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Analysis of peak asymmetry in chromatography

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Abstract

The knowledge of the symmetry of chromatographic peaks is extremely important regarding the digital signal processing. The significant deviation of the peak shape from the symmetrical peak makes hardly possible the acquisition of chromatographic signal information, such as the retention time, the peak area, the peak width at half peak height, the peak overlapping, etc. In the literature one can find many methods for the determination of the asymmetry factor. For example it is suitable to calculate the skewness from the third central moment. However in case of noisy baseline the value of the skewness oscillates highly depending on the number of points used for the mathematical calculation. In this work a new method is presented for the determination peak shape asymmetry. We order mathematical function to the chromatographic peaks by fitting, and then symmetrical curve is generated with the same peak maximum position and height, the peak width is fitted. The difference of the two functions is constituted and areas of the data differences are calculated, which are really characteristics of the peak asymmetry. Correlation between the area of the difference signal and the asymmetry factor is established. The method was applied for different types of chromatographic peak shapes and the results were interpreted.

Keywords: Peak asymmetry; Curve fitting; Chemometrics; Signal processing

1. Introduction

Most of the real chromatographic peaks are not symmetrical, so it is necessary to introduce an asymmetric factor (skew), which characterizes the peak shape. Before the asymmetric factor examination it is important to know the reasons of the real asymmetry.

The most important reasons for the presence of asymmetry are the column overload, the heterogeneity of the stationary phase surface, the heterogeneity of the column packing, and the extra-column effects. Golshan-Shirazi and Guiochon [1] has demonstrated that significant tailing due to extremely slow mass transfer kinetics can only be observed when the apparent plate number is uncommonly small. The slow mass transfer may explain the peak asymmetry in affinity chromatography, but generally the tailing or fronting presence has to be attributed to the other factors.

Dondi and co-workers [2,3] and Cavazzini et al. [4] have discussed the role of the surface heterogeneity by means of the characteristic function method. Heterogeneity will not result always strongly tailing profiles when the isotherms are linear, the elution profile is approaching a Gaussian peak in this case. The effect of slow mass transfer, adsorption–desorption kinetics and dispersion on the band profile were characterize by Felinger [5] combining the

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stochastic model of chromatography with mobile-phase dispersion.

Extra-column, or instrumental effect are another major source for the emergence of asymmetrical peaks observed in chromatography. Most of the instrumental broadening effects are considered as contributions from continuous mixing. For instance, the volume of any connections, fittings within the chromatograph, or the time constant of the detector may increase the width and the asymmetry of the peak. Tubings, in which sample smoothly travels, introduce Gaussian-type band broadening; and mixer-type volumes introduce exponential tailing. Tailing injection profile can appear in gas chromatography, when the temperature of the injection port is not sufficiently high [6].

Peak asymmetry can be evaluated by means of the empirical peak shape models. There exist several methods that do not require the calculation of the skewness or other exact peak shape parameters. Often quantities can be obtained with other types of calculation or with simple graphical measurements, which describe quickly the peak asymmetry.

In the literature can be found many methods for determination of asymmetry factor [7,8]. The asymmetry factor b/a is a widely used empirical measure to characterize the tailing of asymmetrical peaks. It is usually measured at $0.1h$ peak height. Foley [9] demonstrated that many chromatographic figures of merit (CFOMs) measured at 10% peak height, give more accurate values with much lower relative standard deviation, than at 50, 30 or 5% in the case of Gaussian-, near Gaussian- and EMG peaks.

