Extraction and UPLC Analysis of Pesticides from Hops

Summary

The EDGE® automated solvent extraction system is the most advanced system available for extraction and clean-up of pesticide residues from difficult matrices, including hops and cannabis. It utilizes a combination of pressurized fluid extraction and dispersive solid phase extraction to drastically reduce the sample preparation time and potential for human error. The system provides a complete pesticide residue extraction in only 5 minutes in a single step, while eliminating matrix interferences. The 5-minute complete sample filtration, including cooling, and system cleaning, allows 12 samples to be extracted in one hour, making EDGE the ideal sample preparation system for high-throughput extraction of samples considered difficult due to background interferences.

Introduction

More and more consumers want to know what is in their food, particularly anything that could be harmful, such as pesticides. There is a driving need for pesticide analysis and the list of pesticides regulated throughout the world continues to increase. The QuEChERS method has become a widely accepted method to extract pesticides from food matrices. Due to the large number of pesticides to monitor and the low method detection limits, pesticide analysis can be a big challenge. Alternative methods can help aid in this challenge, giving improved recoveries for difficult matrices with a faster and simplified method.

The manual, multi-step process of the QuEChERS method requires multiple sample transfers and generates a lot of consumable waste. With the EDGE, the sample and sorbents are together in a single sample cell, meaning extraction and cleanup are performed in one step. The food sample is extracted in under 5 minutes, using the patent pending Q-Cup™ technology which performs both extraction and cleanup of the sample. The collected extract is filtered, cooled, and ready for analysis. Included in the run time is both the rinsing of the sample and washing of the system, ensuring no carryover. EDGE offers the fastest pesticide extraction possible in one simple method.

Materials and Methods

Reagents

A 454 g (16 oz.) bag of YCHHOPS Cascade Hops Pellets, Alpha: 6.3% was obtained from Alternative Beverage (Charlotte, NC). Acetic acid, glacial (CH_3COOH) HPLC grade 99.7% and UPLC-MS Optima™ grade water (H_2O) were obtained from Fisher Chemical (Fair Lawn, NJ). Acetonitrile (C_2H_3N) anhydrous 99.8% and UPLC grade methanol (CH_3OH) were obtained from Sigma-Aldrich (St Louis, MO). Ammonium Acetate (C_2H_7NO2) 1 M, pH 5.0 was obtained from Waters (Milford, MA). ACS grade magnesium sulfate ($MgSO_4$) anhydrous > 99.5% and Supel™ QuE Citrate/Sodium Bicarbonate tubes were also obtained from Sigma-Aldrich (St Louis, MO). The primary secondary amine and Siliabond C18 (17%) sorbents were obtained from Silicycle (Quebec, Canada). The Canadian Pesticide Mix 4 in LCMS grade acetonitrile was obtained from SPEX CertiPrep (Metuchen, NJ).

Extraction

All samples were extracted, filtered, and cleaned on an EDGE system from CEM Corporation (Matthews, NC). The extraction solvent used was 1% glacial acetic acid in acetonitrile; each sample was extracted with 30 mL of solvent. The Q-Cup™ was assembled using the M2 and C9 Q-Discs™. This robust combination of M2 and C9 Q-Discs ensures full support of the plant sample while providing <0.25 µm filtration. The C9 Q-Disc should first be placed in the bottom cap, followed by the M2 Q-Disc with the textured side up. Following Q-Cup assembly, 5.0 +/- 0.02 g mix of $MgSO_4$, citrate/sodium bicarbonate, primary secondary amine and Siliabond C18 (1:1:1:1) were added to the Q-Cup. This mix of salts and sorbents was used to remove matrix interferences from hops. Approximately 0.5 +/- 0.02 grams of hops was weighed out and added to the Q-Cup. The hops were added directly on top of the sorbent mixture, creating a top layer with the sorbent layer beneath it. The samples were then spiked with 100 µL of 100 µg/mL Canadian Pesticide Mix 4 and left in the hood for approximately 30 minutes to allow the solvent to evaporate. The samples were then loaded into the EDGE sample rack and queued. The EDGE extraction method created for this extraction used 30 mL total volume, divided up with 15 mL top volume, 10 mL bottom, and 5 mL rinse. The extraction utilized a 30-second hold at 100 °C. After the hold, the solvent collected into a 40 mL graduated glass vial. Once all samples were complete, their final volume of 30 mL was confirmed and an aliquot was transferred to an LC vial direct to analysis. Between each sample, a 20 mL wash of clean solvent was applied throughout the system to prevent sample-to-sample carry-over.

