Sensitive Detection of 2-Methoxy-3-Isobutylpyrazine (IBMP) and Trichloroanisole (TCA) in Wine Using Triple Quadrupole GC/MS

Application Chemists: Stephan Baumann, Anna Hjelmeland

General recommendations for wine analysis

Extraction:

- Headspace-solid phase microextraction (HS-SPME) is an inexpensive and simple extraction procedure

- Other options are stirbar sorptive extraction (SBSE) or headspace stirbar sorptive extraction (HSSE)

Increasing headspace vapor pressure improves response:

- Saturating the solution with a salt like sodium chloride increases the concentration for some analytes in the headspace depending on the sample matrix.
- Increasing the temperature but beware of degradation/artifacts
- Use agitation, if possible

Optimizing the stationary phase selection

- PDMS, PDMS/DVB, PDMS/DVB/CAR

HS-SPME advice

- Maximize extraction/equilibration time for less volatile compounds
- Use Internal Standards that mimic the physicochemical properties of analytes to normalize for variations in extraction efficiency
- Try to use a single fiber for an experiment. This is especially important if there are no internal standards.

3-Alkyl-2-Methoxypyrazines
Sauvignon Blanc, Sémillon and Cabernet Sauvignon and other Bordeaux varietals

MALB

Types:

- IBMP: 2-Isobutyl-3-methoxypyrazine; green bell pepper aroma
- IPMP: 2-Isopropyl-3-methoxypyrazine; green pea earthy nutty
- SBMP: 2-Sec-butyl-3-methoxypyrazine; musty vegetative
- EMP: 2-Ethyl-3-methoxypyazine; raw potato earthy bell pepper nutty

Sources:

- Natural biosynthesis in plants and microbes
- Ladybugs (IBMP)
- Grapevine Stems (SBMP)

Methoxypyrazines
Concentrations need to be managed to make good wines

Canopy management steps practiced to reduce presence of methoxypyrazines:

- Expose grape clusters to sunlight before the onset of ripening
- Reduce the number of leaves between grape clusters
- Vines with excessive growth will generate more methoxypyrazines
- Grapes picked before sugar maturity will have higher concentrations of methoxypyrazines

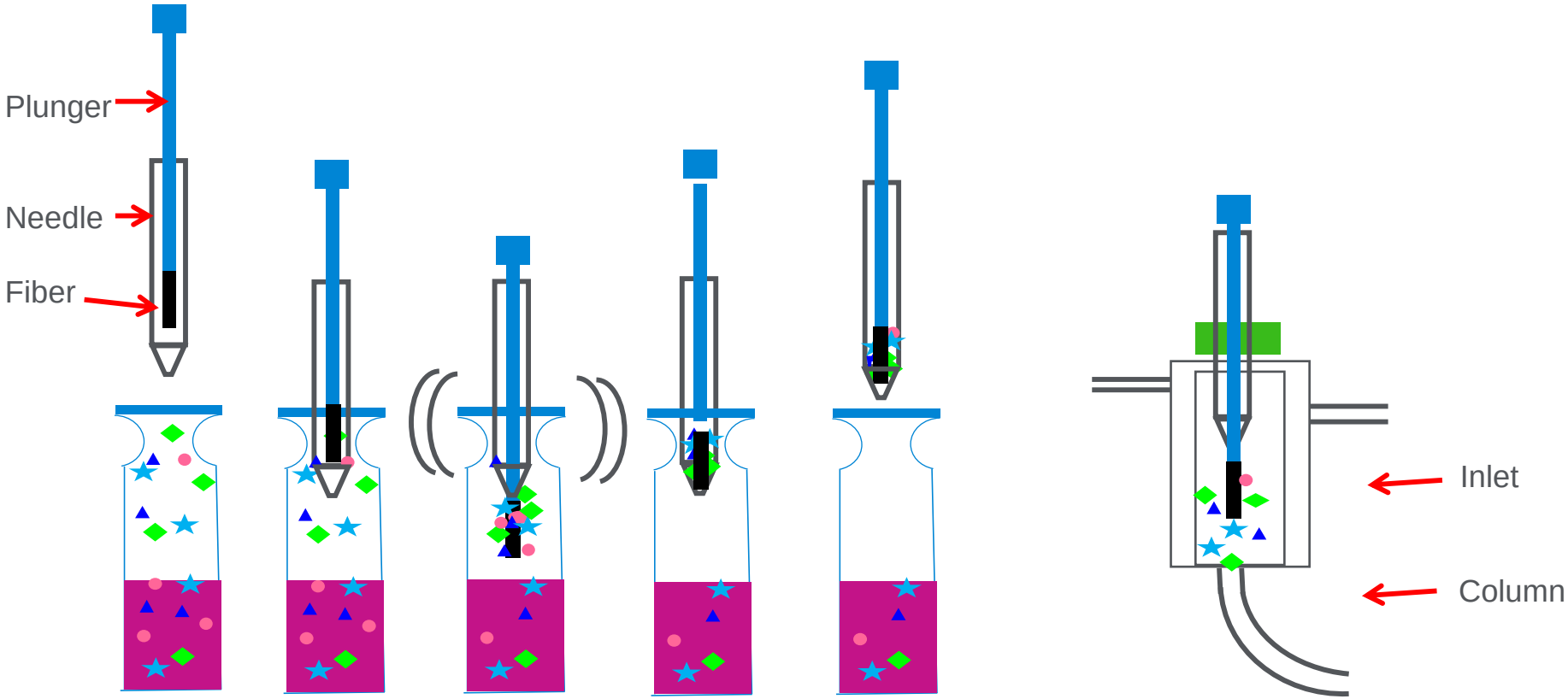
Reference:
Justin J. Scheiner, Gavin L. Sacks, Justine E. Vanden Heuvel, “How Viticulture Factors Affect Methoxypyrazines”, Wines & Vines, 117-120 (2009).

