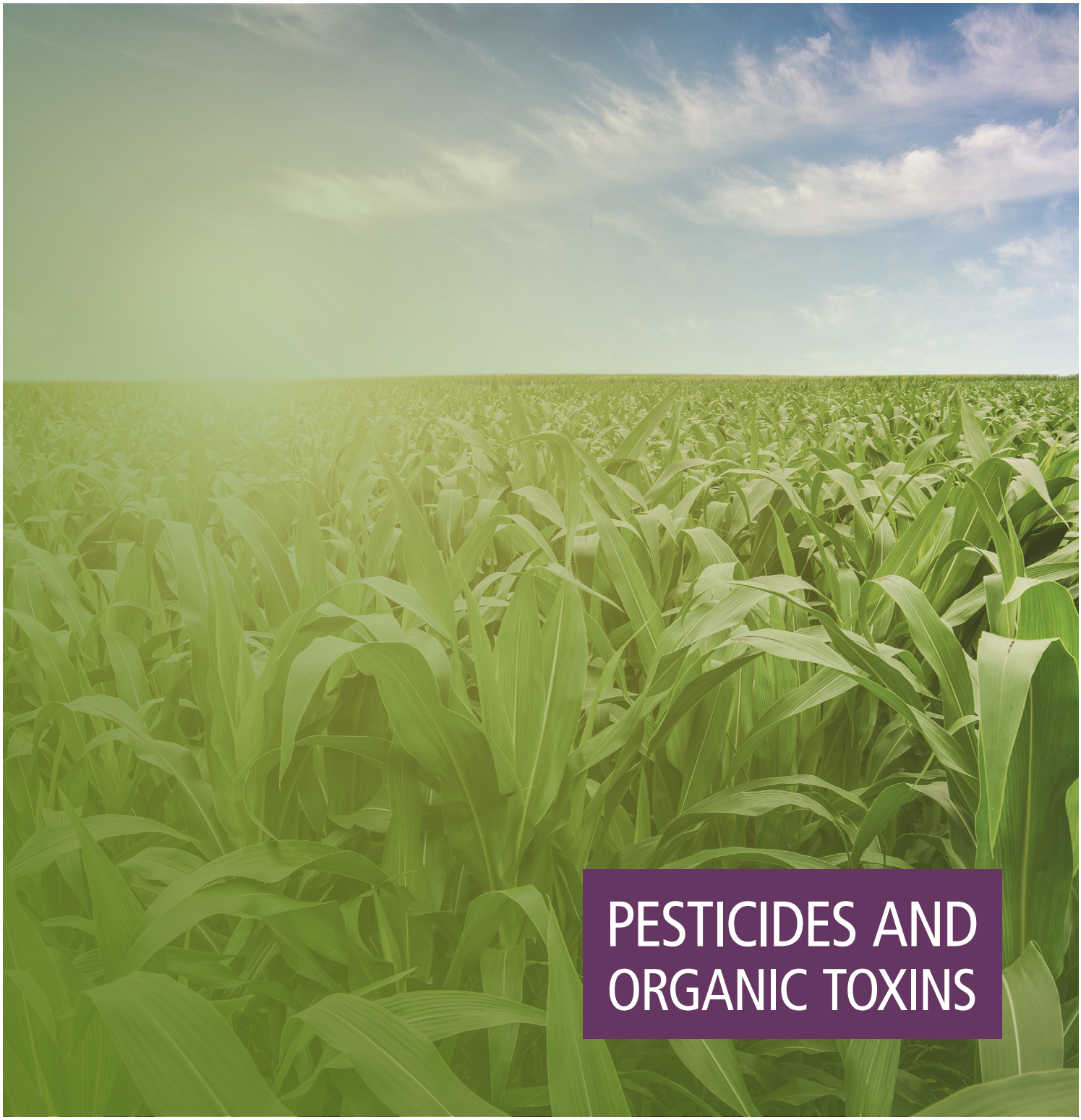


PESTICIDES AND ORGANIC TOXINS

Pesticides and Organic Toxins Compendium

PerkinElmer®
For the Better

Table of Contents

APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

Authors:

Jingcun Wu

Josh Ye

Erasmus Cudjoe

Feng Qin

Shixin Sun

PerkinElmer, Inc.
Woodbridge, Ontario, Canada

Analysis of Multi-Residue Pesticides in Rice by LC/MS/MS

Introduction

Rice is one of the most commonly consumed foods in the world. A variety of pesticides have been used in rice production to control pests, weeds and diseases to increase crop yield. Pesticides applied in rice crops are often country/region specific due to the differences in legislation, weather and production system. Pesticide residue in rice not only affects the quality of the rice, but also threatens the health of general consumers. To prevent health risks, it is important to monitor the presence of pesticides and regulate their levels in rice. Several countries including the United States, China, Brazil, India, Japan and European Union (EU) have established maximum residue levels (MRLs) of pesticides for food and feed including rice.[1-3] The EU MRLs for pesticide residues in rice mostly range from 10 µg/kg to 8000 µg/kg depending on the pesticide.[1] To determine low levels of pesticides in rice, highly sensitive, selective and accurate analytical methods are needed. Due to the large number of pesticides potentially used in rice production, the use of multi-residue methods capable of determining many pesticides in one single run is the most efficient approach. Traditionally, pesticide residues were analyzed mainly by gas chromatography/mass spectrometry (GC/MS) methods,[4,5] but GC is not a suitable technique for ionic and polar compounds, especially for compounds that are thermally labile in the GC injection port. Liquid chromatography tandem mass spectrometry (LC/MS/MS) has become the method of choice for pesticide analysis due to its high selectivity and sensitivity as well as its suitability for a wide range of compounds in various sample matrices.[6-10]

PerkinElmer®
For the Better

QuEChERS extraction method has been widely applied for analysis of multi-residue analytes in food samples including rice.[4,8,9,10] In this study, a fast, sensitive and selective multi-residue method has been developed for analysis of over 200 pesticides in rice samples by coupling a modified QuEChERS extraction method with LC/MS/MS. Using time-managed-MRM™ in the QSight® triple quadrupole mass spectrometer, the optimum dwell time of multiple MRM transitions can be generated automatically for the targeted analytes. This not only saves time in method development but also improves data quality and analytical performance, as demonstrated in this study by the results of multi- residue pesticide analysis in rice samples.

Experimental

Hardware/Software

Chromatographic separation of pesticides was conducted by a PerkinElmer UHPLC System and analyte determination was achieved using a PerkinElmer QSight 220 triple quadrupole mass detector with a dual ionization source. All instrument control, data acquisition and data processing was performed using Simplicity 3Q™ software.

Method

Sample Preparation

Pesticide standards were obtained from ULTRA® Scientific (North Kingstown, RI). Rice samples were purchased from local grocery stores in Ontario, Canada. Different rice samples such as brown rice, black rice and white rice (including Jasmine, Basmati and Calrose) as well as two brands of organic rice samples were tested. These rice samples were originally produced in Thailand, Vietnam, India, Italy and the U.S. Rice samples were prepared according to a published procedure with minor modifications using QuEChERS kits (AOAC 2007.01 method) without dispersive SPE clean-up.[10] One (1) µL of extract was injected directly onto the QSight LC/MS/MS system for quantification.

An organic brown rice sample was used as a controlled blank matrix. Recoveries from the rice sample matrix were evaluated by fortifications of pesticides at concentrations of 10 and 100 µg/kg. Calibration curves were built by eight levels of standards prepared in a neat solution (acetonitrile) and in the rice sample matrix (matrix-matched calibration). Matrix effects were evaluated by comparing the slopes of calibration curves obtained from the neat solution and rice sample matrix. To reduce false positives and negatives, at least two MRM transitions were monitored for each pesticide. LOQs (limits of quantification) were calculated based on a minimum S/N of 10 for both transitions.[12]

LC Method and MS Source Conditions

The LC method and MS source parameters are shown in Table 1. A partial list of the multiple reaction monitoring mode (MRM) transitions of the studied pesticides are shown in Table 2. The acquisition MS method is generated automatically by selecting the pesticides of interest from the built-in compound library in the time-managed-MRM module of the Simplicity software, including both positive and negative analytes.

Table 1. LC Method and MS Source Conditions.

LC Conditions	
LC Column	Brownlee, SPP Phenyl-Hexyl, 100 x 2.1 mm, 2.7 µm
Mobile Phase A	5 mM ammonium formate in water
Mobile Phase B	5 mM ammonium formate in methanol
Mobile Phase Gradient	Start at 10% mobile phase B and hold it for 1 min., then increase B to 95% in 15 min. and keep at 95% B for 2 min. Finally equilibrate the column at initial condition for 3 min.
Column Oven Temperature	40 °C
Auto Sampler Temperature	15 °C
Injection Volume	1.0 µL
MS Source Conditions	
ESI Voltage (Positive)	5000 V
ESI Voltage (Negative)	-4000V
Drying Gas	140
Nebulizer Gas	350
Source Temperature	325 °C
HSID Temperature	200 °C
Detection mode	Time-managed MRM™

Results and Discussion

Analytical Challenges for Multi-residue Pesticides Analysis in Food Samples

Since the pesticides tested in this study contain both polar and non-polar compounds, to extract all the analytes from sample matrices, acetonitrile, an organic solvent, was used. However, the reverse phase LC method used aqueous mobile phase at the beginning of the LC run to retain the polar compounds on the column. Injecting a larger volume of organic solvent such as an acetonitrile sample extract on the LC would lead to poor chromatographic peaks for early eluting polar compounds. To overcome this problem, small sample volume was injected in this study.

Traditional MRM method development is not suitable for analysis of a large number of analytes such as hundreds of pesticide residues in a single run. It is both time-consuming and labor intensive to input all the mass transitions to a method manually. In addition, the dwell time for each transition cannot be optimized easily by traditional method. Therefore, a time-managed-MRM was applied for method development in this study to improve efficiency, data quality and method performance.

Sample matrix effect is the main concern for LC/MS/MS method development, especially for food analysis due to the diversity and complexity of food sample matrices. To overcome sample matrix effects, several approaches have been used, such as sample dilution, use of stable isotope internal standards, matrix-matched calibration, standard addition, sample clean-up, use of high efficiency columns for improved separation, and the use of alternative ionization sources.[11] In this study, sample matrix effects were evaluated by comparing the slopes (X) of calibration curves obtained from standards prepared in solvent (neat

solution) with slopes (Y) obtained from standards prepared in the rice sample matrix. Sample matrix effect (%) can be calculated by the percentage difference between the slopes, i.e. (Y-X) × 100/X. When the percentage of the difference between the slopes of the two curves is positive, there is a signal enhancement effect, whereas a negative value indicates signal suppression effect. As shown in Table 3 and Figures 1 and 2, sample matrix effects are compound dependent. For example, some pesticides, such as acephate and propiconazole, showed signal enhancement (positive values), while others, such as chlorpyriphos and tricyclazole, showed ion suppression (negative values). As shown in Table 3, sample matrix effects for most of the pesticides studied are less than 20% and thus, calibration curves built from neat solutions could be used for their quantification without significant error according to EU regulation.[12] However, significant ion suppression effects were observed for chlorpyriphos (-55%) and tebuconazole (-18%). Therefore, to overcome matrix effects and reduce variations in analytical results, matrix-matched calibrations were used in this study for quantification of all analytes.

Method Performance

All calibration curves built from both the neat solution and rice sample matrix (matrix-matched calibration) showed good linearity (0.1 to 200 ng/mL) with correlation coefficient (R^2) larger than 0.99 (see Figures 1 and 2 for typical examples of calibration curves).

The recoveries of pesticides were evaluated by spiking the analytes to the samples at two concentration levels of 10 and 100 µg/kg, respectively. As shown in Table 3, the recoveries of analytes ranged from 70% to 120% with RSD < 20% for most of the pesticides studied.

The limits of quantification (LOQs) were determined by taking into account the signals of both quantifier and qualifier ions (S/N > 10 for both) and ensuring that the product ion ratios were within 20% tolerance windows of the expected.[12] Most of the tested pesticides have LOQs ranging from 0.5 to 20 µg/kg, which are well below the EU MRLs.

Table 2. MRM Transitions (partial list of the 213 pesticides studied).

Compound Name	Polarity	Q1 Mass	Q2 Mass	CE	EV	CCL2
Acephate	Positive	184.1	143.1	-12	25	-29
Acephate-2	Positive	184.1	125.1	-25	25	-41
Acetamiprid	Positive	223.2	126.1	-30	25	-49
Acetamiprid-2	Positive	223.2	99.1	-56	25	-73
Azoxystrobin	Positive	404.1	372.1	-18	25	-57
Azoxystrobin-2	Positive	404.1	344.1	-34	25	-71
Buprofezin	Positive	306.2	201.1	-18	25	-47
Buprofezin-2	Positive	306.2	116.2	-24	25	-52
Chlorantranilprole	Positive	484	452.8	-20	25	-66
Chlorantranilprole-2	Positive	484	285.8	-18	25	-65
Chlorpyriphos	Positive	350	198	-20	25	-53
Chlorpyriphos-2	Positive	350	97	-32	25	-64
Clothianidin	Positive	250.1	169.1	-16	25	-39
Clothianidin -2	Positive	250.1	132.2	-26	25	-48
Cumyluron	Positive	303.1	185	-20	25	-48
Cumyluron-2	Positive	303.1	125	-43	25	-69
Fenbutatin-oxide	Positive	519.3	197	-67	25	-112
Fenbutatin-oxide-2	Positive	519.3	350.9	-50	25	-97
Fenobucarb	Positive	208	152	-12	25	-32
Fenobucarb-2	Positive	208	95	-19	25	-38
Fluopyram	Positive	397	173	-35	25	-71
Fluopyram-2	Positive	397	145	-70	25	-103
Halofenozide	Positive	331.1	275	-18	25	-49
Halofenozide-2	Positive	331.1	104.9	-25	25	-56
Imazalil	Positive	297.1	201	-25	25	-52
Imazalil-2	Positive	297.1	159.2	-31	25	-58
Imidachloprid	Positive	256.2	175.2	-26	25	-49
Imidachloprid-2	Positive	256.2	209	-18	25	-42
Isoprothiolane	Positive	291.1	231	-16	25	-44
Isoprothiolane-2	Positive	291.1	189	-28	25	-54
Malathion	Positive	331.1	127.1	-22	25	-53
Malathion-2	Positive	331.1	99.1	-24	25	-55
Methamidophos	Positive	142	124.9	-20	25	-32
Methamidophos-2	Positive	142	94.1	-20	25	-32
Piperonyl butoxide	Positive	356.2	177	-13	25	-47
Piperonyl butoxide-2	Positive	356.2	119	-37	25	-69
Pirimiphos-methyl	Positive	306.1	164.1	-28	25	-56
Pirimiphos-methyl-2	Positive	306.1	108.1	-40	25	-67
Profenophos	Positive	375	304.8	-50	25	-75
Profenophos-2	Positive	375	346.8	-42	25	-113
Propiconazole	Positive	342.1	159.1	-42	25	-72
Propiconazole-2	Positive	342.1	69.1	-26	25	-58
Tebuconazole	Positive	308	70	-30	25	-58
Tebuconazole-2	Positive	308	125	-50	25	-76
Thiamethoxam	Positive	292	181	-28	25	-54
Thiamethoxam-2	Positive	292	211	-18	25	-45
Triazophos	Positive	314.1	161.9	-22	25	-51
Triazophos-2	Positive	314.1	118.9	-50	25	-76
Tricyclazole	Positive	190	163	-28	25	-44
Tricyclazole-2	Positive	190	136	-36	25	-51
Trifloxystrobin	Positive	409	186	-26	25	-64
Trifloxystrobin-2	Positive	409	206	-20	25	-59
Fludioxonil	Negative	246.6	125.9	40	-25	60
Fludioxonil-2	Negative	246.6	179.9	39	-25	60

