Optimized Procedures for Analyzing Primary Alkylamines in Wines by Pentafluorobenzaldehyde Derivatization and GC−MS

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Biogenic primary alkylamines in wines are toxicologically significant and affect sensory properties. An optimized method for analysis in wines involving derivatization with pentafluorobenzaldehyde (PFB) to corresponding pentafluorobenzylimines, liquid−liquid extraction, and gas chromatography with mass selective detection is presented. Reaction parameters including pH, temperature, time, and derivatizing agent and amine concentration were varied in simulated wine solution (15% ethanol) to determine effect on reaction efficiency. Optimal reaction efficiency was characterized (pH 12, 24 °C, 30 min, and 10 mg/mL PFB), and parameters were used for the analysis of 10 biogenic alkylamines in 12 California wines. Alkylamine concentration in wines ranged from 0.048 to 91 mg/L. Amine recoveries from wines at five fortification levels (0.1−85 mg/L) were generally 81−100%.

Keywords: *Amines; wines; pentafluorobenzaldehyde; pentafluorobenzylimines; derivative*

INTRODUCTION

More than 30 biogenic amines have been identified in wines (Busto et al., 1995a,b), with total concentrations ranging from a few to about 50 mg/L (Lehtonen, 1996). They are produced by enzymatic degradation or fermentation processes (Busto et al., 1997; Cilliers and Van Wyk, 1985) and may be related to unsanitary winemaking conditions (Vidal-Carou et al., 1991; Zee et al., 1981). Biogenic amines have been shown to affect the sensory properties of wines and to be toxicologically significant (Busto et al., 1995a,b, 1997; Lehtonen, 1996). They are typically found as odorless salts in wines, but higher ambient pH in the mouth produces characteristically distasteful flavors (Lehtonen, 1996). When consumed with ethanol and acetaldehyde in alcoholic beverages, biogenic amines may contribute to symptoms of intoxication (i.e., headache, vomiting, diarrhea) (Jarisch and Wantke, 1996; Lehtonen, 1996) and may also play a role in alcohol dependence (Suomalainen et al., 1974). In addition, 4-(2-aminoethyl)phenol (tyramine), isopentylamine, 3-(2-aminoethyl)indole (tryptamine), and 2-phenylethylamine produce hypertension, while 1,5-diaminopentane (cadaverine) and 1,4-diaminobutane (putrescine) enhance the anaphylactic activity and toxicity of histamine (Subden et al., 1978). As a result, a rapid, sensitive analytical method is necessary to determine biogenic amine content in wines.

Amine derivatization with pentafluorobenzaldehyde (PFB) to corresponding pentafluorobenzylimines (PFBimines) (Figure 1) is compatible with gas chromatography with mass selective detection (GC−MS) and affords picogram level sensitivity by selected ion monitoring (SIM) of the characteristic α-cleavage products. Comparable sensitivity is achieved by GC with an electron capture detector (GC−ECD), but unreacted PFB saturates the ECD unless additional cleanup is applied (Hoshika, 1977). While PFBimine analysis by GC−ECD and GC−MS has been applied to various matrixes such as water, urine, and plasma samples (Avery and Junk, 1985, 1987; Payne et al., 1989; Durden, 1991; Hoshika, 1977; Roberts, 1984; Roberts and Oates, 1984), wines have never been examined. In fact, only a few GC methods have been developed for analyzing biogenic wine amines (Daudt and Ough, 1980).

The majority of studies use high-performance liquid chromatography (HPLC) with UV/vis, fluorescence, or electrochemical detection, following derivatization with various agents (i.e., orthophthalaldehyde, dansyl chloride, fluorescamine) (Lehtonen, 1996). Because of higher detection limits (nanogram level) relative to GC, wine amines or their corresponding derivatives must first be concentrated (Almy et al., 1983; Busto et al., 1994, 1995a,b; Daudt and Ough, 1980; Lehtonen, 1986), making sample preparation more laborious. Also, HPLC packed-column separation may not allow sufficient resolution of closely eluting analyte peaks, possibly sacrificing detectability. The advantages of PFB derivatization with GC−MS over conventional HPLC methods include higher sensitivity, selectivity, analyte resolution, and sample throughput. However, optimal reaction parameters for PFB derivatization have not been determined in wines.

The objectives of this work include the following: (i) determining optimal reaction parameters (i.e., pH, reaction temperature, reaction time, and concentrations

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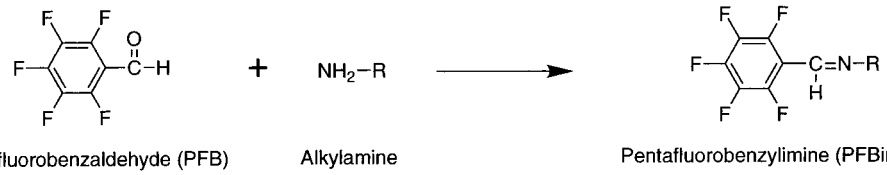

Figure 1. Formation of pentafluorobenzylimine derivatives (PFBimines) from pentafluorobenzaldehyde (PFB) and alkylamines.

of PFB) for the analysis of biogenic primary aliphatic amines by GC−MS−SIM; (ii) applying these parameters for determining 10 primary alkylamines in 12 California wines.