Using the EMG [10] function, the peak shape asymmetry is characterized by the τ/σ ratio. Increasing the value of parameter τ/σ, it can obtained much more tailing peaks, and τ/σ going to zero, the peak shape becomes symmetrical Gaussian one. As both the skew and the excess of the EMG peak shape are determined by the ratio τ/σ, it is very useful to establish a relationship between τ/σ and the empirical determined b/a. The empirical relationship that relate the peak shape parameters of the EMG function and the asymmetry factor are based on the measurements at $0.1h$ peak height fraction. The variance of the asymmetrical peak is estimated as

$$\mu_2' = \sigma^2 + \tau^2 = \frac{w_{0.1}^2}{1.76(b/a)^2 - 11.15b/a + 28} \qquad (1)$$

where $w_{0.1} = a + b$ is the peak width at 10% of the peak maximum. The Gaussian standard deviation can be calculated as

$$\sigma = \frac{w_{0.1}}{3.27b/a + 1.2} \qquad (2)$$

Parameter τ can be calculated from Eqs. (1) and (2). From the location of the peak maximum t_m, the center of the Gaussian part t_R is calculated by

$$t_R = t_m - \sigma\,[-0.19(b/a)^2 + 1.16\,b/a - 0.55] \qquad (3)$$

The absolute relative error of the peak shape parameters determined by Eqs. (1) and (2) is less than 1.5% provided that $b/a \leqslant 2.76$. The b/a ratios measured at various peak height fractions show similar tendency, but the lower the peak height fraction the higher the sensitivity of the dependence on the ratio τ/σ [11].

McWilliam and Bolton [12] have examined the peak area under the EMG curve at various peak heights, and concluded that the relative peak area is a very important characteristic in determining the asymmetry and peak shape.

The determination of the retention time of asymmetrical peaks is not clearly defined, either. It can be interpreted as maximum (apex), the median, or the mean of the peak. The median means the time when 50% of the solute has eluted from the column, and is on the descending part of the peak; the mean is at still larger time. With the nowadays routine integrators, the location of the median and the mean is inconceivable, although by the integration of the peak, the median is easily obtained, since that point corresponds to 50% of the peak area. The maximum, the median, and the mean can deviate in case of modest asymmetry, too.

The US Pharmacopeia (USP) recommends the use of another factor for the characterization of peak asymmetry. The tailing factor (T) is based on the measurement of the half-width parameters a and b at 5% of the peak height, and is calculated as

$$T = \frac{a + b}{2a} \qquad (4)$$

The factor T is very close to the asymmetry factor when the tailing is moderate. The relationship between the asymmetry factor and the USP tailing factor depends on the peak shape. If the asymmetrical peak can be modeled with the EMG peak shape, the following relationship exists:

$$T = 0.6(b/a)_{0.1} + 0.4 \quad (5)$$

In case of biGaussian peak shape model will shall have

$$T = 0.5(b/a)_{0.1} + 0.5 \quad (6)$$

Farnan et al. [13] introduced an asymmetry factor, which is independent of peak shape models. The asymmetry factor is defined as

$$\chi = \frac{t_m - t_0}{\mu_1 - t_0} \quad (7)$$

where t_m is the location of peak maximum, μ_1 is the first moment (mean) of the peak, and t_0 is the hold-up time. The asymmetry factor is unity when the peak maximum position is identical to the first moment of the peak, and zero when $t_m = t_0$.

A directly measurable parameter was selected by Cai and Wu [14] to describe the asymmetry of the peak. It was determined from the leading half of the peak and defined as

$$\beta = \frac{W_{0.1(f)}}{W_{0.5(f)}} \quad (8)$$

where $W_{0.1(f)}$ and $W_{0.5(f)}$ are the front half-width of the peak measured at peak height fractions of 0.1 and 0.5, respectively. Parameter β is well defined for indicating the asymmetry of an EMG peak in those cases where overlapping peaks were concerned.

The distribution function method (DFM) [15] is very sensitive method of comparison between different peak shapes, which compares their normalised integrals. This method has been used to detect small variations in shape of the chromatographic peaks when the mass of the solute injected is increased. To compare peak shapes of the different widths by the DFM, the time scale must be normalised so that peaks span over equal space. To achieve this, the times t_1 (when the signal reaches 10% of the peak maximum on the front side of the peak) and the t_2 (when the signal decreases to 5% of the peak maximum on the rear side) are calculated. Then, on the normalised time scale

$$\theta = \frac{t - t_1}{t_2 - t_1} \quad (9)$$

the peak shapes can be compared after an area normalisation

$$g(\theta) = \frac{f(|t_2 - t_1)\theta + t_1|)}{\int_{t_1}^{t_2} f(t)\mathrm{d}t} \quad (10)$$

The distribution function is

$$F(\tau) = \int_0^{\tau} P(\theta)\mathrm{d}\theta \quad (11)$$

where τ is the normalised time corresponding to the elution of a certain fraction of the total peak area. The same procedure should be followed for the second peak profile, yielding the second normalised distribution function, and the deviation between the two peak shapes can be evaluated from the curvature of the plot.

