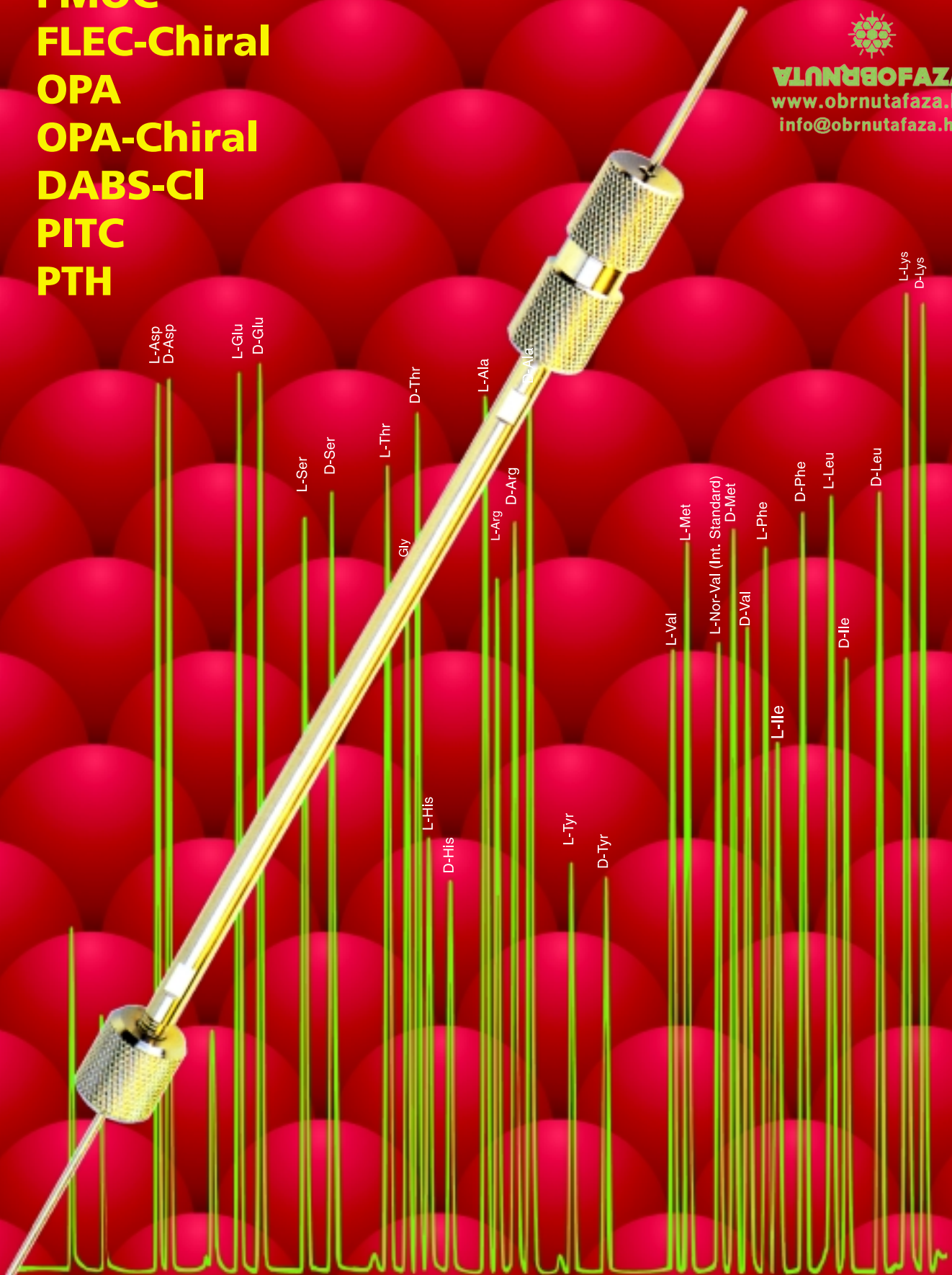


Amino Acid Analysis by HPLC

FMOC
FLEC-Chiral
OPA
OPA-Chiral
DABS-Cl
PITC
PTH



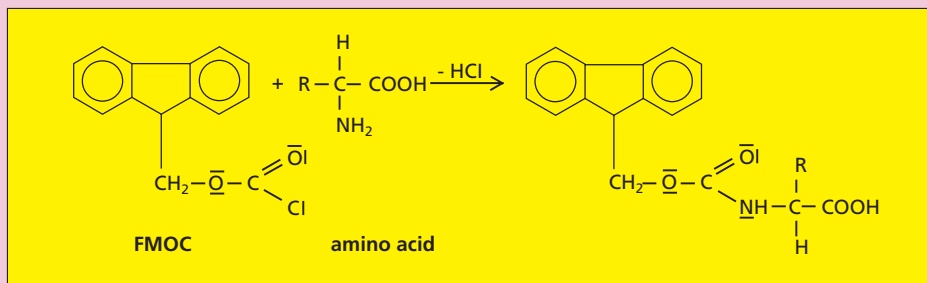
AZAFABRNTA
www.obrnutafaza.hr
info@obrnutafaza.hr



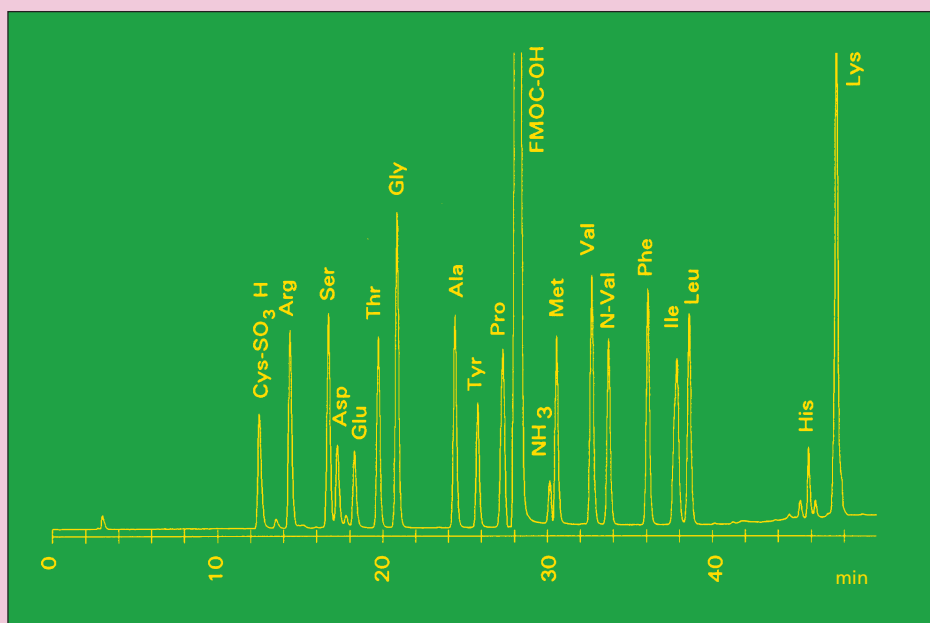
Amino acid analysis by precolumn derivatisation using **FMOC/ADAM**

9-fluorenyl-methoxycarbonyl chloride / 1-aminoadamantane

- ➔ High resolution and high sensitivity
- ➔ Rapid, simple derivatisation
- ➔ Primary and secondary amines
- ➔ Stable derivatives
- ➔ Fully automated or manual derivatisation

