Journal of Chromatography, 75 (1973) 124–125

Note

CHROM. 6318

On the densitometric estimation of R_F values in plane chromatography

Determination of the R_F value is an essential part of the identification procedure for any component separated by plane chromatography but it is often difficult to decide the precise position within the spot which should be taken as the reference point for this evaluation. From theoretical considerations the "centre" of the spot should be taken and this is actually the most concentrated portion of the spot rather than the more easily determined geometric centre. Photodensitometry of the chromatogram will provide an objective estimation of the most intense point on the spot and we have been investigating this method as a routine means of producing more accurate R_F values. However, it was noticed that when the R_F value was estimated from the most intense point on the densitometric trace, by dropping a line perpendicular to the baseline, this value decreased at high sample loading. It was decided to examine this more closely to determine whether a procedure could be found which was independent of the sample load.

Two-microlitre samples of aqueous solutions containing 5–100 $\mu g/\mu l$ Amaranth LNSP were chromatographed on pre-coated cellulose thin-layer plastic films (Macherey & Nagel CEL 300) using ethyl methyl ketone–acetone–water (7:3:3) as eluent[1]. After development the positions of the origin and solvent front were marked by scoring the layer so that they produced negative densitometric peaks. The light

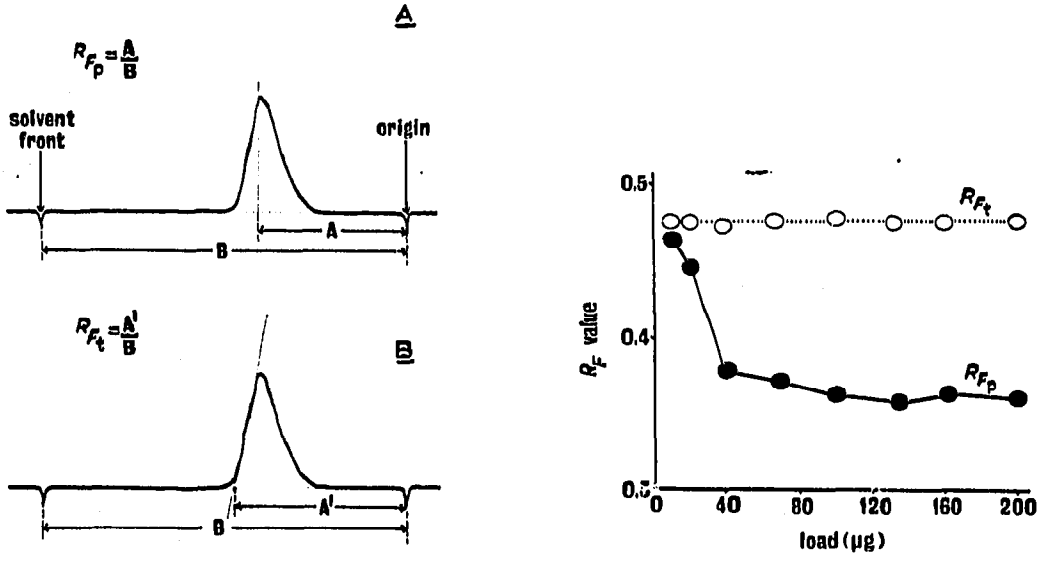

Fig. 1. Calculation of R_{Fp} (1A) and R_{Ft} (1B) values from the densitometric traces.

Fig. 2. Relationship between load and R_{Fp} (●—●) and load and R_{Ft} (○ · · · · · ○) values.

absorption of the dried chromatogram at 525 nm was measured by scanning the film by transmission in a Vitatron TLD 100 flying spot densitometer in the log mode at a scan speed of 1 cm/min using a 0.25 mm aperture, strike length of 14 mm, and span of 8.00. Use of the flying spot system overcame the difficulties, such as multiple peak maxima, sometimes encountered when scanning irregularly shaped spots with a slit system.

The R_F value was first estimated by dropping a line perpendicular to the baseline from the most intense point on the densitometric trace (Fig. 1A). In this case the R_F value (R_{F_p}, Fig. 2) dropped rapidly with increasing load then stabilised at a lower value. Visual inspection of the densitometric traces indicated increasing tailing at the higher concentrations, with a consequent shift in the intensity maximum, whilst the leading edge of the peak appeared to remain in a more or less constant position. The tangent to the leading edge of each peak was therefore constructed and the point of intersection of the tangent with the baseline used to determine a second R_F value (R_{F_t}, Fig. 1B). It can be seen (Fig. 2) that this R_{F_t} value was only marginally affected by the sample load and it is suggested that the use of this value would produce a worthwhile increase in the accuracy of the estimation of R_F values.

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1 K. R. BRAIN, T. D. TURNER AND B. E. JONES, *J. Pharm. Pharmacol.*, 23 (1971) 250S.

Received August 28th, 1972