2. Results and discussion

In this work we have examined the tendency of change of the asymmetry factor of a previously presented mathematical function [16]. The used chromatographic function for the data acquisition and fitting is as follows:

$$f(t) = \begin{cases} 0, & \text{if } t < M - \dfrac{D(4-a^2)}{2a} \\ H \cdot \exp\left\{ \left(\dfrac{4}{a^2} - 1 \right) \cdot \left[\ln\left(1 + \dfrac{2a(t-M)}{D(4-a^2)} \right) - \dfrac{2a(t-M)}{D(4-a^2)} \right] \right\} \end{cases} \quad (12)$$

where M is the peak maximum (s); H is the peak height ($H > 0$); a is the asymmetry factor ($0 < a < 2$); and D is standard deviation ($D > 0$), s.

We started from the concept that the asymmetry factor characterises the deviation of the chromatographic peak shape from the symmetrical one. Constituting the difference of the asymmetrical and symmetrical peaks, and the areas of the difference curve are calculated on the ascending and descending half part of the peak, correlations concerning about the asymmetry factor can be established.

First the curves of the chromatographic peaks with different asymmetry factor (a) are simulated. Then a symmetrical curve is fitted with the same peak maximum position and height. Fig. 1 shows the

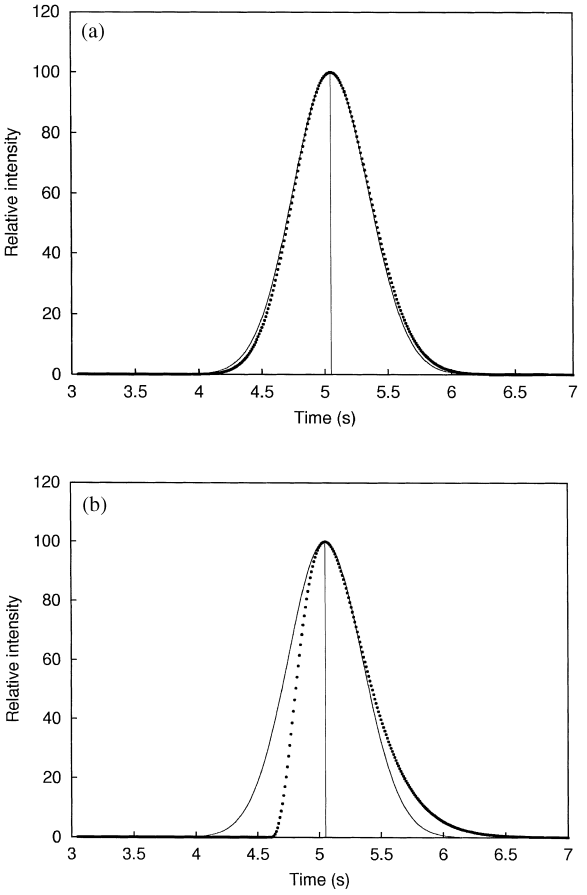

Fig. 1. The simulated curves of function (9) of diverse asymmetrical peaks (a) $a=0.15$; (b) $a=1.0$ (pointed line) and the generated symmetrical ($a=10^{-6}$) curve (solid line) of the same function with the same parameters.

