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Short communication

High-performance liquid chromatography coupled with fluorescence detection for the determination of *trans*-astringin in wine

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Abstract

In this study a high-performance liquid chromatography (HPLC) method was developed for the determination of *trans*-astringin in wine using fluorescence detection. This is the first time the occurrence of *trans*-astringin has been reported in wine. The method allows analysis of both red and white wine samples with no prior treatment. The quantification threshold is 0.03 mg/l. Levels of *trans*-astringin in the French wines analyzed ranged from 0.09 mg/l to 0.29 mg/l. The reproducibility of the method was measured and the CV was less than 4.8% for both red and white wines.

Keywords: Wine; Astringin

1. Introduction

Stilbenes occur naturally in a number of plant families [1] but grapes and related products are considered the most important dietary sources of these substances [2,3]. *Trans*-resveratrol and the 3-O-β-glucoside of *trans*-resveratrol, *trans*-piceid, were recently identified in wine [4–6]. Another compound 3′-OH-*trans*-piceid (*trans*-astringin) has recently been reported to be a constituent of *Vitis vinifera* cells [7] (Fig. 1).

The beneficial role that moderate wine consumption may have in preventing cardiovascular disease is still under debate [8], and great interest has been focused on stilbenes. *Trans*-astringin has been reported to have antioxidant properties, inhibiting in particular the oxidation of human LDL in vitro [9], but its occurrence in wine has never been reported. The aim of the present study was to develop a simple and sensitive method to determine this occurrence. A HPLC method coupled with fluorimetric detection was developed for this.

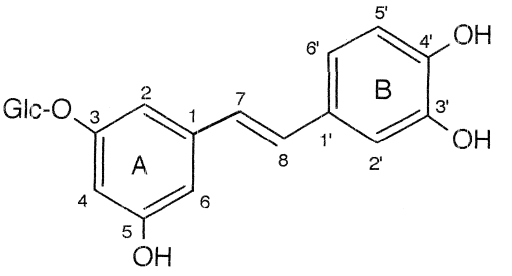

Fig. 1. Structure of *trans*-astringin.

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2. Experimental

Methanol and acetic acid were purchased from Carlo Erba (Val de Reuil, France) and Merck (Nogent sur Marne, France). *Trans*-astringin was obtained from cell suspension cultures [10] and identified unambiguously by MS and NMR as previously described [7]. The fluorescence properties of *trans*-astringin were determined with the 1064 Hewlett-Packard spectrofluorimeter. Calibration was performed with solutions of different concentrations of *trans*-astringin obtained by suitable dilution of methanolic solution at 10 mg/l. Standard solutions were stored at −20°C and protected from light. The linear regression was $Y=499.9x-0.8$ ($r=0.999$; CV<4.71).

2.1. HPLC analysis

A Hewlett-Packard Model 1100 with two low pressure pumps and a 1064 Hewlett-Packard fluorimetric detector coupled to an HP Chem Station were used for solvent delivery system and detection. A Hewlett-Packard column, packed with Nucleosil 100 C18, 250×4 mm, 5 μm particle size was used for the stationary phase. The eluents were for solvent A, an aqueous solution of 20 m*M* acetic acid, and for solvent B, methanol (HPLC grade); both were filtered through 0.45 μm Millipore filters. Elution was performed with the following gradient: 0–5 min 98% solvent A; 5–12 min from 98 to 93% solvent A; 12–18 min from 93 to 89% A; 18–28 min from 89 to 85% A; 28–38 min from 85 to 70%; 38–48 min from 70 to 60%; 48–55 min from 60 to 50% A, 55–75 min 50% A. The flow rate was 0.5 ml/min. Ten French wines (5 red, 5 white) from the South of France were analyzed. Wine samples were directly injected into the HPLC system after dilution in bi-distilled water and filtration (Millex-FH13; Millipore; St. Quentin, Yvelines, France). Dilution of 1/3 was used, and 20 μl were injected.

3. Results and discussion

The maximum excitation and emission wavelength in a methanolic solution as well as in a mixture of 20 m*M* methanol–acetic acid (v/v, 50:50) (the composition of the *trans*-astringin gradient was measured when in the spectrofluorimeter cell) were 298 and 400 nm respectively. The threshold of detection was defined as the amount of the compound resulting in a peak 10-fold higher than the standard deviation of the baseline noise. This was found to be 0.03 mg/l.

