JORDAN FOOD AND DRUG ADMINISTRATION	
AMMAN FOOD LABORATORY	**Edition: 1** **Review: 1** **Date: 01-10-2017** **Page 1 of 6**
DETECTION & QUANTIFICATION OF PESTICIDES RESIDUES IN CEREALS BY LC-MS/MS **Commodity Group (5) according to SANTE/11945/2015**	**No.: AFFsop 2**

Content	**Page**

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DETECTION & QUANTIFICATION OF PESTICIDES RESIDUES IN CEREALS ON LC-MS/MS

Commodity Group (5) according to SANTE/11945/2015

1. OBJECTIVE

The following document describes the procedure for:

1. Food samples preparation
2. Extraction and clean-up of pesticides from Cereal samples
3. LC-MS/MS analytical conditions
4. Standards and calibration levels preparation
5. Identification and results reporting

2. SCOPE

This procedure applies to cereal samples received by Contaminants Monitoring Division (CMD)

3. RESPONSIBILITIES

It is the responsibility of the CMD staff to follow the instructions & ensure adherence to this procedure

4. REFERENCES

1. EN 15662:2008. Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE-QuEChERS-method

2. ANALYSIS OF PESTICIDES IN CEREALS USING THE QUECHERS METHOD AND DETECTION BY GCMS, GC-MS/MS AND/OR LC-MS/MS. E_FP417.1 3rd edition.

3. Document SANTE/11945/2015, “Guidance document on analytical quality control and method validation procedures for pesticide residue analysis in food and feed”, European Commission Directorate General for Health and Food Safety, effective on 01 Jan 2016

5. INSTRUMENTS

1. Sample processing equipment (Blender and grinder)
2. Centrifuges for 50 mL, 15 mL and 2 ml centrifuge tubes
3. Vortex and/or shaker
4. Automatic pipettes (e.g. for 20-200 µL, 100-1000 µL and 1-10 mL)
5. Centrifuge tubes with screw caps (50 mL and 15 ml)
6. Solvent-dispenser (for acetonitrile)
7. Vials with caps for LC auto sampler (2 ml)

6. CHEMICALS

1. Acetonitrile (MeCN) (LC-MS grade)
2. 1% glacial acetic acid in acetonitrile
3. 5% Formic acid solution in acetonitrile
4. Distilled water

7. SAMPLE PREPARATION

1. For cereals: Take around 200 g of the sample and store at (-20 ˚C) for at least 1 hour or overnight
2. After freezing, weigh 100 g of the sample and transfer to a blinder (preserve the other 100 g at -20 ˚C)
3. Homogenize into a fine free flowing powder
4. For powder samples: Take around 200 g of the sample and store at (-20 ˚C) for at least 1 hour or overnight

8. EXTRACTION PROCEDURE

1. Weigh (5 g) of sample into a clean (50 mL) tube
2. Add internal standard or spiking mix if required
3. Add 10 g of cold water and shake briefly
4. Add 10 ml acetonitrile
5. Vortex or shake vigorously by hand for (1 minute)
6. Add buffered QuEChERS extraction packet according to the EN method 15662 (4 g $MgSO_4$, 1 g NaCl, 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate)
7. Vortex or shake vigorously by hand for (1 minute)

8. Centrifuge for (10 minutes) at 4500 rpm
9. Transfer an aliquot (6 ml) of the acetonitrile phase (upper layer) into a 20 ml screw capped tube
10. Place the tube containing the extract for at least 2 hours in the freezer at (-80 ℃)

9. CLEAN-UP PROCEDURE

1. Transfer (1 ml) of the supernatant to the required ready to use dSPE tube (2 ml capacity) contains 150 mg MgSO4, 25 mg PSA
2. Vortex or shake vigorously by hand for (30 seconds)
3. Centrifuge for (5 minutes) at 4500 rpm
4. Take (0.5 ml) of the supernatant and add (0.5 ml) acetonitrile (Dilution factor of 2)
5. Acidify with formic acid 5% in acetonitrile (10 µL to every 1 mL)
6. The final extract has a concentration of ca. 0.5 g/mL (Set sample amount as 0.5)
7. Sample is ready to be injected