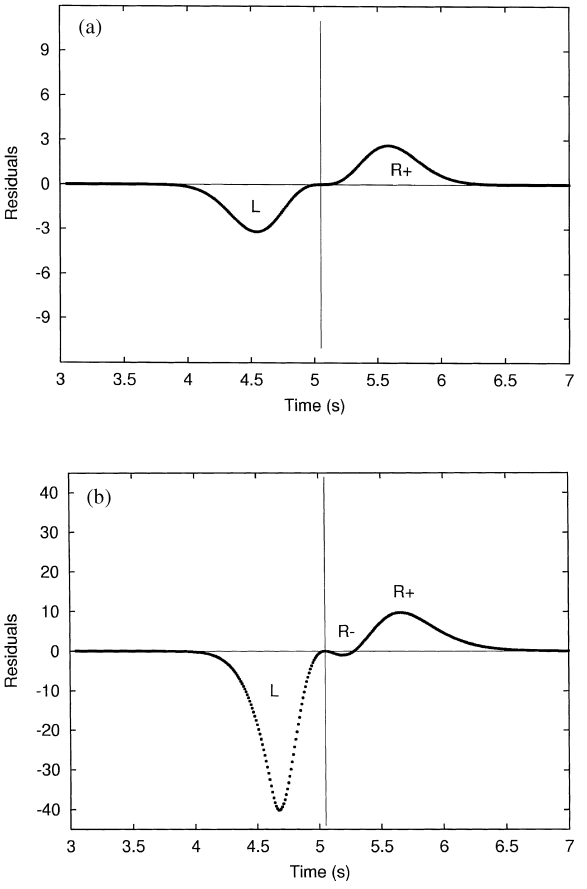

Fig. 2. The difference of the signals of asymmetrical (a) $a=0.15$, (b) $a=1.0$ and symmetrical ($a=10^{-6}$), ascending and descending half parts of the peaks.

simulated curves of diverse asymmetrical peaks (a) $a=0.15$, (b) $a=1.0$, and the generated symmetrical ($a=10^{-6}$) curves of the same function with the same parameters. The difference of the signals of asymmetrical ((a) $a=0.15$; (b) $a=1.0$) and symmetrical curves is constituted, and the areas of the ascending and descending half parts of the peaks are calculated (Fig. 2a,b). It is observable that increasing the asymmetry factor, the absolute value of the left part area $|L|$ (left from the peak maximum) increases, and the right part $|R|$ (right from the peak maximum) has only a positive dominion at the beginning and then it is divided into a negative $|R_-|$ and a positive $|R_+|$ part. Representing graphical the sum of the absolute values of the left and right part areas ($|L|+|R_+|+|R_-|$) in function of asymmetry factor, it is observable that when starting, the sum increases linearly, and arriving at the value $a=1.2$ the sum curve increases slightly exponentially (Fig. 3). The function fitted to the sum curve is $y=ax+b+\exp(cx+d)$; the final sum of square residuals is r.s.s. $=0.2797$. To explain the reason of this tendency it is necessary to examine the areas of the difference curve separately. Representing graphically the absolute value of the left part area $|L|$ in function of asymmetry factor, it is noticeable that the fitted curve is a slightly ascending parabola (r.s.s. $=0.1383$) (Fig. 3); that is not sufficient to explain the exponential ascending part of the sum curve. Examining separately the right hand side parts of the difference curve

