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Note

An improved technique for obtaining reproducibility of R_F values in impregnated paper chromatography

A. M. AHSAN

Department of Chemistry, Northern Illinois University, De Kalb, Ill. 60115 (U.S.A.)

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Difficulties in obtaining reproducible R_F values in impregnated paper chromatography, a technique of great value especially in the separation of steroids[1], have been reported earlier[1–3]. We also encountered them during the course of our work on the pregnane ester glycoside amplexoside A[4]. In agreement with the views of Gasparič[2] and Churáček[3], the main cause for it seemed to us to be lack of constancy in degree of impregnation due to non-standardized experimental techniques.

The methods of impregnation may be broadly classed according to whether it is done (A) before[1] or (B) after[1] preparing the chromatogram (spotting etc.). The latter has the distinct advantage that the time between impregnation and start of run is reduced, thus minimizing loss of stationary phase due to evaporation, with corresponding improvement of results[1]. The utility of this technique is particularly noticeable when spotting 0.5–1 mg of crude extractives per spot[5], which takes considerable time. It consists chiefly in preparing the chromatogram first in the usual way and then dipping it twice in the impregnating medium up to the starting line, once from each end, so that it is fully wet[1].

With it as a starting point we devised the following procedure which differs from that of Bush[1] in that (1) the paper is dipped once only, the portion on which the run is to take place being immersed and the other left dry, (2) constancy in degree of impregnation is obtained by letting excess stationary phase evaporate and weighing to ensure it.

METHOD

The prepared chromatogram was weighed and then impregnated by drawing only the portion on which the run is to take place, up to 1 cm from the starting line, back and forth once through the desired medium (this is important as this gives approximately the desired uptake of stationary phase)[6,7], taken out and hung (top end up) to drain for 30 sec. The whole paper is not impregnated with obvious advantages in the manipulation of the wet paper. After usual blotting and waving in the air to remove volatile carrier solvent, it was rolled up with the dry end outside (to minimize evaporation of stationary phase), this being another advantage of partial immersion of the paper, and weighed again. Most often there was a little excess of impregnating liquid and this was removed by allowing it to lie on the spotting bench

(a wooden frame with glass rods at suitable intervals to support the paper). When water is the stationary phase, as in our case, 2 or 3 min exposure usually sufficed. If there was less impregnating liquid than desired, the paper was immersed again likewise, but allowed to drip for a little less time (15 sec) and then worked up as above (*cf.* Gasparič's Method B[2]). The process of evaporation exposed to air and weighing is repeated till the desired impregnation is obtained. Thereafter the paper is kept rolled and the run started as soon as possible. The time between impregnation and the beginning of development is kept constant from run to run. The rest of the technique of chamber saturation etc. is usual[1,5,7].

RESULTS AND DISCUSSION

On Whatman No. 1 paper with water/*n*-butanol, *k*-strophanthin (commercial) separated into 8 spots, which indicated that the technique was quite effective. This system was selected as water is one of the most volatile of stationary phases generally used, and if this method should work with water, it could be expected to work with other comparatively less volatile agents, given sufficient draft of air to enhance rate of evaporation. The results for 5 representative spots, fast to slow moving, are given in Table I.

TABLE I

$R_F \times 100$ VALUES AND STANDARD DEVIATIONS FOR REPRESENTATIVE SPOTS IN THE SEPARATION OF *k*-STROPHANTHIN ON PAPER AT CONSTANT IMPREGNATION*

System: water/*n*-butanol.

Run No.	*Extent of impregnation (percentage weight of paper)*	*Observed $R_F \times 100$ values for spot Nos.*				
		1	*2*	*3*	*4*	*5*
1	108 (ref. 7)	81	63	40	31	14
2	110	83	65	42	32	13
3	110	82	64	41	30	15
4	109	83	64	38	31	14
5	110	81	62	38	28	16
6	109	81	62	40	30	13
Standard deviation		0.98	1.21	1.60	1.36	1.20

* The solvent front is normally not visible during the run. It was visualized by running the yellow dye, *p*-dimethylaminoazobenzene, which travels almost with the solvent front.

It may be observed that the standard deviations compare well with those of Gasparič[2] and Churáček[3] and incidentally, we confirm the findings of the former[2] who notes that the spots about the middle of the chromatogram show higher scatter.

Two further runs (Table II) were made with N-methylformamide/tetrahydrofuran–benzene–cyclohexane (1:2:5) (*cf.* ref. 6) to demonstrate that the separating power was not vitiated by this technique and impure amplexoside A[4] was chosen to ascertain it. As in these experiments the developing solvent drips from the paper, it is not possible to give R_F values. Both runs ended 40 h after commencement.

TABLE II

DISTANCE OF TRAVEL OF AMPLEXOSIDE A[4] AND IMPURITY ON PAPER AT CONSTANT IMPREGNATION

System: N-methylformamide/tetrahydrofuran–benzene–cyclohexane (1:2:5) (*cf.* ref. 6). Time of run: 40 h.

Run No.	*Extent of impregnation (percentage weight of paper)*	*Distances to which spots travel (cm)*	
		Amplexoside A	*Impurity*
1	35 (ref. 6)	8.4	10.8
2	35	7.0	9.6

With N-methylformamide as the stationary phase the rate of volatilization is much less (about 10 mg/min in still air from 10×46 cm paper) but 15–20 min in a hood with a good draft sufficed.

From the above results it may be seen that constancy in degree of impregnation from run to run is obtained in this technique within reasonable limits, giving good reproducibility in R_F values; and at the same time it is simpler and more convenient to use.

REFERENCES

1 I. E. Bush, *The Chromatography of Steroids*, Pergamon, New York, 1st ed., 1961, pp. 162–168.
2 J. Gasparič and J. Borecký, *J. Chromatogr.*, 5 (1961) 466; J. Gasparič, *Acta Chim. (Budapest)*, 27 (1961) 221.
3 J. Churáček, *J. Chromatogr.*, 33 (1968) 45.
4 A. M. Ahsan, D. M. Piatak and P. D. Sorensen, *Experientia*, 29 (1973) 789.
5 O. Schindler and T. Reichstein, *Helv. Chim. Acta*, 34 (1951) 108; F. Kaiser, *Chem. Ber.*, 88 (1955) 556.
6 L. Sawlewicz, Ek. Weiss and T. Reichstein, *Helv. Chim. Acta*, 50 (1967) 504.
7 E. Schenker, A. Hunger and T. Reichstein, *Helv. Chim. Acta*, 37 (1954) 680.