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Determination of gluconic acid in wine using high pressure liquid chromatography with pulsed amperometric detection

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Summary

The paper establishes a new HPLC method for measurement of the total content of gluconic acid (GA) in wine using a pulsed amperometric detector equipped with a gold electrode cell and an anion exchange column. A NaOH solution was used at a flow rate of 1.5 ml·min^{-1} as a mobile phase under isocratic conditions. Analysis took 30 min. The linearity of the method was shown up to 500 mg·l^{-1}, with a LOQ of 2.0 mg·l^{-1} (10σ). Precision (as RSD%) was under 2.5 % in the usual range of GA concentration in natural wines. Accuracy was checked in comparison with the usual enzymatic method.

K e y w o r d s : wine, gluconic acid, HPLC-PAD.

Introduction

As reported in the exhaustive and updated review of Ramachandran and co-workers (2006), gluconic acid (pentahydroxycaproic acid) (GA) is a noncorrosive, mildly acidic, less irritating, odourless, nontoxic, easily biodegradable, water soluble, nonvolatile organic acid characterised by a refreshing taste and a degree of sourness of about 1/3 as compared to citric acid. It is present - naturally or added as generally permitted food additive or generally recognised as a safe product in Europe and the USA respectively - in several foodstuffs, e.g. fruit and related juices, rice, meat, yogurt, cheese, bread, confectionery, honey, wine, vinegar, balsamic vinegar and sauces.

Of the microorganisms infecting grape berries, *Botrytis cinerea*, *Aspergillus niger* and *Gluconobacter oxydans* have the enzymatic pool to produce GA present in musts and wine, this acid not being assimilable by *Saccharomyces cerevisiae* in reductive winemaking conditions (Sponholz and Dittrich 1985, Donèche 1989, Peinado *et al*. 2003). GA was shown in wine to be in equilibrium with the 2 intramolecular esters δ-gluconolactone (glucono-5-lactone) and γ-gluconolactone (glucono-4-lactone), which were found in amounts of around 40 and 60 mg·g^{-1} GA respectively, at the pH of wine (Barbe *et al*. 2002, Bertrand and Barbe 2002).

Several methods have been proposed for the quantification of GA (Mato *et al.* 2005). Paper chromatography and precipitation using phenylhydrazide were used by Rentschler and Tanner (1955) to establish the presence of GA in botrytised grapes for the first time. A hydroxamate method was proposed by Lien (1959) and an isotachophoretic method was proposed by Everaerts *et al*. (1976). Gas chromatography was used to analyse the silylate derivatives of GA and its δ- and γ-lactones (Laker and Mount 1980, Bertrand and Barbe 2002). ^{13}C-NMR-spectroscopy was applied by Rapp *et al*. (1989) whereas capillary electrophoresis was used by García Moreno *et al*. (2002). Gump and Kupina (1979) proposed a HPLC approach using refractometric and UV detectors, Barbe *et al*. (2000) applied ionic chromatography with conductimetric detection and Seliverstova *et al*. (2003) used ion-exclusion chromatography with UV detection. More recently, a method based on a dedicated electrochemical biosensor in a flow-injection system was proposed (Campuzano *et al*. 2007). Probably, nowadays, the enzymatic method (Moellering and Bergmeier 1988) is the most widely used method in wine analysis while the Fourier transformed infrared method (FT-IR) is used for the evaluation of grape/must soundness (Fischer and Berger 2006).

The paper establishes a new method to determine the total content of gluconic acid in wine using HPLC with pulsed amperometric detection, without sample preparation and alternative to the enzymatic approach.

Material and Methods

R e a g e n t s : D-gluconic acid sodium salt (> 99 %), α-lactose monohydrate (Reagent grade), Glycerol (> 99.5 %), D-(+)-Trehalose dihydrate (> 99 %), D-(−)-Fructose (> 99 %), D-(−)-Ribose (> 99 %), D-(+)-Galactose (> 99 %), D-(+)-Glucose (> 99.5 %), D-(−)-Arabinose (> 98 %), D-(+)-Xylose (> 99 %), Sucrose (99.5 %), D-Sorbitol (> 98 %), Inositol (> 99 %), Mannose (> 99 %); L-Rhamnose monohydrate (> 99 %), D-(+)-Melibiose (> 98 %), D-(+)-Maltose monohydrate (> 98 %) and D-(+)-Raffinose penthahydrate (> 98 %) were purchased from Sigma Aldrich (St. Louis, USA) and used to test the selectivity of the method. Sodium hydroxide (> 97 %) was from Carlo Erba Reagenti (Rodano, Italy). The enzymatic kit for D-gluconic acid/D-gluconic-δ-lactone was from R-Biofarm (Darmstadt, Germany). HPLC grade water (Milli-Q system, Millipore; Bedford, MA, USA) was used for reagents and the HPLC mobile phase.