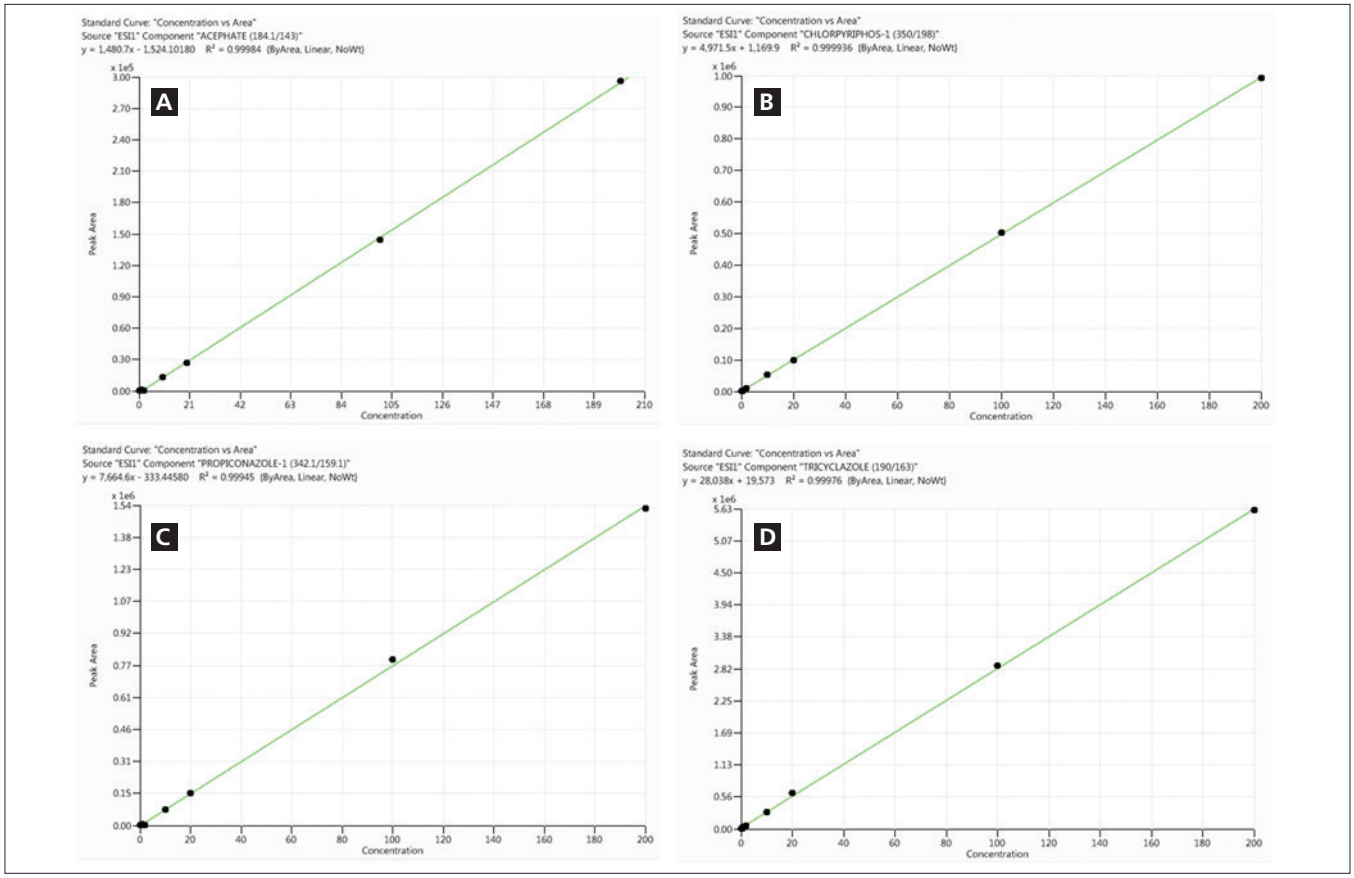


Figure 1. Calibration curves for acephate (A), chlorpyriphos (B), propiconazole (C) and tricyclazole (D) obtained from standards prepared in neat solutions (analyte concentrations range from 0.1 to 200 ng/mL).

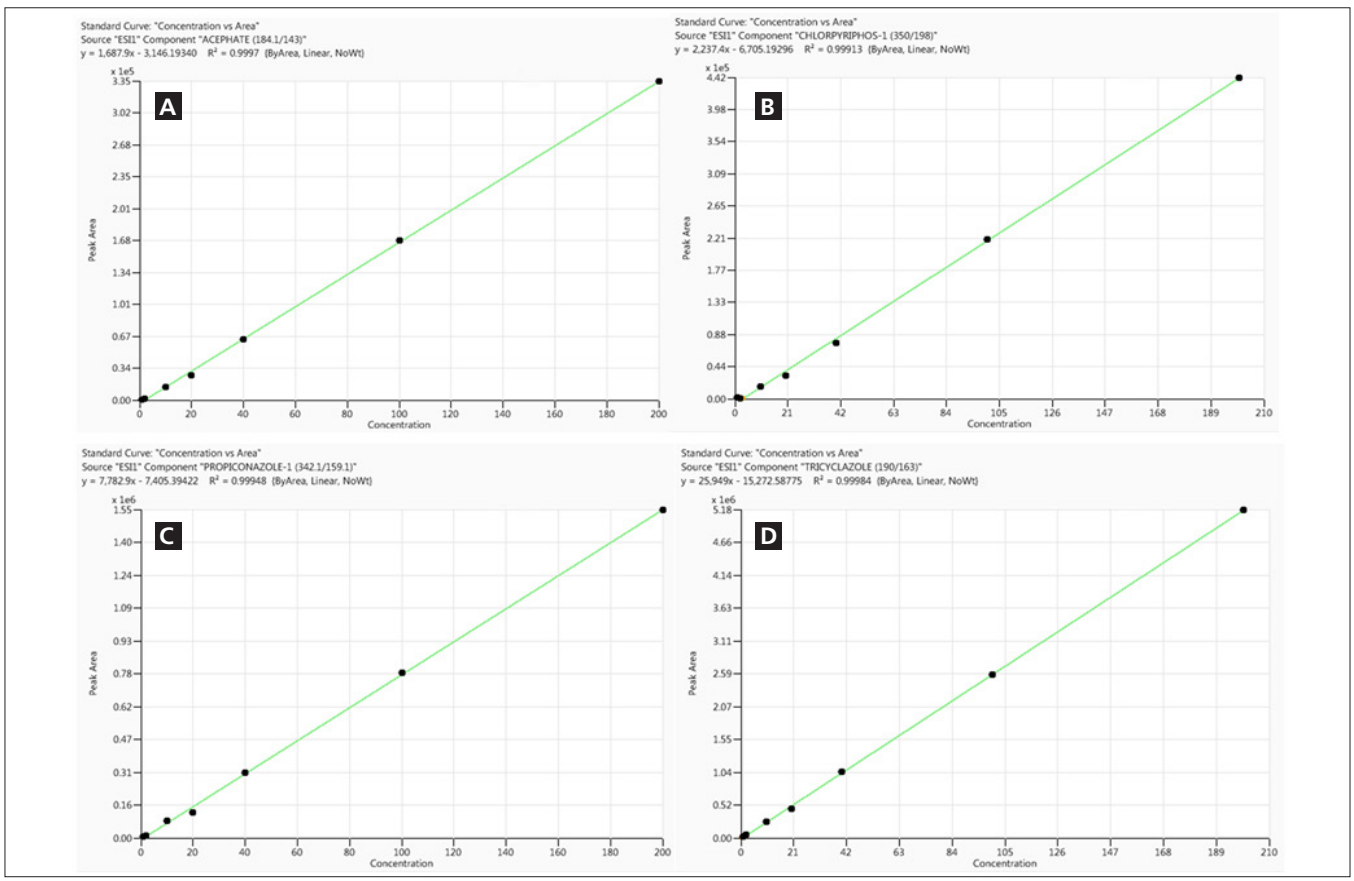


Figure 2 . Calibration curves for acephate (A), chlorpyriphos (B), propiconazole (C) and tricyclazole (D) obtained from standards prepared in rice sample matrix (analyte concentrations range from 0.1 to 200 ng/mL).

Table 3. Results of retention time, recovery, reproducibility (%RSD), matrix effect and linearity for the most commonly detected pesticides in rice samples.

Pesticide	Retention Time (min)	% Recovery(%RSD) at 10 µg/kg	% Recovery (%RSD) at 100µg/kg	Matrix Effect (%)	Correlation Coefficient (R^2)
Acephate	1.88	101.1 (11.8)	81.9 (4.3)	14.0	0.9997
Acetamiprid	8.15	106.5 (2.6)	98.7 (2.3)	2.7	0.9996
Buprofezin	15.05	103.3 (2.9)	98.8 (3.5)	-3.1	0.9996
Chlorpyriphos	15.54	109.6 (10.4)	98.7 (5.0)	-55.0	0.9991
Clothianidin	6.70	105.7 (5.9)	111.2 (8.6)	17.0	0.9995
Cumyluron	12.74	98.9 (7.2)	96.1 (2.5)	-2.6	0.9984
Fenbutatin-oxide	16.90	69.5 (18.6)	78.8 (12.7)	13.1	0.9997
Fenobucarb	11.20	101.6 (2.9)	94.8 (1.9)	2.6	0.9976
Fluopyram	13.00	104.8 (3.6)	101.1 (3.1)	-2.7	0.9991
Halofenozide	12.26	89.4 (15.2)	88.3 (11.4)	-4.4	0.9980
Imazalil	14.33	89.6 (13.6)	95.3 (4.1)	-6.1	0.9996
Imidacloprid	7.57	77.5 (10.8)	112.2 (7.9)	-5.7	0.9991
Isoprothiolane	13.01	111.5 (2.7)	101.1 (2.3)	-0.4	0.9983
Malathion	13.25	92.0 (12.0)	86.0 (4.3)	-9.9	0.9995
Methamidophos	1.41	82.8 (10.1)	76.4 (14.3)	13.3	0.9978
Piperonyl Butoxide	15.26	106.0 (5.0)	105.2 (3.4)	-6.3	0.9977
Pirimiphos-methyl	14.71	107.5 (3.7)	98.8 (5.3)	-0.1	0.9997
Profenophos	14.82	110.7 (6.9)	103.0 (6.5)	-2.5	0.9988
Propiconazole	14.32	106.6 (7.1)	98.3 (2.8)	1.5	0.9994
Tebuconazole	13.72	102.2 (6.9)	104.2 (5.5)	-18.9	0.9993
Thiamethoxam	6.43	116.4 (10.0)	114.0 (14.9)	1.9	0.9991
Triazophos	13.46	117.8 (5.7)	99.5(3.0)	2.7	0.9979
Tricyclazole	9.27	84.2 (5.8)	80.7 (7.8)	-7.5	0.9998
Trifloxystrobin	14.91	106.7 (2.4)	106(4)	-5.8	0.9991

Sample Analysis

The developed method was applied for the analysis of pesticide residues in different food samples, including eleven rice samples; one wheat sample and one veggie straw sample. Figure 3 showed the overlapped MRM chromatograms of pesticides identified and quantified from a brown rice sample. Table 4 lists the pesticide residues determined in the eleven rice samples and the EU MRLs in µg/kg (NA*; some pesticides that are not included in the EU MRLs list were also determined by this method). As shown in Table 4, many of the pesticides identified from sample 4 (S4) and sample 10 (S10) are quite similar because these two rice samples were produced from the same region, which indicates that pesticides applied to rice crops during production are country or region specific due to the regulation and weather conditions in that region.

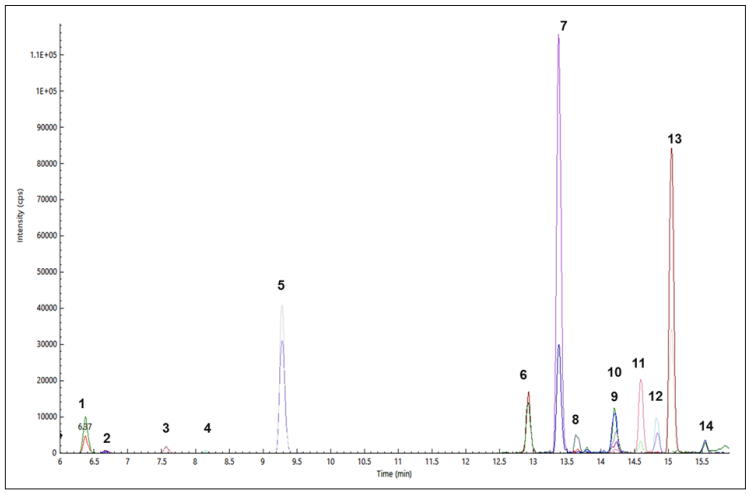


Figure 3. Pesticides determined from a brown rice sample (S10): thiamethoxam (1), clothianidin (2), imidacloprid (3), acetamiprid (4), tricyclazole (5), isoprothiolane (6), triazophos (7), tebuconazole (8), imazalil (9), propiconazole (10), profenophos (11), trifloxystrobin (12), buprofezin (13), and chlorpyriphos (14).

Conclusion

A LC/MS/MS method for multi- residue pesticides analysis in rice was developed by coupling a UHPLC system to a QSight 220 triple-quad mass spectrometer. The method can be applied for the analysis of over 200 pesticides in rice with LOQs well below the limits set by regulatory agencies. The time-managed-MRM module has simplified the creation of MS method with optimum dwell time for monitoring a large number of analytes in food samples. The QuEChERS sample extraction utilized in this study demonstrated good recovery (70-120%) and reproducibility (RSD <20%) for most pesticides. The developed method showed excellent linearity with $R^2 > 0.99$ for all the studied pesticides in rice matrix. A number of pesticide residues were identified and quantified from eleven rice samples with concentrations at or below the EU MRLs. This LC/MS/MS method has also been applied for other food analyses such as wheat and veggie strews samples with good performance. The method presented here can be easily adapted for multi-analyte screening and quantification, providing a single method for more cost-effective analysis of pesticides in rice and other food samples.

Table 4. Pesticide residues determined from eleven rice samples (S1 to S11), in µg/kg.

Pesticide	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	MRL
Acephate				2.0								10
Acetamiprid							0.3			0.8		10
Buprofezin				9.1						46.5		500
Chlorpyriphos				0.5	0.3		1.4			8.7		50
Clothianidin				7.0						3.0		500
Fenobucarb				4.1								NA*
Fluopyram					0.5							10
Halofenozide						5.0						NA*
Imazalil	1.4			2.5		4.6				2.6	1.6	50
Imidacloprid				2.8			1.1			9.2		1500
Isoprothiolane				4.4	9.3		2.9			14.7		5000
Malathion							1.8		2.2			8000
Methamidophos				0.5								10
Piperonyl Butoxide		0.6	1.3					0.8				NA*
Pirimiphos-methyl			1.4									500
Profenophos										5.2		10
Propiconazole				8.3	8.4	6.7	4.1			18.1		1500
Tebuconazole				5.9	5.2		0.9			12.0		1000
Thiamethoxam				10.6						11.0		10
Triazophos				0.6			0.5			17.6		20
Tricyclazole			16.4	5.8	7.6	20.6	0.6			40.2		1000
Trifloxystrobin										1.6		5000

NA*: pesticides not listed in the EU MRLs database, but can be determined by this method.

