Deconvolution of Composite Chromatographic Peaks by Simultaneous Dual Detections

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Abstract

Composite chromatographic peaks are deconvoluted by a method that uses ratio formation from signals of simultaneous double detection. The method is generally suitable if two detector signals can simultaneously be acquired and their uses do not need any a priori assumption or mathematical shape analysis. A simple deduction makes the compound- and detector-specific intensive parameters explicit, which allows for the digital construction of directly invisible component peaks. The simultaneous double detection is shown to be superior to coupled detectors, sequentially fixed chromatograms, and subsequently synchronized peaks. The combination of circular dichroism and ultraviolet (UV) detection is shown to be especially advantageous in the analysis of enantiomers for which the other ratio-forming peak-deconvolution techniques have inherently been insensitive. The double chiroptical UV detection can be of further use to decompose overlapping peaks of nonenantiomeric compounds that are highly similar. The capacity of the method is exemplified by the analysis of morphine alkaloids, steroid oximes, and synthetic heterocycles.

Introduction

Peak overlapping is a common problem in every separation technique. In cases of symmetrical peaks, the extent of overlapping can usually be easily estimated. However, in cases of asymmetrical peaks, the real shape of the component peaks needs to be independently estimated to assess the extent of the overlapping and determine the related component concentrations.

In regards to the deconvolution of composite peaks in multicomponent systems, several methods have been reported and reviewed. Felinger surveyed both mathematical methods (1) and ratio-forming treatments (2), which are the antecedents to this work. Sharaf (3) has sorted the peak homogeneity tests into two classes.

The first class contains methods that assess the peak purity on the basis of monochannel signals from exponentially modified Gaussian (EMG) functions (4–5). Hanggi et al. (6) pointed out the erroneous uses of this method. Berthod (7) applied various functions to simulate chromatograms. Besides EMG, polynomial-modified Gaussian functions were also used to describe, simulate, and decompose chromatograms (8). Further improvements in the shape analysis of chromatograms were achieved by using Fourier-transformation (9) and Kalman filter methods (10). Shao et al. (11) developed an immune algorithm for the resolution of multicomponent overlapping chromatograms.

The second class of methods processes signals from di- or multichannel detectors for the purpose of obtaining information on peak purity by spectrum correlation (12) and the ratio formation of spectra (13) or chromatograms (14–15). The chromatographic ratio formation is most feasible by the use of diode-array detectors (16), but the circular dichroism (CD)–ultraviolet (UV) detectors have been gaining more and more ground (17–18). Recent reviews by Zukowski (19) and Gergely (18) surveyed the advantages and drawbacks of the former and the applications of the latter method. Chiroptical detection has now been used for a decade, and even the first purpose-built commercially available CD detector has only appeared in 1998 (20), indicating the demand to use and develop such detectors.

In an earlier study, we described the principles and some applications of the simultaneous double CD–UV detection in order to study peak homogeneity (15). That study provided means to determine analytes from pure sections of overlapping peaks only and has not been used to analyze optical isomers.

A similar method has been published earlier by Mannschreck et al. (21,22). Nevertheless, the equations reported by Mannschreck et al. can only be used to decompose peaks of enantiomers. The method described here enhances the scope of use by the incorporation of two arbitrarily chosen detector signals.

In this study, we report its improvements in complicated cases for the analysis of composite distorted peaks of highly similar compounds, including enantiomers.

The separation of materials *A* and *B* (detected by methods 1 and 2) can be characterized in terms of the S_{1A}, S_{1B}, S_{2A}, and S_{2B} compound- and detector-specific contributions and the S_1 and S_2 observed overall detector signals as follows:

$$S_1 = S_{1A} + S_{1B} \quad \text{Eq. 1}$$

$$S_2 = S_{2A} + S_{2B} \quad \text{Eq. 2}$$

The S_{1A}, S_{1B}, S_{2A}, and S_{2B} compound- and detector-specific terms are typically Lambert–Beer-type quantities, in particular

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products of concentration (e.g., molarity), a compound-intensive parameter (e.g., molar-absorption coefficient), and an instrument-specific quantity (e.g., pathlength of the light).