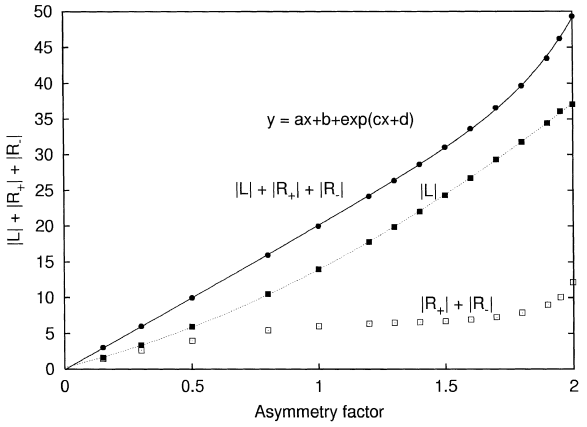

Fig. 3. Representation of the sum of absolute value of the area of difference curve ($|L|+|R_+|+|R_-|$) in function of a factor (solid line). L-area of the difference curve of asymmetrical and symmetrical peak on the *ascending half part* of the chromatographic peak (left from the peak maximum). R-area of the difference curve of asymmetrical and symmetrical peak on the *descending half part* of the chromatographic peak (right from the peak maximum). The exponents of the fitting curve are: $a=20.099\pm0.147$; $b=-0.0752\pm0.111$; $c=4.590\pm0.231$; $d=-6.977\pm0.479$. Plot of $|L|$ area of the difference curve of asymmetrical and symmetrical peaks in function of a factor (dotted line). The curve was fitted with a parabolic function, the exponents of which are: $e=4.803\pm0.083$; $f=8.895\pm0.188$; $g=0.2397\pm0.092$.

in function of asymmetry factor, it can observe that $|R_-|$ slightly increases at the beginning, than goes up abrupt exponentially (r.s.s.=0.4324) (Fig. 4); the

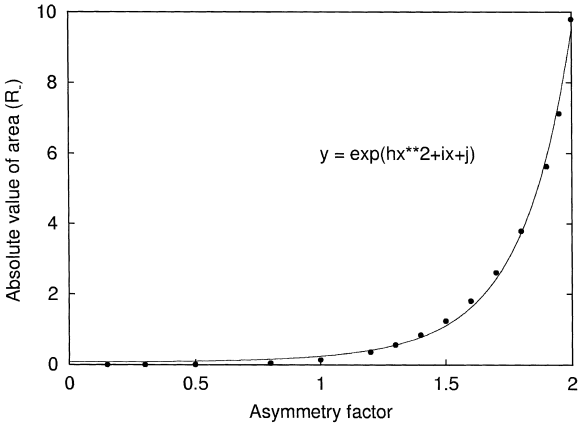

Fig. 4. Representation of $|R_-|$ area in function of a factor. The fitting function is an exponential one, its exponents are: $h=1.252\pm0.352$; $i=-0.0923\pm1.252$; $j=-2.571\pm1.113$.

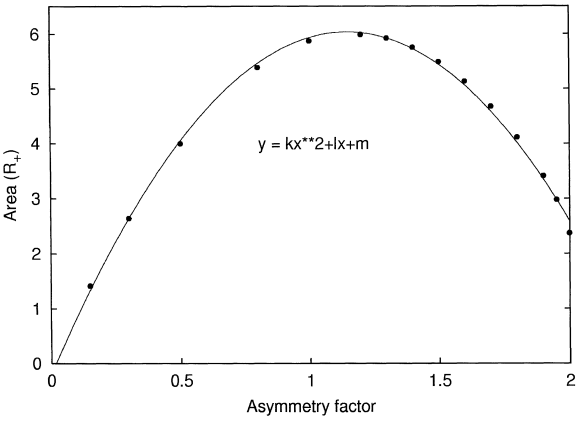

Fig. 5. Representation of $|R_+|$ area in function of a factor. The fitting curve is a parabola, its exponents are: $k=-4.729\pm0.071$; $l=10.850\pm0.161$; $m=-0.1887\pm0.079$. The maximum of the parabola is at $a=1.2$.

fitted curve of the $|R_+|$ in function of asymmetry factor is a parabola, which goes through a maximum at value $a=1.2$ (r.s.s=0.1013) (Fig. 5). Even if the descending part of the parabola ($|R_+|$) compensates the exponential ascending part ($|R_-|$) of the curve, this can be state only to $a=1.2$ when the exponential curve slightly increases; on further values of asymmetry factor the exponential function is dominant opposite to the quadratic one. This is the explication of sum curve tendency ($|L|+|R_+|+|R_-|$) in function of asymmetry factor, that at the beginning the fitting curve is a linear one, and than it goes into an exponential one.

