Histaminol: Identification and HPLC Analysis of a Novel Compound in Wine

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Histaminol, a minor histamine metabolite originating from imidazole acetaldehyde, has been detected in a food matrix as complex as wine. The standard molecule was synthesized, and subsequently the chemical structure was confirmed by ESI-MS and NMR measurements. The development, optimization, and in-house validation of a HPLC-DAD chromatographic method for the quantitative determination of histaminol in wine are described and discussed. The expanded uncertainty ($U_{(k=2)}$) of the procedure was estimated as 11.06%. Twenty commercial Italian wine samples were selected. All samples (16 red and 4 white wines) were analyzed after a C-18 SPE cartridge fractionation procedure. The content of this alcohol was in the range of 0.289−1.094 mg/L (minimum and maximum values were obtained for Nero d'Avola vintage 2007 and Barolo vintage 1969, respectively).

KEYWORDS: Histaminol; wine; HPLC-DAD; validation

INTRODUCTION

Wines and, in general, alcoholic beverages are fermented products obtained through the metabolic action of different microorganisms, such as yeasts and lactic acid bacteria (*1*). During the fermentation process a large number of new compounds are formed, giving different characteristics to the wine when compared to the initial fruit (*Vitis vinifera* L.) (*2, 3*).

Sugars and proteins are the main targets of this process that provides for microorganisms the energy and nutrients required for their development (*4*). The conversion of amino acids is an important topic in the field of fermented foods. There are two main molecular groups through which the starting amino acids are converted: amines or alcohols.

Among microorganisms, lactic acid bacteria can carry out malolactic fermentation and cause various degradative phenomena, even when they are present in low amounts. In any case, their desirable or undesirable activity can lead to a biogenic amines accumulation (*5*). The main biogenic amines in wine are histamine, tyramine, putrescine, cadaverine, and phenylethylamine; these are important because they are responsible for health risks to sensitive individuals (*6*).

The second catabolic way adopted by microorganisms produces alcohols. Fusel alcohols (or fusel oils) derive from amino acid catabolism via a pathway that was first proposed by Felix Ehrlich (a century ago) (*7−9*). This pathway consists of three enzymatic steps: transamination to form an α-keto acid, decarboxylation to an aldehyde, and reduction to the fusel alcohol (*10−12*). *Saccharomyces cerevisiae* can use tryptophan, phenylalanine, or tyrosine as the only source of cellular nitrogen, with the main products of their catabolism being tryptophol, phenylethyl alcohol,

or tyrosol, respectively (*13, 14*). Tyrosol is a compound of pharmaceutical interest showing antioxidant activity (*15*). Tyrosol and hydroxytyrosol, formed by hydroxylation of the aromatic ring of tyrosol and which are present also in virgin olive oil, may be responsible for the cardioprotective bioactivity of wine (*16, 17*).

However, concerning a well-known fermented product such as wine, the analysis of histaminol, the corresponding alcohol derived from catabolism of L-histidine, is not reported.

The metabolic pattern of histamine is well-known from several studies (*18, 19*), the major routes of catabolism being methylation and oxidation. Histaminol is a minor histamine metabolite originating from imidazole acetaldehyde detectable in human urine (in a healthy subject, about 2%) (*20*). With regard to its biological activity this alcohol has a weak inhibitory activity against diamine oxidase, affecting only enzymes of mammalian origin (*21, 22*).

Among microorganisms it is reported that several mycobacteria (*M. diernhoferi, M. fortuitum, M. chelonei*) oxidize histamine to histaminol without further change, transforming histamine into histaminol and imidazolylacetic acid (*23*). Also, some yeast strains (*Saccharomyces rouxii*) are able to transform histamine into histaminol (*24*).

On the basis of the assumptions described previously, the purpose of this work was to synthesize the histaminol standard molecule and subsequently to develop and validate a HPLC-DAD chromatographic method for its qualitative and quantitative characterization. Then, after the optimization of this protocol of analysis, our research work was planned to investigate if this molecule was really present because the existence of this compound in wine has never been reported.

MATERIALS AND METHODS

Chemicals. Chromatographic solvents were of HPLC-MS or HPLC grade, according to their application, and were purchased from Sigma-Aldrich

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(Milan, Italy). Water was obtained by Milli-Q instrument (Millipore Corp., Bedford, MA). All reagents were purchased from Sigma-Aldrich.

Wine Samples. All samples (16 red and 4 white wines) were purchased in a local wine shop. Eight types of wine were from the 2008 vintage, nine from 2007, one from 2006, and, to assess the presence of histaminol in mature samples, two aged red wines, respectively from 1977 and 1969 vintages, were selected. For all samples, histaminol analysis was done immediately after bottle opening.