In cases of overlapping peaks, the S_1/S_2 detector signal ratio (R) is a function of the experimental retention time:

$$\frac{S_1}{S_2} = \frac{S_{1A} + S_{1B}}{S_{2A} + S_{2B}} = R \qquad \text{Eq. 3}$$

In the limiting case of homogenous peaks (the elute containing one component only), the division of equations 1 and 2 results in a constant (R_A or R_B), which is a quantity independent of concentration but inherently characteristic of the compound in question and the detection method.

$$\frac{S_{1A}}{S_{2A}} = R_A \qquad \text{Eq. 4}$$

$$\frac{S_{1B}}{S_{2B}} = R_B \qquad \text{Eq. 5}$$

If compounds A and B are available in pure form, the R_A and R_B values can be determined in simple nonchromatographic experiments. Nevertheless, the "ratio chromatograms" can also be used to determine the R_A and R_B values, provided that the chromatograms contain "horizontal" sections characteristic of the pure components. Thus, in principle, if R_A is not equal to R_B, the values of S_{1A}, S_{1B}, S_{2A}, and S_{2B} can be calculated from equations 1, 2, 4, and 5. Analysis-oriented results of higher confidence can be obtained if all five equations above are utilized.

It can be seen that the relationships of the five equations are symmetrical for the S_{1A}, S_{1B}, S_{2A}, and S_{2B} quantities. Thus, explicit forms for each of them can be reached in several ways providing, however, equivalent results. One of the concluding set of equations is given here:

$$S_{2B} = S_2 \frac{(R - R_A)}{(R_B - R_A)} \qquad \text{Eq. 6}$$

$$S_{2A} = S_2 - S_{2B} \qquad \text{Eq. 7}$$

$$S_{1B} = S_1 \left[(R_A R_B / R) - S_1)\right] / (R_A - R_B) \qquad \text{Eq. 8}$$

$$S_{1A} = S_1 - S_{1B} \qquad \text{Eq. 9}$$

It can be noted that equations 6 to 9 constitute the mathematical basis of the peak deconvolution, because all the component

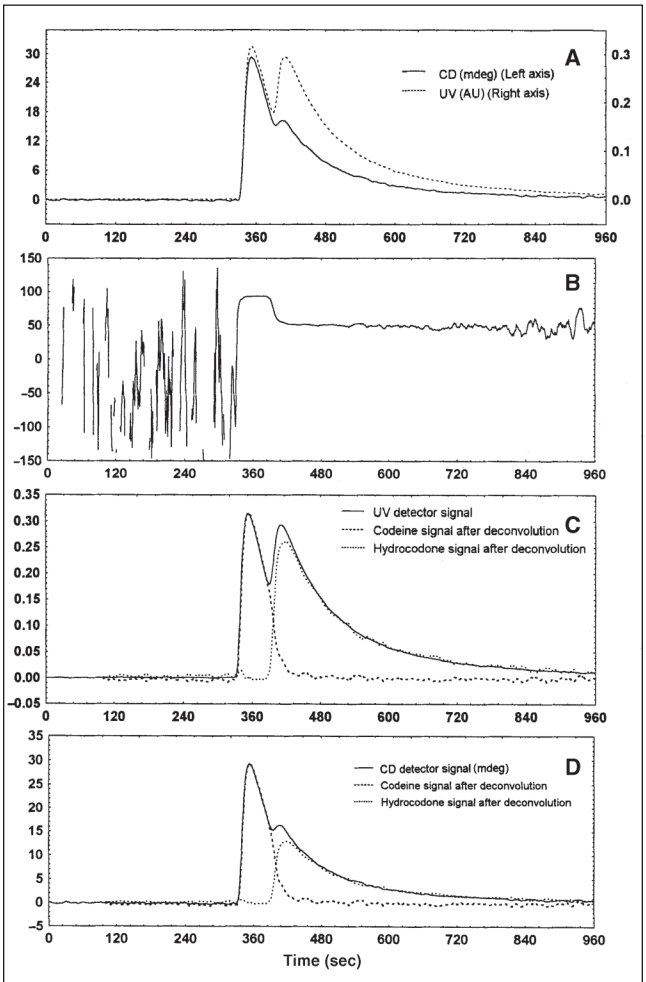

Figure 1. Experimental chromatogram (A), derived ratio chromatogram (B), and unfolded peaks of codeine and hydrocodone (C,D).

