September 7, 2019

Non-target High Throughput Screening Method for 765 Multigroup and Multiclass Pesticides and Chemical Contaminants in Fruits and Vegetables by GC/LC-Q-TOF/MS

—"Shrunken" AOAC Collaborative Study Method

AOAC Study Director Guo-Fang Pang
Chinese Academy of Inspection and Quarantine, Beijing, 100176. P. R. of China
Email: panggf@caiq.org.cn Fax:+86-10-53898011

1 Scope

The method is applicable to qualitative and quantitative determination of 765 pesticide and chemical contaminants in fruits and vegetables by GC-Q-TOF/MS and LC-Q-TOF/MS.

Regarding GC-QTOF/MS: investigate the spiked recoveries and precision of 494 pesticides at concentration levels of 10 μg/kg, 50 μg/kg and 100 μg/kg respectively. At the same time, make matrix spiked experiments at concentrations of 1 μg/kg, 5 μg/kg, 10 μg/kg, 20 μg/kg, and 50 μg/kg to investigate the screening limit of the method. The pesticides with screening limit being 5 μg/kg is the most in all eight matrices, accounting for 40.0%-52.5% of the total pesticides; for pesticides with the screening limit of 100 μg/kg, the type of pesticides in eight matrices is lower than 20, accounting for less than 4.0%. The total types of pesticides with screening limit lower than 10 μg/kg in eight matrices range from 348 to 399, accounting for 71.7%-82.0%

Regarding LC-QTOF/MS: investigate the spiked recoveries and precision of 565 pesticides at concentration levels of 5 μg/kg, 10 μg/kg and 20 μg/kg respectively. At the same time, investigate the screening limit of the method by conducting matrix spiked experiment at concentrations of 1 μg/kg, 5μg/kg, 10 μg/kg, 20 μg/kg, and 50 μg/kg. Except for apples and spinaches in eight matrices, the percentage of pesticides with 1 μg/kg screening limit in other six matrices is greater than 50%. Pesticides with screening limit lower than 10 μg/kg account for 73.2%-81.9% in these eight matrices.

2 Safety precautions

Acetonitrile, toulene and other organic reagents used in the experiment pose certain harm or hazard to human bodies. Emulsified gloves should be worn in the experiment and operation be conducted with care in a well-ventilated environment.

3 Principle

Make a homogeneous extraction of fruit and vegetable testing samples with 1% acetate acetonitrile, clean up with Carb/NH_2 SPE column, elute the pesticides with acetonitrile-toulene (v/v: 3/1), analyze the samples with GC-Q-TOF/MS and LC-QTOF/MS, respectively, and quantify with the matrix-matched calibration curve method.

4 Instrument

4.1 GC-Q-TOF/MS:-7890A GC in tandem with 7200 time-of-flight mass spectrometer and equipped with electronic bombardment ion source, 7693 auto sampler and Mass Hunter data processing software (Agilent Technologies, Wilmington, DE). Chromatographic column:

VF1701 ms（30 m × 250 μm × 0.25 μm, Agilent J&W Scientific, Folsom, CA）or equivalent.

4.2 LC-Q-TOF/MS:-1290 Infinity II UHPLC system in tandem with 6545 quadrupole time-of-flight mass spectrometer, equipped with JetStream ESI ion source. The LC system includes G7120A 1290 Infinity II high speed pump, G7167B autosampler, and G7116B temperature control compartment. MassHuner data acquisition and processing software package (Agilent Technologies, Santa Clara, California, USA) is used for data acquisition and process. Chromatographic column: Zorbax SB-C18, 2.1×100 mm, 3.5 μm (Agilent Technologies, Santa Clara, California, USA) or equivalent.

4.3 Homogenizer-revolutionary speed not less than 13500 r/min.T-25B (Janke & Kunkel, Staufen, Germany) or equivalent.

4.4 Rotary evaporator-Buchi EL131 (Flawil, Switzerland) or equivalent.

4.5 Centrifuger-revolutionary speed not less than 4200 r/min, Z 320(B. HermLe AG, Gosheim, Germany) or equivalent.

4.6 Nitrogen blower-EVAP 112 (Organomation Associates, Inc. New Berlin, MA) or equivalent.

4.7 5 vacuum SPE device—RS-SUPELCO 57101-U, (Sigma Aldrich Trading Co., Ltd) or equivalent.

4.8 Pipette—10 μL, 200 μL,1 mL.

4.9 Analytical balance-range of 0.05-100 g, accurate to ±0.01 g

5 Reagents and testing materials

5.1 Solvents: acetate, acetonitrile, ethyl acetate, toulene, all of which should be HPLC grade.

5.2 Aacetonitrile-toulene (3:1,v/v).

5.3 anhydrous sodium sulfate:analytical grade, 550 ℃, burn 4 h, store in dryer for spare use after cooling.

