THE USE OF AN EXPERT SYSTEM BASED ON TOTAL LUMINESCENCE SPECTRA FOR THE IDENTIFICATION OF DRUGS SEPARATED BY HPLC

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Although uv-absorption is the most common mode of detection in high-pressure liquid chromatography (HPLC), fluorescence spectroscopy can significantly enhance both sensitivity and selectivity for the detection of drugs, degradants and metabolites, especially at the trace level. Moreover, by interfacing the spectrofluorimeter to a microcomputer, it is possible to acquire the total luminescence data as an emission-excitation matrix (EEM) under stop-flow conditions. This can be used for the confirmation of identity or for the validation of peak purity (Warner et al, 1976; Clark et al, 1985). The total luminescence data is represented by a matrix of $(I_f, \lambda_{em}, \lambda_{ex})$ and can be employed to establish a spectral library, which can then serve as a knowledge base for use in an expert system to aid drug identification. The present work describes the establishment of a knowledge base and the associated rule base for the identification of drugs of forensic interest, separated by HPLC.

Chromatography was based on a reversed-phase column of 5-μm ODS-Hypersil (100 x 4.6 mm ID), the mobile phase being acetonitrile-water (50:50 v/v) at a flow rate of 1.0 ml/min. Elution was stopped automatically at the peak apex by a peak recognition routine and the EEM collected. The expert system is implemented in Pascal and micro-Prolog on an Advance 86b microcomputer, interfaced with a Perkin-Elmer LS-5 spectrofluorimeter.

In the rule base, the principal features are considered (i.e. peak maxima, valleys and saddle points) together with the selectivity conferred by retention data. For an unkown compound an initial screen of the key features in the excitation and emission spectra can be obtained in less than 10 min, to yield a short list of candidate compounds for further detailed examination. The expert system selects and controls successive experiments designed to acquire the most characteristic confirmatory data for the analyte. The experiments exploit a number of digital techniques including synchronous luminescence spectroscopy (André et al, 1977), variable-angle synchronous scanning (v.a.s.s.; Clark et al, 1985), derivative spectroscopy (Fell, 1983), which all generate characteristic profiles, data density matrix measurement and spectral stripping. Synchronously scanned spectra are obtained by cutting through the EEM at trajectories of 45° to give sharpened profiles as exemplified by the phenothiazine derivatives and their highly fluorescent sulphoxide degradation products (Clark & Fell, 1983). Further enhancement of the selectivity imparted by the synchronous technique can be achieved by cutting the EEM at any angle as in the v.a.s.s. technique (Clark et al, 1985).

The performance of the expert system developed has been characterised on a small repertoire (n = 12) of fluorescent drugs eluted by HPLC and found to be very satisfactory. The rule base can be extended to encompass a larger knowledge base of fluorophores (n>50) including the carcinogenic polynuclear hydrocarbons. The expert system developed should be well-suited for use with the new generation of fluorescence detectors in HPLC based on the linear photodiode array.

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