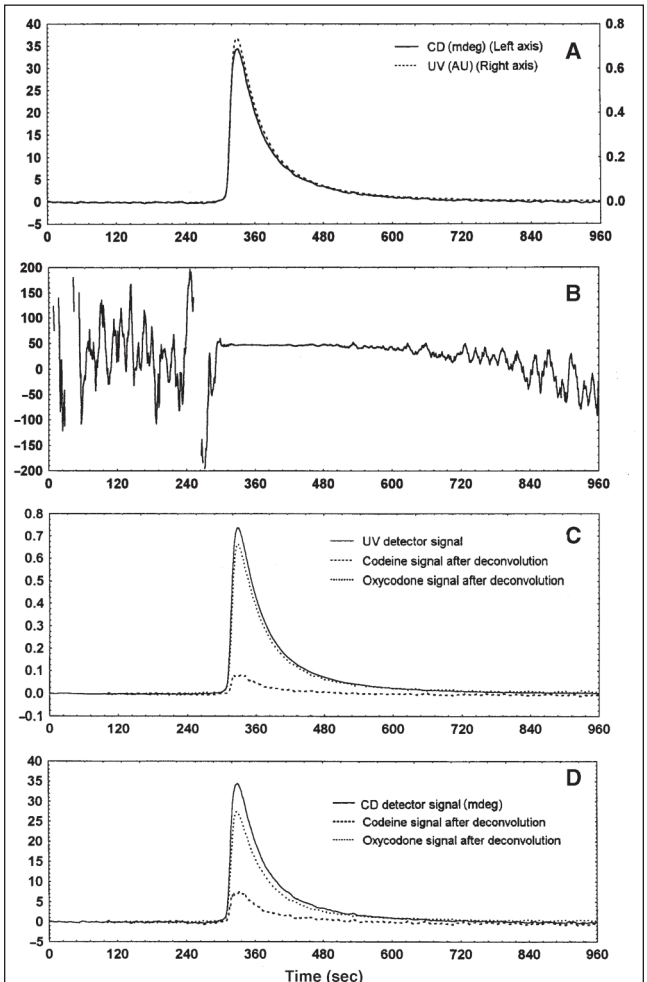

Figure 2. Experimental chromatogram (A), derived ratio chromatogram (B), and unfolded peaks of codeine and oxycodone (C,D).

contributions can be obtained. Provided that molar-intensive properties (such as the molar-absorption coefficient and molar ellipticity) are known, the deconvoluted concentration profile of the peak can also be obtained.

Feasibility of the method is exemplified in three systems—specifically the alkaloid pairs codeine–hydrocodone and codeine–oxycodone, the geometrical Z–E isomers of methyl-testosterone-oxime, and the enantiomers of a synthetic heterocyclic 1-methyl-2-phenyl-1,2,3,4-(6*H*)-pyrazino[2,1-*b*]-quinazo- line-3,6-dione (MM285a)—in which all have eluted in overlapping fashion in the chromatographic systems used. All our studies used simultaneous CD–UV detection modes.

Experimental

Double detection (the key component of our study) was carried out by a Jasco 720 spectropolarimeter, which can simultaneously record the absorption (UV–visible) and the chiral — CD or optical rotatory dispersion (ORD)—signals. Its Jasco LCCD-311 flow-through cuvette (l = 5 mm, v = 16 µL) was linked to the HPLC units, the Jasco PU-980 intelligent pump, and the Rheodyne 7725i injector with a 20-µL loop.

Separation circumstances of the codeine–hydrocodone and codeine–oxycodone samples were identical with those described earlier (14). Separation of the methyltestosterone-oxime isomers was carried out using a reversed stationary phase (ODS C_{18}, 250 × 4.6 mm, Jones Chromatography) column with methanol–water (60:40, v/v) as the mobile phase at 40°C and a 1-mL/min flow rate. Detection took place at 245 nm. Methyltestosterone was the product of Gedeon Richter Co. (Hungary); the oxime derivative was formed with hydroxyl-ammonium-chloride (Merck) in an ethanol–acetate buffer medium.

The synthesis and characterization of the compound MM285a (Figure 4) have been published earlier by Kökösi et al. (23). Enantiomers of the 0.2 m-per-volume racemic mixtures were separated on a Chiralcel OD (250 × 4.6 mm, DAICEL) column, using a 1-mL/min flow rate and detection at 243.2 nm wavelength. One-hundred milliliters of the mobile phase (*n*-hexane 75 v/v%, *i*-propanol 23 v/v%, and methanol 2 v/v%) contained 12.5 µL triethylamine. Determination of the elution sequence for both the methyltestosterone and MM285a isomers was in progress by ^{1}H NMR. Base-line correction and peak-deconvolution calculations were done using MS Office97 Excel programs.

