1.0 Purpose

The purpose of this procedure is to describe the method and conditions for the qualitative analysis of defined or purified chemicals by using LC/MS and MS.

2.0 Scope

This method is intended to identify defined or purified chemicals by using their specified molecular weight. Due to each chemical's specific molecular weight, this method will be directly used without further validation by reference standards. The method and conditions will be listed in detail unless it is otherwise stated.

3.0 Responsibility

3.1 It is the responsibility of QC and Analytical Chemists to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management and/or Analytical Development to keep this procedure current with latest Ion Labs practices.

4.0 Definitions

4.1 **MS** – Mass Spectroscopy

4.2 **HPLC** – High Performance Liquid Chromatography

4.3 **LC/MS** – Liquid Chromatography / Mass Spectroscopy

4.4 **ESI** – Electrospray Ionization

4.5 **SIM** – Selected Ion Monitoring

4.6 **M+1** – This is the mass most likely to be seen in positive mode. This correlates to the

mass of the product plus 1 as charged molecules fly through the quadrapole.

4.7 **M-1** – This is the mass most likely to be seen in negative mode. This correlates to the mass of the product minus 1 as charged molecules fly through the quadrapole.

4.8 **M/Z** – Mass to charge ratio; This corresponds to the mass divided by the overall charge of the molecule being analyzed. Since the quadrapole will deflect ions with a +2 charge twice as heavily, it will be interpreted as M+2/2 by the software. Consequently ions with a +3 charge will be interpreted as M+3/3. This effect is seen with -2, -3 etc. charges as well.

4.9 **QC** – Quality Control

5.0 References

None

6.0 Supplies and Equipment

6.1 Supplies and Glassware

6.1.1 HPLC vials, 12mm X 32mm with screw cap enclosures w/ septa.

6.1.2 1L mobile phase container

6.1.3 Volumetric flask, 500mL, 100mL, 50mL, 25mL

6.1.4 Beaker, 50mL, 100mL

6.1.5 Pipette tips, 10mL, 1mL, 200uL

6.1.6 Eppendorf Tubes, 1.5mL, 1.7mL or 2.0mL

6.1.7 Weigh boats

6.1.8 Plastic luer lock syringe, 10mL, 6mL or 3mL

6.1.9 0.2 or 0.45µm 25mm Nylon syringe filters

6.1.10 0.2 or 0.45µm 25mm HDPE syringe filters

6.2 Equipment

6.2.1 Agilent 1100 Series HPLC with DAD detector

6.2.2 Agilent 6120 MS with ESI source, MSD detector

6.2.3 Analytical Balance

6.2.4 Vortex

6.2.5 Stir Plate

6.2.6 Eppendorf Centrifuge

6.2.7 Wrist action shaker

6.2.8 Pipette 10mL, 1mL, 200uL, 10uL

7.0 Method Parameters

7.1 HPLC - If separation is required to identify multiple component raw materials the following parameters need to be defined: (Note: use accurate and up-to-date tune file)

7.1.1 Column type

7.1.2 Mobile phase

7.1.3 Dissolution Buffer

7.1.4 Column temperature

7.1.5 Wavelength (optional)

7.1.6 Gradient (optional)

7.1.7 Injection volume

7.1.8 Flow rate

7.1.9 Run time

7.1.10 Sample concentration

7.1.11 MS polarity

7.1.12 Scan/SIM settings

7.2 Direct MS Injection - If no separation is required to identify raw materials the following parameters need to be defined: (Note: use accurate and up to date tune file)

7.2.1 Injection volume

7.2.2 Flow rate

7.2.3 Sample concentration

7.2.4 Wavelength (optional)

7.2.5 MS Polarity

7.2.6 Scan/SIM settings

8.0 Sample Preparation

8.1 Samples for identification should be dissolved in Mobile Phase or another suitable solvent or solvent mixture. Most analytes will generate a suitable response at a concentration of 0.1 mg/mL. To manage large volumes the sample can be initially dissolved in a smaller volume that is within the solubility range and a portion further diluted to bring the analyte concentration into a range to give an adequate signal. The sample must be filtered or centrifuged before analyzing by MS.

8.2 All samples must be shaken on a wrist action shaker for 30 minutes at RT in two thirds their initial volumes then brought up to final volume.

