

NOTE NO. 30 - April 1 1989

DIRECT-INJECTION ANALYSIS OF PLASMA PHENYLALANINE*

Analytes: Phenylalanine

Sample Matrix: Human Plasma

Sample Size: 20 microliters

Column: 5 micron GFF ISRP, 15 cm x 4.6 mm ID column coupled to a 25 cm x 4.6 mm ID column.

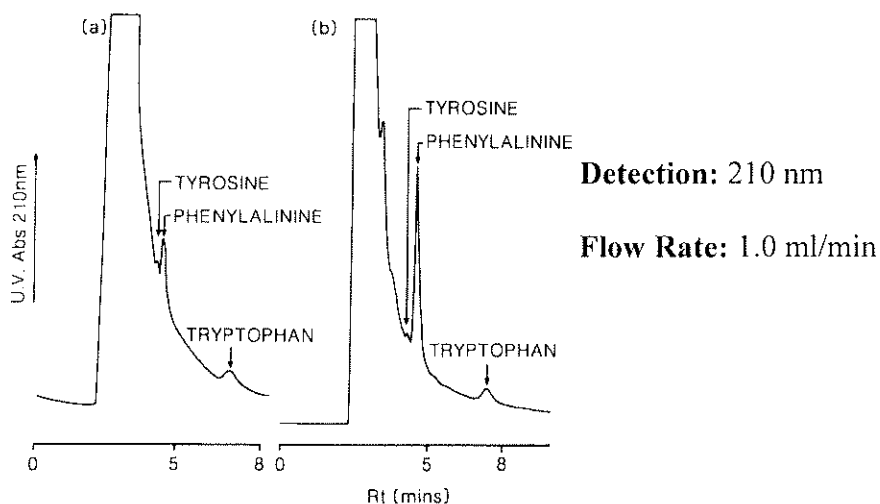
Regis Product Numbers: 731471 and 731472

Mobile Phase: 0.5 M KH_2PO_4 (pH 6.5)

Chromatograms:

(a): Plasma from a healthy adult (non-phenylketonuria; phenylalanine 90 micromoles/liter)

(b): Plasma from a phenylketonuria patient on diet (undertreated; phenylalanine 1104 micromoles/liter)



DIRECT-INJECTION ANALYSIS OF PLASMA PHENYLALANINE*

Results: Correlation of HPLC-ISRP (y) with HPLC/protein precipitation (x); $y = 0.931x + 46.4$, $r = 0.951$, $n = 41$, range = 78 - 1344 micromoles/liter. Linear range; 10 - 1250 micromoles/liter (using a 20 microliter injection loop). Coefficients of variation (intra-batch); $n = 10$ each.

Phenylalanine		micromoles/liter
Mean	SD	CV%
217	11.7	5.4
373	11.2	3.0
965	51.1	5.3
1108	66.5	6.0

Conclusions: The above data show that this HPLC-ISRP method performs well. The linear range could be extended by using a 10 microliter sample injection loop. This would also be expected to increase column lifetime by reducing protein loading. The good correlation with an HPLC technique requiring precipitation of plasma proteins suggests that interference is not a problem. It must be noted that creatinine elutes between tyrosine and phenylalanine and in patients with substantially elevated plasma creatinine interference will occur. However, experience in assessing this method for application to the diagnosis and treatment of phenylketonuria patients has shown that this did not present a problem in PKU patients with normal plasma creatinine.

**N.D. Atherton, Dept. of Clinical Chemistry, The Children's Hospital,
Ladywood Middleway, Birmingham. B16 8ET. United Kingdom.
Present Address; Fisons plc, Research and Development Laboratories, Pharmaceutical Division,
Bakewell Road, Loughborough, Leicestershire. LE11 ORH. United Kingdom
*Provisionally accepted for publication in Clinical Chemistry.**