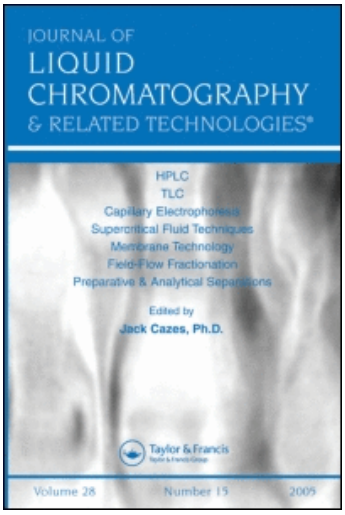

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Wine Phenolics: Optimization Of HPLC Analysis

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

To cite this Article Roggero, Jean-Pierre , Coen, Serge and Archier, Paul(1990) 'Wine Phenolics: Optimization Of HPLC Analysis', Journal of Liquid Chromatography & Related Technologies, 13: 13, 2593 — 2603
To link to this Article: DOI: 10.1080/01483919008049057
URL: http://dx.doi.org/10.1080/01483919008049057

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(13), 2593-2603 (1990)

WINE PHENOLICS: OPTIMIZATION OF HPLC ANALYSIS

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

ABSTRACT

Without previous extraction wine phenolics could be analysed by RP-HPLC via direct injection of the wine samples into the column. In order to optimize the analytical procedure the results obtained with two different columns of slightly different polarity and three different gradient elution systems have been compared.

The separated phenolics were further tentatively identified by means of their retention times and UV spectra which were recorded with a Photodiode Array detector

INTRODUCTION

A new direct RP-HPLC method suitable for the analysis of phenolic compounds in wines has recently been described (1).

In comparison with previous methods (2,3) the new technique shows two important advantages because:

1° The extractions are not exhaustive (4) nor reproducible, perhaps in reason of the important associations between the different phenols. Moreover, the calculations

of phenolic contents upon an extract involve the determination of recovery percentages from a "synthetic wine" which has not the colloidal properties of a real wine. The results may give rise to important analytical errors (e.g. the actual concentrations of some phenolic wine components may be largely underestimated).

2° The extraction causes some degradations to the sample. During the changes in pH values required for extraction of both neutral phenols (pH 7) and phenolic acids (pH 2) (3) hydrolysis of esters and glycosides, oxidation of highly reactive phenols, *cis-trans* isomerisation of cinnamic acids or esters may occur.

However, due to the complexity of a wine sample, the direct injection method requires an efficient RP-HPLC system. Therefore, the results of the analysis of a series of wine phenolics using two slightly different HPLC columns and three different gradient profiles will be described and compared in this paper.

MATERIALS AND METHODS

Wine sample : The used wine was a very young sample (1 month) originating from the Mourvèdre variety and produced in Bandol, Var-France (Bandol Label).

Instrumentation : The chromatograph equipment consisted of a ternary HPLC pump, Model SP-8800 (Spectra-Physics. San Jose. California), a sample injector, Model 7125 (Rheodyne Inc. Cotati. California) and a diode array detector, Model 990 (Waters Millipore Corp. Milford. Mass.). The loop volume was 50 µl.

Two columns have been used :

- a 250-4 Superspher cartridge 100 RP-18. réf 16056 (Merck. Darmstadt. Germany)
- a 250-4 Superspher cartridge 100 RP-18 E. réf. 16858 (Merck).

The RP-18 E column is fully endcapped and therefore slightly more apolar than the RP-18 column.

Chromatographic conditions :

Three solvent mixtures have been used to constitute the eluents :

- A : 1% acetic acid in water
- B : 5% acetic acid in water
- C : acetic acid/acetonitrile/ water 5/30/65 (v/v/v)

The gradient profiles were the following :

	Gradient 1		Gradient 2			Gradient 3		
Time (mn)	B %	C %	A %	B %	C %	A %	B %	C %
0	100	0	100	0	0	100	0	0
15	-	-	-	-	-	0	100	0
20	99	1	-	-	-	-	-	-
30	97	3	0	100	0	0	100	0
40	95	5	0	95	5	0	95	5
50	90	10	0	90	10	0	90	10
60	80	20	0	80	20	0	80	20
80	70	30	0	70	30	0	70	30
120	0	100	0	0	100	0	0	100

The flow rate was 0.5 ml/mn and the column temperature 20°C.

