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Journal of Chromatography A, 909 (2001) 147–154

JOURNAL OF CHROMATOGRAPHY A

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Chiroptical detection during liquid chromatography 7 The rotation angle/absorbance ratio of chiral molecules. Its possible use for on-line analysis during preparative separations of enantiomers☆

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Received 19 June 2000; received in revised form 7 September 2000; accepted 1 November 2000

Abstract

The rotation angle/absorbance ratios $C_+ = \alpha_+ / A_+$ and $C_- = \alpha_- / A_-$, determined via detection by a polarimeter and a photometer, were checked for the first time with reference to their use for on-line analysis during preparative separations. For this purpose, (+)-, (−)- and (±)-carvones were investigated by liquid chromatography (LC) on microcrystalline tribenzoylcellulose. It turned out that the ratios C_+ and C_- depend only slightly upon concentration (Table 1). Overlapped peaks of enantiomers were successfully submitted to computer deconvolution (e.g. Fig. 2, bottom). A procedure for on-line analysis during preparative LC is proposed.

Keywords: Enantiomer separation; Preparative chromatography; Detection, LC; Polarimetric detection; Carvone

1. Introduction

Preparative liquid chromatography (LC) on enantioselective sorbents has remarkably improved during the last years [3]. For all types of separations – batch, recycling and simulated moving bed (SMB) techniques – control during the chromatographic procedure is essential, particularly if the latter is performed in an industrial scale. Such control can in principle be carried out on the basis of a computer simulation of the whole process and/or on the basis of an experimental analysis of solutions of interest, e.g. an eluate or a liquid occurring during an SMB separation. This analysis means that the enantiomeric purity of such solutions be determined continuously. Its knowledge as a function of the retention factor, the retention time or the retention volume v would be important for forthcoming manipulations which might be necessary during a preparative LC run and, therefore, for its performance. To our knowledge, such an on-line analysis of enantiomers is not described in the literature yet [4].

Chiroptical detectors can be used to determine enantiomeric composition. Although analytical *single* detection, based upon polarimetric peak heights or peak areas, has been published [5,6], *dual* detection, e.g. a combination of chiroptics with photometry, can result in more information. Circular dichrographs provide all data in a single cell at one wavelength

☆For Part 6 see Refs. [1,2].

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0021-9673/01/$ – see front matter
PII: S0021-9673(00)01105-5

[7–15], whereas the combination of polarimetry and photometry with two unequal wavelengths and two cells with different path lengths is common practice [5,16–19]. At first glance, this seems to be a serious drawback of the latter method. However, especially for preparative applications, it turns out that the number of prospective analytes is increased by the chance to optimize the working parameters for the two detectors *independently*. Circular dichroism (CD) is limited in a way similar to photometry concerning linear and dynamic ranges which extend to much higher concentrations in polarimetry [17,19–21] than in CD. For these reasons, we decided to try polarimetry first when choosing a chiroptical method for on-line analysis during preparative LC of enantiomers. On the other hand, there are no publications on the use of CD or of polarimetry with the aim of quantitative evaluation of measurements at elevated concentrations.

We have performed many successful computer deconvolutions of overlapped peaks of enantiomers, in the framework of exclusively analytical applications. For this purpose, we used polarimetric/photometric detection for biaryls [8,10,22], helicenes, e.g. [23], 2*H*-pyrans [18,19,24,25], and thiobenzamides, e.g. [8], as well as circular dichroic/photometric detection for 2*H*-pyrans, e.g. [13,26], and thioacrylamides, e.g. [13]. Our approach does not apply Gaussian [6,27] or any other peak shapes but merely treats overlapped peaks as the *sum* of their components, which means their *difference* in the case of polarimetric detection because of the unequal signs of the rotation angles α_+ and α_-. In addition, a relation between these angles and the corresponding absorbances A_+ and A_- is required for deconvolution. In this respect, we had chosen [16,28,29] the rotation angle/absorbance ratios $C_+ \equiv \alpha_+/A_+$ and $C_- \equiv \alpha_-/A_-$. Very often they are easily obtainable from a plot of the experimental α_- and A_--values, examples for the resulting $\alpha(A)$ diagrams being given in Fig. 1, top and centre.