S a m p l e p r e p a r a t i o n a n d i n s t r u m e n t s : The sample for the HPLC-pulsed amperometric detector (PAD) analysis was diluted 10 times, with α-lactose added as internal standard to a final concentration of 200 mg·l^{-1} in

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order to minimise electrode fouling problems, filtered with a 0.45 µm cellulose acetate syringe filter (Alltech; Deerfield, IL, USA) and collected in a 2 ml glass screw-top vial. A HPLC Alliance 2695 (Waters Corp.; Milford, MA, USA) equipped with autosampler mod. 540 and an RCX/10 Anion exchange column (250 x 4.6 mm; Hamilton; Bonaduz, Switzerland) was used. The PAD detector was a Coulochem II 5200A used in pulsed mode and equipped with a 5040 gold electrode cell (ESA Inc.; Chelmsford, MA, USA).

Sample preparation and measurement of the total amount of GA (free plus lactone forms) was carried out enzymatically according to the producer's technical note using a U-2000 Spectrophotomer (Hitachi Ltd.; Tokyo, Japan).

Chromatographic separation: A degassed 0.1 M sodium hydroxide solution was used as a mobile phase in isocratic condition with a flow rate of 1.5 ml·min^{-1}. The injection volume was 10 µl and the column temperature 20 °C. Separation took about 16 mins followed by 13 mins of column cleaning and conditioning. The pulse setting of the gold electrode was + 200, + 700, and - 900 mV for 300, 100, and 100 ms respectively. Sample quantification took place using external calibration in a range from 1 to 500 mg·l^{-1} of GA.

The performance of the method was studied in a range from 0 to about 500 mg·l^{-1}, suitable for 1/10 diluted natural wines. The linearity and precision (as RSD%) of the method was checked using standard solutions at 12 concentration levels (0, 7.5, 12.7, 37.5, 62.0, 87.5, 157, 229, 297, 386, 434 and 504 mg·l^{-1}) and with 10 independently prepared repetitions/level. The limit of detection (LOD) was estimated both with the usual approach (3σ of 10 replicates of a blank) and according to HUBAUX and VOS (1970), studying the regression line of the signal in a range of concentrations between 0 and 37.5 mg·l^{-1}. The second approach defined the LOD avoiding false negatives with a probability of 1-β = 0.95. Similarly, the limit of quantification (LOQ) was estimated both as 10σ and according to EURACHEM (1993), as the concentration value when the RSD% of the method was 12 %.

The accuracy of the method was estimated through comparison with the usual enzymatic method, analysing 55 natural wines. The data were statistically evaluated using STATISTICA® 8.0 (StatSoft, Inc.; Tulsa, OK, USA).

Results and Discussion

The chromatogram in Fig. 1 shows the HPLC-PAD peak pattern measured in a natural red wine fortified with GA. GA elutes at 14.24 mins in a zone not interfered with by the compounds normally present in wine and listed in "reagents".

The linearity of the method was demonstrated up to 500 mg·l^{-1}, corresponding to 5000 mg·l^{-1} GA in wine, as shown by the following parameters of the regression analysis: n = 120; Intercept 0.0002 µA; S.D. intercept 0.0025 µA; Slope 0.005458 µA l·mg^{-1}; S.D. slope 0.000010 µA l·mg^{-1}; S.D. regression 0.0186 µA; R^2 0.9996.

The precision (RSD %) at 12 concentration levels is shown in the Table. The LOD calculated as 3σ was 0.6 mg·l^{-1}, whereas it was 4.4 mg·l^{-1} when evaluated according to HUBAUX and VOS (1970). LOQ was 2 mg·l^{-1} (10σ) or 9 mg·l^{-1} when estimated according to EURACHEM (1993). The LOQ values are suitable for the measurement of the lower concentration of GA (109 mg·l^{-1}) quantified in 100 Italian wines on sale. Accuracy was evaluated by comparing the analytical performance of the HPLC meth-

