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Original Paper

A liquid chromatographic method optimization for the assessment of low and high molar mass carbonyl compounds in wines

Carbonyl compounds (CC) play an important role in beverage aroma since they may affect flavor of wines, brandies, and beers, among others. For this reason, it is necessary to identify and quantify CC through adequate analytical techniques. This study is a proposal of both developing and optimization of a new analytical methodology that allows investigate $C_1 - C_8$ CC in wines simultaneously by quantifying even those ones that are predominantly present in the adduct form hydroxylalkylsulfonic acids (HASA). The HASA dissociation is undertaken by specific alkaline media (pH 11). The developed methodology employed the LC with UV/VIS detection (λ = 365 nm) technique under gradient elution in the way to reach both free-CC and bound-CC quantification. Results showed that binary gradient system using eluent A (MeOH/ACN/H_2O 74.5:0.5:25% v/v/v) and eluent B (MeOH) reached the best separation condition of both lower and higher molecular mass CC. This proposed method allowed simultaneous quantification of formaldehyde, acetaldehyde, propanone, furfuraldehyde, butyraldehyde, benzaldehyde, hexanaldehyde, 2-ethyl-hexanaldehyde, E-pent-2-en-1-al, and cyclohexanone – all of them were found in white wine (*Moscato Canelli*) and red wine (*Shiraz*) produced in the São Francisco Valley, in the Northeastern Region of Brazil – although this optimized method may probably be suitable for quantification of propionaldehyde, isobutyraldehyde, heptanaldehyde, octanaldehyde, benzaldehyde, and E-hex-2-en-1-al as well. We could not prove if this method is also able to determine the latter CC group since we have not found these substances present in detectable levels in our real samples considered in this study.

Keywords: Carbonyl compounds / Liquid chromatography / Method development and optimization / São Francisco valley / Speciation / Wines

Received: April 22, 2009; revised: July 9, 2009; accepted: July 10, 2009

DOI 10.1002/jssc.200900281

1 Introduction

Nowadays the assessment system of the flavor quality of wines uses a quite descriptive terminology whose parameters are generally excessively subjective, seldom matching to those ones utilized by wine consumers or researchers. For this reason, the development of new analytical methodologies is necessary in order to isolate, identify, and quantify the analyte or group of analytes who are related to wine flavor then supporting scientifically the subjective wine characterization system.

Among volatile organic compounds already found in wines there are the carbonyl compounds (CC) that are frequently cited. Some researchers highlight the major role that CC play in aroma characterization of fermented beverages, for instance, acetaldehyde, if present in excess, gives oxidized-like aroma. The kind and concentration level of found CC in wine and other fermented beverages can vary from pleasant to undesirable notes to flavor beverage yet even a same carbonyl can either impact positively or negatively the sense character of wine depending mainly on its concentration level [1] or molecular structure. Moreover, CC interact with bisulfite ion from wine then forming the hydroxylalkylsulfonic acids (HASA) that will also influence aroma characteristics [2, 3].

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Abbreviation: CC, carbonyl compounds; **HASA,** hydroxylalkylsulfonic acids

The currently adopted methodology by Organisation Internationale de la Vigne et du Vin (OIV) only provides total CC quantification from alcoholic beverages [4]. In this way, it is not possible to know individual aldehydes and ketones concentration levels present in beverages. Indeed, most studies which utilize LC and UV–VIS detection for separation and identification of CC in wines refer to necessity of quantifying both free-CC and bound-CC after converting them to colored derivatives before chromatographic analysis. This sample preparation step is necessary due to the relative chemical instability of carbonyl group in complex media such as beverage and food matrices [5–11]. Derivatization is therefore performed in order to enhance detector response and CC selectivity making CC analysis reliable even to low molecular mass aldehydes such as formaldehyde, acetaldehyde, and propionaldehyde which are very volatiles and reactive [10, 12]. Such characteristics make direct analysis of CC quite difficult thus the indirect methods *via* derivatization reactions, are more appropriate and mostly utilized [10].

Several derivatizing agents such as 5,5-dimethyl-1,3-cycle-hexanodione, 2,4-dinitro-3,5,6-trideuterophenylhydrazine, hydroxylamine, semicarbazine, and 2,4-dinitrophenylhydrazine are frequently employed in indirect methods of CC analyses. Their main derivatization reactions are based on the addition of the nucleophilic nitrogen atom to the carbonyl carbon in this way yielding stable products [10, 13–15]. Among all derivatizing agents, the most used one is the acidified 2,4-dinitrophenylhydrazine solution (2,4-DNPH) which was firstly described by Allen in 1930 [cited by reference 9]. According to de Andrade and Tanner [16] free aldehydes can be directly analyzed in a liquid chromatograph after derivatization with 2,4-DNPH solution. Quantification of total-CC, however, is based on the decomposition of HASA adduct. In this way, literature reports suggest that samples should be alkalinized in order to reach complete HASA decomposition. This is followed by derivatization step and then quantification as same as described to free-CC. HASA levels (bound-CC) are found by the difference of free-CC level from total-CC level, for every CC [2, 3, 5, 16, 17].

