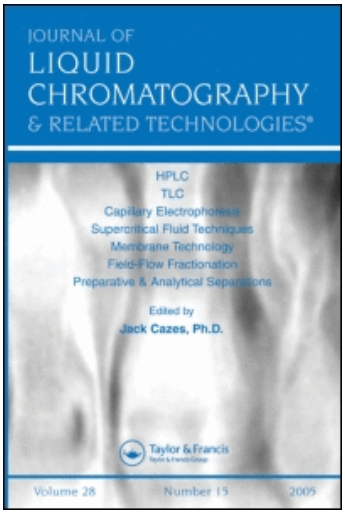

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Parametric Studies on the Determination of Enantiomerization Rate Constants from Liquid Chromatographic Data by Empirical Peak Shape Equations for Multi-Step Consecutive Reactions

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PARAMETRIC STUDIES ON THE DETERMINATION OF ENANTIOMERIZATION RATE CONSTANTS FROM LIQUID CHROMATOGRAPHIC DATA BY EMPIRICAL PEAK SHAPE EQUATIONS FOR MULTI-STEP CONSECUTIVE REACTIONS

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ABSTRACT

On-column enantiomerization is a well documented and practically important special case of the occurrence of a reversible reaction in a chromatographic reactor. The present paper explains the possibility to obtain enantiomerization rate constants from liquid chromatographic data using analytical equations derived for multi-step consecutive reactions. The results are compared with results from a finite difference method, and the application to experimental data is demonstrated.

INTRODUCTION

Enantiomerization is a special case of reversible reactions, which, when carried out in a chromatographic column, starts from especially well defined conditions. Since normally a racemic mixture is injected on the column (which is then pushed out of equilibrium by the chromatographic separation), the molar inlet amounts of both reactants are always the same. Moreover, despite the action of the principle of microkinetic reversibility, the rate constants are always commensurate[1] because of the impossibility to achieve large separation factors, and the detector responses of non-chiralic detectors are equal for both reactants. Therefore, enantiomerizations are attractive model reactions for studying reversible reactions on chromatographic columns. There is, on the other hand, also a pharmaceutical interest in rate constants of enantiomerizations, which are consequently the best investigated complex reactions on chromatographic columns. With retention times in the range from about one minute to about one hour, rate constants in the range from about 10^{-3} min^{-1} to about 10 min^{-1} can be measured; higher rate constants will lead to the formation of one single peak of interconverting material, lower rate constants will give two separated (i.e. mainly unchanged) peaks.

The determination of enantiomerization rate constants by chromatographic reactor investigations was essentially introduced by Schurig et. al.. They predicted the possibility theoretically,[2] proved the feasibility experimentally,[3] and finally evaluated rate constants based on numerical simulations by the plate model,[4,5,6] which is, in a mathematical sense, a special case of finite difference calculations. Hochmuth and Koenig[7] published data as well, which are based on the same algorithm. Mannschreck et al.[8] and Vecania and Crespo[9] started from the application of the stochastic model of chromatography to reversible reactions as introduced by Keller and Giddings[10] and Kramer,[11] deriving an explicit solution for the peak shapes.

There is, however, at least a third model of chromatography, the so called continuous or dynamic model, which was extensively used in the past for the investigation of on-column reaction kinetic processes by use of solutions from Laplace transformations or simplifications. One of the main problems with the determination of rate constants from chromatographic reactor data is the impossibility to separate the reaction mixture, since the reaction occurs along the whole length of the column. Therefore, the simple determination of conversions from reactant peak areas will be at least more or less inaccurate, causing errors in the calculation of rate constants from the conversions, the equations for which are given by the solutions of the continuous model. Especially in reversible reactions the consideration of the 'coalescence range' (range, in which a mixture of reactants elutes) cannot easily be neglected.

The present paper is devoted to parametric investigations of the analytical approximate peak shape equation for reversible first order reactions derived in detail elsewhere (cf. refs. 13,14) on the basis of the continuous model, especially considering the well-documented case of enantiomerizations.

