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Letter to the Editor

Quantitative techniques in thin-layer chromatography

Sir,

In a recent detailed survey of the literature on quantitative thin-layer chromatography (TLC) we came across a paper in this journal[1] which, in our opinion, contains a misinterpretation of previous work by Bethke *et al.*[2] on the subject of the data pair technique and transferable calibration factors in TLC–densitometry[3]. Surprisingly, similar work has been also published in two other journals[4,5], but with differing conclusions regarding the relative merits of external *vs.* internal calibration.

Not considering the statistical aspects of the reported data, as the authors implicitly acknowledged the limitations associated with a lack of optimal working conditions and sufficient replicates[1], the following points should have been taken into account in writing or reviewing this paper.

(1) As stated by Grijalba *et al.*[1], in the data pair technique "a series of standard concentrations is applied on a plate. The procedure is repeated on the same plate with the same series of standards but with an equal amount of the unknown. The concentration of the unknown is then calculated from the intercepts of the curves with the concentration axis". However, the data pair technique, the only purpose of which is to compensate for fluctuating chromatographic conditions, requires the application of standards and unknowns in a paired fashion for each concentration and in such a way that the two spots of each pair are placed about one half-width apart[2]. In other words, the data pair method by itself is not a technique that quantitates TLC spots, but rather an ingenious approach to compensate for sources of variability across a TLC plate, thus eliminating systematic errors. Consequently, one should not confuse the simple standard additions method[6,7], which is what the authors are really using, with the data pair technique. The fact is that the latter could be used to improve the precision of the first or of any other quantitative methods for that matter.

(2) One of the methods also evaluated by Grijalba *et al.*[1] is that of transferable or external calibration, and in this regard it is not clear why, after determining the mean slope using five plates, *another* plate should be necessary for calculating the intercept on the ordinate and five more to calculate the precision and accuracy.

In fact, the ordinate intercept could be derived from any of the first five plates, which in any case would likewise have been incorrect as this value varies from plate to

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plate. What one needs to do instead is to "achieve a maximum of external information and a minimum of individual calibration", working with *one* calibration point on each plate[3].

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1 A. Grijalba, D. Fos, L. Martinez Valls, A. Idoate and F. A. Vega, *J. Chromatogr.*, 178 (1979) 443.
2 H. Bethke, W. Santi and R. W. Frei, *J. Chromatogr. Sci.*, 12 (1974) 392.
3 H. Bethke and R. W. Frei, *Anal. Chem.*, 48 (1976) 50.
4 M. J. Perez Garcia, M. Ferrer, A. Grijalba, D. Fos and F. Vega, *Cienc. Ind. Farm.*, 10 (1978) 187.
5 M. Ferrer, J. C. Basarte, M. Fernandez, D. Fos and F. A. Vega, *Farmaco, Ed. Prat.*, 35 (1979) 32.
6 H. Bethke and R. W. Frei, *J. Chromatogr.*, 91 (1974) 433.
7 A. Shatkay, *J. Chromatogr.*, 198 (1980) 7.

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