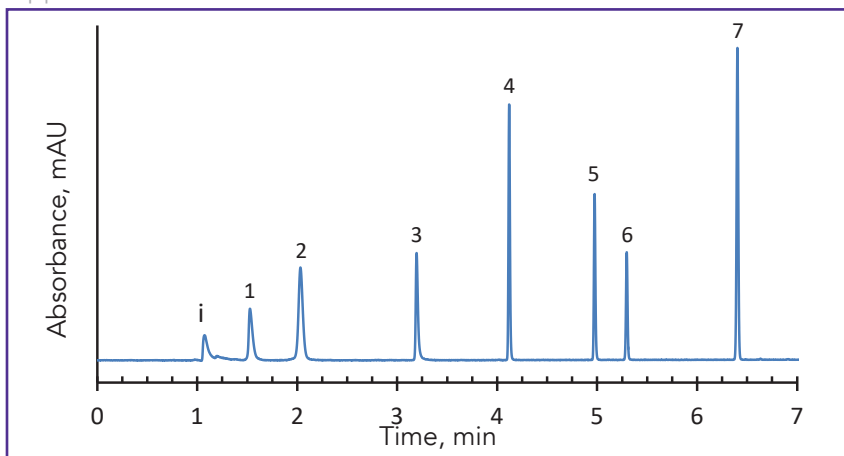




Separation of Melatonin and Related Compounds on HALO® RP-Amide

Application Note 143-B



PEAK IDENTITIES:

- i. Impurity
- 1. Serotonin
- 2. 5-hydroxy-L-tryptophan
- 3. L-Tryptophan
- 4. N-Acetyl-5-hydroxytryptamine
- 5. Melatonin
- 6. 3-Indoleacetic acid
- 7. Indole

Serotonin and melatonin are bioactive amines and are found in plant and animal tissues. In this application a mixture containing serotonin, melatonin and related amine compounds is well separated in less than 10 minutes using a HALO® RP-Amide column. The gradient may be adjusted to accommodate possible interfering peaks from sample matrices.

TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 2.7 µm,
4.6 x 150 mm

Part Number: 92814-707

Mobile Phase: A/B

A: 0.1% formic acid in water

B: 0.1% formic acid in acetonitrile

Gradient: Time (min) % B

0.0	5
1.5	5
7.0	70
8.5	95

Flow Rate: 1.5 mL/min

Pressure: 273 bar

Temperature: 35 °C

Detection: UV 280 nm, VWD

Injection Volume: 2.0 µL

Sample Solvent: Methanol

Response Time: 0.02 sec

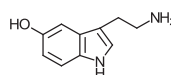
Data Rate: 25 Hz

Flow Cell: 2.5 µL semi-micro

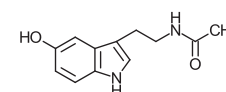
LC System: Shimadzu Prominence UFLC XR

Extra Column Volume: ~14 µL

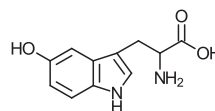
STRUCTURES:



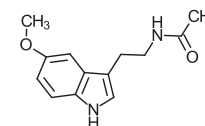
Serotonin



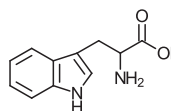
N-Acetyl-5-hydroxytryptamine



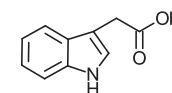
5-Hydroxy-L-tryptophan



Melatonin



L-Tryptophan



3-Indoleacetic acid



Indole

