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Data Acquisition and Analysis of Derivatized Hexose/Glucose by LC-QQQ-MS

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We use this protocol and it's working

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Abstract

This protocol outlines the steps for the separation, detection, and quantification of derivatized hexose/glucose using LC-QQQ-MS. Samples are analyzed with a C18 column coupled to a 1290 II LC system and an Ultivo triple quadrupole mass spectrometer. The procedure includes chromatographic conditions, MS settings, and data analysis workflows for high-throughput metabolite quantification.

Materials

Materials:

- Derivatized hexose/glucose samples
- Eclipse Plus C18 column (4.6 × 100 mm, 3.5 µm, Agilent)
- Agilent 1290 Infinity II LC system
- Agilent Ultivo triple quadrupole (QQQ) MS with ESI source
- LC/MS-grade water, acetonitrile, isopropanol
- Ammonium formate (10 mM)
- Formic acid (0.1%)
- MassHunter Qualitative Analysis Software
- Quant-My-Way QQQ Quantitative Analysis Software
- Microsoft Excel

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Reagents and Mobile Phases:

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- **Mobile Phase A:**
  10 mM ammonium formate + 0.1% formic acid in 60:40 (v/v) water:acetonitrile
- **Mobile Phase B:**
  10 mM ammonium formate + 0.1% formic acid in 90:10 (v/v) isopropanol:acetonitrile

Procedure:

3 Connect Eclipse Plus C18 column to the LC system.

4 Ensure column oven is set to 30°C.

5 Set autosampler temperature to 4°C.

6 Perform external mass calibration with standard mix every 7 days.

Sample Injection:

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- Inject **3 µl** of each sample with fast polarity switching enabled.

LC Gradient:

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	Time (min)	%B
	0.0	50
	3.0	100
	6.0	100
	6.1	50
	8.0	50

Flow rate: 0.400 ml/min

Mass Spectrometer Settings

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- Spray Voltage: 3.6 kV (positive mode), 2.5 kV (negative mode)
- Gas Temperature: 250°C
- Sheath Gas Temperature: 400°C
- Gas Flow: 11 l/min
- Sheath Gas Flow: 12 l/min
- Nebulizer: 28 psi

MRM Transitions

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	Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (eV)
	Benzoyl-Hexose/Glucose	718.0	231.0	100	11

	Benzoyl-Hexose/Glucose	718.0	105.0	100	37
	Benzoyl-Hexose/Glucose	718.0	579.1	100	5

Data Analysis

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Use MassHunter Qualitative Analysis software to validate peak alignment and retention times. Confirm identification by comparing MS/MS spectra to standards and evaluating both transitions for consistent relative intensity. Use Quant-My-Way for QQQ quantification with MRM data. Export raw peak areas to Microsoft Excel for further processing and normalization.

Consistent peak profiles for derivatized hexose/glucose with reproducible retention times and MRM transition ratios. Accurate relative quantification should be observed when using validated transitions and verified against standard fragmentation patterns.