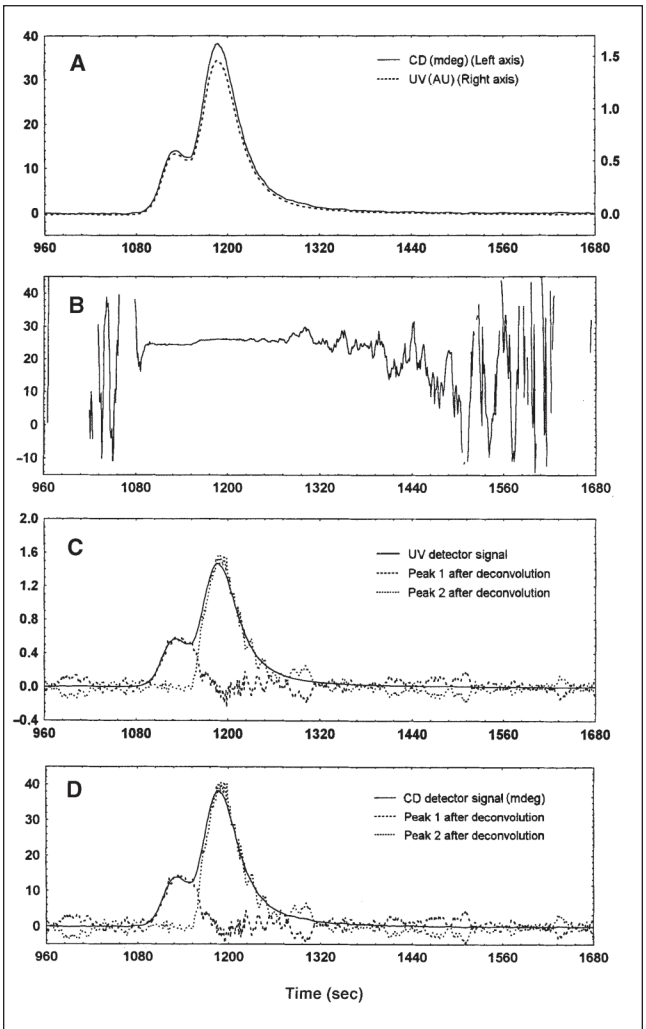

Figure 3. Experimental chromatogram (A), derived ratio chromatogram (B), and unfolded peaks of methyltestosterone-oxime (C,D).

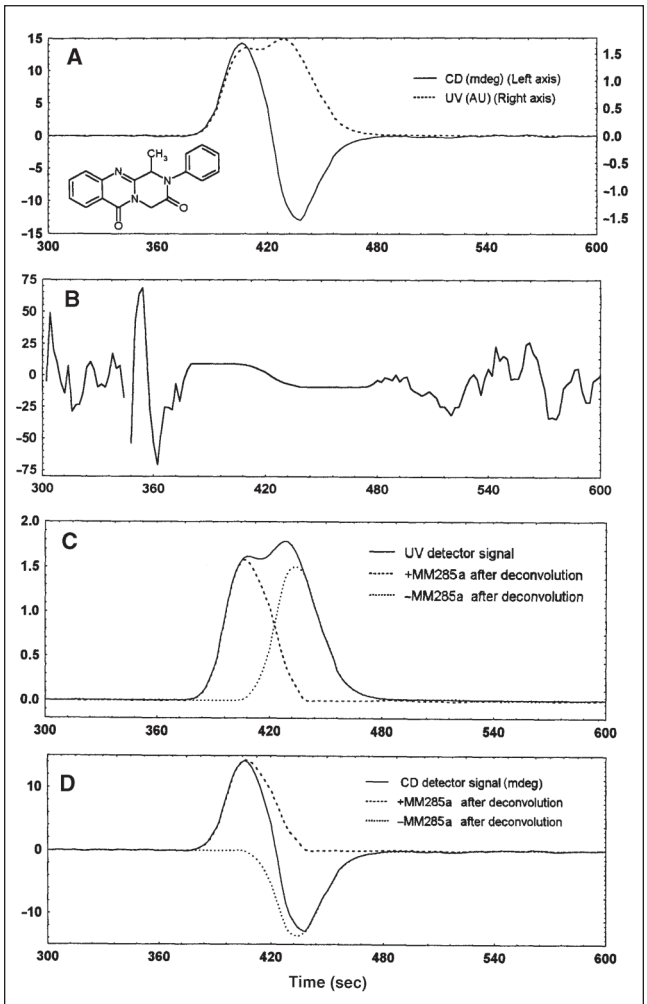

Figure 4. Experimental chromatogram (A), derived ratio chromatogram (B), and unfolded peaks of the separated enantiomers of the racemic MM285a substance (C,D). The molecular constitution is also given (A).