5.4 SPC cartridge: Carbon/NH2 (1 g, 6mL, waters,USA) or equivalent

5.5 SPE cartridge adaptor: fit for 6 mL SPE cartridge (57020-U) (Sigma Aldrich Trading Co., Ltd) or equivalent.

5.6 Solid phase extraction unit one-time control flow rate hose (57059) (Sigma Aldrich Trading Co., Ltd).

5.7 Pear-shaped flask: 80 mL (Z680346-1EA, Sigma Aldrich Trading Co., Ltd) or equivalent.

5.8 Reservior: 30 mL(A82030, Agela, China) or equivalent.

5.9 Centrifugal tube: 80 mL.

5.10 Micro-hole filtering membrane (nylon): 13 mm × 0.2 μm.

5.11 Pesticide mixed standard stock solutions: Study director supplies pesticide mixed standard stock solutions for use in the collaborative study with the volume of 1.0 mL, which needs to be diluted into standard working solutions before use. The detailed steps are as follows:

5.12 Internal standard heptachlor-epoxide (used for GC-QTOF/MS) standard working solution' concentration is 10 mg/L, while internal standard Atrazine D5 (used for LC-QTOF/MS) working solution concentration is also 10 mg/L, and all the standard working solutions should be kept from any exposure to light and stored in refrigerator at 4 ℃.

Table1. The concentrations of pesticides mixing standard stock solution

No.	LC-QTOF/MS			GC-QTOF/MS		
	Name	CAS No.	Con. mg/L	Name	CAS No.	Con. mg/L
1	Acetochlor	34256-82-1	10	Ametryn	834-12-8	10
2	Ametryn	834-12-8	10	Atrazine	1912-24-9	10

3	Benalaxyl	71626-11-4	10	Benalaxyl	71626-11-4	10
4	Diphenamid	957-51-7	10	Benzoylprop-ethyl	22212-55-1	10
5	Esprocarb	85785-20-2	10	Cyflufenamid	180409-60-3	10
6	Fenothiocarb	62850-32-2	10	Diphenamid	957-51-7	10
7	Mepanipyrim	110235-47-7	10	Isoprothiolane	50512-35-1	10
8	Metalaxyl	57837-19-1	10	Kresoxim-methyl	143390-89-0	10
9	Methoprotryne	841-06-5	10	Methoprotryne	841-06-5	10
10	Metolachlor	51218-45-2	10	Metolachlor	51218-45-2	10
11	Orbencarb	34622-58-7	10	Orbencarb	34622-58-7	10
12	Pentanochlor	2307-68-8	10	Penconazole	66246-88-6	10
13	Picoxystrobin	117428-22-5	10	Picoxystrobin	117428-22-5	10
14	Pirimiphos-methyl	29232-93-7	10	Pirimicarb	23103-98-2	10
15	Propisochlor	86763-47-5	10	Quinalphos	13593-03-8	10
16	Quinalphos	13593-03-8	10	Simeconazole	149508-90-7	10
17	Sebuthylazine	7286-69-3	10	Tebufenpyrad	119168-77-3	10
18	Simeton	673-04-1	10	Terbuthylazine	5915-41-3	10
19	Terbuthylazine	5915-41-3	10	Tetraconazole	112281-77-3	10
20	Tetraconazole	112281-77-3	10	Thiazopyr	117718-60-2	10

5.13 Preparing the matrix-matched internal standard calibration mixed solution and establishing matrix-matched internal standard calibration curve: Following the operational procedures in 6.1, 6.2.1 and 6.2.2 in the method, prepare 8 portions of concentrated and purified liquids of fruit and vegetables, add respectively 0.5, 2.5, 5, 25 and 50 μL pesticide mixed standard working solutions (relative to 5 g sample matrix) (see Table 1) and prepare them into the standard working curve of 1, 5, 10, 50 and 100 μg/kg.

For GC-QTOF/MS, add 20 μL heptachlor-epoxide internal standard working solutions; for LC-QTOF/MS, add 20 ul Altrazine D5 working solutions. Place in a water bath at 35 ℃ and nitrogen blow to dryness, and dissolve the residues with 1.0 mL acetate ethyl for GC-QTOF/MS, dissolve the residues with 1.0 mL acetonitrile-water (3:2,v/v) for LC-QTOF/MS, homogenize uniformly with ultrasonication, pass through 0.2 μm filtering membrane to become a 5 point matrix-matched internal standard calibration mixed solutions used for establishing 5 point matrix-matched internal standard calibration curve, and the matrix-matched internal standard calibration mixed solutions should be prepared for immediate use. The linear correlation coefficient (R^2) should be greater than 0.98 for each pesticide matrix-matched internal standard calibration curve.

