Application Brief

Food Testing and Agriculture

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Use of Salt to Increase Analyte Concentration in SPME Headspace Applications

Author

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Abstract

Static headspace gas chromatography is one of the most frequently used techniques for the analysis of flavor components in foods and beverages. Samples must be prepared to maximize the concentration of the volatile components in the headspace and minimize unwanted contamination from other compounds in the sample matrix. The use of solid phase microextraction (SPME) allows for a fast, solvent-less, selective analysis of the headspace compounds. The addition of salt to the sample matrix will often lower the partitioning coefficient (K) for some target analytes, thus increasing the concentration of analytes in the headspace, which is the key advantage of this methodology.

Experimental

Amount of salt

The magnitude of the salting-out effect on K is not the same for all compounds. Compounds with K values that are already relatively low will experience little change in partition coefficient after adding a salt to an aqueous sample matrix. The addition of salt, however, will assist by lowering the compounds with higher K values and increase their concentration in the headspace. Each application is different. As a rule, the amount of salt added should be enough to saturate the sample (20 to 40% wt/wt salt/sample ratio). Saturation will maintain the same ionic strength from sample-to-sample and ensure reproducibility.

For example, water salinity is 35 g/L, which equates to 3.5 g in 10 mL of sample. In this case, 4 g (±0.5 g) of salt to a 10 mL water-based sample will ensure that enough salt has been added to saturate the sample.

Type of salt

Sodium chloride (NaCl) is the most used salt to adjust ionic strength. However, other salts such as ammonium chloride (NH_4Cl), sodium sulfate (Na_2SO_4), or sodium hydroxide (NaOH) may have different salting out capabilities, particularly when dealing with complex matrices such as food. It is important to note that while salt may improve the SPME extraction of the desired analytes, it could also cause co-extraction of more matrix interferences or undesired compounds.

Method

Guaiacol and 4-methylguaiacol are main target compounds implicated in smoke-affected grapes and wines. The use of the DVB/carbon WR/PDMS SPME phase was chosen due to its selective extraction of odor and flavor compounds.

Sample preparation

- 20 mL headspace vial and cap (part numbers 5188-6537 and 5188-2759)
- 10 mL sample with 4 g of NaCl
- Samples (n = 5) spiked at 50 ppb
- Agilent SPME Arrow DVB/carbon WR/PDMS, 1.10 mm, 120 µm (part number 5191-5861)

Table 1. SPME headspace parameters.

Parameter	Setting
Predesorption Time	3 min
Predesorption Temperature	250 °C
Incubation Time	5 min
Heatex Stirrer Speed	1,000 rpm
Heatex Stirrer Temperature	40 °C
Sample Extract Time	10 min
Sample Desorption Time	3 min

Table 2. Agilent 8890 GC settings.

Parameter	Setting
Inlet Liner	Agilent Ultra Inert inlet liner, splitless, straight, 0.75 mm id, recommended for SPME injections (p/n 5190-4048)
Injection Mode, Temperature	Splitless, 250 °C
Control Mode	Constant flow (1.2 mL/min)
Column	Agilent J&W DB-HeavyWAX GC column, 30 m × 0.25 mm, 0.25 µm (p/n 122-7132)
Oven Program	120 °C (hold 1 min); 10 °C/min to 250 °C (hold 0 min); 60 °C/min to 280 °C (hold 0 min)

Table 3. Agilent 7000D triple quadrupole GC/MS conditions.

Parameter	Setting
Transfer Line	280 °C
Acquisition Mode	dMRM
Solvent Delay	3.0 min
Tune File	Atune.eiex
Gain	10
MS Source Temperature	280 °C
MS Quadrupole Temperature	150 °C

An Agilent PAL3 autosampler with robotic tool change (RTC) was installed on an Agilent 8890 GC system with an Agilent 7000D triple quadrupole GC/MS. The SPME headspace parameters, GC method settings, and MS conditions are listed in Tables 1, 2, and 3, respectively.

Results and discussion

The increase of response of smoke impact volatiles is seen with the addition of 4 g of NaCl. Figure 1 shows the TIC scan of multiple smoke impact compounds when analyzed with and without the addition of NaCl. Figures 2 and 3 show the area differences of guaiacol and 4-methylguaiacol by analyzing their MRM transitions. Table 4 provides the area counts for both guaiacol and 4-methylguaiacol with and without the addition of NaCl.