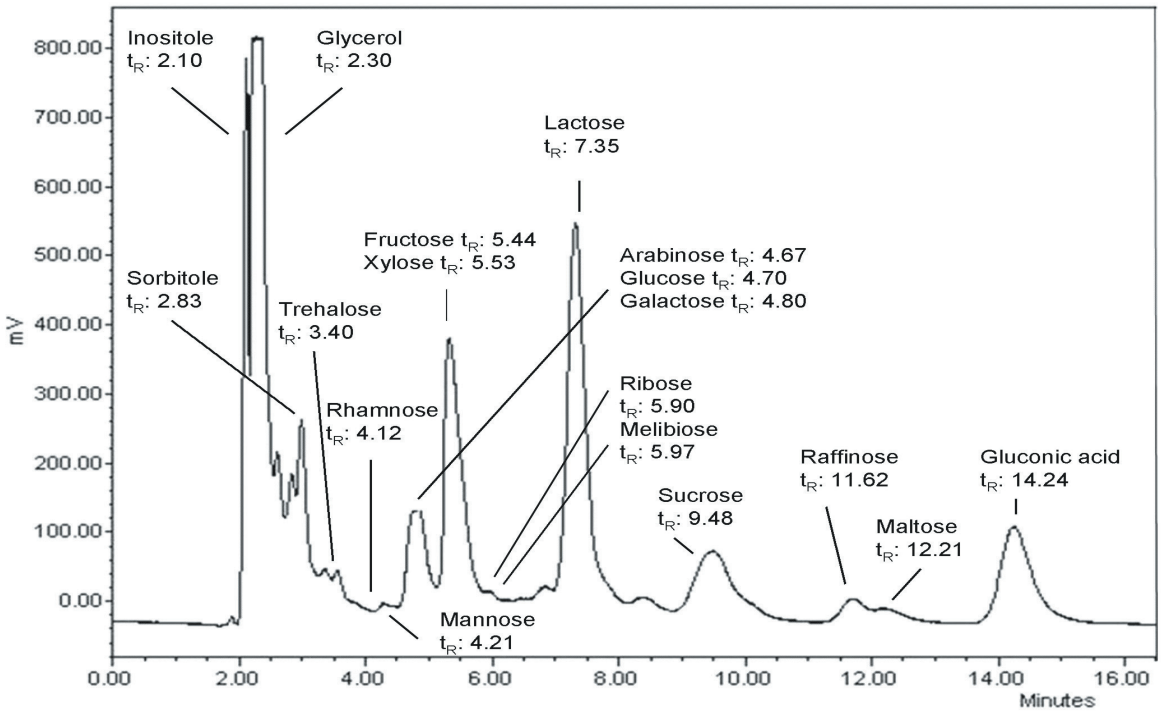

Fig. 1: Chromatographic peaks and corresponding retention time (t_R), related to a red wine fortified with gluconic acid, measured using a pulsed amperometric detector.

T a b l e

Precision (as RSD%) of the proposed method at 12 concentration levels of gluconic acid

Level	GA concentration ($mg \cdot l^{-1}$)	RSD%
1	0	37.9
2	7.5	13.3
3	12.7	6.1
4	37.5	2.0
5	62.0	2.2
6	87.5	2.5
7	157	0.8
8	229	1.4
9	297	1.9
10	386	1.8
11	434	1.4
12	504	1.0

od proposed in relation to the enzymatic method (Fig. 2), applying the regression model proposed by Passing and Bablok (1983). The methods were compared taking into account the analytical errors of each and seeking the linear regression model that minimises the sum of the squares of the deviation for each. The equation that best describes the measurements obtained with the two methods has an intercept = - 34.34 $mg \cdot l^{-1}$, significantly different from the zero value, fixing a confidence interval of 95 %. The slope was 0.9424. The difference between the methods was statistically significant (t test, $p < 0.05$). This means that on average the analytical results obtained using the enzymatic model were around 6 % lower than those obtained using the HPLC-PAD method. It is reasonable to suppose that the differences between the two methods can be attributed to the difficulties in obtaining complete hydrolysis of the lactonic δ and γ forms by following the producer's instructions before enzymatic measurement. At all events these differences are not technologically significant.

The HPLC-PAD method was applied to the analysis of 100 wines on sale. The GA content in 58 white (25^{th} percentile 433 $mg \cdot l^{-1}$; median 589 $mg \cdot l^{-1}$; 75^{th} percentile 1,249 $mg \cdot l^{-1}$) and 22 red (369 $mg \cdot l^{-1}$; 715 $mg \cdot l^{-1}$; 1,118 $mg \cdot l^{-1}$) wines from the 2006 vintage covered an interval of between around 100 and 2,700 $mg \cdot l^{-1}$, with 50 % of the samples containing up to 600 $mg \cdot l^{-1}$ and 30 % of the samples over 1 $g \cdot l^{-1}$. There were no significant differences between red and white wines, whereas as expected the 20 raisin wines analysed, produced in different years, had significantly higher levels of content (1,297 $mg \cdot l^{-1}$; 2,059 $mg \cdot l^{-1}$; 3,979 $mg \cdot l^{-1}$; HSD Tukey's test for an unequal number of samples, $p < 0.001$). The measured contents fit with those reported in the literature (Postel *et al*. 1972, McCloskey 1974, Holbach and Woller 1978).