3. Application

The method can be applied for different types of chromatographic peak shapes (gas, liquid). The steps of determination of real asymmetry factor of chromatographic peaks are as follows:

(1) Function (12) is fitted to the measured digitised data.
(2) The peak width of the symmetrical curve is fitted to the measured digital data with the same peak maximum position and height of the fitted asymmetrical curve.
(3) The difference of the fitted asymmetrical and symmetrical peak is calculated.

(4) The areas of the difference signal of the asymmetrical and symmetrical curve on the ascending and descending half part of the peak are determined.
(5) The $|L|$ and $|L|+|R_+|+|R_-|$ areas (after normalisation) characterise very faithfully the real asymmetry of the measured chromatographic peak.

Using our concept, next we determine the asymmetry of different phenol derivatives resolved by HPLC (Fig. 6).

Experimental conditions:

Stationary phase:	LiChrosorb RP-18
Column:	250×3.0 mm
Temperature:	25 °C
Detector:	UV 280 nm
Mobile phase:	Methanol–water (50/50)+0.001 *M* $HClO_4$
Sample concentration:	0.25×10^{-2} *M*
Injection volume:	20 μl

To obtain the asymmetry factor of the peaks, we obey the above steps (1–5) of determination. As an example Fig. 7 shows the fitted asymmetrical and symmetrical curves of the peak number 4 of the chromatogram from Fig. 6 (steps 1–2). The results of the chromatogram evaluation of different flow velocities and the data obtained from the curve fittings are summarised in Table 1.

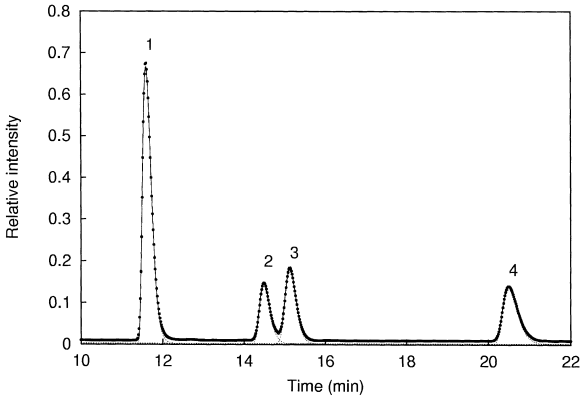

Fig. 6. Chromatogram of the resolution of different phenol derivatives using HPLC. The compounds of the peaks are the following: (1) 2-nitrophenol, (2) 4-ethylphenol, (3) 2,4-dimethylphenol, and (4) 2,3-dichlorophenol.

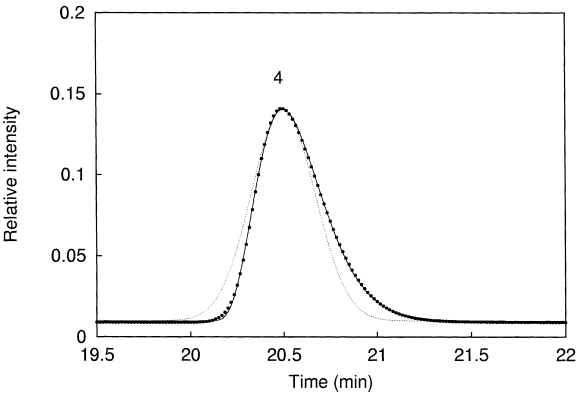

Fig. 7. Fitted asymmetrical (solid line) and symmetrical curve (dotted line) of the peak number 4 of the chromatogram from Fig. 6 (flow velocity, 0.9 ml/min).

Fig. 8 illustrates the difference signal of the asymmetrical and symmetrical curve of the peak number 4 of the chromatogram from Fig. 6. It is noticeable that the area of the difference signal on the descending part of the peak (right from the peak maximum) has only a positive dominion (R), and this tendency still remain at all other peaks from the chromatogram. Table 2 shows the calculated normalised areas of the difference signals left and right from the peak maximum position. It is observable, that increasing the flow velocity the areas $|L|$ and $|R|$ decrease linearly, which correspond to the reality (Fig. 9).