MATERIALS AND METHODS

Reagents. Primary aliphatic amines and internal standards (IS) including methylamine hydrochloride (99+%), ethylamine hydrochloride (98%), *n*-propylamine hydrochloride (99+%), *n*-butylamine (99.5%), *n*-hexylamine (99%), *n*-heptylamine (99%), 2-phenylethylamine hydrochloride (99%), 1,4-diaminobutane•2HCl (97%), 1,5-diaminopentane•2HCl (99%), pentafluoronitrobenzene (IS; 98%), and methyl-d_3-amine hydrochloride (IS; 98+ atom % D) were purchased from Aldrich (Milwaukee, WI). *n*-Pentylamine (98%) was from Lancaster (Pelham, NH). Pentafluorobenzaldehyde (98+%) was from Oakwood Research Chemicals (West Columbia, SC); acetonitrile and hexane were Optima grade solvents from Fisher (Pittsburgh, PA); and ethanol (200 proof) was from Quantum Chemical (Tuscaloola, IL). Cross-linked poly(vinylpyrrolidone) resin (PVPP) was from Aldrich; anhydrous sodium sulfate (ACS certified) was from Fisher. Purified deionized water was prepared with a Corning MegaPure apparatus (Dubuque, IA).

Wines. Wines were made in the Univeristy of California−Davis Department of Viticulture and Enology winery using standard procedures with grapes obtained from the UC Davis experimental vineyards (Oakville, CA, and Davis, CA) or were commercial wines donated to the UC Davis Department of Viticulture and Enology.

Instrumentation. A Hewlett-Packard 6890 series GC and 5972A MSD (Wilmington, DE) were used for all PFBimine analyses. The MSD was operated at full scan (*m*/*z* 50−500) for ion selection and SIM (*m*/*z* 208.0, 211.0, and 213.0; dwell time of 35 s) for samples. The *m*/*z* 208 and 211 ions correspond to the α-cleavage products of undeuterated PFBimines and methyl-d_3-PFBimine, respectively, while *m*/*z* 213 is the molecular ion for pentafluoronitrobenzene. Separation was achieved with a 30 m × 0.25 mm i.d. × 0.25 μm film DB-5 capillary column (J&W Scientific, Folsom, CA); carrier gas (He) flow rate was 2 mL/min. A split/splitless injector was used in splitless mode; the injector and transfer line were maintained at 250 and 280 °C, respectively; and 3 μL of the sample was injected for each run. The temperature program was as follows: 45 °C for 4 min, increased at 15 °C/min to 280 °C, held for 15 min.

Mass Spectra and Retention Times. The 70-eV electron impact mass spectra (with retention time) of internal standards and PFBimine derivatives (*m*/*z*) are shown relative to the base peak as follows: pentafluoronitrobenzene (IS; 5.747 min), 117 (100), 213 (47), 167 (33), 155 (30), 183 (23); methyl-d_3-amine (IS; 10.190 min), 211 (100), 212 (94), 117 (33), 180 (32), 193 (19); methylamine (10.288 min), 208 (100), 209 (88), 117 (26), 161 (19); ethylamine (12.826 min), 208 (100), 181 (33), 194 (19), 223 (16); *n*-propylamine (16.226 min), 208 (100), 181 (40), 209 (40); *n*-butylamine (19.761 min), 208 (100), 181 (64), 209 (50), 190 (47), 222 (23); *n*-pentylamine (23.035 min), 208 (100), 181 (96), 250 (74), 190 (63), 194 (31), 222 (24), 161 (21); *n*-hexylamine (26.174 min), 250 (100), 208 (96), 181 (89), 190 (62), 194 (25), 222 (19), 196 (18), 161 (17), 236 (13); *n*-heptylamine (29.123 min), 250 (100), 208 (73), 181 (58), 190 (46), 209 (37), 210 (21), 222 (19); 2-phenylethylamine (33.266 min), 208 (100), 181 (20); 1,4-diaminobutane (42.965 min), 249 (100), 181 (77), 208 (55), 194 (54), 221 (50), 230 (31), 161 (19); 1,5-diaminobutane (44.166 min), 181 (100), 208 (83), 263 (78), 244 (61), 190 (54), 222 (39), 161 (29).

