

Liquid-Liquid Extraction Based Simple Trans-Resveratrol Analysis In Wine Samples

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Abstract: 3,5,4-trans-trihydroxystilbene (trans-resveratrol) is quite soluble in CH₂Cl₂ and CHCl₃. Wine samples are shaken in and rinsed with CHCl₃ to obtain fine extracts of trans-resveratrol. The obtained CHCl₃ solutions were directly injected to HPLC system for analysis. Determinations were made in 60% MeCN: 40% EtOH, and by trans-resveratrol standard addition. The resolution is calculated to be about 1.0 in the chromatogram. In 9 wine samples produced in Turkey, resveratrol concentrations were detected in between 1.46 and 6.53 ppm. By consecutive extractions, extraction ratio of resveratrol was determined and by increasing the amount of the samples, accuracy of the method was tested. It was found that the recovery is about 100.42 %.

Key words: Resveratrol determination, wine, HPLC, liquid-liquid extraction.

Şarap Örneklerinde Sıvı-Sıvı Ekstraksiyonu İle Trans-Resveratrol Analizi

Özet: 3,5,4-trans-trihidroksistilben (trans-resveratrol)'in CH₂Cl₂ ve CHCl₃ içindeki çözünürlüğü oldukça fazladır. Trans-resveratrolü tam ekstrakte etmek için, şarap örnekleri CHCl₃ içinde çalkalandı. Elde edilen CHCl₃ çözeltisi HPLC sistemine direk olarak enjekte edildi. Analiz, %60 MeCN: %40 EtOH içinde ve trans-resveratrol standart eklenmesiyle gerçekleştirildi. Kromatogramdaki rezolüsyon yaklaşık 1.0 olarak hesaplandı. Türkiye de üretilmiş olan 9 şarap örneğinde resveratrol konsantrasyonu 1.46-6.53 ppm olarak bulundu. Resveratrolün ekstraksiyon oranı, ardışık ekstraksiyon ile tespit edildi ve örnek miktarı artırılarak, metodun doğruluğu test edildi. Geri kazanım % 100.42 olarak bulundu.

Anahtar kelimeler: Resveratrol tayini, şarap, HPLC, sıvı-sıvı ekstraksiyonu.

1. Introduction

Trans-resveratrol is an anti oxidant material which validates the French Paradox as well. It's positive effects on heart diseases and anti tumor properties are well known in the time being [1-3]. For these reasons, it is probably the most important phenolic ingredient of wine. A number of studies have been reported on analysis of resveratrol in wine in the late years [4-21]. All analyses in the fore mentioned studies are based on chromatographic methods. In some of them, pre concentrations were conducted by solid phase extractions and the determinations were made possible by gradient programming in HPLC [5-10]. Some other studies were conducted using LCMS or GCMS working at high temperatures between 250-300 °C [13, 15, 18, 19]. In all of the HPLC employing studies, an acidic buffer was used in the gradient programming process. A typically in this study, with the help of an isocratic mobile phase, analyses were done without employing a buffer. By utilizing the fact that trans-resveratrol is finely soluble in

CH₂Cl₂ or CHCl₃, wine samples were shaken in particular volumes of CHCl₃ and liquid-liquid extractions were done. The only disadvantage of this proposed method is that at this point, shaking process is time consuming taking at least 2.5 hours for completion.

2. Experimental

In this study, different wine samples from varying regions in Turkey were taken for analysis. 50.0 mL samples of these wines were mixed with 10.0 mL of CHCl₃ and the mixtures were constantly stirred for 150 minutes. The aqueous phases were extracted with the help of an extraction funnel and the remaining CHCl₃ solutions were directly injected to 85% MeCN – 15% EtOH mobile phase. In the study, a system consisting of a Shimadzu LC-20AT pump, a Shimadzu SPD-20A UV detector and a Inertsil ODS-3 (250 × 4.6 mm, 5 μm) column, Shimadzu CTO-20A oven was employed.

Analysis conditions are given as: 0.25 mL/min flow rate, 40 °C column temperature, 45-68 bar column entry pressure and λ= 310 nm detection wavelength.

After the chromatograms of the phenols in CHCl₃ were obtained, standard additions from 50.0 ppm stock solution, accounting for 1.0, 2.0, 3.0, 4.0 and 5.0 ppm rise in resveratrol concentrations, were done to inject the final solutions to the system again. Calibration curves were obtained from the increases in the peak areas of trans-resveratrol which were observed at minutes 11.1-11.2. The X-axis intercepts were taken into account for resveratrol amount in the wine samples.