10. ANALATYCAL CONDITION

High-Speed Analysis Method (for 646 Residual Pesticide Components) - LC/MS/MS Method Package - Residual Pesticides Version 2- SHIMADZU

1. UPLC Conditions:

Instrument	Nexera (2040c) LC system, shimadzu
Analytical Column	Restek Raptor Biphenyl (100, 2.1mm, 2.7 um) (Cat#: 9309A12)
Solvent A	2 mmol/L ammonium formate + 0.002 % formic acid - Water
Solvent B	2 mmol/L ammonium formate + 0.002 % formic acid - Methanol
Injection Volume	2 µl
Flow rate	0.4 ml / min
Column Oven Temperature	35°C

2. Gradient Program:

Step	**Time (min)**	**Mobile Phase A (%)**	**Mobile Phase B (%)**
1	0.01	97	3
2	1	90	10
3	3	45	55
4	10.5	0	100
5	12	0	100
6	12.01	97	3

7	15	97	3
Stop	15.01	97	3

3. MS Conditions:

Nebulizing Gas Flow Rate	3 L/min
Drying Gas Flow Rate	10 L/min
Heating Gas Flow Rate	10 L/min
Interface Temperature	350°C
DL Temperature	150°C
Block Heater Temperature	300°C
Ionization mode	ESI

11. STANDARDS PREPARATION

1. Prepare working solution one (**W1**) (1 ppm) by taking 100 µl from the pesticide mix (100 ppm) and complete with acetonitrile (MeCN) to 10 ml
2. When mixing more than one pesticide mix of (100 ppm), take 100 µl from each mix and complete with acetonitrile (MeCN) to 10 ml to get (1 ppm) final concentration
3. Prepare working solution two (**W2**) (100 ppb) by taking (1 ml) from (**W1**) and complete with MeCN to (10 ml)

12. CALIBRATION LEVELS PREPARATION

Use matrix matched external standard calibration curve

1. Level 1 (10 ppb): Direct in an auto sampler vial add 100 µl of (**W2**) + 900 µl 1% glacial acetic acid in MeCN
2. Level 2 (20 ppb): Direct in an auto sampler vial add 200 µl of (**W2**) + 800 µl 1% glacial acetic acid in MeCN
3. Level 3 (50 ppb): Direct in an auto sampler vial add 50 µl of (**W1**) + 950 µl 1% glacial acetic acid in MeCN
4. Level 4 (100 ppb): Direct in an auto sampler vial add 100 µl of (**W1**) + 900 µl 1% glacial acetic acid in MeCN
5. Level 5 (150 ppb): Direct in an auto sampler vial add 150 µl of (**W1**) + 850 µl 1% glacial acetic acid in MeCN

6. Level 6 (200 ppb): Direct in an auto sampler vial add 200 µl of (**W1**) + 800 µl 1% glacial acetic acid in MeCN

13. IDENTIFICATION

1. The retention time of any analyte in tested sample extract should correspond to that of the matrix-matched calibration standard with a tolerance of ±0.1min
2. Extracted ion chromatograms of tested sample extracts should have peaks of similar shape and response ratio to those obtained from matrix-matched calibration standards analyzed at comparable concentrations
3. Chromatographic peaks from different selective ions for the analyte must fully overlap
4. The ion ratio should not deviate more than 30% (relative)

14. REPORTING RESULTS

1. The results must always be reported and expressed in mg/kg
2. Where the residue definition includes more than one analyte, the respective sum of analytes must be calculated as stated in the residue definition and must be used for checking compliance with the MRL
3. Residues for individual analytes below the reporting limits must be reported as <RL mg/kg
4. Where good mixing of samples has been undertaken, the RSD of replicate results of the test portions should normally not exceed 30%
5. In general, residues data do not have to be adjusted for recovery when the mean recovery is within the range of 70-120%
6. If residues data are adjusted for recovery, then this must be stated in the report
7. The result should be reported together with the expanded measurement uncertainty (MU) as follows: Result = x ± U (units), with x representing the measured value
8. The sample is considered non-compliant if x-U > MRL