LC/MS analysis of hydrophilic compounds by a polymer-based amino HILIC column

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Abstract

The effectiveness of the polymer-based amino HILIC column, Shodex Asahipak NH2P, for the analysis of saccharides and their related compounds is well established. However, proven applications for other types of hydrophilic compounds using this HILIC column were limited.

The use of high organic content mobile phase in HILIC mode is suitable for the LC/MS high sensitive analysis. In this study, we present LC/MS methods for the analysis of various hydrophilic compounds using NH2P series column.

1. Analysis of amino acids

An LC/MS method was developed for the analysis of 20 amino acids without derivatization steps. Separation suitable for the LC/MS identification of all amino acids was obtained. Signals detected by an ESI-MS provided linear calibration curves (exception of few) with low detection limit. Developed method was applied to determine amino acids in a commercially available product.

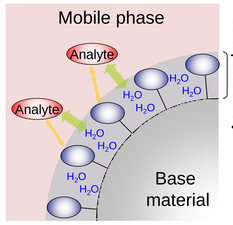
2. Analysis of other hydrophilic compounds

Methods for the separation of various hydrophilic compounds including, creatine and creatinine, melamine and cyanuric acid, were developed. Baseline separation of these compounds were accomplished. Additionally, in the case of creatine and creatinine analysis, no interference from the urea was observed, providing a good sensitivity.

Overview of HILIC

HILIC = Hydrophilic Interaction Chromatography

It is one type of normal phase mode
- Stationary phase: Contains polar functional group
- Mobile phase: High water-miscible organic solvent ratio, water acts as a strong solvent
- Higher the hydrophilicity, longer the retention
- Reversed order of elution compared to reverse phase mode



Separation mechanisms
- Partition of analytes between mobile phase and aqueous layer formed on the surface of stationary phase
- Interaction between analytes and polar functional group; e.g. Ion exchange and hydrogen bond interaction

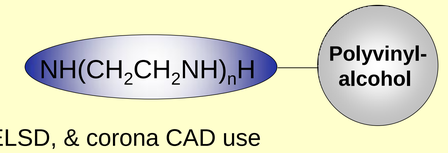
= Polar functional group
e.g. Hydroxy, Carbamoyl, Amino

Advantages of HILIC
- Hydrophilic compounds such as drugs, metabolites, and nutrients, which elute at void volume on reverse phase mode are retained
  - → Hydrophilic compounds can be separated from matrix and other components, provides an accurate analysis of them
  - → No derivatization or additional ion-pair reagent is required
- Use of high organic ratio solvent as an eluent
  - → High sensitivity on ESI-MS
  - → Possible to inject samples pretreated with high organic solvent directly

Asahipak NH2P series

Product specifications and Line up

- Polyamine modified on PVA base gel
- Excellent chemical stability
- Wide usable pH range (pH 2 -13)
- Washable with alkaline solution
- Because of the low bleed, suitable for MS, ELSD, & corona CAD use