RESULTS AND DISCUSSION

The first analyses were performed using a 100 RP-18 column and the binary gradient 1.By means of this system many phenolics could be perfectly separated (fig. 1) and quantified but as indicated previously (1) the separation of certain of the compounds e.g. protocatechuic, *p*. hydroxybenzoic and vanillic acids or epicatechin proved to be difficult. Moreover some unknown peaks were unresolved and therefore their identification and quantification seemed at first sight to be impossible.

However, in such a case the Photodiode Array Detector may be very useful. For example, the identification of protocatechuic acid is very easy at 260 nm where the peak of the phenolic acid becomes visible. Moreover, in the case of critical pairs one of the components may be quantified on the sole condition that the co-eluting compound does not absorbe UV-light in the vicinity of the λmax of the phenolic

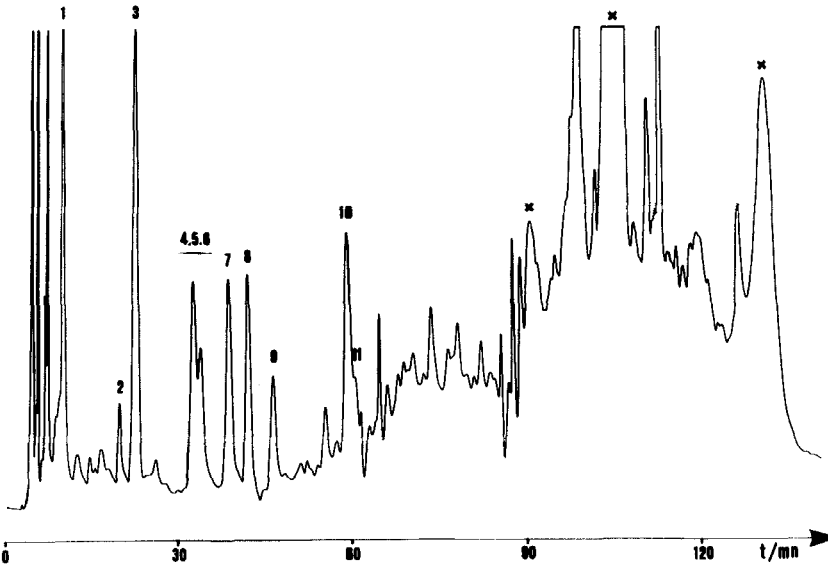

Figure 1 : Chromatogram of wine phenolics using a RP-18 column and gradient 1. Detection : 280 nm. 1:Gallic acid. 2:Protocatechuic acid. 3: Caffeoyl-tartaric acid. 4,5 and 6 : Compounds X,Y, and Z. 7:*p*-Coumaroyl tartaric acid. 8: Tyrosol. 9:Catechin. 10:unresolved peak containing Proanthocyanidin B1. 11:Vanillic acid. x:Anthocyanins.

substance under consideration. For example a flavonol may be quantified even when unseparated from an anthocyanin. Important additional advantages of the Photodiode Array Detector are the capacity for recording the UV-spectrum of each peak of the chromatogram and the possibility to perform baseline corrections of the obtained spectra.

In the chromatogram shown in fig. 1 two peaks, appearing between 32 and 34 minutes, are in fact due to three different components (viz. compounds n° 4, 5 and 6).

From two of these compounds, respectively called X and Y, U.V. spectra have been recorded while from the third compound Z only the λmax value could be obtained. In addition , the separation of these three compounds has been achieved

