Sensitive Detection of 2-Methoxy-3-Isobutylpyrazine (MIBP or IBMP) in Wine Using Triple Quadrupole GC/MS in PCI Mode

Application Brief

Foods and Flavors

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Abstract

A method for the detection and quantification of 2-methoxy-3-isobutylpyrazine in wine at a concentration as low as 2 ppt was developed using the Agilent 7890A/7000 Series Triple Quadrupole GC/MS in PCI mode with a Pressure Controlled Tee configuration.

Introduction

Many wine varieties contain 3-alkyl-2-methoxypyrazines, which are aroma substances with very low threshold values. They appear in high concentrations and contribute to the characteristic aroma in Sauvignon Blanc, Sémillon and Cabernet Sauvignon. One of the most important members of this family of compounds is 2-methoxy-3-isobutylpyrazine (known as both MIBP or IBMP). It has been reported in green pepper and helps to lend a vegetative, green pepper character to wine. The olfactory threshold value of MIBP is lowest in white wine, at 1 ng/L, and the optimal concentration of MIBP is in the very low range of 8–15 ng/L. Concentrations above 30 ng/L create unpleasant aromas. MIBP also functions as a pheromone in some species of ladybugs, and inclusion of these insects in the grape crush could lead to high levels and bad-tasting wine.

Detection of MIBP in wine is accomplished by headspace solid-phase-microextraction (HS-SPME) GC/MS analysis, and an electron ionization (EI) based HS-SPME GC/MS/MS method has recently been reported with a limit of detection (LOD) of 8.6 ng/L and a limit of quantification (LOQ) of 33 ng/L in wine [1]. This application brief describes a method using the Agilent 7000 Series Triple Quadrupole GC/MS which significantly extends the sensitivity of detection. The method utilizes positive chemical ionization (PCI) and GC backflushing to enable detection at levels as low as 2 ng/L (2 ppt) without a change in sample extraction procedures.

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Experimental

Standards and Reagents

The standards and reagents used are listed in Table 1. Stock solutions of MIBP and isotopically labeled MIBP, in concentrations of 10,000 µg/ml were prepared in 10% ethyl alcohol, stored at 4 °C in the dark, and diluted as required for calibration standards.

Table 1. Standards and Reagents

Standard	3-Isobutyl 2-methoxy-pyrazine	Sigma-Aldrich	99% purity
Reagents	Absolute alcohol	Malinckrodt	200 proof
	Water	Millipore	5 ppb TOC
	Sodium chloride	Sigma-Aldrich	99.5 % purity
	Tartaric acid	Sigma-Aldrich	99% purity

Instrument

The experiment was performed on an Agilent 7890A gas chromatograph (GC) equipped with a split/splitless capillary inlet and an Agilent 7000 Series Triple Quadrupole GC/MS with Triple-Axis Detector. The split/splitless inlet is fitted with a long-lifetime septum (p/n 5183-4761) and a deactivated, splitless single taper injection liner (p/n 5181-3316). HS-SPME injections were made using a manual SPME Holder (Agilent p/n 391896401 or equivalent). The instrument conditions are listed in Table 2.

Table 2. Gas Chromatograph and Mass Spectrometer Conditions

GC Run Conditions	
Analytical Column	2 × Agilent J&W HP-5ms UI, 15 m × 0.15 mm, 0.25 µm (p/n 19091S-431UI)
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 (2.25 min hold), 8 °C/min to 130 °C
Column velocity	39.8 cm/s
Injection	SPME; 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
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

A 1-meter Agilent J&W HP-5 ms Ultra Inert (UI) column was used as a guard, and a backflushing configuration was employed to allow for easy inlet and column maintenance. The guard column was connected to the first analytical column via an ultra-low dead volume Ultimate Union. Two 15-m Agilent J&W HP-5ms UI analytical columns were connected by a Pressure Controlled Tee configured with a Purged Ultimate Union [2-4]. The guard column protects the analytical column from any contaminants that are adsorbed onto the SPME fiber and allows servicing of the inlet without the potential to oxidize the analytical column.