The aim of the present paper is to check how these procedures can be used for an on-line analysis during *preparative* separations of enantiomers. The main problem will be how to determine the ratio C_+ or C_- for the deconvolution of chromatograms measured *at high concentrations* [19]. As a first step to answer these questions, we selected carvone (Fig. 1), both enantiomers and the racemate of which are well

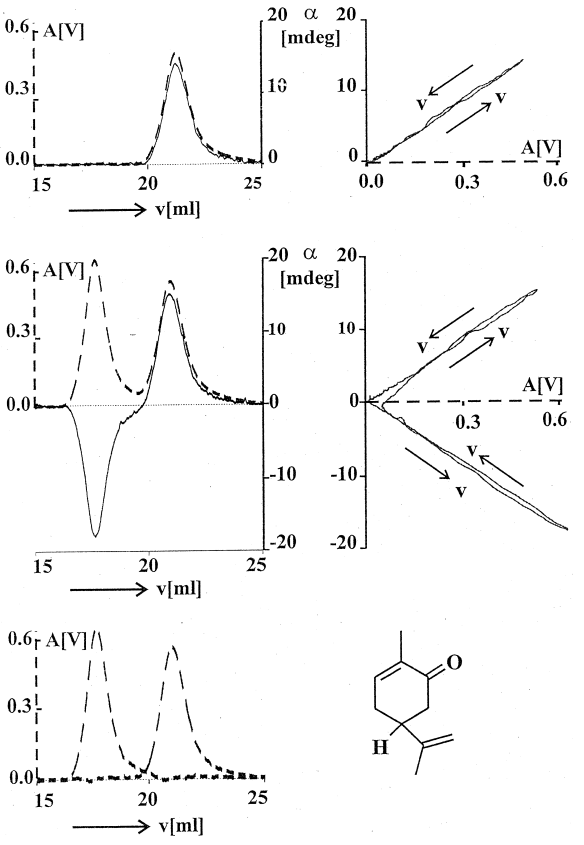

Fig. 1. Liquid chromatography of carvones with a photometer cell of 10 mm path length. Top: 0.20 mg of (+)-carvone in 40 μl of MeOH. Experimental chromatograms $A(v)$ and $\alpha(v)$ as well as diagram $\alpha(A)$, resulting in the ratio $C_+ = \alpha_+/A_+ = +28.8$ mdeg/V (Table 1, entry 3). Centre: 0.40 mg of (±)-carvone in 40 μl of MeOH. Experimental chromatograms $A(v)$ and $\alpha(v)$ as well as diagram $\alpha(A)$, resulting in the ratio $C_+ = \alpha_+/A_+ = +28.9$ mdeg/V (Table 1, entry 7). Bottom: Computer deconvolution of the experimental chromatogram $A(v)$ of (±)-carvone by means of $C_+ = 28.9$ mdeg/V.

characterized and therefore suitable for checking our procedures. We chose LC conditions under which, at low concentration, almost complete separation of the peaks of (+)- and (−)-carvone (Fig. 1, centre) occurs which will overlap at high concentration.

2. Experimental

(+)-Carvone: Fluka Chemie AG, Buchs, Switzerland, ≥99% (Fluka). $[\alpha]_{589}^{20} = +61 \pm 2$, $[\alpha]_{546}^{20} = +$

74±3 deg ml $g^{-1}dm^{-1}$, neat, $d^{20}=0.960$ g cm^{-3}. Enantiomeric purity 98±2% (HPLC, microcrystalline tribenzoyl cellulose; see below for details).

(−)-Carvone: Sigma-Aldrich Chemie GmbH, Steinheim, Germany, 98% (Sigma-Aldrich). $[\alpha]_{589}^{20}=-60\pm2$, $[\alpha]_{546}^{20}=-73\pm3$ deg ml g^{-1} dm^{-1}, neat, $d^{20}=0.959$ g cm^{-3}. Enantiomeric purity 98±2% (HPLC).

(±)-Carvone: Equal amounts of (+)- and (−)-carvone mixed. Enantiomeric purity 1±2% (HPLC).