IBMP Sample Preparation

- 20 mL headspace vials
- IBMP calibration standards are made in model wine (0.5 % w/v tartaric acid in 12 % v/v ethanol).
- Sauvignon Blanc and Cabernet Sauvignon wine were used to spiked samples
- 5 mL Aliquots taken
- Standards and spikes made at 0, 5, 20, and 100 ng/L in MIBP, and 80 ng/L in isotopically labeled IBMP
- 2 g NaCl added

Reference:
R. Godelman, S. Limmert, T. Kuballa, “Implementation of headspace solid-phase-micro-extraction-GC-MS/MS methodology for determination of 3-alkyl-2-methoxypyrazines in wine”, Eur Food Res Technol 227, 449-461 (2008).

Solid Phase Microextraction (SPME) Steps



The 1 cm 50/30 µm DVB/Carboxen/PDMS fiber SPME samples were equilibrated at ambient temperature ~25 °C for 30 min. The fiber was then lowered into the headspace and the samples were extracted for 30 min before being desorbed in the inlet for two minutes. At that time the inlet was purged. The fiber was left in the inlet to clean for an additional nine minutes.

IBMP GC Run Conditions

GC Run Conditions	
Analytical Column	Two 15 m x 0.25 mm x 0.25 µm HP-5msUI columns (P/N 190915-433UI)
Inlet temperature	250°C
Inlet pressure	9.5 psi
Carrier gas	Helium, constant flow mode, 1.2 mL/min
Splitless	Purge 50 mL/min @ 2 min
Oven program	45°C (2.25min hold), 8°C/min to 130 °C
Column velocity	39.8 cm/s
Injection	SPME (PDMS/DVB/CAR) ; 2 min.; 250°C
Transfer line temperature	250 °C
GC Post-Run Conditions	
Backflush device	Purged Ultimate Union (P/N G3186-60580) controlled by a Pressure Control Module (P/N G3476-60501)
Backflush conditions	-1.2 mL/min@200 °C for 2 min

Note the SPME fiber was equilibrated for 30 min at room temperature since I didn't have any fancy equipment to heat, agitate (automate) sample adsorption.

IBMP MS Conditions

MS Conditions	
Tune	PCI Autotune
Delta EMV	800V
Acquisition parameters	PCI; selected reaction monitoring
Reagent Gas Flow	20 % Methane
Collision Gas Flows	Nitrogen at 1.5 mL/min, Helium at 2.35 mL/min
Solvent delay	3.75 minutes
MS temperatures	Source 300 °C; Quadrupoles 150 °C

Compound	Triple Quadrupole GC/MS			
	RT (min)	SRM	Dwell Time (ms)	Collision Energy (EV)
IBMP	11.5	167→94	60	35
		195→124	60	30
		195→106	60	35
$^{2}H_{3}$-IBMP (Internal Standard)	11.5	170→127	20	30
		170→128	20	30
		170→100	20	30