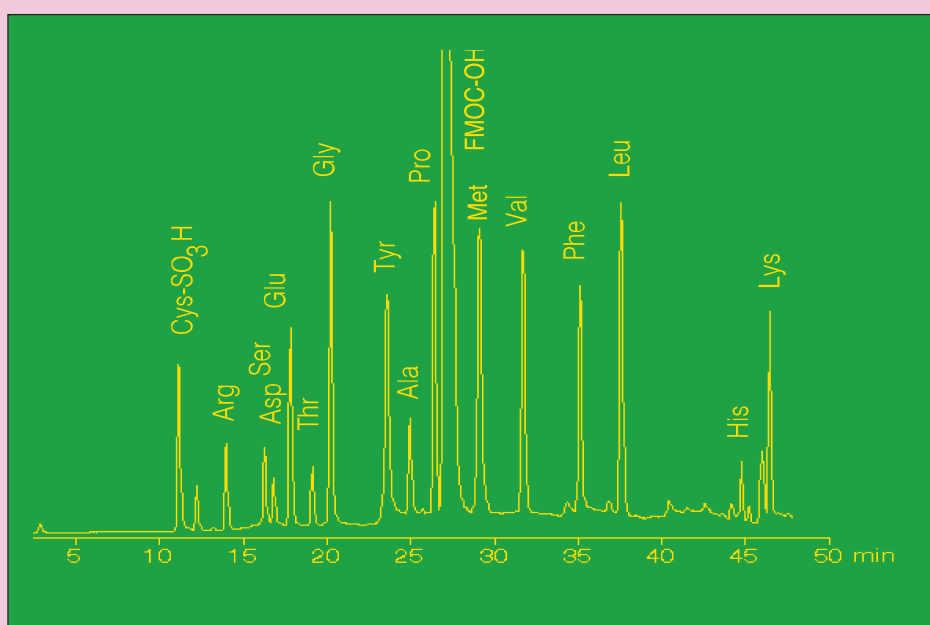


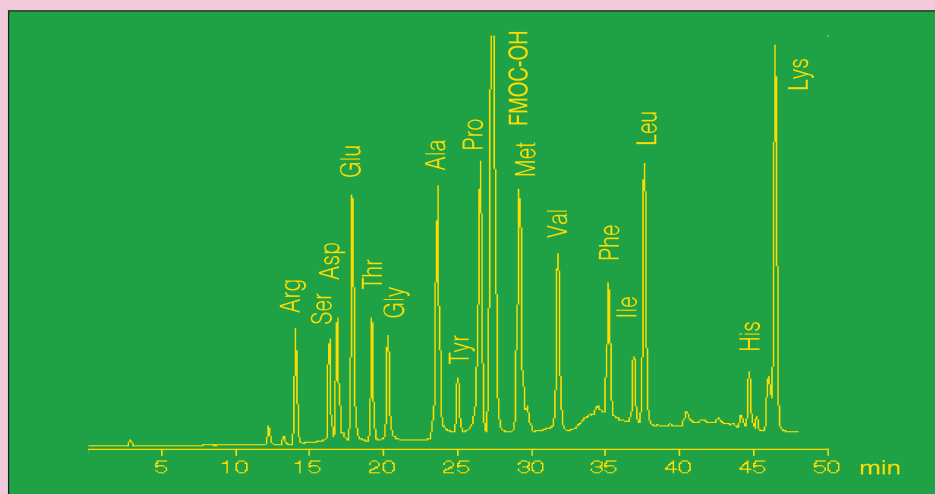
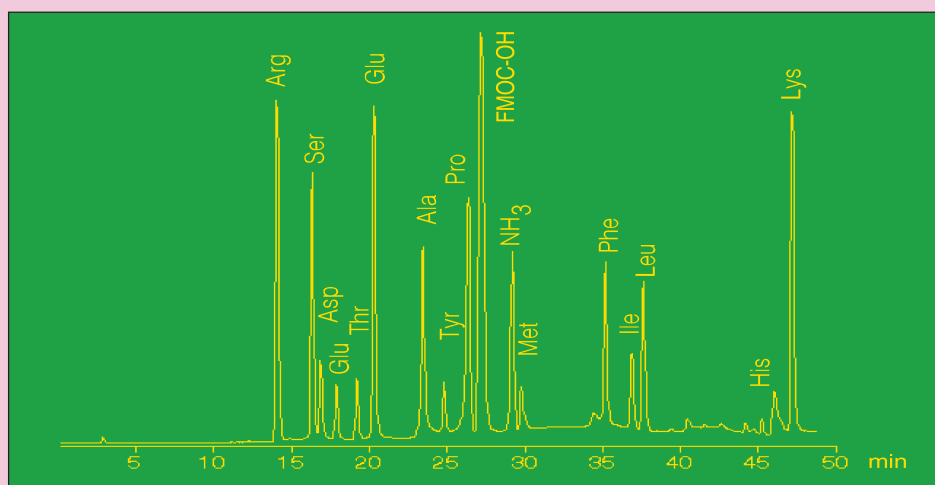
10 054 Protein Hydrolysate Standard (6 N HCl / 15 hrs)



10 055 Amino Acid Analysis - β -Chain of Insulin (hydrol. with 6 N HCl / 15 hrs)

Column phase:
GROM-SIL FMOC-2
Column size:
250 x 4 mm
Eluent A:
50 mM Na-acetate, pH 4.2 / ACN = 80 / 20
Eluent B:
50 mM Na-acetate, pH 4.2 / ACN = 20 / 80
Gradient:
0-40% B (0-35 min),
40-70% B (35-45 min),
70-100% B (45-50 min)
Flow rate:
1.0 ml
Pressure:
17 MPa
Temperature:
40°C
Detection (Fluor.):
263 nm (exc.), 313 nm (em.)
Injection:
10 μ l (2 μ M, each a. a.)



10 056 Amino Acid Analysis of Bovine Serum Albumin (hydrolyzed with 6 N HCl, 15 hrs)**10 057 Amino Acid Analysis of Human Urotilatin (hydrolyzed with 6 N HCl, 15 hrs)****References**

I. Betner, P. Földi, LC+GC 6, 832 - 840 (1988)

B. Gustavsson, I. Betner, J. of Chromatogr. 507, 67-77 (1990)

J. Maier-Rosenkranz, A. Maisch, A. Kupka, P. Földi, LC+GC INT. 7, 509-516 (1994)

Derivatisation instructions, E.Grom, Application Service 001

Columns and reagent kits**Complete kit**

984.0000 Complete kit including 4 mm i.d. special column as a cartridge, all necessary reagents and detailed working instructions for amino acid analysis by precolumn derivatisation with Fmoc/ADAM.(consisting of order nos. GS FM2 0512K2504, GS OD2 2010V0204, GS FM2 0512V0104V, 2100, 4100, 900.0001, 900.0101, 984.0500, 984.0600)

Columns* and single items for reordering

GS FM2 0512K2501 tailor-made, customised 250 x 1.0 mm analytical Fmoc column (cartridge)

GS FM2 0512K2502 tailor-made, customised 250 x 2 mm Fmoc Microbore column (cartridge)

GS FM2 0512K2503 tailor-made, customised 250 x 3 mm analytical Fmoc column (cartridge)

GS FM2 0512K2504 tailor-made, customised 250 x 4 mm analytical Fmoc column (cartridge)

GS OD2 2010V0204 Clean-up precolumn (20 x 4 mm)

2100 guard column holder for clean-up guard column

GS FM2 0512V0104V 5 Guard columns (10 x 4 mm)

4100 Guard column combination

900.0000 L-amino acid standard solution, 17 amino acids (ala, arg, asp, (cys)₂**, glu, gly, his, ile, leu, lys, met, phe, pro, ser, thr, tyr, val); 2.5 µMol/ml each / ** = 1.25 µMol/ml, 1 ml

900.0001 L-amino acid standard solution (17 amino acids; 2.5 µMol/ml each), 10 x 1 ml

900.0100 L-norvaline (2.5 µMol/ml), as internal standard, 1 ml

900.0101 L-norvaline (2.5 µMol/ml), as internal standard, 10 x 1ml

984.0500 1-amino-adamantane (ADAM/HCl), 1 g (to be dissolved in a solution of borate buffer and acetone; see instruction sheet)

984.0600 Fmoc-reagent II (100 ml)

The Fmoc/pentane column and reagent kit has the order number 981.0000, see application number 10 130, page 40

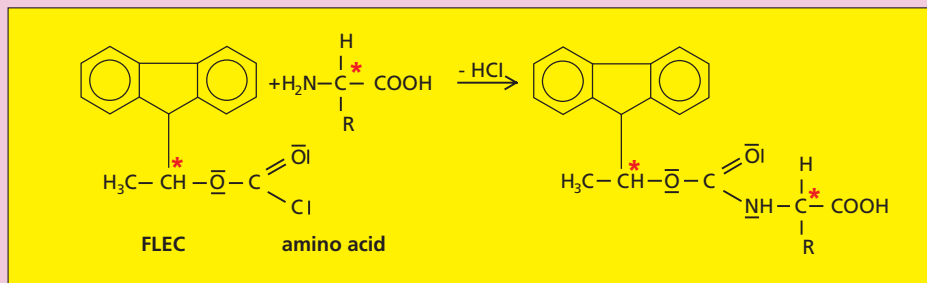
* Cartridges and Columns of other dimensions, e.g., capillary columns, available upon request

Separation and analysis of **D-** and **L-amino** acids and other optically active amines by precolumn derivatisation with

FLEC/ADAM or **EVE**

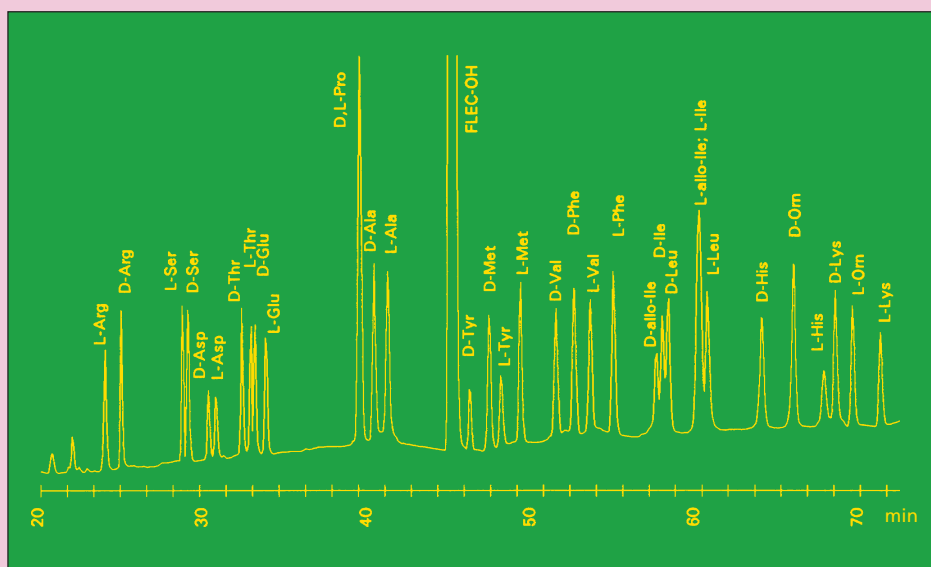
(+)-1-(9-fluorenyl)ethyl chloroformate / 1- aminoadamantane or glycine

- ➔ Separation of all D- and L-amino acids
- ➔ Primary and secondary amino acids
- ➔ High sensitivity
- ➔ Simple, fully automated (or manual) derivatisation
- ➔ High resolution



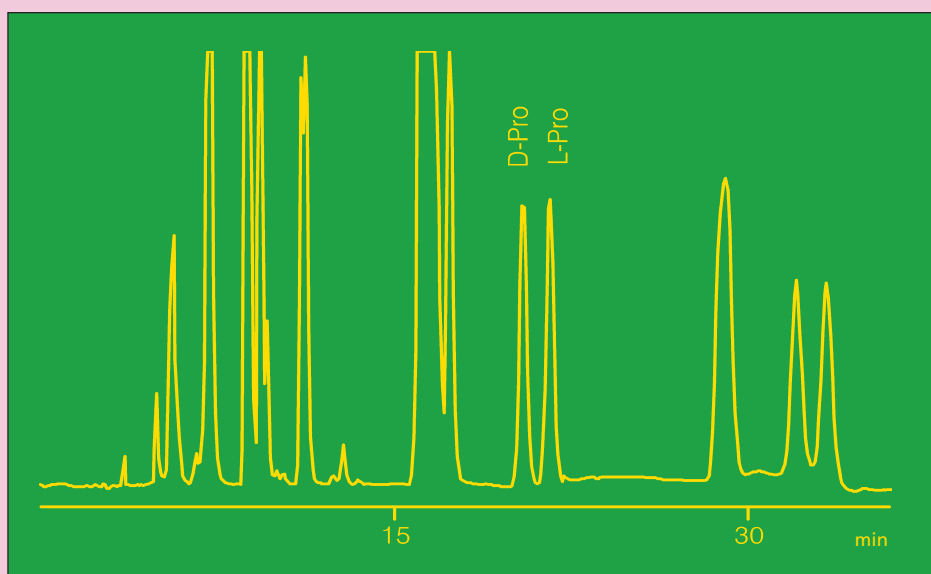
10 058 a Separation of a D-, L-Amino Acid Standard by HPLC employing the FLEC-ADAM Approach

