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Comprehensive two-dimensional liquid chromatography–tandem mass spectrometry for the simultaneous determination of wine polyphenols and target contaminants

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ABSTRACT

A novel system for comprehensive two-dimensional liquid chromatography coupled to a triple quadrupole mass spectrometer is described for the analysis of wine components. The first dimension consisted of a 250-mm microbore cyano column utilizing 5 μm diameter particles, interfaced to a 50-mm superficially-porous particle C_{18} column with 2.7 μm diameter particles. Both columns were operated under reversed-phase conditions. Correlation between the two chromatographic separation modes was decreased by designing a 60-s shift gradient program in the second dimension, and the increase in orthogonality was evaluated quantitatively utilizing a number of orthogonality metrics. The system was employed for the analysis of a red wine sample, without preliminary clean-up procedures, and a total of 43 polyphenols were separated and identified. Comparison with a one-dimensional LC system showed a large increase in the number of identified components with the two-dimensional system. Optimized multiple reaction monitoring experiments allowed for the determination of *trans*-resveratrol, which is one of the most active antioxidant component of wine, and for monuron, a plant protection product (herbicide) of interest to regulatory agencies. The estimated limits of detection and of quantification were 0.3 μg L^{-1} and 1 μg L^{-1}, respectively, well below the minimum detection limit (10 μg L^{-1}) set by current regulation.

1. Introduction

The technique of comprehensive two-dimensional liquid chromatography (LC × LC) has undergone a dramatic development over the last decade, with applications to environmental, clinical, biological, and food samples spanning from high to very high complexity [1–5]. In contrast to multidimensional heart-cutting approaches (LC–LC), the LC × LC technique transfers the whole column effluent from a first chromatographic dimension (^{1}D) to a second chromatographic dimension (^{2}D) for further separation. This is accomplished by utilizing a switching valve with sample loops or micro-cartridges that hold each collected fraction. Continuous on-line techniques like LC × LC have clear advantages over other instrumental arrangements of two-dimensional LC, which may involve off-line fraction collection between the two dimensions, and/or flow-interruption (stop-flow techniques) in ^{1}D. The advantages of on-line comprehensive LC × LC compared to off-line LC × LC methods include faster analysis time, the ease of implementing automation, and the reduced risk of sample loss, sample deterioration, and artefact formation.

The increase in peak capacity, n_c, with respect to one-dimensional LC, will depend primarily on the adequate choice of the columns used in the two dimensions, as the maximum gain in n_c will result from the coupling of independent retention mechanisms. This is best accomplished by selecting stationary

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phases with different retention mechanisms (e.g., normal-phase and reversed-phase columns or other approaches). In this case the sample components are spread out through multiple retention patterns and identification is easier due to the formation of group-type patterns of homologous series of compounds [6–8]. However, a number of requirements need to be fulfilled including mobile phase miscibility and instrumental (flow rate, transfer loop size/volume) compatibility. Such difficulties may be minimized by the use of narrow- (2.1 mm ID) or microbore (1.0 mm ID) ^{1}D columns, for which the minimum plate height occurs at relatively low mobile phase flow rates (around 500 and 50 μL min^{-1}, respectively) [9,10]. Furthermore, ^{1}D columns can be conveniently operated under sub-optimal flow rates, typically, down to 100 and 10 μL min^{-1}, for the 2.1 mm and the 1.0 mm ID columns, respectively. This facilitates larger peak widths in ^{1}D thus allowing for more complete sampling of the ^{1}D-separated components onto the second dimension [11]. Compared to the use of a conventional ^{1}D column with a 4.6 mm inner diameter, the quantity of solvent transferred onto the second dimension column is substantially reduced facilitating peak compression and peak focusing [12] in ^{2}D.

The coupling of similar columns (e.g., RP-LC × RP-LC) may offer superior resolution [13,14] when different mobile phase pH values [15–17] are utilized. This may also be implemented by varying the gradient composition between subsequent ^{2}D runs [18], according to the properties of the analytes being separated. The evolution of the LC × LC technique has been greatly advanced by the availability of commercial instrumentation capable of ultrahigh pressure (UHPLC) operation and higher performance columns such as those made of superficially-porous particles. These innovations allow satisfactory efficiency even at high flow rates (up to 4–5 mL min^{-1}) typically employed in ^{2}D, and the establishment of quick reconditioning (in as little as a few s) between subsequent gradient runs [19–21]. Whatever the front-end separation, RP-LC represents the most widespread choice for use in ^{2}D, being amenable to the direct connection to a mass spectrometry (MS) system. From a quantitative standpoint, the coupling of LC × LC separation alleviates ion suppression effects found in MS detection which may cause a higher abundant species to obscure the detection of a less abundant one. Quantitative analysis is therefore more reliable than with one-dimensional LC–MS; such benefits are even more evident for LC × LC platforms based on on-line coupling to MS via an electrospray ionization (ESI) source, where quantification is often significantly affected by matrix effects [22].

