A TLC DETERMINATION OF THE UNKNOWN SUBSTANCES USING HETP RATIOS BETWEEN THE UNKNOWN AND THE REFERENCE COMPONENT

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SUMMARY

The authors give an account of the experiments concerning the effect of the amount of sample on the increase in HETP value. The results obtained show that the relation can be represented by the equation

$$\log \mathrm{HETP}_{(A)} = \log \mathrm{HETP}_0 + Km_A$$

The HETP value for each spot can be calculated by means of the equation:

$$\mathrm{HETP} = \frac{(d_{1/2} - d_{\max})^2}{2 \cdot \ln 2 \cdot L\,(R_F - R_F{}^2)}$$

where

$d_{1/2}$ = distance from the start to the half-maximum concentration
$d_{\max}$ = distance from the start to the maximum concentration
L = distance from the start to the front

By adding the reference component to the mixture, the constant K is obtained, and HETP_0 is calculated by means of extrapolation. The amount of the unknown component (i) is calculated by the equation:

$$m_i = \frac{\log \mathrm{HETP}_i - \log \mathrm{HETP}_0}{K}$$

Methods of quantitative determination in thin layer chromatography are usually divided in two groups. The first group comprises the methods bases on the spot extraction followed by one of the conventional microchemical analyses. In this case TLC is merely a method of quantitative separation. In the second group we classify procedures based either on direct measurement of the intensity of the spot, colouring *in situ* or by determining the optical density of the spots on a photograph. Apart from the optical methods which are most commonly used, other physical methods can also be applied. Again, two different methods are distinguished, *viz* either the determination of the integral intensity of the spot or differential measurements. In the latter case the area under the curve is proportional to the amount of substance.

The object of the present study, which belongs to the latter group, was to find out the relationship between the amount of substance and its HETP value.

PRINCIPLES

The HETP value of the spot of a particular component in the developed chromatogram depends on the system solvent–adsorbent, initial spot area at the start, longitudinal diffusion of molecules during the process of development and the rate of establishing the equilibrium between molecules and the stationary phase which is related to "mass-transfer" factor. VAN DEEMTER gives the following equation

$$\text{HETP} = A + B/v + Cv \qquad (1)$$

where A represents the "eddy diffusion" effect comprising the pore size (grain size) factor, layer thickness and the solvent viscosity; B/v is the longitudinal diffusion which according to SNYDER is negligible in TLC; Cv is the mass transfer factor depending on the mass ratio between the substance and the amount of adsorbent bound to it[1].

In a simple form and in the first approximation, the equation (1) can be expressed as:

$$H = H_0 + H' \qquad (2)$$

where H_0 is obtained by extrapolating H to zero concentration.

$$H_0 = \lim H_{m_A \to 0}$$

H' depends on the mass transfer factor only,

$$H' = \mathrm{f}\,(m_A/m_{\text{ads}}) \qquad (3)$$

Therefore if we know the relationship between m_A/m_{ads} and HETP it becomes possible to determine quantitatively unknown substances by measuring and calculating the HETP values of different spots.

EXPERIMENTAL

The object of the present study was to determine:

(a) deviations from the mean values of the HETP for different substances,

(b) the relationship between the amount of the substance and the HETP,

(c) the error of the method.

Experiments were carried out in a usual way on 20 × 20 cm plates. Desaga apparatus was used to spread the silica layer (0.25 mm) of Silica Gel G. (E. Merck). Samples of the same volume (0.1 μl) were applied by means of a micropipette; the total quantity of the substances under study were varied by changing the concentration,

Plates were activated for 2 h at 110°. The development was discontinued after the solvent front had reached a distance of approximately 10 cm from the start. Substances used were:

Sudan brown	(SBr),	R_F 0.47
Sudan red III	(SR),	R_F 0.21
Fettrot	(FR),	R_F 0.38
Sudan blue	(SBl),	R_F 0.24
Sudan green	(SG),	R_F 0.53
Fettreingelb	(FG),	R_F 0.84