References

1. Commission Regulation (EC) 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin, J. Eur. Union. L70/1 (2005).
2. U.S. Environmental Protection Agency, Electronic code of federal regulation: Title 40: part 180-tolerance and exemptions for pesticide chemical residues in Food. http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr180_main_02.tpl
3. China National Standard GB 28260-2011. 2011. Maximum residue limits for 85 pesticides in food, Ministry of Health of the People's Republic of China.
4. X. Hou, M. Han, X. Dai, X-F. Yang and S. Yi, A multi- residue method for the determination of 124 pesticides in rice by modified QuEChERS extraction and GC-MS/MS. Food Chemistry, 2013, 138, 1198-1205.
5. M. Kirchner, E. Matisova, S. Hrouzkova, and J. D. Zeeuw, Possibilities and limitations of quadrupole mass spectrometric detector in fast gas chromatography. J. Chromatogr. A, 2005, 1090 (1-2), 126–132.
6. J. Wu, Quantitative Method for the Analysis of Tobacco-Specific Nitrosamines in Cigarette Tobacco and Mainstream Cigarette Smoke by Use of Isotope Dilution Liquid Chromatography Tandem Mass Spectrometry, Anal. Chem., 2008, 80 (4), 1341–1345.
7. K. Zhang, M.R. Schaab, G. Southwood, E.R. Tor, L.S. Aston, W. Song, B. Eitzer, S. Majumdar, T. Lapainus, H. Mai, K. Tran, A. El-Demerdash, V. Vega, Yanxuan Cai, J.W. Wong, A.J. Krynitsky, and T.H. Begley, A Collaborative Study: Determination of Mycotoxins in Corn, Peanut Butter, and Wheat Flour Using Stable Isotope Dilution Assay (SIDA) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS), J. Agric. Food Chem. 2017, 65 (33), 7138-7152.
8. A. Wilkowska and M. Biziuk, Determination of pesticide residues in food matrices using the QuEChERS methodology, Food Chemistry, 2011, 125, 803-812.
9. L. Pareja, A.R. Fernandez-Alba, V. Cesio and H. Heinzen, Analytical methods for pesticide residues in rice, Trends in Anal. Chem. 2011, 30 (2), 270-291.
10. L. Pareja, V. Cesio, H. Heinzen and A.R. Fernandez-Alba, Evaluation of various QuEChERS based methods for the analysis of herbicides and other commonly used pesticides in polished rice by LC-MS/MS, Talanta, 2011, 83, 1613-1622.
11. A. J. Krynitsky, J. W. Wong, K. Zhang and H. Safarpour, Focus on Food Analysis: Important considerations regarding matrix effects when developing reliable analytical residue methods using mass spectrometry, LCGC North America, 2017,Vol. 35, No. 7, 444-451.
12. European Commission, SANCO. 2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed, SANTE/11945/2015 https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_11945.pdf.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

013845_01 PKI

APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

Authors:

Li-Zhong Yang, Zhuo Man, Xiangdong Zhou

PerkinElmer, Inc.
Shanghai, China

Feng Qin

PerkinElmer, Inc.
Bolton, Canada

Direct Analysis of Glyphosate and Similar Polar Pesticides in Oatmeal by UHPLC-MS/MS

Introduction

Glyphosate (N-(phosphonomethyl) glycine), an organophosphorus compound, is used to kill weeds (e.g. annual broadleaf weeds and grasses) that compete with crops. Since its introduction to market approximately 40 years ago, glyphosate has become one of the world's most widely used herbicides due to its relatively low toxicity in comparison with other herbicides towards mammals. The adoption of glyphosate by farmers intensified after the introduction of genetically engineered "glyphosate tolerant" crops, such as corn and soybeans, that can withstand glyphosate treatment unlike the weeds the herbicide is meant to destroy. Like other pesticides, glyphosate is directly administered to food products and can come in contact with both food workers and the environment, resulting in the bio burden of exposure in uncontrolled regional populations. As a registered herbicide product under a number of regulatory organizations, glyphosate has been considered nontoxic with minimal risk to human health with persistent exposure at trace levels. However, recent toxicity evaluations by different organizations have put glyphosate at the center of a dispute. The World Health Organization's (WHO) International Agency for Research on Cancer classified it as "probably carcinogenic to humans" in March of 2015[1]. However, in November of 2015, the European Food Safety Authority (EFSA) published a report claiming that there was no scientific evidence linking glyphosate to cancer[2].

PerkinElmer®
For the Better

Independent of the dispute in the scientific community, federal regulations have been established by food authorities in several countries. The typical maximum residual level for glyphosate is between 0.05 to 500 mg/kg, but may vary depending on the food commodity.

Glyphosate is a very polar compound with high solubility in water and low solubility in most organic solvents. These properties mean that these compounds do not retain well on conventional C18 LC columns and non-polar GC columns. Therefore, the derivatization with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) is a common procedure to improve extraction and separation of glyphosate and other related compounds with LC and GC based methods. These methods based on derivatization are labor-intensive, time-consuming and less reproducible.

There is a growing need to develop a method for analysis of glyphosate and other related polar compounds without derivatization. Recently, the EU Reference Laboratories (EURL) published two methods that can directly analyze glyphosate (GLY), its metabolite, aminomethylphosphonic acid (AMPA), and glufosinate (GLU) without derivatization. One method used an ion exchange column with a long run time (23 min), while the second method utilized a Hypercarb column, which requires a special priming/reconditioning procedure and showed significant chromatographic peak tailing[3]. Our study reports a 12 minute LC/MS/MS method with an amino-based column to analyze glyphosate and other related polar compounds in underivatized states, with exceptional selectivity and sensitivity.

Experimental

A PerkinElmer Altus® A-30 UPLC® system was used with a PerkinElmer QSight™ 210 triple quadrupole mass spectrometer. Instrument control, data acquisition and processing was performed using the PerkinElmer Simplicity 3Q™ software.

The LC method conditions are provided in Table 1.

Table 1. LC method.

Column	Shodex NH2P-50 2D column, 2.0 x 150 mm, 5 µm
Mobile Phase	A: 5 mM ammonium acetate (pH11.0) in water; B: acetonitrile
Flow Rate	0.25 mL/min.
Oven Temp.	35 °C
Injection Volume	10 µL
Gradient Conditions	

Time(min)	Mobile Phase	
	A (%)	B (%)
0.00	20	80
2.00	20	80
2.01	80	20
8.00	80	20
8.01	20	80
12.00	20	80

The mass spectrometer was equipped with an electrospray ionization source operating in negative ion mode. The mass spectrometer source conditions are shown in Table 2:

Table 2. Mass spectrometer source conditions.

Parameter	Setting
Dry Gas	150
Nebulizer Gas	220
Heating Gas Temp	500 °C
Electrospray Voltage	-4500 V

MRM settings for each analyte were optimized by infusing neat standard solutions. The parameters for each analyte's MRM transition are listed in Table 3. The dwell time for each MRM was set at 30 ms.

Table 3. Optimized MRM settings.

Compound	Transitions m/z	EV /V	CE /eV
(GLY)	167.6/62.9*	-19	35
	167.6/149.6		14
(AMPA)	109.7/63.0*	-27	-30
	109.7/78.9		37
(GLU)	179.6/63.0*	-20	53
	179.6/84.9		27

** Quantifier ion*

Sample Preparation

1.0 g of oatmeal sample was weighed into a centrifuge tube, 10 mL of water/ acetonitrile (V/V, 2/1) was added to the tube and the mixture was then shaken/vortexed for one minute, ultra-sonicated for 15 minutes and centrifuged for five minutes at 6000 rpm. The recovered supernatant was filtered through a 0.22 µm nylon membrane filter for LC/MS/MS analysis. To avoid possible interaction between analytes and glass surfaces, plastic sample vials were used during the analysis and samples were analyzed immediately after preparation.

Standards Calibration Solutions

Matrix matched calibration standards were prepared by adding different levels of analytes (5.0, 10.0, 100.0, 200.0 and 500.0 ng/mL, respectively) in oatmeal matrix extract.

Results and Discussion

Figure 1 shows typical MRM chromatograms for the three analytes spiked to 10 ng/mL (0.1 mg/kg) in oatmeal extract. All three analytes were well retained on the column and showed good peak shape and signal to noise. GLY and AMPA were eluted at very similar retention times due to their similar chemical structure. GLU was baseline separated from the other analytes. No matrix interferences, which can affect peak integration, were observed.

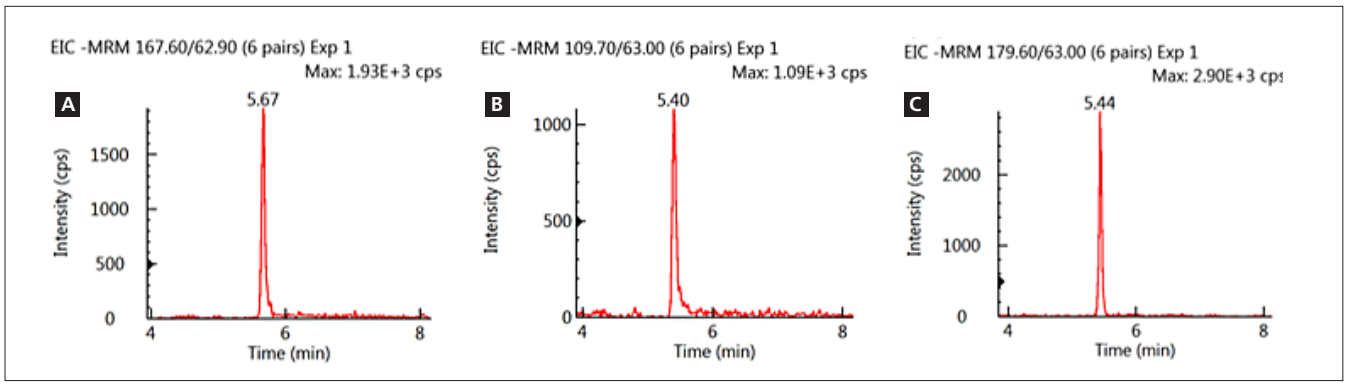


Figure 1. MRM Chromatograms of GLY (A), AMPA (B), and GLU (C) spiked at 10 ng/ml in oatmeal extract.

It is commonly known that LC/MS/MS, especially when working in ESI mode, is susceptible to matrix effects, affecting quantitational accuracy. In this study, signal intensities of standards in neat solution were compared with those of standards in matrix-matched solution at different concentration levels to calculate matrix effects (ME). An ME value of less than 100% indicates matrix suppression, whereas an ME value larger than 100% indicates matrix enhancement. As seen in Table 4, both GLY and AMPA show matrix suppression, while GLU shows matrix enhancement. Using matrix-matched standards, one can often compensate for matrix effects, which may allow for good quantitational accuracy without the use of internal standards. Therefore, calibration curves were generated by running matrix-matched calibration standards as described in the experimental section.

Figure 2 shows the calibration curves for GLY, AMPA and GLU. Good linear correlation coefficients ($R^2 \geq 0.997$) were obtained between concentrations of 5 to 500 ng/mL (0.05-5 mg/kg in real sample). For the 5 ng/mL calibrant, the signal-to-noise ratios (S/N) for GLY, AMPA and GLU were 432, 165, and 325, respectively. From these values, the limits of quantitation (LOQs; S/N ≥ 10) were calculated to be 0.12, 0.30 and 0.15 ng/mL. As the EU has set the maximum residue limit (MRL) for glyphosate in oatmeal at 20 mg/kg, the method developed in this study easily meets this requirement.

Table 4. Matrix effect result in oatmeal matrix.

Compound	GLY	AMPA	GLU
Matrix effect (%)	67.3	87.3	107.6

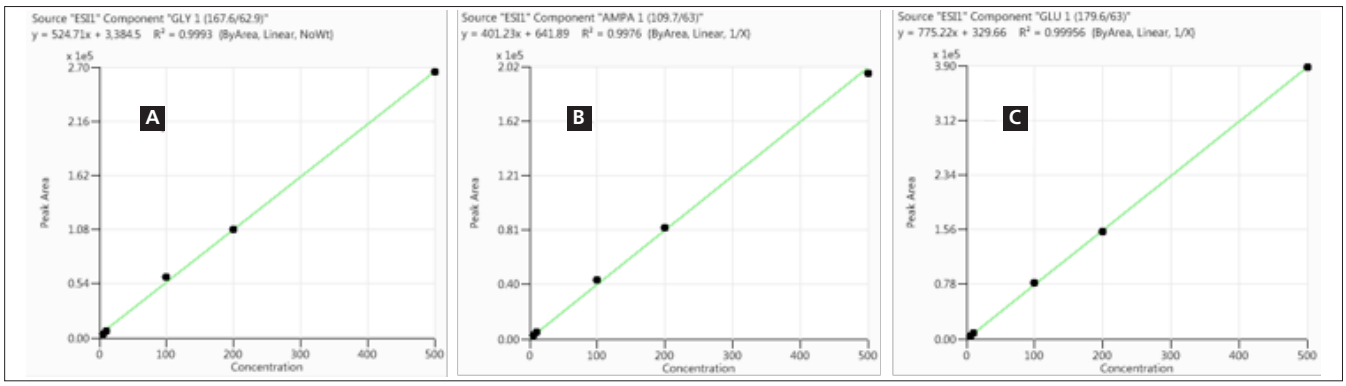


Figure 2. Calibration curves for GLY (A), AMPA (B) and GLU (C) in oatmeal extract, respectively

Table 5. Linear dynamic range, regression coefficients, LOQ and S/N at LOQ level for analytes.

Compound	Range (ng/mL)	R^2	S/N at 5 ng/mL	LOQ in matrix (S/N ≥ 10) Ng/mL
GLY	5-500	0.999	432	0.12
AMPA	5-500	0.997	165	0.30
GLU	5-500	0.999	325	0.15

Recovery of the analytes was evaluated at concentrations of 0.05 and 1 mg/kg. All recoveries were satisfactory, with mean values ranging from 85% to 130%, and relative standard deviations less than 13% for all three analytes (Table 6).

Table 6. Recovery of the analytes from oatmeal sample at different concentration levels.

Compound	Spiked Level (50 µg/kg)		Spiked Level (1 mg/kg)	
	Recovery /%	Recovery /%	Recovery /%	RSD/%
GLY	118	8.74	85	6.91
AMPA	130	11.8	94	8.72
GLU	122	12.9	96	1.93

Conclusion

In this study, we reported a rapid, sensitive and reliable 12 min LC/MS/MS method that allowed direct analysis of GLY, AMPA, and GLU in oatmeal without derivatization. The sample preparation method was a simple water/acetonitrile extraction, which showed good recoveries and minimal matrix effects for all three compounds. The calibration curves for three analytes exhibited good linearity over three orders of magnitude with calibration fit of R^2 greater than 0.997. The LOQs for glyphosate and other related polar compounds were much lower than the EU's MRL of 20 mg/kg in oatmeal.

References

1. http://monographs.iarc.fr/ENG/Monographs/vol112/mono112-09.pdf. Accessed on Aug 2nd, 2016
2. Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA Journal 2015; 13(11):4302.
3. Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction with Methanol (QuPPe-Method). http://www.crl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf. Accessed on Aug 2nd, 2016

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

013031_01 PKI

APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

Authors:

Josh Ye, Feng Qin, Frank Kero, Craig Young, Jason Weisenseel, Jamie Foss

PerkinElmer, Inc.
Downers Grove, IL

Matteo Maglioli. Ph.D.
Quebec, Canada

"No Dilute" Just Shoot: Robustness of a QSight LC-ESI-MS/MS for Low Level Pesticide Residue Analysis in Wine

Introduction

Traditional analysis by chromatography and mass spectrometry often requires sample cleanup to minimize matrix effects and to avoid contamination of the ion source in the mass spectrometer. However, sample preparation is usually labor intensive and requires trained analysts with specialized skills. Strategies to redesign the front end of mass spectrometers to minimize source contamination and thereby avoid the need for extensive sample cleanup, led to the invention of a hot surface induced desolvation (HSID™) interface[1]. The PerkinElmer QSight™ LC/MS/MS mass spectrometer contains the HSID interface coupled to a Laminar flow ion guide™, both of which prevent accumulation of contamination along the ion path making it a very sensitive and maintenance free instrument.

In this study, we used the QSight LC/MS/MS system to evaluate the potential of eliminating sample preparation for trace level pesticide analysis in a complex sample such as wine[2]. We injected undiluted red and white wine samples into the mass spectrometer and studied reproducibility in analysis of the spiked pesticides over 200 injections. The instrument showed excellent reproducibility with minimal signal drift during the duration of the study (over a week), confirming the robustness of the QSight mass spectrometer.

PerkinElmer®
For the Better

What is the HSID Interface and How Does it Work?

The HSID apparatus is a multiorthogonal channel interface directly heated up to 300 °C that is present immediately after the sampling orifice in the source and connects the orifice to the Laminar flow ion guide of the QSight mass spectrometer. Unlike traditionally designed interfaces, the HSID with its multi-channels orthogonal to each other (Fig. 1) produces turbulent and Laminar flow and disrupts the free jet expansion of the sample ions. The orthogonal channels prevent neutrals from entering the mass spectrometer reducing chemical noise, and any solvated charged clusters entering the HSID are entrained and desolvated in the hot flow of gas, further contributing to reduction in chemical noise.

The ions from the HSID interface are gently transferred by gas flow to the Laminar flow ion guide™, which is not subject to the traditional axial fields, but is at zero potential. The ion guide has multiple pumping stages to generate several pressure regions from the sample interface to the mass analyzer. In these regions, pressure gradually drops, creating a well-defined flow pattern along the ion path enabling ions to be gently extracted into the analyzer. Both the HSID and laminar flow ion guide prevent accumulation of contamination along the ion path making the QSight maintenance free. Among many benefits of the HSID interface include high sensitivity due to an inherent reduction in chemical background (i.e. S/N, reduced N) and the ability to perform analysis at high LC flow rate (3 mL/min) without reduction in signal.

