

# A Comparative Study of Targeted Screening Method by LC/MS/MS and Un-targeted Screening Method by LC-TOF in Residual Pesticides Analysis

**ASMS 2015** WP 054

Zhaoqi Zhan, Jie Xing, Zhe Sun, Edwin ZhiWei Ting and  
Yin Ling Chew  
Customer Support Centre, Shimadzu (Asia Pacific) Pte Ltd,  
79 Science Park Drive #02-01/08, Singapore 118264

# A Comparative Study of Targeted Screening Method by LC/MS/MS and Un-targeted Screening Method by LC-TOF in Residual Pesticides Analysis

## Introduction

Advanced screening methods based on liquid chromatography-mass spectrometry (LC-MS) for detection of un-predicted residues of pesticides and veterinary drugs in agriculture products and food have been reported in recent years [1]. Without spectrum libraries like in GCMS, LC/MS/MS was initially not used in screening analysis. Whereas, high resolution LC-TOF was selected for screening analysis due to its accurate mass capability [1-2]. However, with rapid progress in data acquisition technique speed like UFMS (ultrafast MS) and high MRM capacity, new generation triple quadrupole LC/MS/MS has been

used for targeted screening, e.g., of over a few hundred of pesticides in one analysis [3]. It is interesting to know the advantages and limitations of the two different screening approaches for pesticide residues in agriculture and food matrixes. We describe here a comparative study on targeted screening analysis based on MRM method on a UFMS-TQ system and un-targeted screening analysis based on high resolution MS full spectrum method on a LC-TOF system using same sample sets of mixed pesticides aiming at unveiling their capabilities and limitations in the challenging screening analysis.

## Experimental

Mixed pesticide samples were obtained from a third party without information of compound number and names before completion of analysis. The unknown pesticide samples were analysed by two different screening methods on two LC-MS systems. A MRM-based targeted screening method was carried out on LCMS-8050, an ultrafast

(UFMS) triple quadrupole system. Un-targeted screening analysis of the same samples was carried out on LCMS-IT-TOF, a high resolution MS system. The two systems, analytical conditions and parameters are compiled into Table 1.

Table 1: Un-targeted screening analysis conditions of LCMS-IT-TOF

System & items		LCMS-8050*	LCMS-IT-TOF
LC conditions	Column	Shim-pack XR-ODS III (150 mmL. x 2mmi.D., 2.2µm)	Shim-pack XR-ODS III (150L x 2.0, 2.2µm)
	Flow Rate	0.4 mL/min	0.3 mL/min
	Mobile Phase	A : Water 5mM ammonium formate with 0.1% formic acid B : MeOHwith 5mM NH4 formate with 0.1% formic acid	A : Water 5 mmol/L NH4 formate, 0.1% formic acid B : MeOHwith 5 mmol/L NH4 formate, 0.1% FA
	Elution Mode	Gradient elution, 20 minute B: 5% (0 min) -> 100% (16min ~ 18min) -> 5% (18.1min ~20min)	Gradient elution, 35 min B: 15% (0min) -> 100% (25mins to 31min) -> 15% (31.1min to 35min)
	Oven Temp.	45 °C	50 °C
	MS conditions	Interface	ESI heated
MS Mode		Schedule MRM, in positive and negative mode	Multi-event TIC, Positive and negative
Interface Temp.		300 °C	RT
Block Temp.		400 °C	250 °C
DL Temp.		250 °C	200 °C
Nebulizing Gas		Nitrogen, 2.0 L/min	Nitrogen, 1.5 L/min
Heating Gas		Zero Air, 10 L/min	N.A.
Drying Gas Flow		Nitrogen, 10 L/min	Nitrogen, 10 L/min
Inj Vol	Inj. Volume	1.0 µL	10 µL

\* Refereed to method 1. The LC conditions of Method 2 and method 3 are different.

# A Comparative Study of Targeted Screening Method by LC/MS/MS and Un-targeted Screening Method by LC-TOF in Residual Pesticides Analysis

