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Aromatic Monomer Analysis by UHPLC-MS/MS

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ABSTRACT

An analysis method was developed to allow for quantitation of aromatic compounds by ultra high pressure liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) detection. This was achieved by using reverse phase chromatography and multiple reaction monitoring (MRM) in negative ion mode using electrospray ionization (ESI).

GUIDELINES

NOTICE
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MATERIALS

Working Solution Preparations:

Acetone **Merck MilliporeSigma (Sigma-Aldrich) Catalog #270725**

Milli-Q Water **Contributed by users**

Internal Standard:

4-Hydroxybenzoic-2356-d4 acid **Contributed by users Catalog #662763**

Aromatic Analytes:

4-Hydroxybenzoic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #240141**

4-Hydroxybenzaldehyde **Contributed by users Catalog #91554**

3-5-Dimethoxy-4-hydroxyacetophenone **Contributed by users**

Ferulic Acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1270311**

Vanillic Acid **Merck MilliporeSigma (Sigma-Aldrich)**

Syringaldehyde **Merck MilliporeSigma (Sigma-Aldrich) Catalog #08319**

Syringic Acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #63627**

Vanillin **Merck MilliporeSigma (Sigma-Aldrich)**

Acetovanillone **Merck MilliporeSigma (Sigma-Aldrich)**

p-Coumaric acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #55823**

Analytical Column:

Equipment	
Kinetex Phenyl-Hexyl	NAME
Analytical Column	TYPE
Phenomenex	BRAND
00D-4500-AN	SKU
https://www.phenomenex.com/products/kinetex-hplc-column/kinetex-phenyl-hexyl#order	LINK
2.1 mm x 100mm (1.7µm pore size)	SPECIFICATIONS

Instrumentation:

Equipment

6470 Triple Quad LC/MS	NAME
LC-QQQ System	TYPE
Agilent Technologies	BRAND
G6470A	SKU

Equipment

1290 Infinity UHPLC	NAME
Ultra-high performance liquid chromatography system	TYPE
Agilent Technologies	BRAND
1290 Infinity UHPLC	SKU
https://www.agilent.com/en/product/liquid-chromatography/hplc-systems/analytical-hplc-systems	LINK

SAFETY WARNINGS

All chemicals used for this procedure are hazardous. Read the Safety Data Sheet (SDS) for all chemicals and follow all applicable chemical handling and waste disposal procedures. Manufacturer specific SDS information can be found by following the CAS numbers of compounds in 'Materials' list.

BEFORE START INSTRUCTIONS

All solvents, analytes, and chemicals used in this protocol are listed in the 'Materials' section. They are excluded from in-line referencing to keep steps clear and concise.

Internal Standard Preparation

1 This analysis uses 4-Hydroxybenzoic-2,3,5,6-d_4 acid (d_4-4HBA) as an internal standard. Prepare a 500 µg/mL internal standard working solution into 50:50 Acetone / MilliQ Water. Plan to prepare enough solution volume to allow for addition of d_4-4HBA to each sample or standard volume at a 1:100 volumetric ratio.

1.1 For example, a 500 µg/mL concentration of internal standard working solution was used for a 10 µL spike into 1000 µL sample volume. In this scheme, the 5 µg/mL concentration added will need to be accounted for in data processing.

Preparation of Standards

2 By weight, create individual 20,000 µg/mL stock solutions of all monomers (listed in Materials section) and use 50:50 Acetone / MilliQ Water as a diluent.

3 Combine the stock solutions to create a 100 µg/mL mixed standard working solution in 50:50 Acetone / MilliQ Water.

3.1 For example, to prepare 10 mLs of mixed standard working solution of aromatic monomers (distinguished in Materials section), add 50 µL of each of the 20,000 µg/mL stock solutions (10 analytes) and add 9500 mL 50:50 Acetone / MilliQ Water.

4 Using the mixed standard working solution at 100 µg/mL, create a calibration curve with a minimum of five points using 50:50 Acetone / MilliQ Water as the diluent.

5 Add 10 µL of the 500 µg/mL d_4-4HBA internal standard working solution per every 1000 µL of calibration standard.

5.1

Calibration Level	Concentration (µg/mL)	Volume of 100 µg/mL Mixed Standard Working Solution (µL)	Volume of 50:50 Acetone / MilliQ Water (µL)	Volume of Internal Standard Working Solution (µL)
1	0.5	5	995	10
2	1	10	990	10
3	3	30	970	10
4	5	50	950	10
5	10	100	900	10
6	25	250	750	10
7	50	500	500	10
8	75	750	250	10
9	100	1000	0	10

example calibration level preparation

Sample Preparation

6 Ensure samples to be analyzed are suspended in a matrix compatible with your instrumentation. Analytes of interest in samples should be expected between 1 µg/mL and 100 µg/mL concentrations, otherwise dilution should be carefully performed to achieve concentrations in this calibration range.

