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Determination of multiresidue pesticides in Arabian dates using LC and GC Triple Quadrupole Mass Spectrometry

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1. Introduction

Arabian dates (hereinafter called “dates”) are the fruits of the date palm tree, which grows in many tropical regions of the world and is high rich in fiber and antioxidants. Dried dates are a nutritive food in Middle East and African countries and form an important part of the Muslim diet during Ramadan, where it is traditionally eaten every evening for break-fasting.

Substances such as chemicals, some organic components, disinfectants used to eliminate the harmful effects of bacteria, viruses and pests are called "Pesticides". In addition to providing benefits such as growing vegetables and fruits in nature without harm and purification of our living spaces from harmful microorganisms, pesticides can pose a great threat to human health due to incorrect use.

As reported in many studies, pesticides are also widely used in dates. It is known that the determination of pesticides in dried dates is difficult due to high sugar content of dried dates matrix.

This study reports a sensitive and robust analytical method for quantitative analysis of multi pesticide residues in commercially available dried dates by using GC-MS/MS and LC-MS/MS triple quadrupole instruments.

2. Materials and methods

Shimadzu LCMS-8060 and GCMS-TQ8050 NX were used in this application.

Fig. 1 Shimadzu LCMS-8060 and Shimadzu GCMS-TQ8050 NX

Table 1: LC-MS method parameters

Column	Shim-pack GISS C18, 2.1 x 100 mm; 3 µm
Mobile phase	A: Water + 2 mM ammonium formate, 0.01 % formic acid
Mobile phase	B: Methanol + 2 mM ammonium formate, 0.01 % formic acid
Flow rate	0.4 mL/min
Gradient	3 %B (0 min) → 10 %B (1 min) → 55 %B (3 min) → 100 %B (10.5 → 12 min) – 3 %B(12.01 →15 min)
Injection volume	1 µL
Column temperature	35 ^{0}C
Ionization mode	ESI (positive, negative)
Gas flow	Nebulizing gas:- 3 L/min; Heating gas:- 10 mL/min; Drying:- 10 L/min
MS temperature	Interface:- 350 ^{0}C; Desolvation:- 150 ^{0}C; Heat block:- 300 ^{0}C

Table 2: GC-MS method parameters

Column	SH-Rxi™-5Sil MS (30 m x 0.25 mm x 0.25 µm)
Injection mode	Splitless
Sampling time	1.00 min
Carrier gas	Helium
Flow control Mode	Constant linear velocity
Linear velocity	55.0 cm/sec
Column flow	2.21 mL/min
Injection volume	1.0 µL
High pressure injection	250.0 kPa (1.5 min)
Column temp. program	90 ^{0}C (1 min.)→(30 ^{0}C /min.)→130 ^{0}C→(10 ^{0}C /min.) →320 ^{0}C (3 min.)
MS Temperature	Ion source:- 230 ^{0}C; Interface:- 290 ^{0}C

A combined system of Shimadzu Nexera series ultra high-performance liquid chromatograph and LCMS-8060 triple quadrupole mass spectrometer and Shimadzu GCMS-TQ8050 NX triple quadrupole mass spectrometer instruments were used in this application. The analysis was carried out using a multiple reaction monitoring (MRM) acquiring 3 transitions for most of the compound.

The sample preparation procedure was based on modified AOAC 2007.01 QuEChERS method, as given below[1].

Analysis time for LC-MS is 15 minutes, while for GC-MS is 24 minutes.

2.1 Sample preparation

- Homogenized the commercially available dried samples with the same amount of water.
- Weigh in 15 g of homogenized sample.
- Add 15 mL of 1% acetic acid solution in acetonitrile (v/v). Shake for one minute.
- Add QuEChERS salt Restek Cat # 25851 (6 g anhydrous magnesium sulfate + 5 g anhydrous sodium acetate)
- Mix well and shake for one minute.
- Centrifuge the 50 mL tube to separate the layers.
- Take 8 mL of supernatant layer and transfer to dSPE tube containing cleanup mixture Restek Cat # 26221 (1.2 g MgSO4 + 400 mg PSA + 400 mg C18)
- Immediately recap the dSPE tube and shake it vigorously by hand for half minute.
- Take 0.5 mL from upper layer in a vial and add 0.5 mL acetone for GC-MS, add 0.5 mL 50:50 methanol/water for LC-MS.
- Analyze by LC-MS/MS and GC-MS/MS.

Fig. 2 Sample preparation method by QuEChERS method for dried dates sample

2.2 Preparation of matrix match standard calibration levels.

A locally purchased dried dates samples were used. It was extracted as described in the sample preparation procedure. Then, the blank extract was spiked with a stock standard solution to prepare a matrix matched calibration curve as shown below. The extracted matrix blank sample was scanned by LC-MS and GC-MS instruments and none of the pesticides included in this study were detected.