## Results and Discussion

### Description of targeted and un-targeted screening approaches

It has been accepted with unanimity that the MRM technique is one of best analytical methods in quantitative analysis of trace level organic compounds in complex matrix. Although MRM method has been used widely in quantitative analysis of thousands of compounds, it had not been used for screening analysis aiming at detection of concerned chemicals like pesticides in agriculture products until a recent time. The conventional method for screening analysis of pesticide residues is by GCMS with well-established spectrum library. However, GCMS with EI or CI ion source could not detect and quantify less and non-volatile pesticides effectively. In recent years, LC-MS with ESI interface has been increasingly used in analysis of pesticide residues using MRM method or high resolution TOF-MS method [1]. The so-called HRMS instruments like LC-TOF with its high mass-resolving power were first adopted in un-targeted screening analysis for pesticides and other chemical contaminants in agriculture products and food. A different methodology from GCMS is employed, in which data analysis of the full spectra data is searched against a compound (molecular formula) database via

accurate mass matching (+/-5ppm or better) to find candidates. The key advantages of this approach are: it does not need to restrict the retention time and the raw data can be re-analysed using different molecular formula database of any concerned compound or compounds group. On the hand, with rapid progress in instrumentation technology in recent years, new generation LC/MS/MS systems with ultrahigh data acquisition speed and extremely high capacity of MRM are invoked to use in screening analysis in food safety field. Targeted screening methods based on pre-loaded MRMs of hundred pesticides have been used increasingly as an alternative. This study is aimed at a comprehensive comparison between MRM-based targeted screening method and HRMS-based un-targeted screening method in detection and identification of pesticides in the unknown samples. Table 2 outlines the two methods used in this study, which were carried out on LCMS-8050, a latest model of LC/MS/MS with the highest performance specification of Shimadzu series, and LCMS-IT-TOF, a high resolution hydride MS system.

Table 2: Comparison of targeted & un-targeted screening methods

Item	Targeted Screening MRM method	Un-targeted screening HRMS method
No. of pesticides	fixed number (347, 167 & 121)*	not limited
Detection and identification method	Two pre-set MRM for each pesticide, intensity ratio	Accurate mass matching mass window (+/-) 5ppm
	Pre-determined RT with (+/-) 0.5 min window	Isotope pattern matching (scope > 50%)
Data analysis	Pre-set method, automated	allow re-process with specific database for data mining

\* three method packages are used with different numbers of pesticides

# A Comparative Study of Targeted Screening Method by LC/MS/MS and Un-targeted Screening Method by LC-TOF in Residual Pesticides Analysis

## Results of targeted and untargeted screening of mixed pesticide samples

Figure 1 shows the MRM chromatograms of targeted screening analysis of the unknown mixed pesticide sample by three methods covering different numbers of compounds on LCMS-8050. The results of the screening by using three methods are shown in Figure 2. The total number of unique pesticides found in the unknown sample is 189.

