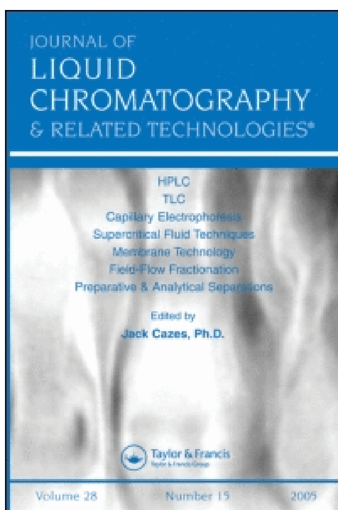
This article was downloaded by:
On: *25 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK




Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Wine Phenolics Analysis Via Direct Injection: Enhancement of the Method

Jean-Pierre Roggero[a]; Paul Archier[a]; Serge Coen[a]
[a] Laboratoire de Chimie Organique et Analytique Faculté des Sciences. 33, Avignon, France

**To cite this Article** Roggero, Jean-Pierre , Archier, Paul and Coen, Serge(1991) 'Wine Phenolics Analysis Via Direct Injection: Enhancement of the Method', Journal of Liquid Chromatography & Related Technologies, 14: 3, 533 — 538
**To link to this Article: DOI:** 10.1080/01483919108049268
**URL:** http://dx.doi.org/10.1080/01483919108049268

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(3), 533-538 (1991)

# WINE PHENOLICS ANALYSIS VIA DIRECT INJECTION: ENHANCEMENT OF THE METHOD

JEAN-PIERRE ROGGERO, PAUL ARCHIER, AND SERGE COEN
*Laboratoire de Chimie Organique et Analytique*
*Faculté des Sciences. 33, rue Louis Pasteur*
*84000 Avignon - France*

Downloaded At: 10:17 25 January 2011

## ABSTRACT

Complete wine phenolics determination via direct injection into the analytical column is obtained by means of two analyses using the same elution profile but slightly different solvents. A perfectly stable column temperature (22.5 °C) is required for good selectivity and reproducible retention times.

## INTRODUCTION

In previous papers we described a new H.P.L.C. method for investigation of phenolics in wines (1) and a stepwise optimization of the analytical procedure in order to settle the best gradient profile with use of an efficient RP-18 column (2).

Using that method very good results were obtained at room temperature in winter (c.a. 20°C) and up to 24-25°C, but a loss of resolution was noted in summer and the necessity to maintain a precise column temperature became evident.



Copyright © 1991 by Marcel Dekker, Inc.

The present paper describes the new survey which has been done to determine the best column temperature and modify consequently the elution gradient.

## MATERIALS AND METHODS

### Instrumentation:

The HPLC system consisted of a Spectra-Physics ternary pump (Model SP-8800), equipped with a filter-mixer and a bypass-valve, a Rheodyne 7125 injector (50µl loop), a Diode Array Detector (Waters 991) and a Superspher RP-18 column from Merck (250-4 mm), protected by a Guard-Pak module (Waters).

A 20 liters thermostat in which were immersed a continuously acting cryogenic coil (250-300 watts) and a regulated heating coil (500-800 watts) was used to produce the thermostated water at 22.5°C which flowed into a bath containing the column.

Downloaded At: 10:17 25 January 2011

### Chromatographic conditions:

*Solvents :*

**A** : acetic acid - water (1/99 v/v)

**B** : acetic acid - water (5/95 v/v)

**B'**: acetic acid - water (6/94 v/v)

**C** : acetic acid - water - acetonitrile (5/65/30 v/v/v)

*Gradient profile :*

| Time (mn) | **A** % | **B(B')**% | **C** % |
|---|---|---|---|
| 0 | 100 | 0 | 0 |
| 15 | 0 | 100 | 0 |
| 30 | 0 | 100 | 0 |
| 50 | 0 | 90 | 10 |
| 60 | 0 | 80 | 20 |
| 80 | 0 | 70 | 30 |
| 120 | 0 | 0 | 100 |

The flow rate was .5 ml/mn and the wine sample directly injected after filtration upon a Dynagard filter (0.2µm . Merck).

