Dr. Maisch
Any Column, Any Size, Any Media

Dr. Maisch HPLC GmbH
Beim Brückle 14
D-72119 Ammerbuch
T: +49 (0) 7073 50357
F: +49 (0) 7073 4216
info@dr-maisch.com

LC-MS/MS Analysis of Tryptophan Metabolites

Technical Notification 0015

Introduction

Tryptophan (TRP) and its metabolites play a central role in numerous physiological and pathophysiological processes, including serotonin and kynurenine biosynthesis, immune regulation and gut-brain axis signaling. Quantitative profiling of 19 TRP metabolites by LC-MS/MS enables comprehensive pathway analysis in biological matrices such as plasma and serum.

The method presented here employs a ReproSil Fluosil 100 PFP column for rapid, high-resolution chromatographic separation of all target analytes within 1.8 min. Detection is performed by triple-quadrupole mass spectrometry in positive ESI mode using multiple reaction monitoring (MRM). The instrument platform was an Agilent 1290 Infinity III LC coupled to an Agilent 6475 Triple Quadrupole MS with Agilent Jet Stream (AJS) ion source.

Recommended Column: fl118.pfp.0502 (ReproSil Fluosil 100 PFP, 1.8 µm, 50 × 2.0 mm)

The ReproSil Fluosil 100 PFP is a pentafluorophenyl (PFP) stationary phase on ultra-pure silica (Pore Size: 100 Å and Particle Size: 1.8 µm) and column dimensions L = 50 mm, ID = 2.0 mm.
The phase provides unique π–π interactions, superior retention of polar and halogenated analytes, outstanding peak symmetry, and excellent batch-to-batch reproducibility (USP L43).

Chromatographic Conditions

Part Number (P/N)	fl118.pfp.0502
Packing Material	ReproSil Fluosil 100 PFP
Column Dimension [mm]	50 x 2.0 mm
Analyte(s)	19 Tryptophan Metabolites
Eluent A	H_2O + 5 mM $NH_4(HCO_2)$ + 0.05% Formic Acid
Eluent B	MeOH + 5 mM $NH_4(HCO_2)$ + 0.05% Formic Acid
Elution Type	Gradient
Flow Rate [ml/min]	0.5
Injection Volume [µl]	5
Temperature [°C]	40
Detection	ESI(+) MRM; Agilent 6475 Triple Quadrupole MS
Ionization	Positive ESI; Capillary 4950 V; Nozzle 1450 V
Gas Parameters	Sheath 12 l/min @ 250 °C; Drying 8 l/min @ 240 °C
Nebulizer [psi]	60

Dr. Maisch HPLC GmbH
Beim Brückle 14
D-72119 Ammerbuch
T: +49 (0) 7073 50357
F: +49 (0) 7073 4216
info@dr-maisch.com

Gradient

Time [min]	Flow Rate [ml/min]	%B
0.00 – 0.09	0.5	30
0.10 – 1.20	0.5	85
1.21 – 1.25	0.5	95
1.26 – 1.80	0.5	30

Analytes (19 Tryptophan Metabolites)

1. 3-Hydroxykynurenine 2. 5-Hydroxytryptophan 3. L-Kynurenine 4. N-Formylkynurenine 5. Serotonin 6. 5-Hydroxyindole-acetate 7. L-Tryptophan 8. Kynurenic acid 9. Indole-3-acetamide 10. Xanthurenic acid 11. Indole-3-pyruvate 12. Tryptamine 13. Quinolinic acid 14. Indole-3-acetate 15. Melatonin 16. Indole-3-aldehyde 17. Indole 18. Indole-3-propionate 19. Indole-3-acrylate