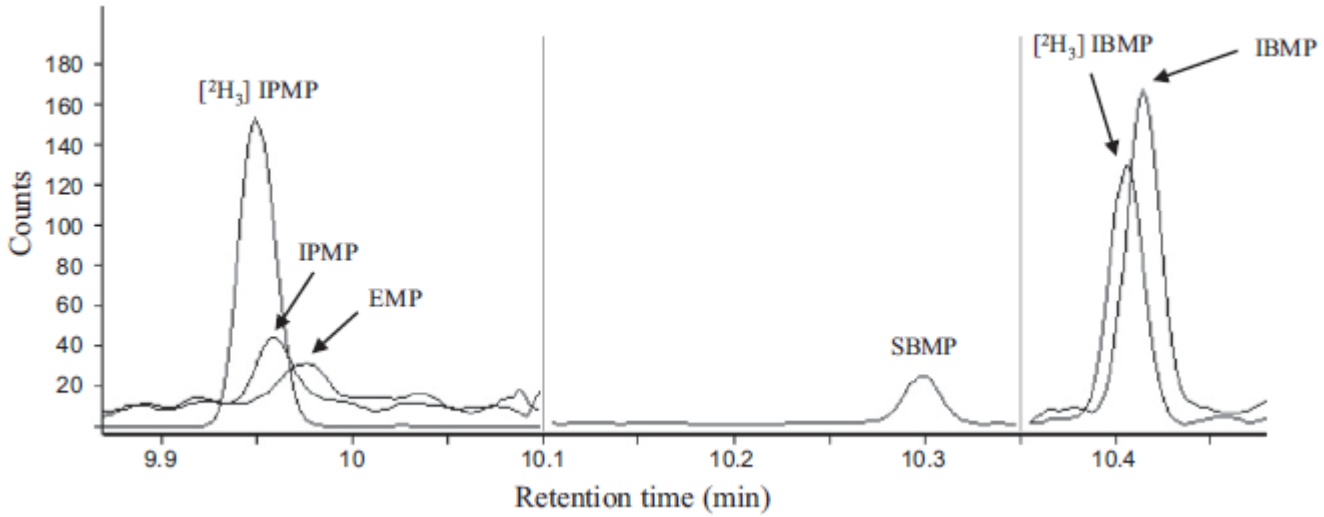
Note that Anna also identified a 167→125 (15 EV) qualifier transition for IBMP.

IPMP, SBMP, EMP MS Conditions
Anna Hjelmeland: Talanta 148 (2016) 336-345

Compound	Triple Quadrupole GC/MS			
	RT (min)	SRM	Collision Energy EV	Quantifier/Qualifier Ratio
$^{2}H_3$-IPMP	9.95	156→121	15	1.17
		156→123	30	
IPMP	9.96	153→121	15	1.04
		153→123	25	
EMP	9.98	139→107	15	0.9
		139→124	20	
SBMP	10.1	167→138	20	1.23
		167→123	30	

In addition to IBMP, three other methoxypyrazines IPMP (2-iso-propyl-3-methoxypyrazine),SBMP (2-sec-butyl-3-methoxypyrazine), and EMP (2-ethyl-3-methoxypyrazine) have been identified in grapes and/or wine and can impact aroma quality. Anna used a 30 m x 0.32 mm i.d. x 1.0 µm film thickness DB-WAXetr capillary column for optimal separation of all four methoxypyrazines.

Chromatographic Separation and Results from Talanta article



Compound	Sensory Threshold In Wine (ng/L)	Standard Curve Range (ng/L)	Correlation Coefficient R	LOQ (ng/L)	LOD (ng/L)
IBMP	8 - 15	0.5 - 50	0.993	0.5	0.25
IPMP	0.3 - 1.6	0.5 - 50	0.986	1	0.5
SBMP	-	0.5 - 50	0.992	0.5	0.25
EMP	-	0.5 - 50	0.99	1	0.5

What is Positive Chemical Ionization?

- Reagent ions are formed from a "reagent gas" by bombardment with electrons
- Reagent gas ions undergo subsequent reactions with sample molecules to form sample ions ("Brönsted acids/bases")

Example:

CH_4 (0.1 - 1 torr) → (e-) → CH_5^+ (M+1), $C_2H_5^+$ (M+29), $C_3H_5^+$ (M+41)

