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Note

Influence of the biological matrix on retention behaviour in thin-layer chromatography: evidence of systematic differences between pure and extracted drugs

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Toxicologists, using thin-layer chromatography (TLC) as a primary tool in systematic toxicological analysis, must frequently consult reference libraries and data bases. Such collections of data are usually a compilation of results obtained in different laboratories. As the retention behaviour of drugs shows considerable inter-laboratory variability, the problem of standardization of TLC data has been recognized and dealt with in several ways [1—5]. The conversion of apparent (observed) R_F values into corrected values (R_F^c values) allowed the use of comprehensive tabulations of reference data available from several sources [5, 6]. In general, however, the reference data are obtained with pure drug standards. In recent work [7], we found that basic drugs extracted from biological materials showed greater intra- and inter-laboratory variability in R_F values than the pure drugs. The size of the variations appeared to be system-dependent, being smallest in methanol, medium in methanol—butanol (60:40, containing 0.1 *M* sodium bromide) and largest in basic chloroform—methanol (90:10). Moreover, the results obtained for the methanol system indicated that there may be a systematic difference between data obtained for

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pure and extracted drugs. If such a difference really exists, then reference drugs, used as correction standards, should be treated in a similar manner to unknown samples.

The purpose of this work was to investigate further the potential differences in the TLC behaviour between pure drugs and extracted drugs and to establish whether the effect of the biological matrix could be circumvented by using reference standards spiked in the biological matrix being examined.

EXPERIMENTAL

Drug-free samples of autopsy blood and liver homogenate (diluted 1:5 with water) were spiked with the appropriate mixture of drugs used as a correction standard. Three combinations of drugs were used: codeine + caffeine + nitrazepam, codeine + caffeine + diazepam and paracetamol + diazepam + phenylbutazone. The final concentration of each drug was 50 mg/l. Drug-free samples of liver were also individually spiked with methotrimeprazine, fluphenazine and lignocaine to a final concentration of 20 mg/l.

Spiked samples were extracted with chloroform at pH 9.0 (codeine + caffeine + nitrazepam, codeine + caffeine + diazepam, lignocaine, methotrimeprazine and fluphenazine) or at pH 7.0 (paracetamol + diazepam + phenylbutazone). Volumes of 1 ml of sample and 5 ml of solvent were used. After rotation for 10 min the samples were centrifuged and 4 ml of organic solvent were collected, dried with sodium sulphate, evaporated and reconstituted with 100 μl of methanol.

Mixtures of pure drugs in the same combinations, containing 2 mg/ml of each drug in methanol, were also used.

Volumes of 5 μl of reconstituted extracts and pure drug solutions were applied to TLC plates (Kieselgel GF_{254} Fertigplatten, 20 × 10 cm; E. Merck). The chromatograms were developed for a distance of 8 cm [8, 9] in one of four systems. Methanol or methanol—butanol (60:40, containing 0.1 *M* sodium bromide) systems [5] were used for the examination of the mixtures of codeine, caffeine and nitrazepam and for the examinationof liver extracts containing lignocaine, methotrimeprazine or fluphenazine. Mixtures of codeine, caffeine and diazepam were developed in chloroform—methanol (90:10, plates dipped in potassium hydroxide) [6] and the mixture of paracetamol, diazepam and phenylbutazone was examined in chloroform—acetone (40:10). The above mixtures are routinely used as correction standards in appropriate systems. The samples of pure drug mixtures and liver and blood extracts were alternately applied to the same plate, each sample being applied three times. For each specimen nine or ten independent observations were made.

RESULTS

Fig. 1 represents the R_F values of the drugs used as correction standards in the four systems. A definite and significant trend can be noticed, viz., drugs extracted from biological materials, particularly from liver, had a lower mobility than pure drugs. This can influence the correction of chromatographic

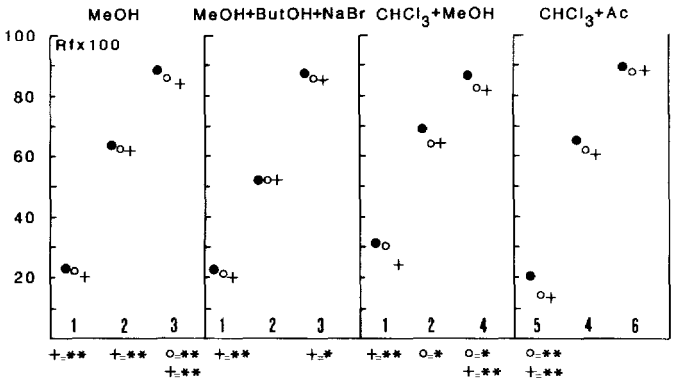

Fig. 1. R_F Values for pure drugs and for drugs extracted from plasma and liver in four different TLC systems. 1 = Codeine; 2 = caffeine; 3 = nitrazepam; 4 = diazepam; 5 = paracetamol; 6 = phenylbutazone. Uncorrected data for pure drugs (•), drugs extracted from plasma (○) and drugs extracted from liver (+) on the same plates. Statistically significant differences at the 90% confidence level (*) or at the 95% confidence level (**).