Results and Discussion

Overlapping is often worsened by the asymmetry of the component peaks. Estimation of the individual peak areas is especially difficult in such cases, as will be illustrated by codeine and hydrocodone.

Figure 1 shows the experimental chromatogram, the derived ratio chromatogram, and the deconvoluted curves of 6.75 mmol codeine and 25 mmol hydrocodone. Besides the incomplete separation, the highly asymmetrical shape of the second peak poses a great burden on the evaluation, partly because of the closely similar UV spectra of the two compounds. Fortunately, the CD intensity at 242 nm of codeine and hydrocodone were sufficiently different, which resulted in a 93 and 49.5 CD–UV ratio for the pure substances of codeine and hydrocodone, respectively. These differences provided a means to decompose the peaks and construct both the "UV-detected" and "CD-detected" component peaks of the individual analytes.

Taking this example, the advantage of the double detection can be generalized: the use of two detection techniques obviously provides a much better chance to find sufficient difference in the physico-chemical properties than only one technique.

Figure 2 is the analogous set of 2.45 mmol codeine and 25.8 mmol oxycodone chromatograms. However, a striking difference is that no apparent deformity of the peak indicates the presence of more than one component. Unlike in Figure 1B with two horizontal ranges, Figure 2B contains one horizontal section only, making the lack of even a partial separation evident.

The codeine contamination of the oxycodone is only indicated by the ratio value, which is higher in the experimental chromatogram than in the case of pure oxycodone. The use of double detection and formation of the ratio chromatogram unfolds the individual peaks of both components and detection techniques (Figures 2C and 2D). Deconvolution and evaluation of the curves provide the following analytical data: 26.3 mmol oxycodone and 2.48 mmol codeine (CD detection) and 26.4 mmol oxycodone and 2.49 mmol codeine (UV detection). Thus, the amount of the nonseparated contaminant codeine could be estimated within 2% error.

Figure 3 shows the partial separation and peak deconvolution of the geometrical isomers of methyl-testosterone-oxime. Upon derivatization of the parent compound in the 3-keto position, the isomeric Z and E oximes can be formed. Figure 3 shows the chromatograms recorded by CD and UV detections and the ratio-type and deconvoluted derivative chromatograms. The unfolded chromatograms are significantly more noisy than in the previous two cases. Furthermore, the CD–UV ratios of the two isomers differ only slightly, and the resulting decomposition is more ambiguous. Thus, the quantitative evaluation from the deconvoluted curves takes further considerations. Nevertheless, the derived unfolded curves certainly provide clear markers of the initiating and terminating parts of the component peaks that can be used at least for further method development of the separation.

There is no doubt that CD–UV or ORD–UV detection can be most powerfully applied in the analysis of enantiomers. This is a consequence of the fact that enantiomers provide ellipticities of identical magnitude, but the opposite sign (the derived CD–UV ratio also inherits the opposite sign). Consequently, a chromatogram of CD–UV ratio detection has sections of positive and negative horizontal ranges in intervals when one of the enantiomers solely elute. Because these ratios are concentration-independent quantities, the numerical absolute values of such sections are identical, regardless of the enantiomeric ratio in the sample. The real concentration of the enantiomers can be calculated from the direct CD and UV intensity data. The subsequently eluting enantiomers are manifested in the CD–UV ratio chromatogram in linear horizontal sections that are identical distance from the time axis. In the overlapping range, one horizontal line transforms into the other nonhorizontal. The point in which this line crosses the axis ($y = 0$) indicates that the elute contains the two enantiomers in identical concentration (racemic mixture). Decomposition of the curves provides the calculated chromatograms with unfolded homogeneous peaks, their areas, and thus the enantiomer-specific composition of the sample.

The above principles are exemplified in the enantiomer-specific analysis of MM285a (shown in Figure 4). This compound contains one chiral carbon atom in position C_1, where the methyl moiety adjoins. Because all achiral physico-chemical properties of the enantiomers are identical, the double detection method must contain a technique that can distinguish between optical isomers. More specifically, it must be a chiroptical method. It should be noted that not even a multichannel diode-array detector after partial separation of the enantiomeric compounds could decompose the peak, because the identical UV spectra of the enantiomers would produce equivalent ratios at any wavelength. Figures 4C and 4D show the enantiomer-specific concentration profile of enantiomers (both in the CD and UV detected versions) following the deconvolution process.

This method provides a means, for example, to monitor the progress of stereoisomeric reactions and to design the fraction collection in preparative chromatography.

Acknowledgments

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