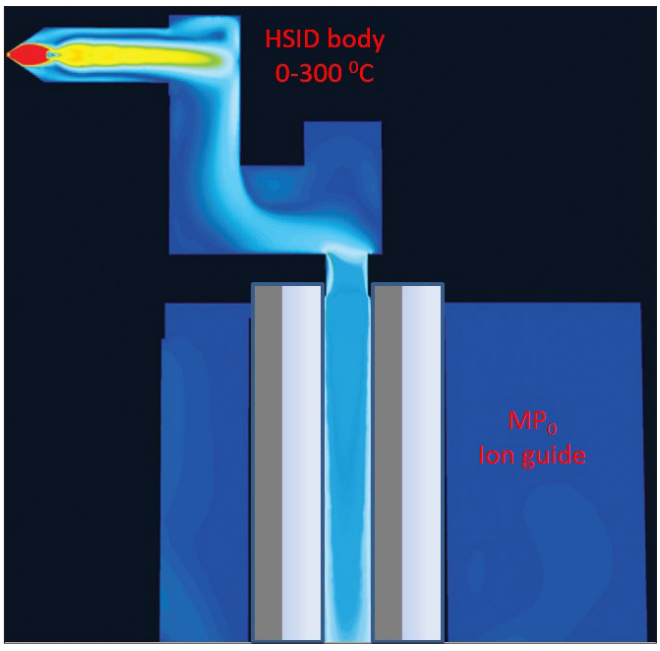


Figure 1. Schematic of an HSID interface for laminar flow tandem mass spectrometry.

Experimental

Hardware/Software

Chromatographic separation was conducted by a PerkinElmer Altus® A-30 UPLC® System and detection was achieved using a PerkinElmer QSight 220 MS/MS detector with a dual ionization source. All instrument control, data acquisition and data processing were performed using the Simplicity 3Q™ software platform. The mobile phase flow rate was at 0.5 mL/min.

Method Parameters

MS settings are shown in Table 1 and Table 2, respectively. Source parameters including gas flows, source temperature and position settings were optimized for maximum sensitivity. The quadrupole peak widths (Q1 and Q2) were set at 0.7 amu. Example compound-dependent parameters for the partial list of MRM transitions are listed in Table 3.

Table 1. MS source settings.

ESI voltage	5000 V
Drying gas	120
HSID Temp	200 °C
Entrance voltage	30 V
Source Temp	325 °C
Nebulizer gas	350
Detection Mode	MRM Mode

Table 2. Optimized compound-dependent parameters for selected pesticides.

Name	Precursor	Fragment	Type	Collision Energy
Dimethenamid	276.1	244.0	Quantifier	18
Dimethenamid	276.1	168.0	Qualifier	30
Benthiavalicarb-isopropyl	382.1	180.0	Quantifier	38
Benthiavalicarb-isopropyl	382.1	197.0	Qualifier	24
Pyriproxyfen	322.0	96.0	Quantifier	22
Pyriproxyfen	322.0	185.0	Qualifier	30

Standards and Samples

Argentina wine samples, including a bottle of Cabernet Sauvignon (Cabernet) and a bottle of Pinot Grigio (Pinot), were purchased from a local grocery store. The samples were fortified to 10 ng/mL of pesticide mixed standards obtained from ULTRA Scientific® (North Kingstown, RI). Then, 10 µL of spiked samples (no further sample preparation and no dilution) were injected for robustness and reproducibility analysis. Linearity and LOQs of the analytes were also evaluated. Matrix-matched standards were prepared at 0.1, 1, 10, and 100 ng/mL levels by diluting the pesticide mix standards stock solution with blank Cabernet and Pinto wine samples.

Results and Discussion

Robustness Study

Commercial wine samples (Pinot and Cabernet) were fortified to 10 ng/mL with a pesticides mixed standard. These fortified samples were then tested with 200 repeat injections over the course of 7 days. Three pesticides (Dimethenamid, Benthiavalicarb-isopropyl and Pyriproxyfen) were selected to demonstrate the stability of the system. Summarized plot of peak area versus injection number for these analytes in spiked pinot and cabernet are presented in Figure 2. As observed, the trace remains flat across the 200 injections, and peak area reproducibility (CV, calculated as relative standard deviation) was ~5%, indicating excellent performance stability during analysis.

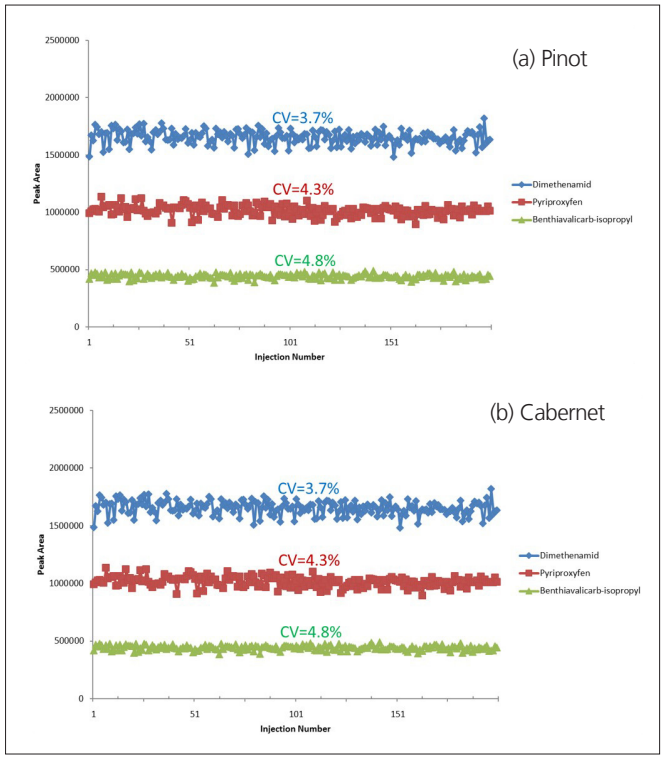


Figure 2. Peak area of three pesticides vs injection number for spiked (a) Pinot Grigio and (b) Cabernet Sauvignon samples.

Comparison of Calibration Curves Before and After 200 Injection Study

Matrix-matched representative calibration curves for quantitative and qualitative ions for Benthiavalicarb-isopropyl, Dimethenamid, and Pyriproxyfen after the wine injection study was performed are shown in Figure 3 (Pinto) and 4 (Cabernet). The selected pesticides have a linear dynamic range of 0.5 to 100 ng/mL and are identical to the calibration curves generated prior to wine injections. Linear regression coefficients were obtained for all the pesticides with R^2 >0.992. There is no maximum residue limit (MRL) of pesticides set for wine yet, according to the EU regulation. However, the limit of quantitation (LOQ) at level of low part per billion or less is considered sufficient.

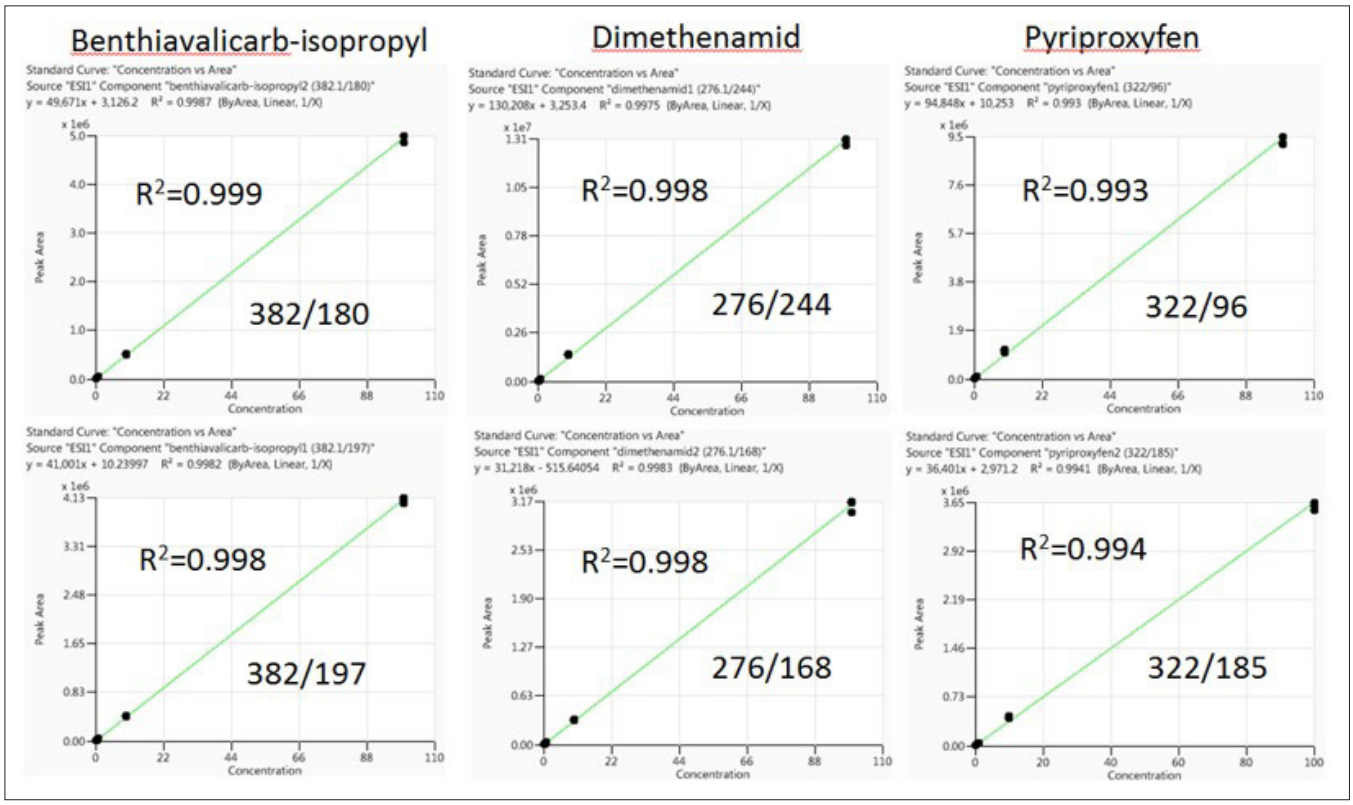


Figure 3. Pinot matrix-matched calibration curves for Benthiavalicarb-isopropyl, Dimethenamid, and Pyriproxyfen.

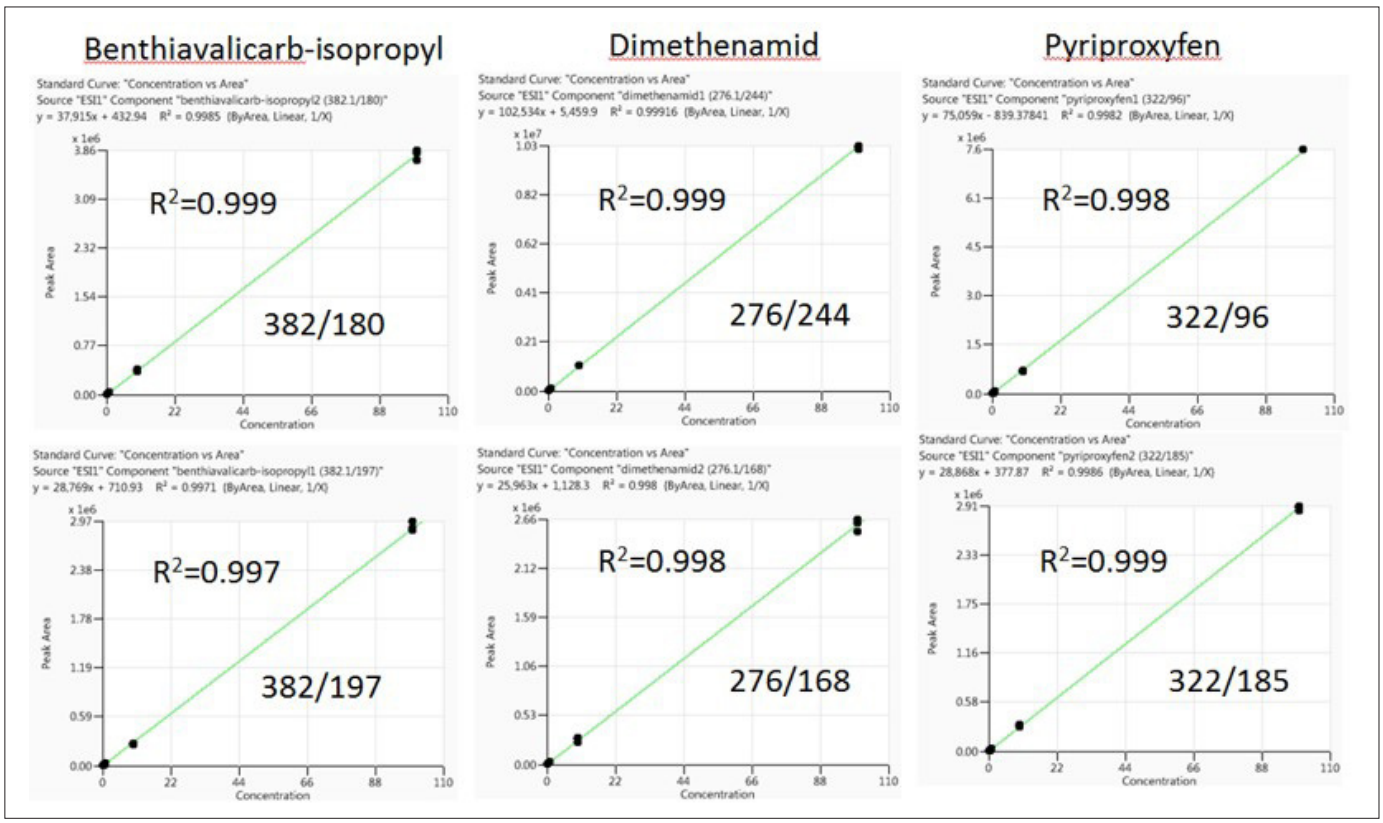


Figure 4. Carbernet matrix-matched calibration curves for Benthiavalicarb-isopropyl, Dimethenamid, and Pyriproxyfen.

Conclusion

A "no-dilute-just-shoot" approach was presented in this study to demonstrate the advantages of an HSID interface on a PerkinElmer QSight 220 LC/MS/MS system. The orthogonal design of the interface, and the turbulent and laminar flows used for ion transportation provide maximum protection for the MS instrument from being contaminated. Over the two matrix variables x200 continuous injections of wine samples without any cleanup steps, the instrument performance remained consistent. Peak area CVs are ~5%, and peak shape and height are nearly the same. Linearity and LOQs of the calibration curves for all analytes using matrix-matched standards was found to be maintained after >200 injections. This study suggests that it is possible to increase the lab productivity and reduce cost on QSight LC/MS/MS system by injecting samples that are prepared with minimal cleanup steps.

References

1. Flow Characteristics of a Laminar Flow Interface for LC-MS/MS. PerkinElmer Tech Note 0129893.
2. Meglioli M, Kero F, Ye J, Young C, Reddy S "No dilute" just shoot LC-ESI-MS/MS : feasibility and robustness of a maintenance-free source for applications in low-level pesticide residue analysis "The Proceedings of the 64th ASMS Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, June 2016.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

013032_01 PKI

APPLICATION NOTE

Liquid Chromatography/
Mass Spectrometry

Authors:
Josh Ye, PhD
Feng Qin, PhD
Sharanya Reddy, PhD
Frank Kero, PhD

PerkinElmer, Inc.
Waltham, MA

Analysis of Target Pesticide Residues in Berries with LC/MS/MS Coupled with a QuEChERS Sample Preparation

Introduction

Pesticides are widely used in agriculture to protect plants from a variety of pests and to increase productivity. However, the extensive use of pesticides can pose a health risk to humans and this has led to worldwide stringent regulations, for maximum allowable limits for these residues in foods. Among the routinely used testing methods, LC/MS/MS has become the method of choice, due to its high sensitivity, reliability and accuracy.

In the present study, a unique laminar flow UPLC-ESI-MS/MS triple quad mass spectrometer was used to identify and quantitate 40 pesticides in four brands of non-organic berries. The QuEChERs extraction method proved both rapid and reliable for extracting pesticide residues in the heavily pigmented berry samples.

PerkinElmer®
For the Better

Experimental

Hardware/Software

Chromatographic separation was conducted by a PerkinElmer Altus® A-30 UPLC® System and detection was achieved using a PerkinElmer QSight™ 220 MS/MS detector with dual ionization source. All instrument control, data acquisition and data processing was performed using the Simplicity 3Q™ software platform.

Method parameters

The LC method and MS source parameters are shown in Table 1.

Table 1. LC Method and General MS Conditions.