Later, since there was not any standard reference material available at the time being, 100.0 – 150.0 mL samples of the same wines were again mixed and shaken with 10.0 mL CHCl₃ and the same analysis procedure was followed in order to understand the accuracy of the method.

The resolution of the trans-resveratrol peaks in the chromatograms and the limit of detection were defined with the help of the literature knowledge. In order to define the LOD, one of the 50.0 mL samples that is extracted three times was taken and standard resveratrol solution was added in account for 1.0, 2.0, 3.0, 4.0, 5.0 ppm concentrations. In this analysis, the finding, where the relative standard deviation is about 10%, was accepted to be the limit of detection [22]. This value is found to be around 0.0835 ppm.

3. Results and Discussion

The chromatogram obtained from one of the wine samples, chromatograms for the respective standard additions and the calibration curve obtained from these results were given in Fig. 1a-e.

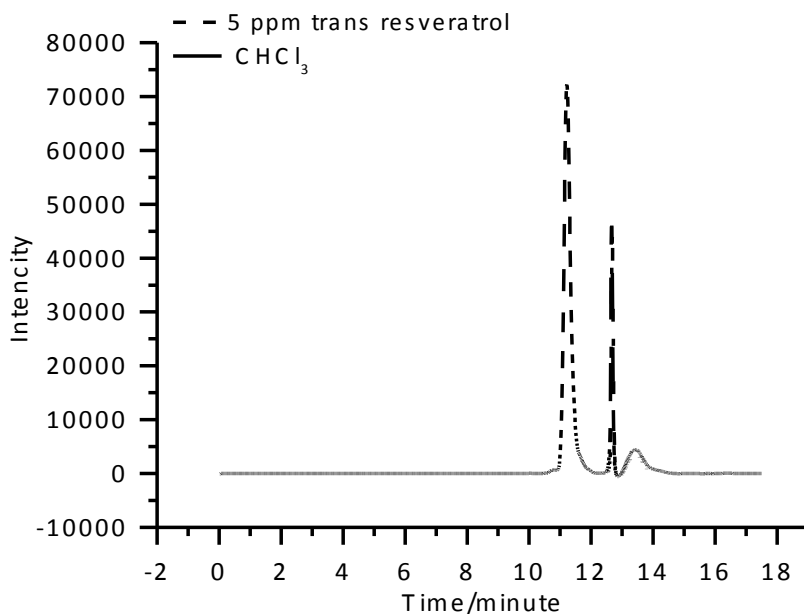


Figure 1a. The chromatogram of standard 5 ppm trans-resveratrol in CHCl_3 (Sigma-Aldrich)
 ————— CHCl_3 (solvent) - - - - - 5 ppm standart in CHCl_3