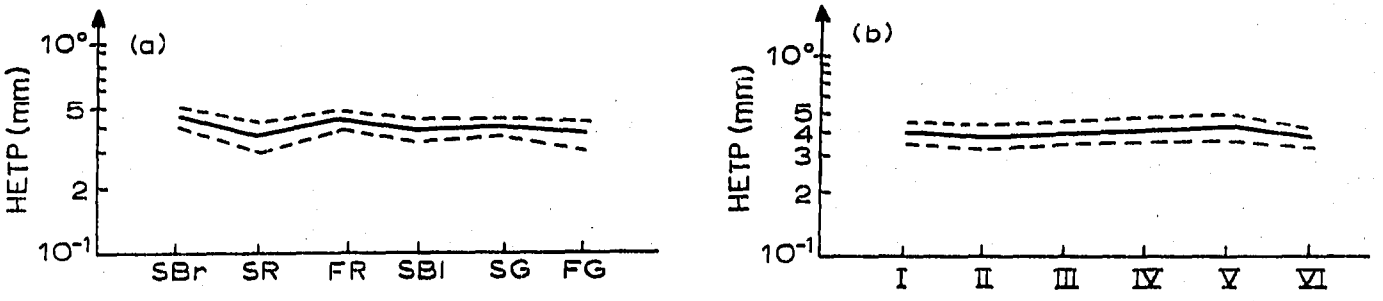

Fig. 1. The diagrams show the mean value of HETP for all substances (b), and the mean HETP value for each substance (a). The amount of each component is 5 μg, according to Table I.

The total amount of each substance varied from 2–20 μg, as shown in the tables.

The measurement of H (HETP) values indicated that satisfactory results could not be obtained by using the equations proposed by GIDDINGS, SNYDER, STAHL AND BRENNER[2,3]. Therefore, we calculated the HETP values by means of the following equation:

$$\text{HETP} = \frac{(d_{1/2} - d_{\max})^2}{2 \cdot \ln 2 \cdot L\,(R_F - R_F^2)} \tag{4}$$

This expression is obtained from the basic CRAIG equation for the component distribution (M_{0A}) in counter-current extraction between the start and the front.

$$C_{dA} = \frac{M_0 \sqrt{n}}{L \sqrt{\{2\pi\,(R_F - R_F^2)\}}} \cdot e^{-\frac{(d - d_{\max})^2}{2HL\,(R_F - R_F^2)}} \tag{5}$$

where

n = number of plates
d = distance from the start
$d_{1/2}$ = distance from the start to the half-maximum concentration
$d_{\max}$ = distance from the start to the maximum concentration, by inserting the values for R_F, H and L.

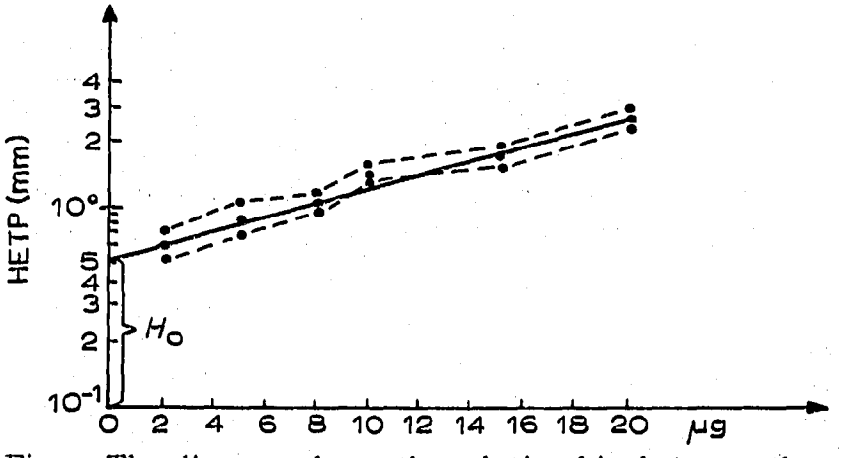

Fig. 2. The diagram shows the relationship between the amount of substance and the HETP value.