Column phase:
GROM-SIL FLEC-1
Column size:
250 x 4 mm
Eluent A:
50 mM Na-acetate, pH 4.0
B: ACN C: THF
Gradient:
17% B + 8% C (0-8 min),
17-30% B + 8-0 % C (8-22 min),
30-50% B + 0% C (22-70 min)
Flow rate:
0.75 ml/min
Pressure:
24 MPa
Temperature:
40°C
Detection (Fluor.):
263 nm (exc.), 313 nm (em.)
Injection:
10 µl (10 nMol/ml, each a.a. 10 µM)

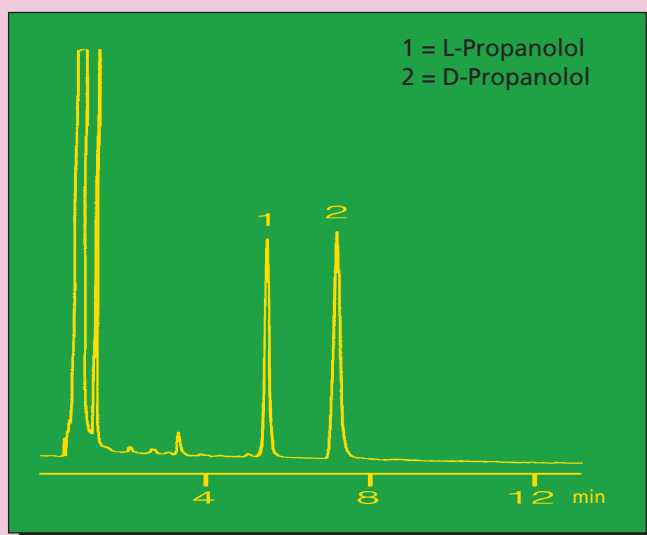


10 058 b Isocratic Separation of D- and L-Proline after Precolumn Derivatization with FLEC/ADAM

Column phase:
GROM-SIL FLEC-1
Column size:
250 x 4 mm
Eluent:
50 mM Na-acetate, pH 4.0 /
ACN = 55 / 45
Flow rate:
0.75 ml/min
Pressure:
24 MPa
Temperature:
40°C
Detection(Fluor.):
263 nm (exc.), 313 nm (em.)
Injection:
10 µl (each a.a. 10 µM)



10 053a Determination of Propanolol - Analysis of Diastereomers of β -Blockers in a pill



Column phase:
GROM-SIL 100 ODS-2 FE
Column size:
125 x 4 mm
Eluent:
water / acetonitrile = 30 / 70
Flow rate:
1.2 ml/min
Pressure:
6 MPa
Temperature:
RT
Detection (Fluor.):
263 nm (exc.), 313 nm (em.)
Injection:
1 μ l (= 500 fmol)

References

S. Einarsson, B. Josefsson,
P. Möller, D. Sanchez, Anal. Chem
59, 1191-1195 (1987)

J. Maier-Rosenkranz, A. Maisch,
A. Kupka, P. Földi, LC+GC INT. 7,
509-516 (1994)

Derivatisation instructions,
E.Grom, Application Service 002

Columns and reagent kits

Complete kit

985.0000 Complete kit including 4 mm i.d. special column as cartridge, all necessary reagents and detailed working instructions for analysis of DL-amino acids and other chiral amines by precolumn derivatisation with FLEC/ADAM.or FLEC/EVE (consisting of order nos. GS FL1 0312K2504, GS OD2 2010V0204, GS FL1 0312V0104V, 2100, 4100, 900.0101, 984.0500, 985.0400, 985.0600, 985.1000).

Columns* and single items for re-ordering

GS FL1 0312K2501	tailor-made, customised 250 x 1 mm FLEC/ADAM analytical column (cartridge)
GS FL1 0312K2502	tailor-made, customised 250 x 2 mm FLEC/ADAM Microbore column (cartridge)
GS FL1 0312K2503	tailor-made, customised 250 x 3 mm FLEC/ADAM analytical column (cartridge)
GS FL1 0312K2504	tailor-made, customised 250 x 4 mm FLEC/ADAM analytical column (cartridge)
GS OD2 2010V0204	Clean-up precolumn (20 x 4 mm)
2100	Guard column holder for clean-up guard column
GS FL1 0312V0104V	5 Guard columns (10 x 4 mm)
4100	Guard column combination
900.0100	L-norvaline (2.5 μ Mol/ml), as internal standard, 1 ml
900.0101	L-norvaline solution (2.5 μ Mol/ml), as internal standard, 10 x 1 ml
984.0500	1-aminoadamantane (ADAM/HCl), 1 g. (to be dissolved in a solution of borate buffer and acetone; see instruction sheet)
985.0400	FLEC reagent (5mg/ml), 1ml
985.0600	Glycine - EVE -, 1g (to be dissolved in a solution of borate buffer and acetone; see instruction sheet)
985.1000	DL- amino acid standard solution (16 pairs): ala, arg, asp, glu, his, ile, leu, lys, met, orn, phe, pro, ser, thr, tyr, val; 2.5 μ Mol/ml each, 5 x 1 ml
985.2000	DL- amino acid standard (24 diastereomeric pairs of amino acids, 100 mg of each): ala, arg, asn, asp, cit, cys, dopa, glu, gly, his, ile, leu, lys, met, nor-leu, nor-val, orn, phe, pro, ser, thr, trp, tyr, val

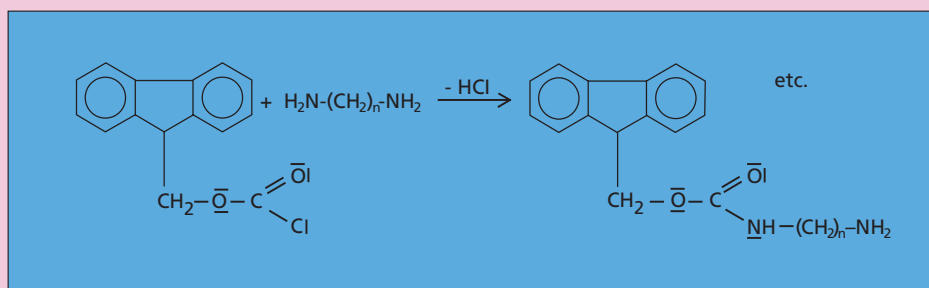
* Cartridges and Columns of other dimensions, e.g., capillary columns, upon inquiry

Analysis of **polyamines** and **biogenic amines** (such as cadaverine, putrescine, spermine, spermidine, histamine, etc.) by precolumn derivatisation with

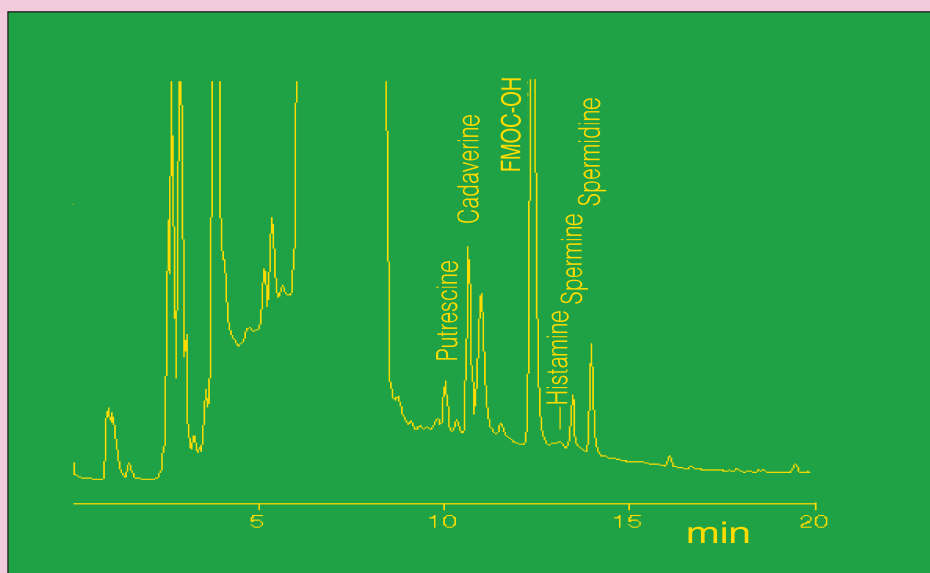
FMOC/EVE

9-fluorenyl-methoxycarbonyl chloride / glycine

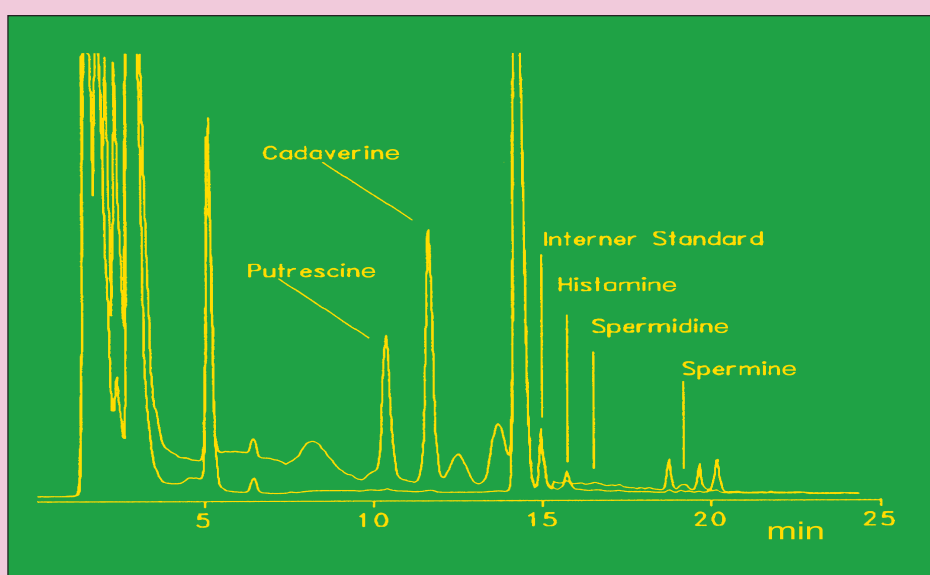
- ➔ Shortest times of analysis
- ➔ Low limits of detection (in femto-Mol range)
- ➔ Simple to perform (no special sample preparation necessary)
- ➔ Stable derivatives
- ➔ Manual or automatic derivatisation



