A Novel Automation Platform in Combination with dSPE for Workflow Improvements in Food Testing prior to GC/MS Analysis

Russell Parry, Lee Williams, Alan Edgington, Geoff Davies, Adam Senior, Helen Lodder, Charlotte Hayes, Claire Desbrow and Dan Menasco.
Biotage GB Limited, Distribution Way, Dyffryn Business Park, Ystrad Mynach, Cardiff, CF82 7TS, UK

Introduction

Food safety testing has grown over the last 10 years. The diverse nature of the samples add complexity making method standardisation challenging. However, default methodology, for solid-matrix pesticide analysis is firmly centred around QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) followed by dSPE (Dispersive Solid Phase Extraction) clean up. Both techniques are manual, labour intensive and typically difficult to automate. This poster will present the use of a dedicated automation platform combined with a prototype "dSPE" column for improved workflow for pesticide extraction from food matrices.

Experimental

Reagents

Pesticide standards were purchased from Sigma-Aldrich (Dorset, UK). Spinach was sourced from local vendors. All solvents were HPLC grade from Fisher Scientific (Loughborough, UK) and Milli-Q (Merck Millipore, Germany) water used throughout.

Sample Preparation

Matrix Preparation:

15 g of sample was weighed into 50 mL Biotage® Lysera centrifuge tubes containing 15 g of ceramic beads.

Maceration Procedure:

Simultaneous maceration of 3 spinach samples was achieved using a Biotage® Lysera equipped with 50 mL tube carriage kit as shown in ***Figure 1.***: 4 x 40s cycles at 5 m/s with 20s dwell.

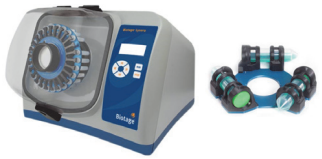

Figure 1. *Biotage® Lysera Bead Mill Homoenizer with 50 mL tube carriage kit.*

ISOLUTE® QuEChERS: 15 mL ACN was added to each sample followed by the standard AOAC QuEChERS Pigmented Fruit and Vegetables, Q0010-15V extraction salts. Further mixing was performed using a single cycle of 60s at 0.8 m/s. Extracts were centrifuged for 5 minutes at 4000 rpm to separate layers.

dSPE: 1-2 mL ACN extract aliquots were further cleaned up using the Pigmented Fruit and Vegetable Q0070 dSPE blends. Manual dSPE processing was performed by mixing the samples for 1 minute, centrifugation for 5 minutes at 4000 rpm followed by transfer to GC vials. An alternative flow-through dSPE approach (prototype product) was performed using the traditional 3 mL SPE column-based format eluting directly into GC vials (1mL extracts).

Post extraction: 1 mL extracts were evaporated in high recovery GC vials at 40 °C, followed by reconstitution in 30 µL of toluene prior to GC/MS analysis.

Biotage® Extrahera™ Automated Sample Preparation Platform

For increased throughput the column-based flow-through dSPE extraction protocol was transferred to an automated sample preparation platform, the Extrahera™ High-Volume HV-5000. The system is equipped with a 4 channel 5 mL pipetting head and utilises positive pressure processing functionality. Columns were processed in 24/48 position arrangements depending on throughput requirements. The Extrahera™ HV-5000 platform is shown in ***Figure 2***.

Figure 2. *Biotage® Extrahera™ HV-5000 automated sample preparation platform.*

GC Conditions

GC: 7890A GC (Agilent Technologies Inc.)
Column: Restek Rxi-5ms, 30 m x 0.25 mm ID x 0.25 µm
Carrier Gas: Helium 1.2 mL/min (constant flow)
Inlet: 280 °C, Splitless, purge flow: 50 mL/min at 1.0 min
Injection volume: 1.3 µL
Oven conditions: Initial temperature 90 °C
Ramp: Available on request
Backflush: 3 void volumes (2.4 mins)
Transfer Line: 300 °C

MS Conditions

MS: 5975C MSD (Agilent Technologies Inc.).
Source Temperature: 230 °C
Quadrupole Temperature: 150 °C
Monitored Ions: EI signals were acquired using selected ion monitoring (SIM) mode. Monitored ions for each panel available on request.