## RESULTS AND DISCUSSION

### Temperature setting:

Temperature is generally not critical in affinity chromatography, but when more than 100 compounds are eluted during 150 minutes that parameter takes some importance. So long as the analytical column is maintained within 0.1°C, the retention times remain constant, even in the last part of analysis, making easier the identification of peaks.

A survey of the variations in retention times shows that some compounds are more temperature dependent than others, leading in some cases to peaks inversions.Starting from 18°C and increasing the temperature the retention times first slightly increase, doubtless in reason of a better penetration into the porous particles ; thereafter these retention times are evidently shorter, due to a weaker adsorption. We chose to set the temperature at 22.5°C, value giving the highest retention times, in order to use the whole specific surface of the phase as well as a sufficient adsorption.

Downloaded At: 10:17 25 January 2011

### Elution gradients:

The change in column temperature from 20 to 22.5°C leads to some variations in selectivity. The third gradient previously settled (2) using solvents **A, B** and **C** allows a satisfactory separation of many phenols, but proanthocyanidin B1 (*) and *p*-coumaroyl tartaric acid co-elute, showing an higher sensitivity of the last phenol to temperature modification.

This failure however leads to the appearence of the *p*-hydroxy benzoic acid peak which lied under B1 and is now particularly obvious on the 254 nm plotting. That phenol being always in very weak concentration its resolution as a single peak is particularly satisfactory.

Moreover the wide "Z" peak previously attributed to di-caffeoyl tartaric acid (2) is clearly resolved in two parts, one of them due to 2-S glutathionyl caffeoyl tartaric acid (or GRP, réf 3);the second one possibly corresponding to the initially proposed structure.

---

(*) : Previously identified to B3 (2). The two compounds having the same U.V. spectra, the identification to B1 is due to the relative abundances of these proanthocyanidins in wine.

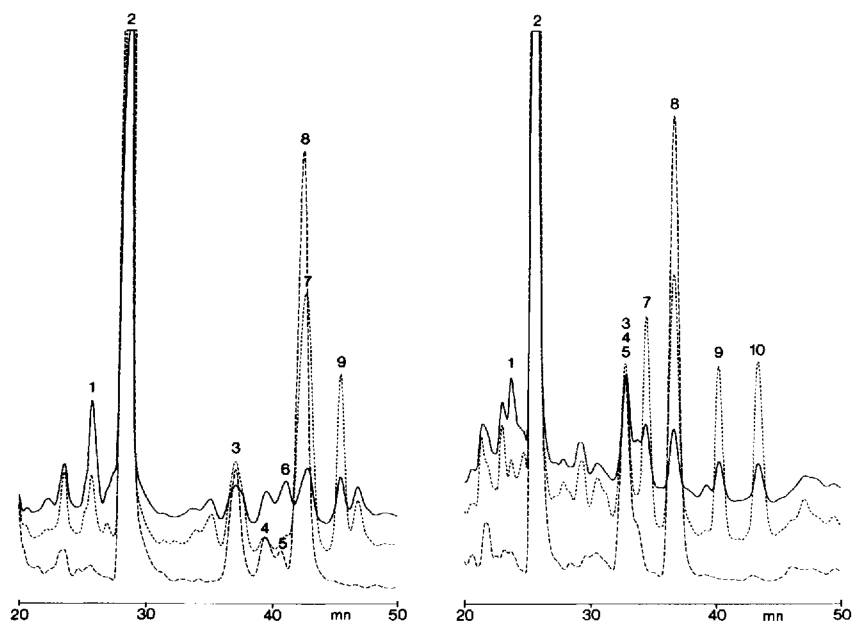



Figure 1:

Parts of two chromatograms of a Cabernet-Sauvignon wine:

Left **A, B, C** solvents - Right : **A, B', C** solvents.
—254 nm .........280 nm ----- 313 nm

**1** : Protocatechuic acid - **2** : Caffeoyl tartaric acid
**3** : *p*-Coumaroyl-tartaric acid glucosidic ester - **4** : GRP - **5** : Di-Caffeoyl tartaric acid (?) - **6** : *p*-Hydroxy-benzoic acid - **7** : B 1 - **8** : *p*-Coumaroyl tartaric acid - **9** : Tyrosol- **10** : Catechin.