Studies about 2,4-DNPH derivatives of CC (by using RP LC and UV–VIS detection) describe many different elution conditions from either environmental, food or beverage sample matrixes. It is usual to employ ACN/water and/or methanol/water into a gradient eluent programming with UV detection, wavelength ranging from 254 to 385 nm [5, 7, 8, 18–30].

In this study, an analytical methodology of separation and identification of both low and high molecular mass (C_1–C_8) aldehydes and ketones by gradient HPLC-UV was developed and applied. Furthermore, a validation approach, based on the establishment of the following analytical parameters such as selectivity, LOD, LOQ, sensitivity, accuracy, precision, robustness, linear range, recovery tests, and real samples analyses [white wine (*Moscato Canelli*) and red wine (*Shiraz*)] was carried out. This methodology can be able to apply for determination of CC (both free-CC and bound-CC) of either colored or transparent beverages.

2 Experimental

2.1 Standards, solvents, solutions, and equipment

Pure CC standards: Carbonyl compound standards (formaldehyde, acetaldehyde, propionaldehyde, propanone, butyraldehyde, isobutyraldehyde, hexanaldehyde, heptanaldehyde, octanaldehyde, 2-ethyl-hexanaldehyde, furfuraldehyde, benzaldehyde, cyclopentanone, cyclohexanone, E-pent-2-en-1-al, and E-hex-2-en-1-al) utilized to obtain their respective hydrazones were purchased from Merck (Germany), Aldrich (USA), and/or Sigma (USA). The 2,4-DNPH standard was acquired from Aldrich (USA).

Solvents: All solvents employed for either sample manipulation, stock standards, analytical standards, or chromatographic eluents (ACN and methanol) preparation were chromatographic grade, purchased from J. T. Baker (USA). Deionized water was produced by Barnstead purification system, NANOpure Diamond model (USA). Just after chromatographic eluents were prepared, they were filtered by using Millipore filters for organic solvents (0.45 μm pore size, 47 mm diameter, USA) and degassed under vacuum and sonication (ultrasonic bath Arruda, model SX-10, Brazil) for 15 min.

0.04 and 0.4% 2,4-DNPH solutions: 0.04% 2,4-DNPH solution was prepared dissolving 0.05 g of this reagent into 60 mL of ACN, 39 mL of deionized water, and 1 mL of concentrated phosphoric acid (final pH around 2.0). Similarly 0.4% 2,4-DNPH solution was prepared by adding 0.50 g of that substance to the same amount of the other reagents (ACN, deionized water, and phosphoric acid). Purification of 2,4-DNPH solutions was performed under liquid–liquid extraction procedure, by using carbon tetrachloride or dichloromethane (both were chromatographic grade, J. T. Baker, USA) as organic phase. Then, a 1 mL aliquot of the freshly purified 2,4-DNPH solution was injected into HPLC-UV system, followed by stocking it under refrigeration into sealed amber flasks [31].

Hydrazone syntheses and stock solutions: Firstly, in a recipient it was added 0.8 g of 2,4-DNPH to 6 mL deionized water and 4 mL sulfuric acid. Secondly, in another recipient, some volume (equivalent to 0.10 g of each substance) of pure standard CC solution was diluted into 20 mL ethanol. Then, both solutions were mixed in order to obtain hydrazone crystals which were vacuum filtered [10, 31]. Next, two or three successive re-crystallizations were done and the hydrazone purity was verified by injecting them in HPLC-UV system. Thirdly, individual

stock solution of each purified hydrazone was prepared by dissolving them into ACN, followed by preparation of the mix stock solution of the 16 CC. Finally, this mix stock solution was stored into sealed amber flasks, under refrigeration. Analytical standards of CC hydrazone derivatives (mix of those 16 CC) were prepared by appropriate dilutions of the latter stock solution.

Equipment: A liquid chromatograph with binary gradient system, equipped with Perkin Elmer pump series 200 (Perkin Elmer, USA), rheodyne injector valve with 20 μL loop, a Perkin Elmer UV/VIS detector series 200 with deuterium lamp (λ = 365 nm) and 4290 Intralab integrator, RP-18 LichroCART 250-4 column (250 × 0.46 mm, 5 μm particle diameter, Merck, Germany) was utilized throughout this work.

2.2 Method optimization

Best conditions for sample preparation adjustments were done under univariate optimization protocols by testing (i) sample volume and (ii) sample : 2,4-DNPH solution ratio.

i. *Sample volume test:* in this test different volumes of white wine (1.0, 5.0, 10, 25, and 50 mL) were submitted to derivatization reaction using the same volume of 0.04% 2,4-DNPH solution (1:1).

ii. *Sample: 2,4 DNPH solution ratio test:* Results from sample volume test showed that it was necessary to increase concentration of 2,4-DNPH solution to 0.4%. In this case, 1 mL white wine and red wine aliquots were used in the ratio test (sample: 0.4% 2,4-DNPH solution) as follows: 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6.