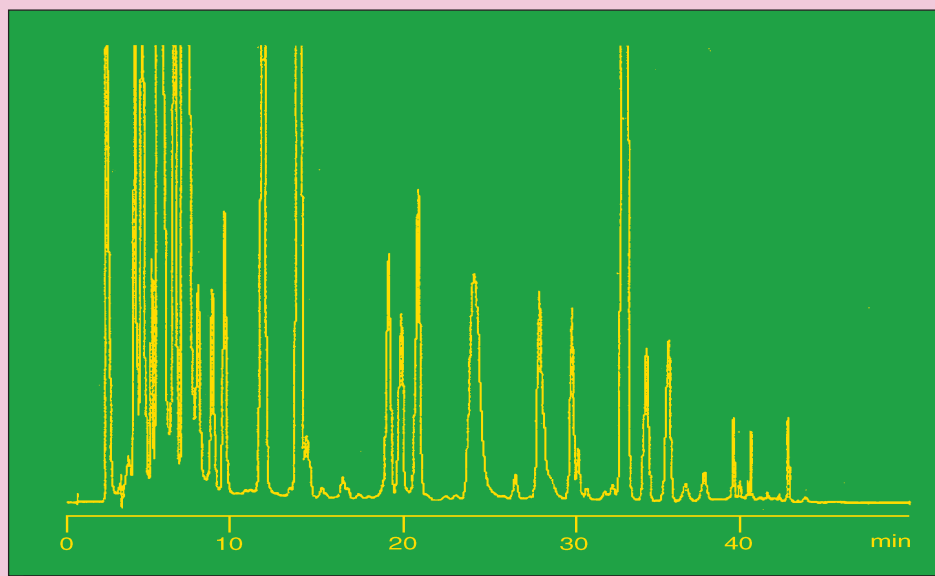
10 059 Determination of Polyamines in Decaying Fish Fillet applying Precolumn Derivatisation with FMOC/EVE and HPLC



10 060 Analysis of Polyamines in Fresh and in Spoiled Meat (pork) by Precolumn Derivatisation with FMOC/EVE



Column phase:
GROM-SIL Polyamine-1, 5 µm
Column size:
200 x 4 mm
Eluent A:
50 mM Na-acetate, pH 4.2 / ACN = 80 / 20
Eluent B:
50 mM Na-acetate, pH 4.2 / ACN = 5 / 95
Gradient:
50% B (0-5 min), 50-100% B (5-20 min),
100% B (20-25 min)
Flow rate:
1.3 ml/min
Pressure:
10.8 MPa
Temperature:
40°C
Detection (Fluor.):
263 nm (exc.), 310 nm (em.)
Injection:
10 µl (0.25 µmol/ml of each)

10 103 Analysis of polyamines in meat employing the FMOC/EVE approach

Column phase:
GROM-SIL Polyamine-2
Column size:
250 x 4 mm
Eluent A:
water / acetic acid / ACN = 400/3/100
Eluent B:
water / acetic acid / ACN = 25/3/475
Gradient:
40% B (0-5 min), 40-50% B (5-15 min), 50% B (15-20 min), 50-65% B (20-27.5 min), 65% B (27.5-35 min), 65-100% B (35-38 min), 100% B (38-50 min)
Flow rate:
1.0 ml/min
Pressure:
15 MPa
Temperature:
40°C
Detection (Fluor.):
263 nm (exc.), 310 nm (em.)
Injection:
10 µl (meat extract)

References

J. Maier-Rosenkranz, A. Maisch, A. Kupka, P. Földi, LC+GC INT. 7, 509-516 (1994)

Derivatisation instructions, E.Grom, Application Service 003

Columns and reagent kits**Complete kit**

983.0000 Complete kit including 4 mm i.d. special column as cartridge, all necessary reagents and detailed working instructions for analysis of polyamines and biogenic amines by precolumn derivatisation with FMOC/EVE (consisting of order nos. GS PO1 0510K2004, GS OD2 2010V0204, GS PO1 V0104V, 2100, 4100, 981.0600, 983.0001, 983.5000, 985.0600).

Columns* and single items for re-ordering

GS PO1 0510K2001	tailor-made, customised 200 x 1 mm POLYAMINE analytical column (cartridge)
GS PO1 0510K2002	tailor-made, customised 200 x 2 mm POLYAMINE Microbore column (cartridge)
GS PO1 0510K2003	tailor-made, customised 200 x 3 mm POLYAMINE analytical column (cartridge)
GS PO1 0510K2004	tailor-made, customised 200 x 4 mm POLYAMINE analytical column (cartridge)
GS OD2 2010V0204	Clean-up precolumn (20 x 4 mm)
2100	Guard column holder for clean-up guard column
GS PO1 0510V0104V	5 Guard columns (10 x 4 mm)
4100	Guard column combination
981.0600	FMOC reagent I (200 ml)
983.0000	Polyamine standard solution (putrescine, cadaverine, spermine, spermidine, histamine; 2.5 µMol/ml each), 1ml
983.0001	Polyamine standard solution (putrescine, cadaverine, spermine, spermidine, histamine; 2.5 µMol/ml each), 10 x 1ml
983.5000	1,8-diamino octane solution (1 µMol/ml) as internal standard, 100 ml concentrate
985.0600	Glycine (EVE), 1g, (to be dissolved in a solution of borate buffer and acetone; see instruction sheet)

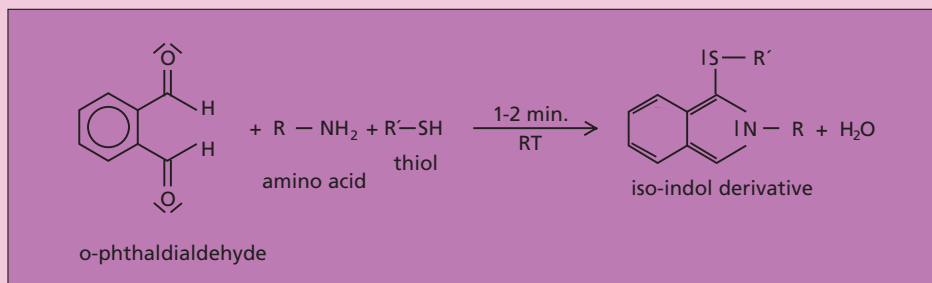
* Cartridges and Columns of other dimensions, e.g., capillary columns, available upon request

Analysis of amino acids by precolumn derivatisation with

OPA/3-MPA

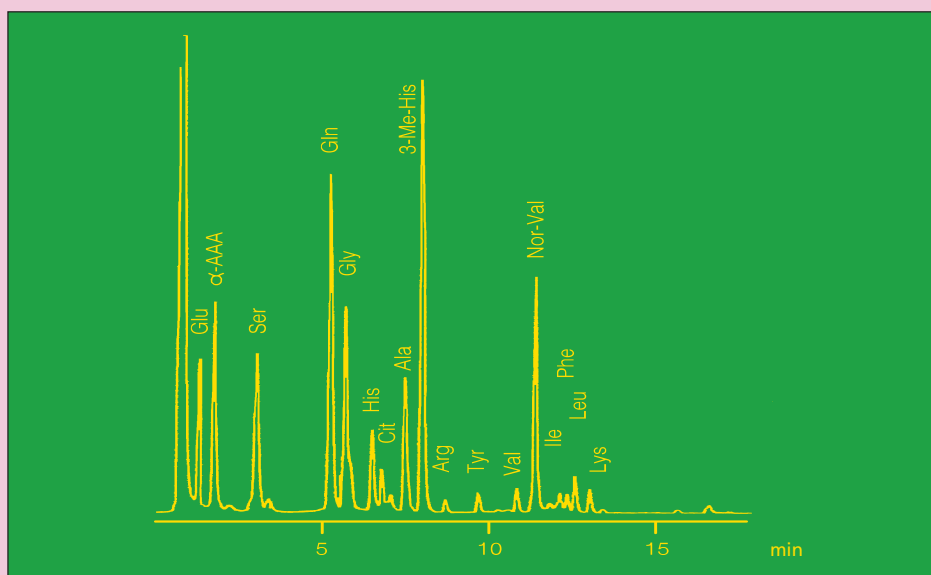
ortho-phthaldialdehyde / 3-mercaptopropionic acid

- ➔ Highest sensitivity (5-10 fMol)
- ➔ Shortest time of analysis
- ➔ Fully automated derivatisation
- ➔ Best resolution
- ➔ Highly reliable



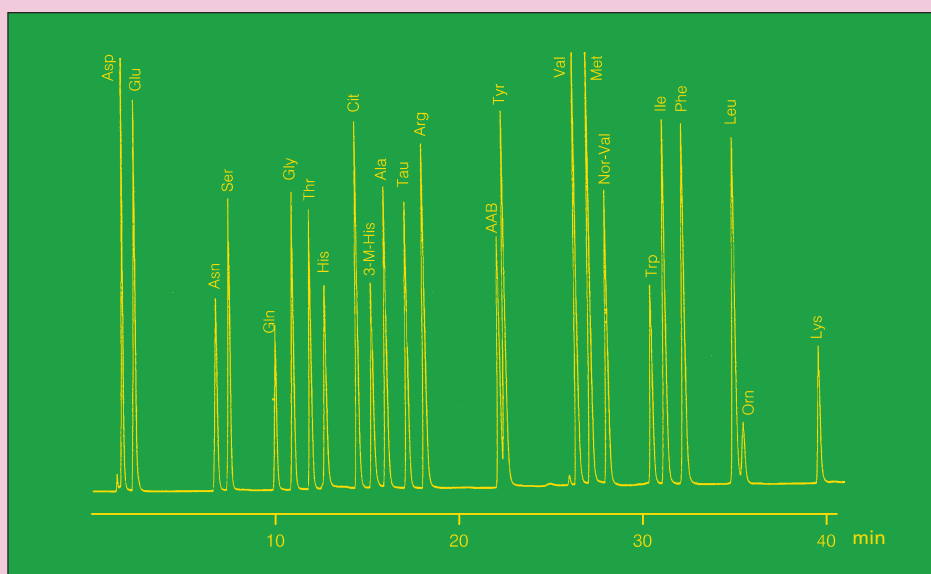
10 061 Amino Acid Analysis of Liver Biopsy Sample using OPA for Precolumn Derivatisation

Column phase:
GROM-SIL OPA-1
Column size:
125 x 4 mm
Eluent A:
12.5 mM Na-phosphate, pH 7.2 /
ACN = 97 / 3
Eluent B:
12.5 mM Na-phosphate, pH 7.2 /
ACN = 50 / 50
Gradient:
0-20% B (0-8 min), 20-55%
B (8-15 min)
Flow rate:
1.1 ml/min
Pressure:
21 MPa
Temperature:
RT
Detection (Fluor.):
330 nm (exc.), 450 nm (em.)
Injection:
20 µl (= 10 pmol, each a.a.)