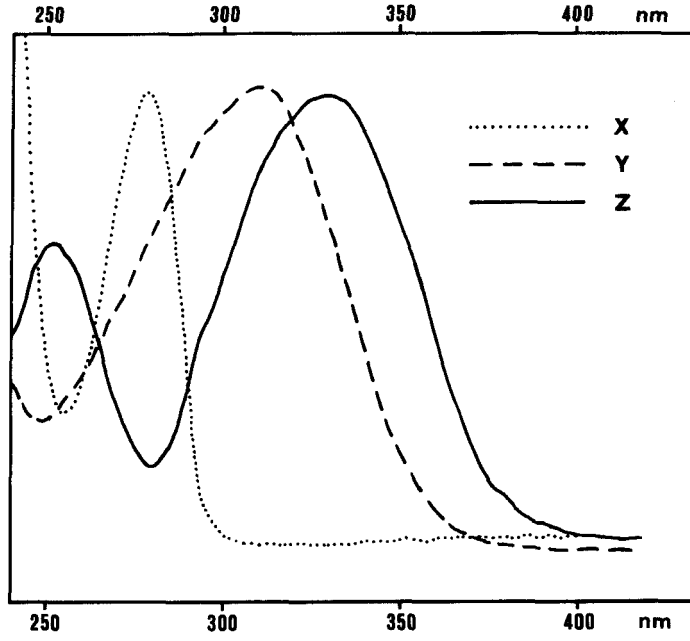

Figure 2 : U.V. Spectra of compounds X, Y and Z. The spectra were obtained by means of the Photodiode Array Detector.

by an additional HPLC-system and as a result the UV spectra of each of the substances has been recorded (fig. 2) The retention times of each of these phenols as well as their λmax value (respectively : 278 nm, 310 nm and 330 nm) suggest further, that compound X could be identical to procyanidin B3, compound Y to *p*.coumaroyl tartaric acid glucosidic ester (the presence of which has recently been noted by several authors (5, 6) and compound Z could correspond to di-caffeoyl tartaric acid.

In order to improve the resolution of the above compounds the use of a slightly less apolar (fully endcapped) 100 RP-18E column, originating from the same manufacturer, has also been tested. The employment of such a column results normally in a weaker retention of several more polar phenolic compounds and therefore its use could possibly improve the separation of certain critical pairs.

TABLE 1

Retention Times of Phenolics Using the Different Analytical Conditions

Gradient	1		2		3	
Column	RP-18	RP-18 E	RP-18	RP-18 E	RP-18	RP-18 E
Gallic acid	9.9	8.9	19.9	18.0	17.1	16.5
Protocatechuic acid	19.6	17.4	33.4	30.1	26.3	25.5
Caffeoyl tartaric acid	22.4	19.5	37.2	34.7	29.6	28.8
X (B_3 ?)	33.7	27.9	50.1	47.0	42.7	40.0
Y (*p*-coumaroyl tartaric acid glucosidic ester ?)	32.4	29.5	45.9	44.1	38.7	38.2
Z (dicaffeoyl tartaric acid)	33.6	27.3	49.0	45.8	41.4	39.4
Coumaroyl tartaric acid	38.5	34.4	50.8	48.2	44.7	43.4
Tyrosol (*)	41.7	38.7	52.7	50.8	47.2	46.2
Catechine	46.2	40.3	57.5	54.2	52.1	50.0

(*) 2. (*p*-hydroxyphenyl) ethanol

Indeed, by using the RP-18 E column, the above X, Y and Z peaks were inverted, Y constituting the second peak while the X and Z compounds were always co-eluting.The retention times shown in the two first columns of Table 1 prove further clearly that the separation of the X and Z components could not be achieved by either column RP-18 or RP-18E and gradient 1. A better resolution of the latter two compounds was therefore pursued by using different gradient profiles. However, in order to simplify the investigation procedure the profiles were only changed during the first 40 min ; thereafter the constitution of the eluent remained the same for the three gradient systems tested.

In gradient profile 2 the content of acetic acid was increased before the addition of acetonitrile. In this experiment, the optimization of the chromatographic process was therefore based on : 1° two different properties of the eluent namely ionic strenght and polarity and 2° on the slight difference in polarity of the two employed RP-HPLC columns. The results obtained with gradient 2 are represented in Table 1 (columns 3 and 4). With the preceding system, the components X and Y are well separated, (especially on the RP-18 column), but the retention time of X is rather close to the retention time of *p*-coumaroyl tartaric acid. Although the latter

TABLE 2

Comparison of the Variations in Retention Times With Changes in Column and (or) Gradient Profiles. Base :Column RP-18 E and Gradient 1