Chromatograms of *trans*-astringin and of a red wine sample monitored by fluorescence detection are reported in Fig. 2A and B. The retention time was 53.7 min. The HPLC separation and the good resolution of the peak without interference allowed the identification of the *trans*-astringin. A co-chromatographic analysis was also performed; it confirmed the identification of *trans*-astringin by HPLC with fluorimetric detection. The chromatogram of the wine in which *trans*-astringin was added is reported in Fig. 2C.

This progressive gradient run developed here has allowed the analysis of other stilbenes: *trans*-resveratrol, *trans*-piceid [11], and other phenolic compounds such as (+)-catechin, tyrosol, gentisic acid [12].

The standard methanolic solution of *trans*-astringin was stable for at least 30 days at −20°C in the dark. When this solution was exposed to wide-spectrum fluorescent lighting at a light intensity of 40 $\mu mol\ m^{-2}\ s^{-1}$ at 25°C, no degradation of *trans*-astringin was observed during the first two h, corresponding to the time of analysis. Therefore, *trans*-astringin can be handled in the laboratory without very stringent precautions.

We found low levels of *trans*-astringin in the wine analyzed, and the results were confirmed by mass spectrometry. *Trans*-astringin was extracted from red wine and FAB-MS spectra showed a $[MH]^+$ peak at m/z 407 ($C_{20}H_{22}O_9$ requires 406).

In this research, *trans*-astringin is reported for the first time to be present in both red and white wines. Concentrations ranged from 0.09 mg/l to 0.29 mg/l for red wines and from 0.10 mg/l to 0.22 mg/l for white wines. To study the reproducibility, the same red and white wine samples were analyzed on five different days. The CV obtained were less than 4.72% for red wines and less than 4.23% for white wines.

Fluorimetric detection evidenced low levels of *trans*-astringin in wine. As already described by Pezet et al. [13] for *trans*-resveratrol and ptero-