Chromatograms – Tryptophan Analysis

Representative Separation of 19 Tryptophan Metabolites

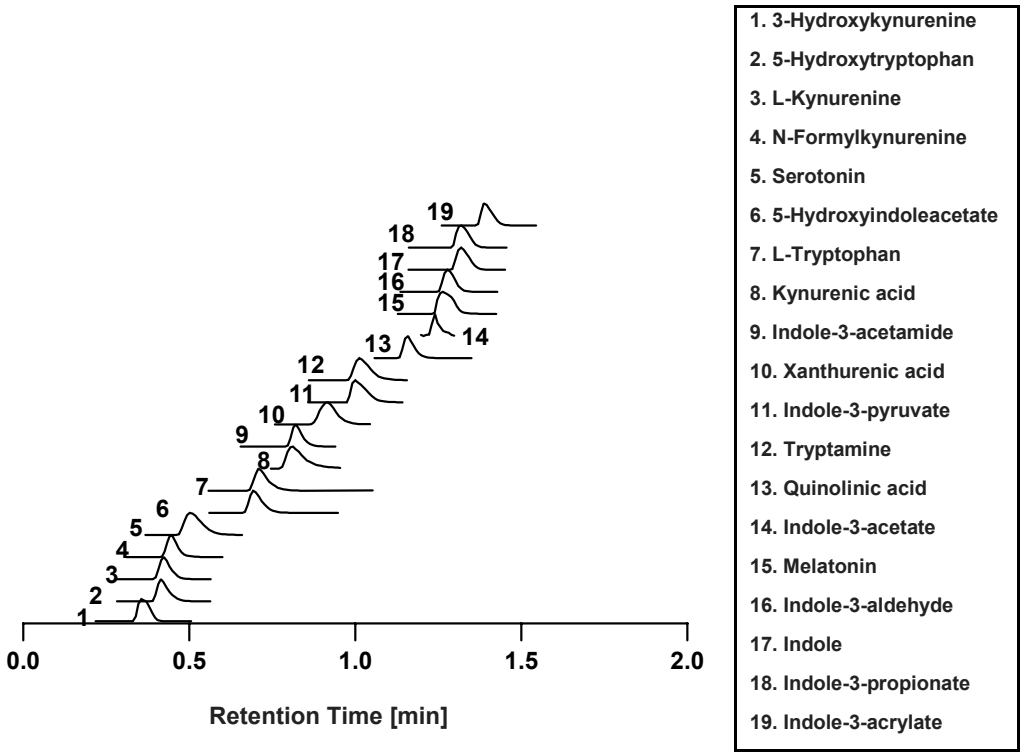


Figure 1: Representative Chromatographic Separation of 19 Tryptophan Metabolites in Retention Time of 0.0 – 1.8 min with ReproSil Fluosil 100 PFP, 1.8 µm, 50 mm x 2.0 mm.

Dr. Maisch HPLC GmbH
Beim Brückle 14
D-72119 Ammerbuch
T: +49 (0) 7073 50357
F: +49 (0) 7073 4216
info@dr-maisch.com

Overlaid Extracted Ion Chromatograms (EICs) of 19 Tryptophan Metabolites

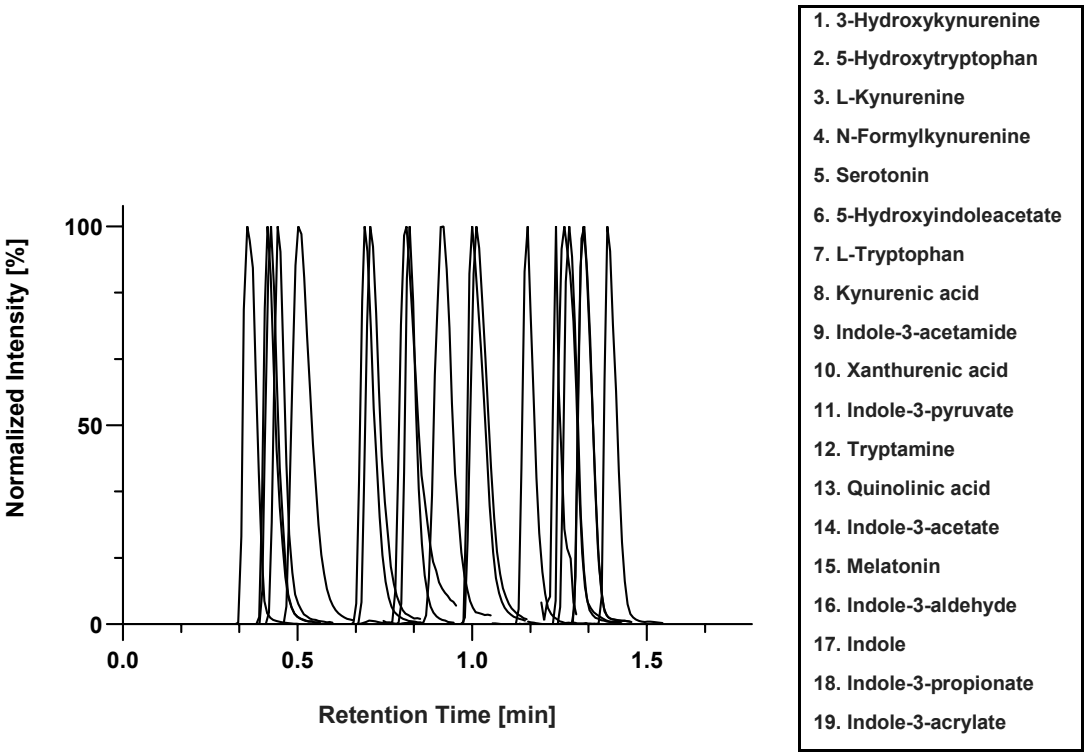
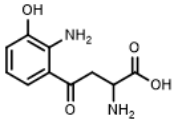