C-18 SPE Cartridge Cleanup/Concentration of Wine Samples. Wine samples were concentrated by C-18 SPE cartridges (SupelClean LC-18 SPE tubes; bed wt, 1 g; volume, 6 mL; Supelco) following the method described by Sun et al. (*25*), with minor modification. Fifty milliliters of wine was treated, using a rotary evaporator at 30 °C, to remove the ethanol. Evaporated ethanol was replaced to the original volume and pH by addition of acidulated distilled water relative to each sample of wine. Ten milliliters of this solution was used for the concentration process. C-18 SPE cartridges were conditioned with methanol (10 mL) and distilled water (2 × 10 mL). The samples were then passed through the cartridges at a flow rate not higher than 2 mL/min. Residual histaminol in the cartridges was eluted by 10 mL of distilled water and collected together with the previous aqueous fraction; the eluate was evaporated to dryness under vacuum and redissolved in methanol, final volume of 1.5 mL (6.67 times more concentrated with respect to the starting wine). The solutions were stored at −20 °C until use (maximum period of storage = 1 month).

Synthesis and Characterization of Histaminol. 4-Imidazolylacetic acid (965 mg, 5.94 mmol) was dissolved in methanol (25 mL), and then thionyl chloride (1.41 g, 11.9 mmol) was added. The mixture was stirred at room temperature for 2 h. After removal of the solvent, the residue was dried under vacuum (methyl 4-imidazolylacetate hydrochloride, white solid, 1.44 g, yield 99%). The methyl ester (1.04 g, 7.31 mmol) was suspended in *tert*-butanol (25 mL) and then treated with sodium borohydride (543 mg, 14.6 mmol) under hot refluxing for 30 min. The solvent was removed, and the solid residue was dissolved in hot methanol. Borates were eliminated, whereas supernatant was evaporated to dryness. The residue (crude histaminol) was purified by gravity column chromatography using $CH_2Cl_2/MeOH/NH_3$ 8:8:0.2 (v/v/v) to obtain histaminol (colorless oil, 110 mg, 13.4% yield).

The purified histaminol ($C_5H_8N_2O$; 112.2 g/mol) was characterized by means of ¹H NMR (CD₃OD, 300 MHz, 298 K), ¹³C NMR (CD₃OD, 75.4 MHz, 298 K) and mass analysis (ESI/MS(+): *m/z* 113 [M − H]⁺) as reported in **Figure 1**. Purity assessed by ¹H NMR (99.25 ± 0.25%) was determined considering the signals of residual solvent.

Working standard solutions in the range of 0.5−10 mg/L for HPLC analysis were prepared by appropriate dilution of the stock standard solution (100 mg/L) in methanol kept at 4 °C for no more than 2 weeks. All working standard solutions were freshly prepared daily prior to use.

HPLC-DAD-ESI-MS/MS. A Surveyor Thermo Finningan chromatography system equipped with a diode array detector (Surveyor PDA) and an ion-trap mass spectrometer (Thermo Finningan LCQ Deca XP Plus MS) was used. Separation was performed on a reversed-phase Supelcosil LC-318 column (250 × 4.6 mm i.d., with particle size of 5 μm, Supelco Park, Bellefonte, PA) at 35 °C. Eluent A was water, eluent B was water/formic acid 0.1% v/v, and solvent C was methanol. The flow rate was kept constant throughout the analysis at 1 mL/min. The elution program was as follows: 1% B isocratic during all of the analysis; 1% C isocratic from 0 to 6 min, 1−99% C linear from 6 to 8 min, 99% C isocratic from 8 to 18 min, 99−0% C linear from 18 to 20 min, and reequilibration of the column from 20 to 35 min under initial gradient conditions. DAD was performed at 211, 215, and 254 nm. The injection volume was 1 μL. The eluate was injected into the electrospray ion source with a splitting of 40%, and the MS and MS/MS spectra were acquired and interpreted using the software Xcalibur. Operating conditions on the ion-trap mass spectrometer in positive polarity were as follows: source voltage, 5.36 kV; source current, 7.27 μA; capillary temperature, 350 °C; capillary voltage, 9.73 V; tube lens voltage, −20.00 V; sheath gas flow rate (N₂), 60 AU. Data were acquired in positive MS and MS/MS scanning mode from *m/z* 50−500; the precursor isolation window was set at 1 atomic mass unit (amu), and the collision energy was optimized at 30%.

HPLC-DAD. A Shimadzu LC-20A Prominence chromatographic system equipped with a diode array detector (DAD; diode array detector SPD-M20A) was used. Separation was performed in the same chromato-
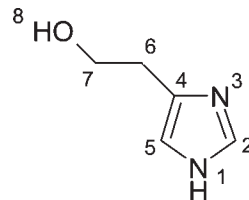
1H NMR	chem shifta (ppm)	13C NMR	chem shift (ppm)
H-1/3	3.30 (m)	C-2	134.3
H-2	7.56 (s)	C-4	134.0
H-5	6.83 (bs)	C-5	117.2
H-6	2.78 (t)	C-6	29.8
H-7	3.76 (t)	C-7	61.3
H-8	2.71 (t)		

Figure 1. ¹H and ¹³C NMR spectroscopic data of histaminol in CD₃OD solutions at room temperature (298 K).

graphic conditions applied and developed in the HPLC-DAD-ESI-MS/MS protocol. A wavelength of 211 nm was set for the in-house validation. The injection volume was 1 μL.

Statistical Analysis. Results were expressed as mean ± standard deviation (SD) of at least three independent experiments. Differences among samples were estimated by analysis of variance (ANOVA) followed by Tukey's "Honest Significant Difference". The statistical significance level was set to 0.05. All statistical analyses were performed using the free statistical software R 2.8.1 version (R Development Core Team, 2008) (*26*).