$M + CH_5^+ \rightarrow MH^+ + CH_4$

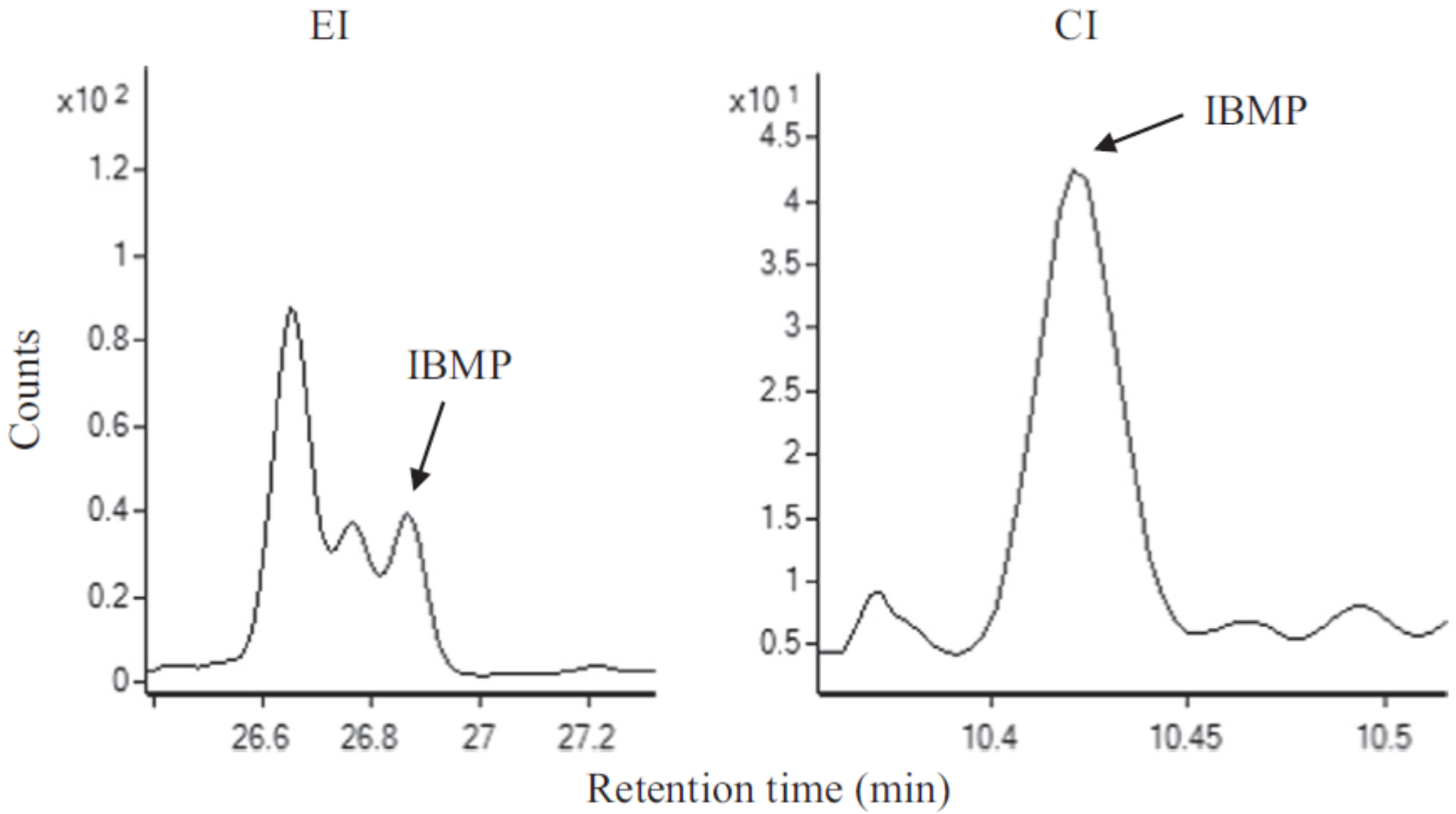
$M + C_2H_5^+ \rightarrow [M+C_2H_5]^+$ (adduct formation)

Chemical Ionization (CI) Attributes

- CI ion formation is much more "gentle" than electron ionization (EI) (less fragmentation) and primarily produces the protonated molecule
- Chemical control of ionization allows selectivity through choosing different reagents gases; methane, isobutane, ammonia, etc.

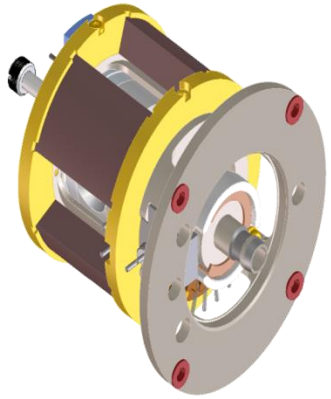
Note that the proton affinity, in kcal/mole, of CH_5^+ is 131.6 while the $C_2H_5^+$ adduct is 162.6. Note that the proton affinity of ammonia is 204. There is much more energy left over for fragmentation when using methane relative to ammonia.

Why not use traditional EI?

