

DETERMINATION OF SYNTHETIC COLORANTS AND NATURAL CARMINE IN WINES

DÉTERMINATION DES COLORANTS SYNTHÉTIQUES ET DU CARMIN NATUREL DANS LES VINS

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Summary : Methods were developed for the determination of synthetic colorants and natural carmine (E120) in wines. The synthetic colorants studied were tartrazine (E102), quinoline yellow (E104), sunset yellow (E110), azo ruby (E122), amaranth (E123), ponceau 4R (E124), and erythrosine (E127). The colorants were extracted using solid-phase extraction (SPE) with NH₂ cartridges. The extracted colorants were then analysed by high-performance liquid chromatography (HPLC). The compounds were identified by comparing the spectrum of extract with spectra in a UV-vis spectral library of known food colorants. The detection limits in red wine ranged from 0.1 mg/l to 0.2 mg/l. Diode array detection and library search makes the method very effective in identifying forbidden colorants in wines. SPE is sufficiently effective in separating and concentrating colorants from wine. The effect of added colorants, e.g. azo ruby (E122), amaranth (E123) and tartrazine (E102) on the UV-vis spectra of red and white wines were studied. The added colorants could be identified in this way but not as accurately as by the HPLC method.

Keywords : synthetic colorant, azo colorant, natural carmine, wine, UV-vis spectrum

Mots clés : colorant synthétique, azo colorant, carmin naturel, vin, spectre de UV-vis

INTRODUCTION

Colour is one of the most important indicators of wine quality. The natural color of red wines ranges from purple-red to tile-red during ageing. The color is caused by polyphenols which originate from the skin of the grape (SOMERS and VÉRETTE, 1988). White wines may have a trace of green color but usually range from nearly colourless to yellow, gold or amber. It has been suggested that the yellow color of white wines is partly formed during pressing when the must is in contact with air (SCHUSTER, 1989). White wines, however, should be as light as possible because yellow color may indicate oxidation (SINGLETON and ESAU, 1969). Although the addition of any kind of colorant to wine is forbidden, colorants have been added to wines to mask defects or to imitate better products. Synthetic colorants, especially azo colorants, have been popular for decades because of their stability, water-solubility and low price.

Many kind of methods have been reported for separating and identifying forbidden colorants in wines. Chromatographic methods are the most common, and in earlier studies thin-layer chromatography (TLC) was often used (BETZ, 1981; ANDERTON *et al.*, 1996 ;

DAMBERGER *et al.*, 1997). DUGAR *et al.* (1994), using TLC with solid-phase extraction (SPE), were able to determine synthetic colorants at concentrations as low as 0.5 mg/l. Recently, FLAK *et al.* (1998) reported that HPLC and differential pulse voltametry (DPV) were both more sensitive than previous methods. Spectrophotometric techniques have also been used to determine forbidden colorants in wines (LAURETTA, 1994).

The aim of this study was to develop rapid and practical methods of identifying forbidden colorants in wines. For this purpose, SPE and HPLC with spectral libraries were employed. We also investigated the possibility of identifying added colorants simply by recording the UV-vis spectra of wines.

MATERIALS AND METHODS

Wine samples: 17 red wines from Spain, France, Chile, Bulgaria, Rumania, Macedonia and Argentina and four white wines from Spain, Israel, Italy and South Africa.

Colorants: tartrazine (E102), quinoline yellow (E104), sunset yellow (E110), azo ruby (E122), ama-

ranth (E123), ponceau 4R (E124), erythrosine (E127), and natural carmine (E120).

Reagents: 1.5 M tetrabutyl-ammoniumhydroxide (TBA), 0.02 M tetrabutyl-ammoniumhydroxide phosphate (TBAP) (adjusted to pH 6.5 with 10 p. cent H_3PO_4), HPLC eluent for synthetic colorants: solvent A: 40 p. cent methanol, solvent B: 70 p. cent methanol; both contain 25 p. cent 0.02 M TBAP; for natural carmine: solvent A: HPLC water (adjusted to pH 3 with 10p. cent H_3PO_4), solvent B: methanol.

Instruments: Hewlett Packard HP 1090 liquid chromatograph; column: LiChrospher 100 RP-18 e (5 μ m) (Merck, Darmstadt, Germany); detector: HP 1040 diode array detector; SPE tube: VAC-Elut (Analytichem International), Bond Elut NH_2 cartridge (size: 1 CC, 100 mg).

Methods: Samples were prepared by the method of DUGAR *et al.* (1994) with the following changes: the amount of wine sample was 15 ml, and in the SPE method 3 ml ethanol and 3 ml distilled water were used in the wash phase and 1.4 ml ethanol: H_2SO_4 (1:1) in the elution phase. Recovery tests were performed by adding colorants to a red wine sample. The concentrations of colorants in red wine were 0.5, 2.5, 10 and 25 mg/l. The samples underwent SPE treatment followed by HPLC analysis. The HPLC gradient for synthetic colorants was 0 min A 100 p. cent, 10 min A 0 p. cent, 14 min A 100 p. cent. The HPLC gradient for natural carmine was 0 min A 80 p. cent, 20 min A 50 p. cent, 23 min A 30 p. cent, 25 min A 30 p. cent. The injection volume was 15 μ l, flow rate 0.8 ml/min and oven temperature 40°C. A UV-vis spectral library was compiled by recording the UV-vis spectra of known food colorants in the UV-vis region 250-600 nm. The HPLC method was validated by measuring linearity, repeatability, limit of detection ($S/N=3$), limit of quantitation ($S/N=10$) and accuracy for amaranth, azo ruby, ponceau 4R and carmine. All analyses were done in duplicate.

