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Analysis of chiral carboxylic acids in wine by high-performance liquid chromatography with coupled UV and circular dichroism detection

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Abstract

A circular dichroism (CD) spectrophotometer, equipped with a flow cell, and a UV detector were connected in series and were used for coupled UV–CD detection of optically active carboxylic acids. The limits of detection and the linear range for the enantiomers of tartaric, malic, lactic and ascorbic acids are reported. Several wines were analysed and CD detection proved to be sensitive enough for accurate determinations. The combined UV–CD detection was helpful for identifying the peaks and allowed the calculation of enantiomeric ratios.

1. Introduction

The study of specific detectors for HPLC and ion chromatography (IC) is currently attracting a great deal of attention [1]. HPLC detectors based on optical activity are potentially advantageous because of their inherent selectivity; in fact, compounds that are not chiral (*e.g.*, solvents, buffers, impurities) will not interfere with analysis even if they co-elute with the analytes. Compared with polarimetric detection, circular dichroism (CD) detection is more sensitive and gives more stable baselines, as it is intrinsically insensitive to refractive index fluctuations [2], but it is necessary to operate in the linear range of the response [3].

Most of the applications developed to date have involved laboratory preparations; the number of real samples investigated is comparatively very small. UV and CD detectors in series have been employed for the determination of the enantiomeric excess of nicotine in leaf extracts [4]; the use of LC with combined UV and either polarimetric or CD detection allowed the identification of enantiomers of the pyrethroid insecticides [5]; the enantiomeric purity of scopolamine isolated from plant extract was determined using achiral–chiral coupled column chromatography with CD confirmation of the individual peaks [6]; a micro-flow cell device was adapted to a CD spectrometer for HPLC separation and structural analysis of proteins [7]; continuous acquisition of circular dichroism spectra during liquid chromatography was successfully performed and it was applied to the resolution of a racemic mixture of 2,2′-spirobi[2*H*-chromene] [8]; and racemic mixtures of alkylarylcarbinols were resolved and the absolute configurations of the fractions eluted were determined by means of CD detection [9].

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Analyses of the organic acid content of wines are routinely conducted; tartaric, malic, lactic and ascorbic acids are among the most important constituents of wines. Different stereoisomers of those acids can be present at a time; in wines, L-forms usually predominate over the D-forms (the concentration of the *meso* form of tartrate is normally negligible in comparison with those of its two optically active forms). D-Tartrate can originate from racemization, or may be added to achieve precipitation of the racemic calcium salt in order to avoid unwanted turbidity. D-Lactate derives from alcoholic fermentation of sugars, whereas its L-enantiomer comes from the conversion of the L-malate (malolactic fermentation), a bacterial process that is very important for the quality of wine; usually, the amount of the D-form is about one fifth of the total lactate [10].

In this paper, we demonstrate that an HPLC–UV–CD system is applicable to the analysis of wines for chiral organic acids, and that this system is also convenient for measuring their enantiomeric excess (e.e.) with adequate precision.

2. Experimental

2.1. Reagents

All aqueous solutions were prepared with ultra-high-quality (UHQ) water produced by an Elga-Stat water-purification apparatus (Elga, High Wycombe, UK). D-(−)-Lithium lactate (97%), L-(+)-lactic acid (>98%), D-(−)- and L-(+)-tartaric acid (>99%), L-(−)- and D-(+)-malic acid (>99%) and L-(+)-ascorbic acid (99.7%), together with C_{18} solid-phase extraction cartridges, were obtained from Merck (Bracco, Milan, Italy). Acetonitrile (HPLC grade) was obtained from Lab Scan (Delchimica, Naples, Italy). The solutions of the investigated substances were prepared with UHQ water immediately before use.

2.2. Apparatus

The HPLC system consisted of a Pye Unicam (Cambridge, UK) PU 4015 pump, a Rheodyne (Cotati, CA, USA) valve fitted with a 20-μl loop and a 250 × 4.6 mm I.D. Adsorbosphere C_{18} 5-μm column (Alltech, Deerfield, IL, USA). The eluent was degassed by means of a stream of helium. The UV detector was obtained from Perkin-Elmer (Norwalk, CT, USA).