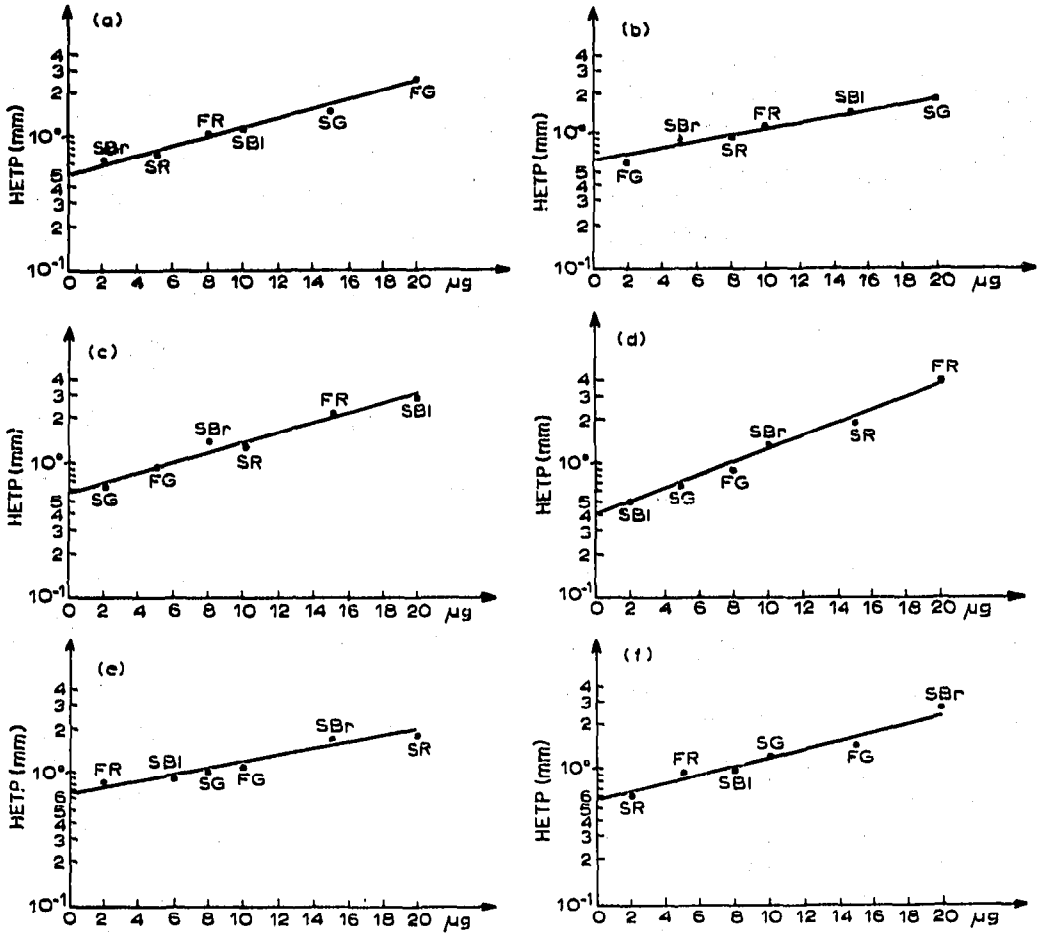

Fig. 3. The 6 diagrams (a–f) show the relationship between the amounts of the components and the HETP values, where the ratios of the amounts of the components in the mixtures are changed according to Table III.

The distance ($d_{1/2} - d_{max}$) was determined from photographs of the original chromatogram by means of a Zeiss Schnellphotometer. Results are summarized and are shown in Tables I–III and Figs. 1–3.

ABLE I

IE AMOUNT OF EACH COMPONENT IN THE MIXTURES APPLIED AT THE START AND THE MEAN VALUE OF HETP BTAINED

II, III, IV, V, and VI denote the positions where the mixture is applied.

ot	*Amounts of components (µg)*						$H \pm \sigma$
	I	*II*	*III*	*IV*	*V*	*VI*	
dan brown (SBr)	5	5	5	5	5	5	0.45 ± 0.05
dan red III (SR)	5	5	5	5	5	5	0.36 ± 0.06
ttrot (FR)	5	5	5	5	5	5	0.43 ± 0.04
dan blau (SBl)	5	5	5	5	5	5	0.38 ± 0.05
dan green (SG)	5	5	5	5	5	5	0.40 ± 0.04
ttreingelb (FG)	5	5	5	5	5	5	0.37 ± 0.06
± σ	0.40 ± 0.05	0.38 ± 0.05	0.40 ± 0.07	0.42 ± 0.06	0.43 ± 0.06	0.38 ± 0.04	

TABLE II

THE QUANTITATIVE RELATIONSHIP BETWEEN THE SUBSTANCES IN THE MIXTURES APPLIED AT THE START AND THE MEAN VALUE OF HETP OBTAINED

Spot	*Amounts of components (μg)*					
	I	*II*	*III*	*IV*	*V*	*VI*
Sudan brown (SBr)	2	5	8	10	15	20
Sudan red III (SR)	2	5	8	10	15	20
Fettrot (FR)	2	5	8	10	15	20
Sudan blau (SBl)	2	5	8	10	15	20
Sudan green (SG)	2	5	8	10	15	20
Fettreingelb (FG)	2	5	8	10	15	20
$H \pm \sigma$	0.45 ± 0.05	0.50 ± 0.07	0.58 ± 0.06	0.73 ± 0.06	0.85 ± 0.07	1.16 ± 0.10