Col umn	RP-18	RP-18 E	RP-18	Additivity of Column and gradient effects
Gradient	1	2	2	
Gallic acid	1.0	9.1	11.0	10.1
Protocatechuic acid	2.2	12.7	16.0	14.9
Caffeoyl tartaric acid	2.9	15.2	17.7	18.1
X	5.8	19.1	22.2	24.9
Y	2.9	14.6	16.4	17.5
Z	6.3	13.5	21.7	19.8
Coumaroyl tartaric acid	4.1	13.8	16.4	17.9
Tyrosol	3.0	12.1	14.0	15.1
Catechine	5.8	13.9	17.2	19.7

resolution is more satisfactory (also because neither compound X nor Tyrosol, which occur both in the surroundings of *p*-coumaroyltartaric acid, are absorbing at 313 nm), a further improvement of the HPLC separation seemed to be possible. Therefore several variations of the chromatographic system have been tested and Table 2 gives a survey of the changes in retention time which as result have been found to occur.

Furthermore, replacement of RP-18E by a RP-18 column indicates the effect of column polarity on the retention times (see Table 2, column n° 1), while a replacement of gradient 1 by gradient 2 (column RP-18E) represents the effect of a lower ionic strenght of the eluent (see Table 2, column n° 2). As can be seen from table 2 (column n° 3 and 4)both effects are nearly additive when a combination of a RP-18 column and gradient 2 are used instead of a RP-18E gradient 1-system. The additivity effect is particularly large for both catechin (which is a strong proton acceptor) and compound X (very likely procyanidin B3) and this finding is in

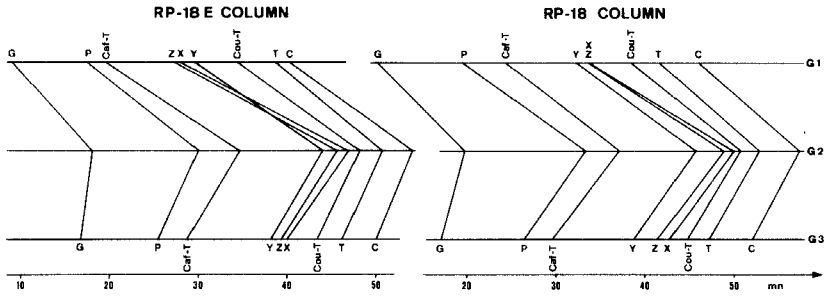

Figure 3 :Repartition of the peaks in the chromatograms obtained with either the RP-18E or the RP-18 column. G : Gallic acid. P:Protocatechuic acid. T : Tyrosol. C : Catechine (see also table 1).

agreement with the structure which has been proposed for the latter phenolic. Moreover the large variation in retention time of component Z with the type of column used is quite suggestive of the presence of two hydroxycinnamic units in the structure of this supposed dicaffeoyl tartaric acid component.

As a result of these findings and in order to obtain a satisfactory separation of the preceding peaks a third gradient was chosen with a variation of ionic strength in between gradients 1 and 2. The results obtained with this new gradient system (gradient 3) are represented in the last columns of Table 1, while fig. 3 shows the separations which have been achieved with the eluents systems and either a RP-18 or RP-18E column.

With regard to the results shown in both Table 1 and fig. 3 several remarks can be made.

1° When changing the column from RP-18 to RP-18E the most pronounced variations in retention times (and this with the exception of the tr's of gallic and protocatechuic acids) have been obtained with gradient 1.

The smallest changes in tr values have further been recorded with gradient 3.

In addition and this with the exception of gallic and protocatechuic acids and component Z (and only when changing from gradient 1 to gradient 2), the largest variations in retention times, with changing gradient system, occurred with a RP-18E column.