6 Analytical procedures

6.1 Extraction

Weigh 10 g sample (accurate to 0.01 g) into a 80 mL capped centrigual tube, add 40 mL 1% acetic acid-acetonitrile, process with high-speed homogenizer at a revolution of 12000 r/min, keep extracting homogeneously for 1 min; add again 1 g NaCl, 4 g anhydrous $MgSO_4$, oscillate 10 min; centrifuge 5 min at a revolution of 4200 r/min, transfer 20 mL of the supernatants into the pear-shaped flask, and rotary evaporate to about 2 mL in a water bath at 40 °C before cleanup.

6.2 Cleanup

6.2.1 Conditioning SPE cartridge: add 2 cm high anhydrous Na_2SO_4 into the Carb/NH_2 cartridge and place on the fixed bracket attached with pear-shaped flask underneath, wash the SPE cartridge with 10 mL acetonitrile-toulene(3:1, v/v) and discard the effluents.

6.2.2 Cleanup the test sample extraction fluid: when the surface of the washing liquid reaches the top layer of anhydrous Na_2SO_4, transfer the above-mentioned test sample concentrates of fruits and vegetables into Carb/NH_2 cartridge, collect the eluates using a clean pear-shaped flask; Use 3×2 mL acetonitrile-toulene(3:1, v/v) to wash test sample concentrate

bottle, and wait till the surface of the test sample concentrate liquid reaches the top layer of anhydrous Na_2SO_4 before transferring the washing liquid into the cartridge. Attach a 30 mL reservoir to the upper part of SPE cartridge, use 25 mL acetonitrile-toulene(3:1, v/v) to wash the SPE cartridge again, rotary evaporate the eluates in the pear-shaped flask in a water bath at 40 ℃ and concentrate to about 0.5 mL.

6.2.3 Diluting and filtering: after adding the relevant internal standard and standard solutions, use nitrogen to dry the concentrates.

For GC-QTOF/MS, use 1.0 mL ethyl acetate to dissolve the residues, homogenize with ultrasonication, and pass through 0.2 μm filtering membrane before determination.

For LC-QTOF/MS, use 1.0 mL acetonitrile-water(3:2, v/v) to dissovle the residues, homogenize with ultrasonication,and pass through 0.2 μm filtering membrane before determination.

Study Director Reminds: during SPE cartridge activation and cleanup, do not let the fluid in the SPE cartridge flow to emptiness in order to avoid the air coming in, which will affect the cartridge efficiency.

6.3 Determination

6.3.1 GC-QTOF/MS instrument conditions

a) Chromatographic column: VF1701 ms (30 m × 250 μm ×0.25 μm) or equivalent
b) Column temperature: maintain at 40 °C for 1 min, programmed temperature increase to 130 °C at 30 °C /min, then increase to 250 °C at 5 °C/min, further increase to 300 °C at 10 °C/min, and maintain at 300 °C for 7 min.
c) Carrier gas: helium gas (purity ≥99.999%), with a flow rate of 1.2 mL/min.
d) Inlet temperature: 270 ℃.
e) Injection volume: 1 μL.
f) Injection mode: without flow splitting, open the purge valve in 1.0 min.
g) Ionization mode: electronic bombardment.
h) Ion source polarity: positive.
i) On source voltage: 70 eV.
j) Ion source temperature: 280 ℃.
k) GC-MS inlet temperature: 280 ℃.
l) Solvent delay: 4 min.
m) Ion monitoring mode: full scan.

6.3.2 LC-QTOF/MS instrumental conditions

a) Chromatographic: ZORBAX SB-C18, 3.5 μm, 100 mm ×2.1 mm or equivalent.
b) Mobile phase ratio and flow rate: see Table 2

Table 2 Mobile phase ratio and flow rate

Step	Time/min	Flow rate (μL/min)	Mobile phase A, (0.1% Formic acid Water) V%	Mobile phase B, (Acetonitrile) V%
0	0.00	400	99.00	1.00
1	3.00	400	70.00	30.00
2	6.00	400	60.00	40.00
3	9.00	400	60.00	40.00
4	15.00	400	40.00	60.00
5	19.00	400	10.00	90.00
6	23.00	400	10.00	90.00
7	23.01	400	99.00	1.00

c) Column temperature: 40 ℃;
d) Injection volume: 10 μL.
e) Ionization mode: ESI.
f) Polarity: positive
g) Nebulizing gas: nitrogen.
h) Nebulizing gas pressure: 0.28Mpa.
i) Ion spray voltage: 4000 V.
j) Drying gas temperature: 325 ℃.
k) Drying gas flow rate: 10 L/min.
l) Sheath gas flow rate:11 L/min.
m) Sheath gas temperature: 375 ℃
n) Collision energy parameters: see Table 3;

'Table 3 Collision energy parameters setting

Time(min)	Collision Energy (V)
0	0
0.5	0
	15
	35

Study Director reminds: the analytical parameters presented in the method are obtained from Agilent instrument for demonstration, which serves only for reference by the collaborator. One may refer to Annex A for instrumental parameters of other brands. Only after he or she pass the fruit and vegetable spiked experiment confirmation and practice examinations with his or her results meeting the acceptance criteria, can these instrumental analytical parameters and database be used in the official collaborative study, and moreover the analytical parameters from the instrument used will be filled out in Table 1 of Annex B.