THEORETICAL

A reversible reaction with a certain distance to equilibrium can be approximated as a limited chain of consecutive reactions. Travelling through the column, the reactant pulse produces one differential product pulse for any local coordinate, and any of those differential pulses produces its own differential pulses and so on, until the end of the chain is reached. The areas of the differential pulses can be evaluated from the continuous model, but their shape must be approximated by a suitable empirical peak shape equation. Since the principle of undisturbed superposition is valid for first order reactions and linear sorption isotherms, the final concentrations can be found by adding all concentration elements involved, i.e., integrating the mathematical terms repeatedly over the spatial coordinate. It should be mentioned that the principle of microkinetic reversibility leads to a certain relation between the individual rate constants in the chiral (stationary) phase: $k_{s1}k_1' = k_{s2}k_2'$, and therefore to an equality of the void time based apparent rate constants, since the rate constants in a non chiral phase are always equal:

$$
\begin{aligned}
k_{a1} &= k_m + k'_1\, k_{s1} \\
k_{a2} &= k_m + k'_2 k_{s2} \\
k_{as} &= k'_1 k_{s1} = k'_2 k_{s2} \\
k_a &= k_m + k_{as}
\end{aligned}
\qquad (1)
$$

Approximating the reversible reaction by a six-step consecutive reaction yields:

$$c = A_0 \{e^{-k_A t_0} \Psi_0(\lambda_0 = 1, t) + \int_0^1 e^{-kat_0} \Psi_1(t, \lambda_0, \lambda_1) + \int_{\lambda_1}^1 e^{-kat_0} \Psi_2(t, \lambda_0, \lambda_1, \lambda_2) + \int_{\lambda_2}^1 e^{-kat_0} \Psi_3(t, \lambda_0, \lambda_1, \lambda_2, \lambda_3) + \int_{\lambda_3}^1 e^{-kat_0} \Psi_4(t, \lambda_0, \lambda_1, \lambda_2, \lambda_3, \lambda_4) + \int_{\lambda_4}^1 e^{-kat_0} \Psi_5(t, \lambda_0, \lambda_1, \lambda_2, \lambda_3, \lambda_4, \lambda_5) d\lambda_0 d\lambda_1 d\lambda_2 d\lambda_3 d\lambda_4 d\lambda_5\} \quad (2)$$

Assuming a Gaussian for the peak shape equations:

$$\Psi_i(t, \lambda_0 \ldots \lambda_i) = \frac{1}{\sqrt{2\pi}\,\sigma_i} e^{-\frac{(t-\mu_i^*)^2}{2\sigma_i^{2*}}} \quad (3)$$

$$\mu_i^* = \mu_1 \lambda_0 + \mu_2 (\lambda_1 - \lambda_0) + \mu_1 (\lambda_2 - \lambda_1) + \ldots + (1 - \lambda_i)\, \mu_{2^{i+1}}$$

The variance is a linear function of the spatial coordinates:

$$\sigma_i^{2^*} = \sigma_1^2 \lambda_0 + \sigma_2^2 (\lambda_1 - \lambda_0) + \sigma_1^2 (\lambda_2 - \lambda_1) + \ldots + (1 - \lambda_i)\, \sigma_{2^{i+1}}^2 \quad (4a)$$

In a further simplification, a linear dependence of the standard deviations on the breakthrough time is assumed

$$\mu_1 < t < \mu_2 : \sigma_i^{2^*} = \frac{\sigma_2^2 (t - \mu_1) + \sigma_1^2 (\mu_2 - t)}{\mu_2 - \mu_1} \quad (4b)$$

$$t < \mu_1 : \sigma_i^2 = \sigma_1^2$$

$$t > \mu_2 : \sigma_i^2 = \sigma_2^2$$

which leads to analytical solutions, resulting in a voluminous formula for the reaction partners, which is available from the authors. The sum of the concentrations (Eq. 2) for both enantiomers yields the total chromatogram.