2.3 Recovery test and establishment of analytical parameters

In order to guarantee analytical quality of results a recovery test was performed following some accepted protocols found in the literature (ANVISA, Ministério da Saúde. Resolução n. 475. Guia para Validação de Métodos Analíticos e Bioanalíticos. Brasilia, DF. Available in: www.anvisa.gov.br/legis/resol/2003/re/899_03re.htm, accessed in: 18/04/2009). Furthermore, the following parameters were established, such as: selectivity, sensibility, linearity, precision, accuracy, LOD, LOQ, and robustness.

2.4 Sample preparation

Quantification of CCs was carried out considering which form CCs are presented in wines, if they are free-CC, bound-CC, or total-CC. First of all, free-CC is literally the free form of each CC, for instance, the CC by itself present in wines. However, it is well known that some of CCs present in wines are bound to bisulfite ion, forming their respective HASA that are what we call bound-CC. In turn, total-CC is the sum of free-CC plus bound-CC (total-CC = free-CC + bound-CC). In this way, bound-CC can be found by the difference between total-CC and free-CC (bound-CC = total-CC − free-CC) [2, 3]. Determination of free-CC was performed by taking different wine aliquots and adding them to 2,4-DNPH solution followed by 15 min sonication. In this way, free CC are converted to their respective hydrazones and then injected into HPLC-UV for quantification. Total-CC were indirectly quantified by promoting the dissociation of HASA − in order to determinate the bound-CC fraction − by alkalinization of the wine sample by adding 2 – 3 drops of 1 mol/L NaOH solution until pH 11. The total-CC fraction quantification was done as same as free-CC [2, 3].

2.5 Tests with real samples

Initially, it was verified which CCs would be found in our target sampless to then perform their quantification afterwards. Identification was done by matching retention time of compounds found in the samples to retention time of CC from standard solution. Samples were also spiked with CC standard solution in order to verify whether or whether not would have increase the CC peak areas from real wine samples. Moreover, at every working day comparisons between retention times of sample peaks and analytical standards peaks were performed [31]. CC quantification was carried out by the method of external calibration curve, composed of 5 to 10 standard concentration levels, considering peak area (peak area *versus* concentration) and linear regression.

3 Results and discussion

Summing the optimized method up, the best results were reached when 1 mL wine is added to 5 mL 0.4% 2,4-DNPH solution in the way that the free-CC present in the sample are derivatized then analyzed. In turn, determination of total-CC request an alkalinization step of wine aliquot until pH = 11, by adding 2 – 3 drops of 1 mol/L NaOH solution, before derivatization be undertaken. The derivatization reaction is favored in acidic medium due to the large excess of derivatizing agent. In both cases the mixes are sonicated for 15 min then injected into RP HPLC-UV. Sample preparation time was under 1 h.

Since no conditions for an isocratic separation were found, that several gradient programs were evaluated. The best separation condition of hydrazones was obtained through binary gradient elution system by using MeOH/ACN/H_2O 74.5:0.50:25.0% (v/v/v) as eluent A and MeOH as eluent B under the following eluent programming: firstly, eluent A only was passed throughout the HPLC-UV system for 12 min, next a gradual change of

Table 1. CC concentration levels (mg/L) in white wine obtained with gradient elution HPLC-UV system

Carbonyl compound	Bound-CC[a]				Total-CC[b]			
	min[c]	mean	max[d]	s[e]	min[c]	mean	max[d]	s[e]
Formaldehyde	0.03	0.04	0.05	0.0064	0.06	0.08	0.10	0.0139
Acetaldehyde	0.31	0.32	0.34	0.0136	1.3	1.3	1.4	0.0326
Furfuraldehyde	0.28	0.31	0.33	0.0287	1.3	1.4	1.5	0.1015
Propanone	0.02	0.02	0.02	0.0034	0.05	0.05	0.05	0.0028
Butyraldehyde	0.02	0.02	0.03	0.0020	0.15	0.17	0.19	0.0151
Cyclopentanone	0.02	0.03	0.03	0.0047	nd[f]	nd	nd	nd
Cyclohexanone	0.07	0.09	0.11	0.0181	0.002	0.003	0.004	0.0004
E-Pent-2-en-1-al	0.03	0.03	0.03	0.0025	0.02	0.02	0.02	0.0002
Hexanaldehyde	0.89	1.1	1.1	0.0943	nd	nd	nd	nd
2-Ethyl- hexanaldehyde	0.05	0.06	0.06	0.0078	0.34	0.37	0.44	0.0556

[a] Bound-CC means carbonyl compound – bisulfite ion adduct (HASA).
[b] Total-CC means total carbonyl compound (total-CC = free-CC + bound-CC).
[c] Minimum.
[d] Maximum.
[e] SD.
[f] Not detected.

eluent A to eluent B for 12 min, then just eluent B for 3 min, and finally returning of eluent A in 10 min. Total chromatographic run was 37 min. Hydrazones detections were done by a UV detector set at λ = 365 nm.