Analysis

The extracts were analyzed under the following LC conditions using Waters Acquity UPLC BEH C18 1.7 µm 2.1 x 50 mm column, with 5 µL injections. Separation was achieved using a gradient elution with the following gradient shown in **Table 1**. The Mobile Phase A: 10 mM Ammonium Acetate in Water, and Mobile Phase B: 5 mM Ammonium Acetate in Methanol.

Table 1. LC Gradient Used for Pesticide Separation

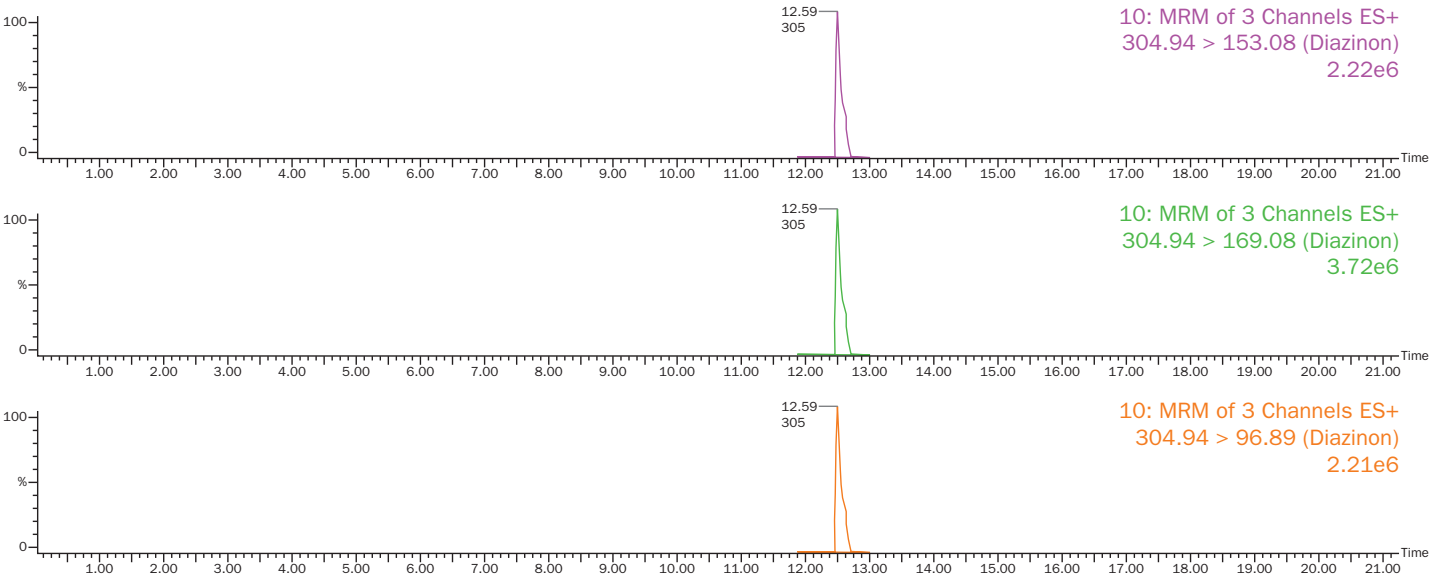
Time	Flow Rate (mL/min)	%A	%B
Initial	0.25	95	5
2	0.25	95	5
6	0.25	60	40
12	0.25	10	90
14	0.25	10	90
16	0.25	95	5

Prior to analysis, each pesticide was tuned using Waters Intellistart and identified with at least two daughters using multiple reactions monitoring (MRM) to accurately identify and quantify each pesticide.

The MS Conditions were as follows, using the Waters Acquity H Class, Xevo TQD:

Ionization Mode: ESI+
Capillary Voltage: 0.10 kV
Source Temp: 150 °C
Probe Temp: 600 °C
Sampling Rage: 10 Hz

Figure 1: Diazinon MRM Transitions



Discussion of Analysis

Figure 1 shows one of the pesticides, Diazinon at 1000 ppb, with its three MRM transitions, shown in the three chromatograms. Each chromatogram is a different transition from the parent ion of Diazinon. The transition for this particular pesticide that was used for quantitation was 304.94 -> 153.08. This is the strongest transition, the transition with the highest intensity. This method of quantitation was used for all 19 pesticides. **Table 2** is a summary of the pesticides with their strongest transition and optimal cone voltage.