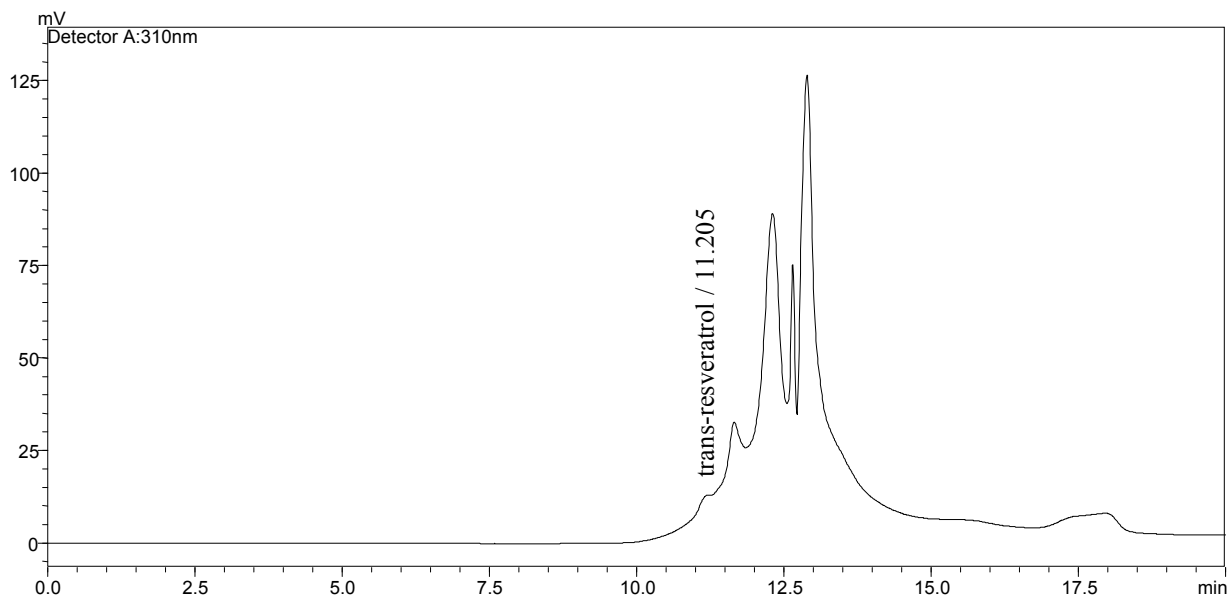


Figure 1b. The chromatogram of Doluca Kalecik Karası 2008 extract in CHCl_3 .
 Trans-resveratrol seems as the shape of a shoulder at 11.205 min

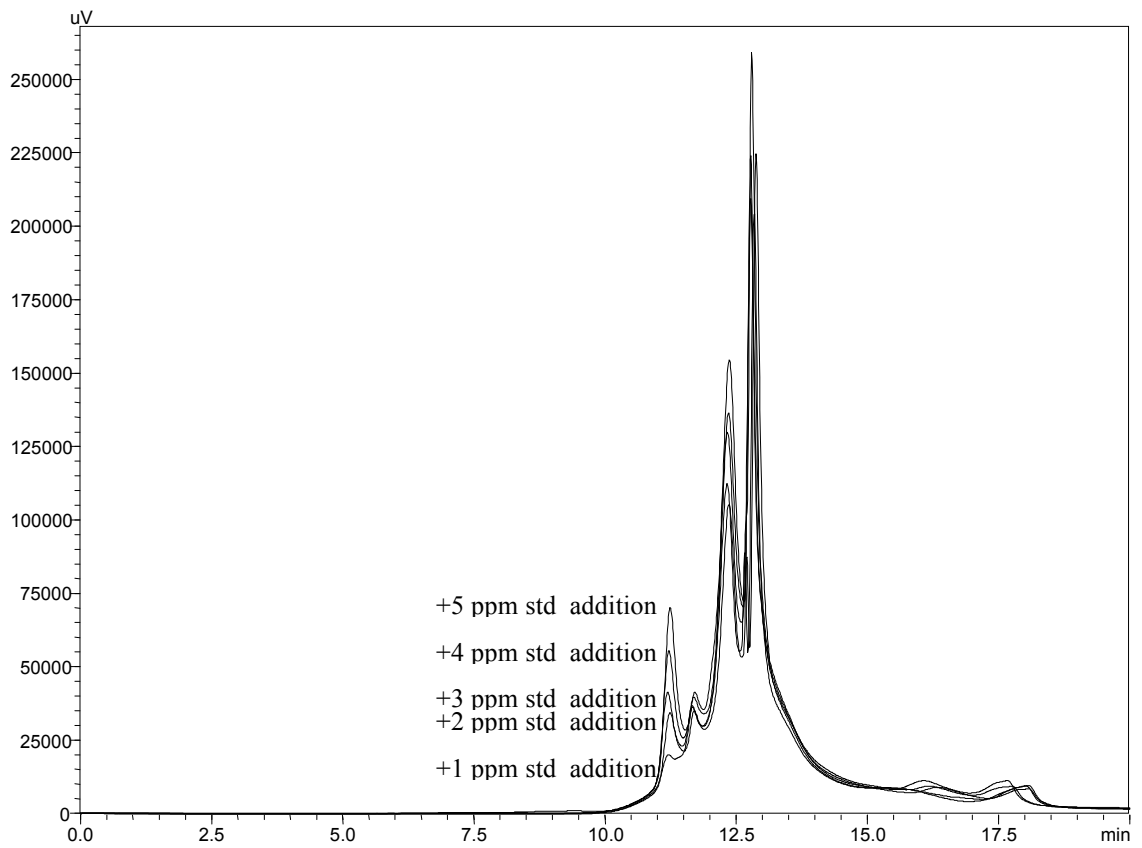


Figure 1c. The chromatograms of standart additions on the Doluca Kalecik Karası extract in CHCl_3