In-House Validation Procedure. *Linearity.* The ordinary least-squares (OLS) regression method was employed. Linearity of the method was evaluated at six concentration levels (0.5, 1.0, 2.5, 5.0, 7.5, and 10.0 mg/L) obtained by dilution of the histaminol standard stock solution. The calibration curve was obtained by analyzing each of these solutions ($n = 9$) on the same day. Blanks were also prepared as a quality control tool, but not used for the regression analysis. Outliers were determined using the Grubbs test. The goodness of calibration function was studied on the basis of requirements such as homoschedasticity (by means of Bartlett test) and linearity (both lack-of-fit test and Mandel fitting test). Normality of the residuals and their independence (no autocorrelation) were determined by Shapiro−Wilk test and Durbin−Watson test, respectively.

Sensitivity, LOD, and LOQ. Sensitivity, limit of detection (LOD), and limit of quantitation (LOQ) were determined from the calibration curve data. Sensitivity was calculated by dividing the residual standard deviation σ_r

$$\sigma_r = \sqrt{\frac{\sum_{i=1}^{n} e_i^2}{n-2}}$$

where e_i = residual (differences between observed and predicted values), by the slope of the curve. LOD and LOQ were calculated by multiplying the sensitivity value by 3.3 and 10, respectively. The LOD concentration has been experimentally confirmed by injecting in triplicate the related solution and observing a signal-to-noise of ≈3. In the same way, the real LOQ was evaluated by three analyses of the standard solution observing a signal-to-noise of ≈10.

Precision. Precision was evaluated in terms of repeatability and intermediate precision. The repeatability and the intermediate precision were investigated using three sample solutions formulated at three different concentration levels of histaminol: 2.5, 5, and 10 mg/L, respectively. For the repeatability each solution was analyzed in triplicate ($n = 3$) during the same day; repeatability was expressed as relative standard deviation percentage (RSD%). The intermediate precision was determined by analyzing the samples at the same concentration levels on three different days ($n = 9$). RSD% values were calculated, and analysis of variance (one-way ANOVA) was performed.

Bias. Bias was proved in terms of recovery. For the recovery assay commercial wine samples were spiked with an appropriate volume of histaminol standard solution to obtain final concentrations of 0.5 and 1 mg/L, respectively. After SPE treatment, each sample was injected three times. The recovery values and RSD values ($n = 3$) were calculated from peak area ratios.
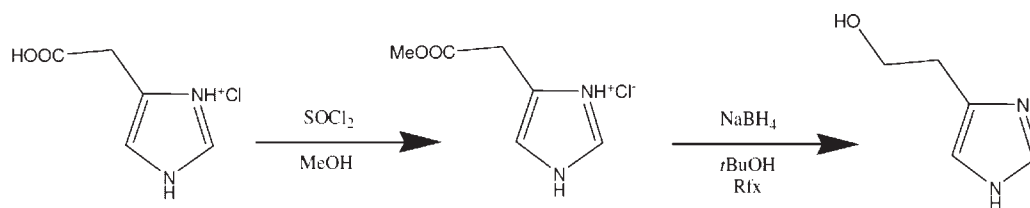
Figure 2. Two-step scheme of histaminol synthesis.

