ULTRA HIGH PRESSURE LIQUID CHROMATOGRAPHY FOR STILBENES SEPARATION AND THEIR DETERMINATION IN BURGUNDY RED WINES•

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1. INTRODUCTION

Stilbenes belong to an important group of polyphenolic secondary metabolites present in various families of plants among which grapes certainly represent the most widespread natural source over the world. Associated products, and in particular wine, are considered as the most important dietary sources of these compounds (Chong *et al.*,2009; Goldberg, 1995). Stilbene derivatives display various distinct chemical structures based on the 1,2-diphenylethylene skeleton. Among the different stilbenes, resveratrol has undoubtedly been the most studied compound since its identification in wine and its association with various health benefits. However, all of the stilbene derivatives have actually shown biological or therapeutic properties including antioxidant and antifungal activities (Jeandet *et al*., 1995). Therefore, several analytical studies have attempted to extract, purify or quantify these species in vine and wine. High performance liquid chromatography (HPLC) coupled with UV or fluorescence detection has obviously largely been used to analyse phenolics and stilbenes in grapes and wines (Sun *et al.*, 2006; Buiarelli *et al*., 2007). HPLC coupled with mass spectrometry (MS) has allowed to detect stilbene derivatives in directly injected wines with high sensitivity using negative mode electrospray ionisation (Luan *et al*., 2000).

Recently, ultrahigh pressure liquid chromatography (UHPLC) has introduced a new era in liquid chromatography. This advanced method, which relies on the use of higher back pressures on columns packed with <2 μm beads, provides very fast analyses with improved resolution, and sensitivity. Various studies already report UHPLC analyses of phenolics or pesticides (Zhang *et al.*, 2009), but so far it has not been used for the simultaneous detection of stilbene derivatives.

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2. MATERIALS AND METHODS

In this study, eight stilbene derivatives (fig. 1) were extracted from the stem of *Vitis vinifera* (Zga *et al*., 2009), and were quantified in four red Burgundy wines (Pinot noir, 2008 vintage) elaborated by the same producer (Maison Louis Jadot, Beaune). These wines differ by the geographic location of grapes they were made of : Clos "Les Avaux" and Clos "Les Couchereaux" are from the Côte de Beaune, and "Clos Saint Jacques" and "Clos Vougeot" are from the Côte de Nuit (Boutegrabet *et al*., submitted).

DryLab 2000 plus software (Molnar-Institut, Berlin, Germany) was used to optimise the separation method of the eight studied stilbenes. An Acquity UPLC system (Waters, Milford, MA, USA) equipped with a model 2996 PDA detector was applied for the analysis. Four columns were tested for the measurements: BEH C18 was purchased from Waters (Eschborn, D) and VisionHT C18, VisionHT C18-HL and VisionHT C18-P were purchased from Alltech Grom (Rottenburg-Hailfingen, D).

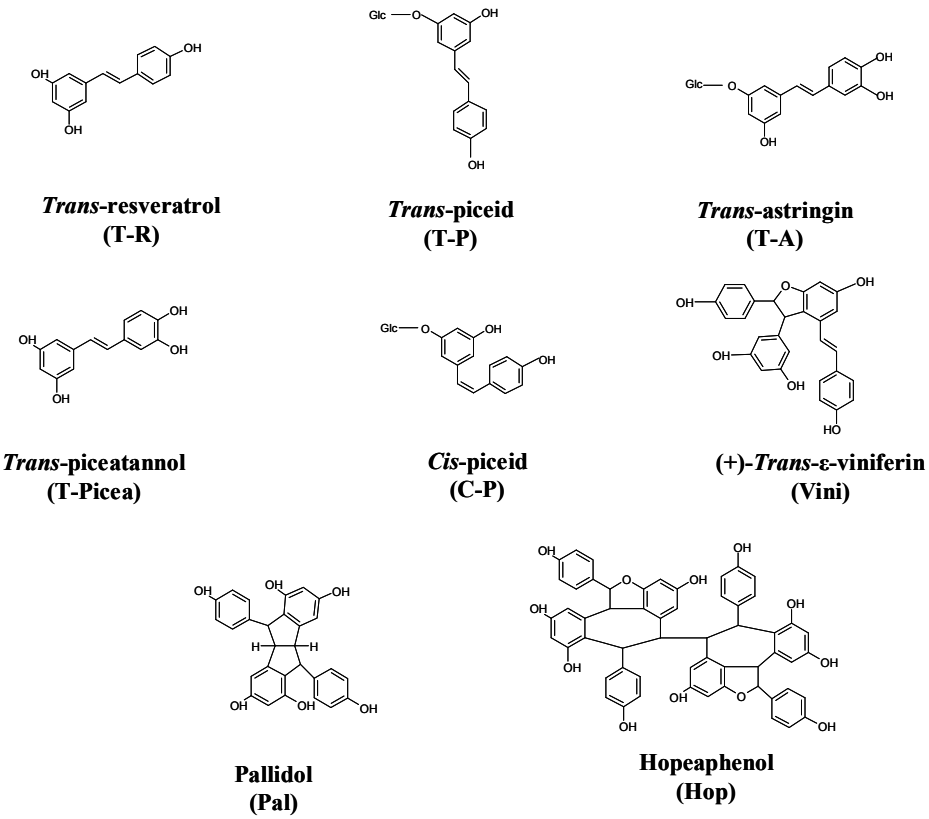

Fig. 1. Chemical structures of the studied stilbenes.