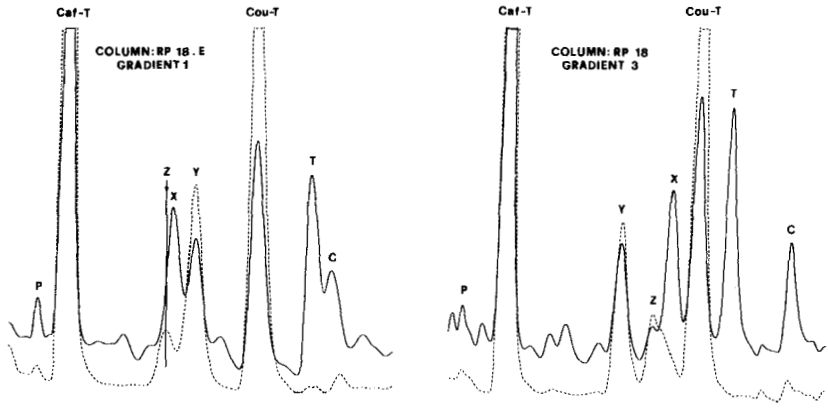

Figure 4 : Comparison of two chromatograms obtained with different columns and gradients. Detection : 280 (———) and 313 (------) nm. (The same abbreviations have been used as in fig. 3).

2° When using gradient system 3 practically the same resolution was obtained with both columns, although a slight preference for column RP-18 may be indicated.

In fig. 4 two identic parts (from caffeoyl tartaric acid to catechin) of two different chromatograms, the first obtained by means of column RP-18 E and gradient 1 and the second recorded by means of column RP-18 and gradient 3, are compared. From this comparison follows clearly that, although the gradient and polarity effects are such that caffeoyl and *p*-coumaroyl tartaric acids show in both case the same difference in retention time, the over-all resolution of the latter system is to be preferred.

Indeed, some phenols (e.g. catechin and compounds X and Z) show increased retention times and this most likely because they are stronger proton acceptors, while other phenols (e.g. Tyrosol and compound Y) show a smaller change in tr-value. The latter finding is further in agreement with the supposed glycosidic ester structure of compound Y.

It is also noteworthy that the Z component could not be obtained in the pure state. However, the separation of Z with a further and so far unknown peak is

achieved by using a RP-18 column and gradient 2. This result represents the only advantage of gradient 2 over gradient 3.

In conclusion, the optimized analytical method elaborated for the analysis of wines of the same origin will certainly allow the investigation of certain phenolic modifications related to wine aging. Indeed, the young wine sample which has been investigated contains no free caffeic nor *p*-coumaric acid but quite large quantities of caffeoyl and *p*-coumaroyl tartaric acid. One year old wine is rich in the two hydroxycinnamic acids but the content of these acids decreases again with further aging. Furthermore, hydroxycinnamoyl tartaric esters and glucosidic combinations decrease continuously during the aging process, whereas the gallic acid content increases. In addition a flavonol glycoside (very likely isoquercitrin) disappears from the wine after two years even though rutin and quercetin contents are stable.

The above results are not in agreement with those previously reported on certain wines of other origins or originating from other varieties (1).

This shows clearly the diversity in behavior of different wines during the aging process.

By means of the described method (RP-18 gradient 3) a survey of the changes occurring in wines during aging in now in this laboratory in progress.

ACKNOWLEDGMENTS

The authors are grateful to Professor C.F. Van Sumere (University of Gent,Belgium) for his contribution to the redaction of the present paper and for helpful remarks on this work.

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 Vin.* **23** (1). p 25-37. 1989.

2) REMINIAC C. and GOIFFON J-P. Etude des composés phénoliques du vin par chromatographie liquide à haute performance. *Bull. Liaison Groupe Polyphénols.* **11.** p 426-431. 1982.

3) SALAGOITY-AUGUSTE M-H. and BERTRAND A. Wine phenolics. Analysis of low molecular weight components by high Performance Liquid Chromatography. *J. Sci. Food agric.* **35.** p 1241-1247. 1984.

4) VISERAS C., GUILLEN D.A., BARROSO C.G., CELA R. and PEREZ-BUSTAMANTE J.A. Liquid-liquid extraction of polyphenolic acids and aldehydes. *Bull. Liaison Groupe Polyphénols.* **13.** p 154-156. 1986.

5) ONG B.Y. and NAGEL W. High Pressure Liquid Chromatographic analysis of hydroxycinnamic acid tartaric esters and their glucose esters in Vitis Vinifera. *J. Chromatogr.* **157.** p 345-355. 1978.

6) SOMERS T.C., VERETTE E., POCOCK K.F. and STRAUSS C.R. Spectral characteristics of hydroxycinnamate esters of Vitis Vinifera. *Bull Liaison Groupe Polyphénols.* **13.** p 502-505. 1986.