LC Method

Column: PerkinElmer Brownlee Phenyl-Hexyl column, 2.7 µm, 2.1 x 100 mm

Mobile Phase: Solvent A: 5 mM ammonium formate in water

Solvent B: 5 mM ammonium formate in methanol

	Time (min)	%A	%B	Flow rate (ml/min)
1	Initial	90	10	0.3
2	1	90	10	0.3
3	15	5	95	0.3
4	17	5	95	0.3
5	17.1	90	10	0.3
6	20	90	10	0.3

Oven Temp.: 40 °C

Injection Volume: 20 µL

General MS Conditions	
ESI voltage:	5000 V
Drying gas:	120
HSID Temp:	200 °C
Entrance voltage:	30 V
Source Temp:	325 °C
Nebulizer gas:	350
Detection Mode:	MRM Mode

Solvents, Standards and Sample Preparation

Berry samples were obtained from a local grocery store in Ontario, Canada. Pesticide standards were obtained from ULTRA Scientific® (North Kingstown, RI). All solvents, reagents and diluents used were HPLC grade.

Samples were prepared using Supra-d™ QuEChERS kits (AOAC 2007.01 method). Briefly, samples were homogenized using a blender at high speed, and 10 g of homogenized samples were weighed and transferred to a 50 mL extraction tube containing 6 g of $MgSO_4$ and 1.5 g of sodium acetate. 10 mL of cold acetonitrile was then added and vortexed until the salt was completely mixed. The solution was then centrifuged at 3500 rpm for 5 minutes. 4 mL of supernatant was then transferred to a 15 mL clean-up tube (AOAC 2007.01 Clean-up Kits), vortexed for 3 minutes and centrifuged for 5 minutes.

0.1 mL of supernatant was transferred to a 1.5-mL centrifuge tube, diluted 10-fold with mobile phase A and then centrifuged at 4000 rpm for 5 minutes. The resulting supernatant was transferred to a 1.5 mL LC vial for direct LC/MS/MS analysis.

Optimizing MS/MS Parameters

Source parameters, including gas flows, source temperature and position settings, were optimized to achieve the best sensitivity. The Q1 and Q2 quadrupole peak widths were set at 0.7 amu. Multiple reaction monitoring mode (MRM) transitions are listed in Table 2.

Table 2. Optimized compound-dependent MS parameters for tested pesticides (partial list).

Compound	Precursor Ion	Product 1	CE1	Product 2	CE2
Atrazine	216.1	174.1	15	132.0	20
Azoxystrobin	404.1	372.1	18	344.1	34
Bifenazate	301.1	198.0	16	170.0	20
Boscalid	343.0	307.0	25	140.0	28
Cyprodinil	226.0	93.0	48	108.0	34
Flonicamid	230.1	203.1	20	174.0	20
Hexythiazox	353.0	228.0	20	168.0	34
Pyraclostrobin	388.0	194.0	16	163.0	36
Pyrimethanil	200.0	107.0	33	82.0	32
Thiamethoxam	292.0	211.0	18	181.1	28

Results and Discussion

Figure 1 shows example chromatograms of the pesticides analyzed in MRM mode at 1 ng/mL. All of the tested pesticides were detected with good signal to noise even at concentrations well below the regulatory limits.

The method showed excellent linearity ($R^2 \geq 0.99$) over three orders of concentration (0.1-100 ng/mL for most analytes). Some of the calibration curves are shown in Figure 2.

The limit of quantification (LOQ) was 0.1 ng/mL for most of the analytes, which is well below the regulatory limits of 10 ng/mL.

Recoveries of the pesticides were determined by spiking 10 µg/kg of pesticides in three different berry samples (raspberry, blackberry and blueberry) in triplicates. Both the mean recovery and reproducibility (RSD) were determined for each berry/analyte combination. The recoveries were between 70 and 115% with a RSD of <20% for the berry/analyte combinations.

Berry samples bought in local grocery stores were tested for pesticide residues using the developed method. Figures 3-5 show the chromatograms of berry samples with positive hits for the target pesticides. The calculated concentrations of those pesticides are listed in Table 3, which ranged from 4.5 to 447.3 µg/kg.

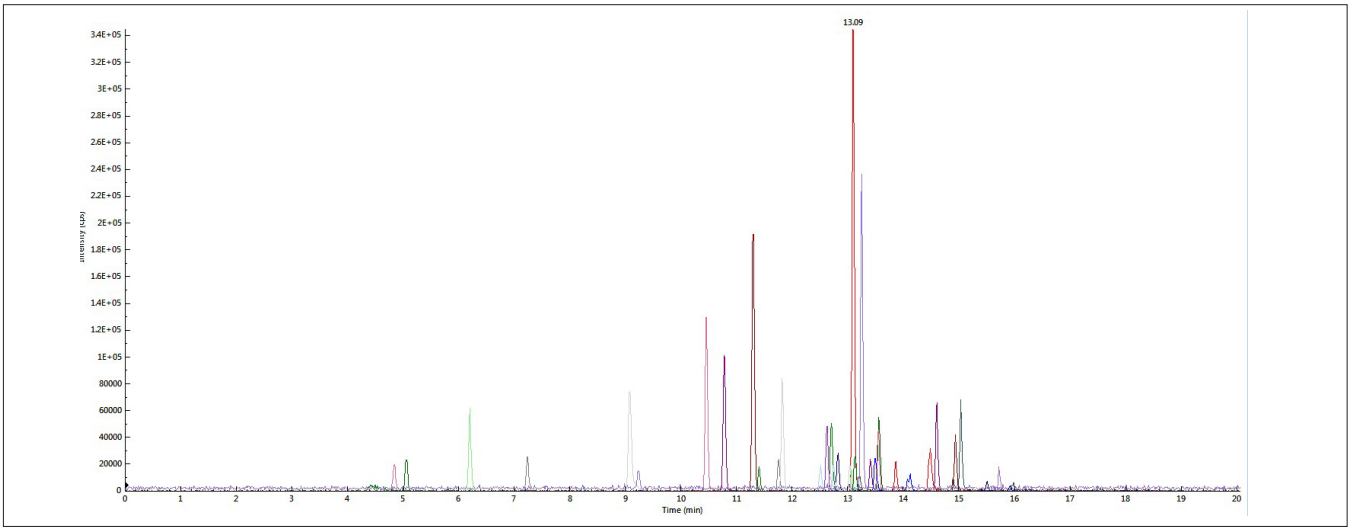


Figure 1. MRM chromatogram of pesticides at 10 ng/mL in neat solution.

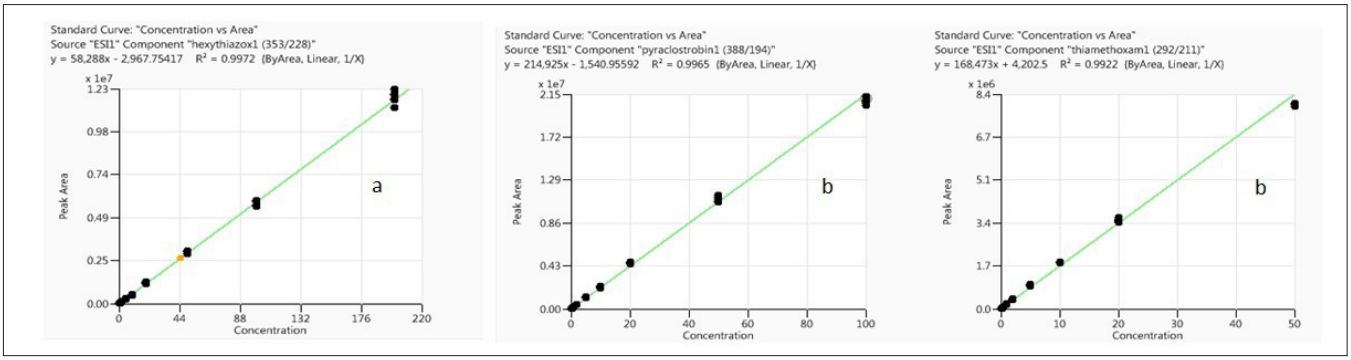


Figure 2. Calibration curves for hexythiazox (a), pyrochlostrobin (b), and thiamethoxam (c) with 6 injections at each concentration level (ng/mL).

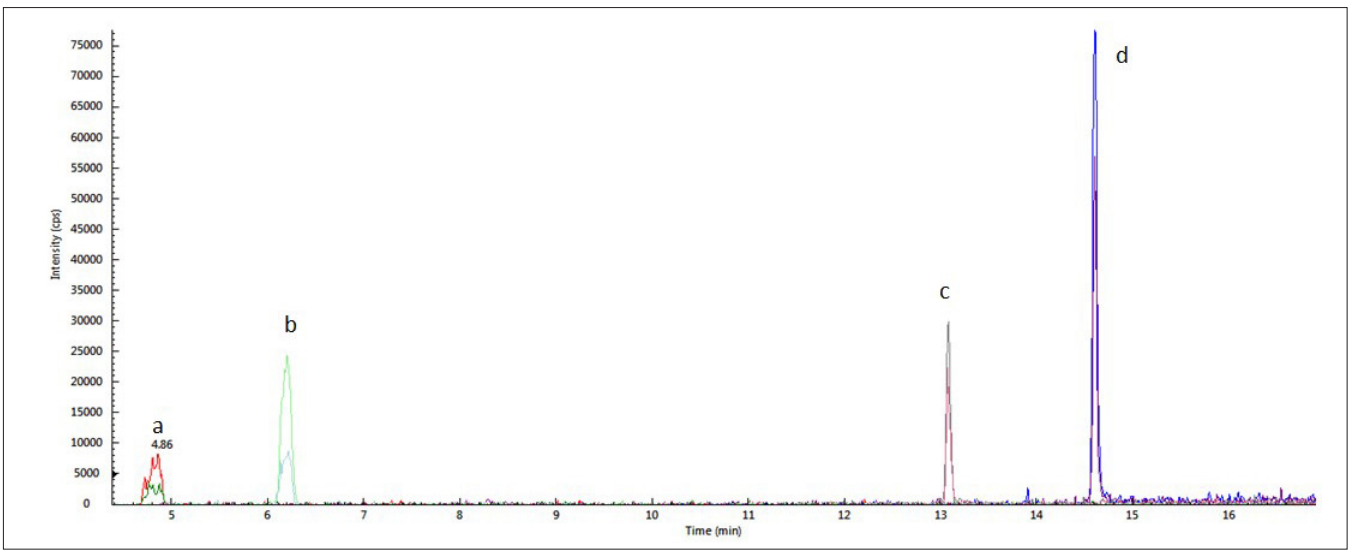


Figure 3. Pesticides identified and quantified from brand A blueberry. The pesticides are flonicamide (a), thiamethoxam (b), pyrimethanil (c), and bifenazate (d).

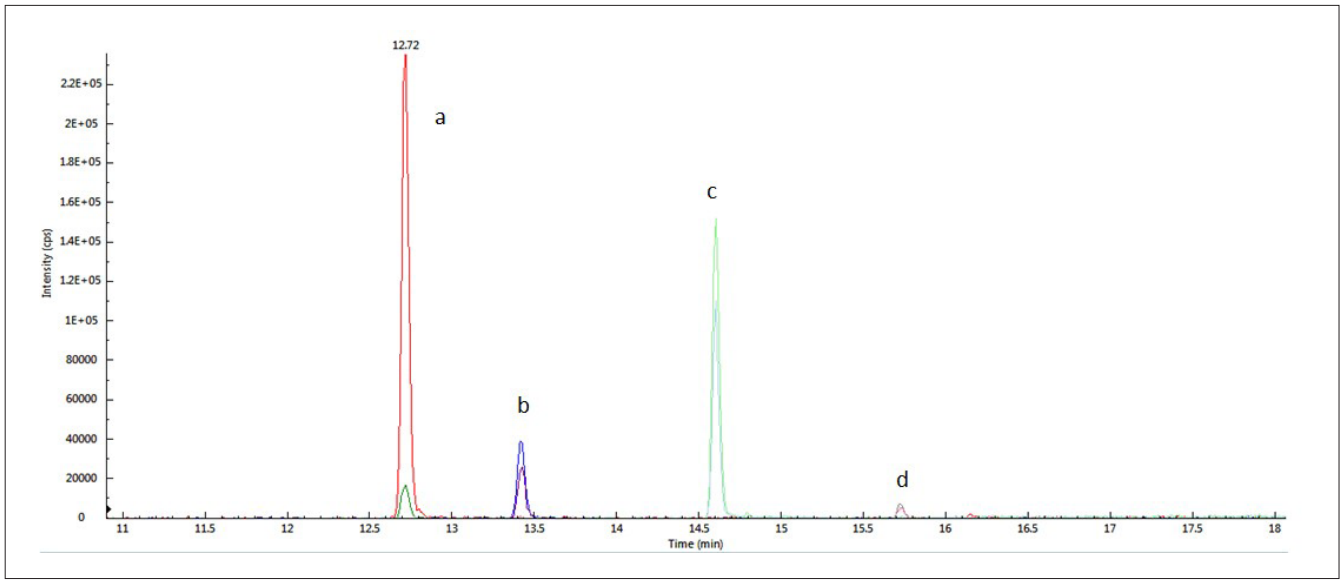


Figure 4. Pesticides identified and quantified from brand B blueberry. The pesticides are boscalide (a), cyprodinil (b), pyrochlostrobin (c), and hexythiazox (d).

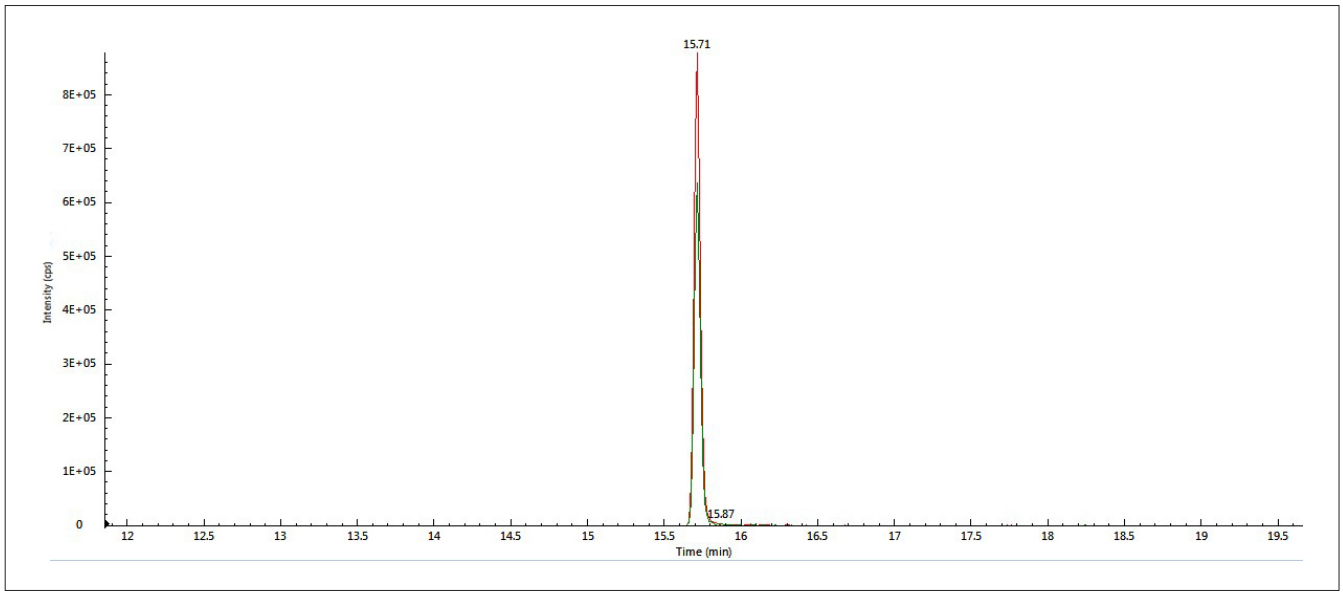


Figure 5. Pesticides identified and quantified from brand D blackberry. Only hexythiazox was detected.

Table 3. Summary of Pesticides found in berry samples.

	Pesticide	Concentration (µg/kg)
Blueberry (Brand A)	Pyraclostrobin	11.8
	Flonicamid	9.7
	Bifenazate	12.6
	Thiamethoxam	9.5
	Pyrimethanil	11.8
Blueberry (Brand B)	Cyprodinil	16.8
	Pyraclostrobin	23.2
	Boscalid	51.1
	Hexythiazox	4.5
Blueberry (Brand C)	N/A	--
Blackberry (Brand D)	Hexythiazox	447.3

Conclusions

A LC/MS/MS method for multi-pesticide residue analysis in berries was developed using a PerkinElmer Altus UPLC® system coupled to a QSight 220 triple-quad mass spectrometer.