Downloaded At: 10:17 25 January 2011

The separation of proanthocyanidin B1 and *p*-coumaroyl tartaric acid is easily obtained by use of a more acidic **B** solvent, namely **B'**, containing 6% of acetic acid, without gradient profile modification.The retention times of the powerful proton acceptors, such as catechins and proanthocyanidins, are thus particularly modified. However , in that analysis *p*-coumaroyl tartaric

**TABLE 1**

**Retention Times of Phenols (mn)**

| Names | Solvents A, B, C | Solvents A, B', C |
|---|---|---|
| Gallic acid | 17 | 17,5 |
| Protocatechuic ac. | 26,5 | 25 |
| *p*-Hydroxy-Bz.ac. | 41,5 | 38 |
| Caffeoyl-Tart.ac. | 29 | 27,5 |
| *p*-Coumaroyl-Tart.ac | 42,5 | 37,5 |
| Vanillic acid | 62 | 57 |
| Caffeic acid | 65 | 60 |
| *p*-Coumaric acid | 89 | 83,5 |
| Ferulic acid | 101 | 98,5 |
| Syringic acid | 74 | 70,5 |
| Di-Caffeoyl Tart.ac(?) | 38,5 | 34,5 |
| G.R.P. | 38 | 35 |
| Tyrosol | 44 | 40,5 |
| Catechin | 51 | 45 |
| Proanthocyanidin B1 | 42,5 | 35,5 |
| *p*-Coumaroyl-Tart.acid glucosidic ester | 36 | 33,5 |
| Rutin | 105 | 104 |
| Isoquercitrin | 106 | 105 |
| Myricetin | 113,5 | 113 |
| Quercetin | 125 | 124,5 |
| Kaempferol | 138,5 | 138 |

Downloaded At: 10:17 25 January 2011

acid glucosidic ester and the two caffeic acid derivatives are not accurately separated; moreover, *p*-hydroxybenzoic acid cannot be identified.

Thus, the complete separation of all phenolics requires two analyses. A partial representation of chromatograms obtained using **A, B, C** or **A, B', C** solvents is shown in Fig 1 and the retention times of important phenols are listed in Table 1. It is noteworthy that the retention times of flavonols and flavonol glycosides, which are only eluted by acetonitrile, are quite the same in the two analyses.

Some results obtained on young or aged Mourvedre and Cabernet-Sauvignon wines have been published elsewhere (4).

## REFERENCES

1) ROGGERO J-P. and ARCHIER P. Mise au point d'une méthode de dosage des phénols simples des vins. Application à des vins d'origines et d'âges différents. *Connaiss.Vigne et Vin.*, **23** (1) p. 25-37. 1989.

2) ROGGERO J-P., COEN S.and ARCHIER P.
Wine Phenolics:Optimization of H.P.L.C.Analysis. *J.Liq.Chromatogr.* *(in press).*

3) SINGLETON V.L., ZAYA J., TROUSDALE E. and SALGUES M., Caftaric acid in grapes and conversion to a reaction product during processing. *Vitis.* , **23** . p. 113-120.1984

4) ROGGERO J-P., COEN S. and ARCHIER P.
Analyse des phenols simples et des flavonols des vins par injection directe en C.L.H.P. *Communication.Groupe Polyphenols 1990 International Conference.Strasbourg, France.To be published in Bull. Liaison Groupe Polyphenols,* **15.,** 1990.

Downloaded At: 10:17 25 January 2011