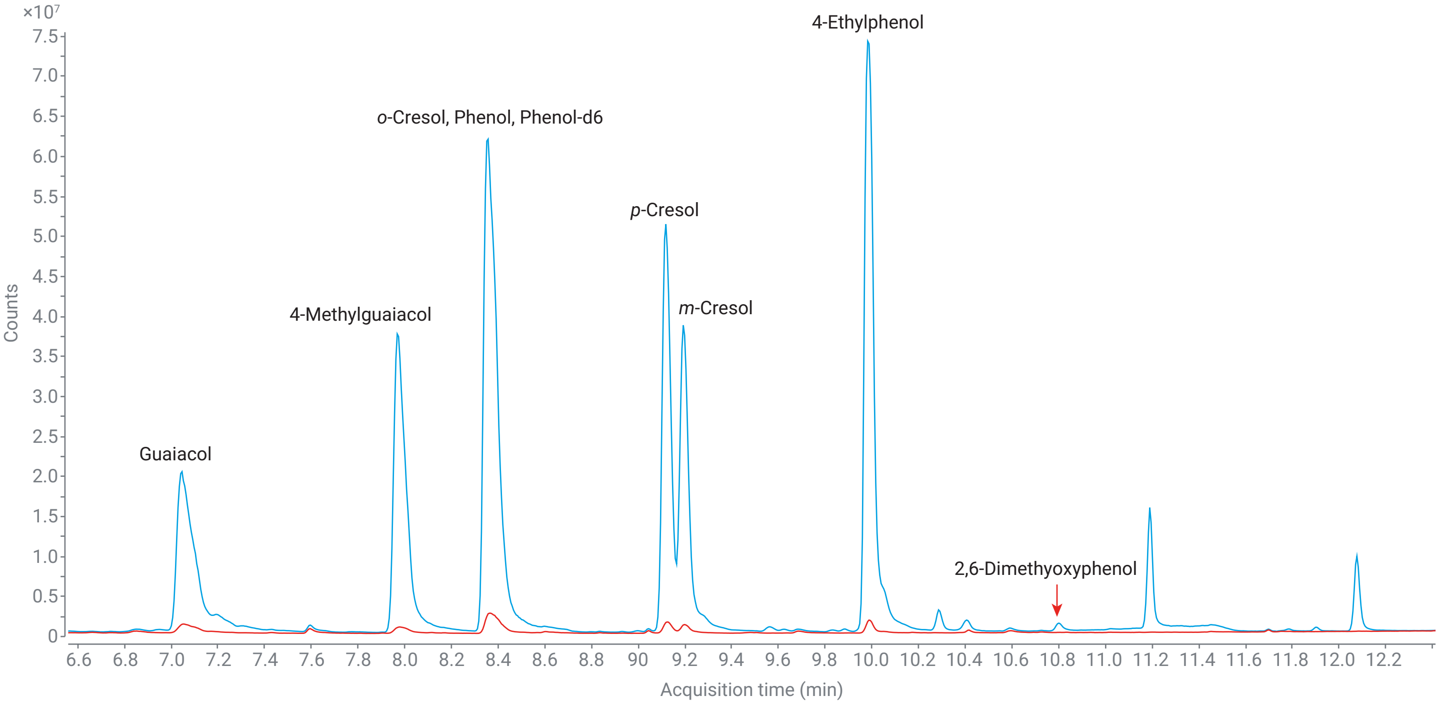
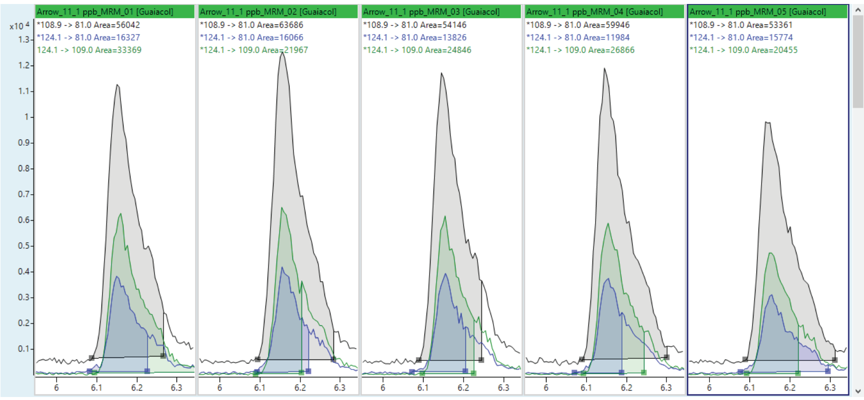


Figure 1. TIC scan of smoke impact compounds at 50 ppb extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861). The red trace indicates standards that were run without salt, and the blue trace indicates standards that were run with 4 g NaCl.

