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PO-CON1329E

Targeted Screening and Quantification of Pesticide Residuals in Tobaccos by Ultra Fast LC/MS/MS

ASMS 2013 ThP-530

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1. Introduction

Pesticides are used widely during cultivation of plants including tobaccos.
Although they are not foodstuffs, there has been a global concern in the tobacco industry and public about pesticide residues being taken into the body. LC/MS/MS methods have been employed increasingly in detection and quantification of pesticide residuals in foods and agriculture products. One of the challenges in food safety analysis is the large numbers of pesticides on the watching lists imposed by authorities, which leads to screening analysis using conventional MRM method on triple quadrupole LC/MS/MS to be difficult.
The new generation ultra-fast LC/MS/MS technique introduced recently features with ultra fast MRM speed (>500 MRM transitions per second), which enhances greatly the capacity of MRM method when it is used for screening analysis [1].
Both screening analysis and quantification could be carried out using a single method on the same system. Here we report an example of screening analysis and quantification of pesticides in tobaccos using a ready-to-use Method Package on LCMS-8040 system.

2. Experimental

Three samples of dried tobacco leaves labelled as A, B and C were obtained from a manufacture for this study.
The QuEChERS (Restek) method was employed for extraction of pesticides from the samples for screening analysis using a ready-to-use MRM based Method Package developed by Shimadzu [2].
The details of the pre-treatment method are described in Fig. 1.

The Method Package includes the complete analytical conditions from retentions and MRM transitions of 167 pesticides which are on the Positive List (Japanese Regulation).
By applying this method directly, screening analysis for the pesticides listed could be easily carried out without any method development efforts.
The results of this direct screening analysis were treated as the preliminary results, which were required to be further confirmed.
The LC/MS/MS system used was LCMS-8040 (Shimadzu Corporation, Japan) coupled with Nexera UHPLC.
The details of the LC and MS conditions are shown in Table 1.

The purposes of this study are in two aspects. Since the pesticides included in the Method Package (167 in current version) may be not sufficient for particular projects running in a testing laboratory, expanding of the compound list of the Method Package may become a task to be carried out for users. Therefore, the first aspect of the study was to practise the procedure of adding new registration of some concerned pesticides which are not in the pesticide list.

The second aspect was to validate and quantify the screening results obtained from the direct screening analysis. Nine concerned pesticides standards were obtained from Sigma-Aldrich.
A mixed stock solution of the nine pesticides standards (Tables 2 & 3) was prepared and spiked into the extraction solution of Samples A.
The pesticide standard in the mixed stock solution was 2500 ng/mL and was further diluted into calibration series.

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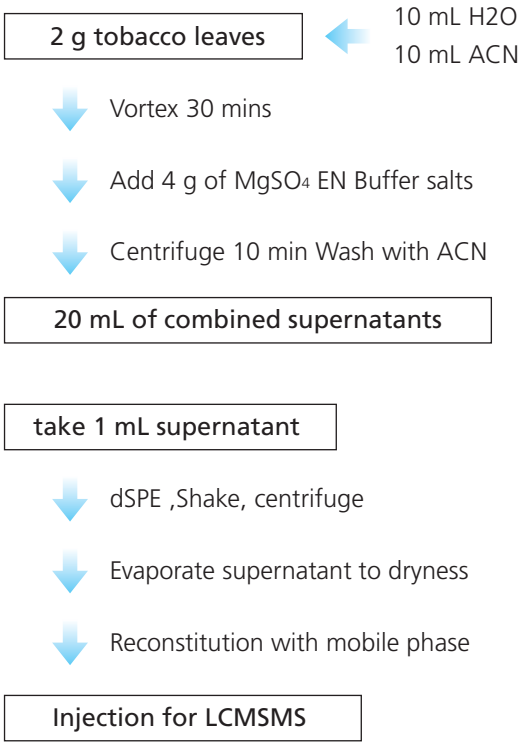

Fig. 1 QuEChERS Extraction

Table 1 MRM Based Screening Method

LC Conditions	
Instrument	Shimadzu LCMS-8040
Column	Shim-pack FC-ODS 150 × 2 mm, 3 um
Mobile Phase	A: 5 mmol/L Ammonium Acetate – Water B : 5 mmol/L Ammonium Acetate – Methanol
Gradient (B Conc.)	15%, 0 min → 40% 1~3.5 min → 50%, 6 min → 55%, 8 min → 95%, 17.5 min ~ 30 min → 15%, 30.01 → 40 min (stop)
Flow rate	0.2 mL/min
Oven temperature	40°C
Injection volume	5 µL
MS Conditions	
MS interface	ESI
MS mode	Two MRM for each compound
Heat Block temp.	400°C
DL temp.	250°C
Nebulizing Gas	1.5 L/min
Drying Gas	10 L/min