Optimization Procedure. Initial reaction conditions for derivatization included pH 7, 60 °C, 100 mg/L mixed amines in 15% aqueous ethanol, 10 mg/mL PFB in acetonitrile, and a 0−5 h reaction time. For each time point, triplicate 5-mL standard taper test tubes (Kimble, Vineland, NJ) containing mixed amine solution (1 mL, pH 7) and PFB solution (0.5 mL) were prepared. Test tubes were glass stoppered and swirled gently to mix. Samples were reacted for t_0, 30 min, 1, 2, 3, and 5 h; t_0 samples were not heated, while the others were placed in a dry bath (Fisher, Pittsburgh, PA). After the appropriate reaction time, samples were immersed in ice water for 1−2 min to inhibit further reaction. Next, hexane (1 mL) containing pentafluoronitrobenzene IS (PFNB, 10 mg/L) was added, and the mixtures were shaken by hand for 90 s; if necessary, anhydrous sodium sulfate (100−200 mg) was added to disrupt emulsions. Finally, extracts were shaken for 30 s with 0.1 N NaOH (1 mL), which was used in a previous study (Hoshika, 1977) to convert PFB to a geminal diol (March, 1985) that is both unreactive toward amines and is presumably removed by aqueous phase partitioning. Derivatization and extraction efficiences of methylamine, ethylamine, *n*-propylamine, *n*-butylamine, *n*-heptylamine, 2-phenylethylamine, 1,4-diaminobutane, and 1,5-diaminopentane were determined by GC−MS−SIM as PFBimine:PFNB abundance ratios. Methyl-d_3-amine, distinguishable from methylamine by SIM, was used identically to undeuterated amines as a surrogate.

Since the pK_a of amines is about 9, pH effect on reaction efficiency was evaluated at 9, 10.5, 12, and 13.5. An Orion 250A meter (Boston, MA) and combination electrode was used for pH measurement. Temperature effects were evaluated separately by comparing reaction efficiencies at ambient room conditions (24 ± 1 °C) and 100 °C (maintained with the dry bath). Reaction times of 0−5 h were again used for these trials. Following optimization of pH, temperature, and time, the effect of PFB concentration (2, 5, and 20 mg/mL) was evaluated.

Because PFNB decomposition would produce misleading reaction efficiencies, its stability in hexane extracted with pH-adjusted water (5, 7, 9, 12, and 13.5) was investigated. Triplicate 1-mL aliquots of water were shaken for 90 s with 1 mL of PFNB−hexane (10 mg/L). Hexane fractions were analyzed in the same manner as the optimization samples and quantified against external standards.

Analysis of Wines. Eleven wines made using standard procedures in the Department of Viticulture and Enology (University of California Davis) and one commercial wine from the University of California Davis cellar were analyzed for primary amine content using optimized parameters [pH 12, room temperature (24 °C), 30 min reaction time, 10 mg/mL PFB]. After wines (70-mL aliquots) were spiked with methyl-d_3-amine (IS, 30 μL, 20 mg/mL), they were decolorized by magnetically stirring with polyvinyl polypyrrolidone (PVPP; 1.4 g) for 15 min and vacuum filtered through a 5.5-cm Buchner funnel and Whatman No. 1 filter paper; wine color following filtration ranged from clear to pinkish. In a prior study, PVPP removed polyphenolic compounds from wines with minimal loss of fortified biogenic amines (Busto et al., 1994). Decolorized wines were then pH adjusted with sufficient NaOH(s) and concentrated HCl. Triplicate aliquots (5 mL) were transferred to 25-mL standard taper test tubes, spiked with PFB solution (2.5 mL), swirled, and left to react. Solutions were quenched in ice water and liquid−liquid extracted with hexane (2 mL). Extracts were cleaned with 0.1 N NaOH (2 mL) prior to GC−MS−SIM analysis.

Table 1. Calibration Curve Information for Derivatized Alkylamines

analyte	*y*-intercept	slope	R^2	linear range (mg/L)
methylamine	0.0991	0.281	0.997	0.01−100
ethylamine	0.195	0.494	0.998	0.01−100
n-propylamine	0.233	0.476	0.998	0.01−100
n-butylamine	0.0461	0.270	0.998	0.01−100
n-pentylamine	0.00343	0.192	0.998	0.025−100
n-hexylamine	−0.0631	0.171	0.998	0.05−100
n-heptylamine	−0.0798	0.147	0.998	0.1−100
2-phenylethylamine	0.627	0.243	0.990	0.05−100
1,4-diaminobutane	−0.0462	0.00719	0.995	0.1−100
1,5-diaminopentane	−0.111	0.108	0.997	0.1−100

Wine amines were quantitated against a calibration curve of similarly analyzed mixed amine solutions (0.01−100 mg/L in 15% aqueous ethanol) using abundance ratios (alkyl-PFBimine:methyl-d_3-PFBimine); the linearity of amine derivatives is shown (Table 1). Standards were not treated with PVPP prior to derivatization and extraction. Also, *n*-pentylamine and *n*-hexylamine were analyzed in wines but had not been used in optimization studies. Wine amine analysis was validated by spike and recovery studies of Cabernet Sauvignon (1995) fortified with 0.1, 1, 10, 40, and 85 mg/L of all amines. The approximate method limit of detection for amines was half that of the lowest calibration curve standard (Table 1).