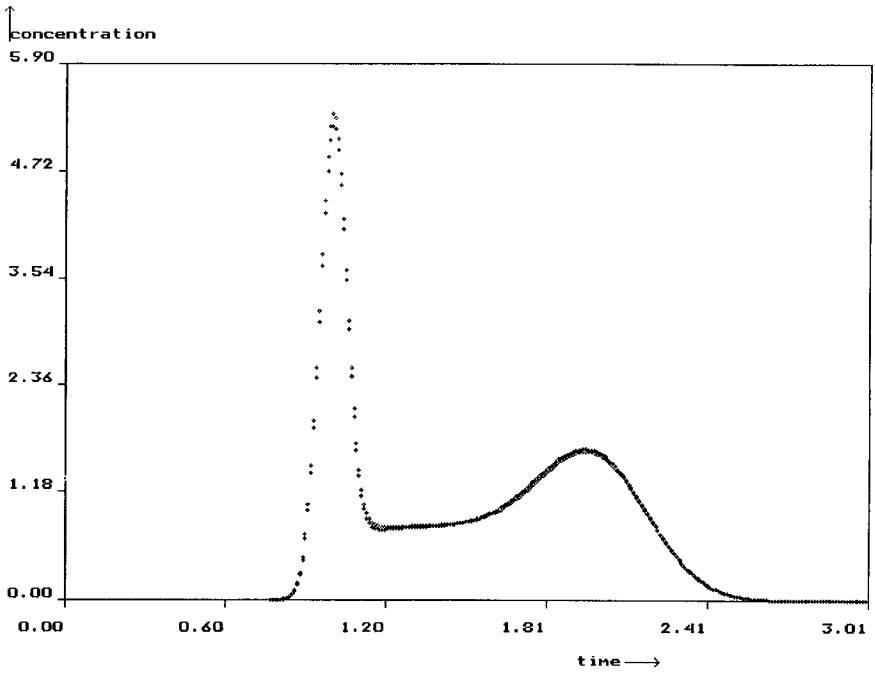

Figure 1. Comparison of theoretical enantiomerization chromatograms as calculated from the numerical integration (crosses) of Eq. 2-3-4a and the analytical solution (circles) from Eq. 2-3-4b (μ_1=1, μ_2=2, σ_1=0.05, σ_2=0.1,$k_a t_0$=0.5).

There are several steps of simplifications to be considered in the following discussion:

- Substitution of the spatial dependence of the variances (Eq. 2, Eq.3 , Eq.4a) by the average temporal dependence on the breakthrough time (Eq. 2, Eq.3, Eq. 4b).

- Agreement of the analytical results from Eq. 2,Eq.3, Eq. 4b with the reference method of finite differences.

- Correspondence of the analytical results from Eq.2, Eq. 3 and Eq. 4b with experimental chromatograms.

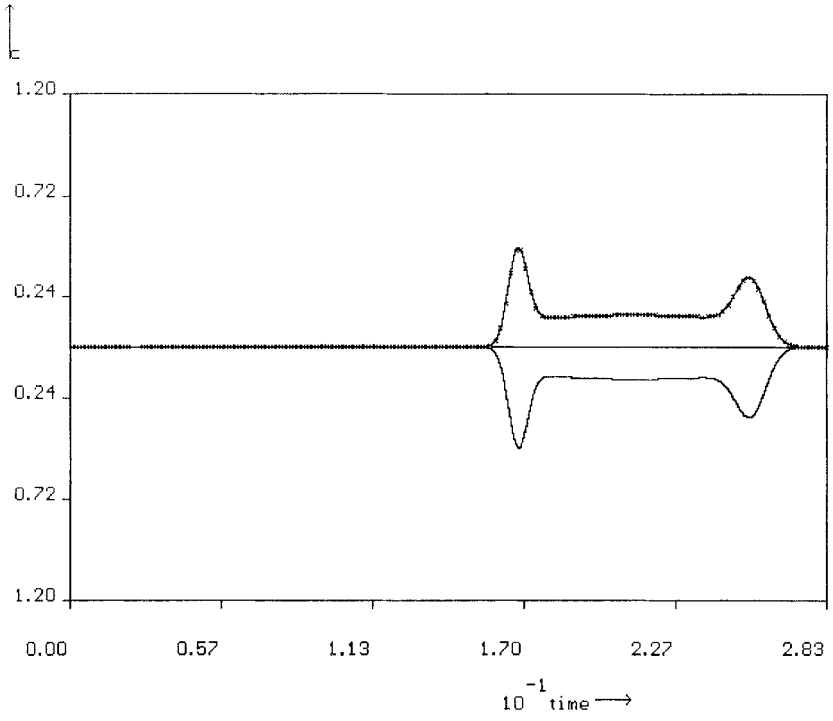

Figure 2. Comparision of theoretical enantiomerization chromatograms as calculated from the finite difference method (upper section) and the analytical equation (crosses in the upper section, and lower section) with μ_1=16.75, μ_2=25.54, σ_1=0.356, σ_2=0.557, $k_a t_0$=1.