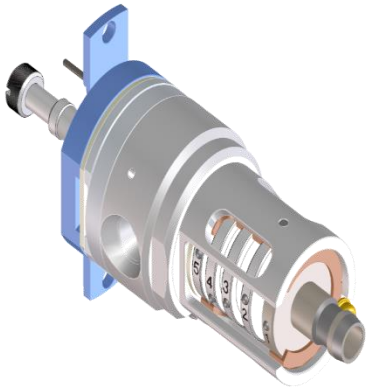


Note that even with a painfully slow chromatographic separation, IBMP is not separated from interferences using the EI source.

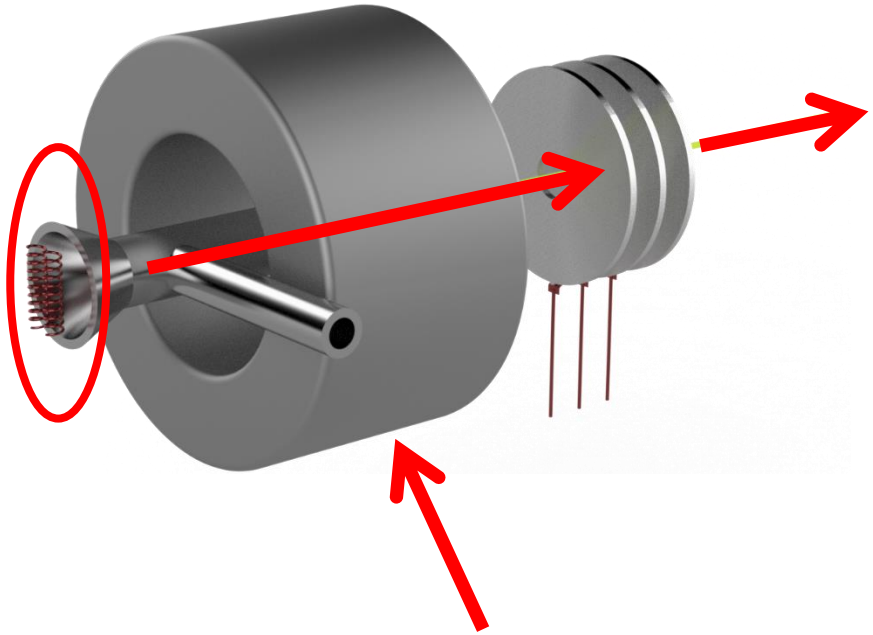
Could Low Energy EI be applied on the 7010?
A definite maybe!