A) 1 ppb guaiacol with no NaCl



```
Arrow_11_1 ppb_MRM_01 [Guaiacol]
*108.9 -> 81.0 Area=56042
*124.1 -> 81.0 Area=16327
124.1 -> 109.0 Area=33369

Arrow_11_1 ppb_MRM_02 [Guaiacol]
*108.9 -> 81.0 Area=63686
*124.1 -> 81.0 Area=16066
*124.1 -> 109.0 Area=21967

Arrow_11_1 ppb_MRM_03 [Guaiacol]
*108.9 -> 81.0 Area=54146
*124.1 -> 81.0 Area=13826
*124.1 -> 109.0 Area=24846

Arrow_11_1 ppb_MRM_04 [Guaiacol]
*108.9 -> 81.0 Area=59946
*124.1 -> 81.0 Area=11984
*124.1 -> 109.0 Area=26866

Arrow_11_1 ppb_MRM_05 [Guaiacol]
*108.9 -> 81.0 Area=53361
*124.1 -> 81.0 Area=15774
*124.1 -> 109.0 Area=20455
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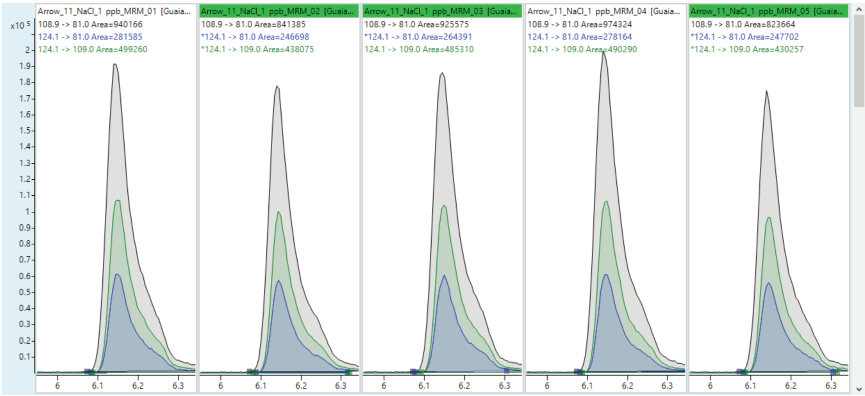
B) 1 ppb guaiacol with 4 g NaCl



```
Arrow_11_NaCl_1 ppb_MRM_01 [Guaia...
108.9 -> 81.0 Area=940166
124.1 -> 81.0 Area=281585
124.1 -> 109.0 Area=499260

Arrow_11_NaCl_1 ppb_MRM_02 [Guaia...
108.9 -> 81.0 Area=841385
*124.1 -> 81.0 Area=246698
*124.1 -> 109.0 Area=438075

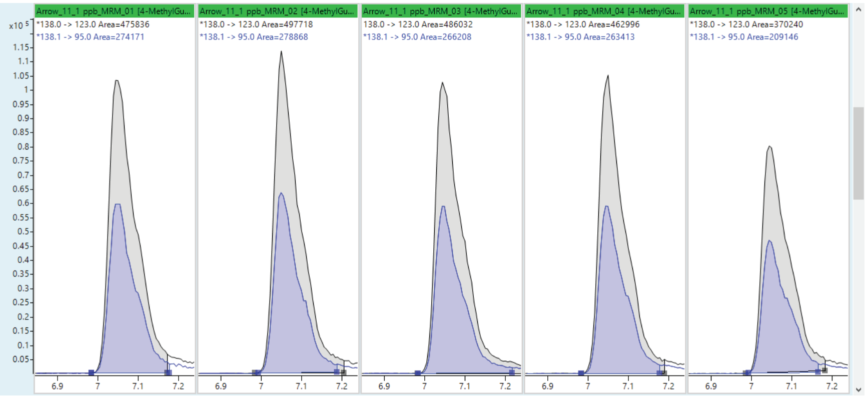
Arrow_11_NaCl_1 ppb_MRM_03 [Guaia...
108.9 -> 81.0 Area=925575
*124.1 -> 81.0 Area=264391
124.1 -> 109.0 Area=485310