The proposed method is suitable to simultaneous quantification of formaldehyde, acetaldehyde, furfuraldehyde, butyraldehyde, benzaldehyde, hexanaldehyde, 2-ethyl-hexanaldehyde, E-pent-2-en-1-al, and cyclohexanone by using a wine aliquot as small as 1 mL. However, this optimized method may probably be suitable for quantification of propionaldehyde, isobutyraldehyde, heptanaldehyde, octanaldehyde, benzaldehyde, and E-hex-2-en-1-al as well. We could not prove if this method is also able to determine the latter CC group since we have not found these substances present in detectable levels in our real samples considered in this study.

The CCs concentration levels in white wine obtained with gradient elution HPLC-UV system are given in Table 1. It can be noted that in some cases the concentrations of bond-CC are higher than their total concentrations (bond + free forms). Two mechanisms are suggested to explain this phenomenon [2]. One of them, the alkaline medium required in Total-CC quantification becomes much easier than the simple addition reactions between α- and β-unsaturated aldehydes or ketones and strong nucleophilic species such as hydroxide ion. In this way, the hydroxide ion will be added to unsaturated CC through the carbonyl double bond, resulting in alcohols or other compounds unidentified by this method. The other mechanism could be related to the conjugated addition reaction between anions and α- and β-unsaturated CCs, known as "Michael addition". In this case, bisulfite would be added to the 1,4-position in relation to the carbonyl group, removing the double bond of the carbon chain without changing the carbonyl group; the new compound will be attacked by hydroxide ion, giving a different adduct. This adduct would not be able to react with 2,4-DNPH to form the hydrazone, and the analytical signal could not be obtained.

Table 2. Obtained peaks during sample volume testing

Volume of wine (mL)	Volume of 2,4-DNPH (mL)	Number of acquired peaks
1.0	1.0	9
5.0	5.0	9
10	10	9
25	25	11
50	50	18

3.1 Sample volume test

The main goal of this test was to optimize the wine volume to be adopted during sample preparation procedure. It was considered the following volumes in this investigation: 1.0, 5.0, 10, 25, and 50 mL of wine, trying to find which wine volume would give more detected peaks of CCs. Table 2 shows how many peaks were obtained in each case. It should happen that the bigger the wine volumes, the more favored would be the carbonyl compound yet taking into consideration that this implies involving a pre-concentration step since a SPE step is utilized during sampling preparations as already described in ref. [2, 5 – 8]. However, when we used 25 and 50 mL of wine, for instance, there were found 11 and 18 peaks, respectively. When using lower volumes just nine peaks were detected. Despite this initial and evident