Equipment: Pump L 6000 A, Merck-Hitachi, Darmstadt, Germany. Injector Rheodyne 7125. Degasser ERC 3312, Erma Optical Works, Ltd., Tokyo, Japan. Polarimeter PE 241, Perkin-Elmer, Überlingen, Germany, cell with path length of 100 mm and volume of 80 μl; all rotation angles α are given in mdeg (millidegree) at 436 nm. Photometer ERC 7215, Erma Optical Works, Ltd., cells with path lengths of 1 and 10 mm, both with a volume of 8 μl; all absorbances A are given in V (Volt) at 330 nm. All measurements had to be performed within the dynamic range of the photometer. For this purpose, a cell of only 1 mm path length had to be used for the higher concentrations. The instrument proved to be linear up to $A=0.3$ absorbance units, corresponding to $A=5$ V when the range setting 0.64 was used. This limit was not exceeded during our measurements, all of which were performed during two consecutive days in order to minimize any changes which the equipment or the column might undergo.

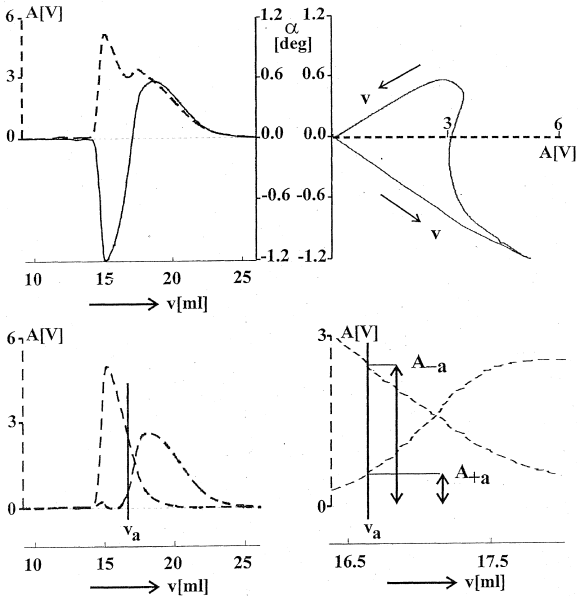

Fig. 2. Liquid chromatography of 50.0 mg of (±)-carvone in 500 μl of MeOH with a photometer cell of 1 mm path length. Under these conditions, the column is overloaded. Top: Experimental chromatograms $A(v)$ and $\alpha(v)$ as well as diagram $\alpha(A)$, resulting in the ratio $C_-=\alpha_-/A_-=-229$ mdeg/V (Table 1, entry 13). Bottom, left and right: Computer deconvolution of the experimental chromatogram $A(v)$ by means of the above C_--value. At any actual elution volume v_a, the actual absorbances A_{-a} and A_{+a} can be obtained numerically in the framework of the computer program used (see text).

Column 250×8 mm; sorbent microcrystalline tribenzoylcellulose [30], 10–15 μm, Riedel-de Haën AG, Seelze, Germany; room temperature.

Eluent MeOH, 1 ml/min, 50 bar. All retention volumes v refer to injection at $v=0$.

Data processing: As the photometer and the polarimeter are put in series, a correction concerning the v scales of the $A(v)$ and $\alpha(v)$ sets of data was applied by a computer program for dual detection [13,18,31]. In addition to recording these experimental chromatograms, it determined diagrams $\alpha(A)$ and performed deconvolutions [8,10,23], e.g. for Figs. 1 and 2. Chromatographic parameters, including the plate heights H_+ and H_-, were also calculated by this program from the experimental $A(v)$ curves.

3. Results concerning (+)-, (−)- and (±)-carvones

We have determined ratios C_+ and/or C_- at low concentrations with a photometer cell of 10 mm path length (e.g. Fig. 1, centre) and with 1 mm path length (e.g. Fig. 2, top) at higher concentrations. The latter experimental chromatograms show lower retentions and higher peak widths, i.e. the column is overloaded in a way similar to preparative LC. The slopes of the linear regions of the diagrams $\alpha(A)$ of (±)-carvone represent C_+ and C_- (Table 1) although the absolute value of the ratio for the *second* peak (C_+ in the cases of Figs. 1 and 2) may sometimes be less precise because of strong tailing of the first one (Fig. 2; Table 1, entry 13). Alternatively, the ratios α/A of many points of the chromatograms can be plotted versus the elution volume v in order to determine C_+ and C_-. In our experience [32], the values found by this particular procedure are less precise than the

Table 1
Ratios $C_+ = \alpha_+ / A_+$ and $C_- = \alpha_- / A_-$ for carvones. *b*: Path length of photometer cell. *m*: Mass injected. c_{max}: Concentration at the peak maximum (see text); in the case of (±)-carvone, the (+) peak was used. H_+, H_-: Plate heights for second and first peak, respectively