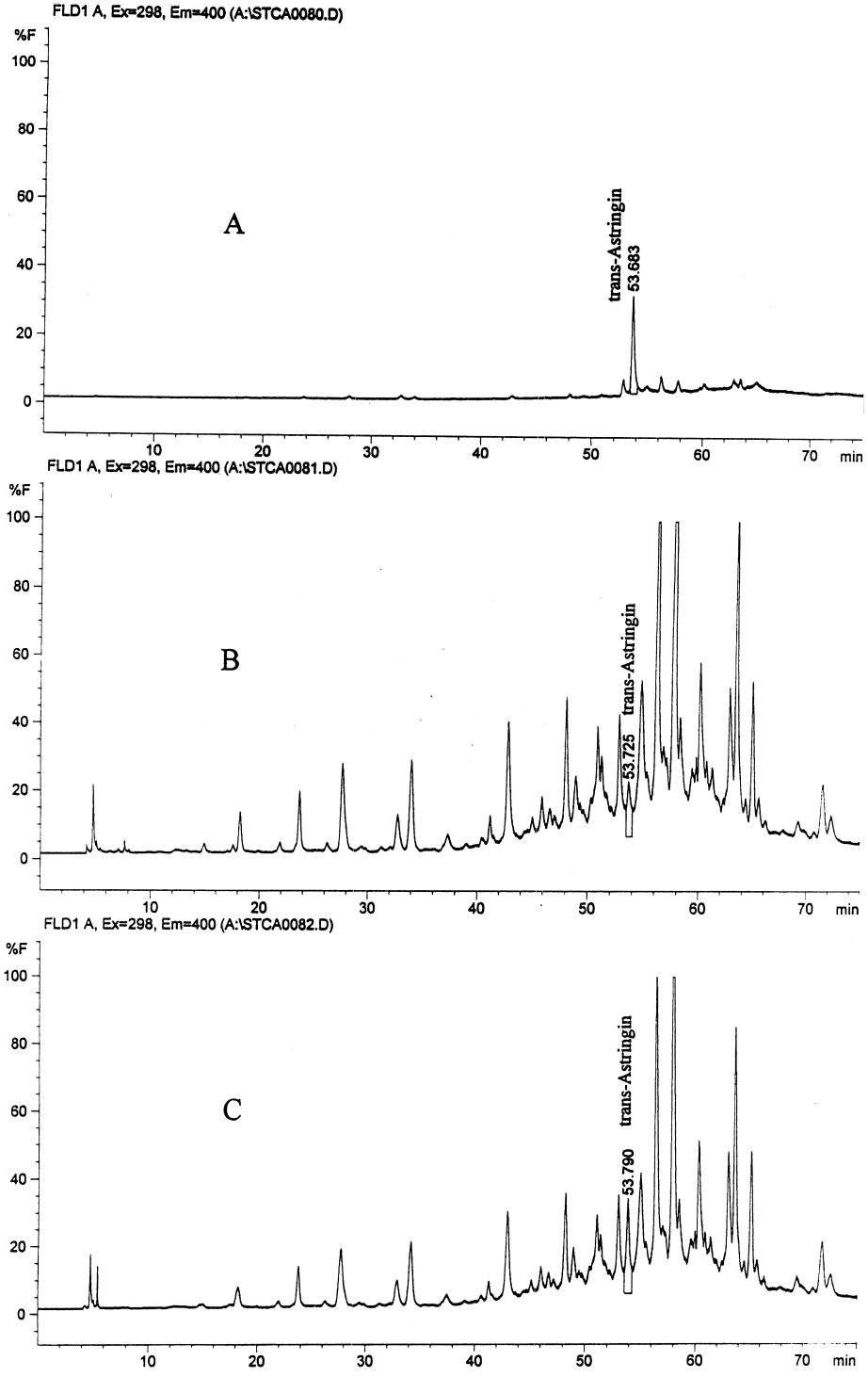

Fig. 2. Chromatogram of *trans*-astringin standard (A), of a red wine sample (B), of the same red wine sample with *trans*-astringin added (C).

stilbene, the use of a fluorimetric detector is well adapted to the low concentrations of stilbenes in wines. In particular, no prior treatment of the wine sample is required. For the ten French wines analyzed here, no large differences were observed between red and white wines, but analysis of more samples of commercial wine is needed to estimate the dietary impact and bioavailability of the *trans*-astringin in the wines of world.

4. Conclusion

The present method for the analysis of *trans*-astringin in wine combines the simplicity of direct HPLC injection with the sensitivity of fluorescence detection.

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References

[1] J.H. Jart, Ann. Rev. Phytopathol. 19 (1981) 437–458.
[2] D.M. Goldberg, Clinic. Chem. 41 (1995) 14–16.
[3] F. Mattivi, F. Reniero, S. Korhammer, J. Agric. Food Chem. 43 (1995) 1820–1823.
[4] E.H. Siemann, L.L. Creasy, Am. J. Enol. Vitic. 43 (1992) 49–52.
[5] P. Jeandet, R. Bessis, M. Sbaghi, P. Meunier, Vitis 33 (1994) 183–184.
[6] J.P. Roggero, P. Archier. Sci. Aliments 14 (1994) 99–107.
[7] P. Waffo-Téguo, S. Krisa, A. Decendit, G. Deffieux, J. Vercauteren, J.M. Mérillon, J. Nat. Prod. 59 (1996) 1189–1191.
[8] S. Renaud, M. deLorgeril, Lancet 339 (1992) 1523–1526.
[9] B. Fauconneau, P. WaffoTéguo, F. Huguet, L. Barrier, A. Decendit, J.M. Mérillon, Life Sciences 61 (1997) 2103–2110.
[10] P. Waffo-Téguo, A. Decendit, J. Vercauteren, G. Deffieux, J.M. Mérillon, Phytochemistry 42 (1996) 1591–1593.
[11] S. Carando, Etudes analytiques des composés phénoliques du vin. Influence de la consommation de vin sur la (+)-catéchine plasmatique. Thèse de Doctorat de l'Université de Montpellier I, Faculté de Pharmacie, 1998.
[12] S. Carando, P.L. Teissedre, J.C. Cabanis, Dosage de certains composés phénoliques du vin par CLHP/fluorescence. Annales des Falsifications, de l'Expertise Chimique et toxicologique. 1999, in press.
[13] R. Pezet, V. Pont, P. Cuenat, J. Chromatogr. A 663 (1994) 191–197.