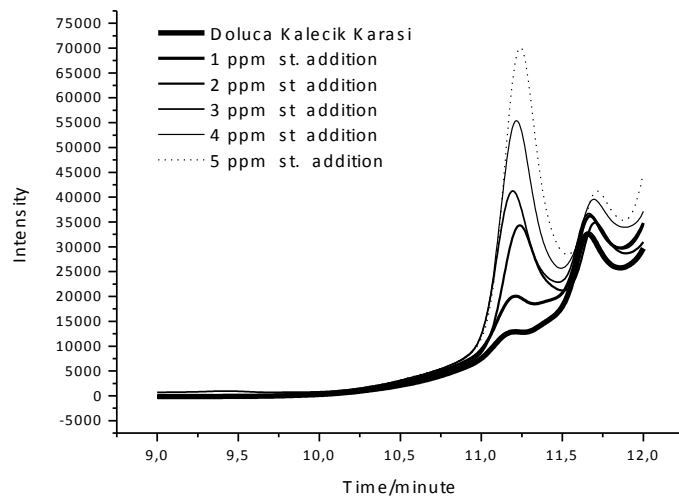


Figure 1d. Chromatogram of standart additions on Doluca Kalecik Karası extract zoomed in around the shoulder area of trans-resveratrol.

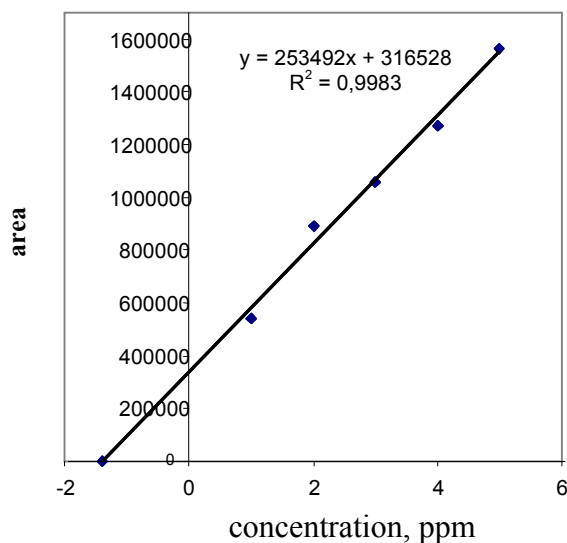


Figure 1e. The calibration curve of standart additions on Doluca Kalecik Karası extract in CHCl_3 .

As it can be seen in the chromatograms, the first observed trans-resveratrol peak (between $t_R = 11.1-11.3$ minutes) is hardly resolved from the peaks of the other phenolics. The R value is about 1.01 ± 0.03 . The peak observed at 12.6 minutes is of CHCl_3 .

The chromatograms obtained from a wine sample that is consecutively extracted three times and from the same wine's 100 mL sample is given in Fig. 2.

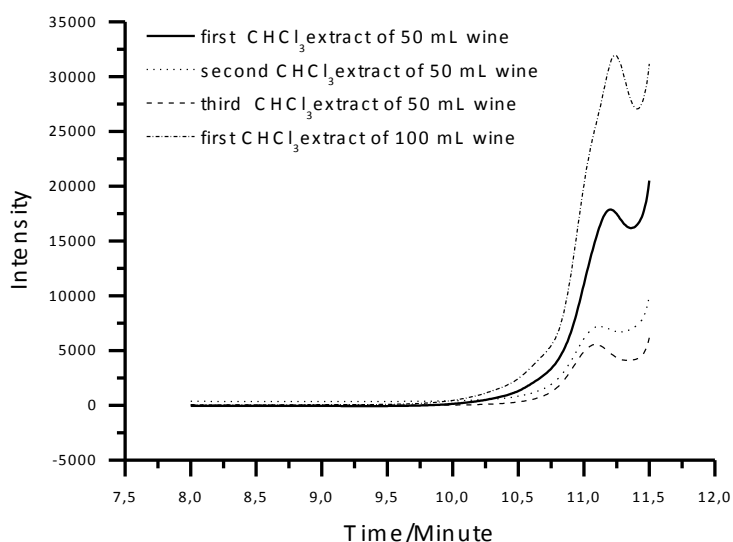


Figure 2. The chromatograms of first, second, third CHCl_3 extracts of 50 mL Kavaklıdere Sade 2007 and CHCl_3 extract of 100 mL same wine, zoomed in around shoulder

For optimum separation, different compositions of MeOH, CHCl_3 , MeCN and EtOH were tried out. However, a mobile phase composition that resolves trans-resveratrol

completely ($R \geq 1.5$) was unable to be found. The most appropriate mobile phase was then defined as 85% MeCN: 15% EtOH.

