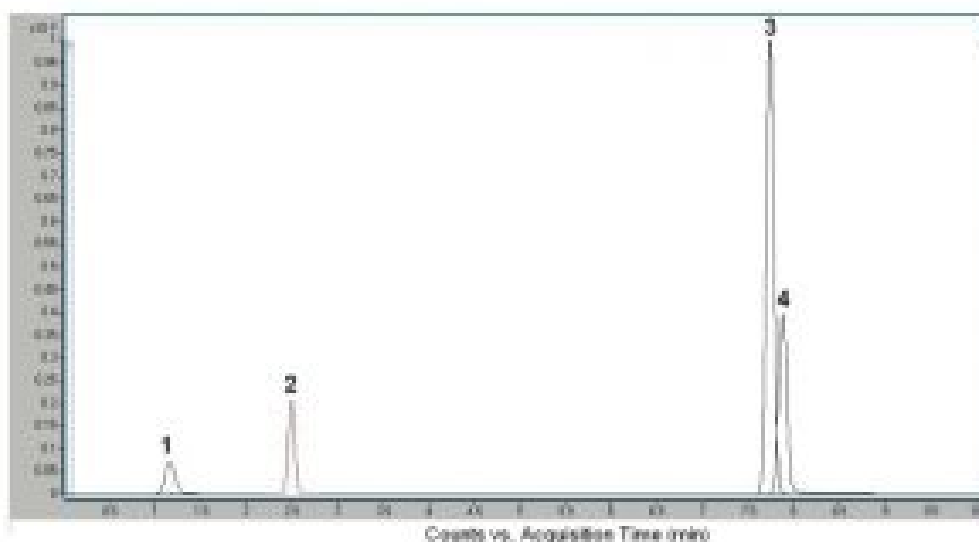


Serotonin, metabolites, analogs analyzed by HPLC with LCMS – AppNote

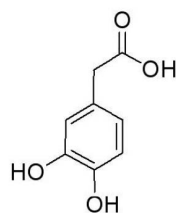
Simple HPLC Separation Method without Fluorescent Tags.

A Method using Aqueous Normal Phase (ANP), coupled with ESI Detection (LCMS), was developed for the analysis of Neurotransmitters and their Metabolites because previous HPLC based Methods which are used to analyze NT and Metabolites in biological fluids have several drawbacks. Among other problems, they often require a derivatization to convert NT into a fluorescent molecule or uses ion pairing reagents in the Chromatographic Process and the ion pair reagents typically are not MS compatible or hinder MS Detection.

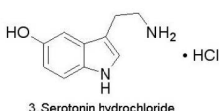
Mass spectrometry (MS) coupled to HPLC with a Diamond Hydride Column is a powerful technique which can be used within the field of Analytical Toxicology or can be used for accurate determination of NT and metabolites in biological samples for routine assessment of physiological or various pathological processes.



1. 5-Hydroxyindole-3-acetic acid (5-HIAA)



2. 3,4-Dihydroxyphenylacetic acid



3. Serotonin hydrochloride



4. (-)-Epinephrine

Peaks:

1. 5-hydroxy-3-indole acetic acid (5-HIAA) Metabolite of serotonin 192m/z
2. 3,4-Dihydroxyphenylacetic Acid (DOPAC) 169m/z
3. Serotonin 177m/z 4. Epinephrine 184m/z

Method Conditions

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

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150mm

Mobile Phase:

A: DI Water / 0.1% Formic Acid

B: Acetonitrile / 0.1% Formic Acid

Gradient:

Time (minutes)	%B
0	95
2	95
4	70
5	70
7	45
8	45

Injection vol.: 1μL

Flow rate: 0.4mL / minute

Detection: ESI – pos – Agilent 6210 MSD TOF Mass Spectrometer.

Sample Preparation: Mix of 4 compounds: 153.5μg / mL of each in Water. Dilution: 30μL of the mix sample into 70μL of 0.1% Formic Acid in Acetonitrile.

t₀: 1.44 minutes

Note: Neurotransmitters are important natural molecules that play significant roles in the mammalian central nervous system. The Chromatographic Method presented here is sensitive enough to be used to quantitate the metabolite concentrations in blood samples and may be applicable to relevant clinical studies.



Attachment - No 52 Serotonin, Metabolites & Analogs Analyzed by HPLC with LCMS pdf 0.3 Mb [Download File](#)

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MicroSolv Technology Corporation

9158 Industrial Blvd. NE, Leland, NC 28451

tel. (732) 380-8900, fax (910) 769-9435

Email: customers@mtc-usa.com

Website: www.mtc-usa.com

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