Screening Method for 30 Pesticides in Green Tea Extract Using Automated Online Sample Preparation with LC-MS/MS

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Overview

Purpose: Develop a rapid and sensitive automated online sample preparation LC-MS/MS method to screen for multiple pesticides in green tea extract.

Methods: Automated online sample preparation using the Transcend system powered by TurboFlow technology coupled with the TSQ Access MAX triple stage quadrupole mass spectrometer.

Results: A TurboFlow method with online multi-residue screening in green tea extract was developed.

Introduction

Analysis of pesticide residues has been one of the most important tasks of food safety laboratories. Mass spectrometers (MS), with liquid chromatography coupled to triple stage quadrupole mass spectrometers (LC-MS/MS), have been the main tools used in pesticide residue analysis. There is a consensus that sample preparation is becoming the bottleneck to the entire workflow. Traditional sample preparation methods, usually involving liquid-liquid extraction (LLE) or solid phase extraction (SPE), can be time-consuming and labor-intensive. In addition, low recovery, matrix interference and poor reproducibility are among other major concerns. In recent years, a rapid processing method, QuEChERS, has gained popularity. The QuEChERS method makes it easier and less expensive for analytical chemists to examine pesticide residues in various food matrices.[1] However, some reports show matrix interference tends to be severe after QuEChERS, and the mass spectrometer is more vulnerable to contamination by highly complex food matrices.[2] In this study, we describe an easy, comprehensive, online screening LC method using a Transcend TLX-1 system powered by TurboFlow technology to analyze multiple pesticide residues in green tea extract. Figure 1 illustrates a typical Transcend TLX-1 system with the TSQ Access MAX triple stage quadrupole mass spectrometer.

Methods

Matrix Standard Curve

One gram of Chinese green tea was extracted using 10 mL HPLC grade acetonitrile followed by 15 minutes of ultra-sonication. The extract was then filtered through a 0.45 µm membrane filter. The resultant solution was used to prepare the matrix calibrators and QC samples. The matrix calibrant concentrations were 6.25 µg/L, 12.5 µg/L, 25 µg/L, 50 µg/L and 100 µg/L, respectively. The matrix QC sample concentration was 10 µg/L.

TurboFlow Method Parameters

System: Thermo Scientific Transcend TLX-1 system controlled by Thermo Scientific Aria OS 1.6.3 software
Column: Thermo Scientific TurboFlow Cyclone 0.5 x 50 mm
Injection Volume: 10 µL
Loading Solvent: 0.1% formic acid in water
Loading Flow Rate: 1.5 mL/min
Eluting Solvent: 0.1% formic acid in methanol

The TurboFlow method schematic is shown in Figure 2.

HPLC Method Parameters

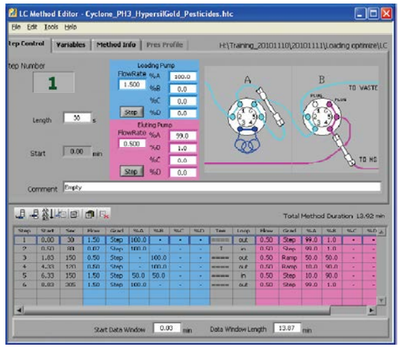
Analytical Column: Thermo Scientific Hypersil GOLD 2.1 x 100 mm, 3 µm
Solvent A: 0.1% formic acid in water
Solvent B: 0.1% formic acid in methanol

Mass Spectrometer Parameters

MS: Thermo Scientific TSQ Quantum Access MAX
MS Ionization Source: Heated Electrospray Ionization (H-ESI)
Ion Polarity: Positive ion mode
Spray Voltage: 2 KV
Sheath Gas Pressure (N_2): 30 arbitrary units
Auxiliary Gas Pressure (N_2): 15 arbitrary units
Vaporizer Temperature: 300 °C
Capillary Temperature: 300 °C
Collision Gas Pressure: 1.5 mTorr

FIGURE 1. Typical layout of a Transcend™ TLX-1 system with a TSQ Access MAX™ triple stage quadrupole mass spectrometer

FIGURE 2. TurboFlow™ method schematic as viewed in the Aria™ OS software



Results

Extraction and Separation of 30 Pesticide Residues

In 2006, Japan released the most stringent pesticide-related regulation in history entitled "Positive List System for Agricultural Chemical Residues in Foods".[3] Since Japan is China's major tea importer, the limits discussed in the current study follow this regulation. As described in the Experimental section, the tea matrix standard samples are 6.25 µg/L, 12.5 µg/L, 25 µg/L, 50 µg/L and 100 µg/L, respectively. The matrix QC samples are 10 µg/L. Figure 3 shows the representative chromatograms at 6.25 µg/L, which has been determined as the lower limit of quantitation (LLOQ). The data demonstrate that 30 pesticides were well separated with good peak shape. The peaks' signal-to-noise ratios are far greater than the required 10:1 at the LLOQ. Table 1 shows the linear curve for these 30 analytes. All R^2 values are between 0.993-0.999. The relative standard deviation (RSD) for 6 consecutive injections of the 6.25 µg/L calibrator was in the range of 2.85% -7.48%.