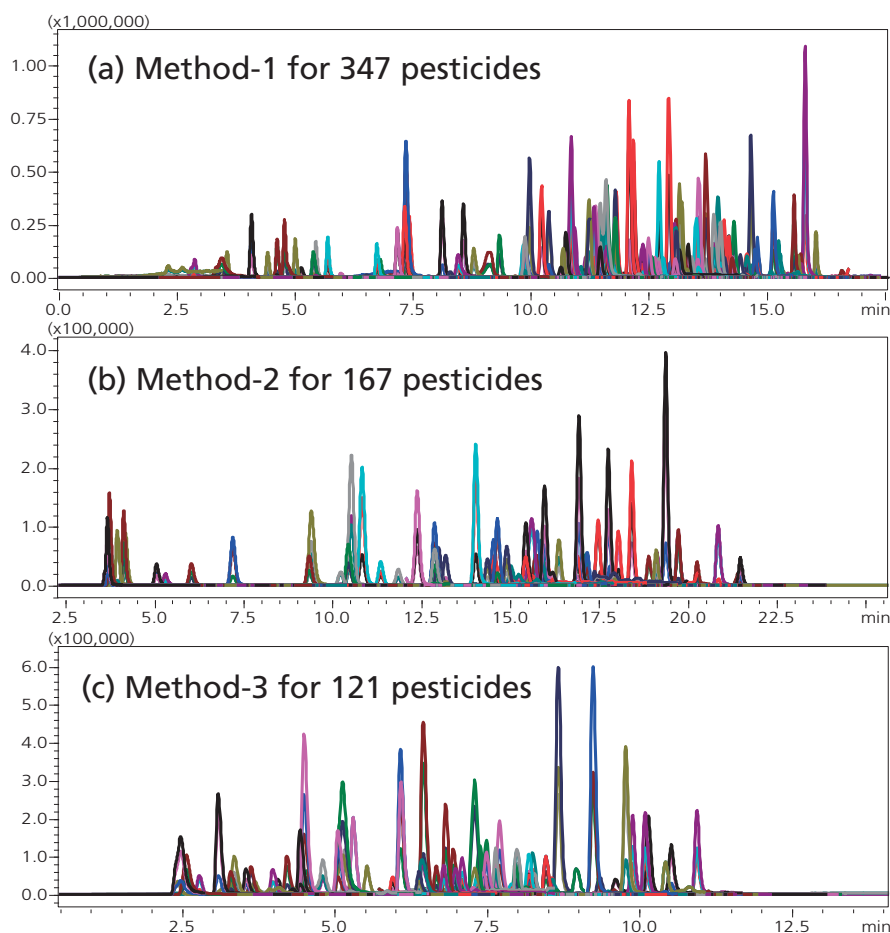


Figure 1: MRM chromatograms of targeted screening analysis of an unknown sample by three methods on LCMS-8050. The numbers of pesticides screened by the three methods are 347, 167 and 121, respectively.

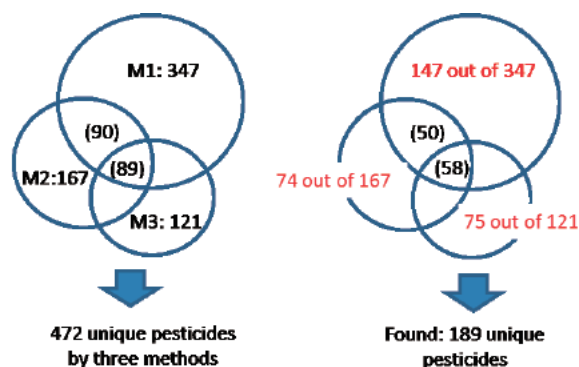


Figure 2: Numbers of pesticides of three MRM method packages covered are Method-1: 347, Method-2: 167 and Method 3: 121 (left). A total of 189 pesticides were found in the unknown mixed pesticide sample by the three methods (right).

## A Comparative Study of Targeted Screening Method by LC/MS/MS and Un-targeted Screening Method by LC-TOF in Residual Pesticides Analysis

Figure 3 shows multi-event TICs of un-targeted screening analysis of the unknown mixed pesticide sample on LCMS-IT-TOF. The so-called multi-event method was described elsewhere [4]. Instead of a single event of full mass range ( $m/z$ 100~900), the data acquisition was performed in ten separate events, each covering a narrow mass range. This method was proven to be more sensitive than a single event method due to a lower baseline [4]. A

total of 83 pesticides was detected and confirmed from this un-targeted screening analysis using a compound database of 450 pesticides (see Table 3). Fifty-three out of the eighty-three (70%) found pesticides were also found in targeted screening by LCMS-8050. In addition, 37 candidates were suspected present in the sample. Nine out of 37 suspected candidates (24%) were also found in the targeted screening analysis by LCMS-8050.

### Comparison of targeted and untargeted screening

As shown in Figure 4, nine pesticides found in the unknown sample by both targeted and untargeted screening methods are selected randomly to compare the detection results individually. A general impression is that for those firmly detected pesticides, the detection results by both HRMS method on LC-TOF and MRM method on LC/MS/MS are qualified as screening candidates. The absolute detection sensitivity of MRM-based targeted screening are obviously higher than that of HRMS-based un-targeted screening. There are instrumentation factors and methodology factors. The LCMS-8050 is a latest model of triple quadrupole system with extremely high sensitivity, which contributes at least partially to the results of more pesticides found. However, this study is focused on the methodology factors. One of the key ideas of un-targeted screening by HRMS is that the method should avoid discrimination of any mass in data acquisition step. This

means that all ions of a sample including matrix and solvent clusters are detected equally. As a result, some peaks of interesting compounds may be interfered to become shoulder or tailing peaks or submerged peaks due to high baseline. These can make peak detection more difficult in the subsequent data analysis step due to inappropriate integration parameters being applied. This was proven by reversed searching of those pesticides that were not found by un-targeted screening but found by MRM method in the LC-TOF raw data. By entering the exact masses of these pesticides to extract the corresponding EIC, additional 69 pesticides were found from the LC-TOF raw data. Figure 5 shows a few examples of the EICs obtained by this method. It can be that all these peaks in TICs are severely disturbed or merged by the baseline.