Table 2 New registration of Pesticides onto the Shimadzu Method Package

Additional Pesticide	RT (min)	MRM	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Clomazone	14.050	240.1 > 125.1	-28	-19	-26
		240.1 > 89.1	-28	-45	-18
Profenofos	18.442	373.0 > 303.0	-27	-17	-23
		373.0 > 345.1	-27	-12	-19
Chlorpyrifos	19.299	350.0 > 198.1	-26	-18	-23
		350.0 > 125.1	-26	-20	-26
Flumetralin	19.416	422.1 > 143.1	-30	-25	-30
		422.1 > 107.0	-30	-60	-22
Pendimethalin	19.439	282.2 > 212.1	-20	-10	-25
		282.2 > 194.1	-20	-18	-22
Butralin	19.656	296.2 > 240.2	-22	-13	-28
		296.2 > 222.2	-22	-21	-27

3. Results and Discussion

3-1 Targeted Screening Analysis using ready-to-use method package

The ready-to-use Method Package for pesticides consists of 167 pesticides for screening analysis.

In addition to these pesticides, six other concerned pesticides as shown in Table 2 were registered onto the Method Package using their standards and tested under the same conditions.
As a result, the modified method package was expanded to include 173 compounds with completed parameters of retentions and optimized MRMs.

The expanded method package was applied to the samples A, B and C for screening analysis of the 173 pesticides. The results were summarized in Table 3.

It can be seen that a total of six pesticides were found in the three samples at significant levels as shown in Fig. 2. The screening results are considerably reliable because of matching of two MRM transitions as well as retention of each pesticide found.
However, further confirmation by spiked samples of the found pesticides is required to reach solid conclusions.

3-2 Validation and Quantification of Screening Results

It is necessary to use spiked samples to validate the screening results above.

At the same time, quantification of the found pesticides could be obtained if calibration curves were established using spiked samples.

A series of spiked samples were prepared by adding mixed standards (six pesticides) into the extact solutions of samples A, B and C. The chromatograms of sample A and its 100 ppb spiked samples are shown in Fig. 3.

It can be seen that the MRM peak pairs of every found pesticide (except Methomyl) in sample A were in accordance with the spiked samples in terms of MRM peak ratio and peak intensity which increased proportionally with the spiked amount. This results firmly confirm the screening results above.

MRM calibration curves of the six found pesticides were established using the spiked samples into Sample A as matrix.
The calibrations curves with excellent linearity for ranges from 1 ppb to 500 (or 2500) ppb are shown in Fig. 4.

The performance of the MRM quantification method was evaluated and the results are summarized in Tables 4-6. The repeatability of the method was evaluated and the RSD (%, n = 6) of peak area obtained for 50 ng/mL and 10 ng/mL concentration were found to be below 5% except chlorpyrifos (11.5%).

Table 6 shows the results of matrix effect (ME), recovery (RE) and process efficiency (PE) of the six pesticides in Samples A, B and C by the QuEChERS pre-treatment and MRM quantification method.

Both post-spiked and pre-spiked samples were prepared and analyzed using the MRM quantification method established. The results indicates that the current method from sample pre-treatment by the modified QuEChERS method and MRM quantification method generated considerably excellent results in low matrix effect, high recovery and high process efficiency.

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Table 3 Screening results using the expanded m ethod package of 173 pesticides

No	Found Pesticides	Result of Screening Analysis		
		Sample A	Sample B	Sample C
1	Methomyl	Detected	-	-
2	Thiodicarb	Detected	-	Detected
3	Azoxystrobin	Detected	-	-
4	Profenofos	Detected	Detected	Detected
5	Chlorpyrifos	-	-	Detected
6	Butralin	-	Detected	-

Note: pesticide No. 4-6 were new registrations into the Method Package in this study