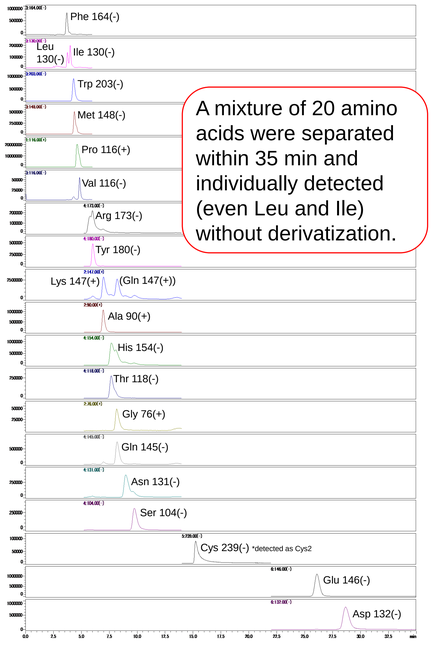


Suitable column dimensions for LC/MS

Product name	Plate Number (TP/Column)	Functional Group	Particle Size (μm)	Pore Size (Å)	Column Size (mm) I.D. × Length
Asahipak NH2P-40 2B	≥ 2,000	Amino	4	100	2.0 × 50
Asahipak NH2P-40 2D	≥ 5,500	Amino	4	100	2.0 × 150
Asahipak NH2P-40 2E	≥ 7,000	Amino	4	100	2.0 × 250
Asahipak NH2P-50 2D	≥ 3,500	Amino	5	100	2.0 × 150
Asahipak NH2P-50G 2A	(guard column)	Amino	5	-	2.0 × 10
Asahipak NH2P-40 3E	≥ 8,500	Amino	4	100	3.0 × 250
Asahipak NH2P-50G 3A	(guard column)	Amino	5	-	3.0 × 10
Asahipak NH2P-50 4B	≥ 1,500	Amino	5	100	4.6 × 50
Asahipak NH2P-50 4D	≥ 5,500	Amino	5	100	4.6 × 150
Asahipak NH2P-50 4E	≥ 7,500	Amino	5	100	4.6 × 250
Asahipak NH2P-50G 4A	(guard column)	Amino	5	-	4.6 × 10
Asahipak NH2P-LF	≥ 1,500	Amino	-	-	8.0 × 75

【Result 1】 Simultaneous analysis of 20 amino acids

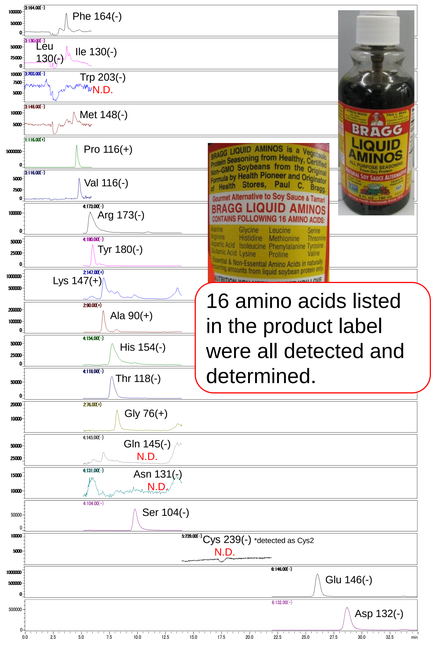
Amino acid standards



A mixture of 20 amino acids were separated within 35 min and individually detected (even Leu and Ile) without derivatization.

Sample : 20 Amino acid standards 5 μg/mL each in H_2O/CH_3CN=25/75, 5μL

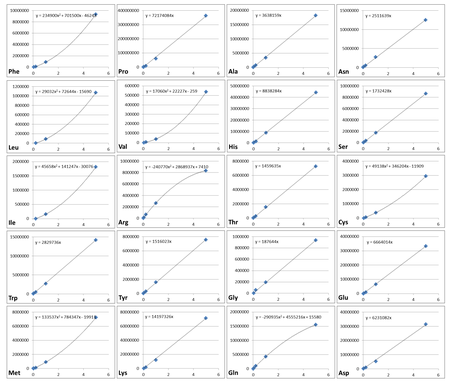
Analysis of commercial product
Bragg Liquid Amino*



16 amino acids listed in the product label were all detected and determined.

Sample : Filtered after 4000 x dilution with H_2O/CH_3CN=25/75, 5 μL
*Bragg Amino Liquid is a product of Bragg Live Foods, Inc.