For quantifying each pesticide, a 7-point matrix matched calibration curve ranging from 50 ng/mL-1000 ng/mL was utilized to account for ion suppression or enhancement that could take place due to the complexity of the matrix. **Figure 2** shows an example of the same pesticide Diazinon and its 7-point matrix matched calibration curve. The correlation coefficient is greater than 0.999, showing excellent linearity.

Figure 2: Matrix Matched Calibration Curve for Diazinon

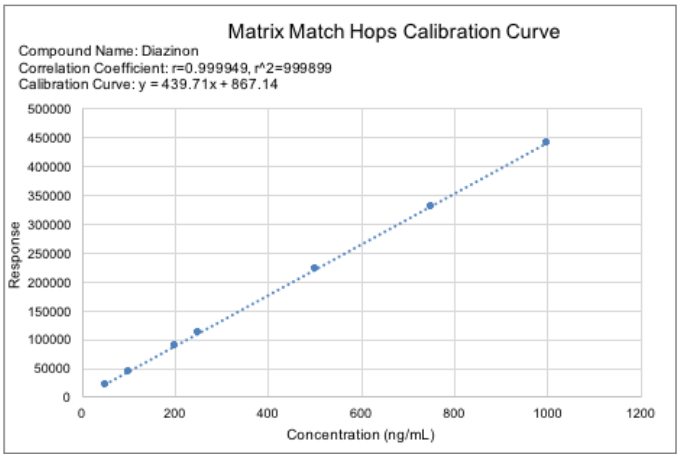


Table 2: Pesticide MS Parameters for Quantitation

Name	Transition	Cone Voltage (V)
Acephate	142.93	16
Chlorpyriphos	96.88	93.20
Coumaphos	226.83	50
Diazinon	169.08	24
Dichlorvos	108.92	34
Dimethoate	198.87	30
Prophos	130.90	36
Etofenprox	177.00	60
Etoxazole	140.97	54
Terrazole	105.01	52
Fensulfothion	281.05	32
Fenthion	168.95	32
Malathion	127.00	30
Methyl Parathion	124.92	36
Mevinphos	127.00	26
Imidan (Phosmet)	160.03	30
Spiroxamine	144.10	36
Tetrachlorvinphos (Z)	126.99	42
Thiophanate-methyl	150.99	28

Results and Discussion:

The extraction for each sample took less than 5 minutes and was performed sequentially. **Table 3** lists the pesticides with their corresponding recoveries and RSD values. Overall, 16 of the 19 pesticides fall within the accepted recovery range of 80-120%. The two pesticides, Acephate and Prophos, that exceed that range above the 120% recovery show peak interference in the blank matrix. This could be optimized either through a further optimized LC method or sorbent mixture. Since graphite carbon black (GCB) was not used, this could be a pigment interference but needs to be confirmed through blank comparisons with and without GCB. The third analyte that falls out of range lower than 80% recovery is fensulfothion. This pesticide is known to have a several metabolites including the oxygen analogue sulfone. This pesticide can be easily quantified if the fensulfothion and its three metabolites can be oxidized to create one single peak of the oxygen analogue sulfone.[1] A degradation study could be performed isolating this pesticide to observe the rate of degradation in acetonitrile at a range of temperature to isolate the optimal temperature.

Conclusion:

The EDGE efficiently extracted the pesticides from hops in 5 minutes, including sample cleanup, filtration, cooling and system washing. Furthermore, hops is a known difficult sample, due to its high lipid content and the EDGE was able to yield an extract with sufficient cleanup in just one automated step resulting in good recoveries. No matter the food sample, EDGE offers a fast and simple extraction method that includes the cleanup process and is a good alternative to the QuEChERS method.

Table 3: Pesticide Recovery

Name	Recovery (n=3)	% RSD (n=3)
Acephate	136	8.8
Chlorpyriphos	98	0.6
Coumaphos	98	2.0
Diazinon	103	0.9
Dichlorvos	97	6.6
Dimethoate	94	3.7
Prophos	121	0.6
Etofenprox	103	0.8
Etoxazole	114	3.0
Terrazole	103	1.3
Fensulfothion	64	14.4
Fenthion	100	2.7
Malathion	88	4.0
Methyl Parathion	96	2.1
Mevinphos	107	0.3
Imidan (Phosmet)	93	0.8
Spiroxamine	88	2.7
Tetrachlorvinphos (Z)	95	2.8
Thiophanate-methyl	117	80.1

References

[1] 1972 Evaluations of fensulfothion pesticide residue in food. AGP:1972/M/9/1; WHO Pesticide Residues Series, No. 2, 1973, no 239 on INCHEM. http://www.inchem.org/documents/jmpr/jmpmono/v072pr17.htm

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