CD detection was performed by means of a Jasco (Tokyo, Japan) J-600 computerized spectropolarimeter, which was equipped with a cylindrical, laboratory-made flow cell of 8 mm I.D. and 0.7 mm optical path length. The cell was constructed with two 10 × 10 mm quartz windows, spaced by a PTFE ring and mounted on a PTFE bearing. No light condenser [3] was employed, in order to avoid reduction of the light-flux energy caused by the system of lenses. The efficiency of the light-flux energy was estimated by comparing the high-tension voltage of the photomultiplier detector, measured with an ordinary 1-mm cell, with that measured with the flow cell; the measurements were corrected for the differences in light paths; both cells were filled with the chromatographic eluent. No decrease in the efficiency of the light-flux energy or an increase in the noise was observed.

2.3. Chromatographic conditions

An isocratic eluent, consisting of 0.1 *M* sodium dihydrogenphosphate adjusted to pH 2.5 by addition of 85% phosphoric acid solution, was used at a flow-rate of 1 ml min^{-1}. The column temperature was 23 ± 1°C.

A 4-s time constant and a 2-s step resolution (corresponding to a 4000-s maximum duration of the chromatographic run) were chosen for the CD spectrophotometer, in order to minimize the noise without sacrificing the definition of the chromatographic peaks.

After being collected, the CD chromatograms were smoothed; quantification of the analytes was performed on the basis of peak height.

2.4. Sample clean-up

A pretreatment of the wine samples was performed in order to decrease the content of

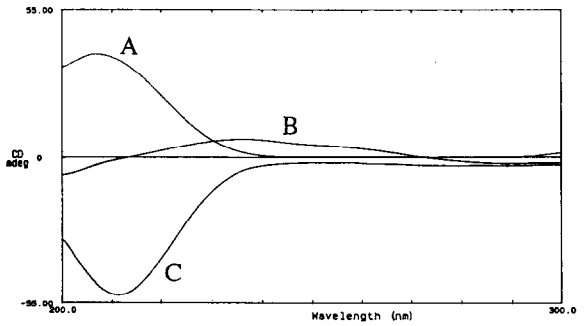

Fig. 1. CD spectra of pure enantiomers of chiral acids. Peaks: A = L-(−)-malic; B = L-(+)-ascorbic; C = L-(+)-tartaric acid. mdeg = 10^{-3} degrees.

non-polar substances potentially dangerous for the chromatographic stationary phase. The pretreatment consisted in a single elution of 1 ml of wine on a 400-mg C_{18} SPE cartridge. Preliminary tests were conducted on standard solutions of the investigated acids, the pH of which was adjusted at 3.5; they showed no evident interference of the pretreatment with the concentrations of the investigated analytes, as the recovery was 100 ± 4% ($n = 3$).

3. Discussion

Some typical CD spectra of the investigated acids are reported in Fig. 1. Tartaric, malic and lactic acid show their CD and UV maxima around 210 nm. Ascorbic acid has its CD maximum around 235 nm, whereas it shows its largest UV absorptivity at 260 nm. Both CD and UV detection of tartaric, malic and lactic acid were performed at 210 nm, whereas CD and UV detection of ascorbic acid were conducted at 235 and 210 nm, respectively. The CD spectrum of D-(+)-glucose was also examined; it was observed that no interference on the CD chromatograms of the acids could have derived from glucose, as it exhibits its CD activity at wavelengths shorter than 200 nm.

Fig. 2 shows the UV and CD chromatograms of a mixture of the L-enantiomers of tartaric, malic and lactic acid. As can be seen, there is no direct relationship between the sign of the optical rotatory power of the enantiomers and the sign of their CD peaks.

A linear correlation between CD signal and concentration was observed in the range 5–100 mM. The parameters of the calibration equa-