Sample Preparation

All samples were prepared in 20-mL headspace vials. Calibration samples were prepared from the MIBP stock solutions in model wine (0.5 % w/v tartaric acid in 12 % v/v ethanol). Spiked samples were prepared in Sauvignon Blanc and Cabernet Sauvignon wine. Calibration and spiked samples were made at concentrations of 0, 5, 20, and 100 ng/L in MIBP, and 80 ng/L in isotopically labeled MIBP. Two grams of sodium chloride was added to increase extraction efficiency. HS-SPME was performed using a conditioned 50/30 µm DVB/Carboxen/PDMS StableFlex SPME fiber, (Agilent p/n SU57329U or equivalent). Samples were subjected to 30 min of static HP-SPME extraction at room temperature, and the fibers were desorbed for 2 min in the GC inlet at 250 °C.

Analysis Parameters

The parameters used in the analysis of MIBP and the internal standard are shown in Table 3.

Table 3. Analysis Parameters

		Triple Quadrupole GC/MS		
Compound	**RT (min**	**SRM**	**Dwell Time (ms)**	**Collision Energy (EV)**
MIBP	11.5	167→94	60	35
		195→124	60	30
		195→106	60	35
Isotopically Labeled-MIBP (Internal Standard)	11.5	170→127	20	30
		170→128	20	30
		170→100	20	30

Results

PCI Improves Sensitivity

The method developed on the Triple Quadrupole GC/MS system using PCI provides ultralow detection of MIBP in a complex matrix with minimal interferences (Figure 1). Using the calibration curve determined with MIBP in model wine (Figure 2), the level of MIBP in the tested Cabernet Sauvignon was measured at 2 ppt (Figure 3). MIBP was not detected in the tested Sauvignon Blanc (Figure 4a) or the model wine blank (4b). The high level of sensitivity achieved is due in large part to the selectivity of positive chemical ionization (PCI).

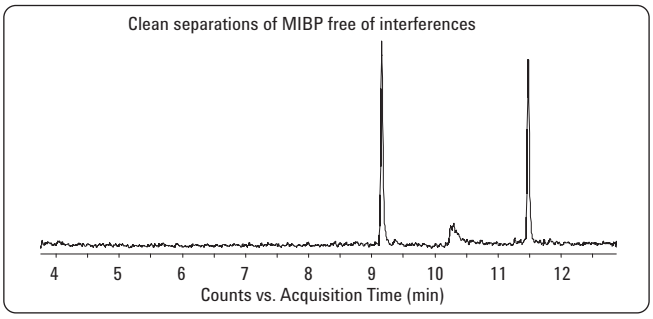


Figure 1. Reconstructed Total Ion Current Chromatogram (RTICC) resulting from SRM analysis, showing the separation of MIBP in a sample of Cabernet Sauvignon wine spiked with 5 ng/L MIBP. Both the isotopically labeled internal standard and MIBP standard elute at 11.5 minutes, and both are well resolved from the interference peaks at 9.2 and 10.4 minutes.

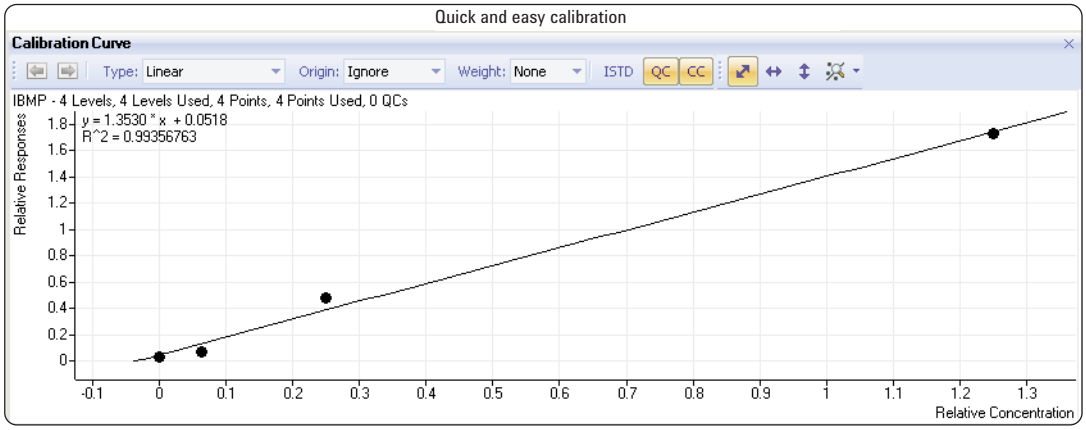


Figure 2. Calibration curve for quantification of MIBP. Samples containing 0, 5, 20 and 100 ng/L of MIBP in model wine were used to construct the curve.