Calibration curves



Quantitative results

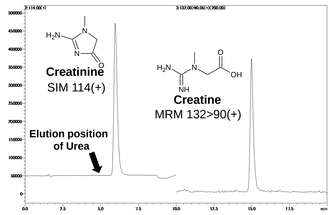
	Phe	Leu	Ile	Trp	Met	Pro	Val	Arg	Tyr	Lys
Concentration in bragg's (wt%)	0.45	0.91	0.38	N.D.	0.05	0.57	0.52	0.68	0.17	0.52
% Recovery rate*	118	97	130	75	122	122	70	96	85	60
Detection limit (μg/mL)	0.2	0.5	0.5	0.01	0.02	0.002	0.2	0.02	0.01	0.1
	Ala	**His**	**Thr**	**Gly**	**Gln**	**Asn**	**Ser**	**Cys**	**Glu**	**Asp**
Concentration in bragg's (wt%)	0.35	0.08	0.53	0.47	N.D.	N.D.	0.47	N.D.	1.5	1.4
% Recovery rate*	109	112	119	104	95	102	102	84	144	168
Detection limit (μg/mL)	0.005	0.02	0.01	0.2	0.05	0.02	0.005	0.2	0.01	0.005

*Calculated based on 25 μg/mL standard spiked into 4000 x diluted sample

Column : Shodex Asahipak NH2P-40 2D (2.0 mm I.D. x 150 mm)
Eluent : (A) 100 mM $HCOONH_4$ (aq) / (B) CH_3CN
Linear gradient; (B%) 75% (0-10 min) → 50% (11-35 min)
Flow rate : 0.2 mL/min Column temp. : 30 °C
Instrument : Shimadzu Nexera / LCMS-8030
Detector : ESI-MS SIM

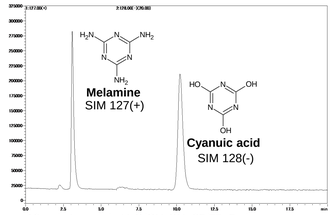
【Result 2】 Analysis of other hydrophilic compounds

Creatine & Creatinine



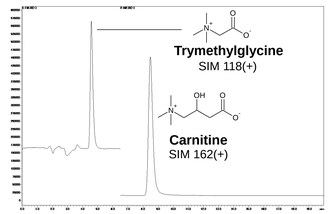
10 ng/mL each in H_2O/CH_3CN=25/75, 5 μL
Linear gradient; (B%) 90% (0-6 min) → 75% (7-20 min)

Melamine & Cyanuic acid



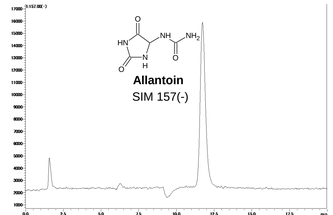
10 ng/mL each in H_2O/CH_3CN=25/75, 5 μL
Isocratic; (B%) 75%

Trymethyl glycine & Carnitine



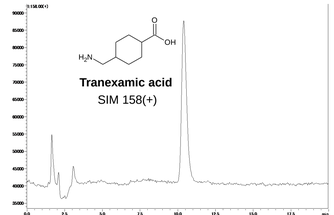
5 ng/mL each in H_2O/CH_3CN=20/80, 5 μL
Isocratic; (B%) 80%

Allantoin



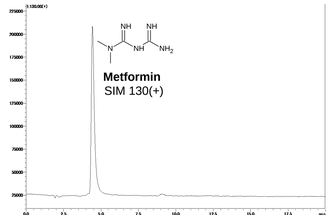
25 ng/mL in H_2O/CH_3CN=25/75, 5 μL
Isocratic; (B%) 90%

Tranexamic acid



5 ng/mL in H_2O/CH_3CN=25/75, 5 μL
Isocratic; (B%) 75%

Metformin-HCl



5 ng/mL in H_2O/CH_3CN=5/95, 5 μL
Isocratic; (B%) 95%

- Mobile phase containing >75% CH_3CN was suitable for the analysis of various hydrophilic compounds; resulted in moderate retention and sharp symmetrical peaks
- NH2P column with 2.0 mm I.D. x 150 mm dimensions is very useful for high sensitive LC/MS analysis of hydrophilic compounds

Column : Shodex Asahipak NH2P-40 2D (2.0 mm I.D. x 150 mm)
Eluent : (A) 50 mM $HCOONH_4$ (aq) / (B) CH_3CN
Flow rate : 0.2 mL/min
Column temp. : 30 °C
Instrument : Shimadzu Nexera / LCMS-8030
Detector : ESI-MS