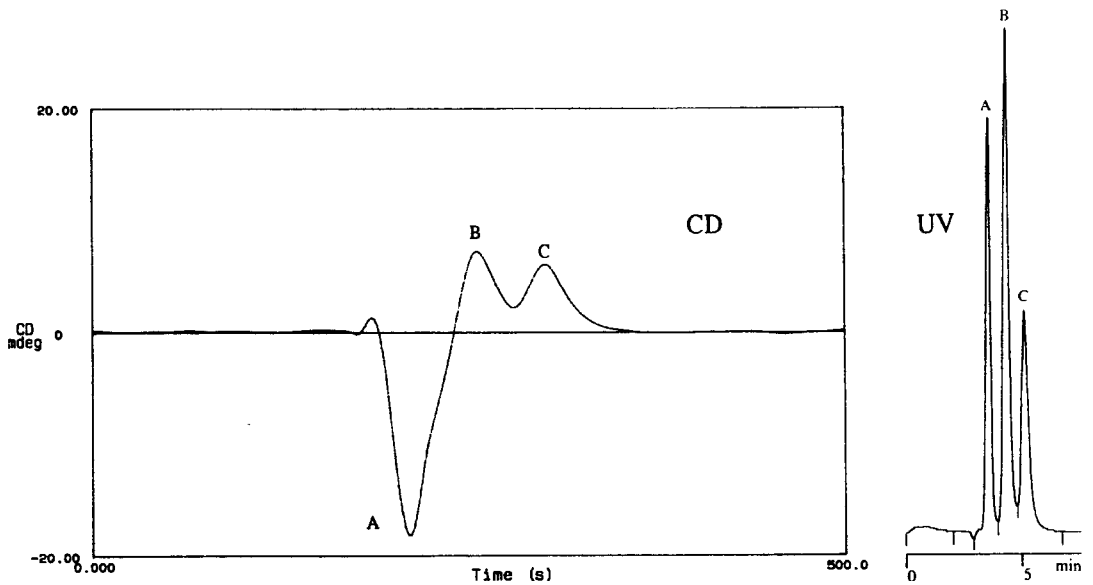

Fig. 2. CD and UV chromatograms of a mixture of three acids. Peaks: A = L-(+)-tartaric; B = L-(−)-malic; C = L-(+)-lactic acid. mdeg = 10^{-3} degrees.

Table 1
CD calibration equations and detection limits

Substance	*A*	*B*	*C*	*D*	*E*
Ascorbic acid	0.3 ± 0.2	87 ± 4	0.3	5	2.5
Tartaric acid	0.6 ± 0.4	700 ± 42	0.6	4	0.4
Malic acid	0.2 ± 0.2	137 ± 9	0.2	4	2
Lactic acid	0.2 ± 0.2	129 ± 5	0.1	4	2

Calibration equation: $y = A + Bx$; A = intercept ± S.D. [mdeg (10^{-3} degrees)]; B = slope ± S.D. (mdeg l mol^{-1}); x = concentration (mmol l^{-1}); C = standard error ($n = 3$); D = number of data points; E = detection limits (mmol l^{-1}) (signal-to-noise ratio = 2, injection volume = 20 μl).

tions, together with the observed detection limits, are reported in Table 1.

As an example of the results obtained, CD and UV chromatograms of an Italian red wine (Lambrusco from Emilia, "amabile" variety) are reported in Fig. 3. Only three of the UV-absorbing compounds were also detected by CD, thus helping in the validation of the peaks; ascorbic acid gave no CD signal, as detection was conducted at 210 nm. The concentrations of the L-enantiomers of tartaric, malic and lactic acid exceeded those of the remaining stereoisomers.

Table 2 reports the results obtained for different wines. The uncertainties in the concentrations of the L-enantiomers (or e.e., for tartaric acid), as given in Table 2, were calculated by considering the uncertainties in the coefficients of the CD calibration equations and those in the concentrations obtained by UV detection. The values of the experimental uncertainties, coupled with the enantiomer concentrations or e.e. in Table 2, allow a significant comparison of different wines. One of the samples was analysed twice, 9 and 11 months after the vintage. In this wine, the persistence of a relevant concentration of malic acid indicated that malolactic fermentation did not occur completely; the concentration of the D-(−)-lactate, due to the alcoholic fermentation, increased with time. Malic acid was not found in the remaining samples, whereas lactic acid was found in all the analysed wines except one.