RESULTS AND DISCUSSION
NUMERICAL AND EXPERIMENTAL DATA

In order to estimate the influence of the approximation with respect to the standard deviation, Eq.2, Eq.3 and Eq. 4a were integrated numerically for medium conversions, a suitable resolution and with varied standard deviations.

It can be seen from Figure 1 that the agreement between the algorithms is fairly good, which supports the plausibility of the medium standard deviation approach (Eq. 4b). The slight differences between the results are mainly due to the accumulation of numerical errors during the sixfold numerical integration. Therefore, also because of the tremendous time required, the latter cannot serve as a numerical fitting method.

Table 1

Correspondence of the Statistical Moments from the Finite Difference Method (FDM) and from the Analytical Solution of the Model in the Laplace-Domaine (LD)

$k_a t_0$	0.5		1.0		2.0	
Moment	**FDM**	**LD**	**FDM**	**LD**	**FDM**	**LD**
μ_{1tot}	18.37	18.37	19.25	19.24	20.07	20.07
μ_{2tot}	23.93	23.92	23.05	23.05	22.23	22.22
σ_{1tot}	2.59	2.55	2.75	2.72	2.52	2.48
σ_{2tot}	2.60	2.56	2.76	2.73	2.52	2.49

Table 2

Correspondence of the Rate Constants and Moments as Obtained from Fitting the Analytical Solution (FAS) to the Chromatograms from the Finite Difference Method (FDM)

	FDM	**FAS**	**FDM**	**FAS**	**FDM**	**FAS**
$k_a t_0$	**0.5**	**0.499**	**1**	**1.006**	**2**	**2.026**
Defect Area ($A_0 = 1$)		$1.2\ 10^{-2}$		$7.6\ 10^{-3}$		$6.0\ 10^{-3}$
μ_1	16.75	16.74	16.75	16.74	16.75	16.73
μ_2	25.54	24.53	25.54	25.53	25.54	25.53
σ_1	0.356	0.361	0.356	0.360	0.356	0.358
σ_2	0.557	0.565	0.557	0.559	0.557	0.567

Fortunately, Figure 2 demonstrates an excellent agreement of enantiomerization chromatograms as evaluated from a finite difference method (similar to Rouchons method[15]) and from the analytical six step equation with the same input data.