4. Conclusion

The '*a*' (asymmetry) factor characterises very faithfully the difference measure from the symmetrical peak shape, but does not give us information about, on which part of the peak appears the difference from the symmetrical one, and how large is the ratio of the differences comparing each to the other on different places.

The method worked out here gives the absolute value and the ratio of the difference between the left and right parts ($|L|/|R|$) of the peak.

According to this method it can also determine the asymmetry after peak maximum position above (R_+)

Table 1
Data obtained from the asymmetrical and symmetrical curve fittings of phenol derivatives in function of flow velocity using function (12)

Peak	Flow velocity (ml/min)																								
	0.5					0.6					0.7					0.8					0.9				
	M (min)	*H*	*a*	*D* (min)	*w*	*M* (min)	*H*	*a*	*D* (min)	*w*	*M* (min)	*H*	*A*	*D* (min)	*w*	*M* (min)	*H*	*a*	*D* (min)	*w*	*M* (min)	*H*	*a*	*D* (min)	*w*
1	21.88	0.6661	0.9618	0.1988	0.0104	17.53	0.6713	0.9807	0.1723	0.0086	14.92	0.6664	0.9626	0.1493	0.0098	13.32	0.6594	0.9653	0.1406	0.0091	11.60	0.6553	0.8797	0.1150	0.0123
1′	21.88	0.6661	10^{-6}	0.1677	0.0104	17.53	0.6713	10^{-6}	0.1444	0.0086	14.92	0.6664	10^{-6}	0.1269	0.0098	13.32	0.6594	10^{-6}	0.1189	0.0091	11.60	0.6553	10^{-6}	0.0996	0.0123
2	27.29	0.1438	0.7714	0.2364	0.0046	21.91	0.1416	0.7797	0.2016	0.0045	18.67	0.1395	0.7684	0.1777	0.0047	16.68	0.1386	0.7491	0.1655	0.0048	14.49	0.1357	0.6473	0.1304	0.0053
2′	27.29	0.1438	10^{-6}	0.2229	0.0046	21.91	0.1416	10^{-6}	0.1889	0.0045	18.67	0.1395	10^{-6}	0.1657	0.0047	16.68	0.1386	10^{-6}	0.1547	0.0048	14.49	0.1357	10^{-6}	0.1190	0.0053
3	28.48	0.1828	0.6177	0.2488	0.0046	22.88	0.1805	0.6472	0.2128	0.0045	19.50	0.1784	0.6483	0.1866	0.0047	17.45	0.1779	0.6403	0.1748	0.0048	15.13	0.1715	0.4890	0.1355	0.0053
3′	28.48	0.1828	10^{-6}	0.2421	0.0046	22.88	0.1805	10^{-6}	0.2067	0.0045	19.50	0.1784	10^{-6}	0.1822	0.0047	17.45	0.1779	10^{-6}	0.1713	0.0048	15.13	0.1715	10^{-6}	0.1349	0.0053
4	38.44	0.1383	0.9348	0.3599	0.0084	31.04	0.1304	0.8464	0.2787	0.0085	26.46	0.1353	0.9367	0.2751	0.0088	23.81	0.1343	0.8861	0.2565	0.0091	20.50	0.1305	0.7702	0.1878	0.0100
4′	38.44	0.1383	10^{-6}	0.3149	0.0084	31.04	0.1304	10^{-6}	0.2466	0.0085	26.46	0.1353	10^{-6}	0.2394	0.0088	23.81	0.1343	10^{-6}	0.2296	0.0091	20.50	0.1305	10^{-6}	0.1699	0.0100

w, linear coefficient of the fitted baseline; 1–4, asymmetrical fitted peaks; 1′–4′, symmetrical fitted peaks.

