

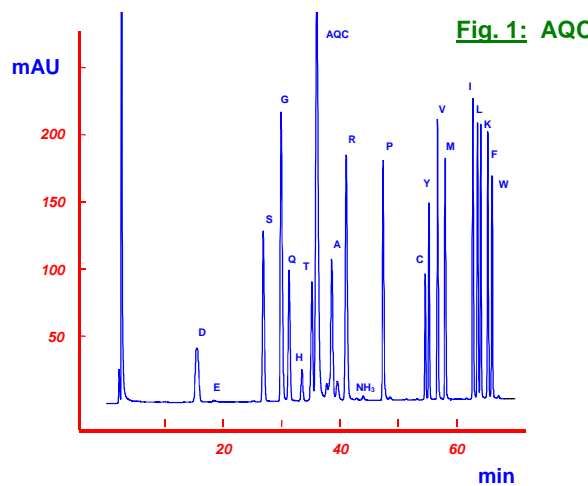
Modern Methodes for Amino Acid Analysis

- Overview -

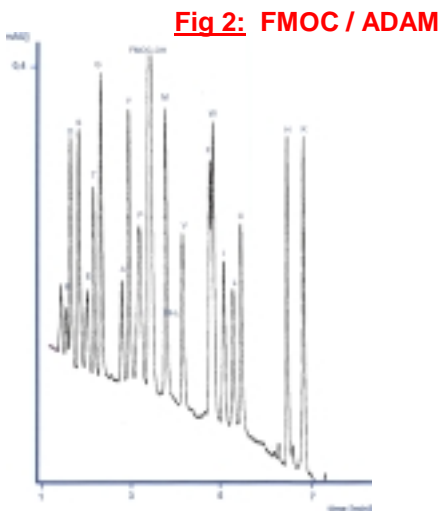
In comparison to the classical analysis of amino acids derivatized with ninhydrin, faster, considerably more sensitive and fully automated methods of precolumn derivatisation with subsequent separa-

tion and identification via reversed phase HPLC have recently been developed and successfully employed, especially for proteinaceous amino acids.

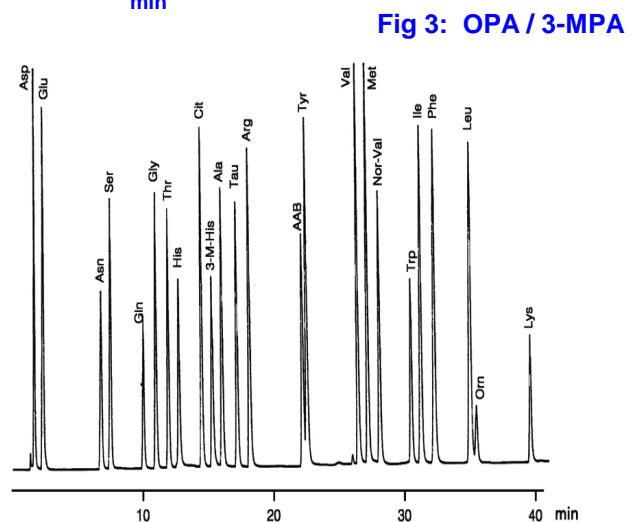
High Sensitivity



Short Analysis Times



Good Resolution



the seemingly best method is always a compromise

The derivatisation and analysis with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC, Fig. 1) is distinguished by its very simple procedure and extremely low detection levels in capillary HPLC, while that with 9-Fluorenylmethoxycarbonyl chloride

/1-adamantanamine (FMOC/ADAM, Fig. 2) is especially rapid and that with o-phthalaldehyde/3-mercaptopropionic acid (OPA/3-MPA, Fig. 3) is remarkable for its combination of high sensitivity and superlative resolution.

Common reagents for the derivatisation of amino acids

	PITC	DABS-Cl	OPA / 3-MPA	OPA / IBCL	FMOC / ADAM	FLEC / ADAM	AQC
Stable reaction products	+	+	-	+	+	+	+
Secondary amines	+	+	-	-	+	+	+
Stereoselectivity analysis of D- And L- aminoacids	-	-	-	+	-	+	-
Automation	±	-	+	+	+	+	+
Detection	UV	VIS	UV and FL	UV and FL	UV and FL	UV and FL	UV and FL
Lower detection limit (UV resp. FL)	≤ 10 pmol	~10 pmol	1 pmol resp. ≤ 10 fmol	10 pmol bzw. ≤ 100 fmol	10 pmol resp. ≤ 50 fmol	10 pmol resp. ≤ 100 fmol	≤ 50 fmol
Analysis time	~ 10 min	~ 35 min	~ 12 min	~ 70 min	~ 7 min	~ 75 min	20 – 60 min
Application	proteins, foodstuffs	proteins, foodstuffs	physiological samples, fermentation broths	foodstuffs	proteins	foodstuffs, pharmaceuticals	proteins

Because of their stereoselectivity, which enables the simultaneous detection and highly sensitive quantification of both D- and L-amino acids, (+)-1-(9-fluorenyl)ethyl chloroformate/1-adamantanamine (FLEC/ADAM, Fig. 4) and o-phthaldialdehyde/N-isobutyl-L-cysteine (OPA/IBCL, Fig. 5) are also

increasingly being used as derivatisation reagents. While not as sensitive as some of the other cited methods, analyses with 4-(dimethylamino)azobenzene-4'-sulfonyl chloride (DABS-Cl, Fig. 6) or phenyl isothiocyanate (PITC, Fig. 7) might be of value where sample amounts are not limiting.