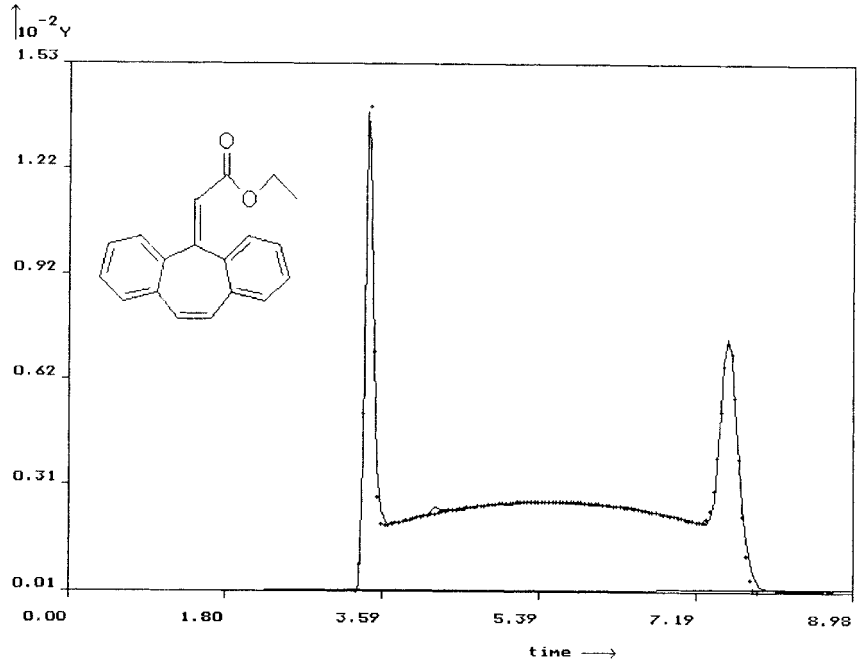

Figure 3. The separation of SLPC-OOH and SLPC-OH. SLPC-OOH was converted to SLPC-OH using PHGPx (0.03 μg protein in total 500μl reaction mixture at pH 7.4, 37°C, 15 min). The minor peaks indicated by arrows (I and II) are probably the 9-OOH and 9-OH isomers of SLPC-OOH and SLPC-OOH.

Despite the impossibility to derive a closed analytical solution for the peak shapes, an analytical solution in terms of the statistical moments can always be derived for linear chromatography reactors. Therefore, the validity of the results from the finite difference method is independently shown by the correspondence of their statistical moments with those analytically calculated from the closed solution in the Laplace-domain (Table 1).

Table 2 compares the results from fitting the analytical equation to the finite difference chromatograms, using Tshebyscheffs minimum criterion (i.e. for equidistant points: minimizing the defect area given by the incongruent parts of the chromatograms). A fitting procedure for experimental chromatograms was written in TURBO PASCAL basing on the following sequence:

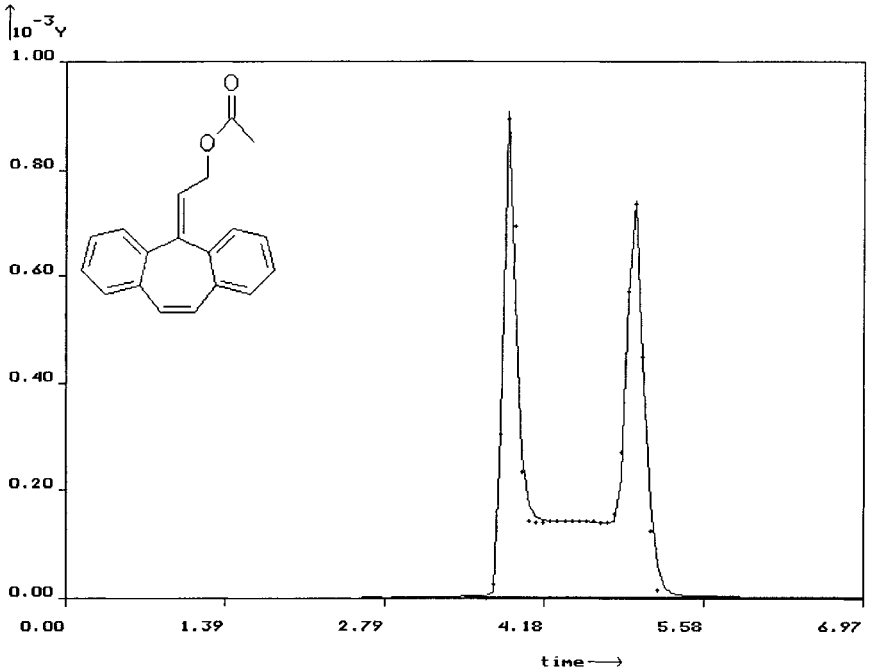

Figure 4. Best fit of an experimental chromatogram (column:Chiralcel OD-H, eluent: hexane/ tert.-butyl-methyl ether 7/3, flow:1.5 ml/min, temperature:30 /C).

In the first and in the second pass, the retention times and the half width values of the reaction chromatograms outer flanks are used for the peak shape data, and the apparent rate constant is systematically varied to minimize the defect area.

Normally, at this stage a reasonable agreement (sufficient to avoid trapping in side minima) is already achieved, and in the final passes the retention times and standard deviations are varied as well, which leads to a considerable improvement of the fitting result. The response factor is evaluated in every pass from the area ratio of the chromatogram referred to and the calculated chromatogram.