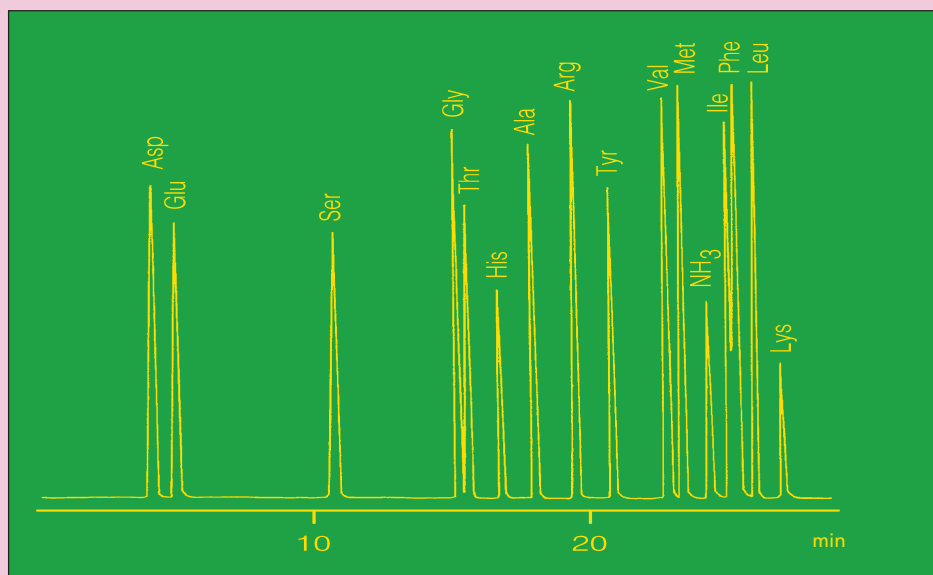
10 062 Amino Acid Analysis after Precolumn Derivatisation with OPA/Mercaptopropionic Acid - Extended Standard -

Column phase:
GROM-SIL OPA-1
Column size:
250 x 4 mm
Eluent A:
25 mM Na-phosphate, pH 7.2 / THF = 995 / 5
Eluent B:
25 mM Na-phosphate, pH 7.2 / MeOH /
ACN = 50 / 35 / 15
Gradient:
0% B (0-3 min), 0-35% B (3-20 min),
35-50% B (20-25 min),
50-60% B (25-36 min),
60-70% B (36-40 min), 70-100% B (40-43 min)
Flow rate:
0.8 ml/min
Pressure:
24 MPa
Temperature:
25°C
Detection (Fluor.):
330 nm (exc.), 450 nm (em.)
Injection:
10 µl (= 10 pmol, each a.a.)



10 063

High Sensitivity Amino Acid Analysis of Protein Hydrolysate by Microbore-HPLC after Precolumn Derivatisation with OPA

**Column phase:**

GROM-SIL OPA-1

Column size:

250 x 1 mm

Eluent A:

12.5 mM Na-phosphate, pH 7.2 / ACN / MeOH = 97/0.5 / 2.5

Eluent B:

12.5 mM Na-phosphate, pH 7.2 / ACN / MeOH = 70 / 25 / 5

Gradient:

0-100% B (0-20 min), 100% B (20-30min)

Flow rate:

0.08 ml/min

Pressure:

30 MPa

Temperature:

30°C

Detection (Fluor.):

330 nm (exc.), 450 nm (em.)

Injection:

1 µl (= 5-10 fmol, each a.a.)

References

H. Godel, T. A. Graser, P. Földi, P. Pfänder, P. Fürst, J. of Chromatogr. 297, 49-61 (1984)

T. A. Graser, H. Godel, S. Anders, P. Földi, P. Fürst, Anal. Biochem. 151, 142-152 (1985)

Derivatisation instructions, E.Grom, Application Service 004

Columns and reagent kits

Complete kit

980.0000 Complete kit including 4.6 mm i.d. special column as cartridge, all necessary reagents and detailed working instructions for amino acid analysis by precolumn derivatisation with OPA/3-MPA (consisting of order nos. GS OP1 0308K1505, GS OD2 2010V0405, GS OP1 0308V0105V, 2100, 4100, 900.0001, 900.0101, 980.0600, 981.0520, 981.0530).

Columns* and single items for re-ordering

GS OP1 0308K1501	tailor-made, customised 150 x 1 mm OPA analytical column (cartridge)
GS OP1 0308K1502	tailor-made, customised 150 x 2 mm OPA Microbore column (cartridge)
GS OP1 0308K1503	tailor-made, customised 150 x 3 mm OPA analytical column (cartridge)
GS OP1 0308K1504	tailor-made, customised 150 x 4 mm OPA analytical column (cartridge)
GS OD2 2010V0204	Clean-up precolumn (20 x 4.0 mm)
2100	Guard column holder for clean-up guard column
GS OP1 0308V0104V	5 Guard columns (10 x 4.0 mm)
4100	Guard column combination
900.0000	L-amino acid standard solution, 17 amino acids (ala, arg, asp. (cys) ₂ ^{**} , glu, gly, his, ile, leu, lys, met, phe, pro, ser, thr, tyr, val); 2.5 µMol/ml each / ^{**} = 1.25 µMol/ml, 1 ml
900.0001	L-amino acid standard solution (17 amino acids; 2.5 µMol/ml each), 10 x 1 ml
900.0100	L-norvaline (2.5 µMol/ml), as internal standard, 1 ml
900.0101	L-norvaline (2.5 µMol/ml), as internal standard, 10 x 1ml
980.0546	Borate buffer (1M, pH 10.7) for high sensitive microanalysis, 200 ml
981.0520	Borate buffer (1M, pH 10.7)
981.0530	Solution for deproteinisation (30% w/v 5-sulfosalicylic acid containing 1.02 mM L-norvaline solution (internal standard), 100ml
980.0600	OPA reagent, 20 ml of 10x stock solution

* Cartridges and Columns of other dimensions, e.g. capillary columns, available upon request

High-sensitivity determination of **D-** and **L-** amino acids after precolumn derivatisation with

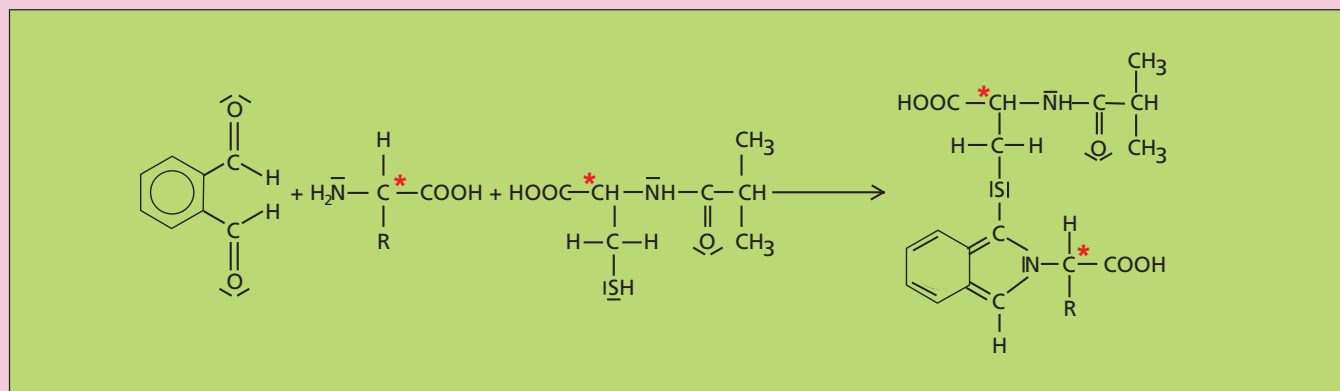
OPA/IBLC

ortho-Phthaldialdehyde / N-isobutryl-L-cysteine

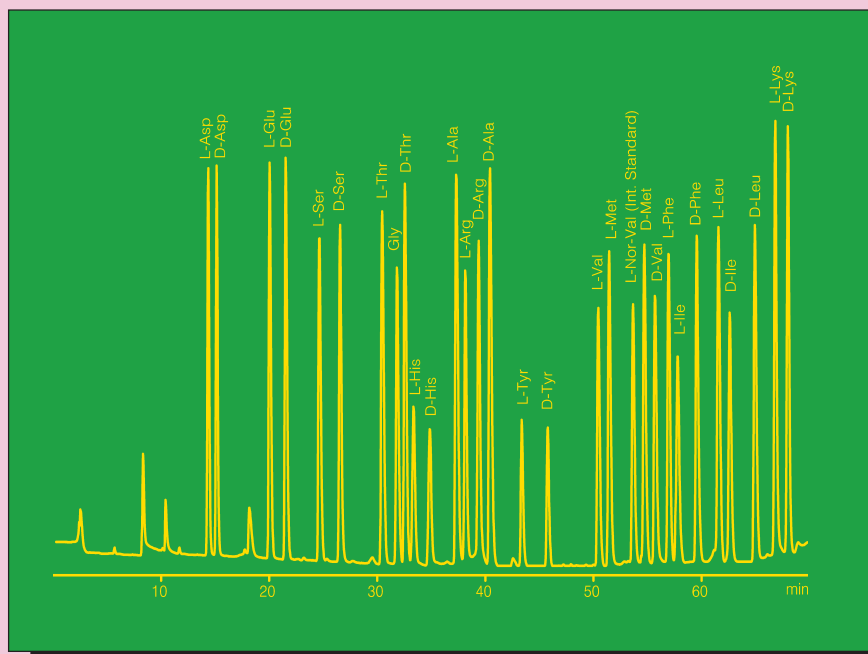
High resolution (both optical antipodes of practically all protein amino acids in a single chromatogram)

Fully automated, simple handling
Very high reproducibility of the results

Outstandingly high sensitivity (fMol range)
Extraordinarily long lifetimes of columns



10 064 Determination of the D- and L-Amino Acids after Precolumn Derivatisation with OPA / N-Isobutryl-L-cysteine



References

- H. Brückner, R. Wittner, H. Godel, *Chromatographia* 32, 383-388 (1991)
- H. Brückner, P. Jack, M. Langer, H. Godel, *Amino-Acids* 2, 271-284 (1992)
- H. Brückner, S. Haasmann, M. Langer, T. Westhauser, R. Wittner, H. Godel, *J. of Chromatogr. A* 666, 259-273 (1994)
- H. Brückner, T. Westhauser, *Chromatographia*, 39 419-426 (1994)

Derivatisation instructions, E. Grom, Application Service 005

Column phase: GROM-SIL OPA-2
Column size: 250 x 4 mm
Eluent: A: 23 mM Na-acetate, pH 6.0
B: 600 ml MeOH + 50 ml ACN
Gradient: 0-53% B (0-75 min)
Flow rate: 0.8 ml/min
Pressure: 18 MPa
Temperature: 24°C
Detection (Fluor.): 230 nm (exc.), 445 nm (em.)
Injection: 10 µl (= 50 pmol, each a.a.)