The simple/routine sample preparation approach used in this work provides the following advantages: 1. dilution of the QuEChERS extract with water makes the sample extract more compatible with typical reversed phase separation, leading to reduced solvent effects; 2. dilution also helps to reduce any potential matrix effects, leading to more accurate and reproducible results.

These results demonstrated this methods applicability and effectiveness in detecting and quantitating pesticides lower than 10 parts per billion (ppb), per regulatory limits set by the EU directive 91/414/EEC.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

013026_01 PKI

APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

Authors:

Wilhad M. Reuter

Avinash Dalmia

PerkinElmer, Inc.
Shelton, CT

Analysis of Mycotoxins in Multi-Grain and Corn Cereals without Derivatization by LC-MS/MS Using Time-Managed MRMs

Introduction

Mycotoxins in feed, crops and various foods are known to pose a serious health hazard to both livestock and humans. They are produced by fungi as toxic secondary metabolites, with grains, maize and cereals being particularly vulnerable. With this in mind, and considering that an estimated 25% of all crops show some signs of mycotoxin contamination, many countries have established regulatory guidelines for maximum mycotoxin limits in not only feed and grain, but also in processed food products, notably cereal and baby food.

The current global regulatory limits for mycotoxins in processed cereals are shown in Table 1. The most demanding of these are for mycotoxin B1 (2 µg/kg maximum in EU) and ochratoxin A (3 µg/kg maximum in EU and Singapore).

PerkinElmer®
For the Better

Table 1. Global maximum regulatory limits (µg/kg; ppb) for mycotoxins in processed cereal products intended for human consumption.[1]

Mycotoxin	EU	USA	China	Singapore	Brazil
Sum of B1, B2, G1 and G2	4	20	NA	5	5
B1 Only	2	NA	5* 20**	5	NA
Sum of T-2 and HT-2	75***	NA	NA	NA	NA
Sum of F-B1 and F-B2	800	NA	NA	NA	400
Ochratoxin A	3	NA	5*	3	10
Ergot Alkaloids	NA				

*Grain products **Corn/peanut products NA = none available at this time
***Indicative level (regulatory level under discussion)

Thereupon, the presented work details an effective, reliable and robust LC-MS/MS method, using time-managed MRM (Multiple Reaction Monitoring) transitions, for the monitoring of mycotoxins in multi-grain and corn cereals at low µg/kg levels, without the need for derivatization.

The analyzed mycotoxins included aflatoxins B1, B2, G1, G2, ochratoxin A, HT-2 and T-2 toxins, ergocristine and fumonisins F-B1 and F-B2.

Experimental

Hardware/Software

For the chromatographic separations, a PerkinElmer UHPLC System was used with a PerkinElmer QSight® 210 MS/MS detector. All instrument control, analysis and data processing was performed using the Simplicity 3Q™ software platform.

Method Parameters

The LC and MS/MS method parameters are shown in Tables 2 and 3, respectively.

Table 2. LC Method Parameters.

Column	PerkinElmer Analytical C18, 3 µm, 4.6 x 100 mm (Part# N9303863)
Mobile Phase	Solvent A: Water; 5 mM NH_4-formate and 0.1% formic acid Solvent B: 90:10 methanol/water; 5 mM NH_4-formate and 0.1% formic acid

	Time (min)	Flow Rate (mL/min)	%A	%B	Curve
1	Initial	1.00	45.00	55.00	
2	3.50	1.00	45.00	55.00	6
3	3.75	1.00	10.00	90.00	6
4	7.00	1.00	10.00	90.00	6
5	7.10	1.00	45.00	55.00	6

Analysis Time	7 min; re-equilibration time: 4 min
Pressure	6200 psi/413 bar (maximum)
Oven Temp.	35 °C
Injection Volume	50 µL

Table 3. MS/MS Method Parameters.

Ionization Mode	ESI (positive)
Drying gas (nitrogen): 120 (arbitrary units); HSID™ Temp: 320 °C; Electrospray V1: 4500 V; EV(V): 30	

Exp er. Group 1 (1.00 - 2.10 min)	MRM Transitions (amu)				
	Quantifier Ion	Qualifier Ion	CCL2(V)	CE(V)	Dwell Time (msec)
Aflatoxin G2	331.1/245.2	331.1/285.1	-100	-35	100
Exp er. Group 2 (1.90 - 3.50 min)	**MRM Transitions (amu)**				
	Quantifier Ion	**Qualifier Ion**	**CCL2(V)**	**CE(V)**	**Dwell Time (msec)**
Aflatoxin G1	329.0/243.2	329.0/283.2	-120	-30	100
Aflatoxin B2	315.1/259.2	315.1/287.2	-120	-32	100
Aflatoxin B1	313.3/285.2	313.3/241.3	-110	-30	100
Exp er. Group 3 (4.90 - 5.62 min)	**MRM Transitions (amu)**				
	Quantifier Ion	**Qualifier Ion**	**CCL2(V)**	**CE(V)**	**Dwell Time (msec)**
HT-2 Toxin	447.3/285.3	447.3/345.4	-120	-23	100
Fumonisin F-B1	722.8/352.4	722.8/334.4	-120	-47	100
Exp er. Group 4 (5.22 - 5.85 min)	**MRM Transitions (amu)**				
	Quantifier Ion	**Qualifier Ion**	**CCL2(V)**	**CE(V)**	**Dwell Time (msec)**
Ergocristine	610.6/223.3	610.6/592.6	-120	-23	50
T-2 Toxin	489.2/245.1	489.2/387.1	-115	-30	50
Ochratoxin A	404.2/239.1	404.2/358.2	-85	-25	50
Exp er. Group 5 (5.55 - 6.10 min)	**MRM Transitions (amu)**				
	Quantifier Ion	**Qualifier Ion**	**CCL2(V)**	**CE(V)**	**Dwell Time (msec)**
Fumonisin F-B2	706.8/336.5	706.8/354.3	-120	-30	50

HSID™ = Hot-surface induced desolvation
EV(V) = Entrance voltage; CCL2(V) = Collision cell lens 2; CE(V) = Collision cell energy

Standards and Sample Preparation

Standards were obtained from Sigma-Aldrich. A working standard (WS) was prepared in 80/20 ACN/water, used for both the initial calibration and as the spiked extraction solvent.

For the initial calibration (in straight solvent), a 5-level calibration set was prepared by diluting the WS 1:1 with water and then serially diluting with 40/60 ACN/water. All calibrants were run in triplicate.

Samples of multi-grain cereal (MGC) and corn cereal (CC) obtained from a local food market were analyzed for mycotoxins. 5 grams of each sample was homogenized for 45 seconds, each sample being prepared in duplicate. The homogenized samples were extracted with either 20 mL of 80/20 ACN/water (as a control) or with 20 mL of WS (serving as the spiked extraction solvent).

For extraction, all mixtures were first vortexed for 25 minutes, with intermittent hand shaking, followed by centrifugation at 3500 rpm for 10 minutes. For the calibration plots of the spiked matrix extracts, each supernatant was diluted 1:1 with water and then serially diluted with 40/60 ACN/water. For recovery testing, 5 grams of homogenized samples were spiked with 5 mL of 40/60 ACN/water containing 5 ppb aflatoxin B1 and ochratoxin A, both having the lowest regulatory limits (2 and 3 µg/kg, respectively). 5 mL of each supernatant was diluted 1:1 with water. For all extractions, the overall sample dilution was 8-fold.

All prepared calibrants and samples were filtered using 0.22-µm filters and then injected (50 µL) in triplicate on column.

Results and Discussion

Figure 1 shows the chromatography and the resulting chromatographic repeatability for 10 replicate injections.

For the calibration plots, developed in either straight solvent (40/60 ACN/water) or as matrix-matched extracts, the R^2 values for all analytes were > 0.995. A representative plot for aflatoxin B1 prepared in solvent is shown in Figure 2, while Figure 3 shows the corresponding plots for aflatoxin B1 prepared in matrix.

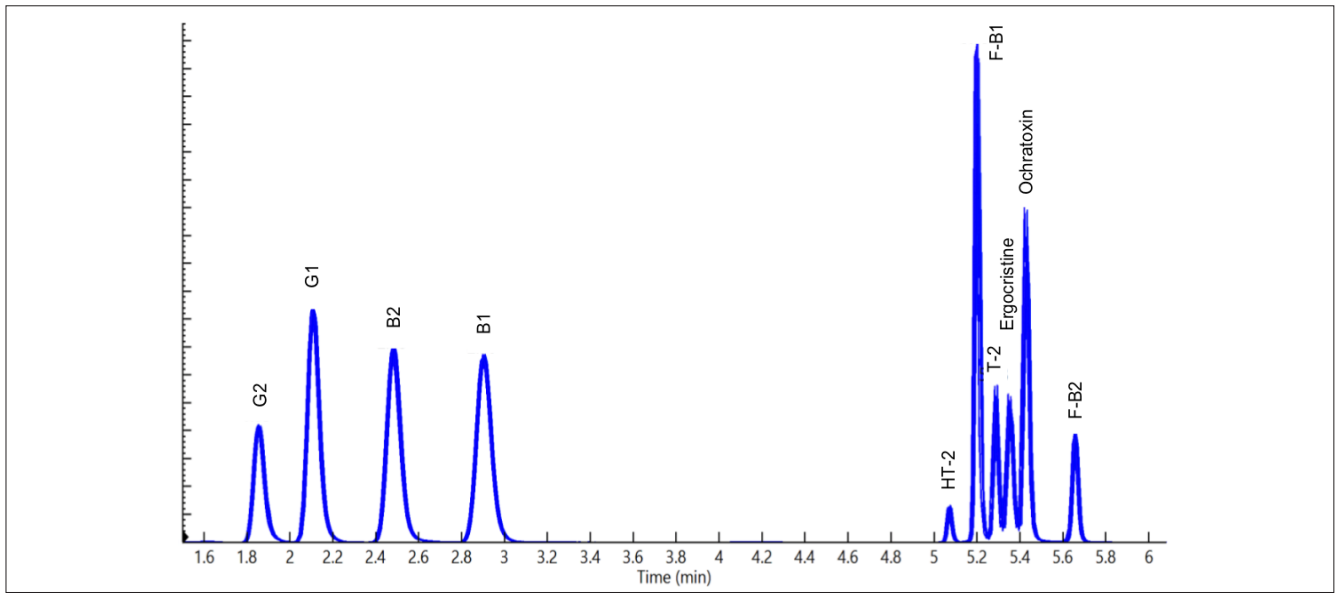
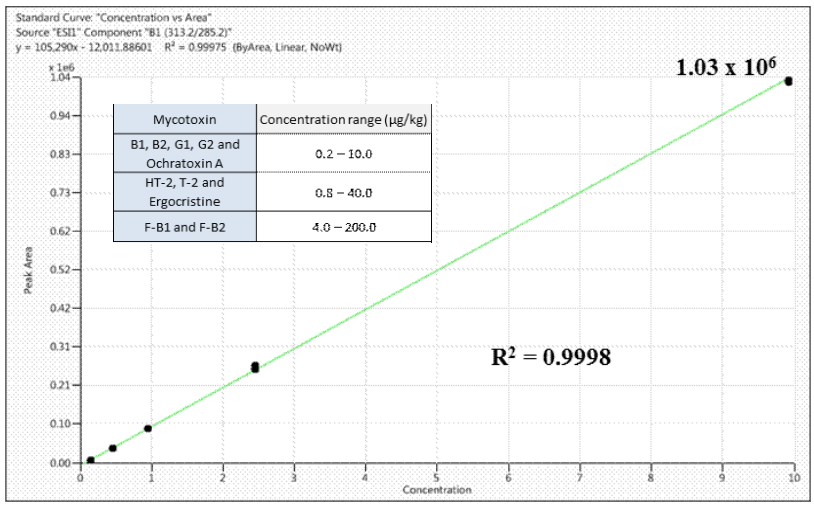


Figure 1. 10-replicate overlay of MRM quantifier transitions for all analytes.

To check for possible ion-suppression, calibration plots prepared in straight solvent were compared with the matrix-matched standards. Comparing the upper level (L5) values in Figures 2 and 3 shows an example of this for aflatoxin B1. Overall, ion suppression of 20-25% was observed from matrix effects.



Mycotoxin	Concentration range (µg/kg)
B1, B2, G1, G2 and Ochratoxin A	0.2 – 10.0
HT-2, T-2 and Ergocristine	0.8 – 40.0
F-B1 and F-B2	4.0 – 200.0

Figure 2. Calibration plot for B1 in 80/20 ACN/water. The B1 concentration range and that of all other analytes are provided in the inserted table.

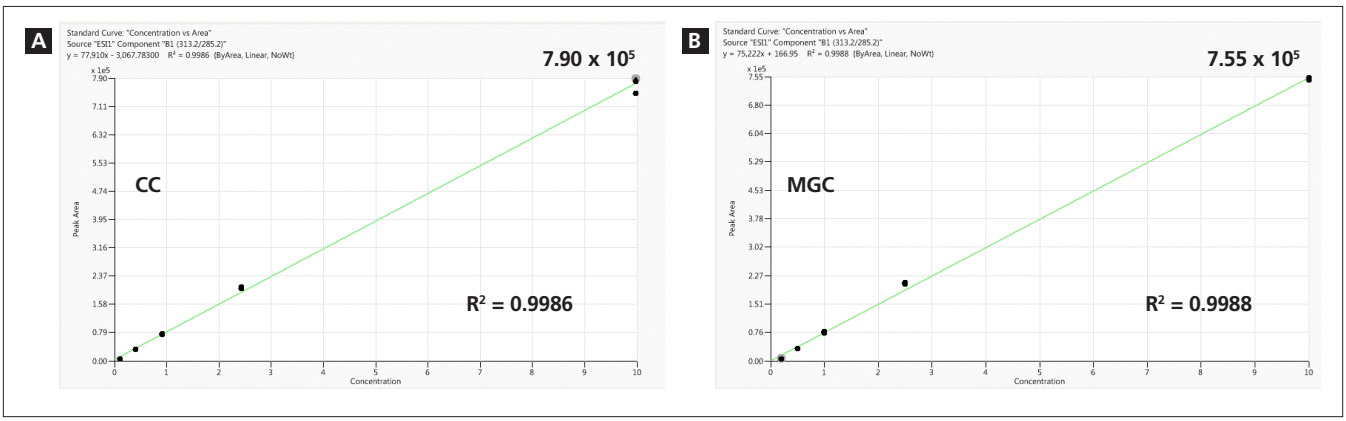


Figure 3. Calibration plots for B1 from supernatant of spiked extracts; A: CC; B: MGC.

However, the extent of ion suppression was considered inconsequential, since, per LOQ, the EU's 2-µg/kg regulatory limit for B1 was easily met. The calculated LOQ for B1, as well as those for all the other analyzed mycotoxins, are shown in Figure 4.

A 80/20 ACN/water blank injection showed no detectable mycotoxins. However, the control, obtained from injecting the supernatant from the unspiked extractions, showed quantitatable T-2 amounts for both MGC and CC (7.92 µg/kg and 13.8 µg/kg, respectively). This is chromatographically highlighted in Figure 4, showing quantitatable amounts of T-2 in the CC sample and confirmed via the qualifier MRM. However, the resulting T-2 amounts for both CC and MGC were well below the EU's regulated limit of 75 µg/kg for the sum of HT-2 and T-2.

For additional analyte ID confirmation, the qualifier/quantifier ion ratios for the lowest level CC supernatant calibration results are shown in Table 4. The asterisked analytes are the qualifiers. All analyte identities were positively confirmed.

The aflatoxin B1 and ochratoxin A recovery results for the 5-µg/kg spiked MGC homogenate are shown in Figure 5. The recoveries for both analytes were quite close to that expected. The bit higher ochratoxin A recovery was interesting but not further investigated.

Table 4. Calculated S/N and LOQ values for the eight analyzed mycotoxins.