Experiments were performed with a Hewlett Packard Liquid Chromatograph 1090, equipped with a DAD (Hewlett Packard) and a Chiralyser (polarimetric detector, IBZ Messtechnik, Hannover), using CHIRALCEL OD-H analytical columns (4.6x250 mm) from Daicel. A Haacke K F3 cryostat was applied to

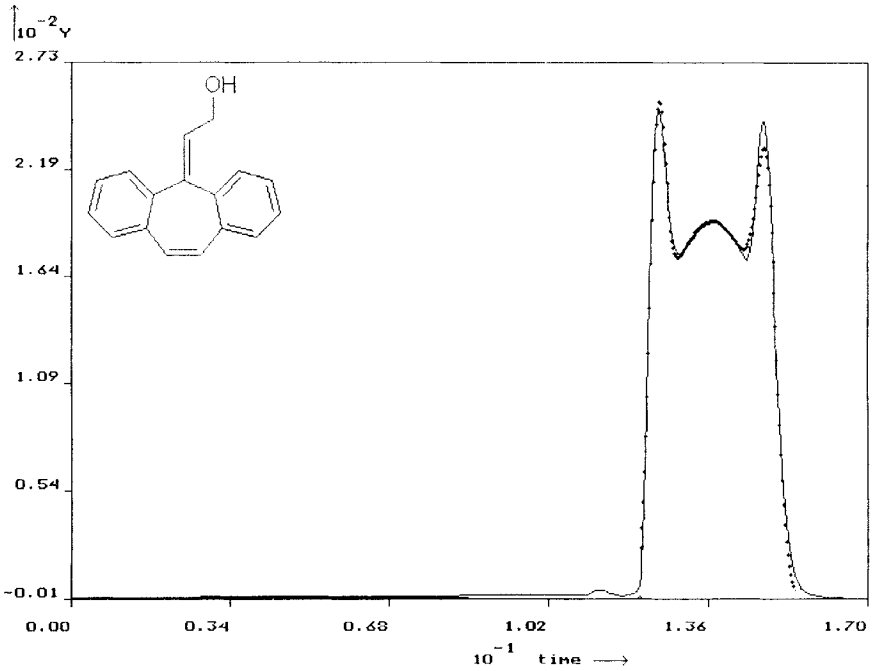

Figure 5. Best fit of an experimental chromatogram (column:Chiralcel OD-H, eluent:hexane/2-propanol 9/1, flow: 0.7 ml/min, temperature:30 /C).

control the column temperature. Solvents were purchased from Merck Darmstadt. Three examples are shown for substances with axial chirality, the donation of which by Professor J.-C- Fiaud (Laboratoire des Synthese asymetrique, URA CNRS 1497 Bat.420, Universite de Paris Sud, Institut de Chimie Moleculaire d'Orsay, F-91405 Orsay Cedex, France) we gratefully acknowledge.

Fig. 3-5 demonstrate excellent or at least sufficient agreement of experimental chromatograms and fitting equation at the possible minimum of the defect area. Table 3 summarizes the names and results of these substances. For comparison, rates constants were evaluated by the Lebl-Gut-Method[16] from the areas of the residual peaks of the injected enantiomer mixture and the area of the intraconverting substance. Though this method produces some systematic error from the incomplete separation of reactant and product, it was chosen for not involving assumptions about the peak shapes or related parameters (plate numbers or standard deviations, mass transfer or diffusion coefficients).

Table 3

Substances Investigated and the Respective Rate Constants

Fig.	Substances	k(FAS) (min^{-1})	k(LEBL-GUT) (min^{-1})
3	dibenzo<a,d>cyclohepten-5-ylidene-acetic acid ethyl ester	0.417 0.189	0.412 0.205
4	acetic acid 2-(dibenzo <a,d>cyclophepten-5-ylidene-)ethyl ester	0.131 0.103	0.119 0.150
5	2-(dibeno <a,d>cyclohepten-5-ylidene-) ethanol	0.109 0.0915	0.148 0.289
6	acetic acid 2-(dibenzo <a,d>cyclohepten-5-ylidene-) ethyl ester	0.132 0.102	corresponds to Fig. 4, however. assuming EMG-peaks for the reactants