Despite the relatively poor resolution, the linearity of the calibration curve shown in Fig. 1e. indicates that such an analysis is credible.

Fig. 2. reveals that most of the trans-resveratrol in wine is extracted in CHCl_3 at the first extraction. The second extraction contributes just a little, the respective peaks of the third and the fourth extractions are obviously transformed into a plateau. It is probable that one or more other phenolic species form a matrix that affects the analysis significantly. In order to deal with this matrix effect, standard additions were made. We were unable to find a wine sample resveratrol amount of which is known and noted. Therefore, wine volumes were doubled and tripled for two other sets of samples while keeping CHCl_3 amount the same to test the method for accuracy. If the method was accurate, these analyses would yield results consistent with the first ones, twice and three times the first results. Fortunately, the outcome was in this manner indeed. A comparison can be done between the figures given in Fig. 2. In 50 mL Kavaklıdere Sade Öküzgözü 2007 wine sample, 2.13 ppm resveratrol amount was recorded and for 100 mL sample of the same wine, 4.28 ppm resveratrol reading was the outcome. In the same manner, for all analysed wine samples, an average recovery of $100.42\% \pm 1.61$ was calculated. In Table 1, the resveratrol amounts and % average recovery values of 9 wines of interest are given. The recovery values are in the range 103.52 ± 6.52 and 98.28 ± 1.133 which was considered acceptable. These findings are also comparable with the data in the literature.

In conclusion, though there are many studies about trans and cis resveratrol analysis in the literature; almost all of the proposed methods require a gradient solvent programming. There has not been any simple isocratic separation reported. Besides, any liquid-liquid extraction based method is absent. This study proposes a new and simple liquid-liquid extraction method that is directly employable for every wine sample. Moreover, this method does not require a solvent gradient, makes it possible to do analysis with just one mobile phase. The only disadvantages of the method are that the shaking process is quite time consuming and the limit of detection is a little high. Probably, the analyte is unable to fully separate by extraction from the other phenolic species absorbing at the same frequency. For this reason, using peak heights or areas directly may as well yield incorrect results. At this point, standard addition is found to be useful in order to get rid of the interferences. However and unfortunately, this application makes sure that the absorbance value is never around zero in between the retention time of trans-resveratrol resulting in a high limit of detection.

A question comes in mind at this point; the composition of the phenolic ingredients in wine differs greatly in various samples; do not the amounts of extracted phenols effect the analysis or do they have the same effect on trans-resveratrol readings? If standard addition method was employed, these concerns should be well satisfied, of course, in exchange for the sacrifice of a better limit of detection value.

Table 1. The results of trans-resveratrol of 9 Turkish wines using standard addition method and their % average recovery

Wine Sample	t-resveratrol concentration, ppm	Recovery %
Kalecik Karası 2008	1.46 ± 0.05	103.52 ± 6.07
Alicante İzmir 2008	2.08 ± 0.07	101.15 ± 4.26
Cinsault Trakya 2008	6.53 ± 0.40	98.69 ± 2.32
Öküzgözü Malatya 2008	2.56 ± 0.12	98.28 ± 1.33
Öküzgözü Denizli 2008	2.52 ± 0.25	100.47 ± 1.67
Öküzgözü Sade 2007	2.13 ± 0.06	99.96 ± 3.88
Boğazkere Denizli 2008	1.68 ± 0.12	99.69 ± 4.28
Shiraz Denizli	3.68 ± 0.52	100.17 ± 3.67
Cabernet Sauvignon Denizli 2008	2.85 ± 0.17	101.89 ± 2.94

4. Conclusion

This paper suggests a simple liquid-liquid extraction based method for the determination of resveratrol in wine samples. The resveratrol amounts in Turkish wine samples were successfully determined by standard addition using an isocratic mobile phase and without the need of any gradient programming or buffer. Although the resolution was not high, the recovery values suggest that the method is applicable on purpose.

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