T: + cESI Full ms [50.00-150.00]
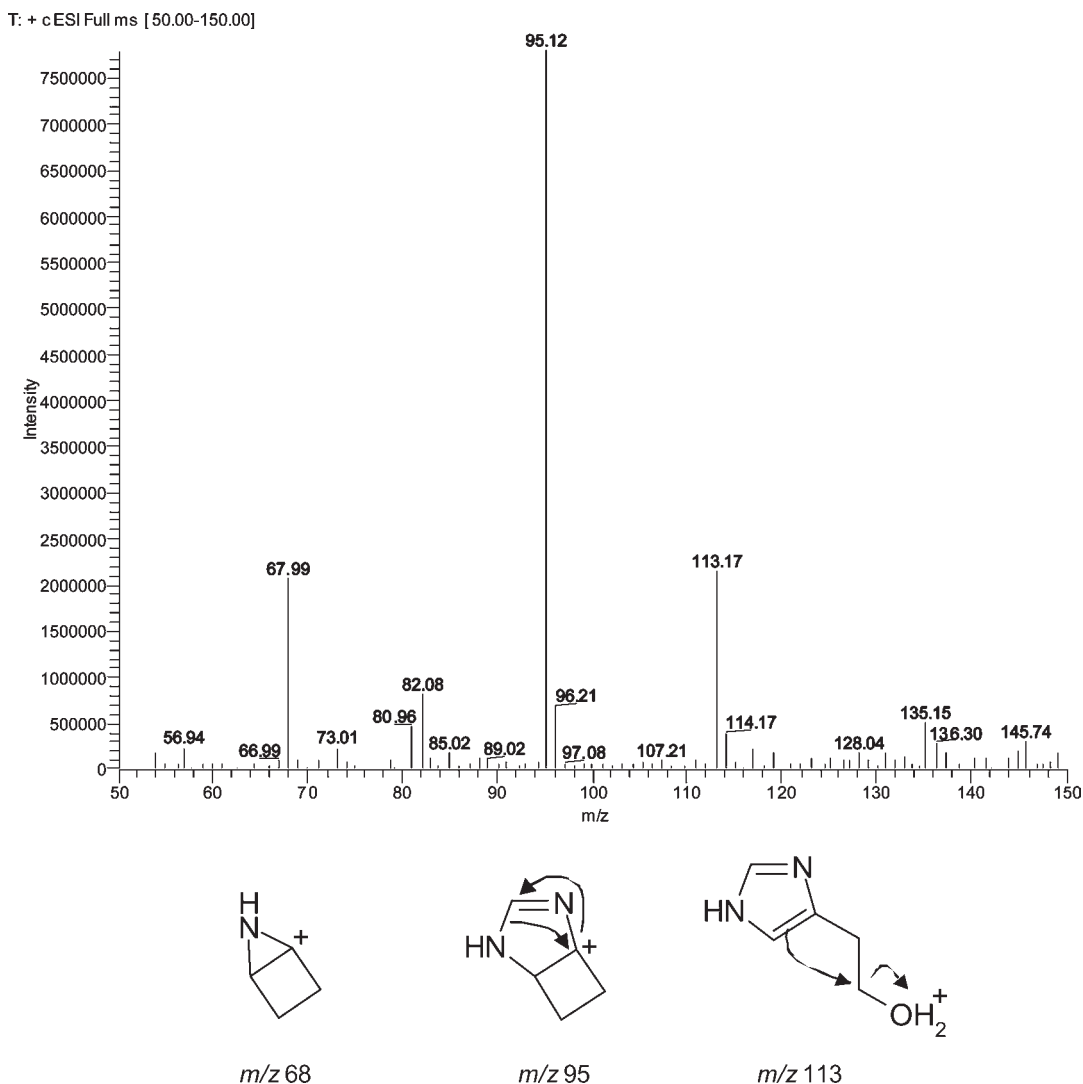
Figure 3. Relative abundance of histaminol fragments obtained using the ESI$^+$-MS source displayed by low-energy collision-induced dissociation of protonated molecules. The fragmentation pattern is dominated by the loss of water ($-H_2O$), *m/z* 95 ions, and the loss of a cyano group ($-HCN$), *m/z* 68 ions.

Uncertainty Estimation. Measurement uncertainty was estimated on intralaboratory data: studies of precision, performance data of the analytical process, and quantification of individual component. Contribution from stock standard solution concentration is not covered by precision data and was estimated individually. After estimation of all sources of uncertainty, they were joined according to their laws of combination obtaining the combined standard uncertainty. To calculate the expanded uncertainty (*U*), a coverage factor (*k* = 2), which corresponds to a 95% confidence interval, was used (*27–30*).

RESULTS AND DISCUSSION

The histaminol molecule is not readily available as a standard; therefore, we plan to obtain it through synthesis. Starting from commercial 4-imidazoleacetic acid hydrochloric, the carboxyl group

was reduced to obtain the corresponding alcohol (**Figure 2**). Purification of the final product was carried out by gravity column chromatography, and then it was fully characterized by NMR and mass direct analyses.

Under ESI$^+$-MS conditions, the mass spectrum of histaminol showed an abundant [M + H]$^+$ ion without fragmentation. The product ion MS/MS spectrum as obtained by low-energy collision-induced dissociation of protonated molecules showed fragmentation patterns dominated by the loss of water ($-H_2O$), leading to product ions at *m/z* 95, and the loss of a cyano group ($-HCN$) to *m/z* 68 ions (**Figure 3**).

Chromatographic characteristics of histaminol (retention time and mass confirmation) were certified by the HPLC-DAD-ESI-MS/MS system. Elution under reversed-phase (C-18) partition
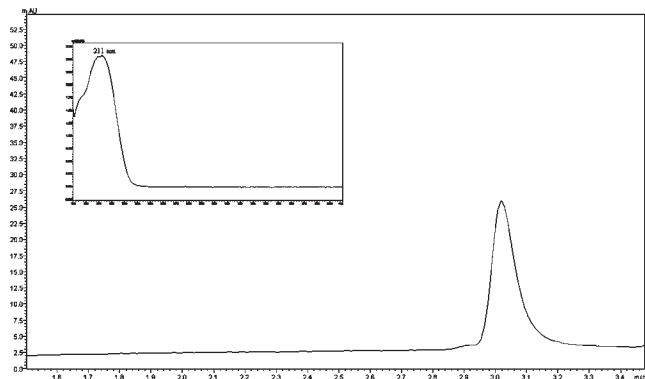
Figure 4. HPLC-DAD chromatogram and spectrum (maximum absorption at 211 nm) of histaminol standard. Elution under reversed phase (C-18) allows molecule separation in 3 min (high repeatability; RSD < 0.5%).

conditions allowed histaminol separation in approximately 3 min (the entire chromatographic analysis requires 35 min) with high repeatability (RSD < 0.19%), and its DAD spectrum showed a maximum absorption at 211 nm (**Figure 4**).

The wine samples were not directly analyzed, but they underwent a phase of cleanup to eliminate anthocyanidinic and proanthocyanidinic components to obtain both a reduction of potential interfering substances and a concentration of histaminol. Sample fractionation was performed using solid-phase extraction cartridges (C-18); optimal working conditions were adequately selected as reported under Materials and Methods.