TABLE III

THE RATIOS OF THE AMOUNTS OF THE COMPONENTS OF THE MIXTURES APPLIED AT THE POSITIONS I–VI

Spot	*Amounts of components (μg)*					
	I	*II*	*III*	*IV*	*V*	*VI*
Sudan brown (SBr)	2	5	8	10	15	20
Sudan red III (SR)	5	8	10	15	20	2
Fettrot (FR)	8	10	15	20	2	5
Sudan blau (SBl)	10	15	20	2	5	8
Sudan green (SG)	15	20	2	5	8	10
Fettreingelb (FG)	20	2	5	8	10	15

DISCUSSION

It is interesting to note that spots of some substances deviate from the theoretical positions and are found at higher HETP values. This phenomenon appears for SBr and FR, in all cases. The possible explanation is that these substances are in fact mixtures of two isomers having very close R_F values so that they do not separate. A semilogarithmic plot of results shows that all the points lie on a straight line and fit the equation:

$$\log \mathrm{HETP} = \log \mathrm{HETP}_0 + Km_A \qquad (6)$$

This expression corresponds to a graphical presentation given by SNYDER for the dependence of HETP values on the mass-transfer factor. However, it is difficult to understand why the spot size should be proportional to the logarithm of the substance quantity. The comparison of the experimental data which fits the empirical eq. (6) with the theoretical expression

$$\mathrm{HETP} = \mathrm{HETP}_0 + \mathrm{f}(m_A)$$

shows certain similarities but not complete identity.

CONCLUSION

The preceding data permit us to conclude that the method is applicable for quantitative determinations of unknown substances in the mixture. The main advantage of the method is that it is not based on the measurement of the total area under the photometrically scanned curve, but on the determination of the half-maximum breadths of the peaks and calculation of the HETP values. The error does not exceed 5%.

REFERENCES

1 L. R. SNYDER, *Anal. Chem.*, 39 (1967) 698.
2 C. GIDDINGS, *Anal. Chem.*, 37 (1965) 60.
3 E. STAHL, *Dünnschichtchromatographie*, Springer-Verlag, Berlin, 1962, S. 100.
4 R. E. TREYBAL, *Liquid Extraction*, McGraw-Hill, New York, 1963, p. 377.

DISCUSSION

DE LIGNY: Dr. TURINA and I used different expressions for the HETP and one might think that at least one of them must be incorrect. However, this is not the case. Both HETP definitions are correct, the difference stemming from the fact that they are based on different physical models of the chromatographic process.

From a counter-current extraction a solute concentration distribution results, characterized by:

$$\mu = np \quad (1)$$
$$\sigma^2 = npq \quad (2)$$

where

μ = mean of the concentration distribution
σ = standard deviation of the concentration distribution
n = number of equilibrations
p = fraction of the solute transported to the next extraction element after each equilibration
q = fraction of the solute residing in the same extraction element

In eqns. (1) and (2) p and σ are expressed in numbers of extraction elements. For use in chromatography, μ and σ should be expressed in [cm]:

$$\mu = npH \quad (3)$$
$$\sigma^2 = npqH^2 \quad (4)$$

where H is the height equivalent to a theoretical plate. From eqns. (3) and (4) it follows that:

$$\frac{\sigma^2}{\mu} = qH = (1 - R_F)\, H \quad (5)$$

which is the definition given by Dr. TURINA.

This definition is based on a physical model of the chromatographic process which is fully analogous to counter-current extraction, *i.e.*, it is assumed that the mobile phase is added discontinuously, in amounts equal to the volume of mobile phase

per theoretical plate. This model was described by S. W. MAYER AND E. R. TOMPKINS, *J. Am. Chem. Soc.*, 69 (1947) 2866.

However, nowadays the model described by A. J. P. MARTIN AND R. L. M. SYNGE, *Biochem. J.*, 35 (1941) 1358, is almost universally adopted. These authors assume that the mobile phase is added *continuously, i.e.*, in *infinitesimal small amounts*. Therefore, the fraction p of the solute, transported after each equilibration, is also infinitesimally small and the fraction q, residing in the extraction element is ≈ 1. So, instead of eqn. (5), one gets:

$$\frac{\sigma^2}{\mu} = H \tag{6}$$

which is the definition used by me.

As this last definition is already generally adopted, I think that the introduction of an alternative definition (5) has only the disadvantage of creating confusion.