Mycotoxin	S/N of L1 calibrant*	LOQs (µg/kg) (per L1 calibrant)**	Sample LOQs (µg/kg)*** (Considering 8-fold dilution)
G2	73	0.03	0.24
G1	122	0.02	0.16
B2	91	0.02	0.16
B1	83	0.02	0.16
HT-2	27	0.30	2.40
F-B1	44	0.91	7.28
Ergocristine	40	0.20	1.60
Ochratoxin A	75	0.03	0.24
T-2	85	0.09	0.72
F-B2	19	2.11	16.84

* Lowest replicate-averaged S/N of CC and MGC L1 calibrants via spiked extraction
** As per lowest replicate-averaged S/N
*** Calculated based on LOQs of L1 calibrants

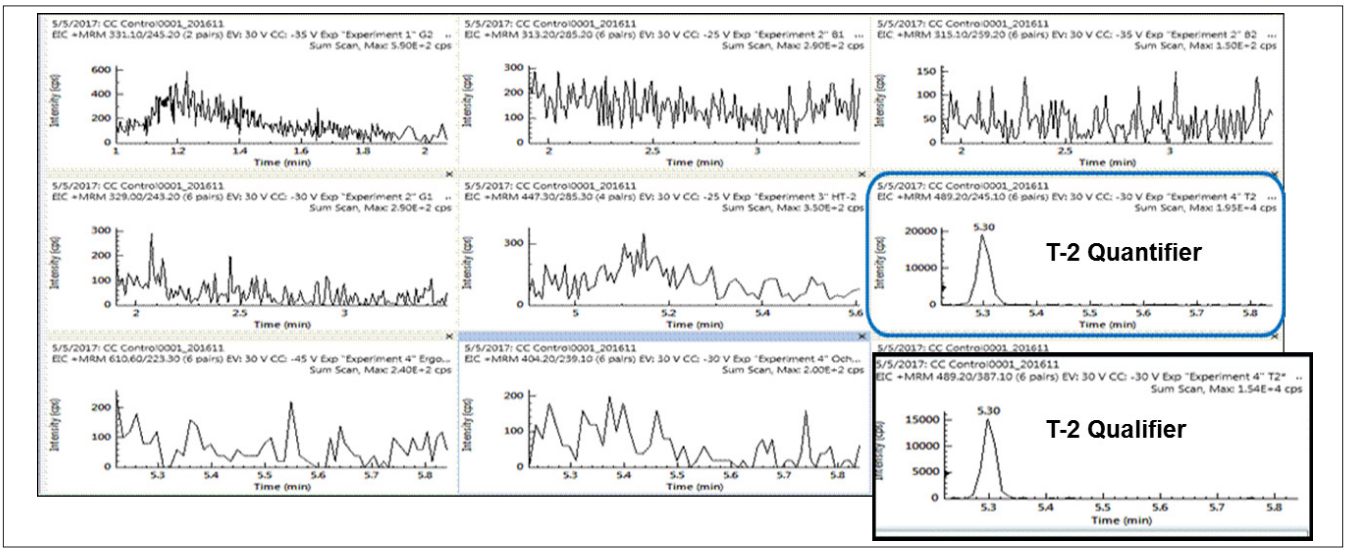


Figure 4. Quantifier MRM chromatograms of CC control, highlighting T-2 (blue); the insert at lower right shows the corresponding T-2 qualifier.

Table 5. Ion ratios for all ten analyzed mycotoxins. The green background indicates a positive match to within 20 % of ion ratio of the lowest calibration standard.

Analyte Component	Group	Mass Transition Q1/Q2	Ion Ratio Area
G2	10	331.1/245.2	-
G2*	10	331.1/285.1	0.67
B1	20	313.2/285.2	-
B1*	20	313.2/241.3	0.91
B2	30	315.1/259.2	-
B2**	30	315.1/287.2	0.92
G1	40	329/243.2	-
G1*	40	329/283.2	0.44
HT-2	50	447.3/285.3	-
HT-2*	50	447.3/345.4	0.92
F-B1	60	722.8/352.4	-
F-B1*	60	722.8/334.4	1.05
ERGOCRISTINE	70	610.6/223.3	-
ERGOCRISTINE*	70	610.6/592.6	0.43
OCHRATOXIN-A	80	404.2/239.1	-
OCHRATOXIN-A*	80	404.2/358.2	0.43
T2	90	489.2/245.1	-
T2*	90	489.2/387.1	0.80
F-B2	100	706.8/336.5	-
F-B2*	100	706.8/354.3	0.80

Mycotoxin B1 and ochratoxin A were chosen as examples herein, as they have the lowest regulatory limits of the analyzed mycotoxins.

Conclusions

- Repeatable chromatography was achieved in under seven minutes with all eight mycotoxins resolved.
- The LOQs were below the established regulatory limits in processed cereal for all analyzed mycotoxins, achieved by LC-MS/MS with time-managed MRMs, without the need for pre- or post-column derivatization.

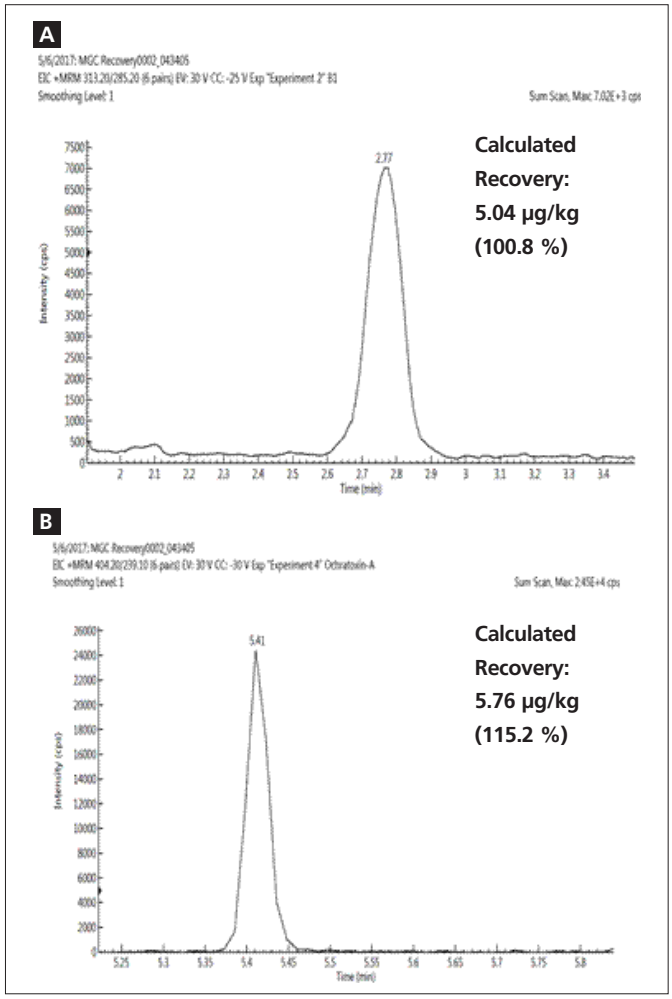


Figure 5. Chromatographic and quantitative recovery results for A) B1 and B) ochratoxin A, both spiked to 5 µg/kg in MGC.

- There was minimal ion suppression in the supernatant from the spiked extractions, allowing for simple, convenient sample preparation by liquid extraction.
- For analyte confirmation, product ion ratios could be used well below the regulatory concentration limits for all the analytes in this study.
- Though quantitatable levels of T-2 were found in both cereals, they were considerably below regulatory limits.

References

1. www.mycotoxins.info/myco_info/consum_regu.html.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

013618B_01 PKI

APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

Authors:

Mingli Zhu, Weifeng Zhang
Guangzhou Agricultural Products Quality and Safety
Guangzhou, China

Lina Tang
Xiamen Entry-Exit Inspection and Quarantine Bureau
Fujian Province, China

Lizhong Yang, Xiangdong Zhou, Chengyuan Cai, Yongming Xie
PerkinElmer, Inc.
Shanghai, China

Feng Qin , Jingcun Wu
PerkinElmer, Inc
Toronto, Canada

Simultaneous Determination of Pesticides Residues and Illegal Additives in Wine

Introduction

Wine derived from grapes can often contain pesticides and fungicides that have been sprayed on the fruits during their growing period. In addition to these contaminants, wine can also contain additives that have been deliberately added during production processes to improve its flavor and color. Both pesticides and illegal additives, if present in significant levels in wine, can pose health risk to consumers. Currently, pesticides and illegal additives are tested using different methodologies[1,2,3,4]. In this study, a simple and sensitive LC-MS/MS method has been developed and applied for the determination of both pesticides and pigments in a single analytical run.

PerkinElmer
For the Better

Experimental

Hardware/Software

Chromatographic separation was conducted on a PerkinElmer UHPLC system, while detection was achieved using a PerkinElmer QSight™ 220 triple quadrupole mass spectrometer with a dual ionization source. All instrument control, data acquisition and data processing was performed using the Simplicity 3Q™ software.

Method

Sample Preparation

1.0 mL of test sample was accurately pipetted into a centrifuge tube and then 9.0 mL of water was added and blended. After centrifugation for five minutes at 6000 rpm, the supernatant was transferred directly into an auto sampler vial without further filtration for LC-MS/MS analysis.

LC Conditions

The analytes were separated using a PerkinElmer Brownlee SPP C18 column (4.6 x 100 mm, 2.7 µm). The temperature of the column oven was set at 30 °C. The mobile phases consisted of (A) 5 mM ammonium acetate in water and (B) acetonitrile. The flow rate was 0.8 mL/min and the mobile phase gradient is shown in Table 1. The injection volume was 10 µL.

Mass Detection Parameters

The mass spectrometer source conditions are listed in Table 2, the compound dependent parameters such as collision energy (CE) and the entrance voltage (EV) were optimized for each analyte by flow injection analysis and their values are shown in Table 3, in which the values of limit of quantification (LOQ) for all the analytes determined under the optimized conditions are also listed.

Results and Discussion

A new UHPLC-MS/MS method was successfully developed for simultaneous quantification of 23 pesticides residues and nine illegal additives of pigments. As illustrated in Figure 1, all target compounds were detected with good peak shape and sensitivity. Using this method, the LOQs of the target compounds ranged from 0.5 to 50 µg/L in wine samples as shown in Table 3.

The effects of sample matrices and dilution factors on the analysis were studied during sample preparation process. Wine samples with different dilution factors (1:2, 1:5, 1:10, and 1:20) and spiked with the same amount of analytes (pigments: 100µg/L, pesticides: 10 µg/L) were analyzed and their responses were compared. As shown in Figures 2 and 3, the responses increased with the increase of the dilutions, indicating that sample matrix effects (mainly ions suppressions) could be reduced by sample dilutions. Thus, a 1:10 dilution of the sample was used in this study for wine analysis. Sample clean up treatments using PSA, C18 and GCB were also investigated for the spiked samples (pigments: 200 µg/L, pesticides: 20 µg/L) with 1:10 dilution of the samples. The results showed that better responses and recoveries were obtained for most of the analytes studied without clean-up, as shown in Figures 4 and 5. The lower responses were obtained from the samples after clean-up steps, especially from samples treated with GCB and C18, possibly because these compounds contain nonpolar components, which could be lost due to retention on the materials during clean up steps. PSA (primary secondary amines) could be used to remove sugars, fatty acids, organic acids, and anthocyanin pigments; C18 was used to remove nonpolar interferences and GCB (carbon) was used to remove pigments, sterols, and nonpolar interferences.

Table 1. Mobile phase gradient.

	Time (min)	A%	B%
1	0.0	95	5
2	3.0	60	40
3	5.0	50	50
4	8.0	20	80
5	9.0	5	95
6	11.0	5	95
7	11.1	95	5
8	13.0	95	5

Table 2. MS Source Conditions.

ESI Voltage (Positive)	5500 V
Drying Gas	70 arbitrary units
Nebulizer Gas	200 arbitrary units
Source Temperature	500 °C
HSID Temperature	320 °C
Detection Mode	Time-managed MRM™

Table 3. Optimized MRM Parameters and the Limit of Quantifications (LOQs).

No.	Analyte	MRM Transition Quantifier / MRM Transition Qualifier		RT/min	CE/eV	EV/V	LOQ/ μg/L
1	Tartrazine	468.9	451.0	1.45	-22	23	50
		468.9	200.1		-33	23	
2	New red	545.9	504.0	1.85	-20	24	50
		545.9	341.1		-34	24	
3	Acid Red-27	538.8	348.1	1.99	-41	27	50
		538.8	223.0		-37	27	
4	Carmine	538.9	158.2	2.29	-49	30	50
		538.9	223.1		-37	30	
5	Sunset Yellow	408.7	392.1	2.56	-26	25	50
		408.7	236.1		-29	25	
6	Allura Red AC	452.9	217.1	2.80	-30	17	10
		452.9	202.2		-54	17	
7	Azorubine	458.8	223.2	3.26	-34	20	10
		458.8	442.0		-22	20	
8	Brilliant Blue	749.2	306.1	3.45	-59	75	10
		749.2	171.2		-71	75	
9	Erythrosin B	836.7	583.0	3.81	-69	67	10
		836.7	329.0		-86	67	
10	Methamidophos	142.0	94.0	1.97	-11	22	10
		142.0	125.0		-18	22	
11	Thiamethoxam	292.0	211.0	3.48	-17	20	0.5
		292.0	181.0		-30	20	
12	Carbendazim	192.0	160.0	3.97	-24	28	0.5
		192.0	132.0		-40	28	
13	Dimethoate	230.0	125.0	4.09	-29	22	0.5
		230.0	199.0		-12	22	
14	Acetamiprid	223.0	126.0	4.19	-29	30	0.5
		223.0	99.0		-54	30	
15	Thiabendazole	202.2	175.2	4.40	-45	33	0.5
		202.2	131.2		-57	45	
16	Dimethomorph	388.0	301.0	6.87/7.11	-26	40	0.5
		388.0	165.0		-41	40	
17	Pyrimethanil	200.0	107.0	7.48	-32	50	5
		200.0	82.0		-32	50	
18	Fenhexamid	302.1	97.2	7.97	-32	57	5
		302.1	55.2		-71	77	
19	Azoxystrobin	404.0	372.0	7.99	-19	25	0.5
		404.0	344.0		-33	25	
20	Epoxiconazole	330.0	121.0	8.04	-22	25	0.5
		330.0	101.0		-50	25	
21	Triadimefon	294.0	197.0	8.08	-20	30	0.5
		294.0	225.0		-16	30	
22	Boscalid	343.0	307.0	8.09	-25	25	1
		343.0	140.0		-28	25	
23	Fluquinconazole	376.0	349.0	8.16	-26	25	1
		376.0	307.0		-34	25	
24	Hexaconazole	314.0	70.0	8.44	-24	25	0.5
		314.0	159.0		-36	25	
25	Imazalil	297.1	159.1	8.49	-42	30	1
		299.1	161.1		-42	30	
26	Penconazole	283.8	70.1	8.51	-23	20	0.5
		283.8	159.1		-48	20	
27	Malathion	331.0	127.0	8.51	-10	20	5
		331.0	285.0		-16	20	
28	Prochloraz	376.0	308.0	8.84	-16	20	0.5
		376.0	70.0		-37	20	
29	Cyprodynil	226.3	93.2	8.87	-51	66	0.5
		226.3	108.3		-35	56	
30	Phoxim	299.0	77.0	9.40	-46	20	1
		299.0	129.0		-18	20	
31	Trifloxystrobin	409.2	186.1	9.52	-43	31	0.5
		409.2	206.2		-33	21	
32	Chlorpyrifos	350.0	198.0	10.00	-23	25	0.5
		350.0	97.0		-47	25	

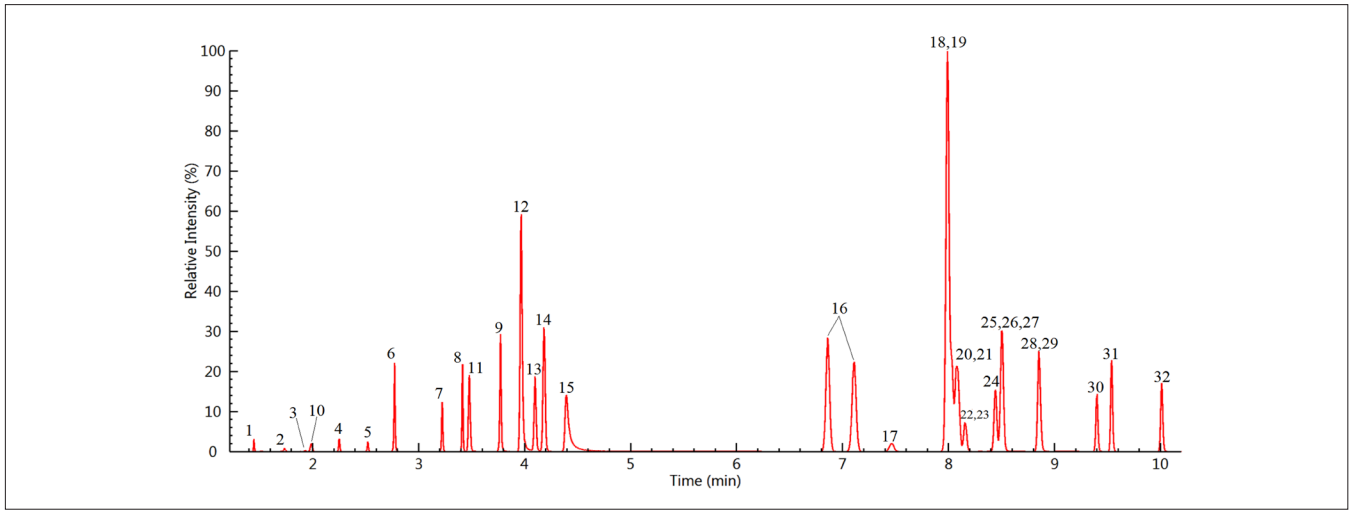


Figure 1. LC–MS/MS chromatograms of the 9 pigments (100 µg/L) and 23 pesticides (10 µg/L) spiked to a wine sample (compound names are shown in Table 3).