In-House Validation. After optimization of the chromatographic conditions and identification of the molecule, an in-house validation method was performed by HPLC-DAD. A calibration curve was constructed by injecting six different concentration levels of histaminol standard solutions in the range of 0.5−10 mg/L. For each concentration, solutions were prepared independently and injected at at least three different times. The linear regression model (OLS method) showed a good correlation between the histaminol concentration and the detector response ($y = 926.94x - 137.98$; $r^2 = 0.9982$). Homoschedasticity, normality, and independence of the residuals were verified. Bartlett test confirmed no significant differences among the variance values at the different concentration levels, showing *p* values > 0.05 at the 95% confidence level (**Table 1**). The Shapiro−Wilk test confirmed no significant deviation of normality for the residuals (*p* > 0.05), and no autocorrelation was observed (Durbin−Watson test, *p* > 0.05). To verify the goodness of linear regression, the lack-of-fit test and the Mandel test were also performed, both indicating that the linear regression model correctly describes the experimental data (*p* > 0.05). The results of the regression statistic obtained for histaminol ratio are reported in **Table 1**.

System suitability parameters were measured to verify the system performances; the value of retention time of histaminol was 3.01 min, theoretical plate number was 5503, and tailing factor was 1.3. All of the values for the system suitability parameters were within acceptable ranges.

LOD and LOQ were determined on the basis of the calibration curve, following the method suggested by the ICH guidelines (method based on the standard deviation of the response and the slope) (*31*). LOD and LOQ obtained for histaminol standard solution were 0.489 and 1.482 mg/L, corresponding to 0.073 and 0.222 mg/L of wine, respectively. These results attest to the potential of this method for the determination of histaminol in wine samples.

Method precision, evaluated in terms of repeatability and intermediate precision, was assessed at three different concentra-

Table 1. Statistical Evaluation of Histaminol Curve Calibration (Linear Regression Model), Precision, and Trueness of the Method for the Histaminol Quantification

	calibration equation ($y = mx + q$)[a]
concentration range (mg/L)	0.5−10
m	926.94
q	−137.98
r^2	0.9982

	regression diagnostic	
	statistic	*p* value
homoschedasticity (Bartlett test)	10.93	0.053
normality of residuals (Shapiro−Wilk test)	0.986	0.797
independency of residuals (Durbin−Watson test)	2.378	0.899
F (variance ratio)	2.939×10^4	$<2.2 \times 10^{-16}$
lack-of-fit test	1.746	0.155
Mandel test	0.012	0.914

Precision

		intermediate precision (*n* = 9)		
concentration (mg/L)	repeatability (*n* = 3) RSD (%)	RSD (%)	*F* value[b]	p^b
2.5	3.275	5.063	4.065	0.076
5	3.658	3.691	0.301	0.750
10	1.231	1.877	1.560	0.285

Bias (*n* = 3)

concentration (mg/L)	type of sample	quantification (mg/L)	recovery (%)	RSD (%)
0.5	blank	0.464	92.87	0.378
	wine	0.460	92.05	0.403
1	blank	0.938	93.80	0.291
	wine	0.925	92.53	0.344

[a] *x* = concentration; *y* = area values. [b] One-way ANOVA.

tion levels (2.5, 5, and 10 mg/L) of histaminol standard solution (**Table 1**). Repeatability (*n* = 3) gave results between 1.23 and 3.66%, obtained for 10 and 5 mg/L concentrations, respectively. Intermediate precision was evaluated for the same concentrations in three different days (*n* = 9), obtaining RSD values in the range of 1.88−5.06%. The analysis of variance showed no statistically significant differences among replicates for each concentration, confirming a high precision of the method.

The bias of the method was proved in terms of recovery at two different concentration levels of histaminol. Commercial red wine samples were spiked with an appropriate volume of histaminol standard solution to obtain final concentrations of 0.5 and 1 mg/L, respectively. These fortified wine samples were then cleaned up/concentrated, using the SPE (C-18) cartridge applied protocol, and analyzed under the optimized chromatographic procedure. Recovery and precision were calculated. Good percentage recoveries were obtained, both at the lower amount spiked (92.05%) and at the higher one (92.53%). In a preliminary stage, white wine samples were also tested, obtaining results comparable with those relative to red ones (data not shown). A good precision of results was observed (RSD in the range of 0.29−0.40%) (**Table 1**). The level of extraction efficiency was considered to be sufficient for quantitative analyses. These results showed a high-quality efficiency of the SPE fractionation technique and a fine precision of developed chromatographic method.

