Waters
Lab Highlights

An Internal Communication
of Applications and Techniques

LAH 0303 2/86
AN/PA/MD/DR/AB

NO. 220 APPLICATION OF A MIXTURE DESIGN STRATEGY FOR ELUENT OPTIMIZATION IN LIQUID CHROMATOGRAPHY WITH SOLUTE RECOGNITION BY MULTIPLE ABSORBANCE RATIOING

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A variety of approaches have been presented for the selection of optimal eluents for LC separations. In general, these have been based on empirical strategies such as sequential simplex optimization or experimental design. One very interesting mixture design strategy has been reported by deGalan and co-workers (1). In this approach, concepts from the solubility parameter model are used to reduce the number of experiments needed to predict the optimal ternary (or quaternary) eluent for a given separation. This approach has the additional advantage of checking for simple (e.g. binary) eluents before moving on to more complex (e.g. ternary) eluents.

In this paper we discuss our recent applications of the deGalan strategy for the optimization of challenging isocratic separations including the cephalosporin antibiotics and other compounds of current pharmaceutical interest. Although the deGalan strategy was originally proposed for reversed-phase separations involving simple aqueous/organic eluents, we have obtained exceptional results in applying this strategy for eluents containing buffers and paired-ion reagents as well. In addition, a variety of column types including C_{18}, cyano, phenyl and unbonded silica have been used with success.

A significant need for most methods development strategies is a reliable, but efficient means of identifying peaks in the various chromatograms. Ideally, the injection of standards should be avoided. Our efforts to use absorbance ratios for this purpose will be discussed.

In particular, we will discuss a technique which we have found useful for the selection of an optimally selective set of wavelengths for absorbance ratio monitoring. The technique is based on principal components analysis and identifies those few wavelengths which are best able to account for the majority of the variation in the spectral data set for the solutes of interest.

All experiments were conducted on a standard liquid chromatograph which included an autoinjector, a microcomputer-based system controller and a programmable multiwavelength detector. Software needed for the eluent optimization and wavelength selection procedures was executed on the same microcomputer which provided system control.

We will discuss the strengths and weaknesses of absorbance ratioing as an approach to solute tracking during the methods development process. As others have noted, spectral changes induced by variations in eluent composition (pH, solvent type) are of considerable importance. Even when these effects are controlled, we find it helpful to monitor two or more ratios for purposes of solute recognition provided that well-chosen wavelengths are monitored. The ratio of two ratios captures limited shape information regarding the solute's UV spectrum and can be obtained with greater precision than a single ratio.

1. A.C.J.H. Drouen, H.A.H. Billiet, P.J. Schoenmakers and L.deGalan, Chromatographia, 16 (1982) 48.