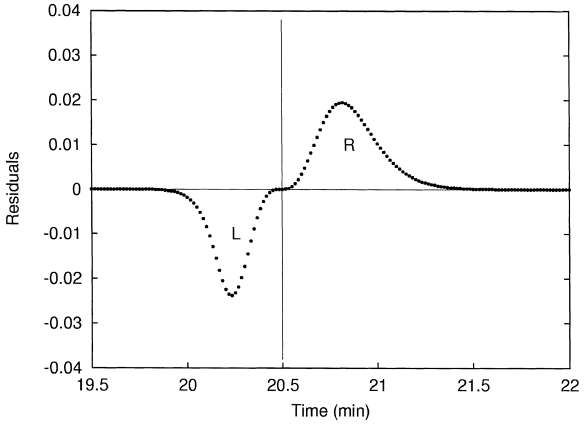

Fig. 8. Representation of the difference signal of fitted asymmetrical and symmetrical curve of the peak number 4 of the chromatogram from Fig. 6 (flow velocity, 0.9 ml/min).

and under (R_-) axe $x=0$, as well as the ratio comparing each to other (R_+/R_-)

The proposed method is very useful for examination of asymmetry factor of a real chromatographic peak (gas, liquid), which determination helps to select the best mathematical function describing the peak shape.

The examination of asymmetry gives the possibility to analyse the physicochemical processes in the column.

As the result of examination it can be proved, that the value of area |L| can also be applied as an asymmetry factor, because it correlates well with the value of '*a*' (Fig. 3).

The difference of the curve of the asymmetrical and symmetrical chromatographic peaks characterise faithfully the asymmetry of the peak and after

Table 2
Normalised calculated areas (|L| and |R|) of the difference signals of phenol derivatives in function of flow velocity

Flow velocity (ml/min)	Peak number 1			2			3			4		
	|L|	|R|	|L|/|R|	|L|	|R|	|L|/|R|	|L|	|R|	|L|/|R|	|L|	|R|	|L|/|R|
0.5	4.909	7.679	0.6394	6.238	5.827	1.0705	5.521	4.667	1.1830	9.740	12.408	0.7850
0.6	4.334	6.778	0.6748	5.254	5.149	1.0204	4.992	4.149	1.2032	6.469[a]	9.117[a]	0.7096[a]
0.7	3.825	5.668	0.6568	4.430	4.609	0.9612	4.506	3.508	1.2845	7.312	9.642	0.7583
0.8	3.539	5.388	0.6465	3.997	4.215	0.9483	4.227	3.199	1.3214	6.860	8.133	0.8435
0.9	2.621	4.054	0.6465	2.100	3.501	0.5998	2.554	1.855	1.3768	4.038	5.203	0.7761

[a] Defective value.

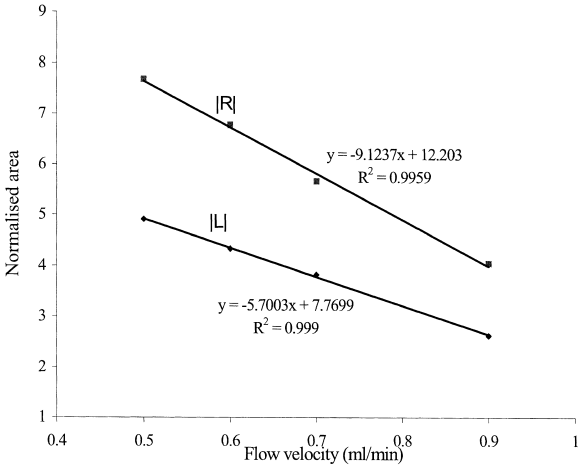

Fig. 9. Dependence of the normalised areas |L| and |R| in function of flow velocity (peak number 1 of the chromatogram from Fig. 6; Table 2); (♦), (■) measured value.

normalisation does not depend on peak maximum position, height and width.

In general the simple models determine the asymmetry factor between two points of the peak at a certain peak height (0.05*h*; 0.1*h*; 0.5*h*; 0.606*h*; etc.), thus in chromatographic practice, mainly in case of distorted shapes, the same asymmetry values can appear for different peak shapes. The method suggested by us shows that at different peak shapes surely different asymmetry values (L; R) can be calculated, because every point of the peak is taken into consideration for the determination of asymmetry.

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