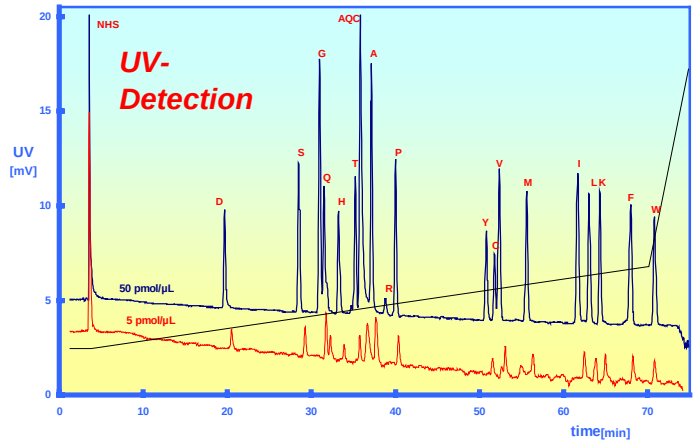
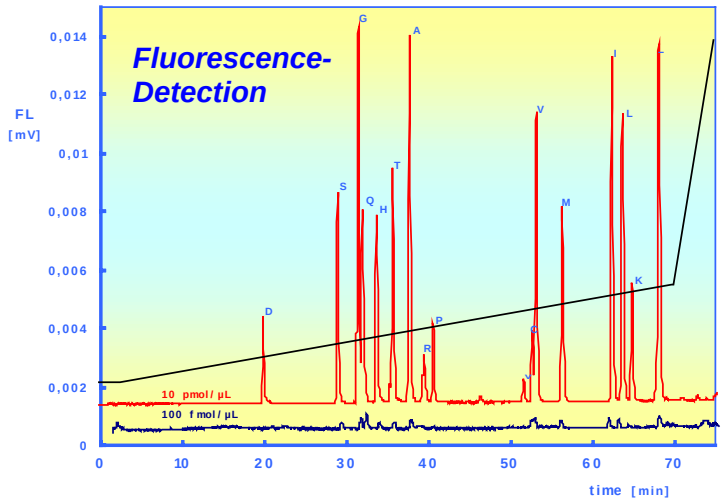
Highly Sensitive Amino Acid Analysis via Microbore- and Capillar-HPLC, by Precolumn Derivatisation with 6-Aminoquinolyl-N-Hydroxysuccinimidyl-Carbamate (AQC)

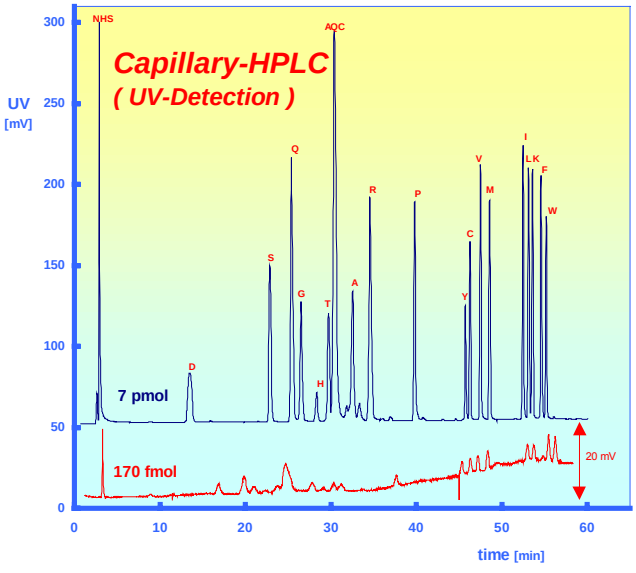
In addition to conventional amino acid analysis via post-column derivatisation with ninhydrin, pre-column derivatisation with o-phthaldialdehyde (OPA) and 9-fluorenyl-methoxycarbonyl chloride (FMOC) have recently pre-vailed as alternative methods. The advantages of both methods include high sensitivity (≥10 fmol) and short analysis times (10-30 min). One of the few serious drawbacks of these pre-column derivation methods is the fact that the sensitive fluorescence detection of tryptophan is difficult at best, due to intramolecular quenching. Furthermore, OPA reacts only with primary amines and therefore can not be used for amino acids containing secondary amines, such as proline or hydroxyproline.





Stationary phase: GROM Sapphire 110 C18, 3 µm
Column: (a, b) 150 mm x 2 mm, (c) 150 mm x 0.3 mm
Eluent: A, 50 mM Na acetate, pH 5.75;
B, 70% acetonitrile/30% 50 mM Na acetate, pH 6.0 (v/v)
Gradient: in % B: (a, b) 2% (0-2.5 min), 2-30% (2.5-70 min), 30-70% (70-75 min); (c) 2-10% (0-30 min), 10-60% (30-80min).
Flow: 1.06 mm/s (linear velocity)
Temperature: 45° C
Detector: UV, 254 nm;
FL, 250 nm excitation, 395 nm emission
Flowcell: (a) 3 mm, 1.2 µl; (b) 2mm, 3 µl; (c) 10 mm, 45 nl
Injection: (a, b) 1 µl; (c) 80 or 150 nl
Sample: standard mixture (~10 pM of each amino acid) diluted accordingly

These disadvantages can be overcome by pre-column derivatisation of amino acids with **6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AGC)** followed by their chromatographic separation via capillary or nano-HPLC. Given the high UV (254 nm) extinction coefficient of the AQC adducts, the same high sensitivity (≥10 fmol) can be achieved with capillary HPLC as with conventional HPLC and fluorescence detection. AQC reacts not only with primary, but also with secondary amino acids. Further factors favoring this method include the very simple derivatisation procedure, excess AQC does not disturb the analysis and the AQC adducts are more stable than those produced with FMOC.



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