4. Conclusions

Combined UV and CD detection was applied to analyses for chiral organic acids in wines. For this purpose, a CD spectrophotometer was equipped with a suitable flow cell. The CD

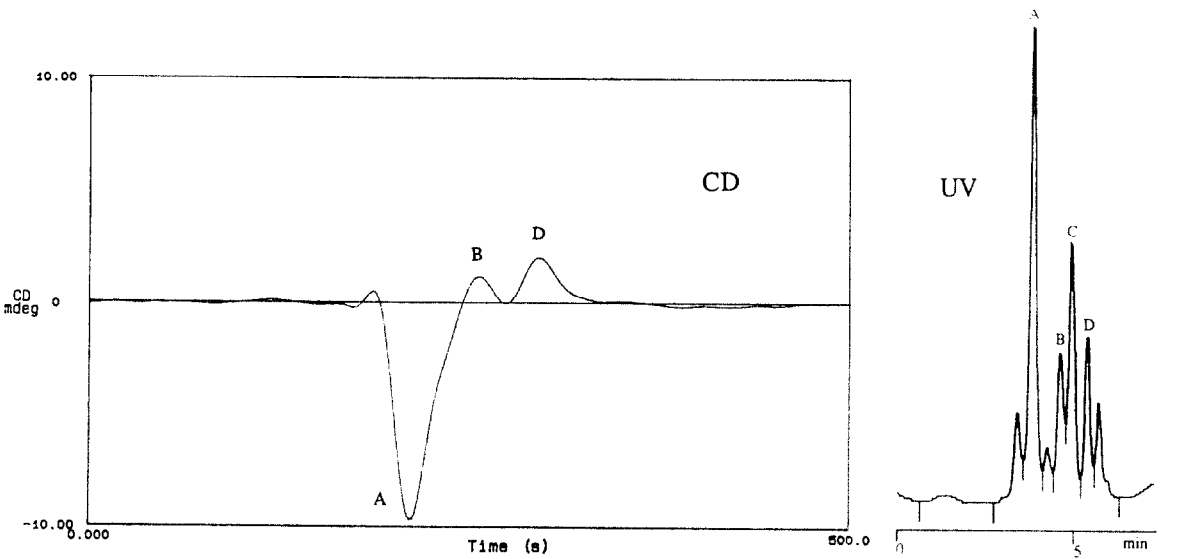

Fig. 3. CD and UV chromatograms of Lambrusco "amabile" wine. A = L-(+)-tartaric; B = L-(−)-malic; C = L-(+)-ascorbic; D = L-(+)-lactic acid. mdeg = 10^{-3} degrees.

Table 2
Analyses of wines

Wine and year	*A*	*B*	*C*	*D*	*E*	*F*	*G*
Lambrusco "amabile", 1992[a]	18.2 ± 0.7	7.5 ± 0.6	14.9 ± 0.6	10 ± 2	35 ± 1	30 ± 5	0.5 ± 0.05
Lambrusco "amabile", 1992[b]	17.4 ± 0.7	12.6 ± 1	17.6 ± 0.9	14 ± 3	46 ± 2	30 ± 4	0.55 ± 0.05
Moscato d'Asti, 1992	16.4 ± 0.7	6.8 ± 0.6	–	–	–	–	–
Barbera d'Alba, 1992	19.7 ± 0.8	10.8 ± 0.9	–	–	55 ± 2	37 ± 5	0.75 ± 0.08
Lambrusco "secco", 1992	22 ± 0.9	16 ± 1.3	–	–	58 ± 2	42 ± 6	1.8 ± 0.1
Limnio, 1989	14.3 ± 0.6	7.1 ± 0.7	–	–	37 ± 2	22 ± 3	2.1 ± 0.1
Côtes du Rhône, 1992	9.3 ± 0.4	3 ± 0.6	–	–	38 ± 2	22 ± 3	2.3 ± 0.1

All concentrations are expressed as mmol l^{-1} ± experimental uncertainty ($n = 3$). *A* = tartaric acid, total; *B* = L-(+)-tartaric, enantiomer excess; *C* = malic acid, total; *D* = L-(−)-malic acid; *E* = lactic acid, total; *F* = L-(+)-lactic; *G* = ascorbic acid, total. Note: the enantiomer concentration of ascorbic acid is not given, as its CD signal was lower than the detection limit.
[a] Analysed 9 months after vintage.
[b] Analysed 11 months after vintage.

detector was helpful in validating the UV peaks. It proved to be sensitive enough to allow the determination of the principal chiral organic acids contained in wine. The comparison of UV and CD quantitative results allowed the calculation of the enantiomer concentrations, or e.e., of tartaric, malic and lactic acid with experimental uncertainties that allowed a significant comparison of different wines.

5. References

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