Malolactic fermentation in the wines used for this study was confirmed using enzymatic analysis of malic acid (Boehringer Mannheim Corp., Indianapolis, IN).

Statistical Analyses. A miminum of three analyses were performed for each sample. Means and standard deviations for replicate analyses were calculated and reported.

RESULTS AND DISCUSSION

Optimized PFB Derivatization. The influence of pH, reaction temperature, reaction time, and PFB concentration on amine derivatization are shown (Figure 2). Optimization study findings demonstrated that pH 12, 24 °C reaction temperature, 30 min reaction time, and 10 mg/mL PFB were most efficient for producing the PFBimine derivatives. Abundance ratios (PFBimines:PFNB) produced at 24 °C and 10 mg/mL PFB from 0 to 5 h typically increased from pH 5 to pH 12 but fell sharply at pH 13.5 (Figure 2A). Higher pH favors deprotonation of cationic amine salts to the corresponding base, which is required for nucleophilic attack of PFB. Decreased concentrations of PFBimines at excessively high pH are not surprising, since degradation of PFB to a geminal diol (March, 1985) prevents its reaction with amines. Increasing the temperature from 24 to 60 °C, while maintaining the optimal pH of 12, lowered the reaction efficiency of the alkylamines (Figure 2B). However, 2-phenylethylamine, 1,4-diaminobutane, and 1,5-diaminopentane were derivatized most effectively at 60 °C (Figure 2B). The aromatic ring of 2-phenylethylamine and the second derivatization required of the alkyl diamines likely demand higher reaction temperatures for optimal reaction relative to the *n*-alkylamines; aromaticity could also influence solubility in aqueous acetonitrile and, therefore, derivatization. Since the majority of our analytes are *n*-alkylamines, room temperature (24 °C) was adopted.

Similarly, while a reaction time of 5 h produced the highest reaction efficiency overall using pH 12 and 24 °C (Figure 2C), only 2-phenylethylamine, 1,4-diaminobutane, and 1,5-diaminopentane derivatization efficiency was hindered by shortening it to 30 min (Figure 2C). Since a 5-h reaction is not feasible, particularly if many samples are to be analyzed, 30 min was adopted.

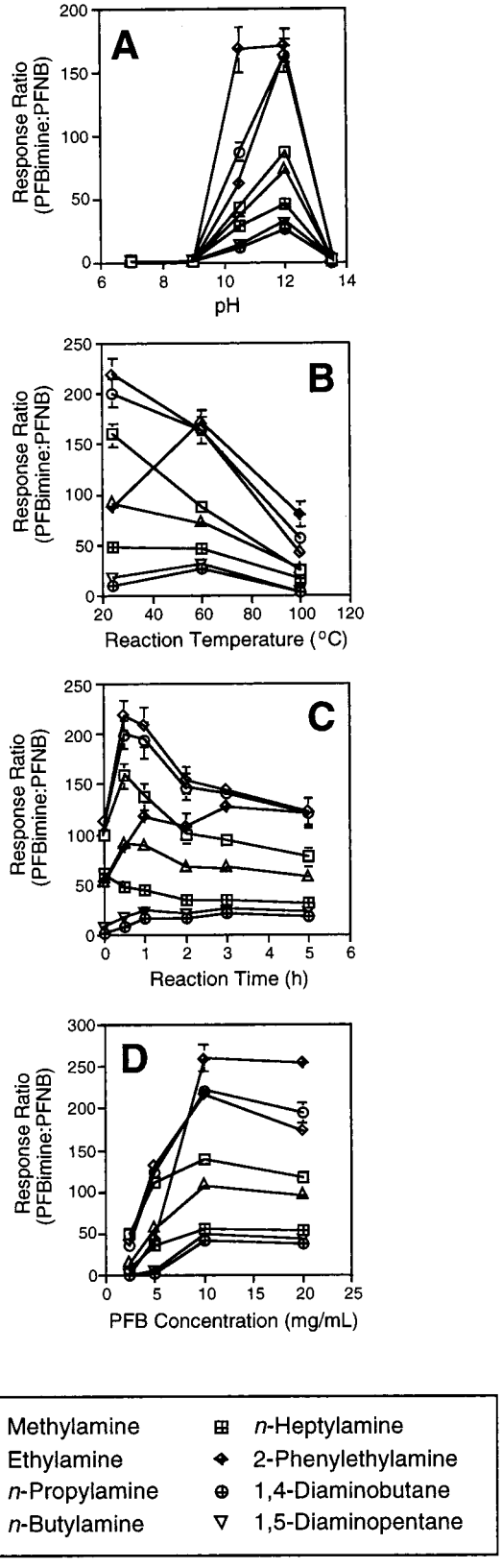

Figure 2. Effect of (A) pH, (B) reaction temperature, (C) reaction time, and (D) pentafluorobenzaldehyde (PFB) concentration on the derivatization of amines, shown as PFBimine:PFNB abundance ratios.