GC-MS standard preparation
- 450 µL matrix
- 50 µL Std
- 500 µL acetone

Calibration Standards: 2-100 ng/mL

LC-MS standard preparation
- 450 µL matrix
- 50 µL Std
- 500 µL water/methanol

Calibration Standards: 2-100 ng/mL

2.3 Preparation of pre-extraction spike samples (Recovery samples)

In order to study the extraction efficiency of the method, recoveries of the pre-extraction spiked samples were studied. 15 g of blank sample was spiked with a stock solvent standard to prepare pre-extraction spike concentration levels of 10 ng/mL and 40 ng/mL. These samples were extracted, analyzed, and quantified against a matrix matched calibration curve to study their recoveries.

2.4 Method Development

For MRM method creation, Shimadzu's Smart Pesticide Database Ver.2 and LC/MS/MS Method Package for Residual Pesticide Ver. 3 were used for GC-MS and LC-MS instruments. Shimadzu database solutions for residual pesticides provides all required information such as MRM transitions, collision energies and other LC and GC parameters. Thanks to the Automatic Adjustment of Retention Time Function (AART) algorithm, the retention times of GC pesticides were easily determined without using a standard. This function, which calculates the retention time of pesticide compounds using the n-alkanes standard, considerably shortened the method development time.

3. Results

405 pesticides were analyzed, of which 200 were in GC-MS and 205 in LC-MS. Extracted sample matrix was quantitated using a developed MRM method.

The calibration curve for LC-MS and GC-MS pesticides is plotted for matrix-matched standards in the 2 ppb to 100 ppb range. Linear response was obtained with r^2 greater than 0.995. Recoveries were checked by spiking at 10 ng/mL and 40 ng/mL. Recovery results of most of the compounds were found in the range of 70 - 120 %. Only two pesticides did not meet the recommendations of the SANTE/11312/2021 guideline[2]. Pre-extraction spiked samples were analyzed six times and their % RSD was found to be less than 20 %.

Separation of Cyfluthrin and Cypermethrin in GC-MS is difficult due to their isomer structure. Adequate separation was provided using a short temperature program for these pesticides. Cypermethrin has 6.7 % RSD at LOQ level and 95.0 - 89.7 % recovery.

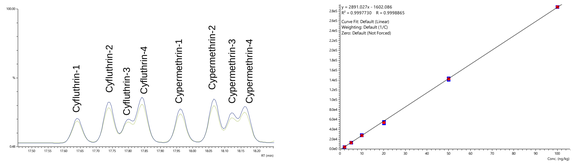

Fig. 3 Separation of Cyfluthrin & Cypermethrin isomers in matrix by GC-MS and total Cypermethrin calibration curve

Shimadzu's Synchronized Survey Scan (SSS) automatically performs a product ion scan whenever a pre-set survey scan threshold is exceeded. SSS allows different collision energies to be applied to the same precursor ion for product ion screening in LC-MS applications.

LabSolutions Insight Screening

Shimadzu's LabSolutions Insight™ is user-friendly software designed to streamline complex data analysis workflows of multiple analytes such as pesticides. In addition to qualitative screening, LabSolutions Insight Screening™ software compares the spectrum with pesticide compounds that have already been registered in the library. Comparing the product ion spectra of pesticide compounds in the library with the actual sample spectra is a very useful method to further increase the reliability of the analysis result. As seen in the example below, Benfuracarb was detected in MRM scanning mode was compared with an LC-MS library containing hundreds of product ion spectra. Using the LC-MS Pesticide library and SSS function reduces the risk of false positives/negatives.

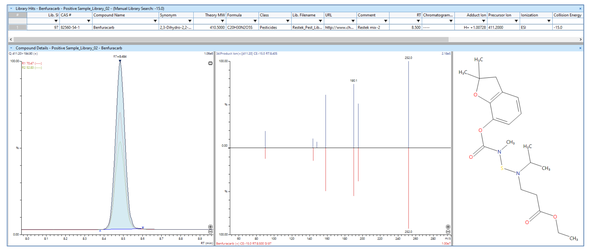

Fig. 3 Benfuracarb MRM chromatogram and MS/MS spectrum comparison with library

4. Conclusions

The results showed that more than 400 pesticide residues in the dried date matrix yielded sensitive, robust, and reproducible results by Shimadzu LCMS-8060 and GCMS-TQ8050 NX.

The developed method in this study was proved to be reliable and accurate and permits rapid determination of pesticides and can be easily applied for quality control of dried dates.

205 pesticides were analyzed simultaneously with 5 msec fast polarity switching of LCMS-8060. The fast 24 minute GC-MS method allowed the quantitative analysis of 200 pesticides.

Thanks to Shimadzu's SSS function and LabSolutions Insight Screening™ software, it is possible to screen compounds that exceed a pre-set threshold level. In this study, we have conducted MRM measurement with full scan dependent product ion scanning with three different collision energies by LC-MS instrument. This allowed us to verify the result of the analysis.

5. References

1.AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate.

2.Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed. Document No. SANTE/11312/2021.