Conclusions

The proposed method represents a new HPLC approach using pulsed amperometric detection for accurate measurement of the content of gluconic acid in wine, from a few dozen up to 5,000 $mg \cdot l^{-1}$. It gives the direct measure of the sum of the open and lactonic forms of GA, without sample preparation. Precision (RSD%) is under 2.5 in the usual range of concentration in natural wines. This method is a valid alternative to the enzymatic method for routine quality control of wine.

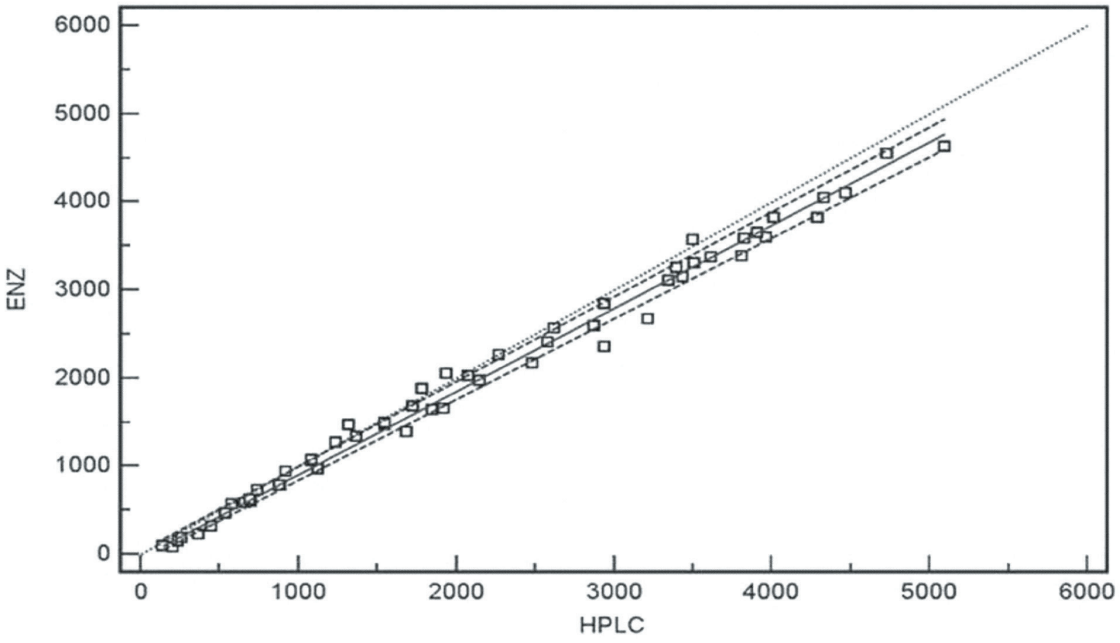

Fig. 2: Regression plot, according to Passing and Bablok (1983), of the gluconic acid content ($mg \cdot l^{-1}$) measured in 55 wines using the enzymatic (ENZ) and chromatographic (HPLC) methods. Legenda: ····· slope = 1; ------- 95 % confidence intervals; —— regression line.

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References

Barbe, J. C.; de Revel, G.; Bertrand, A.; 2002: Gluconic acid, its lactones, and SO_2 binding phenomena in musts from botrytized grapes. J. Agric. Food Chem. **50**, 6408-6412.

Barbe, J. C.; de Revel, G.; Joyeux, A.; Bertrand, A.; Lonvaud-Funel, A.; 2000: Role of carbonyl compounds in SO_2 binding phenomena in musts and wines from botrytized grapes. J. Agric. Food Chem. **48**, 3143-3149.

Bertrand, A.; Barbe, J. C.; 2002: Formation of γ-gluconolactone in a wine-like model system. J. Sci. Food Agric. **82**, 1571-1573.

Campuzano, S.; Gamella, M.; Serra, B.; Reviejo, A. J.; Pingarrón, J. M.; 2007: Integrated electrochemical gluconic acid biosensor based on self-assembled monolayer-modified gold electrodes. Application to the analysis of gluconic acid in musts and wines. J. Agric. Food Chem. **55**, 2109-2114.