Arrow_11_NaCl_1 ppb_MRM_04 [Guaia...
108.9 -> 81.0 Area=974324
124.1 -> 81.0 Area=278164
124.1 -> 109.0 Area=490290

Arrow_11_NaCl_1 ppb_MRM_05 [Guaia...
108.9 -> 81.0 Area=823664
*124.1 -> 81.0 Area=247702
*124.1 -> 109.0 Area=430257
```

Figure 2. MRM comparison with area counts for 1 ppb guaiacol replicates with A) no addition of salt and B) 4 g NaCl. Extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861).

A) 1 ppb 4-methylguaiacol with no NaCl



B) 1 ppb 4-methylguaiacol with 4 g NaCl

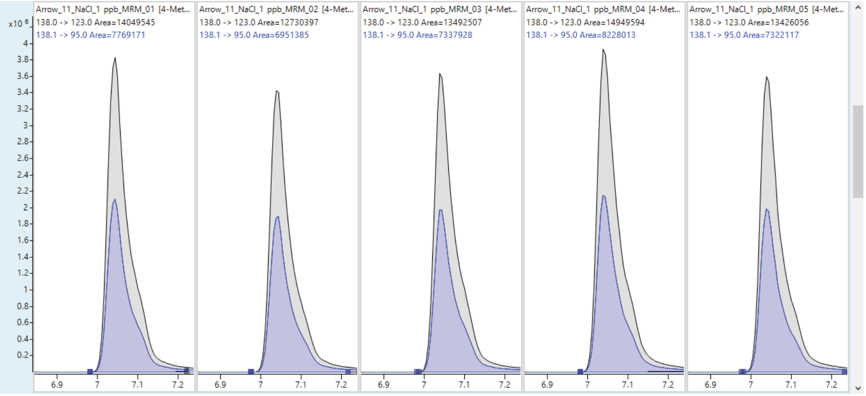
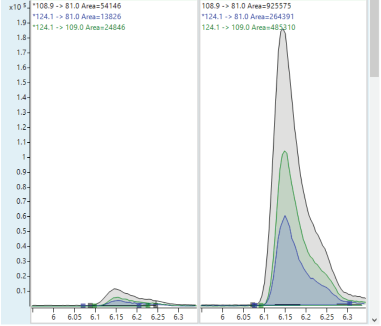


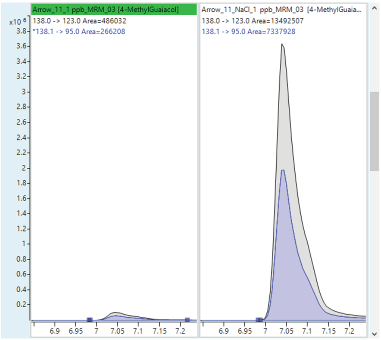
Figure 3. MRM comparison with area counts for 1 ppb 4-methylguaiacol replicates with A) no addition of salt and B) 4 g NaCl. Extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861).

A) Guaiacol



94% increase in response with NaCl

B) 4-Methylguaiacol



96% increase in response with NaCl

Figure 4. SPME comparison of wine impact compounds with and without NaCl for A) guaiacol and B) 4-methylguaiacol.

Table 4. Area counts of 1 ppb guaiacol and 4-methylguaiacol extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861).

Compound	Amount of NaCl	Replicate 01	Replicate 02	Replicate 03	Replicate 04	Replicate 05	% RSD
Guaiacol	0 g	56,042	63,686	54,146	59,946	53,361	7.04
	4 g	940,166	841,385	925,575	974,324	823,664	6.50
4-Methylguaiacol	0 g	475,836	497,718	486,032	462,996	370,240	10.67
	4 g	14,049,545	12,730,397	13,492,507	14,949,594	13,426,056	5.40

Conclusion

With the addition of NaCl to saturation, there is an average of 95% increase in response for the target compounds implicated in smoke-affected grapes and wines.

References

1. Westland, J.; Abercrombie, V. Analysis of Free Volatile Phenols in Smoke-Impacted Wines by SPME. *Agilent Technologies application note*, publication number 5994-3161EN, **2021**.

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