THE ANALYSIS OF AROMATIC AMINO ACIDS BY SECOND AND FOURTH DERIVATIVE UV-SPECTROSCOPY

A.F. Fell, Department of Pharmacy, Heriot-Watt University, 79 Grassmarket, Edinburgh EH1 2HJ

The assay by UV-spectroscopy of mixtures of aromatic amino acids in solution is complicated by serious spectral overlap of the chromophores. The advent of simple electronic analog devices for rapidly processing UV-visible spectra to generate the first, second or higher derivative of spectral band absorbance with respect to wavelength, presents an alternative means for deconvolution of overlapping bands (O'Haver & Green 1976; Fell 1978). Derivative spectroscopy offers enhanced resolution of L-phenylalanine (Phe), L-tyrosine (Tyr) and L-tryptophan (Try), and permits their quantitative assay in injections for parenteral nutrition.

The second derivative UV-spectra of individual aqueous solutions of Phe and Try display a characteristic series of inverted, sharp peaks. In binary mixtures the amplitudes at 254 nm (Phe) and 286 nm (Try) are linear with concentration and pass through the origin. The 95% confidence limits ($n = 7$) for Phe were 1.00 ± 0.017 mM and for Try they were 0.300 ± 0.006 mM. At a constant level of Try (0.294 mM) the amplitude measure at 286 nm was unaffected by Phe concentration from 0.2 to 1.4 mM. At a constant Phe level of 1.00 mM, the amplitude at 254 nm increased by 2.0% when the Try concentration was increased to 0.4 mM. A batch of injections containing these amino acids was assayed by second derivative spectroscopy, using a composite standard at declared formulation strength (10.0 mM Phe, 2.94 mM Try) to compensate the slight dependence of the Phe amplitude on Try concentration. Recoveries relative to label strength were 96.1% (Phe) and 101.0% (Try), the relative standard deviations ($n = 8$) being 1.74% and 0.66% respectively. Low levels of pyridoxine and nicotinamide in the formulation did not interfere.

Ternary mixtures of the aromatic amino acids may be assayed by second derivative UV-spectroscopy after dilution with 0.1 M NaOH, when Tyr undergoes a bathochromic shift, displacing its band away from Try and thereby reducing their mutual interference. At 240 nm Tyr displays a broad peak with an amplitude which is linear with concentration in ternary mixtures, but which yields a large positive intercept on the Y-axis. The respective amplitudes at 254 nm and 286 nm for Phe and Try were linear with concentration, the 95% confidence limits being 1.00 ± 0.055 mM (Phe) and 0.250 ± 0.007 mM (Try). The amplitude for Phe showed a slight dependence on Try and Tyr concentrations. When applied to ternary aqueous standards, recoveries were as follows: 1 mM Phe 95.1%; 0.25 mM Try 99.3%; 0.06 mM Tyr 94.4%.

In fourth derivative spectra of ternary amino acid mixtures in 0.1M NaOH, the Tyr signal at 240 nm was absent, whereas the derivative peaks for Phe at 254 nm and Try at 286 nm were sharper, better resolved and independent of each other. Under these conditions, recoveries from ternary mixtures of standards were 102.1% (Phe) and 99.8% (Try). Second and respectively fourth derivative spectroscopic assays of a series of injections containing the three aromatic amino acids gave average recoveries as follows: Phe 94.3% (4th: 93.1%); Try 91.5% (4th: 90.1%); Tyr 58.1%.

Second and fourth derivative spectra of some proteins, including dihydrofolate reductase, DNA-ase, RNA-ase and bovine serum albumin, present a sensitive qualitative profile for confirmation of identity (Fell 1979). The free aromatic amino acid levels in deproteinised plasma may also be assayed in cases of phenylketonuria and hypertyrosinaemia.

Fell, A.F. (1978) Proc. Analyt. Div. Chem. Soc. 15: 260-267
Fell, A.F. (1979) UV Spectrometry Group Bulletin (in press)
O'Haver, T.C., Green, G.L. (1976) Analyt. Chem. 48: 312- 318