8.3 For filtration, withdraw 10mL using a 10mL plastic syringe. Attach the appropriate hydrophilic or hydrophobic filter to the end of the syringe. Filter and discard the first 0.5mL of sample before collecting. From the collected sample dilute as needed then add 1mL to an HPLC vial for analysis.

8.4 For centrifugation, add 1.7 to 2.0mL of sample to an Eppendorf tube. Using an equivalent tube add an equal volume of water for use as a counter balance. Place the two tubes in opposing positions in the Eppendorf centrifuge rotor. Centrifuge for 10 minutes at 3000 RPM. Withdraw an aliquot of supernatant, dilute as needed and add 1mL to an HPLC vial for analysis.

8.5 If the sample has been previously tested by MS or LC/MS, the system can be set up using the parameters defined in the electronic MS Parameters for Chemical ID spreadsheet located in the F:\Laboratory folder. Otherwise, the following can be used as a general starting point. For pure compounds, separation by HPLC is not necessary.

8.5.1 Positive Ion Mode

8.5.1.1 Column: C18 or C8, 150 mm X 4.6 mm, 5 µm particle size

8.5.1.2 Mobile Phase A: 0.1% formic acid in water

8.5.1.3 Mobile Phase B: Acetonitrile

8.5.1.4 Gradient: Initial 10% B, ramp to 80% B over 15 min

8.5.1.5 Injection Volume: 10 µL

8.5.2 Negative Ion Mode

8.5.2.1 Column: C18 or C8, 150 mm X 4.6 mm, 5 µm particle size

8.5.2.2 Mobile Phase A: 20 mM ammonium acetate in water

8.5.2.3 Mobile Phase B: Acetonitrile

8.5.2.4 Gradient: Initial 10% B, ramp to 80% B over 15 min

8.5.2.5 Injection Volume: 10 µL

9.0 Criteria

9.1 Chemical or chemicals are positively identified when the corresponding molecular weight of the expected analyte(s) is identified in the MS chromatogram (taking into account M+1, M-1 and M/Z if present) and the other principle detected ions can be explained.

10.0 Revision History

Revision	Date	Description of Changes	CCR	By
0	04/21/14	New	14-0335	B. Johns
1	09/21/16	Biennial review: Updated responsibilities. Removed form.	16-0848	N. Zhang
2	11/26/19	Update responsibilities. Update formatting. Add general guidance for analytes that have not been previously tested. Added M+1, M-1 and M/Z.	19-0910	S. Sassman
3	01/30/23	Update logo. Update formatting. Revise responsibilities section.	CC-23-0051	K. Burris

Mass Spectroscopy Parameters for Chemical ID

Raw Material	Chemicals to detect	Molecular Weight(s)	Known Background Chemicals	Observed m/z	HPLC Conditions	MS Conditions
Magnesium Aspartate	*Aspartate*	133.11	Magnesium	Pos+: **134.0**, 135.0, 199.1 Neg-: **132.0**, 133.0, 201.1	1) [Sample]: 1 mg/mL 2) Injection volume: 10uL 3) Column type: No 4) Mobile phase: A) 20mM Ammonium Acetate B) ACN (60:40) 5) Flow rate : 1 mL/min 6) Dissolution Buffer: Water 7) Run Time- 5 minutes 8) Temperature 30°C	1) Polarity : +/- 2) Scan/SIM settings: 20.0 - 800.0 dalton
Dimagnesium Malate	*Malate*	134.1	Magnesium	Pos+: 76.2 199.1 Neg-: 133.1,	1) [Sample]: 0.5mg/mL 2) Injection volume: 10uL 3) Column type: No 4) Mobile phase: A) 0.1% Formic acid B) MeOH (50:50) 5) Flow rate : 0.5 mL/min 6) Dissolution Buffer: Water 7) Run Time- 5minutes 8) Temperature 30°C	1) Polarity: - 2) Scan/SIM settings: 20.0 - 800.0 dalton

Acronyms Used

MS- Mass Spectroscopy
MeOH - Methanol
HCOOH- Formic Acid
CF_3COOH- Trifluroacetic Acid
AcOH - Acetic Acid
NH_4COOH- Ammonium Formate
$CH_3CO_2NH(CH_2CH3)_3$- Trimethylammonium acetate