3. RESULTS AND DISCUSSION

A useful and fast separation method for stilbenes was optimised with quality-by-design based software (Fekete *et al*., 2009) and validated by means of UHPLC target approach and

used for direct analysis of wine after selection of the VisionHT C18-HL column as the best stationary phase having the optimum resolution and retention to the stationary phase. The obtained results indicate that this method is more sensitive (5 ppb without preconcentration) and reproducible (4-6 %) and has no matrix effect. The application of this method to real wines (fig. 2) indicated that the simultaneous quantification of stilbene derivatives allows to pinpoint geographic expressions (Gougeon *et al*., 2009) in wines at a spatial resolution as low as that of Burgundy appellations, thus emphasising possible terroir effects resulting from interplays between several environmental factors such as soil microbiology, UV exposure or vine status (Morlat, Bodin, 2006).

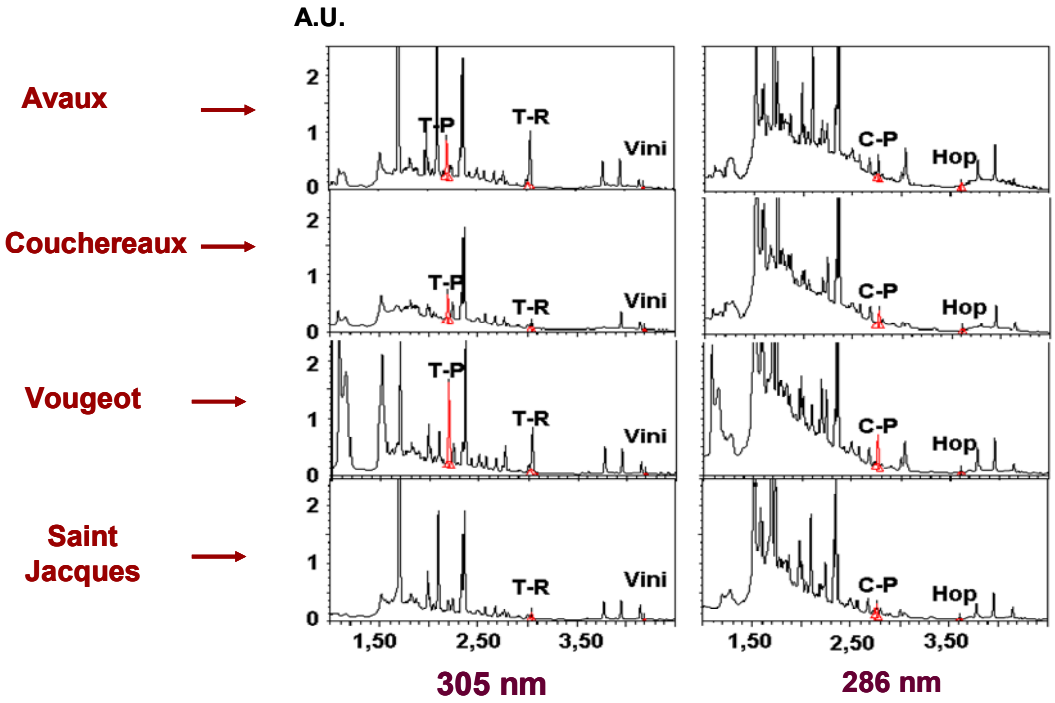

Fig. 2 - Wine chromatograms obtained with the VisionHT C18-HL column, with detection at 286 nm (right) and 305 nm (left), which represent absorption maxima. Abbreviation: T-P: *trans*-piceid, C-P: *cis*-piceid, T-R: *trans*-resveratrol, Hop: hopeaphenol, Vini: (+)-*trans*-ε-viniferin.

Abstract

In this study for the first time, eight natural stilbenes (*trans*-resveratrol, *trans*-piceid, *cis*-piceid, *trans*-astringin, *trans*-piceatannol, (+)-*trans*-ε-viniferin, pallidol and hopeaphenol) isolated and purified from *Vitis vinifera*, were simultaneously separated and analysed within 5 mn by ultra high pressure liquid chromatography coupled with photodiode array detection. The separation has been optimized using quality-by-design software allowing to get the quality by design and fast optimization of UHPLC and to study the influence of the ultra high pressure on the prediction. Four different reversed phase materials of subparticle size were tested and compared on the basis of retention behaviour, separation efficiency and matrix effect of the directly injected wine. Characteristics of the method showed high repeatability of retention times (RSD 0.03-0.07 %) and

peak areas (RSD=3-6 %) on the determined linear range (between 0.005-50 mg L^{-1}) for most components. Five of these stilbenes were identified and quantified in Burgundy red wines at different concentrations without any sample preparation steps, thus enabling a straightforward stilbene-based metabologeographical approach of wine analysis.

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