# A Comparative Study of Targeted Screening Method by LC/MS/MS and Un-targeted Screening Method by LC-TOF in Residual Pesticides Analysis

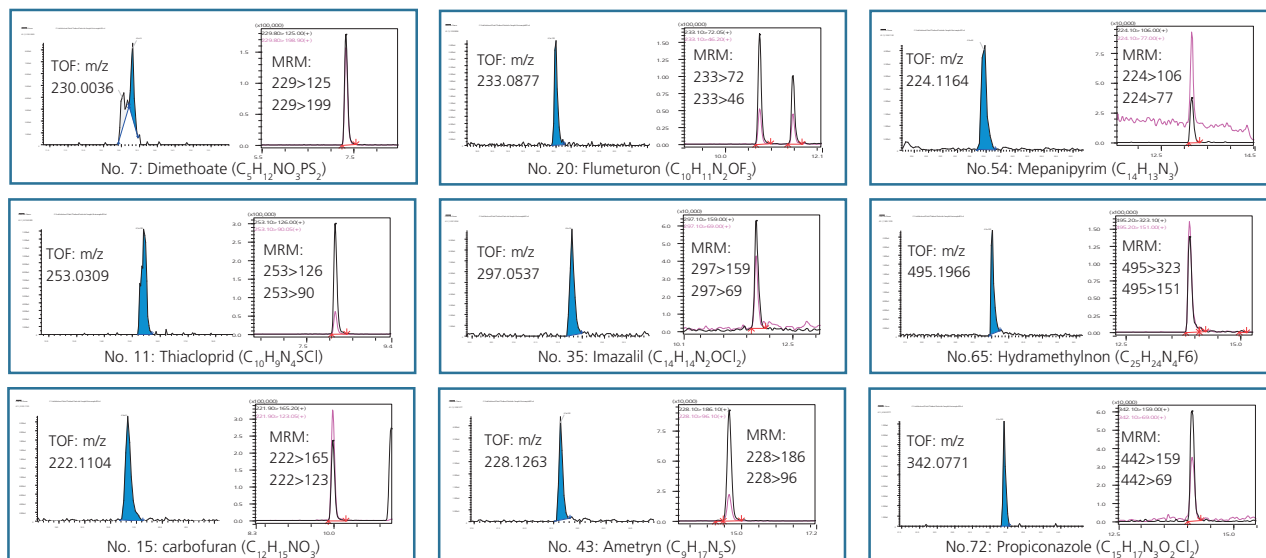


Figure 4: Individual comparisons of detection of nine pesticides (out of 83) in an unknown sample by targeted and un-targeted screening methods.

But, they could be detected if their EICs were extracted successfully. This results indicate that HRMS based un-targeted screening is highly depending on the data analysis method. The current peak picking algorithm

could not find effectively the distorted and submerged peaks. More sensitive peak picking programs (different algorithm) are required for effective detection of distorted and submerged peaks in LC-TOF data.

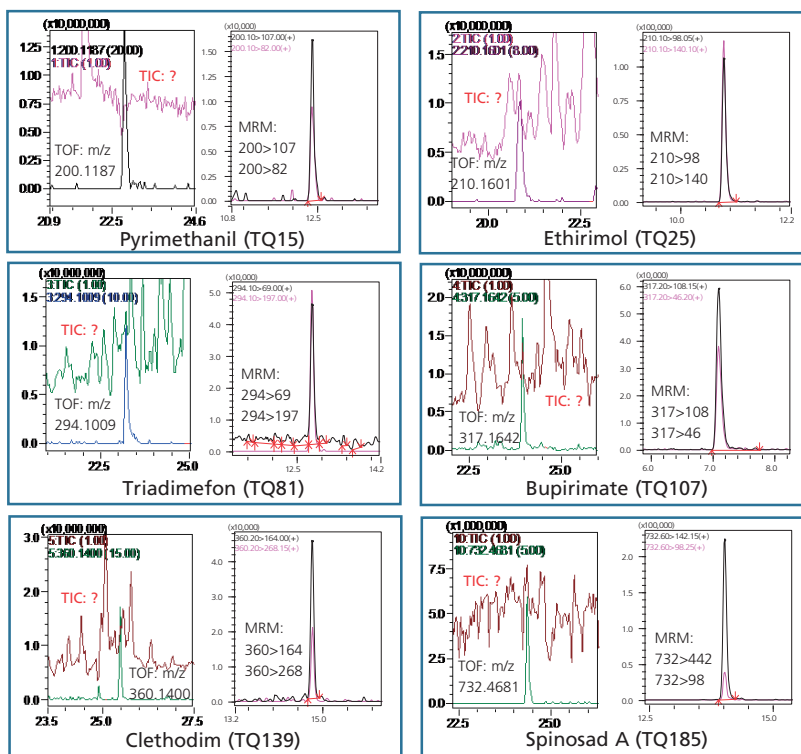


Figure 4 Sixty-nine additional pesticides were confirmed exist in the unknown sample by re-exploring the LC-TOF raw data through extracting the exact masses of pesticides found by MRM method (displaying 6 only).