High Efficiency Source



High Efficiency Source with Magnet Removed

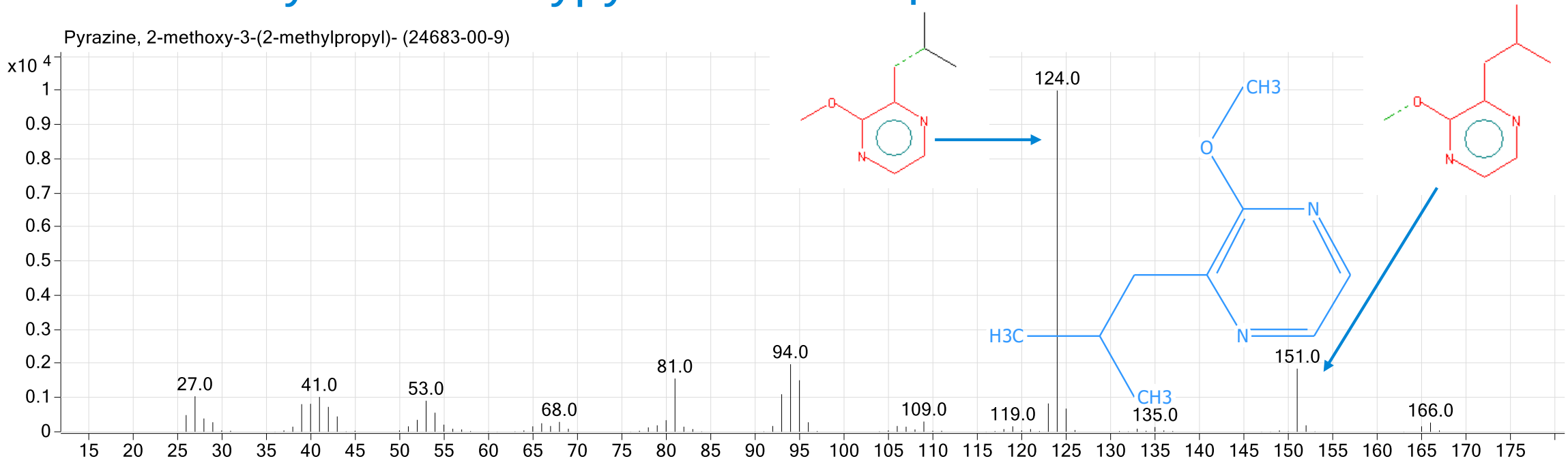


High emission filaments for more electron current

Powerful cylindrical magnet collimates electrons

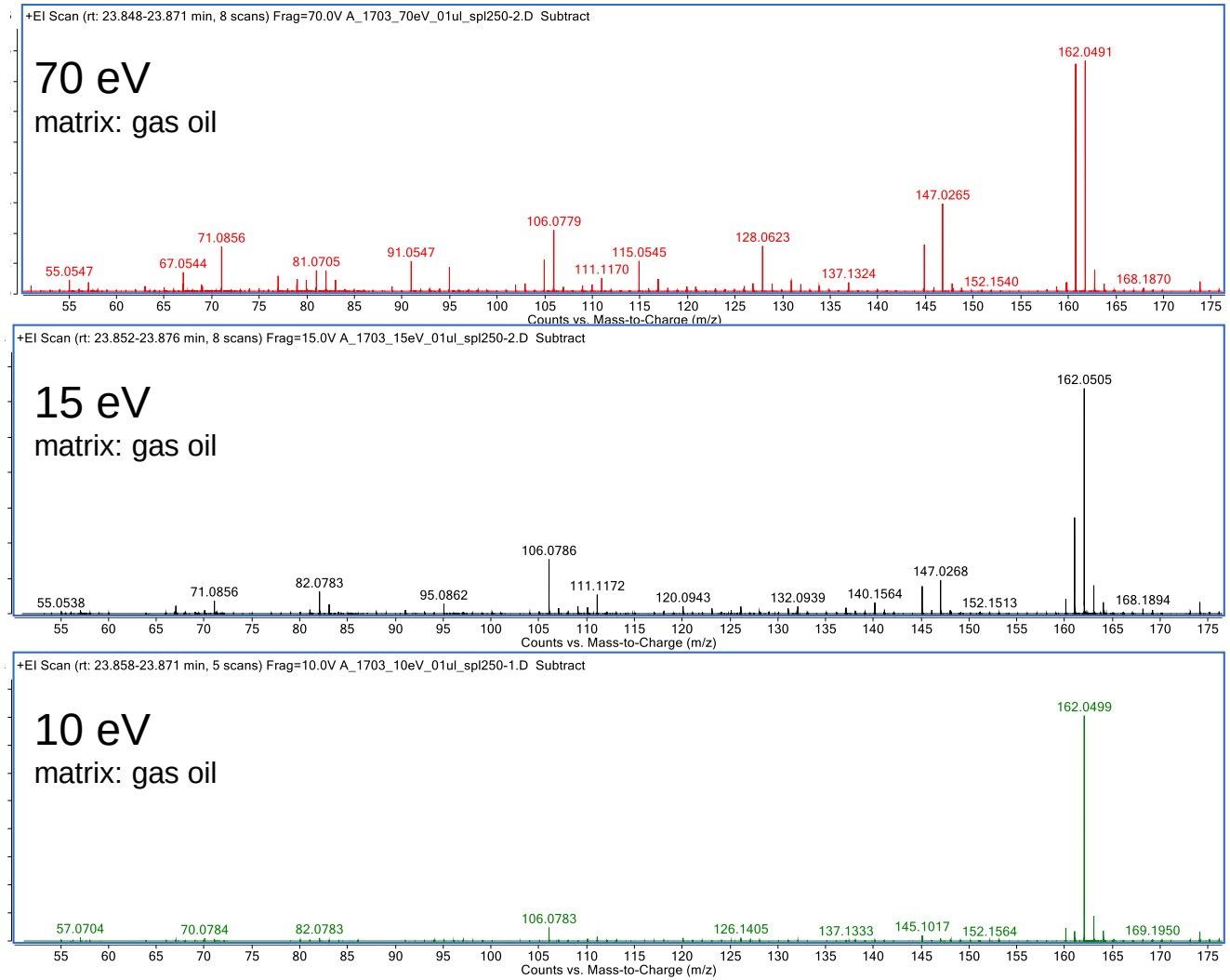
Long path length for ionization

Could Low Energy EI be applied on the 7010?
2-Isobutyl-3-methoxypyrazine example



Typically in EI ionization, high-energy electrons are created through thermionic emission at 70 eV. The most abundant EI fragments found at 70 eV are at 151 and 124 Da. These fragments should be greatly reduced in abundance relative to the parent ion if we reduce the ionization energy to 30, 20 or 15 eV. This should simplify the fragmentation pattern for both IBMP and the numerous interferences found in wine matrix.