Results

A selection of 50 analytes were chosen as a representative panel, incorporating pesticides, fungicides and insecticides. Spinach extraction was performed using AOAC QuEChERS blend and methodology in traditional tube formats. Sample maceration using the Biotage® Lysera bead mill homogenizer and resultant extracts are demonstrated in ***Figure 3.***

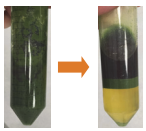

Figure 3. *Macerated Spinach sample and initial ISOLUTE® QuEChERS extract.*

1-2 mL ACN extracts were then removed and further cleaned up using dSPE. Traditional dSPE tube processing and centrifuged extracts are demonstrated in ***Figure 4.***

Figure 4. *Traditional dSPE processing and centrifuged extract.*

Figure 5. demonstrates an alternative flow-through dSPE column approach and processed extract.

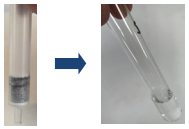

Figure 5. *Alternative flow-through dSPE column processing and extract.*

Final 1 mL ACN spinach extracts comparing traditional dSPE and column flow-through processing approaches are demonstrated in ***Figure 6.*** Pre-concentration extracts are depicted on the left while post evaporated and reconstituted extracts are shown on the right. Increased pigment removal was obtained using the column flow-through approach.

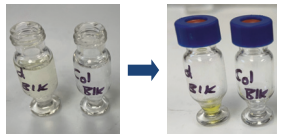

Figure 6. *Final pre and post evaporated dSPE extracts.*

The Extrahera™ High-Volume HV-5000 automated platform was simply optimized for pipetting and processing flow control for dSPE columns.

Traditional and automated column-based flow-through dSPE were compared for method performance along with optimized processing and throughput. Timing comparison was performed processing 12 extracts as demonstrated in ***Table 1.*** Time saving of 25 minutes were realized compared to traditional dSPE processing. However, the EH HV-5000 is capable of processing 24/48 samples simultaneously in 3 mL column format, thus exponentially increasing time savings for larger sample sets.

Table 1. *Timing comparison for specific dSPE step.*

	Timing Comparison (mins)	
Step	***Classic dSPE***	***EH HV-5000***
QuEChERs Extract Transfer	3	3
Shake/Centrifuge Tubes	25	-
EH Processing	-	5
Transfer 1mL to vials	5	-
Evaporate	20	20
Recon	2	2
Total	***55***	***30***

Pesticide analyte performance comparisons in terms of recoveries, RSDs, matrix factors and signal are shown in ***Figures 7-10.***

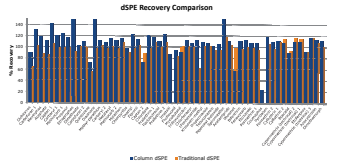

Figure 7. *Pesticide recovery comparison for dSPE processing.*

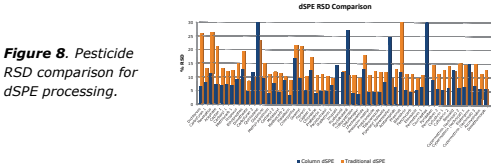

Figure 8. *Pesticide RSD comparison for dSPE processing.*

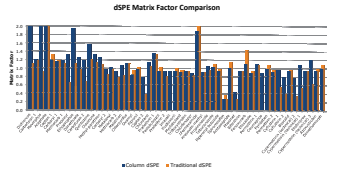

Figure 9. *Pesticide Matrix Factor comparison for dSPE processing.*

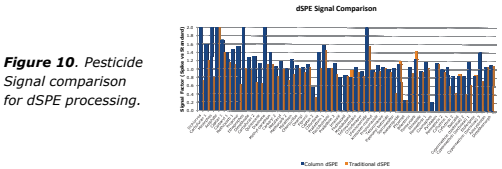

Figure 10. *Pesticide Signal comparison for dSPE processing.*

Final SIM chromatograms are presented in ***Figure 11.***

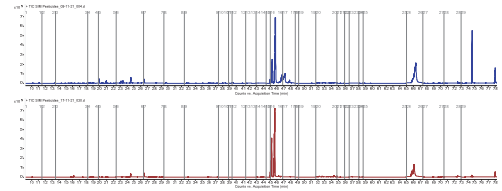

Figure 11. *GC/MS SIM chromatograms: traditional dSPE (top) and column flow through (bottom).*

Conclusion

- This poster demonstrates improved workflow and speed for processing food samples using a combination of automation and column flow-through dSPE formats.
- We have demonstrated equivalent pesticide performance with improved sample cleanliness using the prototype dSPE columns.Improved sample turnaround times are realized through simultaneous multi-sample processing.

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