Only a few applications involving quantitative LC × LC work have been reported so far. One major issue is that dilution takes place at the injection of the fractions into ^{2}D by means of the interface, resulting in a loss of sensitivity with respect to one-dimensional techniques [9,23,24]. The scarce availability of commercial software for 2D data handling, visualization and zone quantitation represents another major limitation to widespread use of these approaches for quantitative purposes. The vast majority of LC × LC applications rely on the use of a single UV wavelength or a photodiode array (PDA) detector for quantitative analysis. In LC × LC–MS systems, the possible need for splitting the LC flow before entering the ion source will further impair the sensitivity and induce additional peak broadening [3]. The high scanning rates and high resolution make time-of-flight (ToF) MS the detector of choice in LC × LC applications. However, MS instruments based on the triple quadrupole technology (QqQ) are capable of scan speeds up to 30,000 amu/s, with very little interscan delay. Furthermore, polarity switching on time scales as fast as 5 ms allows the detection of fast eluting peaks with greater confidence and precision. Maximizing dwell times further helps to optimize the cycle time of MS/MS methods, allowing targeted multicomponent analysis through time-segmented multiple reaction monitoring (MRM) with unprecedented sensitivity and speed.

The scope of this research was the development of a novel LC × LC–PDA–MS/MS instrument based on a triple quadrupole (QqQ) design, and capable of delivering extremely high separation power. The coupling of two separation dimensions with the capability to afford enough selectivity and sensitivity as needed for the quantification of low-level or trace components will be described. The system will be demonstrated for the simultaneous separation and identification of bioactive phenolic constituents of a red wine sample, and the quantitative determination of a phenylurea-type pesticide. This offers far greater capabilities than would be possible with a standard one-dimensional LC–MS instrument, especially for applications which require higher resolution and quantitative accuracy.

2. Experimental

2.1. Samples and chemicals

Polyphenol reference materials used for method optimization and identification: 3,4,5-trihydroxybenzoic acid (gallic acid), 3,4-dihydroxybenzoic acid (protocatechuic acid), (1*S*,3*R*,4*R*,5*R*)-3-{[(2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,4,5-trihydroxycyclohexanecarboxylic acid (chlorogenic acid), (2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol (catechin), 4-(2-hydroxyethyl)phenol (tyrosol), (2*S*,3*S*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol (epicatechin), 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyloxy]-4*H*-chromen-4-one (rutin), (*E*)-3-(3,4-dihydroxyphenyl)-2-propenoic acid (caffeic acid), 4-hydroxy-3-methoxybenzoic acid (vanillic acid), 4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid), 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one (isoquercitrin), 7-[[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2,3-dihydro-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (naringin), ethyl 3,4,5-trihydroxybenzoate (ethylgallate), 2-(4-hydroxy-3-methoxy-phenyl)acetic acid (homovanillic acid), 3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid (sinapic acid), 4-hydroxy-3-methoxybenzaldehyde (vanillin), 4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde), (*E*)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid (ferulic acid), 5-[(*E*)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol (*trans*-resveratrol), 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one (quercetin), 2-methoxy-4-prop-2-enylphenol (eugenol), 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromenone (luteolin), 5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (apigenin), 4-(2-hydroxyethyl)-1,2-benzenediol (hydroxytirosol), and 3-(4-chlorophenyl)-1,1-dimethylurea (monuron) used in the quantitation assay were from Sigma-Aldrich/Supelco (Milan, Italy). LC–MS grade methanol (MeOH), water (H_2O), acetonitrile (ACN), and acetic acid were from Sigma-Aldrich/Supelco (Milan, Italy).

The red wine (Cabernet Sauvignon) was purchased in a local market.

2.2. Sample preparation

The wine sample was filtered through a 0.45 μm Acrodisc nylon membrane (Pall Life Sciences, Ann Arbor, MI, USA) prior to LC injection. For LC × LC method optimization and compound identification by ESI–MS data, a mixture of the polyphenol standard compounds was prepared by weighing the appropriate amount of each standard and diluting with MeOH/H_2O to a final concentration of 30 mg L^{-1},