Entry	Carvone	*b* (mm)	*m* (mg)	c_{max} (mg/ml)	H_+ (μm)	H_- (μm)	C_+ (mdeg/V)	C_- (mdeg/V)
1	(−)	10[a]	0.10	0.10	–	164		−27.7
2	(+)	10[a]	0.10	0.10	163	–	+27.1	
3			0.20	0.16	161	–	+28.8[d]	
4			1.2	0.72	231	–	+31.2	
5			2.5	1.5	290	–	+31.9	
6	(±)	10[a]	0.20	0.08	164	165	+27.3	−27.2
7			0.40	0.15	174	160	+28.9[d]	−29.0[d]
8			2.5	0.82	244	202	+30.4	−30.3
9			5.0	1.4	335	267	+30.8	−31.2
10	(+)	1[b]	2.0	1.1	335	–	+204	
11			25.0	8.1	1310	–	+228	
12	(±)	1[b]	4.0	1.1	339	264	+203	−204
13			50.0	6.6[c]	2100[c]	721[c]	+216[e,f]	−229[e]

[a] Concentrations upon injection: 5.0 mg/ml of (+)- or (−)-carvone or 10 mg/ml of (±)- carvone.
[b] Concentrations upon injection: 50.0 mg/ml of (+)-carvone or 100 mg/ml of (±)- carvone.
[c] Determined after deconvolution (Fig. 2).
[d] Cf. Fig. 1, centre.
[e] Cf. Fig. 2, top.
[f] The absolute value $|C_+| = 216$ of the *second* peak is less reliable than the value $|C_-| = 229$ of the first one because of strong tailing of the latter (Fig. 2).

ones from $\alpha(A)$ diagrams. This statement is also true for the detection by the differential absorbance ΔA instead of the rotation angle α: In our experience [32,33], the values found by plots of $\Delta A/A$ versus v, first performed by Drake, Gould and Mason [7], are not as precise as the ones from our $\Delta A(A)$ diagrams [13,26].

The concentrations c_{max} at the peak maxima (Table 1) were estimated according to $c_{max} \approx m_e / w_h$, where m_e is the injected mass of the enantiomer and w_h the width of the peak at half height, measured in units of volume. This relationship was derived using a general expression [34] and the approximation of a triangle instead of a Gaussian peak shape. When *m* of (±)-carvone is increased by a factor of 25 and c_{max} increases correspondingly, the ratio C_+ increases [21] gradually by 13% (Table 1, entries 6–9). This finding means that C_+ or C_- should be known for the concentration at which an analysis by deconvolution is intended. We shall follow this advice when designing an on-line procedure in Section 4. The probable reasons for this slight concentration dependence of the above ratio are slow response [35] of our type of polarimeter at higher concentration and also gradual deviations from the ideal law of Beer for the absorbance [36] and/or from the ideal law of Biot for the rotation angle [37–39]. Such nonlinear effects may be caused by RR–SS and RS–SR interactions [40] and will be taken into account in Sections 4 and 5. The above deviations may also represent some of the reasons for the fact that C_+ does not increase exactly ten times (Table 1) when the path length *b* is decreased ten times. Such an exact behaviour is expected from the equation [28,29]:

$$C_+ \equiv \frac{[\alpha_+]\, l}{\epsilon\, b} \qquad (1)$$

$[\alpha_+]$: Specific rotation of the (+) enantiomer; ϵ: Absorption coefficient; *l*: Path length of the polarimeter cell; *b*: Path length of the photometer cell.

However, the main reasons for the deviation from this factor of ten are probably the unknown precisions of the two path lengths *b*, an aspect which is not further discussed here.

According to Eq. (1), C_+ depends upon the two unequal path lengths and the parameters $[\alpha_+]$ and ϵ. Considering the analogy to CD, a parameter g'_+ (or g'_-) is given by

$$g'_+ \equiv \frac{\Delta\epsilon_+ \, d}{\epsilon \, b} \qquad (2)$$

$\Delta\epsilon_+$: Differential absorption coefficient; *d*: Path length of the dichrograph cell

As it is possible to measure $\Delta\epsilon_+$ and ϵ in the *same* cell along the same path [7], $d=b$ leads to the anisotropy factor $g_+ \equiv \Delta\epsilon_+/\epsilon$, a fundamental [41] parameter of chiral molecules. Since the introduction [28,29] of the ratio C_+ (or C_-), it has turned out that it represents another useful parameter characterizing a chiral molecule. For the present preparative application, it would be desirable to use a *single* cell providing a longer path for the polarimetric measurement and a shorter one, in perpendicular direction, for photometry.