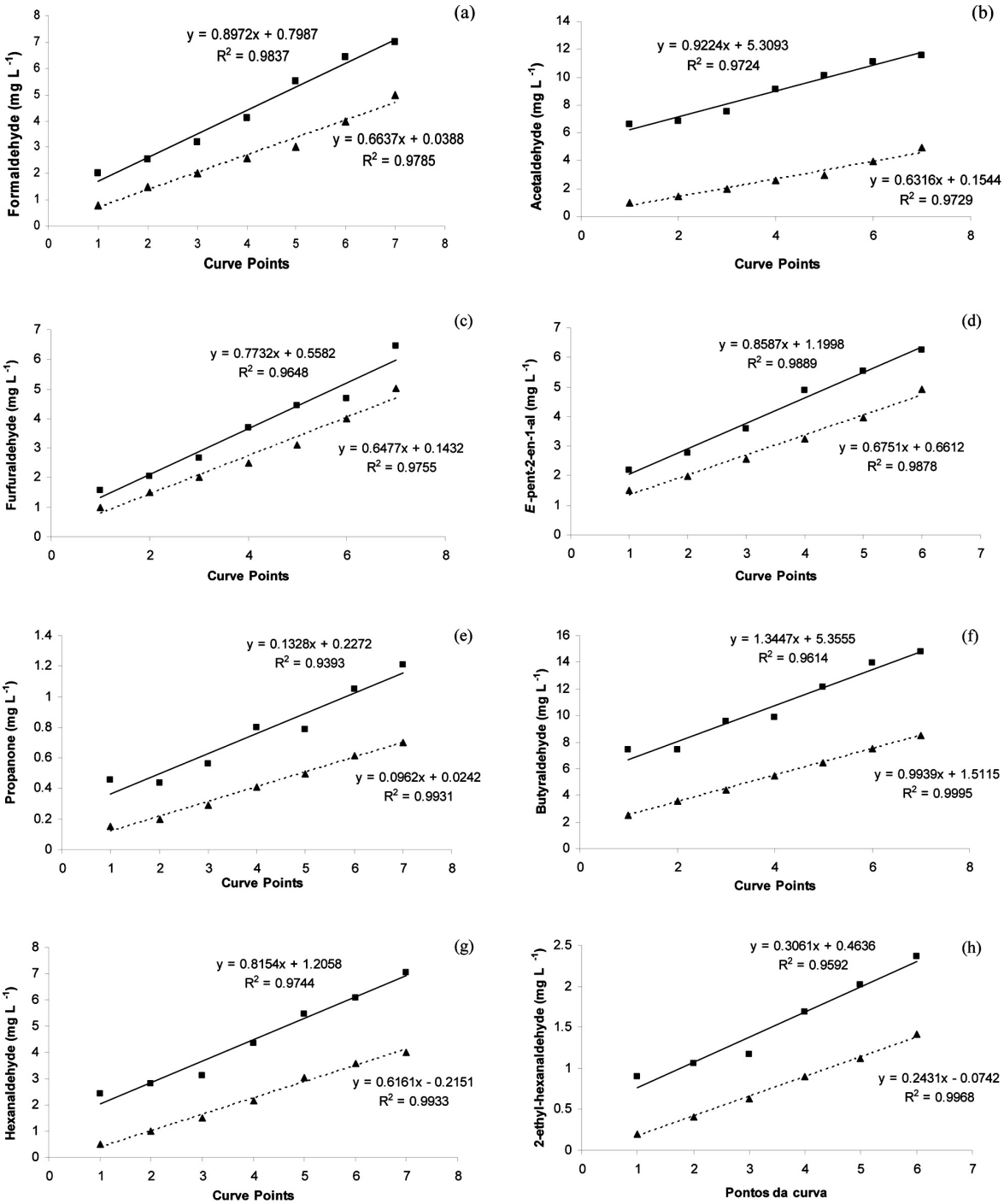

Figure 1. Standard addition (___) and pure standard (– –) analytical curves utilized in the selectivity method test [(a) formaldehyde, (b) acetaldehyde, (c) furfuraldehyde, (d) E-pent-2-en-1-al, (e) propanone, (f) butyraldehyde, (g) hexanaldehyde, (h) 2-ethyl-hexanaldehyde].

advantage of utilizing bigger volumes of wine (25 and 50 mL) for extraction of a higher number of CC, we noticed whose conditions provoked big losses of both formaldehyde and acetaldehyde due to C-18 cartridge saturation in the pre-concentration step. Moreover, it became necessary that large volumes of 2,4-DNPH solution resulted in a more laborious and expensive sample preparation as well as larger waste production. Since it was not noted losses of formaldehyde and acetaldehyde nor differences in the number of detected peaks from the other tests (1.0, 5.0, and 10 mL) we have opted to work with aliquots of 1 mL of wine by using more concen-

trated 2,4-DNPH solutions (0.4%). In this way, it was possible to enhance peak detections and reduce sample and reagent volumes adopted during analyses as well.

3.2 Sample/2,4 DNPH solution ratio test

Just after defining the ideal sample volume to be taken for analyses, new tests were done by combining different ratios of wine to 0.4% 2,4-DNPH solution trying to establish an adequate ratio between 1 mL aliquot-to-2,4-DNPH solution for both white and red wines. There were some tested ratios such as 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6, taking as reference condition the best response of formaldehyde, acetaldehyde, 2-ethyl-hexanaldehyde, and cyclohexanone concentrations, all of them previously known to be present in detectable levels in the wine samples considered in this study. Figure 1 shows fluctuations of CC concentrations among all tested proportions, for both white and red wines.

Considering white wine (Fig. 1a) it is observable an increasing trend as the higher 2,4-DNPH solution into the ratio the bigger the analyte responses, expressed as mg/L. The ratio 1:5 was found to give the highest concentration levels of cyclohexanone, 2-ethyl-hexanaldehyde, formaldehyde, and acetaldehyde and, therefore, the chosen ratio to be employed during white wine sample preparation. In the ratio test for red wine, the rise of 2,4-DNPH solution did not significantly influence the analytical response. Except for cyclohexanone, concentrations of acetaldehyde, formaldehyde, and 2-ethyl-hexanaldehyde did not demonstrate any trend of increasing while there were rise in 2,4-DNPH solution volume that was undertaken. Because acetaldehyde signal was discreetly higher in 1:5 ratio, this also was the chosen ratio for red wine samples even though when using 1:6 ratio we observed a decreasing tendency in the signal of every CC considered in this test (Fig. 1b). We therefore conclude that the best response (expressed as the highest CC concentration extracted from wine) is reached when using 1:5 sample-to-2,4-DNPH solution ratio for both wines.