Reaction efficiency increased with 2−10 mg/mL PFB using optimized pH, reaction temperature, and reaction time but rose no higher with 20 mg/mL PFB (Figure 2D). PFB is apparently saturated at low levels, while

Table 2. Concentrations of Alkylamines (mg/L) in California Wines[a]

wine	methyl-amine	ethyl-amine	*n*-propyl-amine	*n*-butyl-amine	*n*-hexyl-amine	2-phenyl-ethylamine	1,4-diamino-butane	1,5-diamino-pentane
Cabernet Sauvignon, ML[b] (1985)	0.0677 ± 0.0008	0.328 ± 0.005	0.0777 ± 0.0007	< LOQ	0.273 ± 0.001	0.277 ± 0.000	5.09 ± 0.28	1.10 ± 0.01
Cabernet Sauvignon, ML (1995)	0.104 ± 0.002	0.860 ± 0.004	0.0794 ± 0.0002	0.0695 ± 0.0008	0.274 ± 0.000	0.276 ± 0.000	6.25 ± 0.19	1.21 ± 0.02
Cabernet Sauvignon, ML (1998)	0.0747 ± 0.0014	0.828 ± 0.011	0.0753 ± 0.0003	<LOQ	0.273 ± 0.001	0.279 ± 0.000	10.2 ± 0.2	1.33 ± 0.02
Cabernet Sauvignon (1998)	0.0780 ± 0.0010	1.85 ± 0.04	0.0757 ± 0.0002	<LOQ	0.274 ± 0.000	0.274 ± 0.000	5.89 ± 0.46	1.04 ± 0.00
Carignane (1997)	0.0939 ± 0.0016	3.50 ± 0.06	0.0762 ± 0.0002	<LOQ	0.273 ± 0.001	0.274 ± 0.001	1.26 ± 0.03	1.14 ± 0.01
Chardonnay, ML (1992)	0.0477 ± 0.0010	0.297 ± 0.001	0.0756 ± 0.0004	<LOQ	<LOQ	0.274 ± 0.000	2.17 ± 0.05	1.03 ± 0.00
Chardonnay, ML (1994)	0.131 ± 0.003	0.497 ± 0.005	0.0748 ± 0.0003	<LOQ	<LOQ	0.312 ± 0.001	1.59 ± 0.03	1.04 ± 0.00
Chardonnay (1997)	0.261 ± 0.001	2.05 ± 0.03	0.0747 ± 0.0004	<LOQ	<LOQ	0.311 ± 0.001	6.42 ± 0.24	1.07 ± 0.04
Chenin Blanc (1997)	0.0678 ± 0.0009	0.747 ± 0.002	0.0752 ± 0.0001	<LOQ	<LOQ	<LOQ	2.74 ± 0.08	1.04 ± 0.00
Pinot Noir (1998)	0.350 ± 0.002	4.15 ± 0.05	0.0777 ± 0.0002	<LOQ	0.277 ± 0.001	0.292 ± 0.001	91.1 ± 2.3	1.26 ± 0.01
Ruby Cabernet (1997)	0.0692 ± 0.0034	0.715 ± 0.023	0.0751 ± 0.0006	<LOQ	<LOQ	0.278 ± 0.000	11.6 ± 0.4	1.11 ± 0.01
Zinfandel, ML (1994)	0.0620 ± 0.0122	1.58 ± 0.67	0.0760 ± 0.0002	< LOQ	<LOQ	0.351 ± 0.041	4.63 ± 0.37	1.12 ± 0.06

[a] *n*-Pentylamine and *n*-heptylamine were below the LOQ for all wines tested. [b] ML refers to wines stated to have undergone malolactic fermentation.

concentrations ≥10 mg/mL sufficiently derivatized the amines. Since the maximum total amine content in wines is about 50 mg/L (Lehtonen, 1996) and since about 90 mg/L was used for optimization, 10 mg/mL PFB was selected. Reaction efficiencies in deionized water were comparable to the above, suggesting that variations in ethanol content in wines (typically <15%) would not effect derivatization.