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Table 2. Histogram of Uncertainties in Procedure and Relative Values[a]
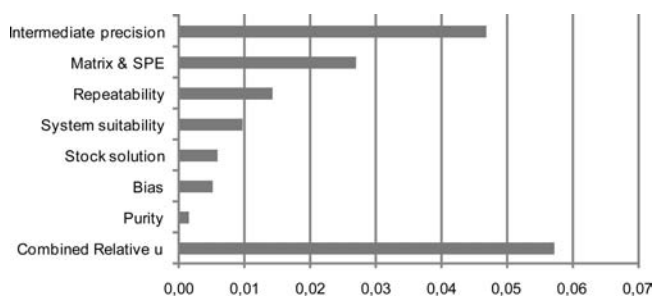
description	corrected u^b	description	corrected u^b
purity	0.0014	repeatability	0.0142
bias	0.0052	matrix and SPE	0.0224
stock solution	0.0058	intermediate precision	0.0469
system suitability	0.0096		
combined u	0.0553	expanded U ($k = 2$)	0.1106

[a] The main part of the total uncertainty comes from the intermediate precision 71%, solid-phase extraction (SPE) step 16%, and repeatability 7%. [b] Corrected standard uncertainty with a coverage Student's *t* factor for 2 degrees of freedom to expand it to a confidence limit of 95%.

Table 3. Histaminol Content in Wine Samples (Mean Value $\pm$ SD, $n = 3$)

wine	type of wine	year	content (mg/L)[a]	RSD%
Barbera	red	2008	<LOQ	
Cabernet	red	2008	0.369 ± 0.009 g,h	2.44
Dolcetto	red	2008	0.436 ± 0.045 g	10.31
Fiano di Avellino	white	2008	0.551 ± 0.015 f	2.76
Greco di tufo	white	2008	nd (<LOD)	
Grignolino	red	2008	<LOQ	
Nero d'Avola	red	2008	0.786 ± 0.032 d	4.12
Sangiovese	red	2008	0.374 ± 0.028 g,h	7.37
Barbera	red	2007	0.550 ± 0.031 f	5.62
Cabernet	red	2007	<LOQ	
Fiano di Avellino	white	2007	0.409 ± 0.023 g	5.72
Greco di tufo	white	2007	0.293 ± 0.018 h	6.08
Grignolino	red	2007	0.680 ± 0.018 e	2.59
Lambrusco	red	2007	0.548 ± 0.006 f	1.16
Nero d'Avola	red	2007	0.289 ± 0.014 h	4.78
Sangiovese (Romagna)	red	2007	0.439 ± 0.034 g	7.81
Sangiovese (Umbria)	red	2007	0.956 ± 0.020 c	2.06
Nebbiolo	red	2006	0.827 ± 0.010 d	1.24
Spanna	red	1977	0.868 ± 0.007 d	0.80
Barolo	red	1969	1.094 ± 0.068 b	6.18

[a] Mean $\pm$ SD values followed by the same letter are not significantly different (*p* > 0.05); nd, not detectable; LOD, 0.073 mg/L; LOQ, 0.222 mg/L.

Uncertainty Estimation. To determine histaminol in wine, the uncertainty (*u*) sources with greater significance were simplified in four groups: (i) *u* associated with histaminol purity and preparation of its standard stock solution; (ii) *u* associated with sample preparation (matrix and SPE cartridges); (iii) *u* associated with performances of HPLC (injection, area, DAD and system suitability); and (iv) *u* of method precision (bias, repeatability, and intermediate precision).

(i) Reference material purity (given by NMR) was 99.25 $\pm$ 0.25%. Assuming a rectangular distribution, the purity had a standard uncertainty of 0.0014 (*27*). For histaminol standard solution preparation, the main sources of uncertainty were the purity, the mass (including the balance uncertainty), and the volume of the volumetric flask, including the variability product of temperature. The uncertainty was calculated by the error propagation approach. In the calculation of standard uncertainty, the purity of histaminol was considered to be a rectangular distribution, whereas for the volumetric flask uncertainty, a triangular distribution was considered. A rectangular distribution was also used for the temperature effect. Therefore, according to the rules of error propagation, the relative *u* of histaminol stock solution preparation is 0.0058 (standard concentration = 100 mg/L) (*27, 29*).

(ii) The uncertainty about both the biological matrix and the SPE cleanup step was assessed by the difference in recovery of wine samples compared to a wine-like blank sample (water/ethanol 88:12, v/v). Both samples were spiked with two known amounts of histaminol (final concentrations of 0.5 and 1 mg/L). Recovery data showed a lower percentage of wine samples than blank ones. This difference adds another source of uncertainty about the procedure; by combining the effect of biological matrix with the cleanup treatment, a relative *u* of 0.0224 was obtained.

(iii) The sources of uncertainty relating HPLC performances were taken into consideration. The injection precision, peak area repeatability, and diode array tolerance were derived from the technical specifications of the instrument. The retention time *u* was obtained through the system suitability parameters report. The combined *u*, relative on these four sources, is 0.0096.

(iv) The bias *u* of the analytical procedure was investigated during the in-house validation study using spiked samples. The

standard uncertainty was calculated as the standard deviation divided by the square root of the number of repetitions. The obtained value is 0.0052 (corrected with a coverage Student's *t* factor, confidence limit of 95%). The repeatability *u*, at three different concentration levels (2.5, 5, and 10 mg/L; (*n* = 3), is 0.0142, and the intermediate precision *u*, evaluated for the same concentrations on three different days (*n* = 9), is 0.0469 (both corrected with relative Student's *t* factor).

