Purdue-UAB Botanicals Center for Age-Related Disease

Lab Exercise: LC/MS and LC/MS/MS Analysis of Isoflavonoids from Kudzu Dietary Supplement

Day 1

1. Observation of precursor ion of isoflavonoids (LC/MS)
2. Observation of product ions (LC/MS/MS)
3. Review of data of kudzu extract
4. Overview of Instrumentation

Day 2

1. Observation of multiple ion monitoring (MRM) using the information gathered on day 1.
2. Discussion of solvent selection and formation of adducts.
3. Quantitation of data.

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Step by Step Analysis of a Botanical Sample

Step 1: scan acquisition

Acquires data from low to high mass within a scan range that is specified by the MS/MS operator.

Scan is used for identification of unknowns and molecular weight determination.

Scanning is not the preferred method of quantification.

Observation of precursor ions of O- and C-glycoside of isoflavones using scan acquisition:

1. Set the mass spectrometer to scan for a mass to charge ratio (m/z) of 200 – 800 in both positive and negative mode.

2. Using the sample loop, inject Puerarin and daidzin into the LC system.

3. Perform an LC/MS experiment (Q1 scan) of Puerarin (daidzein–8–C–glucoside) and daidzin (daidzein–7–O– glucoside).

4. The precursor ion for Puerarin and daidzin is observed at 417 *m/z* in the positive mode, indicating that it has a molecular weight of 416.

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Step by Step Analysis of a Botanical Sample

Step 2: Determination of Product Ions (LC/MS/MS)

MS/MS system fragments the precursor ions
in a collision cell to produce product ions.

The controlled fragmentation of the precursor ion
is reproducible and may be used to identify unknowns.

The operator specifies the mass
for which the mass spectrometer will look for
product ions.

Observation of the product ions of O- and C-glycoside of isoflavonoids.

A. Daidzin

1. Set the mass spectrometer system to product (or daughter) scan.
2. Enter the precursor (parent) ion mass.
3. Using the sample loop, inject O-glycoside into the LC system.
4. The product ion profile will be displayed on the computer screen.

B. Puerarin

Follow the same procedure as above for Puerarin.

Determination of Product Ions Cont.

LC/MS and LC/MS/MS Analysis of Genistein

Inject a 50 uM sample (previously prepared) of genistein into the LC System and perform LC/MS (Q1 scan) followed by LC/MS/MS (observation of product ions.) Follow the same procedure from above.

LC/MS and LC/MS/MS Analysis of Crude Kudzu Extract

Inject a crude extract of kudzu, perform LC/MS with a full Q1 scan. Demonstrate the data collected from a prior LC/MS/MS analysis of the kudzu extract.

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Step by Step Analysis of a Botanical Sample

Overview of Instrumentation

- HPLC Pumps with a Degassing Module
- Autosampler
- Spray Chamber Assembly
- Orifice
- Curtain Gas
- Tandem Quadrupole MS/MS consists of four quadrupoles linked in a series:

    Q0 - First in series just behind the orifice.
    Focuses ions into the next quadrupole, Q1
    Q0 operates in total ion mode

    Q1 - Mass Filter
    Quadrupole where scanning usually occurs

    Q2 - Collision Cell (total ion mode)
    Quadrupole where daughter ions are formed

    Q3 - Last quadrupole in the series.
    May operate as a mass filter to direct daughter ions to the detector in total ion mode.

- Detector
- High Vacuum System

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Step by Step Analysis of a Botanical Sample

Solvent Selection and Adduct Formation

The parent ion may appear in decreased abundance or in some cases not be seen.

The mass of greatest intensity may be the parent ion coupled with another ion, called an ***adduct***.

The following examples of adduct formation are taken from "Isoflavones and Their Conjugates in Soy Foods: Extraction Conditions and Analysis by HPLC-Mass Spectrometry"

Stephen Barnes, Marion Kirk and Lori Coward, Depts. Of Pharmacology
And Biochemistry and UAB Comprehensive Cancer Center Mass Spectrosopy
Core Facility, University of Alabama at Birmingham,
Birmingham, AL 35294

Journal of Agricultural Food Chemistry, 1994, 42, 2466-2474

Solvent:
Gradient of 0-50 % Acetonitrile in 10 mM Ammonium Acetate

Positive Electrospray	Observed Mass (m/z)	% Relative Abundance
$[M + H]^+$	503	100
$[M + NH_4]^+$	520	9.8
$[M + K]^+$	541	44.1
$[M + 2K]^+$	579	8.2
Negative Electrospray		
$[M - H]^-$	501	100
$[M + CH_3COO]^-$	561	9.6

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Step by Step Analysis of a Botanical Sample

Adduct Formation Cont.

Solvent Containing Sodium Acetate:

Positive Electrospray	Observed Mass (m/z)
$[M + H]^+$	M + 1
$[M + Na]^+$	M + 22

Negative Electrospray

$[M - H]^-$	M – 1
$[M + CH_3COO]^-$	M + 59

Solvent Containing Ammonium Formate:

Positive Electrospray	Observed Mass (m/z)
$[M + H]^+$	M + 1
$[M + NH_4]^+$	M + 17

Negative Electrospray

$[M - H]^-$	M – 1
$[M + HCOO]^-$	M + 45

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Adduct Formation Cont.

Organic solvent ions may also form adducts. It is possible to see acetonitrile [M + 40] or methanol adducts [M + 31].

Chlorine adducts may be formed in negative electrospray. Chlorine adducts form a 3:1 ratio of two peaks; M + 35 : M + 37 (3 : 1)

Cations (NH_4^+, K^+, Na^+) are formed during positive electrospray. Anions (CH_3COO^-, $HCOO^-$) are formed during negative electrospray.

Some isoflavones such as genistein may form a temporary aggregation of two molecular ions that will appear as [2M – H]. This 2M ion is not a dimer. It is formed as a result of the voltage to which the molecular ion is exposed during electrospray.

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Step by Step Analysis of a Botanical Sample

Step 3: multiple reaction monitoring

Brings together the data generated during the initial scan and the daughter scan.

Enhances sensitivity by lowering the possibility of interference.

Is the most common method for quantitation on a MS/MS system.

The MS/MS operator will enter the *m/z* for both the parent and daughter ion.

Observation of MRM Analysis of Genistein

1. Set the MS/MS system to perform multiple reaction monitoring (also known as MRM) and enter the appropriate parent and daughter masses for genistein.

2. Using the sample loop, inject genistein into the LC system.

3. A single peak should be observed for genistein during MRM.

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Step by Step Analysis of a Botanical Sample

Step 4: Quantitation of Data

Performed by Mr. Kenneth Jones

1. Retrieval of stored data.
2. Manual integration of peaks.
3. Use of internal standard to normalize data.
4. View a representative standard curve.