Columns and reagent kits

Complete kit

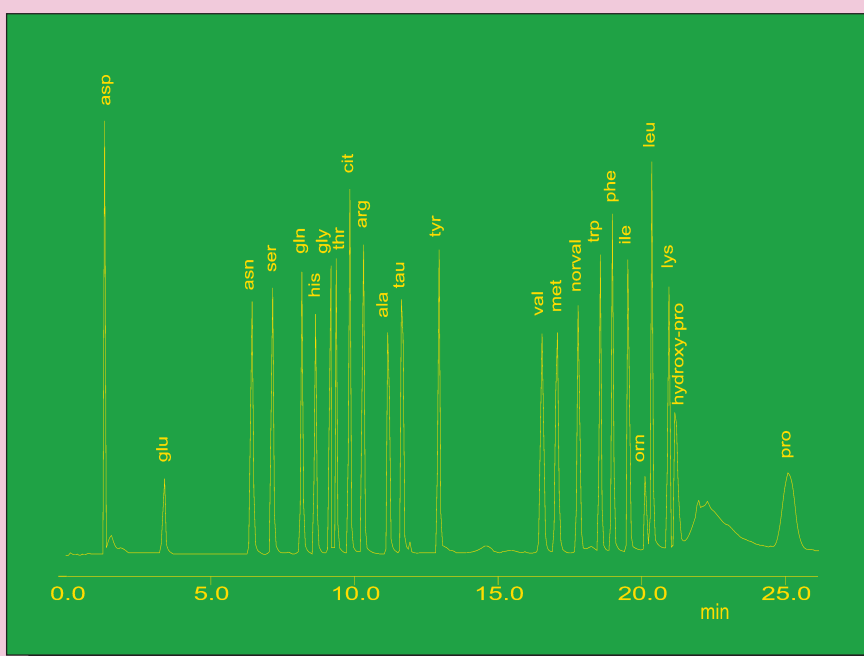
986.0000 Complete kit including 4 mm i.d. special column as cartridge, all necessary reagents and detailed working instructions for analysis of DL-amino acids after precolumn derivatisation with OPA/IBLC (consisting of order nos. GS OP2 0512K2504, GS OD2 2010V0404, GS OP2 0512V0104V, 2300, 4100, 900.0101; 981.0520; 981.0530, 986.0600, 986.1000)

Columns* and single items for re-ordering

GS OP2 0512K2501	tailor-made, customised 250 x 1 mm OPA analytical column (cartridge)
GS OP2 0512K2502	tailor-made, customised 250 x 2 mm OPA Microbore column (cartridge)
GS OP2 0512K2503	tailor-made, customised 250 x 3 mm OPA analytical column (cartridge)
GS OP2 0512K2504	tailor-made, customised 250 x 4 mm OPA analytical column (cartridge)
GS OD2 2010V0204	Clean-up precolumn (20 x 4 mm)
2100	Guard column holder for clean-up guard column
GS OP2 0512V0104V	5 Guard columns (10 x 4 mm)
4100	Guard column combination
900.0001	L-amino acid standard solution, 17 amino acids (ala, arg, asp, (cys) ₂ **, glu, gly, his, ile, leu, lys, met, phe, pro, ser, thr, tyr, val), 2.5 µMol/ml each / ** = 1.25 µMol/ml, 10 x 1 ml
900.0100	L-norvaline (2.5 µMol/ml), internal standard 1 ml
900.0101	L-norvaline (2.5 µMol/ml), internal standard, 10 x 1 ml
981.0520	Borate buffer (1M, pH 10.7)
981.0530	Solution for the deproteinization of proteins (30% w/v 5-sulfosalicylic acid in 1.02 mM L-norvaline solution (internal standard), 100 ml
985.2000	DL- amino acid standard (24 diastereomeric pairs of amino acids, 100 mg of each)- ala, arg, asn, asp, cit, cys, dopa, glu, gly, his, ile, leu, lys, met, nor-leu, nor-val, orn, phe, pro, ser, thr, trp, tyr, val -
986.0600	OPA-chiral reagent (10ml of concentrate, to be diluted 1:10)
986.1000	DL- amino acid standard solution, - ala, arg, asp, glu, gly, his, ile, leu, lys, met, L-nor-val, phe, ser, thr, tyr, val: 2.5 µMol/ml each, 5 x 1 ml

New! **987.0000** Complete kit including 4 mm i.d. special column as cartridge, all necessary reagents and detailed working instructions for analysis of amino acids after precolumn derivatisation with OPA/3-MPA.

10 165 Analysis of Primary and Secondary Amino Acids by Precolumn Derivatisation with OPA and FMOC



Column phase: GROM-SIL OPA-3
Column size: 125 x 4 mm
Eluent: 25 mM Na-Phosphate, pH 7.2 / THF = 99.2 / 0.8
Gradient: 0% B (0-2 min), 0-50% B (2-10 min), 50-60% B (10-15 min), 60-100% B (15-20 min), 100% B (20-25 min)
Flow rate: 1.1 ml/min
Pressure: 29 MPa
Temperature: RT
Detection (Fluor.): 330 nm (exc.), 450 nm (em.), after lys (~22 min)
Injection: 10 µl (10 pmol, each a.a.)

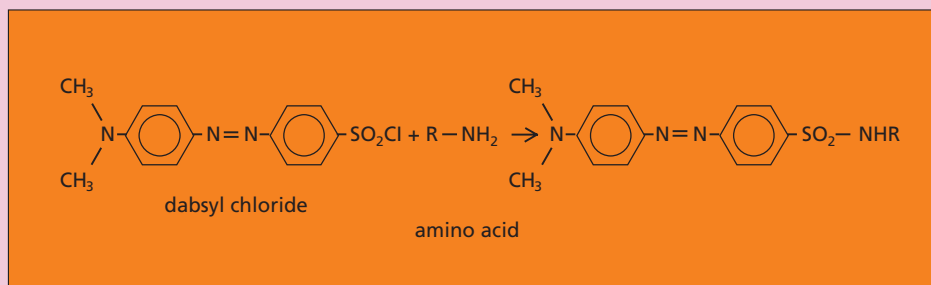
* Cartridges and Columns of other dimensions, e.g. capillary columns, available upon request

Analysis of **amino acids** after precolumn derivatisation with

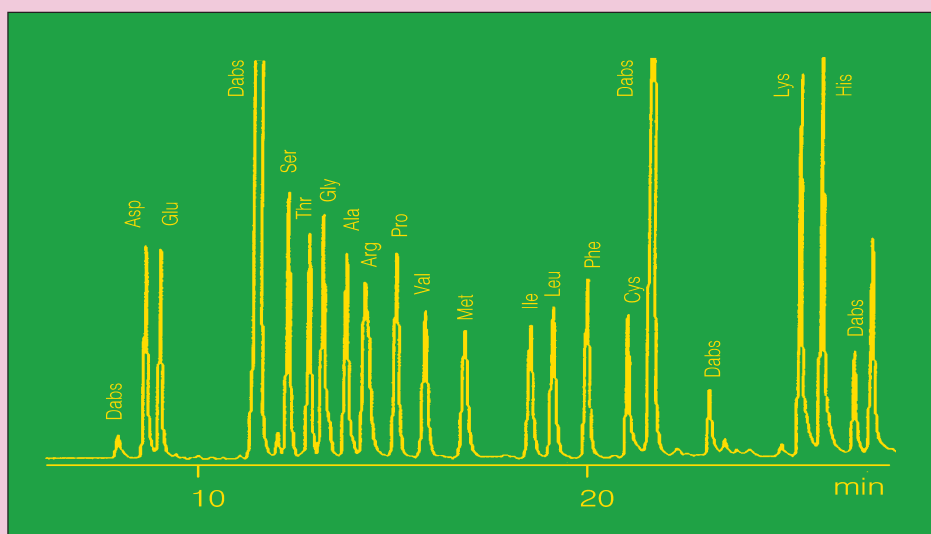
DABS-Cl

4-Dimethylaminoazobenzene-4'-sulfonyl chloride

- ➔ Derivatisation of primary and secondary amines
- ➔ Simple spectrophotometric detection at 436 nm
- ➔ Stable derivatives
- ➔ Excellent resolution

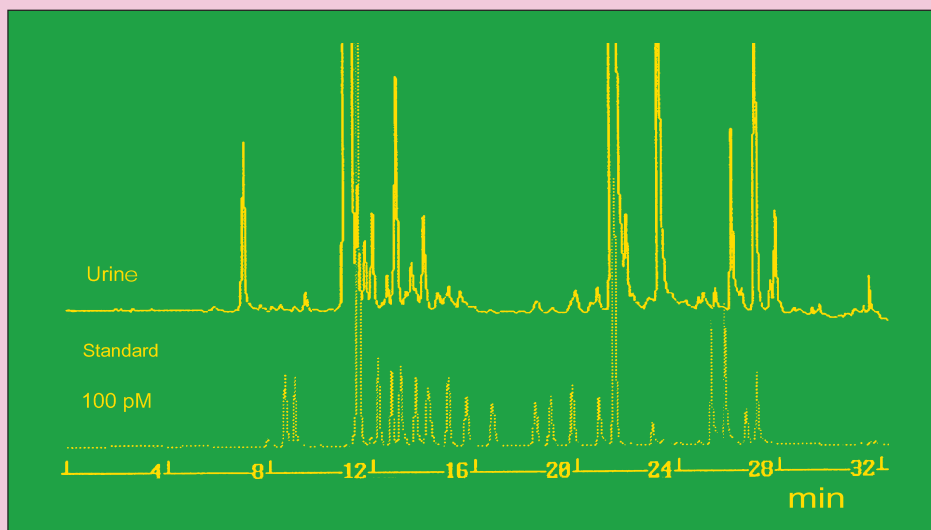


10068 a Separation of Amino Acids by HPLC after Precolumn Derivatisation with DABS-Cl - protein hydrolysate standard -