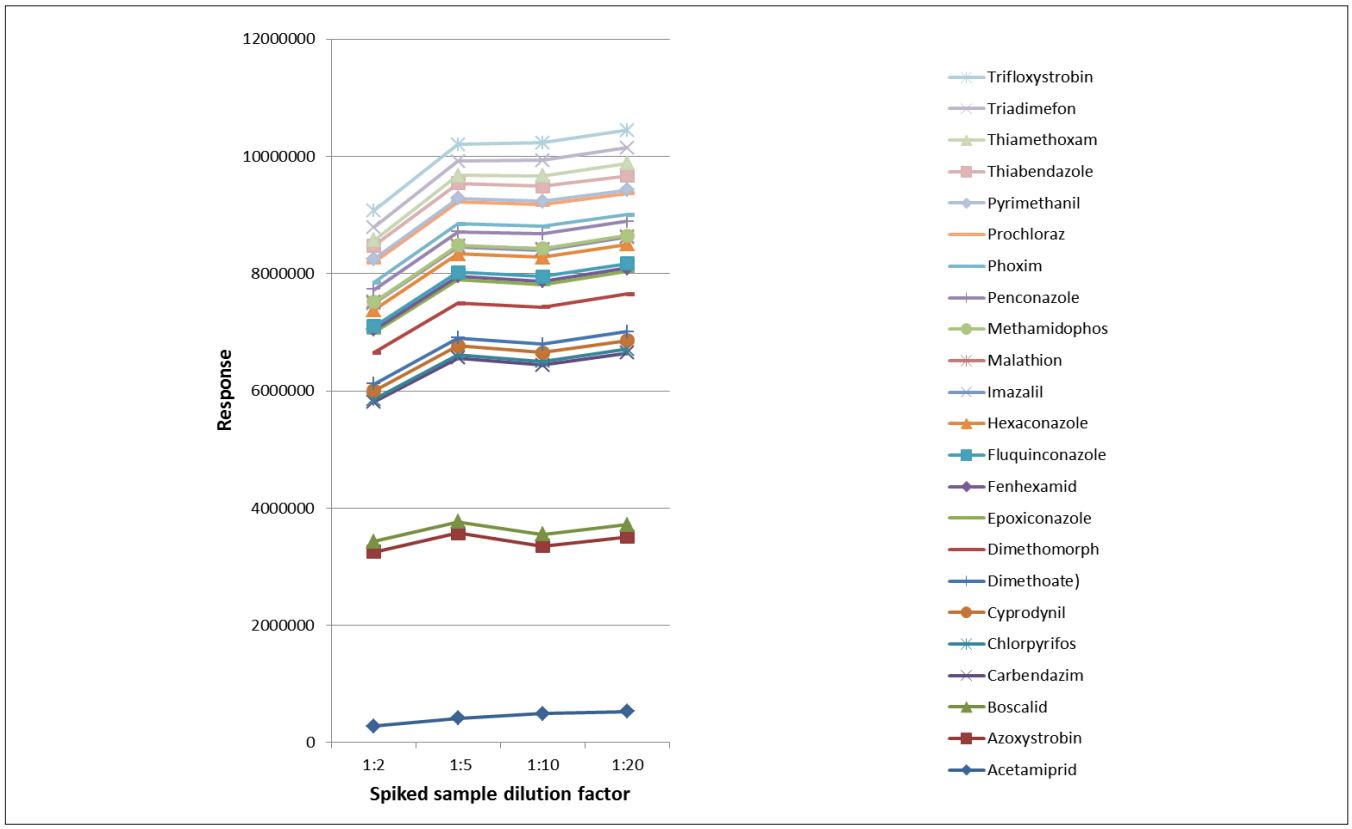


Figure 2. Result of pesticides (10µg/L) with different dilution factors.

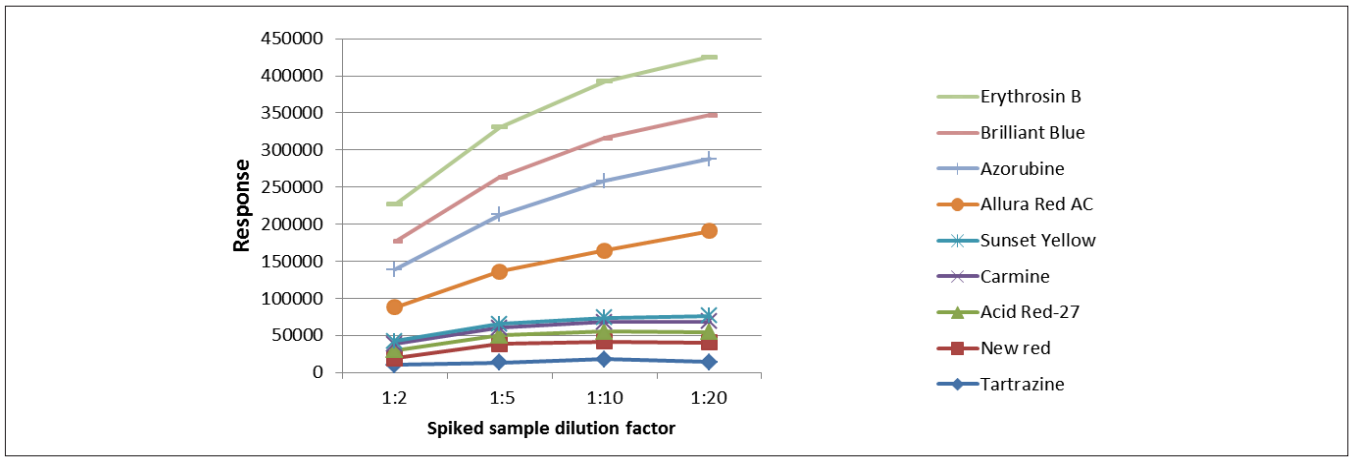


Figure 3. Result of pigments (100µg/L) with different dilution factors.

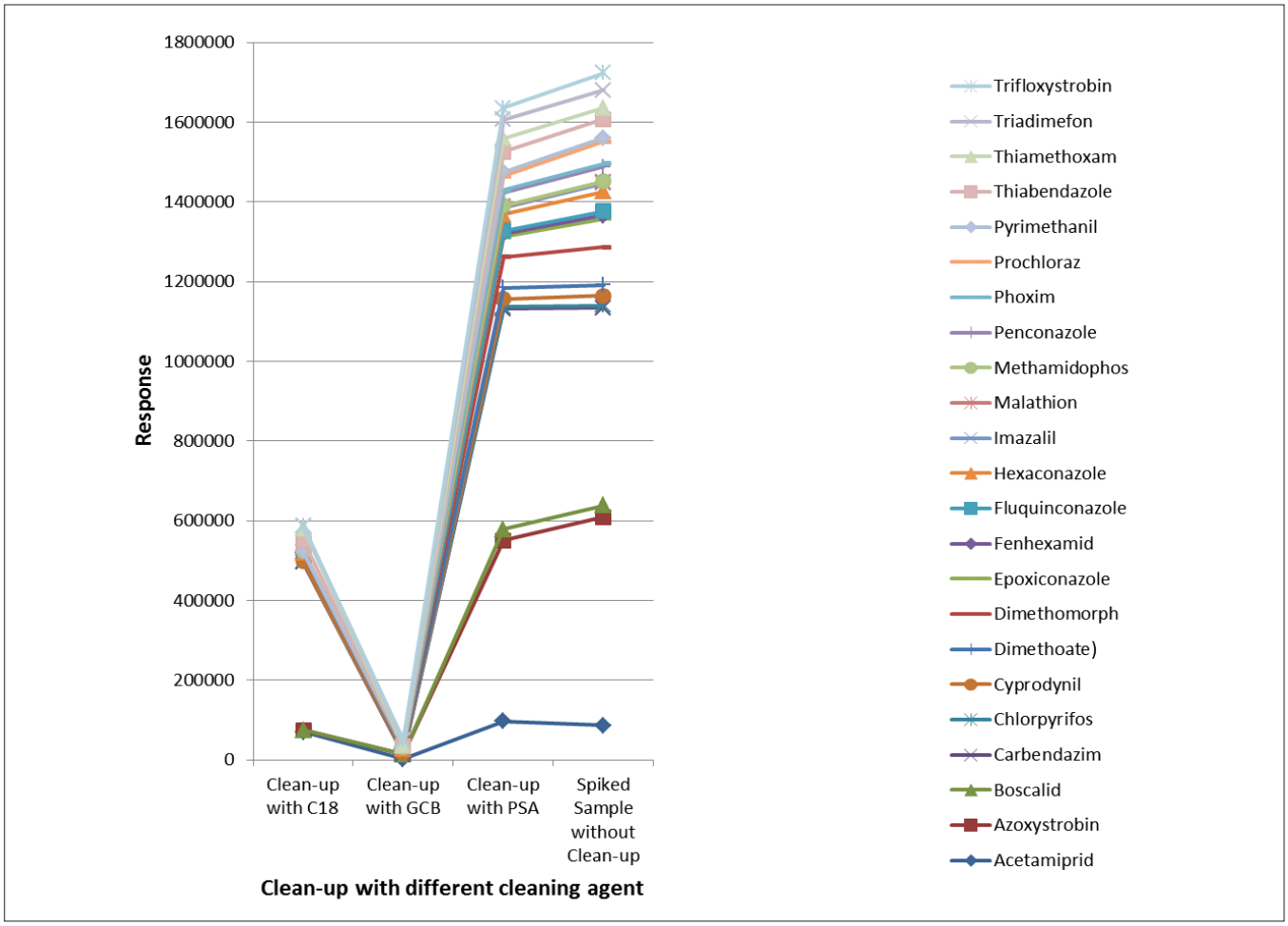


Figure 4. Effects of clean-up steps on responses of the pesticides (20µg/L).

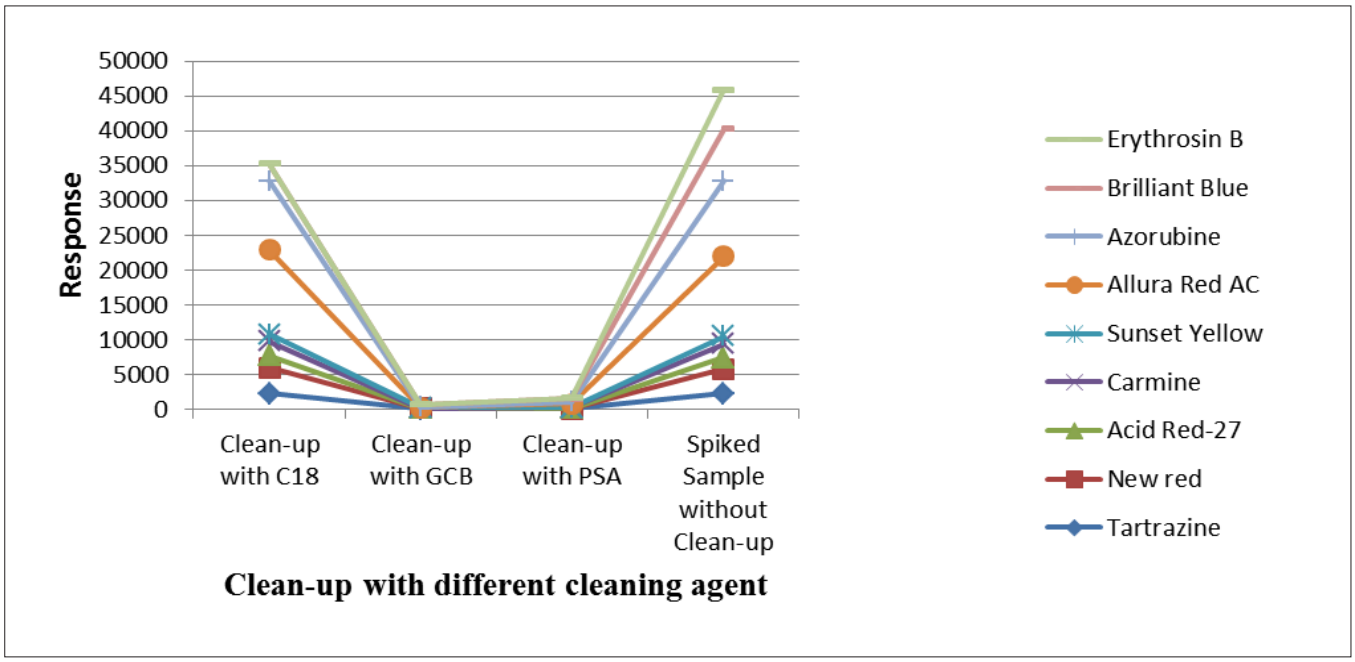


Figure 5. Effects of clean-up steps on response of pigments (200µg/L).

In this study, matrix-matched calibration curves were used for quantification. The matrix-matched calibration curves showed good linearity over three orders of magnitudes with regression coefficients (R^2) greater than 0.99, from 1 µg/L to 1000 µg/L for the nine pigments and from 0.5 µg/L to 100 µg/L for the 23 pesticides, respectively. The recoveries of the analytes were evaluated at concentrations of 50, 100 and 500 µg/L, and the mean recovery values ranging from 85.0% to 115.0% with RSD <11%. The developed method has been applied for the analysis of 10 real wine samples and the results are summarized in Table 4.

Conclusions

A rapid, sensitive and selective 'dilute-n-shot' method has been developed and validated for simultaneous determination of 23 pesticides and nine pigments in wine. The method has the advantage of analyzing pesticides and pigments in a single run using UHPLC-MS/MS method. The results demonstrated that the accuracy and precision of the method were acceptable for routine monitoring of these compounds in analytical laboratories.

Table 4. Results of the analytes determined from the 10 real wine samples (in µg/L).

Compound	Sample 01	Sample 02	Sample 03	Sample 04	Sample 05	Sample 06	Sample 07	Sample 08	Sample 09	Sample 10
Acetamiprid	-	-	4.1	10	-	-	-	-	-	-
Azoxystrobin	-	-	11.5	-	-	-	-	-	-	-
Boscalid	-	33.5	173.1	87.3	53.1	345.8	10.7	245.5	16.1	42.4
Carbendazim	2.8	-	-	-	-	-	164	16	-	-
Chlorpyrifos	3.4	-	-	-	-	-	-	-	-	-
Cyprodynil	33.9	-	-	10.8	-	82.6	-	-	-	-
Dimethoate	-	-	-	5.7	-	-	27	-	-	-
Dimethomorph	-	-	-	-	-	134.3	90.2	100.2	-	-
Fenhexamid	-	-	-	180.3	-	275.8	-	434.4	-	-
Pyrimethanil	-	-	-	43.2	-	-	115	136.4	-	-
Thiabendazole	-	-	-	6.1	-	-	-	-	-	-
Thiamethoxam	-	-	-	-	-	20.2	-	-	-	-

References

1. Guo J, Zhu K, Zheng S, Chen Q, Lin M. Food and Fermentation Industries, 2017, 43(1):192-198.
2. Wang J, Chow W, Leung D. Anal. Bioanal. Chem., 2010, 396:1513–1538.
3. Li Y, Zheng Y, Xiong C, Zeng Y,Chen S. Chinese Journal of Chromatography, 2013, 31(8):729-733.
4. Gui Q, Liu H, Xu W, Gong Y. Journal of Chinese Mass Spectrometry Society, 2015, 36(2):148-155

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

013708_01 PKI

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

014073_01 PKI