The stability of PFNB during the extraction of derivatized optimization samples was confirmed by similar quantified levels (about 7 mg/L) in 15% aqueous ethanol solutions ranging from pH 5 to pH 13.5. As a result, the pH optimization results were not influenced by PFNB decomposition and reflect true reaction efficiencies. The concentration difference relative to the hexane stock (10 mg/L) can be explained by aqueous partitioning during extraction.

Amine Content of Wines. Ten amines were determined in 12 red and white varietal California wines (Table 2), and the total ion chromatogram for derivatized and extracted Cabernet Sauvignon with ML (1998) wine is shown (Figure 3). Overall, concentrations of amines ranged from 0.062 to 91 mg/L. Methylamine (0.048−0.35 mg/L), ethylamine (0.33−4.2 mg/L), *n*-propylamine (0.075−0.079 mg/L), 1,4-diaminobutane (1.3−91 mg/L), and 1,5-diaminobutane (1.0−1.3 mg/L) were found in all wines. The 91 mg/L of 1,4-diaminobutane in Pinot Noir (1998) appears anomalous, as the next most abundant amine was also 1,4-diaminobutane [12 mg/L in Ruby Cabernet (1997)]. This assumption was consistent with maximum reported values ranging from 10 to 17 mg/L in various wines analyzed by HPLC methods (Bauza et al., 1995; Busto et al., 1994; Soleas et al., 1999). Ranges for methylamine, ethylamine, *n*-propylamine, and 1,5-diaminobutane were consistent with those reported in previous wine studies using HPLC methods (Bauza et al., 1995; Busto et al., 1994, 1995a, 1996, 1997; Soleas et al., 1999).

Levels of 2-phenylethylamine were present in all wines (0.27−0.35 mg/L), except Chenin Blanc (1997); *n*-hexylamine (0.27−0.28 mg/L) was found in all Cabernet Sauvignon wines, Carignane (1997), and Pinot Noir (1998); *n*-butylamine was below the limit of quantification (LOQ) for all wines, except Cabernet Sauvignon with malolactic fermentation (w/ML) (1995) (0.069 mg/L). The absence of *n*-butylamine in all but one wine

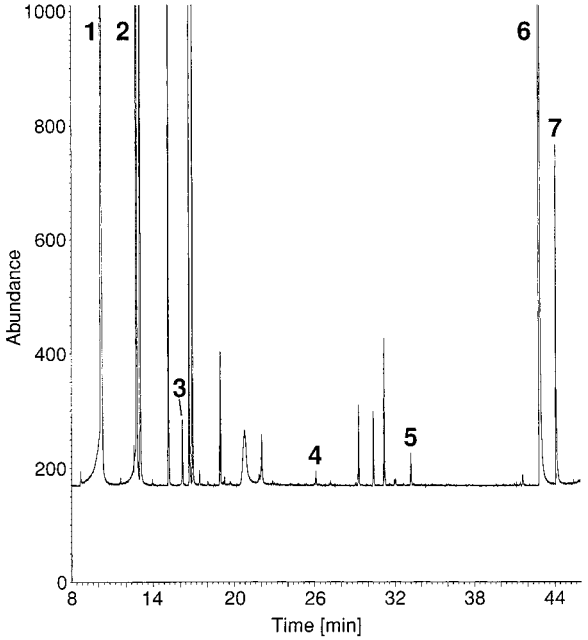

Figure 3. Total ion (*m*/*z* 208, 211, 213) chromatogram from derivatized Cabernet Sauvignon w/ML (1998) wine, showing methyl-d_3-amine and methylamine (1; coeluted at about 10.2 min), ethylamine (2; 12.8 min), *n*-propylamine (3; 16.2 min), *n*-hexylamine (4; 26.2 min), 2-phenylethylamine (5; 33.3 min), 1,4-diaminobutane (6; 43.0 min), and 1,5-diaminopentane (7; 44.2 min).

is indicative of its relative scarcity. Previous studies showed levels <0.01 mg/L (Ough, 1984) consistent with our findings, as our method LOQ is about 0.010 mg/mL, and we would not have detected *n*-butylamine at such levels (Table 1). Concentrations of 2-phenylethylamine and *n*-hexylamine are consistent with previously reported ranges (Busto et al., 1995a, 1996; Soleas et al., 1999). Both *n*-pentylamine and *n*-heptylamine were below the LOQ in all wines. Contrary to our findings, *n*-pentylamine has been reported in wines at 0.02−0.43 mg/L (Ough, 1984) and <0.45 mg/L (Busto et al., 1994). Levels of *n*-heptylamine were below the LOQ, supporting data from a prior study (Zee et al., 1981) which showed that this amine is not found naturally in wines.