3.3 Establishment of analytical parameters

Selectivity: Selectivity study of a method that works with a complex matrix sample such as wine should be done through standard addition method [32]. For this reason, in this present study the selectivity test was done by comparing angular coefficient (a) of two linear regression curves acquired both with and with no standard addition. The first curve was done with seven different concentration levels of CC standards ($a_{standard}$) while the second curve was done with wine sample addition to the same seven different concentration level of CC standards ($a_{wine + standard}$). Studied CC were formaldehyde, acetaldehyde, butyraldehyde, furfuraldehyde, hexanaldehyde, E-pent-2-en-1-al, propanone, and 2-ethyl-hexanaldehyde. When inclination of both is equal or very close one to another, $a_{wine + standard}/a_{standard}$ tends to approach 1, meaning that there is not matrix effect therefore the method is considered selective [33]. (INMETRO. Instituto Nacional de Metrologia, Normalização e Qualidade Industrial. *Orientações sobre validação de métodos de ensaios químicos*. DOQ-CGCRE-008, March 2003. Available in http://www.inmetro.gov.br/infotec/publicacoes/Acredita%C3%A7%C3%A3o.pdf, accessed in 18/04/2009.) Curves with concentration levels of either standard addition to samples and pure standards are shown in Fig. 2. The developed method from this study is considered selective for formaldehyde, acetaldehyde, butyraldehyde, furfuraldehyde, hexanaldehyde, E-pent-2-en-1-al, propanone, and 2-ethyl-hexanaldehyde since $a_{wine + standard}/a_{standard}$ values were 1.3518, 1.4604, 1.1938, 1.2719, 1.3804, 1.3529, 1.3235, and 1.2591, respectively.

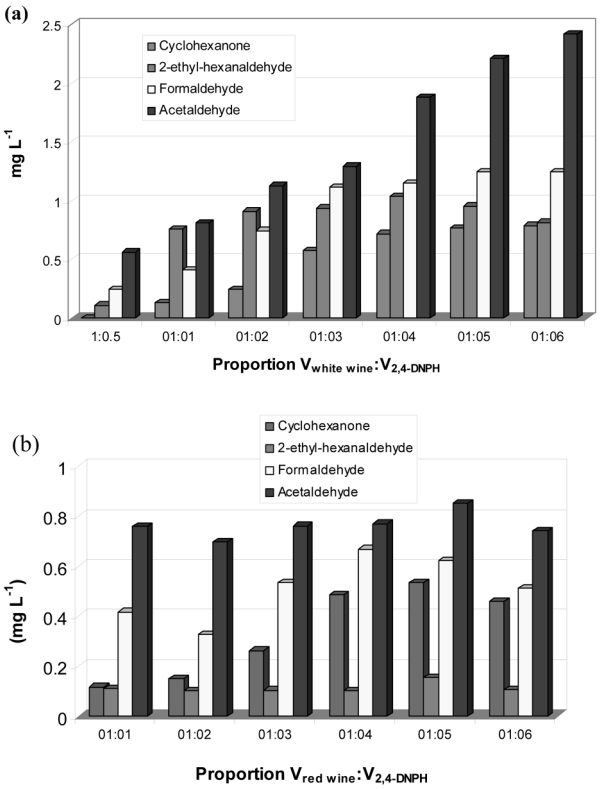

Figure 2. Concentration levels of formaldehyde, acetaldehyde, 2-ethyl-hexanaldehyde, and cyclohexanone in white wine (a) and red wine (b), during sample/0.4% 2,4-DNPH solution ratio tests.

Linearity and linear range: The mathematical relationship between signal (response) and analyte concentration is expressed as curve equation and correlation coefficient (R^2). In this study we considered an evidence of ideal data fit whenever $R^2 > 0.9900$. In Table 3 is found all correlation coefficients from analytical curves of CCs.