Donèche, B.; 1989: Carbohydrate metabolism and gluconic acid synthesis by Botrytis cinerea. Can. J. Bot. **67**, 2888-2893.

EURACHEM Secretariat (Ed.); 1993: Accreditation for Chemical Laboratories. Teddington, London.

Everaerts, F. M.; Beckers, J. L.; Verheggen, T. P. E. M.; 1976: Isotachophoresis – Theory, Instrumentation and Applications. Elsevier, Amsterdam, The Netherlands.

Fischer, U.; Berger, T.; 2006: Objektive Bestimmung der Traubengesundheit. Der Deutsche Weinbau **13**, 16-19.

García Moreno, M. V.; Castro Mejias, R; Natera Marín, R.; García Barroso, C.; 2002: Analysis of sugar acids by capillary electrophoresis with indirect UV detection. Application to samples of must and wine. Eur. Food Res. Technol. **215**, 255-259.

Gump, H. B.; Kupina, S. A.; 1979: Analysis of gluconic acid in botrytised wines. In: G. Charalambous (Ed.): Liquid chromatographic analysis of food and beverages **2**, 331-352. Academic Press. Inc., New York.

Holbach, B.; Woller, R.; 1978: Der Gluconsäuregehalt von Wein und seine Beziehung zum Glyceringehalt. Die Weinwissenschaft **33**, 114-127.

Hubaux, A.; Vos, G.; 1970: Decision and detection limits for linear calibration curves. Anal. Chem. **42**, 849-855.

Laker, M. F.; Mount, J. N.; 1980: Mannitol estimation in biological fluids by gas liquid chromatography of trimethylsilyl derivatives. Clin. Chem. **26**, 441-443.

Lien, O. G. Jr.; 1959: Determination of gluconolactone, galactolactone and their free acids by hydroxamate method. Anal. Chem. **31**, 1363-1366.

Mato, I.; Suárez-Luque, S.; Huidobro, J. F.; 2005: A review of the analytical methods to determine organic acids in grape juices and wines. Food Res. Int. **38**, 1175-1188.

McCloskey, L. P.; 1974: Gluconic acid in California wines. Am. J. Enol. Vitic. **25**, 198-201.

Moellering, H.; Bergmeyer, H. U.; 1988: D-gluconate (D-glucono delta lactone) and D-gluconate-6-phosphate. In: H.U. Bergmeyer (Ed.): Methods of enzymatic analysis **4**, 220-227. VCH Publishers, Cambridge, UK.

Passing, H.; Bablok, W.; 1983: A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in Clinical Chemistry, Part I. J. Clin. Chem. Clin. Biochem. **21**, 709-720.

Peinado, R. A.; Mauricio, J. C.; Ortega, J. M.; Medina, M.; Moreno, J.; 2003: Changes in gluconic acid, polyols and major volatile compounds in sherry wine during aging with submerged flor yeast cultures. Biotechnol. Lett. **25**, 1887-1891.

Postel, W.; Drawert, F.; Maccagnan, G.; 1972: Über den Gehalt an D-Gluconsäure in Weinen verschiedener Qualitätskategorien. Chem. Mikrobiol. Technol. Lebensm. **1**, 151-155.

Ramachandran, S.; Fontanille, P. ; Pandey, A.; Larroche, C.; 2006: Gluconic acid: properties, applications and microbial production. Food Technol. Biotechnol. **44**, 185-195.

Rapp, A.; Markowetz, A.; Spraul, M.; Humpfer, E.; 1989: Nachweis und quantitative Bestimmung von Zuckern, Zuckeralkoholen und Zuckersäuren im Wein mit ^{13}C-NMR-Spektroskopie. Z. Lebensm. Unters. Forsch. **188**, 138-143.

Rentschler, H.; Tanner, H.; 1955: Über den Nachweis von Gluconsäuren in Weinen aus edelfaulen Trauben. Mitt. Lebensmittelunters. Hyg. **46**, 200-208.

Seliverstova I. V.; Ivanov, A. A.; Ivanova, L. A.; 2003: Determination of carboxylic acids in wines by liquid ion-exclusion chromatography. Appl. Biochem. Microbiol. **39**, 87-89.

Sponholz, W. R.; Dittrich, H. H.; 1985: Origin of gluconic acid, 2- and 5-oxogluconic, glucuronic and galacturonic acids in musts and wines. Vitis **24**, 51-58.

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