THE ANALYSIS OF CATHARANTHUS ALKALOIDS BY COMPUTER-AIDED HIGH-PRESSURE LIQUID CHROMATOGRAPHY WITH LINEAR PHOTODIODE ARRAY DETECTION

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The extraction of alkaloids from the leaves of *Catharanthus roseus* requires careful control of the residual alkaloid levels. Analysis of the principal therapeutically-active alkaloids vinblastine (VB) and vincristine (VC) by reversed-phase high-pressure liquid chromatography (HPLC) is complicated by the co-elution of numerous related alkaloids (Görög et al, 1977). The various digital methods proposed to interrogate overlapping peak systems (Fell et al, 1983; Clark et al, 1984), include the method of spectral suppression, which has found limited application since it was only defined for binary systems (Carter et al, 1982). This report describes a novel extension of the method to encompass multiple peak overlap problems, as observed for the catharanthus alkaloids.

Chromatography was performed on a 100 x 4.6 mm ID column packed with 5-µm SAS-Hypersil. The mobile phase composition for optimum resolution and performance was: acetonitrile - 0.01 M ammonium carbonate (46:54, v/v). Rapid-scanning UV-detection was based on the Hewlett-Packard HP-1040A linear photodiode array detector, interfaced with the HP-85B microcomputer, provided with dual disc drive and printer-plotter peripherals. The standard software for the detector was supplemented by programs developed by the authors for multiple spectral suppression. UV-spectra of the individual alkaloids were acquired on a Perkin-Elmer Lambda 5 spectrophotometer and six suitable wavelengths chosen for use in the spectral suppression algorithm, for which the relevant epsilon ratios were calculated. The alkaloids of interest overlapped in the chromatogram and eluted between 200 and 270 seconds after injection; vindoline, k' = 3.99 (VIND, 10.0 µg/ml); VC, k' = 4.26 (10.0 µg/ml); and catharanthine, k' = 4.59 (CA; 10.0 µg/ml) The (n x n) matrix algorithm developed for n components is based on an extension of the expression for spectral suppression (Eq.1), to give the corresponding formulation in matrix notation (Eq.2), where $A_1(t)$ and $A_2(t)$ represent the absorbance at λ_1 and λ_2, at time t, while ε_1 and ε_2 represent the absorptivity values of a defined component at these wavelengths:

$$\Delta A(t) = A_1(t) - \frac{\varepsilon_1}{\varepsilon_2} \cdot A_2(t) \qquad \text{(Eq.1)} \qquad \det(M) = \begin{vmatrix} \varepsilon_1 & A_1(t) \\ \varepsilon_2 & A_2(t) \end{vmatrix} \qquad \text{(Eq.2)}$$

The data are processed so that for the ternary system of overlapping peaks, one, two or all three of the co-eluting peaks can be selectivity suppressed. A qualitative indication of residual impurity is indicated if, when all three known alkaloids are suppressed, a non-zero residual baseline is observed. Moreover, any given pair of alkaloids can be suppressed, to permit the quantitative assay of a third. The calibration curves for each of the alkaloids, examined in the presence of typical amounts of the others, were linear over the range 0 - 10.0 µg/ml and regressed through or close to the origin.

The method proposed has been applied to extracts of *Catharanthus roseus* for the routine characterisation of samples. Currently up to 4 co-eluting components can be suppressed either individually or simultaneously. The method is sensitive and involves little or no sample clean-up. Although exemplified here as a technique for quality control during the processing of natural products, the algorithm is expected to be generally applicable in cases where overlapping chromatographic peak systems need to be interrogated.

Carter, G.T. et al (1982) J. Pharm. Sci. 71: 317-321
Clark, B.J. et al (1984) J. Chromatogr. 286: 271-283
Fell, A.F. et al (1983) J. Chromatogr. 282: 123-140
Görög, S. et al (1977) J. Chromatogr. 139: 203-206