ANALYSIS OF AMINO ACIDS IN PHENYLKETONURIA BY NARROW BORE LIQUID CHROMATOGRAPHY WITH SINGLE- AND MULTI-CHANNEL DETECTION

E. M. Kirk, B. J. Clark, R. J. Dolphin* and A. F. Fell, School of Pharmaceutical Chemistry, University of Bradford, BD7 1DP, UK and *Pye Unicam Ltd., York Street, Cambridge, CB1 2PX, UK.

The assay of free serum tyrosine (Tyr) and phenylalanine (Phe) is of clinical relevance in studies on in-born errors of metabolism in neonates and children, as in the detection of tyrosinaemia and phenylketonuria (PKU). In PKU it is necessary to monitor the response to the dietary treatment required to prevent severe mental retardation. The advent of narrow-bore columns and of rapid-scanning UV-detectors in high-pressure liquid chromatography (HPLC) gives the advantage of small sample requirement, and the ability to validate peak purity. This new column technology has been compared with conventional columns and utilised in the design of a highly sensitive method for the micro-assay of serum Phe and Tyr in paediatric samples for clinical biochemistry.

The method utilises zinc hydroxide generated *in situ* for deproteinisation (Somogyi, 1930), followed by separation on reversed-phase HPLC using a 100 x 2.1 mm column (Spherisorb-5 RP-18 OD-102) with an in-line filter. The optimised eluent composition was: 0.05 M KH_2PO_4-methanol at pH* 4.0 (87:13, v/v); the optimised flow rate was 80 µl/min. The filled-loop volume injected was 500 nl. For single-channel detection using the LDC spectromonitor 3000 with a 1-µl flow cell at 0.05 AUFS, the high absorptivity at 210 nm was utilised for high sensitivity detection. The phase capacity ratios (k') were: Tyr, 0.78; Phe, 2.28; and tryptophan (Trp), 4.84 (N > 35,000/metre). As an internal standard (IS) β -2-thienyl-DL-alanine was used at 697 µM (k', 1.14). Peak height response ratios for Tyr: IS and for Phe:IS in spiked human serum were linear with concentration over the clinical ranges of interest (Tyr, 15-300 µM; Phe, 75-1500 µM): Tyr, $y = 0.0043x - 0.0381$ ($r = 0.9971$; $n = 6$); Phe, $y = 0.0028x - 0.0260$ ($r = 0.9996$; $n = 6$). The recovery data for Phe at normal and elevated levels were: 80.5% (70 µM) and 94.7% (1120 µM), respectively. At 62 µM recovery of Tyr was 90.5%.

The procedure is fast ($t_R < 8.5$ min) and sensitive, the intrinsic limits of detection (LOD) (for signal equivalent to twice baseline noise) being: Phe, 31 pg; Tyr, 24 pg (n = 4). Using the same detector with a regular 100 x 4.6 mm column, packed with the same material, the LOD values were: Phe, 83 pg; Tyr, 31 pg (20 µl injected). The within-batch RSD was: Phe, 1.25% at 1010 µM (n = 6); Tyr, 1.44% at 257 µM (n = 6). Results of the method applied to 21 paediatric samples correlated well with the independent fluorimetric method of McCaman and Robins (1962) over the range 162-1110 µM Phe: $y = 1.0147x - 15.97$ ($r = 0.9946$).

Combination of this method with a linear photodiode array (LDA) UV-detector (Pye Unicam PU4021 with PU4850 computer) permits confirmation of the peak identities. The LDA generates isometric three-dimensional spectrochromatograms of absorbance, wavelength and time which can be used to assess peak purity by a number of digital techniques (Fell et al, 1984). The LOD using this detector was comparable with that of the single-channel detector: Phe, 24 pg; Tyr 19 pg (n = 4). Moreover, sensitivity can be readily increased by combining the outputs of adjacent diode resolution elements: $\Delta\lambda$ = 10 nm; Phe 13 pg; Tyr 9 pg. The narrow-bore column thus permits nanolitre samples to be assayed at higher sensitivity than for the regular column. When coupled with the LDA detector this new technology yields even higher sensitivity and has significant potential for applications in other areas of neonatal biochemistry and in forensic toxicology, where the requirements for handling small samples can present particular difficulties.

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