A recent study by Goldberg and co-workers observed cultivar related differences in the concentration of

Table 3. Percent Recoveries of Alkylamines from Cabernet Sauvignon (1995) Wine

	fortification levels (mg/L)				
analyte	0.1	1	10	40	85
methylamine	89.0 ± 3.1	91.4 ± 0.9	85.0 ± 0.4	83.7 ± 0.1	81.1 ± 0.4
ethylamine	82.8 ± 4.8	94.8 ± 2.2	97.4 ± 0.6	88.4 ± 0.1	86.1 ± 0.9
n-propylamine	90.1 ± 1.0	89.2 ± 1.5	98.0 ± 1.5	88.3 ± 0.7	86.6 ± 2.0
n-butylamine	97.2 ± 2.6	85.2 ± 1.3	95.8 ± 1.8	83.4 ± 0.6	84.9 ± 3.3
n-pentylamine	101 ± 3	101 ± 2	94.6 ± 1.5	91.9 ± 0.5	83.0 ± 3.5
n-hexylamine	113 ± 5	95.5 ± 1.6	96.3 ± 1.6	81.3 ± 0.4	81.5 ± 4.1
n-heptylamine	190 ± 7	100 ± 5	98.2 ± 1.5	81.1 ± 0.4	81.2 ± 4.5
2-phenylethylamine	85.7 ± 2.2	90.8 ± 4.5	98.7 ± 2.6	121 ± 3	136 ± 9
1,4-diaminobutane	2020 ± 455	94.5 ± 5.0	102 ± 6	77.3 ± 1.0	82.8 ± 4.9
1,5-diaminopentane	144 ± 4	81.8 ± 0.5	94.4 ± 5.3	71.2 ± 1.1	71.5 ± 4.2

biogenic amines in wines from the Niagara region of Ontario, Canada (Soleas et al., 1999). From our limited sampling of 12 California wines made from seven different grape varieties, we cannot make any conclusions regarding amine content associated with these varieties.

No enhancement in amine production was evident in wines prepared with malolactic (ML) fermentation. Prior work with 184 South African wines demonstrated that ML fermentation increased the concentrations of histamine and 4-(2-aminoethyl)phenol (tyramine) (Cilliers and Van Wyk, 1985). Our 12 wines do not provide a comprehensive enough sampling to distinguish any differences. In addition, malic acid levels did not always correlate with the stated fermentation conditions employed for these wines. Malic acid concentrations ranged from 0.027 to 1.7 mg/L and from 0.027 to 1.48 mg/L without and with malolactic fermentation, respectively. The effects of other enological variables, including length of skin contact time, barrel aging, and sur-lie fermentation, also require further study for their effects on biogenic amine concentrations in wines.

The high recoveries of amines spiked into Cabernet Sauvignon w/ML (1995) between 0.1 and 85 mg/L (Table 3) validated the use of our method for authentic wines, and the excellent separation of the derivatized amines at 40 mg/L spike level is shown in its total ion chromatogram (Figure 4). Overall, recoveries were >82% for spiked concentrations of 0.1−10 mg/L but diminished at 40 and 85 mg/L fortifications; variability for all levels was typically <5%. PFB depletion at these high amine levels could reduce the conversion of amines to PFBimines, producing lower recoveries. From optimization studies, we previously determined that 10 mg/L of PFB was suitable for the derivatization of 100 mg of total mixed amines. Considering that a total of about 400 and 850 mg were derivatized at 40 and 85 mg/L fortifications, respectively, the resulting recoveries of >71% at these levels are reasonable. At these high fortifications, the *n*-alkylamines were derivatized and extracted with moderately high efficiency (81−92%). The diamines 1,4-diaminobutane and 1,5-diaminopentane demonstrated lower recoveries (71−83%), due to the second derivatization occurring on each. Excessive recoveries of 2-phenylethylamine at 40 and 85 mg/L (121% and 136%, respectively) indicate that the wine itself may enhance derivatization, relative to that which occurs in preparing calibration standards from 15% ethanol solution. Certainly, the phenyl group is unique among the amines used in this study, and this could contribute to differing and, perhaps, higher solubility in wines. Lesser sorption of 2-phenylethylamine to PVPP during wine cleanup as compared to *n*-alkylamines is another explanation, since the abundance