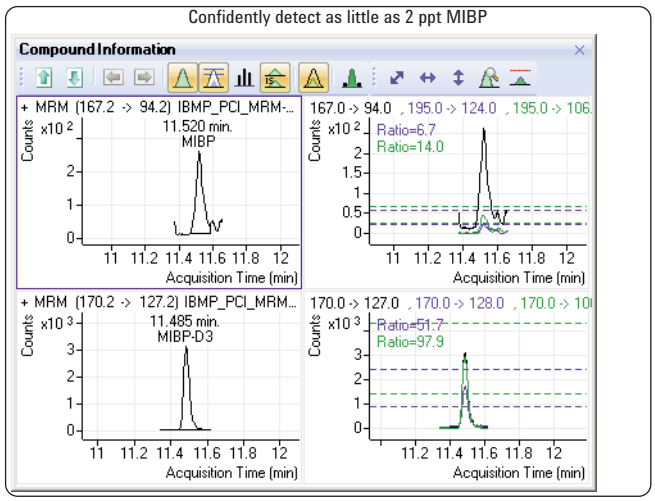
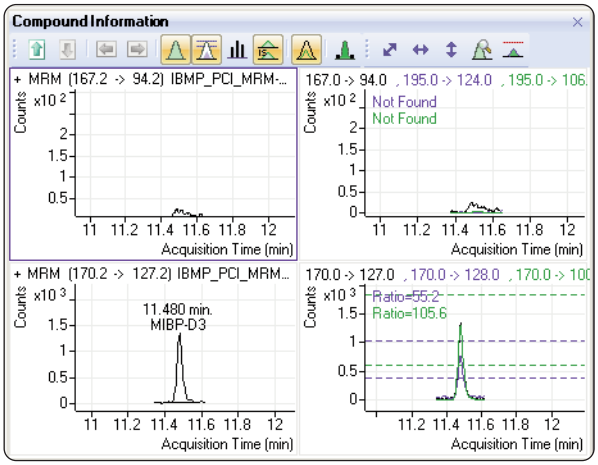
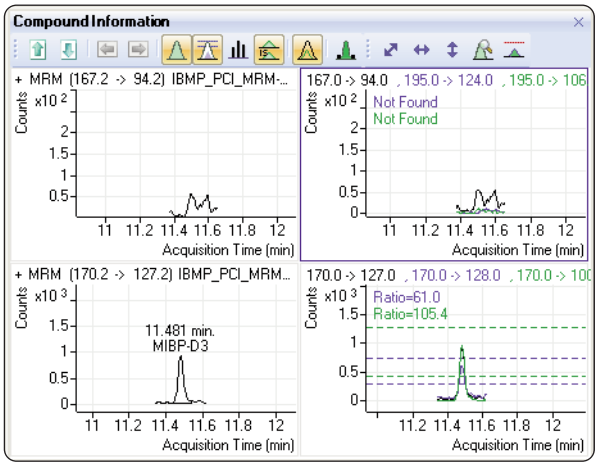


Figure 3. Detection of 2 ng/L (2 ppt) of native (unspiked) MIBP in Cabernet Sauvignon wine, quantified using the calibration curve in Figure 2. The upper traces show the quantifying transition (left) and the two qualifying (right) transitions for MIBP, with no significant interferences. The qualifying transition traces also show the uncertainty bands and the ratios for the two qualifying transitions, relative to the quantifying transition. The lower traces show the quantifying and qualifying transitions for the internal standard, isotopically labeled MIBP.

Elimination of chemical interferences enables low backgrounds and high sensitivity



a.



b.

Figure 4. Analysis of Sauvignon Blanc wine (a), as well as model wine containing no MIBP (b). The upper traces show the quantifying transition (left) and the two qualifying (right) transitions for MIBP, with no significant interferences. No measurable level of MIBP is detected in either sample. The lower traces show the quantifying and qualifying transitions for the internal standard, isotopically labeled MIBP. The qualifying transition traces also show the uncertainty bands and the ratios for the two qualifying transitions, relative to the quantifying transition.

Conclusion

The use of PCI mode with backflushing configuration on the new Agilent 7000 Triple Quadrupole GC/MS minimizes interferences and enables the detection of 2 ppt of MIBP in wine.

References

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2. H. Prest, C. Foucault and Y. Aubut, Capillary Flow Technology for GC/MS: Efficacy of the Simple Tee Configuration for Robust Analysis Using Rapid Backflushing for Matrix Elimination, Agilent Technologies Publication 5989-9359EN.
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Printed in the USA
June 13, 2013
5990-4935EN

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