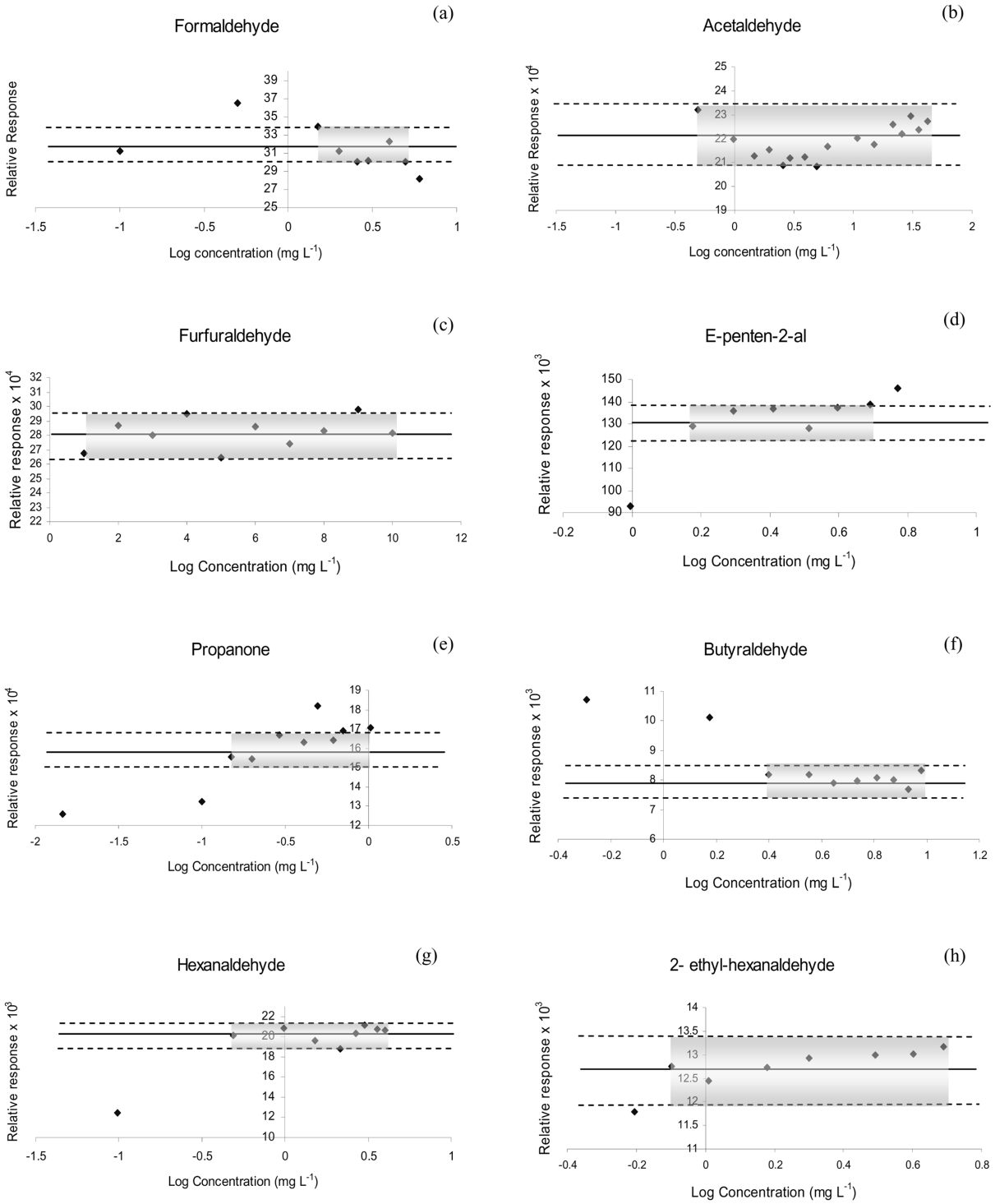

Figure 3. Working linear range of CC identified in wine samples: (a) formaldehyde, (b) acetaldehyde, (c) furfuraldehyde, (d) E-pent-2-en-1-al, (e) propanone, (f) butyraldehyde, (g) hexanaldehyde, (h) 2-ethyl-hexanaldehyde.

Except for cyclohexanone, all R^2 were above 0.99 that indicates good linearity of this CC quantification method. Additionally, it was done a graph of relative response (peak area/analyte concentration) in *y*-axis and logarithm concentrations in *x*-axis (Fig. 3) [32]. Linear ranges for CC studied in this work are found in Table 4. Among studied CC, the one that showed broader linear range was acetaldehyde (0.50 – 42.0 mg/L) while the narrower linear range was found for propanone (0.15 – 1.00 mg/L).

Table 3. Analytical curves ($y = ax + b$) for quantification of 2,4-dinitrophenylhydrazone CC derivatives, by HPLC-UV

CC	Equations	R^2
Formaldehyde	$Y = 285921x + 51841$	0.9932
Acetaldehyde	$Y = 213294x - 1295.7$	0.9991
Furfuraldehyde	$Y = 29327x - 2245.7$	0.9965
Propanone	$Y = 175376x - 2607$	0.9981
Butyraldehyde	$Y = 7901{,}6x - 921.91$	0.9943
Cyclopentanone	$Y = 121747x - 16995$	0.9979
E-Penten-2-al	$Y = 151335x - 41885$	0.9956
Cyclohexanone	$Y = 24217x + 1891.9$	0.9674
Hexanaldehyde	$Y = 21072x + 1657.4$	0.9987
2-Ethyl-hexanaldehyde	$Y = 12995x + 162.51$	0.9986

Table 4. Working linear range (units in mg/L) of found CC in wine samples

Carbonyl coumpound	Working linear range (mg/L)	
	lower limit	higher limit
Formaldehyde	1.50	5.00
Acetaldehyde	0.50	42.0
Furfuraldehyde	0.10	6.00
Propanone	0.15	1.00
Butyraldehyde	2.50	9.50
E-Pent-2-en-1-al	1.50	5.00
Cyclohexanone	1.50	5.00
Hexanaldehyde	0.50	4.00
2-Ethyl-hexanaldehyde	0.80	5.00

Precision: Table 5 shows concentration values, mean values, SD, and RSD for all studied CC. RSD was below 5%, except for cyclohexanone (RSD = 14.41%). Taking into account that a RSD < 20% is acceptable for trace analysis of complex matrices, this method could be considered quite precise for CC quantification in wine samples.