6.1 If dilutions are required, add internal working solution (ISWS) at a 1:100 (ISWS volume / final sample volume) scheme.

7 Add 10 µL of the 500 µg/mL d_4-4HBA internal standard working solution per every 1000 µL of sample volume. This is best done when sample volume is normalized, either following dilution or by pulling precise volumes of sample into a new sample analysis vial.

UHPLC-MS/MS Analysis

8 Prepare an Agilent 1290 UHPLC system according to the following parameters:

Binary Pump Configuration	
Flow Rate	0.5 mL/min
Maximum Pressure	1200 bar
Mobile Phase A	0.1% Formic Acid in Water (v/v)
Mobile Phase B	0.1% Formic Acid in Methanol (v/v)

Gradient Configuration

Time (min)	Composition A (%)	Composition B (%)
0.0	99.0	1.0
1.0	99.0	1.0
7.0	70.0	30.0
7.5	70.0	30.0
9.0	40.0	60.0
9.0	40.0	60.0
9.0	99.0	1.0
12.0	99.0	1.0

Multisampler Parameters	
Injection Volume	0.5 µL
Draw Speed	200 µL/min
Eject Speed	200 µL/min
Wait time after draw	0 sec
Bottom Sensing	enabled

Column Compartment Parameters	
Temperature	40°C

Use the analytical column listed here:

Equipment	
Kinetex Phenyl-Hexyl	NAME
Analytical Column	TYPE
Phenomenex	BRAND
00D-4500-AN	SKU
https://www.phenomenex.com/products/kinetex-hplc-column/kinetex-phenyl-hexyl#order	LINK
2.1 mm x 100mm (1.7µm pore size)	SPECIFICATIONS

9

Analyze samples using an Agilent 6470A Triple Quadrupole Mass Spectrometer equipped with dual Agilent jet stream electrospray ionization (AJS ESI) per the method parameters listed below:

Analyte Name	Precursor Ion	Ion	MRM quantifying transition	CE (V)	Fragmentor (V)	MRM qualifying transition	CE (V)
4-Hydroxybenzoic-2,3,5,6-d4 acid (ISTD)	141.1	$[M-H]^-$	141.1 → 97.0	16	60	141.1 → 69.1	36
4-Hydroxybenzoic Acid	137	$[M-H]^-$	137.0 → 93.0	16	70	137.0 → 65.1	36
4-Hydroxybenzaldehyde	121	$[M-H]^-$	121.0 → 92.0	28	98	121.0 → 120.0	20
Vanillic Acid	167	$[M-H]^-$	167.0 → 152.0	12	89	167.0 → 108.0	20
Vanillin	151	$[M-H]^-$	151.0 → 136.0	12	65	151.0 → 92.0	20
Syringic Acid	197	$[M-H]^-$	197.0 → 182.0	12	98	197.0 → 123.0	24
Syringaldehyde	181.1	$[M-H]^-$	181.1 → 166.0	12	93	181.1 → 151.0	20
p-Coumaric Acid	163	$[M-H]^-$	163.0 → 119.0	16	74	163.0 → 93.0	36
Ferulic Acid	193.1	$[M-H]^-$	193.1 → 134.0	16	89	193.1 → 178.0	12
Acetovanillone	165.1	$[M-H]^-$	165.1 → 150.0	16	65	165.1 → 122.0	28
Acetosyringone	195.1	$[M-H]^-$	195.1 → 180.0	12	190	195.1 → 164.9	20

Optimized multiple reaction monitoring (MRM) transitions for quantification of aromatic monomers as listed in 'Materials'. Fragmentor voltages (V) and corresponding collision energies (CE) for LC-MS/MS analysis are supplied for quantifier and qualifier transitions respectively.

Mass Spectrometer Parameters	
Ion Source	AJS ESI

Time Segments (min)	Scan Type
0.0	To Waste
1.2	Dynamic MRM
12.0	Stop Time

Source Parameters	Value
Gas Temp (°C)	300
Gas Flow (L/min)	7
Nebulizer (psi)	35
Sheath Gas Temp (°C)	350
Sheath Gas Flow (L/min)	11
Capillary (V)	3000
Nozzle Voltage (V)	2000

10 Data analysis utilizes Agilent Quantitative Analysis for QQQ Build Version B.08.

Analytical Quality Control

11 Several strategies are utilized when performing this analysis to ensure instrument stability and reproducibility.

11.1 Calibration Curves

All compounds must have a correlation coefficient (r^2) of 0.995 or greater using a quadratic calibration fit.

11.2 Calibration Verification Standards (CVS)

A calibration verification standard (CVS) is a standard from the calibration curve that is re-analyzed every 20 or fewer samples to ensure instrument drift remains within the determined acceptance criteria. Acceptable CVS recoveries for this analysis are within 15% of the expected amount. Acceptance criteria may differ between instruments and should be determined experimentally.