Fig 4: FLEC / ADAM

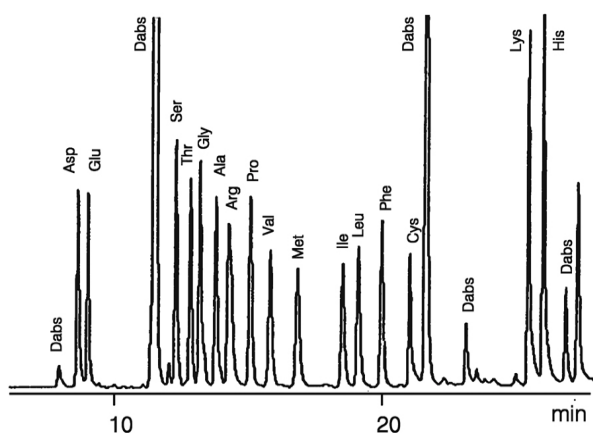


Fig 5: OPA / IBCL

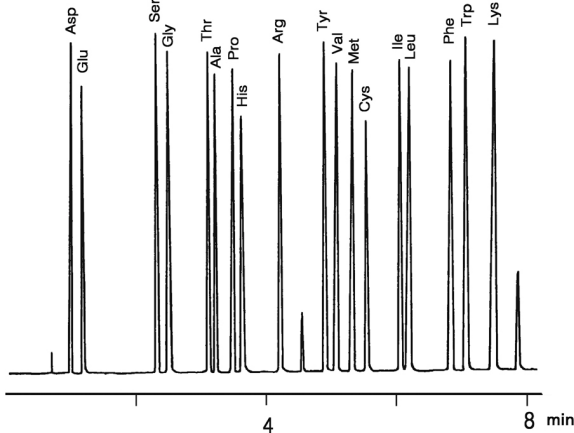


Fig 6: DABS-Cl

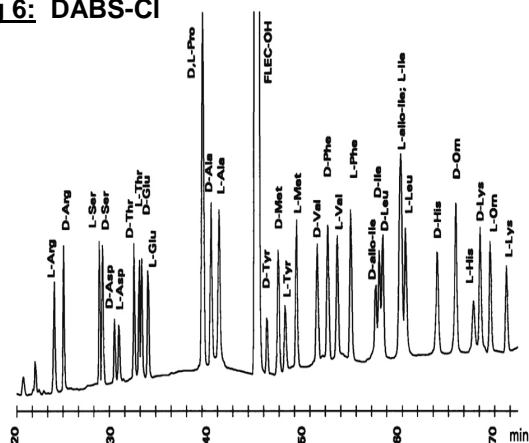
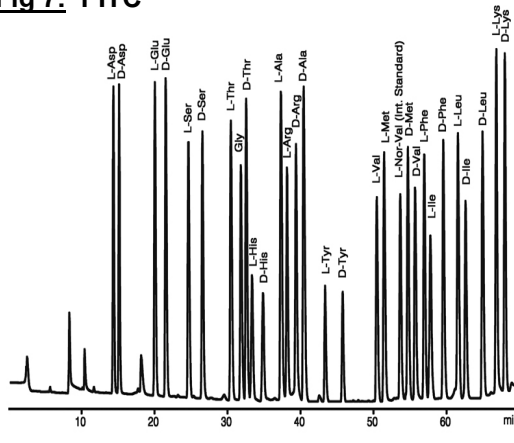


Fig 7: PITC



Selected primary literature references:

- 1) S.A. Cohen et al., Anal. Biochem. 211, 279-287 (1993)
- 2) J.Y. Chang, R. Knecht, D.G. Braun, Biochem. J. 199, 547-555 (1981)
- 3) S. Einarsson, B. Josefsson, P. Möller, D. Sanchez, Anal. Chem. 59, 1191-1195 (1987)
- 4) I. Betner, P. Földi, Chromatographia 22, 381-387 (1986)
- 5) T.A. Graser, H. Godel, S. Anders, P. Földi, P. Fürst, Anal. Biochem. 151, 142-152, (1985)
- 6) H. Brückner, P. Jack, M. Langer, h. Godel, Amino Acids 2, 271-284 (1992)
- 7) B.A. Bidlingmeyer, S.A. Cohen, T.L. Tarvin, J. Chromatogr. 336, 93-104 (1984)