Table 2 summarizes the seven relative uncertainties in procedure, the combined *u* calculated (0.0553), and the expanded *U* (*k* = 2) with a value of 0.1106 (corresponding to 11.06%). This value is within 15%, which is the limit of uncertainty concerning the biological matrices (*30*). In this chromatographic method the main part of the total uncertainty comes from the intermediate precision, 71%, solid-phase extraction (SPE) step, 16%, and repeatability, 7%. These three components are responsible for 95% of the total uncertainty.

Analytical Application. The HPLC-DAD-ESI-MS/MS method was then applied to certify the molecule of histaminol in wine; this step (exclusively qualitative) was based on both the retention time and the comparison of MS and UV spectra. Afterward, the quantification of histaminol in wine was performed by applying the in-house validated HPLC-DAD method.

A set of 20 commercial Italian wine samples, both red and white samples of different vintages, was analyzed. The quantification values of histaminol are presented in **Table 3**. The histaminol content was in the range of 0.289−1.094 mg/L (minimum and maximum values were obtained for Nero d'Avola vintage 2007 and Barolo vintage 1969, respectively). All wines considered contained histaminol (as confirmed by HPLC-DAD-ESI-MS/MS analysis); however, it was not possible to quantify four of them: three samples showed values lower than LOQ and one showed a histaminol content lower than LOD.

Subsequently to Barolo 1969, the highest values were from Sangiovese (Umbria) 2007, Spanna 1977, and Nebbiolo 2006 samples (0.956, 0.868, and 0.827 mg/L, respectively). Among white wines, Fiano di Avellino 2008 showed the highest value of histaminol content (0.551 mg/L).

Considering other alcohols produced during the wine fermentation, the literature reports values of concentration that in some cases may be comparable to those relative to histaminol. With

regard to tryptophol, its average range reported (0.51−2.20 mg/L wine) can be considered essentially uniform and in line with the histaminol values (*32*, *33*). Values for the formation of hydroxytyrosol were reported ranging from a minimum of 1.72 to a maximum of 4.20 mg/L (*34*, *35*). On the other hand, tyrosol, characterized by a greater variability of concentration (1.42−36.00 mg/L wine), is present in higher amount than the previous alcohols (*32*, *35*).

The existence of a certain variability in the quantification data of these substances in a complex fermented matrix such as wine still has a justifiable explanation. Both the relative components of the grape (genetic and environmental) and the winemaking process (fermentation conditions) are responsible for a number of variables that inevitably affect the formation of bioactive substances.

In conclusion, this preliminary study was focused on the development of a HPLC-DAD chromatographic method for the quali/quantitative determination of a novel compound in wine. The application of this method on commercial wines proved the presence of histaminol in the samples analyzed. By increasing the number of samples, it will be possible to provide a significant characterization on its presence in wine. Future studies will aim to investigate if this molecule is derived from histamine and produced during the fermentation in winemaking process. It will be important to understand, if resulting from this biogenic amine, how to promote the formation of histaminol while reducing the amount of a harmful bioactive substance such as histamine. In this context a validated method may prove to be a valuable analytical support to assess changes of this molecule during the fermentation.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Kris-Etherton, P. M.; Hecker, K. D.; Bonanome, A.; Coval, S. M.; Binkoski, A. E.; Hilpert, K. F.; Griel, A. E.; Etherton, T. D. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* **2002**, *113*, 71–88.

(2) Stevens, R. Formation of phenethyl alcohol and tyrosol during fermentation of a synthetic medium lacking amino-acids. *Nature* **1961**, *191*, 913–914.

(3) Moreno-Arribas, M. V.; Gómez-Cordovés, C.; Martín-Álvarez, P. J. Evolution of red wine anthocyanins during malolactic fermentation, post-fermentative treatments and ageing with lees. *Food Chem.* **2008**, *109*, 149–158.

(4) Millet, V.; Lonvaud-Funel, A. The viable but non-culturable state of wine micro-organisms during storage. *Appl. Microbiol.* **2001**, *30*, 136–141.

(5) Lonvaud-Funel, A. Lactic acid bacteria in the quality improvement and depreciation of wine. *Antonie Leeuwenhoek* **1999**, *76*, 317–331.

(6) Halasz, A.; Barath, A.; Simon-Sarkadi, L.; Holzapfel, W. Biogenic amines and their production by microorganisms in food. *Trends Food Sci. Technol.* **1994**, *5*, 42–49.

(7) Ehrlich, F. Uber die Bedingungen der Fuselolbildung und uber ihren Zusammenhang mit dem Eiweissaufbau der Hefe. *Ber. Dtsch. Chem. Ges.* **1907**, *40*, 1027–1047.

(8) Webb, A. D.; Ingraham, J. L. Fusel oil. *Adv. Appl. Microbiol.* **1963**, *5*, 317–353.

(9) Henschke, P. A.; Jiranek, V. *Wine Microbiology and Biotechnology*; Fleet, G. H., Ed.; Harwood Academic: Langhorne, PA, 1993.

(10) Vuralhan, Z.; Luttik, M. A. H.; Tai, S. L.; Boer, V. M.; Morais, M. A.; Schipper, D.; Almering, M. J. H.; Kotter, P.; Dickinson, J. R.; Daran, J. M.; Pronk, J. T. Physiological characterization of the ARO10-dependent, broad-substrate specificity 2-oxo acid decarbox-

(11) Dickinson, J. R.; Salgado, L. E. J.; Hewlins, M. J. E. The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **2003**, *278*, 8028–8034.