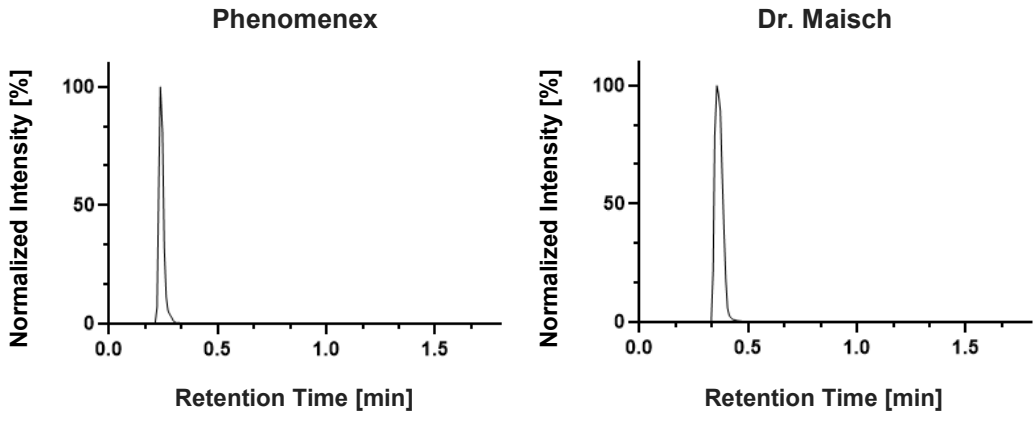
Figure 2: Extracted Ion Chromatograms of 19 Tryptophan Metabolites. Illustrated is signal intensity in relation to retention time. All analytes elute within 0.3 – 1.5 min.

Comparison Between Phenomenex Kinetex® 1.7 µm PFP 100 Å, LC Column 30 x 2.1 mm and Dr. Maisch ReproSil Fluosil 100 PFP, 1.8 µm, 50 x 2.0 mm:

Extracted Ion Chromatograms (EICs), illustrated in normalized signal intensity in relation to retention time.

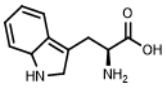
3-Hydroxykynurenine

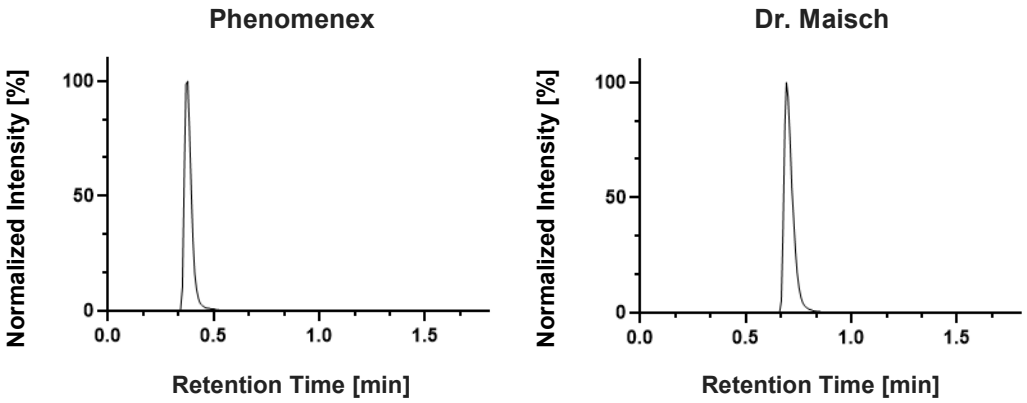




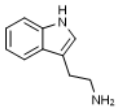
Dr. Maisch HPLC GmbH
Beim Brückle 14
D-72119 Ammerbuch
T: +49 (0) 7073 50357
F: +49 (0) 7073 4216
info@dr-maisch.com