TABLE 1. Standard curve linearity and QC results for the 30 pesticides in tea extract

Compound	RT (min)	Parent ion (m/z)	Product ion (m/z)	Collision Energy (V)	Linear Curve	RF	CV% (n = 6) QC = 10 µg/L
Fenuron	4.82	226.0	184.0 142.1	20 27	Y=1673434x906593X	0.999	2.99%
Ametryn	5.07	228.0	186.0 96.0	20 34	Y=63264.1+1944610X	0.999	2.85%
Dimethamotryn	5.68	256.1	186.1 158.1	21 27	Y=166875+6050656X	0.999	3.29%
Metfenoxam	5.79	280.0	220.0 192.0	17 20	Y=400169+2724281X	0.998	4.23%
Monolinuron	5.85	215.0	126.0 99.0	17 36	Y=-10985.6+18335.3X	0.998	6.51%
Isoprocarb	5.94	194.0	95.0 137.0	16 11	Y=-18662+12428.2X	0.999	6.49%
Dimethachlor	6.01	256.0	224.0 148.0	15 28	Y=-23531.9+98341.3X	0.997	5.53%
Clomazone	6.05	240.0	125.0 89.0	20 37	Y=-43417.5+121181.6X	0.998	6.37%
Furalaxyl	6.21	302.0	242.0 270.0	15 10	Y=358161+2672757X	0.998	4.85%
Azoxystrobin	6.33	404.0	372.0 329.0	15 33	Y=-538888+377645X	0.997	4.06%
Triadimefon	6.39	294.0	197.0 225.0	19 19	Y=-20167.4+16685.6X	0.997	7.31%
Ethoprophos	6.41	243.0	131.0 97.0	21 33	Y=-13814+14313.8X	0.997	7.48%
Isoxaben	6.52	289.0	205.0 91.0	12 23	Y=53008.6+137376X	0.999	6.28%
Isoprothiolane	6.57	291.0	189.0 231.0	22 12	Y=123106+97284X	0.998	6.00%
Flutolanil	6.60	324.0	242.0 262.0	26 18	Y=45077+47886X	0.998	6.56%
Propiconazole	6.65	342.0	159.0 69.0	30 31	Y=17113.2+36428.7X	0.997	6.74%
Benalaxyl	6.78	326.0	148.0 208.0	25 20	Y=172291+126493X	0.997	5.92%
Pirimiphos-methyl	6.81	306.0	164.0 108.0	22 33	Y=227752+204491X	0.994	4.73%
Picoxystrobin	6.82	368.0	145.1 205.0	22 7	Y=320993+78661.5X	0.993	4.03%
Diazinon	6.90	305.0	169.0 153.0	24 26	Y=182249+3962477X	0.998	4.96%
Thiazopyr	6.95	397.0	335.0 275.0	30 40	Y=-5052.12+18434.8X	0.997	6.47%
Piperophos	7.09	354.0	171.0 143.0	25 33	Y=142671+143459X	0.996	4.68%
Trifloxystrobin	7.13	409.0	186.0 206.0	21 10	Y=-18755.2+43150.6X	0.998	6.22%
Tebufenpyrad	7.16	334.0	145.0 117.0	28 36	Y=-3267.09+9390.51X	0.998	7.01%
Piperonyl butoxide	7.25	356.0	177.0 119.0	13 33	Y=-300922+175086X	0.996	4.16%
Pyriproxyfen	7.34	322.0	96.0 185.2	16 27	Y=-19160.9+56881.6X	0.999	4.73%
Trifloxydim	7.39	338.0	284.0 138.0	15 20	Y=-8119.47+46536.4X	0.997	5.01%
Fenazaquin	7.55	307.0	161.0 57.0	18 23	Y=-56587.3+97365.3X	0.998	2.76%
Butralin	7.58	296.0	240.0 222.0	15 20	Y=-2485.92+25797.9X	0.998	4.72%
DEF	7.85	315.0	169.0 113.0	15 25	Y=-8658.91+7992.12X	0.998	3.89%