(12) Hazelwood, L. A.; Daran, J. M.; van Maris, A. J. A.; Pronk, J. T.; Dickinson, J. R. The Ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. *Appl. Environ. Microbiol.* **2008**, *74*, 2259–2266.

(13) Kradolfer, P.; Niederberger, P.; Hutter, R. Tryptophan degradation in *S. cerevisiae*: characterization of two aromatic aminotransferases. *Arch. Microbiol.* **1982**, *133*, 242–248.

(14) Shin, M.; Shinguu, T.; Sano, C.; Umezawa, K. Metabolic fates of L-tryptophan in *Saccharomyces uvarum* (*Saccharomyces carlsbergensis*). *Chem. Pharm. Bull.* **1991**, *39*, 1792–1795.

(15) Bycroft, B. W. *Dictionary of Antibiotics and Related Substances*; Chapman and Hall: London, U.K., 1988.

(16) Covas, M. I.; Mirò-Casas, E.; Fitò, M.; Farrè-Albadalejo, M.; Gimeno, E.; Marrugat, J.; De La Torre, R. Bioavailability of tyrosol, an antioxidant phenolic compound present in wine and olive oil, in humans. *Drugs Exp. Clin. Res.* **2003**, *29*, 203–206.

(17) Bertelli, A. A. E.; Migliori, M.; Panichi, V.; Longoni, B.; Origlia, N.; Ferretti, A.; Cuttano, M. G.; Giovannini, L. Oxidative stress and inflammatory reaction modulation by white wine. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 295–301.

(18) Granerus, G. Effects of oral histamine, histidine and diet on urinary excretion of histamine, methyl histamine and l-methyl-4-imidazoleacetic acid in man. *Scand. J. Clin. Lab. Invest.* **1968**, *22*, 49–58.

(19) Maslinski, C.; Fogel, W. A. *Histamine and Histamine Antagonists Handbook of Experimental Pharmacology*; Springer: Berlin, Germany, 1991.

(20) Bergmark, J.; Granerus, G. Ion exchange chromatography for quantitative analysis of radioactive histamine metabolites in human urine. *J. Clin. Lab. Invest.* **1974**, *34*, 365–373.

(21) Nakajima, T.; Sano, I. A metabolite of histamine: 4(5)-imidazoyl-ethane-2-ol. *Biochim. Biophys. Acta* **1964**, *82*, 260–265.

(22) Bieganski, T.; Osinska, Z.; Maslinski, C. Inhibition of plant and mammalian diamine oxidase by substrate analogues. *Agents Actions* **1982**, *12*, 1–2.

(23) Tarnok, Z.; Tarnok, I. Histamine (HIS) degradation in microorganisms: imidazole ring splitting and formation of imidazolyl ethanol (IMET) and imidazolyl acetic acid (IMAC) in mycobacteria. *Agents Actions* **1986**, *18*, 1–2.

(24) LaRue, T.; Spencer, J. F. T. Production of 4-imidazole ethanol from histamine by *Saccharomyces rouxii*. *Cell. Mol. Life Sci.* **1966**, *22*, 729–730.

(25) Sun, B. S.; Leandro, C.; Ricardo da Silva, J. M.; Spranger, I. Separation of grape and wine proanthocyanidins according to their degree of polymerization. *J. Agric. Food Chem.* **1998**, *46*, 1390–1396.

(26) R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2008.

(27) *EURACHEM/CITAC Guide CG4: Quantifying Uncertainty in Analytical Measurement*, 2nd ed.; 2000; www.eurachem.org

(28) ISO/CEI 17025: general requirements for the competence of testing and calibration laboratories, International Organization for Standardization (ISO), Geneva, Switzerland, 2005.

(29) ISO 5725 - 1 to 6. Accuracy (trueness and precision) of measurement methods and results. International Organization for Standardization, Geneva, Switzerland, 2004.

(30) Guidance for Industry: Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Rockville, MD, May 2001.

(31) International Conference on Harmonisation (ICH) Validation of analytical procedures: text and methodology ICH Q2 (R1), 2007; http://www.ich.org/LOB/media/MEDIA417.pdf.

(32) Chamkha, M.; Cathala, B.; Cheynier, V.; Douillard, R. Phenolic composition of Champagnes from Chardonnay and Pinot Noir vintages. *J. Agric. Food Chem.* **2003**, *51*, 3179–3184.

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(33) Mattivi, F.; Vrhovsek, U.; Versini, G. Determination of indole-3-acetic acid, tryptophan and other indoles in must and wine by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr., A* **1999**, *855*, 227–235.

(34) Dudley, J. I.; Lekli, I.; Mukherjee, S.; Das, M.; Bertelli, A. A. E.; Das, D. K. Does white wine qualify for French paradox? Comparison of the cardioprotective effects of red and white wines and their constituents: resveratrol, tyrosol, and hydroxytyrosol. *J. Agric. Food Chem.* **2008**, *56*, 9362–9373.

(35) Di Tommaso, D.; Calabrese, R.; Rotilio, D. Identification and quantitation of hydroxytyrosol in Italian wines. *J. High Resolut. Chromatogr.* **1998**, *21*, 549–553.