According to the method of Galanos and Kapoulas [1] as modified by De Zeeuw et al. [5], the equations for the correction of R_F values are as follows:

$$R_F^c = aR_F + b \tag{1}$$

$$a = \frac{R_{F_1}^o - R_{F_2}^o}{R_{F_1} - R_{F_2}} \tag{2}$$

$$b = R_{F_1}^o - aR_{F_1} \tag{3}$$

where $R_{F_1}^o$ and $R_{F_2}^o$ are listed values for the correction standards and R_{F_1} and R_{F_2} are observed values for the correction standards.

In general, a mixture of three reference substances and the start and the front are used as reference points for correction with the above equations. Spots between the start and R_{F_1} are corrected by means of these two points;

TABLE I

R_F VALUES OF DRUGS BEFORE AND AFTER CORRECTION BY TWO METHODS AND R_F VALUES FOR CORRECTION MIXTURE

R_F = Observed uncorrected value in liver extracts. $R_{F_p}^c$ = corrected value obtained by means of pure drug correction mixture. $R_{F_e}^c$ = corrected value obtained by means of extracted drug correction mixture. Ref. = R_F value listed in the data bank [5]

Drug	R_F	$R_{F_p}^c$	$R_{F_e}^c$	Ref.
Methotrimeprazine	27	24	28	28
Fluphenazine	35	32	36	42
Lignocaine	64	63	66	68

Correction mixture	R_F of pure drugs	R_F of extracted drugs	Ref.
Codeine	23	19	20
Caffeine	64	61	63
Nitrazepam	89	84	84

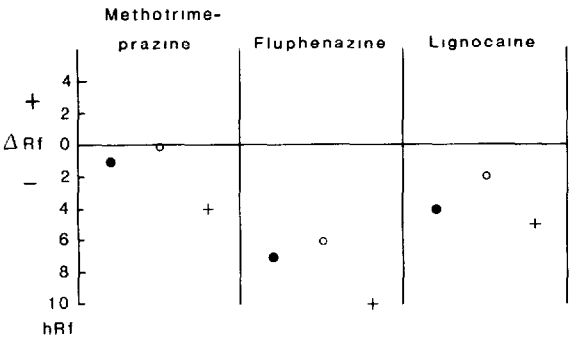

Fig. 2. Differences between experimentally found and corrected R_F values and R_F values listed in a data base for three drugs extracted from liver. $\Delta R_F = R^0_{F_e} - R^0_F$ (see text). (•) Observed, uncorrected R_F values; (+) corrected values by means of pure drug correction mixture; (○) corrected R_F values by means of extracted drug correction mixture. R^0_F data from ref. 5. Solvent, methanol.

spots for which $R_{F_1} < R_F < R_{F_2}$ are corrected by means of R_{F_1} and R_{F_2}, etc.

The following example shows how one can arrive at erroneous results if observed R_F values for an extracted biological sample are corrected by using a mixture of pure drug standards. On one TLC plate were applied liver extracts containing either methotrimeprazine, lignocaine of fluphenazine, in addition to correction standard mixtures (codeine + caffeine + nitrazepam) prepared from pure drugs and from a liver extract. The chromatogram was developed in methanol for a distance of 8 cm. Table I gives the retention data of the drugs before and after correction and the standards. It can be seen that corrected R_F values obtained by means of pure standards and extracted standards may differ substantially; moreover, the corrections made with pure drug standards are in the wrong direction, as shown in Fig. 2.

The results of these investigations prompted us in our routine case work to use drugs extracted from appropriate tissues and biological fluids as correction standards. These extracted drug standards are stored in small portions at −20°C in methanolic solution and are used within four to six weeks.

REFERENCES

1 D.S. Galanos and V.M. Kapoulas, J. Chromatogr., 13 (1984) 128.
2 J.H. Dhont, C. Vinkenborg, H. Compaan, F.J. Ritter, R.P. Labadie, A. Verweij and R.A. de Zeeuw, J. Chromatogr., 47 (1970) 376.
3 J.H. Dhont, C. Vinkenborg, H. Compaan, F.J. Ritter, R.P. Labadie, A. Verweij and R.A. de Zeeuw, J. Chromatogr., 71 (1972) 283.
4 A.C. Moffat, J. Chromatogr., 110 (1975) 341.
5 R.A. de Zeeuw, P. Schepers, J.E. Greving and J.P. Franke, Proceedings of the International Symposium on Instrumental Applications in Forensic Drug Chemistry, Government Printing Office, Washington, DC, 1979, p. 167.
6 A. Stead, R. Gill, T. Wright, J.P. Gibbs and A.C. Moffat, Analyst (London), 107 (1982) 1106.
7 M. Bogusz, M. Klys, J. Wijsbeek, J.P. Franke and R.A. de Zeeuw, J. Anal. Toxicol., 8 (1984) 149.
8 J.P. Franke, P. Schepers, J. Bosman and R.A. de Zeeuw, J. Anal. Toxicol., 6 (1982) 131.
9 J.P. Franke, W. Kruyt and R.A. de Zeeuw, J. High Resolut. Chromatogr. Chromatogr. Commun., 6 (1983) 82.