Background Reduction Effects Using the Transcend TLX-1 system

By using the Transcend TLX system with TurboFlow technology, the background noise and interference peaks are reduced significantly. Figure 4 compares chromatograms of Clomazone at 6.25 µg/L in tea extract using standard HPLC (top) and the TurboFlow method (bottom). The left panel shows the primary transition of *m/z* 240 → 125. The right panel shows the secondary transition of *m/z* 240 → 89. It clearly shows the effectiveness of background reduction using TurboFlow technology while the signal-to-noise ratio increased by 3 and 4 times for *m/z* 125 and 89 transitions, respectively. The area responses of both peaks also increase by more than 50% due to the minimization of ion suppression incurred by matrix. We also noticed the mass spectrometry response become more stable across the entire tested concentration range, thus improving the method reliability.

FIGURE 3. Selected ion chromatograms at LLOQ of 6.25 µg/L for all 30 analytes (in the same order as in Table 1)

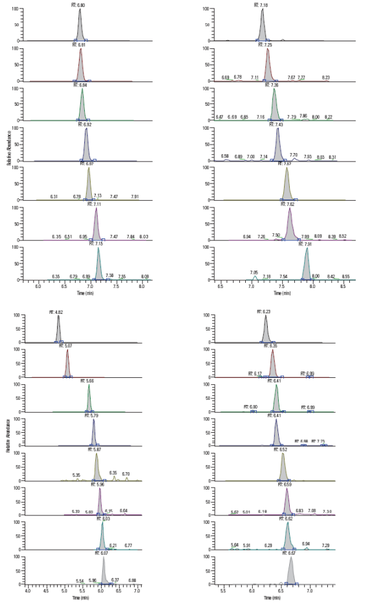
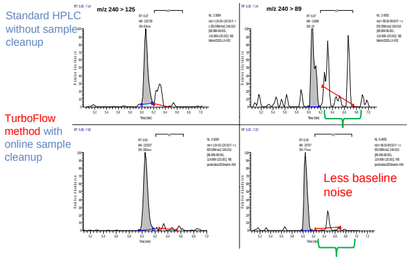


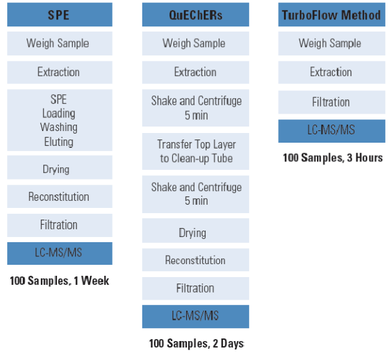
FIGURE 4. Comparison of chromatograms of Clomazone at 6.25 µg/L in tea extract using standard HPLC (top) and the TurboFlow method (bottom)



Comparison of TurboFlow Method with Two of the Most Popular Pesticide Sample Preparation Methods

As shown in Figure 5, we compared a TurboFlow method and two currently popular methods for pesticide residue sample preparation, SPE and QuEChERS. A typical SPE method involves equilibrating the cartridge, loading, washing and eluting analytes. It usually takes about 1 week to process 100 samples. Although QuEChERS was designed to simplify sample preparation, it still requires two-step centrifugation and concentration. A few days are typically required to prepare 100 samples with QuEChERS. TurboFlow technology minimizes preparation of 100 samples to less than 3 hours, dramatically improving the efficiency and throughput of this routine lab test.

FIGURE 5. Comparison of the TurboFlow method to SPE and QuEChERS



Conclusion

A quick, automated online sample preparation LC-MS/MS method has been developed that is sensitive enough to screen the tested pesticides in tea extracts. The method detection and quantitation limits are significantly lower than the strictest limits set by the Japanese government. The use of the Transcend TLX-1 system with TurboFlow technology eliminates the need for time-consuming sample preparation procedures such as SPE and QuEChERS.

References

1. Steven J. Lehotay, Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Approach for Determining Pesticide Residues. Methods in Biotechnology, 2006, 19, 239-261.

2. Steven J. Lehotay, Michelangelo Anastassiades, Ronald E. Majors, QuEChERS, a Sample Preparation Technique that Is "Catching On": An Up-to-Date Interview with the Inventors, LCGC North America, July 2010.

3. The Japanese Positive List System for Agricultural Chemical Residues in Foods (enforcement on May 29, 2006).

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