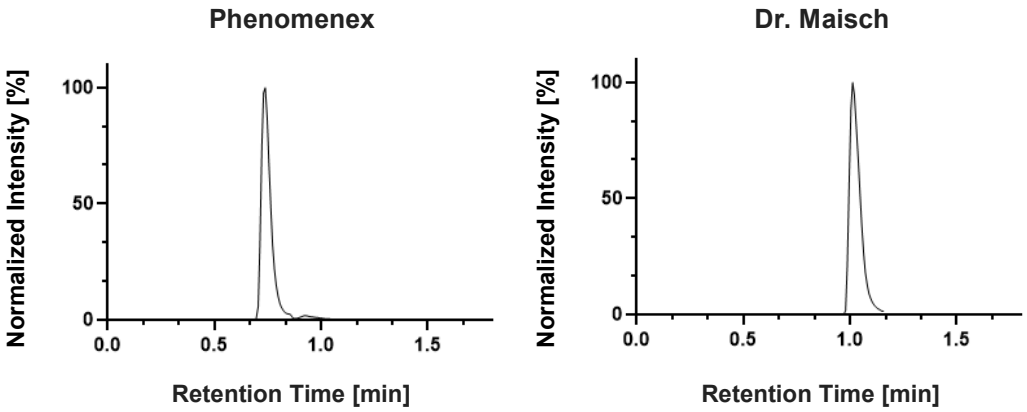
L-Tryptophan



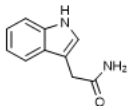


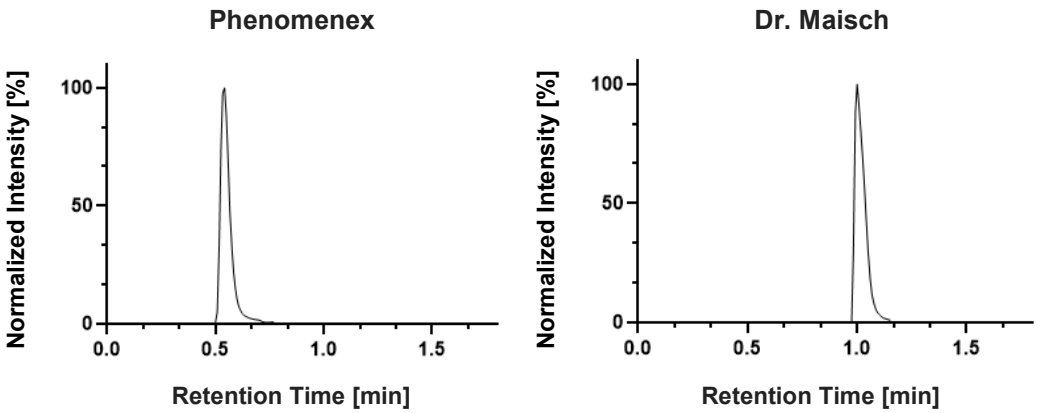
Tryptamine





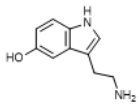
Indole-3-acetamide

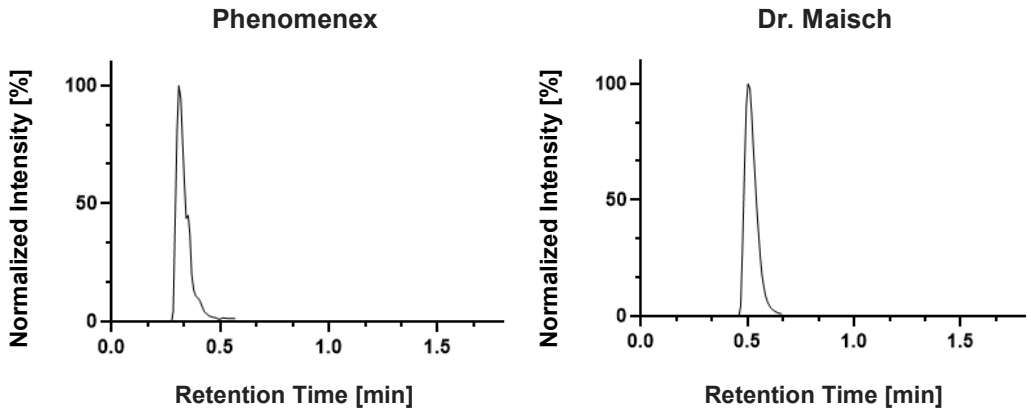




Dr. Maisch HPLC GmbH
Beim Brückle 14
D-72119 Ammerbuch
T: +49 (0) 7073 50357
F: +49 (0) 7073 4216
info@dr-maisch.com

Serotonin





Application courtesy of Prof. Dr. Oliver Schmitz and Team, Applied Analytical Chemistry (AAC) University Duisburg-Essen, Universitätsstr. 5, 45141 Essen, Germany. We gratefully acknowledge their support!

Dr. Maisch products are available globally. For more information contact your Dr. Maisch representative at ***info@dr-maisch.com***