The larger deviation of the rate constants from both methods in case of Figure 5 is due to the less effective separation, which increases the systematic error of the Lebl-Gut-method. The main difference between experimental and theoretical chromatograms results from skewed reactant peaks, which could be minimized by introducing EMG-pulses[17] for the original reactants (as shown in Fig. 6) instead of simple Gaussian distributions, i.e. ψ_0 in Eq. 2 becomes:

$$\Psi_0 = \frac{1}{\tau}\exp\left(\frac{1}{2}\frac{\sigma_1^2}{\tau^2} - \frac{t-\mu_1}{\tau}\right)\int_{-\infty}^{\frac{t-\mu_1}{\tau\sqrt{2}}} e^{-\xi^2}\,d\xi$$

However, since the rate constants are mainly 'encrypted' in the bridge between the reactants (the very product curve), they are only slightly changed by this approach.

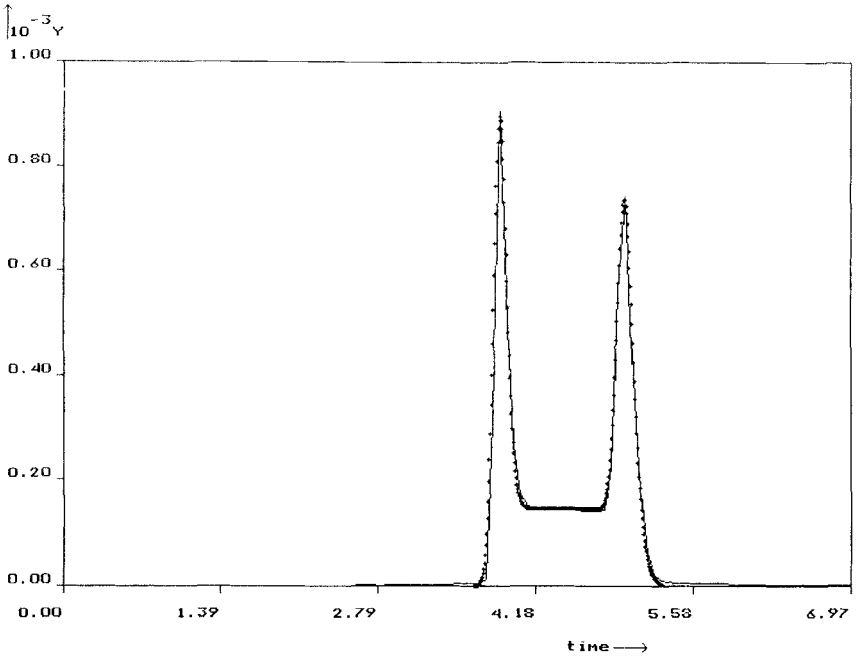

Figure 6. Best fit of the experimental chromatogram in Fig. 4, but using EMG peaks for the reactants.

CONCLUSIONS

The usefulness of the analytical peak shape equation derived from Eq.2, Eq.3 and Eq. 4b could be shown for the special case of enantiomerizations using numerical and experimental examples. In case of skewed reactant peaks the substitution of the simple Gaussian distribution by the EMG distribution improves the agreement between the approximate solution and the chromatogram. However, a considerably skewed peak indicates a slow mass transfer, which could impair the precision of the results. Therefore, experimental conditions should be chosen to avoid noticeable skewing, whenever possible.

SYMBOLS

A_0: inlet peak area
c: concentration (mobile phase)
k_a: apparent rate constant: $k_a=k_s+k'k_s$

k_m: rate constant in the mobile phase
k_s: rate constant in the stationary phase
k': retention capacity
l: column length
λ_i: reduced spatial coordinate for the i-th consecutive reaction step
μ_1: first absolute moment of the primary forward reactant
μ_2: first absolute moment of the primary backward reactant
μ_{1tot}: first absolute moment of the total forward reactant
μ_{2tot}: first absolute moment of the total backward reactant
σ_1: standard deviation of the primary forward reactant
σ_2: standard deviation of the primary backward reactant
σ_{1tot}: standard deviation of the total forward reactant
σ_{2tot}: standard deviation of the total backward reactant
τ: skewness factor of the exponential modyfied Gaussian (EMG)

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