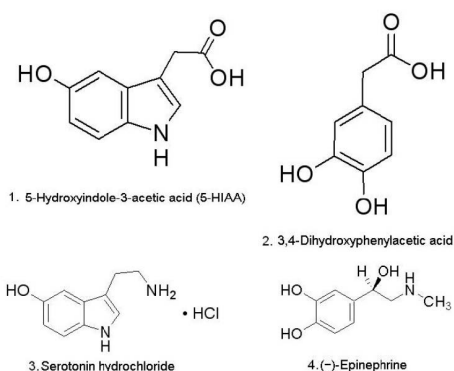
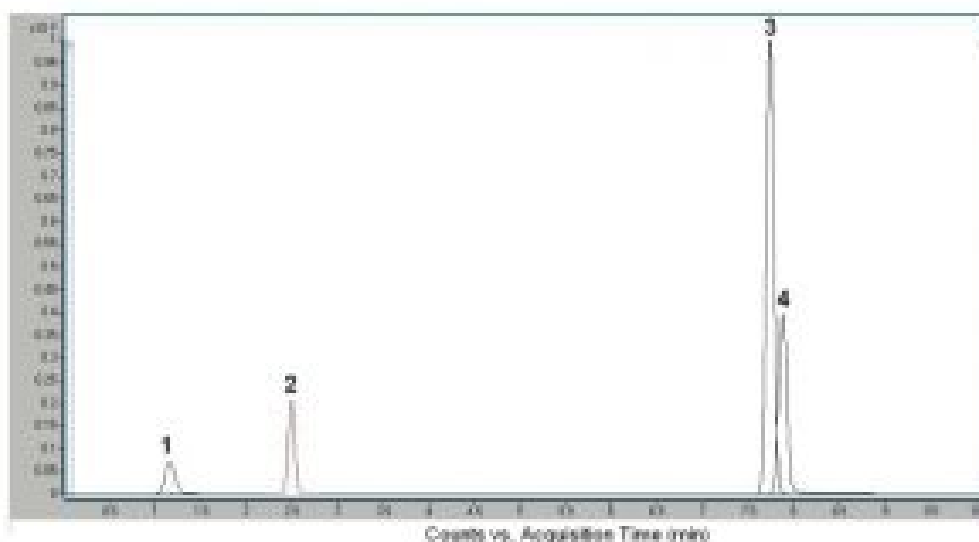


## Serotonin, metabolites, analogs analyzed by HPLC with LCMS - AppNote

### 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.



### 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.

**t<sub>0</sub>:** 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](#)