We also determined the ratios C_+ or C_- by using pure (+)- or (−)-carvones (Fig. 1, top; Table 1, entries 1–5 and 10–11) because this will be important for on-line analysis (Section 4). In order to meet the conditions applied to (±)-carvone, we used the same set-up, although an *enantioselective sorbent* was not essential for this particular purpose. It turned out that these results agree excellently, i.e. within 4%, with the ratios obtained for (±)-carvone at similar concentrations c_{max}. For instance, at $b=1$ mm and $c_{max}=1.1$ mg/ml, the ratios determined using (+)- and (±)-carvones amount to +204 and −204 mdeg/V, respectively (Table 1, entries 10 and 12).

Besides the determination of C_+ or C_- on line with LC (Figs. 1 and 2; Table 1), the latter values can be obtained by *off-line* measurements of α_+ and A_+. This was performed for (+)-carvone in MeOH (0.69 mg/ml; room temperature) with the following results: $\alpha_+ = +79.6$ mdeg, corrected for an enantiomeric purity of 100%, at 436 nm and a path length of 100 mm; $A_+ = 2.49$ V, given for the Volt scale of our LC experiments, at 330 nm and a path length of 10 mm; $C_+ = \alpha_+/A_+ = +32.0$ mdeg/V. This off-line finding fits excellently with the on-line value of +31.2 mdeg/V (Table 1, entry 4) at $c_{max}=0.72$ mg/ml, thus again showing the reliability of the α/A-diagrams and the ratios obtained from them.

Computer deconvolutions [8,10,23] of the experimental chromatograms $A(v)$ and $\alpha(v)$ were performed (e.g. Figs. 1 and 2, bottom) by means of the C_+- or C_--values determined. Upon overlap of peaks, the actual absorbances A_{-a} and A_{+a} of the first and second peak, respectively, at any actual elution volume v_a (e.g. Fig. 2, bottom) are obtainable numerically via the software applied. From these absorbances, the actual optical purity P_a [42] at any elution volume can be calculated, the adjective ''optical'' indicating that the application of any optical method to concentrated solutions does not necessarily result in the true enantiomeric purity [37–39] (cf. Section 5). For instance, at $v_a=16.6$ ml, $A_{-a}=2.50$ V and $A_{+a}=0.625$ V (perpendicular line drawn in Fig. 2, bottom), corresponding to $P_a = (2.50-0.625)/(2.50+0.625)=60\%$. In addition to our program [13,18,31], P_a has been visualized by the computer as a function of v in real time *during* the chromatographic run [19,32].

The use of ratios and deconvolutions has been successful for several classes of compounds (see Introduction) in the framework of exclusively analytical applications. As far as preparative separations are concerned, our choice of carvone as a first step to check our procedure must be followed by the selection of substrates showing stronger UV absorptions and stronger solute–solute interactions than carvone.

4. Discussion with reference to an on-line analysis during preparative liquid chromatography

The above results show that 50 mg of (±)-carvone can be analyzed on line during LC via deconvolution of experimental chromatograms (Fig. 2). However, there are $\alpha(A)$ diagrams of mixtures of enantiomers in which, for several reasons, a linear region does not appear and, therefore, the ratio C_+ or C_- required cannot be determined in this way. In such cases, the $\alpha(A)$ diagram of a *pure* enantiomer, measured under the same experimental conditions, will result in the

ratio required. This was shown by the excellent agreement of ratios C_+ or C_-, obtained for (±)-carvone and for its pure enantiomers (Fig. 1; Table 1) by using the same concentrations and the same detectors for both measurements. Contrary to the procedure used for the carvones, *continuous* analysis during preparative LC may require switching between the pure enantiomer, e.g. (+), and the mixture of the (+) and (−) enantiomers (Fig. 3).

Under certain conditions, the whole chromatographic flow of a preparative separation can be conducted through the detectors which, in addition, may have to serve for the investigation of a pure enantiomer. In other cases, it will be necessary to split that flow and to conduct only a small portion of it through the detectors [6] (Fig. 3). This means that the *amount* of pure enantiomer required may also be small.

