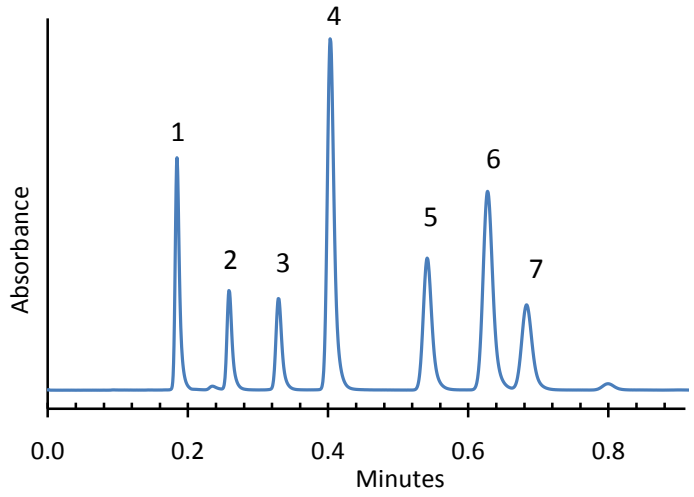
Application Note: 20-B

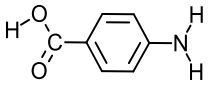
# Isocratic Separation of Anilines on HALO C18



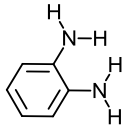

**PEAK IDENTITIES:**
1. p-Aminobenzoic acid
2. 1, 2-Phenylenediamine
3. p-Anisidine
4. Aniline
5. 3-Nitroaniline
6. 2-Nitroaniline
7. 4-Chloroaniline

**TEST CONDITIONS:**
Column: HALO C18, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 60/40-- A/B
A=0.02 M sodium phosphate buffer, pH=7.0
B= Acetonitrile
Flow Rate: 2.0 mL/min.
Pressure: 211 Bar
Temperature: 25°C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: ACN/ water-50/50
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

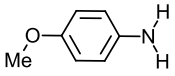
**STRUCTURES:**



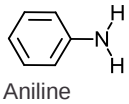

p-Aminobenzoic acid



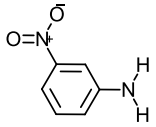

1,2-phenylenediamine



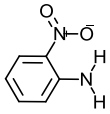

p-Anisidine



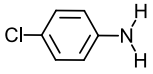

Aniline




3-Nitroaniline



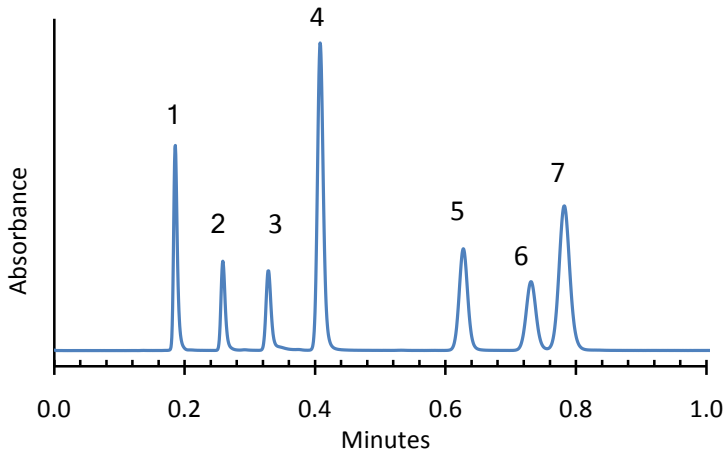

2-Nitroaniline




4-Chloroaniline

Application Note: 21-B

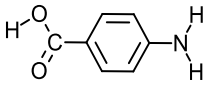
# Isocratic Separation of Anilines on HALO RP-Amide

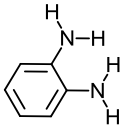


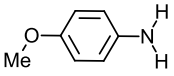

**PEAK IDENTITIES:**
1. p-Aminobenzoic acid
2. 1, 2-Phenylenediamine
3. p-Anisidine
4. Aniline
5. 3-Nitroaniline
6 4-Chloroaniline
7. 2-Nitroaniline

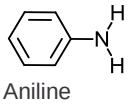
**TEST CONDITIONS:**
Column: HALO RP-Amide, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 60/40-- A/B
A=0.02 M sodium phosphate buffer, pH=7.0
B= Acetonitrile
Flow Rate: 2.0 mL/min.
Pressure: 180 Bar
Temperature: 25°C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: ACN/ water-50/50
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

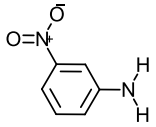
**STRUCTURES:**

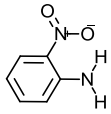

p-Aminobenzoic acid

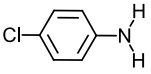

1,2-phenylenediamine


p-Anisidine


Aniline

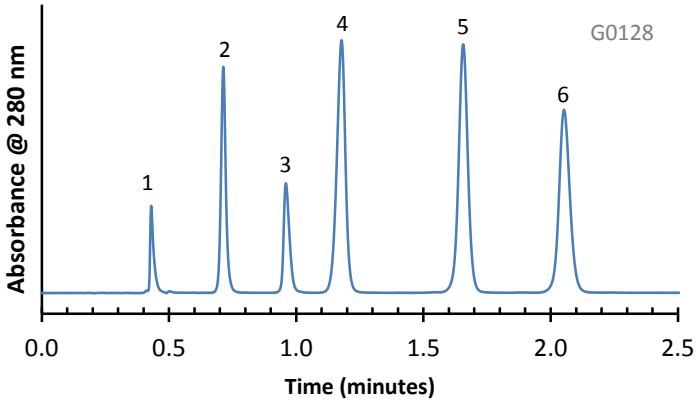

3-Nitroaniline


2-Nitroaniline


4-Chloroaniline

Application Note: 140-B

# Separation of Biogenic Amines on HALO 5 Phenyl-Hexyl by Ion-Pairing




**PEAK IDENTITIES:**

1. System peak, $t_0$
2. L-Tyrosine
3. Octopamine
4. ± Synephrine
5. Tyramine
6. Hordenine

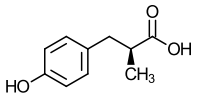
## TEST CONDITIONS:

Column: HALO 5 Phenyl-Hexyl, 3.0 x 100 mm, 5 µm
Part Number: 95813-606
Mobile Phase: 78/22 A/B
A= 0.05 M Phosphate buffer, (pH=3.0)
with 2.7 g/L of sodium hexanesulfonate
B= Methanol
Gradient:

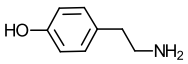
| Time (min.) | %B |
|---|---|
| 0.0 | 22 |
| 4.0 | 30 |

Flow Rate: 0.8 mL/min.
Pressure: 170 Bar
Temperature: 30°C
Sample Solvent: 90/10: water/methanol
Injection Volume: 2.0 µL
Detection: UV 280 nm, VWD
Response Time: 0.02 sec.
Data rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

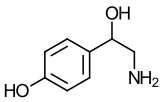
## STRUCTURES:



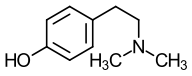

L-Tyrosine



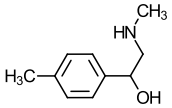

Tyramine




Octopamine




Hordenine




± Synephrine

These five biogenic amines can be rapidly separated with excellent peak shape on a HALO 5 Phenyl-Hexyl column using a methanol/buffer mobile phase containing an ion-pairing reagent.