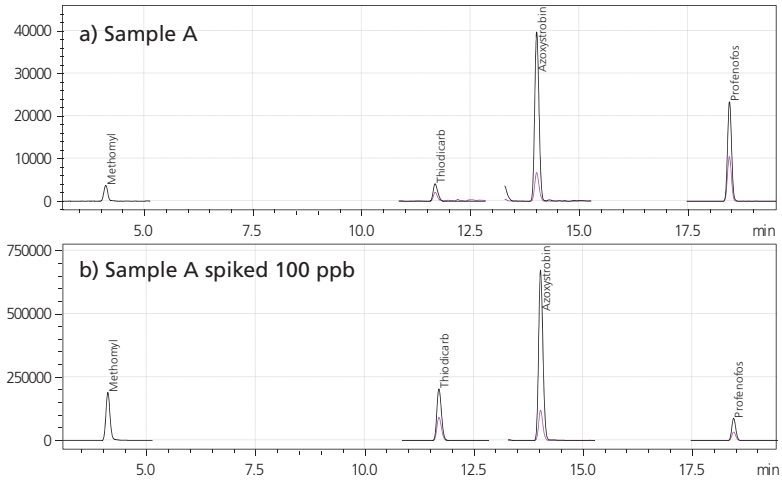

Fig. 3 MRM Chromatograms of Sample A (a) and Sample A spiked with 100 ppb pesticide standards (b)

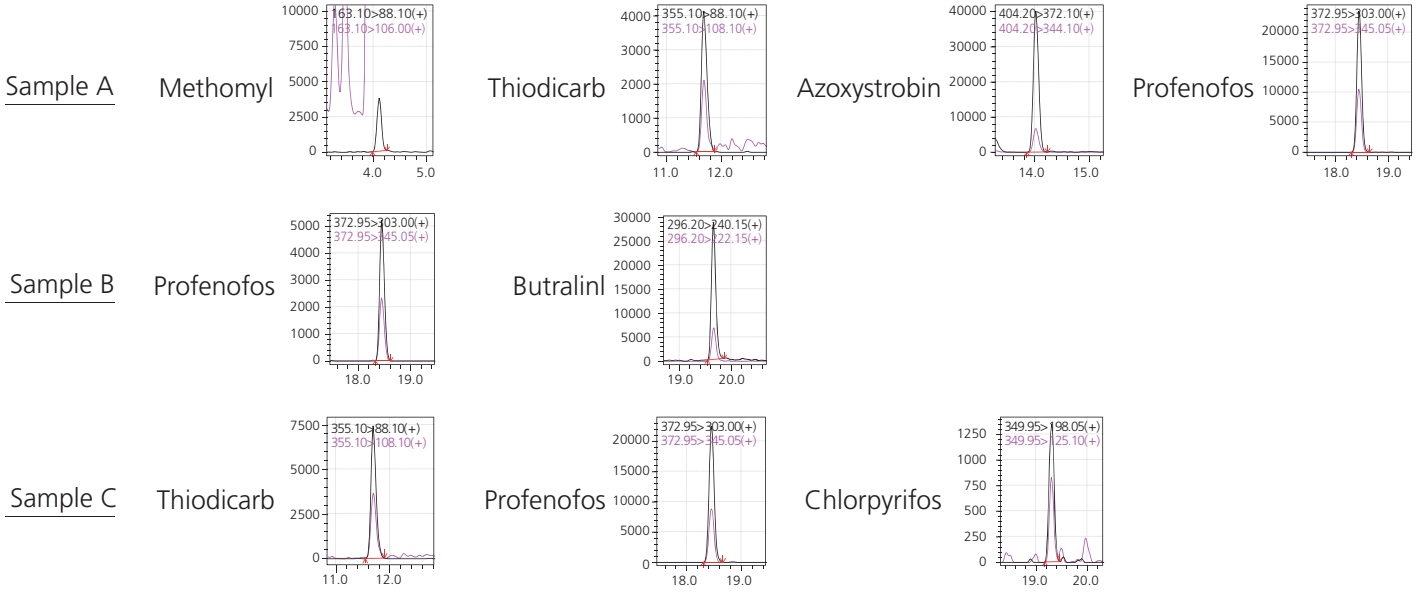

Fig. 2 MRM peak pairs of found pesticides in Samples A, B and C using the expanded method package of 173 pesticides on LCMS-8040

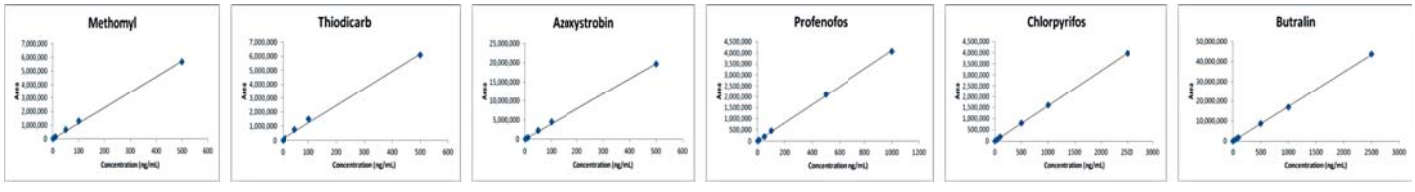

Fig. 4 Calibration Curves of six pesticides in tobacco leave extract by MRM method