HES Low Energy EI example from the new 7250 GC/QTOF



Note that the response goes down with source voltages but the chemical interferences go down by more, often enhancing sensitivity. So please try this with methoxypyrazines and publish the results!

Haloanisoles TCA and TBA implicated in Cork Taint
TeCA and PCA are associated with musty aromas in beverages

Types:

- TCA: 2,4,6-trichloroanisole
- TBA: 2,4,6-tribromoanisole
- TeCA: Tetrachloroanisole
- PCA: Pentachloroanisole

Sources:

- Biomethylation of halophenols
- Halophenols are often anthropogenic although there are some that come from natural sources – cork bleaching, industrial pollution.

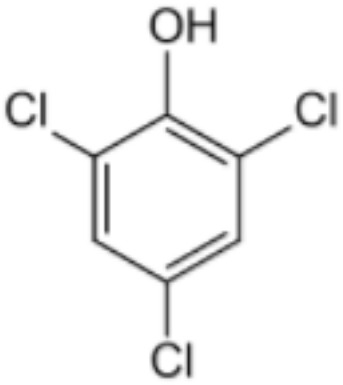
Typical Odor:

- musty, moldy (wet newspaper) aroma

Haloanisoles Contamination typically starts with a Halophenol
TCP and TBP Uses

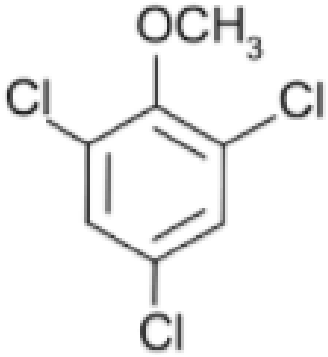
2,4,6-Trichlorophenol

- Also known as TCP, phenaclor, Dowicide 2S, Dowcide 2S, omal.
- It has been used as a fungicide, herbicide, insecticide, antiseptic, defoliant, and glue preservative.



2,4,6-Trichlorophenol

- Also known as Tribromophenol, 2,4,6-TBP, TBP.
- It is often used as a wood preservative and a flame retardant.



These ubiquitous halophenols are toxic to molds and fungi so they methylate the phenols to make them biologically harmless. This also makes the phenols more volatile!

Reference:
Robert Tracy, “2,4,6-TBA - Next 2,4,6-TCA in U.S. Wine Industry”, Practical Winery & Vineyard Journal, November-December (2008).

TCA Sample Preparation

- 20 mL headspace vials
- TCA calibration standards are made in 13 % alcohol Cabernet Sauvignon
- 5 mL Aliquots taken
- Standards and spikes made at 1, 5, 10, 15, and 20 ng/L in TCA, and 10 ng/L in 2,3,6-TCA as an internal standard.
- 2 g NaCl added

TCA GC Run Conditions

GC Run Conditions	
Analytical Column	Two 15 m x 0.25 mm x 0.25 µm HP-5msUI columns (P/N 190915-433UI)
Inlet temperature	250 °C
Carrier gas	Helium, constant flow mode, 3 mL/min
Splitless	Purge 50 mL/min @ 2 min
Oven program	45°C (2 min hold), 25 °C/min to 215 °C
Injection	SPME (PDMS/DVB/CAR) ; 2 min.; 250°C
Transfer line temperature	280 °C
GC Post-Run Conditions	
Backflush device	Purged Ultimate Union (P/N G3186-60580) controlled by a Pressure Control Module (P/N G3476-60501)
Backflush conditions	-5 mL/min @ 250 °C for 2 min

TCA MS Conditions

MS Conditions	
Tune	Autotune
Gain	20
Acquisition parameters	EI; selected reaction monitoring
Collision Gas Flows	Nitrogen at 1.5 mL/min, Helium at 2.35 mL/min
Solvent delay	6.5 minutes
MS temperatures	Source 300 °C; Quadrupoles 150 °C

Compound	Triple Quadrupole GC/MS			
	RT (min)	SRM	Dwell Time (ms)	Collision Energy (EV)
2,4,6-Trichloroanisole	7.594	210→195	25	15
		167→83	25	20
2,3,6-Trichloroanisole (IS)	7.867	210→195	25	10
		210→167	25	20
		167→83	25	20