The limit of detection by the spectrophotometric method was determined by recording spectra by injecting 10 μ l of wine sample into the HPLC apparatus operated in the columnless mode at a flow rate of 0.5 ml/min with water as eluent.

RESULTS AND DISCUSSION

The SPE-HPLC method yielded the following detection limits and determination limits for amaranth, azo ruby, ponceau 4R and carmine in red wines: 0.2/0.7 mg/l, 0.2/0.6 mg/l, 0.2/0.6 mg/l and 0.1/0.4 mg/l, respectively. Erythrosine was observed to precipitate when added into wine and therefore this compound was not studied any further.

Detection limits were also determined for the UV-vis spectrum method by adding azo ruby, amaranth and tartrazine to wines and recording the spectra by columnless HPLC. The UV-vis spectrum of old and young red wines containing amaranth at 10 mg/l differed clearly from that of authentic corresponding red wines. With ageing or oxidation, the absorbances of red wines decrease at 520 nm and increase at 420 nm (SOMERS and VÉRETTE, 1988). The UV-vis spectrum of white wine containing tartrazine at 2.8 mg/l differed little from that of authentic white wine. The spectrophoto-

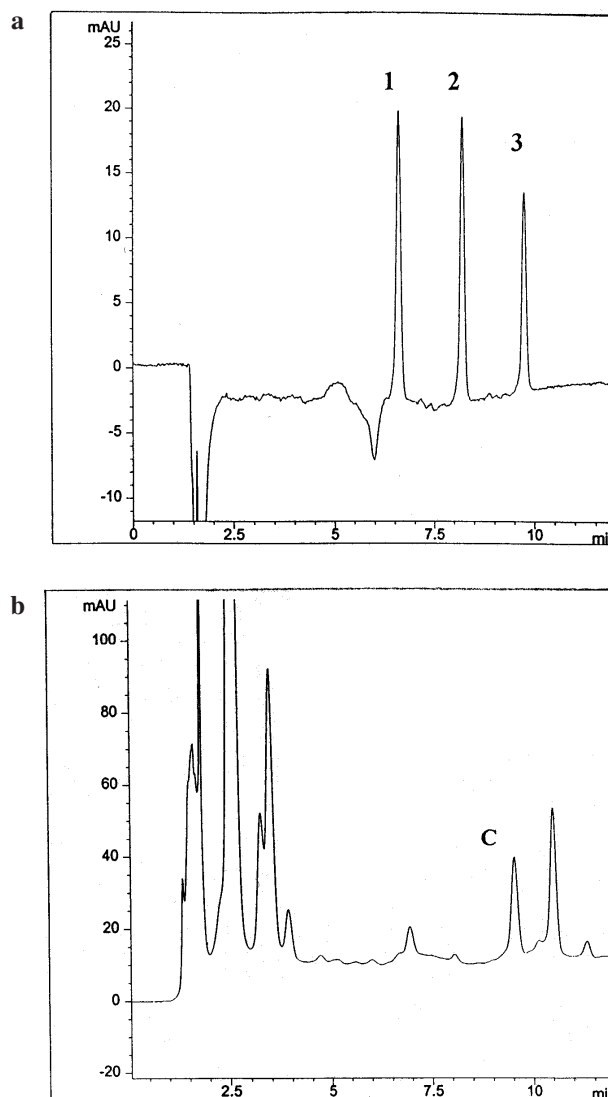


Fig. 1 - a : Amaranth (1), azo ruby (2) and ponceau 4R (3) added to red wine at concentrations of 0.5 mg/l. **b:** Carmine (c) added to red wine at a concentration of 2.5 mg/l. For chromatographic conditions see the Materials and methods section.

Fig. 1 - a - a : Amaranthe (1), azorubine (2) et ponceau 4R (3) ajoutés au vin rouge à une concentration de 0,5 mg/l chacun. **b :** Carmin (c) ajouté au vin rouge à une concentration de 2,5 mg/l.

TABLE I
Retention time and recovery of colorants

	Retention time (min)	Recovery (%)*	
		at 0,5 mg/l	at 2,5 mg/l
Amaranth	6.60	91	81
Azo ruby	8.21	78	89
Ponceau 4R	9.76	131	112
Carmine	9.54	64	64
Tartrazine	6.10	nm**	75
Quinoline yellow	7.62	nm**	45
Sunset yellow	6.46	nm**	41

*mean of two independent measurements ; **nm = not measured

metric method was not as accurate as the HPLC method. According to LAURETTA (1994), the method is suitable for qualitative analysis whereas quantitative analysis by HPLC is very accurate and sensitive.

The accuracy of the methods was studied by adding amaranth, azo ruby and ponceau 4R to red wines in different concentrations. The retention times and recoveries are given in table I. Even at the lowest concentration, 0.5 mg/l, peaks were clearly observable (figure 1a). The determination of carmine was not as simple because disturbing compounds in red wine overlapped the peak of carmine to some extent. Therefore, carmine was analysed using a different gradient and eluents (see Materials and methods). In red wine, a carmine concentration of 0.5 mg/l produced a discernible peak. A chromatogram of a recovery experiment of carmine at a concentration of 2.5 mg/l is given in figure 1b.

In the actual wine analyses, the following wavelengths were used: amaranth 530 nm, azo ruby 530 nm, ponceau 4R 530 nm, tartrazine 430 nm, quinoline yellow 410 nm, and carmine 280 nm. The analyses did not reveal any illegal colorants.

In conclusion, the methods presented are of sufficient sensitivity and accuracy to allow qualitative and semiquantitative determination of forbidden colorants in wines.

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