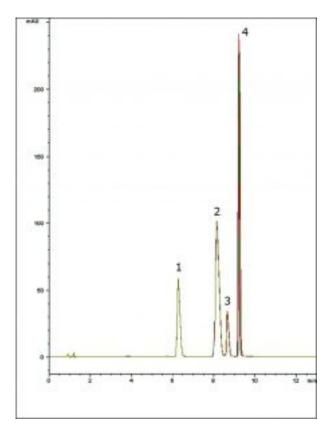


Organic Bases and Isomers at a Low pH - AppNote

Separation of Four Amine Containing Test Solutes

The four test solutes selected for this application are both well-retained and well-resolved. In particular, the separation between the two isomers is readily accomplished, which may be difficult to achieve in Reversed Phase.

This Method uses only Formic Acid as the Mobile Phase additive and is LCMS compatible. This is in contrast with many Reversed Phase methods, which may require Ion Pair Agents in the Mobile Phase.



Peaks:

- 1. 2-Amino-5-Methyl-1,3,4-Thiazole
 - 2. 4-Amino-2-Chloropyridine
 - 3. 5-Amino-1H-Indole
 - 4. 4-Amino-3-Chloropyridine



Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å

Catalog No.: 70000-7.5P Dimensions: 4.6 x 75mm

Mobile Phase:

A: DI Water / 0.1% Formic Acid (v/v)
B: Acetonitrile / 0.1% Formic Acid (v/v)

Gradient:

| Time (minutes) | %B |
|----------------|----|
| 0 | 95 |
| 2 | 95 |
| 9 | 65 |
| 10 | 95 |

Post Time: 3 minutes **Flow rate:** 1.0 mL/minute **Detection:** UV @ 254 nm

Injection vol.: 1µL

 $\textbf{Sample Preparation:} \ 1 \text{mg/mL stock solutions of the four test solutes were prepared using a 50 / 50 Solvent A / 2 mg/mL stock solutions of the four test solutes were prepared using a 50 / 50 Solvent A / 2 mg/mL stock solutions of the four test solutes were prepared using a 50 / 50 Solvent A / 2 mg/mL stock solutions of the four test solutes were prepared using a 50 / 50 Solvent A / 2 mg/mL stock solutions of the four test solutes were prepared using a 50 / 50 Solvent A / 2 mg/mL stock solutions of the four test solutes were prepared using a 50 / 50 Solvent A / 2 mg/mL stock solutions of the four test solutes were prepared using a 50 / 50 Solvent A / 2 mg/mL stock solutions of the four test solutes were prepared using a 50 / 50 Solvent A / 2 mg/mL stock solutions of the four test solutes were prepared using a 50 / 50 Solvent A / 2 mg/mL stock solutions of the four test solutes were prepared using a 50 / 50 Solvent A / 2 mg/mL stock solutions of the four test solutes were prepared using a 50 / 50 Solvent A / 2 mg/mL stock solutions of the four test sol$

Solvent B diluent. 100µL Aliquots of each were mixed and diluted with 600µL of the diluent.

to: 0.9 minutes

Note: Organic bases have a tendency to tail using many HPLC Columns due to interactions with residual silanols on the stationary phase surface. With TYPE- C^{TM} Silica based columns these silanols are mostly replaced with Si-H groups, which often leads to more symmetrical peak shapes for these kinds of analytes.



Attachment

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