matrices and wine in particular. Evaluation of this technique is in progress but it already showed good linearity and repeatability as well as extremely low LOD for a wide range of compounds. Moreover sample preparation is simple and minimal and the analysis is fully automated from extraction to detection, completing the HPLC measurement in an easy way. The only drawback may lie in the memory effect which alters the quantification at ultra low concentrations with the risk of giving false-positives.
- The SPME-Arrow-GC-MS/MS will next be tested for the simultaneous analysis of haloanisoles (HA) and their precursors, halophenols (HP), responsible for cork taint and dusty, moldy odors. A rapid and efficient quantification method is needed as these compounds could have an impact on wine quality even at concentration below their sensory thresholds masking the wine fruity aroma. The new method should be able to reduce the time for sample preparation and lower the LODs of HA and HP.

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