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Table 4 Range and linearity of calibration curves

No.	Compounds	Ret. Time	Calibration	
			Range (ppb)	R^2
1	Methomyl	4.110	1 – 500	0.9984
2	Thiodicarb	11.693	1 – 500	0.9965
3	Azoxystrobin	14.019	1 – 500	0.9987
4	Profenofos	18.442	1 – 1000	0.9996
5	Chlorpyrifos	19.299	1 – 2500	0.9998
6	Butralin	19.656	1 – 2500	0.9998

Table 5 Repeatability of MRM method

No.	Compounds	% RSD (10 ppb, n = 6)		% RSD (50 ppb, n = 6)	
		Ret Time	Area	Ret Time	Area
1	Methomyl	0.127	3.22	0.025	2.39
2	Thiodicarb	0.055	1.18	0.030	1.74
3	Azoxystrobin	0.031	0.97	0.009	2.13
4	Profenofos	0.032	2.92	0.024	2.47
5	Chlorpyrifos	0.042	11.46	0.019	2.08
6	Butralin	0.017	3.22	0.014	4.33

Table 6 Matrix effect (ME), recovery (RE) and process efficiency (PE)

No.	Compounds	Average (%), n = 3		
		ME	RE	PE
1	Methomyl	98.0	84.7	82.9
2	Thiodicarb	113.4	89.2	101.0
3	Azoxystrobin	115.4	81.0	91.2
4	Profenofos	104.5	85.7	89.4
5	Chlorpyrifos	95.1	83.2	79.1
6	Butralin	88.3	80.0	70.7

The calculation of ME, RE and PE

$$\text{Matrix Effect (\%): ME} = \frac{\text{Conc. of Post Spiked - Conc. Sample blank}}{\text{Conc. of Standard}} \times 100\%$$

$$\text{Recovery (\%): RE} = \frac{\text{Conc. of Pre Spiked - Conc. Sample blank}}{\text{Conc. of Pre Spiked - Conc. Sample blank}} \times 100\%$$

$$\text{Process Efficiency (\%): PE} = \frac{\text{Conc. of Pre Spiked - Conc. Sample blank}}{\text{Conc. of Standard}} \times 100\%$$

The quantitative MRM method established was then applied to the samples by processing the raw data in the Postrun program of the LabSolutions. The quantification results in extract solutions and converted to tobacco leaves in ng per gram are shown in Table 7.

Table 7 Quantification results of pesticides in tobacco leaves samples by MRM method

No	Found pesticide	Quantification result in extract solution (ppb)			Quantification result in tobacco leaves (ng/g)		
		Sample A	Sample B	Sample C	Sample A	Sample B	Sample C
1	Methomyl	2.20	-	-	22.00	-	-
2	Thiodicarb	2.48	-	4.06	24.80	-	40.60
3	Azoxystrobin	6.92	-	-	69.20	-	-
4	Profenofos	36.41	8.18	36.10	364.10	81.80	361.00
5	Chlorpyrifos	-	-	6.20	-	-	62.00
6	Butralin	-	10.10	-	-	101.00	-

4. Conclusions

This study shows that the ready-to-use MRM based Method Package provides an easy and reliable workflow for both screening analysis and quantification of residual pesticides in tobacoo leaves on the LCMS-8040. Because of the expandability of the Method Package, users can add easily any desired new registration of pesticides or other concerned compounds.

This flexibility makes it even more valuable in research, manufacture and testing laboratories targeting for different groups of pesticides in tobacco leaves etc. The procedure as well as the strategy of using the Method Package for both targeted screening analysis followed by quantification of found pesticides can be applied to other food safety analysis.

5. References

1. Shimadzu Corporation, Catalogue, LC/MS/MS Method package for Residual Pesticides, C146-E160
2. Michelle Misselwitz, Jack Cochran, Julie Kowalski.
   The QuEChERS Sample Preparation Approach with Dispersive and Cartridge SPE Cleanup, GCxGC-TOFMS, and LC-MS/MS for the Analysis of Pesticides in Tobacco. Retrieved from:
   http://www.restek.com/pdfs/pcon2012_2340-8_pesttobacco. pdf

First Edition: June, 2013

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