6.3.3 Qualitative determination

1) Retention time deviation is ±0.2 min.
2) There are at least two ions. For LC-QTOF/MS, one is preferably molecular ion and the other is secondary fragment ion; for GC-QTOF/MS, both can be fragment ions; mass deviation is less than 5ppm for molecular ion and 10 ppm for fragment ion; for those less than 200 Da, mass deviation can be within ±1 mDa.
3) S/N≥3.
4) For GC-QTOF/MS, ion ratio within ±30% of average of calibration standards from the same sequence.

6.3.4 Quantitative determination

Use the data processing software of GC-QTOF/MS and LC-QTOF/MS to establish matrix-matched internal standard calibration curve and calculate the pesticide content in the samples.

Please follow the requirements to fill out the analytical results of practice exam samples in Tables 2 and 3 of Annex B Excel worksheet; the analytical results of the collaborative samples are tabulated in Tables 4 and 5 of Annex B Excel worksheet.

To ensure the accuracy of qualitative and quantitative results, one must pay special attention

to the following key control points:

a) Before analyzing the collaborative samples, dilute the pesticide mixed standard working solutions 100 times to check whether the sensitivity and stability of the instrument have met the requirements.
b) After completion of one batch of samples, use again the pesticide mixed standard working solutions that have been diluted 100 times to check the sensitivity and stability of the instrument in order to see the consistency before and after determination.
c) Follow strictly the qualitative and quantitative requirements of the method and verify retention time and ion abundance ratio of each pesticide peak so as to ensure that each target pesticide peak is within the integration window and accurately identified.
d) Check the correctness of each pesticide peak integraton line, and adopt uniformly manual integration mode from valley to valley for pesticides with incorrect integration lines.

7 Blank experiment

To prevent any interference from the reagents, reagent blank experiment should be conducted during the entire process of the analytical method in order to confirm there is no interference from the reagents. Blank experiment will be executed per the analytical procedures in Section 6 of the method except no addition of test samples.

Study Director reminds: each collaborator will have to submit total ion chromatograms of the reagent blank samples to the Study Director.

8 Reference

(1) http://www.flworkshop.com/Community/pesticides.html
(2) EU SANTE/11813/2017 guidance document, http://www.ec.europa.eu/food/plant/docs/plant_pesticides_mrl_guidelines_wrkdoc11813en.pdf.
(3) AOAC Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals
(4) Youden W.J.& Steiner E.H.(1975) Statistical Manual of the AOAC, Arlington, VA.USA
(5) Standardization Administration of P.R.China (2008) GBT 23204-2008 , Determination of 519 pesticides and related chemicals residues in tea—GC-MS method
(6) Standardization Administration of P.R.China (2008) GB/T 23205-2008, Determination of 448 pesticides and related chemicals residues in tea—LC-MS-MS method
(7) Wang Z, Chang Q, Kang J, et al. Screening and identification strategy for 317 pesticides in fruits and vegetables by liquid chromatography-quadrupole time-of-flight high resolution mass spectrometry[J]. Analytical Methods, 2015, 7(15): 6385-6402.
(8) Wang Z, Cao Y, Ge N, et al. Wide-scope screening of pesticides in fruits and vegetables using information-dependent acquisition employing UHPLC-QTOF/MS and automated MS/MS library searching[J]. Analytical and bioanalytical chemistry, 2016, 408(27): 7795-7810.
(9) Pang G F, Fan C L, Chang Q Y, et al. Screening of 485 Pesticide Residues in Fruits and Vegetables by Liquid Chromatography-Quadrupole-Time-of-Flight Mass Spectrometry Based on TOF Accurate Mass Database and QTOF Spectrum Library[J]. Journal of AOAC International, 2018, 101(4): 1156-1182.
(10) Li J X, Li X Y, Chang Q Y, et al. Screening of 439 Pesticide Residues in Fruits and Vegetables by Gas Chromatography-Quadrupole-Time-of-Flight Mass Spectrometry Based on TOF Accurate Mass Database and Q-TOF Spectrum Library[J]. Journal of AOAC International, 2018.