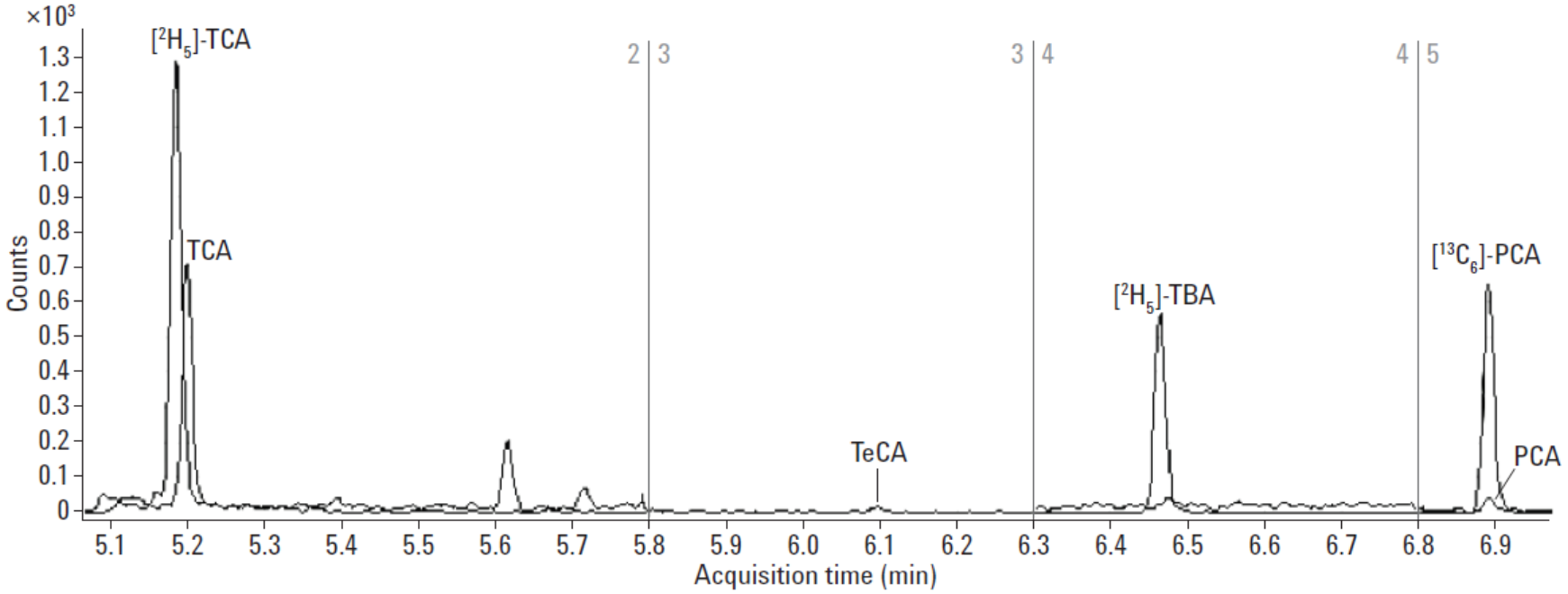
Note that Anna also identified a 212→197 (10 EV) qualifier transition for TCA.

Haloanisole MS Conditions
See Agilent Application note 5991-3812EN for details

Compound	Triple Quadrupole GC/MS		
	RT (min)	SRM	Collision Energy (EV)
$^{2}H_5$ TCA	5.20	215→197	10
		217→199	10
TeCA	6.10	246→203	25
		231→203	15
TBA	6.50	344→329	10
		346→331	10
$^{2}H_5$ TBA	6.48	351→333	15
		349→331	15
PCA	6.91	265→237	10
		280→237	25
$^{13}C_6$ PCA	6.91	286→242	25
		286→271	10

A 30 m DB-5 column was used for this study, like the TCA methodology. The flow rate was dropped from 3 to 1.2 mL/min. The oven parameters were also similar but without an initial hold time.

Chromatographic Separation and Results



Compound	Sensory Threshold in Wine (ng/L)	Standard Curve Range (ng/L)	Correlation Coefficient (R)	LOQ (ng/L)	LOD (ng/L)
TCA	3	0.1 - 50	0.999	0.5	0.1
TeCA	15	0.1 - 50	0.999	0.1	<0.1
PCA	3	0.1 - 50	0.999	0.25	0.1
TBA	10,000	0.5 - 50	0.999	1	0.5

In Summary

- Headspace-solid phase microextraction (HS-SPME) is an inexpensive and sensitive extraction procedure
- Canopy management steps can reduce presence of methoxypyrazines
- 2-Alkyl-3-Methoxypyrazines work well by methane PCI
- 2-Alkyl-3-Methoxypyrazines don’t work well by electron ionization (EI) without derivatization
- It is probable that Low Energy EI with the High Efficiency Source on the 7010 GC/QQQ would also work
- Most haloanisole contamination is anthropogenic
- TCA is the most prevalent haloanisole contaminant but TBA is also becoming an issue in wines
- With a sensitive extraction procedure, haloanisoles can be detected well below their odor threshold