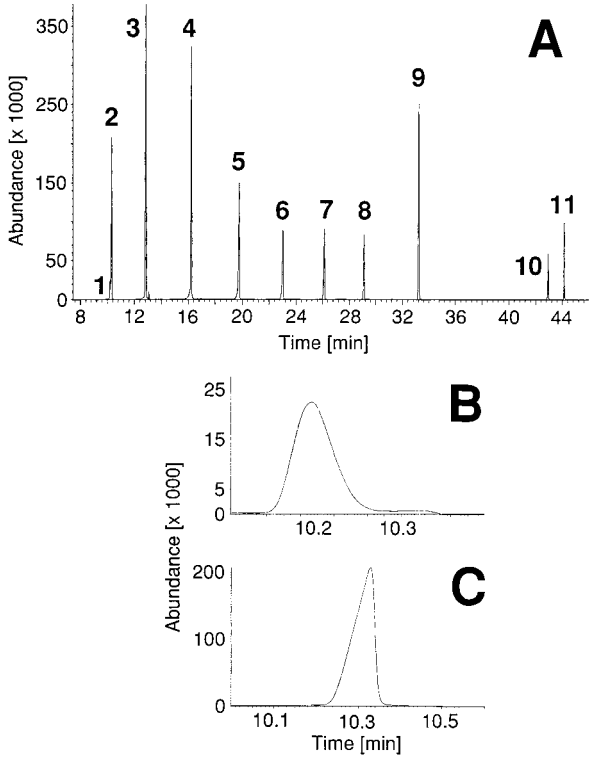

Figure 4. GC−MS total ion (*m*/*z* 208, 211, 213) chromatogram (A) of methyl-d_3-amine (1; 10.2 min), methylamine (2; 10.3 min), ethylamine (3; 12.8 min), *n*-propylamine (4; 16.2 min), *n*-butylamine (5; 19.8 min), *n*-pentylamine (6; 23.0 min), *n*-hexylamine (7; 26.2 min), *n*-heptylamine (8; 29.1 min), 2-phenylethylamine (9; 33.3 min), 1,4-diaminobutane (10; 43.0 min), and 1,5-diaminopentane (11; 44.2 min) fortified in Cabernet Sauvignon w/ML (1995) wine at 40 mg/L each. The separation of coeluting methyl-d_3-amine (B) and methylamine (C) by ion extraction (*m*/*z* 211 and 208, respectively) is also shown.

ratio (phenylethylPFBimine:methyl-d_3-PFBimine) would be higher. Increasing 2-phenylethylamine recoveries from 0.1 to 85 mg/L does support the possibility of sorption occurring, as the proportion of nonsorbed 2-phenylethylamine would increase with concentration.

Fortifications of mixed amines from 0.1 to 10 mg/L demonstrate the applicability of this method to the analysis of wine amines at typical levels. The *n*-alkylamines from methylamine to *n*-pentylamine, as well as 2-phenylethylamine, were recovered with excellent efficiency (85−101%). While recoveries of *n*-hexylamine and *n*-heptylamine at 1 and 10 mg/L were consistent (95−100%), excessive values were observed at 0.1 mg/L. Recoveries of 113% and 190% were obtained from *n*-hexylamine and *n*-heptylamine, respectively, and are indicative of quantification error near the analyte LOQ. The *n*-heptylamine recovery also reflects quanti-

ties in the Cabernet Sauvignon wine that were below the LOQ and, consequently, could not be subtracted, but this contribution is minor. The diamines 1,4-diaminobutane and 1,5-diaminopentane were recovered effectively from the 1 mg/mL fortification at 95% and 82%, respectively. However, excessive values at the 0.1 mg/L level are again explained by calibration curve extrapolation error near the LOQ.

Difficulty with quantification at low and high fortifications for certain amines was not a problem in analyzing our 12 wines. Since *n*-heptylamine levels were below the LOQ in all samples, the issue of overestimating concentrations at or below 0.1 mg/L was not considered. Similarly, levels of 2-phenylethylamine (<0.35 mg/L) were far below the 40 and 85 mg/L fortifications exhibiting excessive recoveries. Also, 1,4-diaminobutane and 1,5-diaminopentane levels (1.3−91 and 1.0−1.3 mg/L, respectively) were higher than the 0.1 mg/L fortifications exhibiting calibration curve extrapolation error and typically lower than the 40 and 85 mg/L fortifications that showed diminished recoveries. These findings, combined with the high amine recoveries overall, validate and attest to the accuracy of wine concentrations determined by this method.

SUMMARY AND CONCLUSIONS

Analysis of amines in wines by PFB derivatization, liquid−liquid extraction, and GC−MS−SIM is a rapid, sensitive, and selective technique. Optimized parameters (pH 12, 24 °C, 30 min, 10 mg/mL PFB) struck a balance between reaction efficiency and sample throughput capabilities. These were applied for the determination of 10 amines in 12 California wines; measured concentrations were comparable to ranges reported in the literature. Spike and recovery studies in wine validated the analysis of amines, particularly at fortifications most applicable to the wines that were analyzed.

ABBREVIATIONS USED

PFB, pentafluorobenzaldehyde; PFBimine, the generic amine derivative; methyl-d_3-PFBimine, amine derivative for methyl-d_3-amine; phenylethylPFBimine, amine derivative for 2-phenylethylamine; SIM, selected ion monitoring; LOD, limit of detection; LOQ, limit of quantification; ML, malolactic fermentation.

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