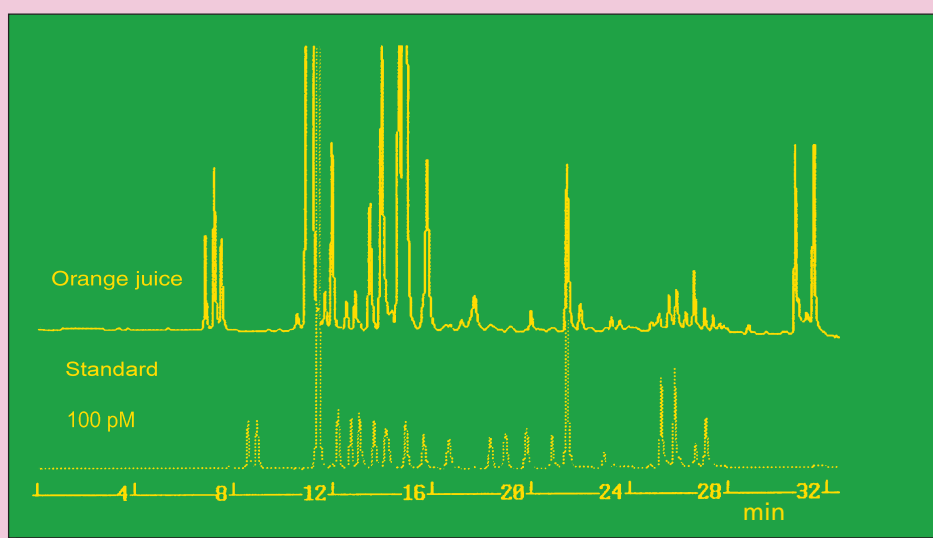


10068 b Determination of Amino Acids in Urine by HPLC after Precolumn Derivatisation with DABS-Cl

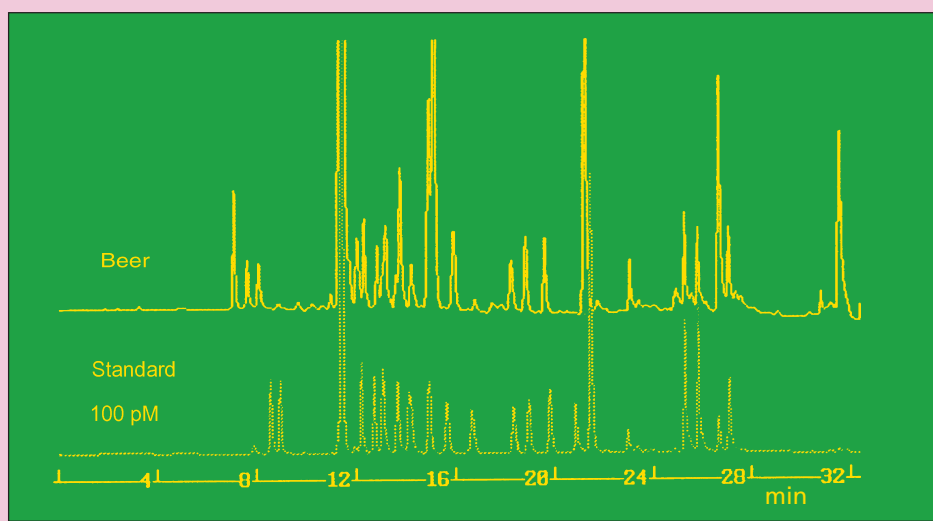
Column phase:
GROM-SIL DABS-1
Column size:
200 x 4.6 mm
Eluent A:
24 mM Na-acetate, pH 6.6 /
ACN = 80 / 20
Eluent B:
ACN / 2-propanol = 40 / 60
Gradient:
5% B (0-4 min), 5-20% B (4-8 min), 20-25%
B (8-15), 25-60% B (15-27), 60-100%
B (27-28 min),
100% B (28-32 min)
Flow rate:
1 ml/min
Pressure:
17 MPa
Temperature:
45°C
Detection (VIS):
436 nm
Injection:
10 µl (~100 pmol, each a. a.)



10068c Determination of Amino Acids in Urine by HPLC after Precolumn Derivatisation with DABS-Cl



10068d Determination of Amino Acids in Beer by HPLC after Precolumn Derivatisation with DABS-Cl



References

J. Y. Chang, P. Martin, R. Bernasconi, D. G. Braun, FEBS Lett. 132, 117-120 (1981)

J. Y. Chang, R. Knecht, D. G. Braun, Biochem.J. 199, 547-555 (1981)

Derivatisation instructions, E.Grom, Application service 006

Columns and reagent kits

Complete kit

982.0000 Complete kit including 4.6 mm i.d. special column as cartridge, all necessary reagents and detailed working instructions for analysis of amino acids by precolumn derivatisation with DABS-Cl (consisting of order nos. GS DA1 0410K2005, GS OD2 2010V0405, GS DA1 0410V0105V, 2100, 4100, 900.0001, 982.0101, 982.0600).

Columns* and single items for re-ordering

GS DA1 0410K2001	tailor-made, customised 200 x 1 mm DABS analytical column (cartridge)
GS DA1 0410K2002	tailor-made, customised 200 x 2 mm DABS Microbore column (cartridge)
GS DA1 0410K2003	tailor-made, customised 200 x 3 mm DABS column analytical (cartridge)
GS DA1 0410K2004	tailor-made, customised 200 x 4 mm DABS analytical column (cartridge)
GS OD2 2010V0204	Clean-up precolumn (20 x 4 mm)
2100	Guard column holder for clean-up guard column
GS DA1 0410V0104V	5 Guard columns (10 x 4 mm)
4100	Guard column combination

900.0000	L-amino acid standard solution, 17 amino acids (ala, arg, asp, (cys) ₂ **, glu, gly, his, ile, leu, lys, met, phe, pro, ser, thr, tyr, val), 2.5 µMol/ml each / ** = 1.25 µMol/ml, 1 ml
900.0001	L-amino acid standard solution (17 amino acids; 2.5 µMol/ml each), 10 x 1 ml
982.0100	S-Carboxyethyl-L-cysteine, internal standard solution, (2.5 µMol/ml), 1 x 1 ml
982.0101	S-Carboxyethyl-L-cysteine, internal standard solution, (2.5 µMol/ml), 10 x 1 ml
982.0600	DABS-Cl reagent, 10 vials, 1.3 mg each

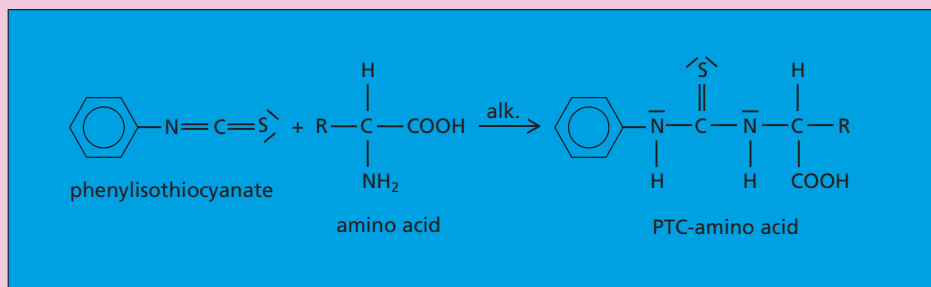
* Cartridges and Columns of other dimensions, e.g. capillary columns, available upon request

Amino acid analysis after precolumn derivatisation with

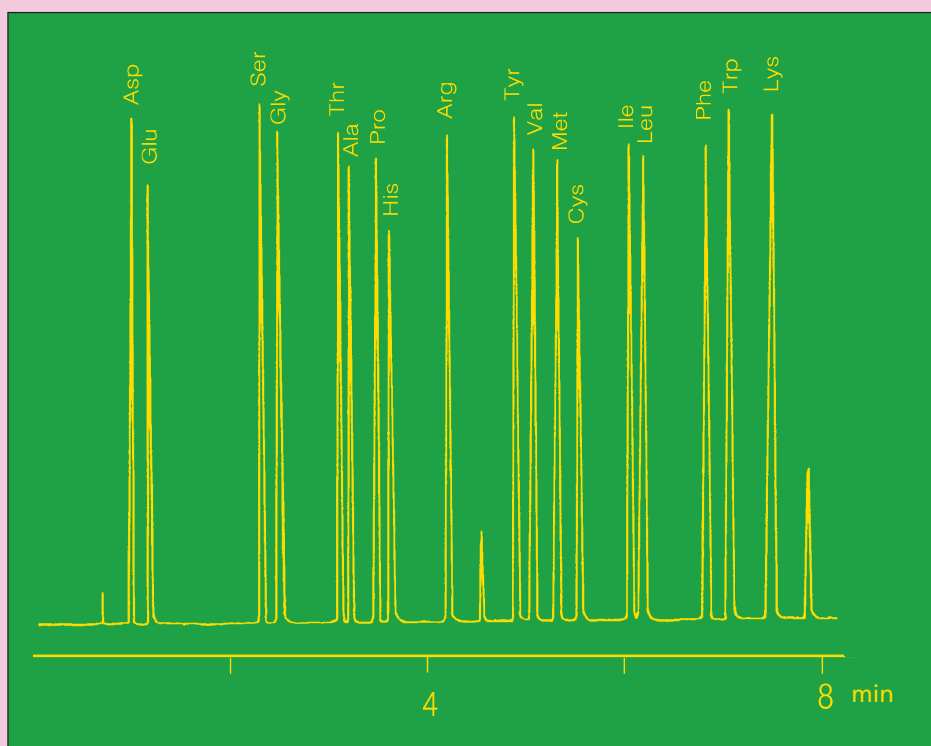
PITC

Phenylisothiocyanate

- Fast separations
- High resolution
- Can be automated
- Stable derivatives
- Simple UV detection at 254 nm



10 065 Amino acid analysis by precolumn derivatisation with PITC



Column phase: GROM-SIL 80 ODS-2 FE, 3 μm
Column size: 125 x 4 mm (with 10 mm guard column)
Eluent A: 50 mM Na-acetate, pH 6.5
B: 50 mM Na-acetate, pH 6.5 / ACN = 50 / 50
Gradient: 0-45% B (0-8 min), 45-100% B (8-10 min)
Flow rate: 1.2 ml/min
Pressure: 15 MPa
Temperature: 55°C
Detection (UV): 254 nm, 0.01 AUFS
Injection: 20 μl (~100 pmol each a. a.)