In principle, the (+) enantiomer in Fig. 3 can be injected directly into the flow of solvent, i.e. flow injection analysis [43] is performed. However, if the enantiomer dissolved in a very small volume of solvent arrives in the detectors, an $\alpha(A)$ diagram (e.g. Fig. 1, top) consisting of a very short straight line will result, the slope C_+ of which cannot be determined precisely. According to our experience [32], artificial broadening of the peak of the pure enantiomer significantly increases the precision of the measurement of C_+ in such cases. A peak-broadening device (Fig. 3) may either consist of a tube with an enlarged inner diameter or a column containing a non-enantioselective or an enantioselective stationary phase. The latter possibility was used for our investigation of the carvones.

In conclusion, we propose computer deconvolution of the experimental chromatograms of the mixture of enantiomers for on-line analysis during preparative LC on nonracemic sorbents [19]. If no linear region appears in the $\alpha(A)$ diagram, a parallel measurement of a *pure* enantiomer (Fig. 3) will result in the C_+- or C_--value, provided that the same concentration and the same detectors as for the above mixture are used. In the latter case, the availability of a certain amount of a pure enantiomer is important.

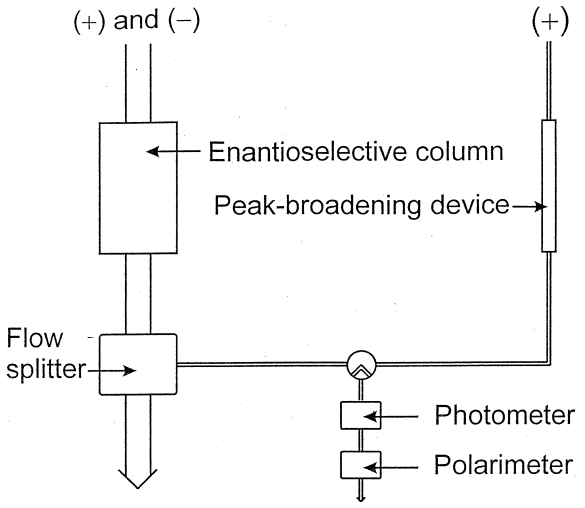

Fig. 3. Schematic set-up for an on-line analysis during preparative liquid chromatography of a mixture of (+) and (−) enantiomers. In order to be able to deconvolve the $A(v)$ chromatogram (cf. Figs. 1 and 2, bottom) of the mixture, the ratio $C_+ = \alpha_+ / A_+$ is determined by using the pure (+) enantiomer (cf. Fig. 1, top). See text for the eventual requirements of a flow splitter and a peak-broadening device.

5. Comparison with an alternative procedure for on-line analysis

For the deconvolution of experimental data, an alternative method would consist of calibrations of the polarimeter and the photometer by means of a pure enantiomer, if a sufficient amount of it is available. Techniques of this type have recently be mentioned [21,44]. The results of such calibrations are valid only for the substrate in question and, therefore, the overall expenditure is considerable. However, this method gives access, at the same time, to the domain of linear response of the photometer and solves the problem of nonlinear interactions in concentrated solutions and of optical/enantiomeric purities.

The procedure proposed in the present publication is less time consuming. It states the domain of linear response of the photometer once for all substrates by using any test sample. Instead of the above calibrations of both detectors for each substrate, the two experimental variables, α and A, of the substrate in question are plotted, one versus the other one. A sufficiently extended linear region in the $\alpha(A)$ dia-

gram may have to be provided by a smaller path length of the photometer cell (e.g. Fig. 2, top). A linear region in the $\alpha(A)$ diagram (e.g. Fig. 1, top) of a parallel measurement for a *pure* enantiomer (Section 4) will, in most cases, indicate the absence of nonlinear effects and the applicability of our procedure.

For both alternatives, the relative importance of their advantages and disadvantages will be shown by future applications.

Acknowledgements

We thank Drs. M. Frieser and M. Schinabeck for many useful discussions concerning on-line analysis. Valuable advice by Mr. F. Kastner and Dipl.-Chem. R. Kießwetter concerning LC and computer hardware is gratefully acknowledged. The present work was supported by Fonds der Chemischen Industrie.

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