# A Comparative Study of Targeted Screening Method by LC/MS/MS and Un-targeted Screening Method by LC-TOF in Residual Pesticides Analysis

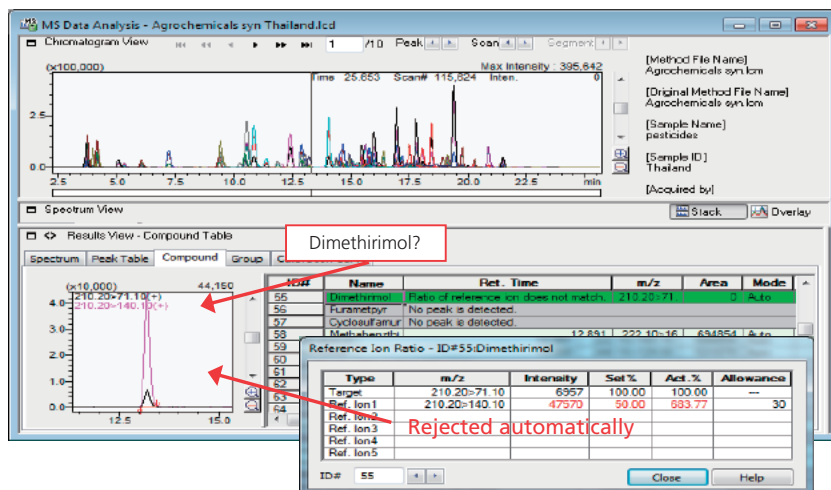


Figure 5: The Identification of a peak for dimethirimol was rejected automatically by the method due to un-matching of intensity ratio of MRMs.

In contrast, the MRM targeted screening method does not affected by data analysis. While, matrix effect is the only factor that may cause false positive and false negative detection. The screening reliability of MRM method is considerably high due to the excellent mass selectivity and specific RT of every compounds. In addition, the confirmation MRM (reference ion) and its intensity ratio with the main MRM (quantifier ion) provides additional selectivity and confirmation, which could reduce further false positive results. Figure 6 shows one example, a peak corresponding to dimethirimol appeared at the expected RT with two MRMs. However, the data analysis program rejected it as a found pesticide due to the intensity ratio of the MRM pair was out of the defined range. This result may have two possibilities: it is

another compound or there is a co-elute peak which MRM is same as the confirmation MRM (210>140) of dimethirimol. The MRM-based targeted screening methods have been increasingly adopted in analysis of pesticides and other chemical contaminants in food safety analysis due to several facts. First, the number of pesticides that can be covered (screened) in a single run has increased drastically due to the improvements in triple quadrupole instrumentation and software technologies. Second, MRM database of most pesticides become available for various LC/MS/MS systems from vendors or research institutes. Third, MRM targeted screening approach can be fully automated from data acquisition to data analysis and reporting, which is favored in routine inspection analysis in food safety labs.

## Conclusions

This is a preliminary comparative study of MRM-based targeted screening method and HRMS based un-targeted screening method for detection of pesticides. HRMS method on LC-TOF is an ideal approach for screening of un-limited pesticides in samples. This approach requires advanced data acquisition method and high sensitivity in full spectrum mode to detect all ions without discrimination. However, data analysis may be very challenging in peak picking of distorted and submerged

peaks due to matrix interference of actual food samples. On the other hand, the MRM-based targeted screening approach has been increasingly adopted in food safety analysis because of its operation easiness and high reliability in detection and identification of targeted pesticides. Further studies with more quantitative comparison of the two screening approaches and their performances for different samples are needed.



# A Comparative Study of Targeted Screening Method by LC/MS/MS and Un-targeted Screening Method by LC-TOF in Residual Pesticides Analysis

## References

1. S. J. Hird, B. P.-Y. Lau, R. Schuhmacher and R. Krska, Trends in Analytical Chemistry, 59 (2014) 59-72.
2. M. Mezcua, O. Malato, M.A. Martinez-Uroz, A. Lozano, A. Aguera, A.R. Fernandez-Alba, J. AOAC Int. 94 (2011) 1674–1684.
3. G.-F. Pang, C.-L. Fan, F. Zhang, Y. Li, Q.-Y. Chang, Y.-Z. Cao, et al., J. AOAC Int. 94 (2011) 1253–1296.
4. Jie Xing, Dinash Aravind, Zhe Sun, Ang May Yen and Zhaoqi Zhan, ASMS2015, Poster, TP201.