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Analysis of *trans*-resveratrol by laser ionization mass spectrometry and HPLC with fluorescence detection Comparison between both techniques

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Abstract

A comparison between two analytical techniques is presented using *trans*-resveratrol as analyte and vine leaf as sample. The employed methods were: (a) laser desorption followed by resonance enhanced multiphoton ionization coupled with time-of-flight mass spectrometry (LD–REMPI–TOFMS), and (b) reversed-phase high performance liquid chromatography (RP-HPLC) with fluorescence detection. While both techniques show a similar range of linearity and reproducibility, marked differences were found in their sensitivity and required time for a single analysis. For example: (i) the chromatographic method required considerable less time (30 min) than the REMPI method to implement the analysis, (ii) the detection and quantification limits of the REMPI technique were 2.1 and 6.7 $\mu g\,L^{-1}$, respectively, while for the chromatographic method they were ten times minor, i.e. 20 and 67 $\mu g\,L^{-1}$, respectively. A critical assessment including advantages and drawbacks of each technique is presented.

Keywords: *Trans*-resveratrol; Laser ionization; Mass spectrometry; HPLC

1. Introduction

Phytoalexins are produced by plants as a defense response to fungal infection, mechanical damage and UV irradiation [1]. *Trans*-resveratrol (3,5,4′-trihydroxystilbene) is one of the major stilbene phytoalexins found in different families of plants such as the *Polygonum Cuspidatum*, a Chinese medicinal plant, whose extract contains *trans*-resveratrol of purity range from 10 to 99%. Also grapes, peanuts and their products are considered the most important dietary sources of resveratrol [2]. The amount of *trans*-resveratrol in red wines is higher than in rose or white wines, partly due to the winemaking process but also depending on the grape variety, environmental factors in the vineyard and wine processing techniques [3].

Trans-resveratrol has received attention in recent years due to its capacity to protect against global cerebral ischemic injury and to ameliorate oxidative damage; it can also inhibit cellular events associated with tumour initiation, promotion and progression [4].

Due to the beneficial health effects of *trans*-resveratrol, several methods have been developed for its detection and quantification. Monitoring of resveratrol and other phenolic compounds requires analytical methodologies capable of performing determinations at trace concentration levels, such as chromatographic techniques, and some pretreatment steps because usually the matrix is too complex.

Trans-resveratrol is usually analyzed by reversed-phase high performance liquid chromatography (RP-HPLC) with standard bore columns. Most HPLC methods perform separation by acidic solvent gradient elution and detection with spectrophotometric UV [5], UV diode array (DAD) [6], or

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fluorimetry [7–9]. Fluorimetry, together with UV-DAD detection [10] and electrochemical detector [11], have been also applied to enhance the sensitivity of detection in HPLC. In addition, some work based on the application of LC coupled with MS [12] has been published. Methods based on gas chromatography mass spectrometry [13] have been proposed for *trans*-resveratrol analysis, but this technique has a major inconvenience: usually extraction, clean-up or a derivatization reaction are required prior to GC analysis of this substance and this handling can enhance the *trans* to *cis* isomerization of resveratrol. In all these techniques, the limiting step in *trans*-resveratrol analysis is the sample preparation, not only because of the need for costly and time consuming operations, but because of the error sources introduced during this analytical step. This has originated some controversy among different laboratories on their respective sample preparation procedures [14–16]. Revisions of some of the methods for the analysis of *trans*-resveratrol [17–19] showed a huge variability in the values published. This was attributed to a possible isomerization during the process of derivatization, losses due to oxidation, isomerization or hydrolysis during the extraction and separation processes, and the presence of some resveratrol derivatives that could interfere in the results. Several sample preparation methods used for the determination of *trans*-resveratrol by HPLC and a comparison of their main features have also been reviewed [20]. Direct analysis of *trans*-resveratrol using no chromatographic techniques as micellar electrokinetic capillary electrophoresis and a new technique based on the combination of laser desorption (LD) followed by resonance enhanced multiphoton ionization (REMPI) and time-of-flight mass spectrometry (TOFMS) has been also performed.

The most common samples analyzed for the determination of resveratrol are wine (mainly red wine), grapes, peanuts or peanut butters, although, the are also studies to a lesser degree in plants like tea and soy, and human tissues. For wine and grape juice samples, several methods have been developed [21–23] for the analysis of *trans*-resveratrol by direct injection on the HPLC system, but in most cases this leads to complex chromatograms which sometimes do not allow reliable identification and/or quantification of the peaks [20]. Micellar electrokinetic capillary electrophoresis has been also used in wine samples with a clear lack of sensitivity attributed to the need for preconcentration techniques [24]. In a previous paper, the analysis of *trans*-resveratrol in plant samples, namely in vine leaves by LD–REMPI–TOFMS was reported [25]. The technique has been further used for *trans*-resveratrol analysis in order to assess its relationship to grape disease resistance and to investigate its activity as a natural pesticide [26,27].

The present paper is dedicated to the comparison between RP-HPLC with fluorimetric detection and LD–REMPI–TOFMS techniques in the analysis of *trans*-resveratrol. Thus, the separation of *trans*-resveratrol from other phenolic compounds in vine leaf extract samples was performed using RP-HPLC. The same type of extract sample and compound was also analyzed by means of the REMPI technique. It should be pointed out that the REMPI technique can be applied without sample preparation; therefore, for the sake of the comparison with the HPLC the same extract sample was used in both experimental methods. After a brief description of those methods, their quality of the results and the relevant analytical parameters were compared and a critical assessment was done in order to establish the main advantages and drawbacks of each experimental method.

2. Experimental

2.1. Reagents and standards

HPLC-grade methanol, from Fluka (Switzerland), ethanol, from Scharlau (Barcelona, Spain), glacial acetic acid from Carlo Erba (Milan, Italy) and purified water with a Milli Q system from Millipore (Milford, MA, USA) were used.

A *trans*-resveratrol standard (99%) from Sigma Aldrich was used.

Standard solutions: 250 mg L^{-1} stock solution in ethanol was prepared. Working standard solutions were prepared by diluting the stock solution in ethanol. The standard solutions were stored at −4 °C in darkness.

2.2. Samples

Vine leaves were directly obtained from the vineyard after harvest in October. They were cut in pieces and introduced in ethanol (ca. 8 L for 4 kg of leaves) allowing 3 weeks of maceration to extract the *trans*-resveratrol. During maceration a sample of the solution was taken every 2 days to follow the extraction process by the evolution of the UV–vis absorption spectrum. The maceration process was carried out in darkness and at room temperature.

After maceration, the solution was filtered through cotton and the residue was analyzed by LD–REMPI–TOFMS to confirm the complete extraction of the *trans*-resveratrol from the leaves.

The obtained solution was directly used for the subsequent analysis. All samples were protected from light to avoid photon-induced isomerization during sample treatment

2.3. LD–REMPI–TOFMS

A full description of the REMPI technique based on the combination of laser desorption with REMPI–TOFMS for the analysis of *trans*-resveratrol in plants has been previously published [25], so only a brief report is given here. Essentially, it consists of two independent high vacuum chambers; the first chamber is used for both laser desorption and laser post-ionization of the sample followed by the ions acceleration towards the second chamber, basically a time-of-flight unit with a two microchannel plate detector. A few nanosecond