References

P. Edman, A. Henschen, „Determination in Protein Sequence“, 2nd ed., (S. B. Needleman, ed.), Springer Verlag, Berlin, (1975)

R. L. Heindrikson, S.C. Meredith, Anal. Biochem. 135, 65-74 (1984)

B. A. Bidlingmeyer, S. A. Cohen, T. L. Tarvin., J. of Chromatogr. 336, 93-104 (1984)

Protein sequencing (Edman-degradation) with HPLC-determination of the

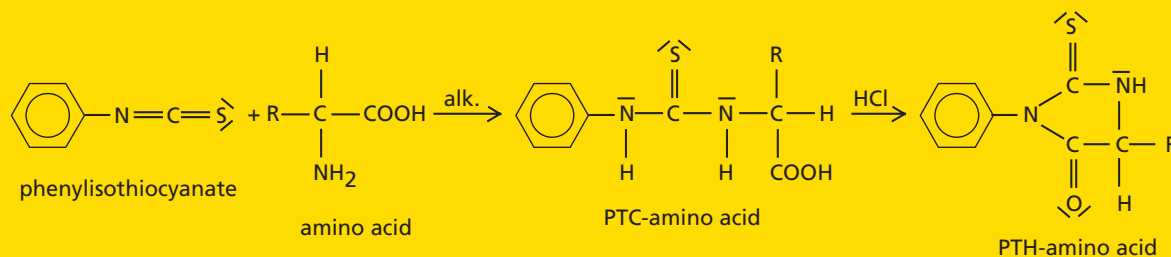
PTH-Amino acids

High sensitivity
(limit of detection
 ≤ 100 fMol)

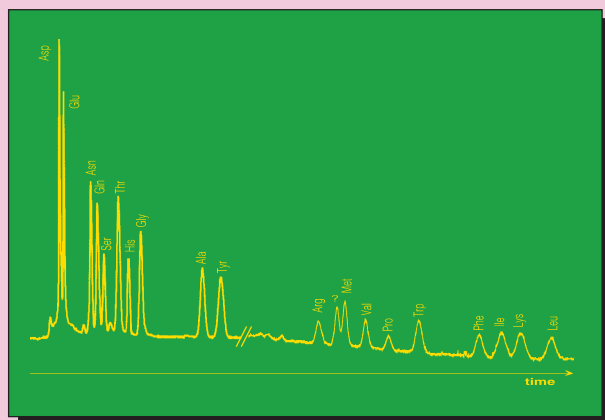
Simple and reliable
separation of all amino
acids under either
isocratic or gradient
elution conditions

Short times of
analysis (≤ 15 min)
Long life-times of
columns

Simple handling of
microbore or
capillary column
hardware
(fingertight)



10 066 Isocratic PTH-Amino Acid Analysis by Capillary HPLC



Column phase:
Superspher C 8 endc., 4 μm
NovoGROM Capillary column:
250 mm x 300 μm
Eluent:
21 mM Na-acetate, pH 4.9 /
ACN / 1,2-dichloroethane =
68.5/31/0.5- v/v
Flow rate:
0.04 ml/min
Pressure:
21 MPa
Temperature:
60°C
Detection (UV):
264 nm, 0.005 AUFS
Injection:
5 μl (~3 pmol, each a. a.)

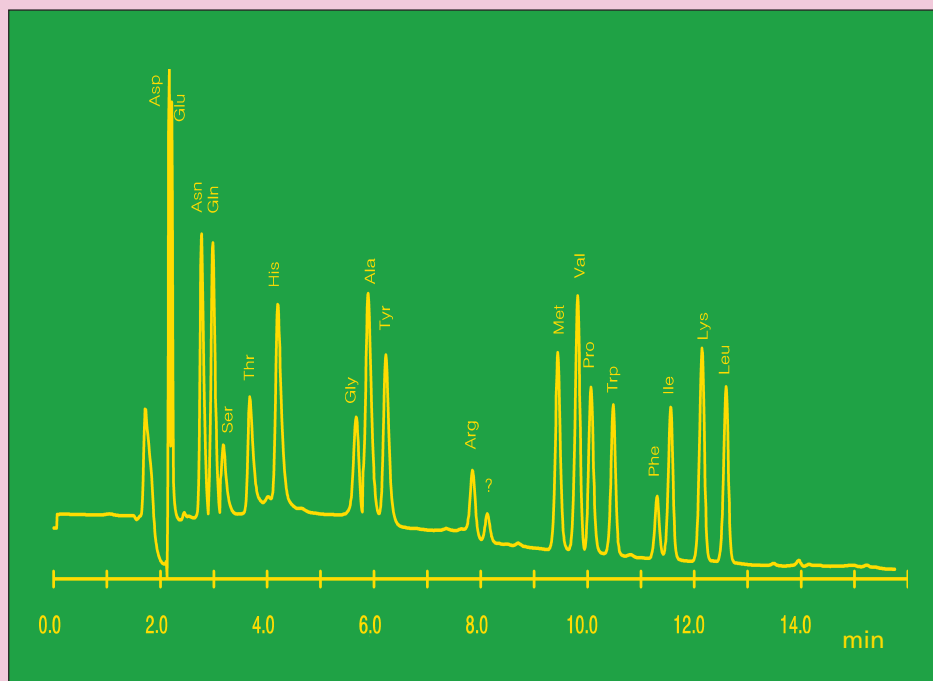
References

P. Edman, Acta Chem. Scand. 4,
283 ff (1949)

F. Lottspeich, Hoppe-Seyler's Z.
Physiol. Chem. 361, 1829-1834
(1980)

F. Lottspeich, J. of Chromatogr,
326, 321-327 (1985)

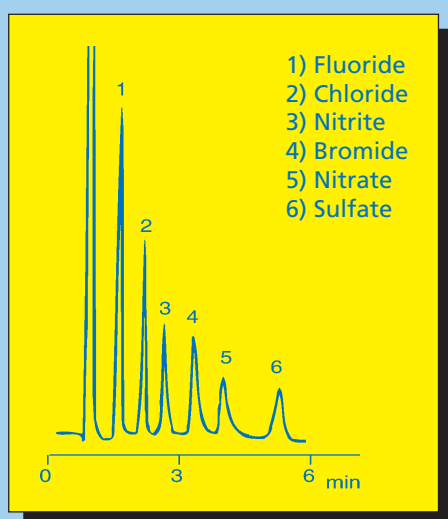
10 067 PTH-Amino Acid Analysis by HPLC applying Gradient Elution



Column phase:
Superspher C 8 endc., 4 μm
NovoGROM-Column:
250 x 4 mm
Eluent A:
21 mM Na-acetate, pH 4.9
Eluent B:
ACN / 1,2-dichloroethane = 993 / 7
Gradient:
30% B (0-1 min),
30-80% B (1-25 min)
Flow rate:
1 ml/min
Pressure:
19 MPa
Temperature:
60°C
Detection (UV):
254 nm
Injection:
10 μl (~3 pmol, each a. a.)

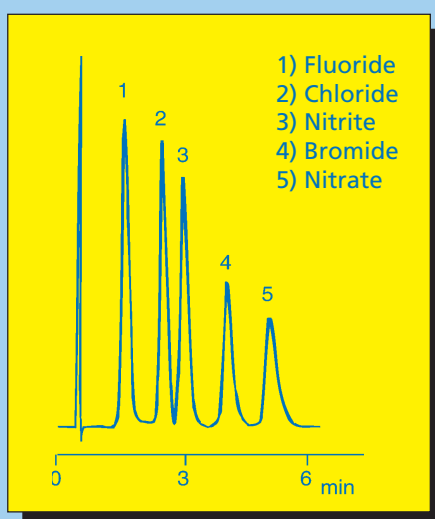
PEEK HPLC Columns

- **fully biocompatible, nevertheless stable up to 40 MPa**
➔ to satisfy even the highest demands of sophisticated biochemical applications
- **all wetted surfaces made from PEEK**
➔ inert against high salt concentrations, even halide-containing buffers
- **stainless steel threads**
➔ countless refilling with any of the stationary phases listed in the HPLC catalogue
- **finger-tight hardware**
➔ convenient, easy to use (without any tools)
- **Low dispersion system with integrated guard columns**
➔ always guarantees high resolution and maximum peak symmetry



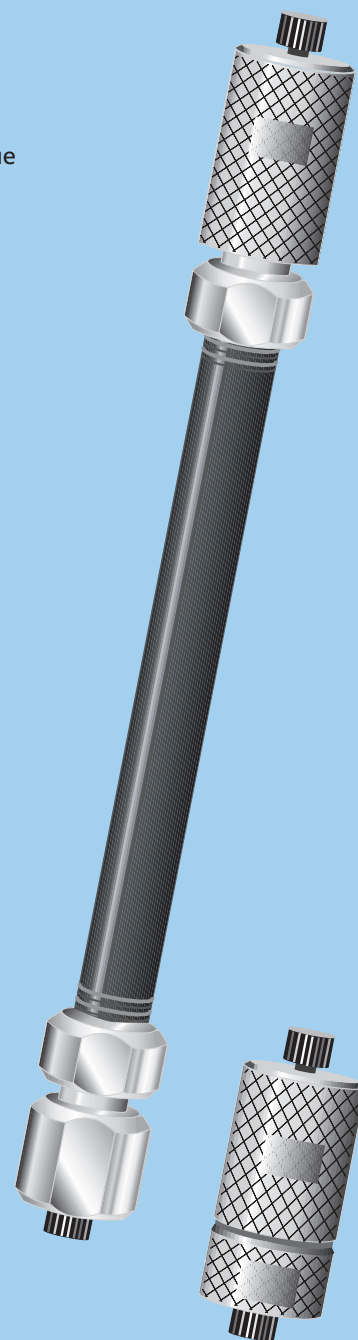
Determination of anions by indirect UV-detection

Column phase: GROM-Anion-1, 10 μm
 Column size: PEEK column 150 x 4.0
 Eluent: 2.0 mM potassium hydrogen phthalate, pH 6.0
 Gradient: 5–50% B (0–12 min)
 Flow rate: 1.2 ml/min
 Injection: 100 μl (10 ppm)
 Detection (UV): 280 nm (indirect)



Determination of anions by conductivity detection

Column phase: GROM-Anion-1, 10 μm
 Column size: PEEK column 150 x 4.0
 Eluent: 2.0 mM sodium benzoate pH 6.5
 Flow rate: 2.5 ml/min
 Injection: 100 μl (10 ppm)
 Detection: Conductivity



Guard-column holder can be used either as a sample enrichment column or for any guard column connected in the traditional way.

PEEK HPLC Column System

- Column inner diameter 4.0 mm
- Guard columns 10 and 20 mm in length
- Guard column head for direct, dead volume free coupling of columns and guard columns
- Guard column holder