LOD and LOQ: the concept of LOD and LOQ may vary according to working area [35]. According to Silva [10] and Swartz and Kruul [35], an alternative to find LOD and LOQ of a method is to calculate them based on response SD-to-inclination of analytical curve rate, $LOD = 3s/a$ and $LOQ = 10s/a$, where s is the SD of linear coefficient and a is the angular coefficient (inclination) from analytical curve [10]. Another way in establishing LOD is to analyze standard solution of the analyte with decreasing concentrations (by successive dilutions) until the least detectable level (analyte signal should be at least three times the background signal) [17], and LOQ equivalent to five times LOD. Both procedures were done in this work in the way to find which one would be more suitable to the developed method. Calculating LOD and LOQ through data from analytical curve (Tables 3 and 6a) resulted to statistically significant higher LOD and LOQ values in comparison to those found by decreasing concentration standard solution method (Table 6b). Therefore, it was concluded that the more appropriate manner of finding LOD and LOQ for this study is the latter procedure. Recommended LOD and LOQ of this work are found at Table 6b.

Table 5. Descriptive statistical data of CC concentration levels

CC	Mean ± s[a] (mg/L)	RSD[b] (%)
Formaldehyde	1.5968 ± 0.0779	4.8761
Acetaldehyde	1.6093 ± 0.0839	5.2168
Furfuraldehyde	0.4145 ± 0.0211	5.0847
Propanone	1.6111 ± 0.0268	1.6665
Butyraldehyde	0.8281 ± 0.0383	4.6270
E-Penten-2-al	1.0874 ± 0.0518	4.7633
Benzaldehyde	1.7471 ± 0.0799	4.5753
Cyclohexanone	0.1371 ± 0.0197	14.411
Hexanaldehyde	1.4427 ± 0.0619	4.2893
2-Ethyl-hexanaldehyde	0.1013 ± 0.0055	5.4326

[a] CC mean concentration ± SD (units in mg/L).
[b] SDRSD.

Table 6a. LOD and LOQ, for CC found in wine samples, calculated through data from analytical curves

Carbonyl compounds	LOD (mg/L)	LOQ (mg/L)
Formaldehyde	0.2784	0.9280
Acetaldehyde	0.0981	0.3271
Furfuraldehyde	0.1997	0.6656
Propanone	0.0229	0.0762
Butyraldehyde	0.4636	1.5447
E-Penten-2-al	0.2200	0.7333
Cyclohexanone	0.4977	1.6590
Hexanaldehyde	0.0734	0.2448
2-Ethyl-hexanaldehyde	0.0953	0.3178

Table 6b. LOD and LOQ, for CC found in wine samples, obtained through decreasing standard solution concentration injection method

Carbonyl compounds	LOD (mg/L)	LOQ (mg/L)
Formaldehyde	0.0064	0.0320
Acetaldehyde	0.0073	0.0365
Furfuraldehyde	0.0127	0.0635
Propanone	0.0176	0.0880
Butyraldehyde	0.0230	0.1150
E-Penten-2-al	0.0087	0.0435
Cyclohexanone	0.0085	0.0425
Hexanaldehyde	0.0432	0.2160
2-Ethyl-hexanaldehyde	0.0071	0.0355

Recovery test: an analytical method can be validated by doing recuperation test, and complemented with standard reference materials analysis or by comparison to another accepted method [34]. Taking into account that there are not reference materials for wines and the simultaneous analysis of CCs in wine is under development, we restricted validation to recovery test. Acceptable recuperation levels ranged from 70% through 120% (Table 7).

Table 7. Recovery test percentages of studied CC

Carbonyl compound	Recovery (%)
Formaldehyde	79.7
Acetaldehyde	75.7
Furfuraldehyde	82.7
E-Pent-2-en-1-al	76.4
Propanone	99.6
Butyraldehyde	93.5
Hexanaldehyde	93.7
2-Ethyl-hexanaldehyde	101

4 Conclusions

The results from this study suggest that the proposed method is suitable to simultaneous quantification of formaldehyde, acetaldehyde, furfuraldehyde, butyraldehyde, benzaldehyde, hexanaldehyde, 2-ethyl-hexanaldehyde, E-pent-2-en-1-al, and cyclohexanone by using a wine aliquot as small as 1 mL. Analysis time is under 1 h, including both the sample preparation and quantification steps. In addition, this method is precise, reliable, selective, and sensitive to determine CCs in wines on a routine basis analysis. LOD and LOQ of formaldehyde, acetaldehyde and furfural were below the olfactory perception limit. Furthermore, this method provides the identification and quantification of volatile organic compounds associated with wine flavor, a property that had only been described subjectively up to now.

Authors wish to thank CAPES, CNPq, PRONEX, FAPESB and FINEP for financial support.

The authors declared no conflict of interest.

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