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Automated QuEChERS Extraction for the Determination of Pesticide Residues in Foods using Gas Chromatography/ Mass Spectrometry

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Abstract
One of the most important aspects of reducing pesticide exposure is monitoring of pesticide residues in foods. A number of analytical methods have been developed, many of them based on traditional liquid-liquid extraction in combination with GC-MS or LC-MS. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation methods have been developed to help monitor pesticides in a range of food samples. These methods, however, still require many manual steps, such as shaking, mixing, centrifugation, and dispersive SPE (dSPE), making them quite labor-intensive. There is a need for automating the dSPE technique to clean up QuEChERS type extracts in order to improve laboratory productivity for monitoring pesticide residue in foods.

This paper describes an automated dSPE cleanup method for QuEChERS extracts that is performed with commercially available kits from Agilent® Technologies. Extraction and clean-up is performed using a micro-scale version of this method and automation is achieved using a GERSTEL MPS autosampler equipped with an Anatune CF-100 centrifuge. The clean-up process is followed by automated injection of the cleaned extract to a GC/MS system. Analytical methodology for confirming the presence of a variety of

pesticides in various food samples was developed using an Agilent 7890 GC with 5975 MSD. Samples were introduced into the gas chromatograph using a GERSTEL Thermal Desorption Unit (TDU) and MPS autosampler with Automated TDU-liner EXchange (ATEX) option. The sensitivity and selectivity of GC/MS combined with the described injection technique, results in method detection limits that meet acceptance criteria for reporting maximum residue levels (MRLs) as established by regulatory agencies.

The ability to automate the dSPE clean-up of QuEChERS extracts and to couple extraction and clean-up directly to GC-MS analysis, results in improved laboratory productivity by streamlining the complete analytical process.

Introduction

Pesticides in food are frequently extracted using acetonitrile and the extracts subsequently cleaned using dispersive solid phase extraction (dSPE), which is the second step in the QuEChERS extraction method. Even after dSPE clean-up, QuEChERS extracts can still be relatively dirty and direct liquid injection can lead to build-up of involatile residue in the GC inlet. A two-stage sample introduction, using thermal desorption and a programmable temperature vaporizer inlet, was employed in this study to help further reduce matrix interference. dSPE kits from Agilent Technologies were used to remove sample matrix residue from QuEChERS type extracts prior to GC/MS analysis. The entire process including mixing, centrifugation, and sample introduction was automated using a GERSTEL MultiPurpose Sampler (MPS) equipped with an Anatune CF-100 centrifuge (Figure 1).

Figure 1. GERSTEL MultiPurpose Sampler (MPS) with Anatune CF-100 centrifuge option.

The complete process was controlled using GERSTEL MAESTRO Software integrated with the Agilent GC/MS software

Experimental

Instrumentation. Analyses were performed on a 7890 GC equipped with a 5975C Inert XL MSD with triple axis detector (Agilent Technologies), PTV inlet (CIS 4, GERSTEL), Thermal desorber (TDU, GERSTEL) and MPS robotic sampler with 10 µL ATEX syringe (GERSTEL) and Anatune CF-100 centrifuge.

Analysis conditions.

TDU:	3 min solvent vent (50 mL/min) 100°C (3 min), 720°C/min, 280°C (3 min)
PTV:	splitless (1.2 min) 25°C (0.2 min), 12°C/sec, 280°C (3 min)
Column:	30 m Rxi®-5Sil MS (Restek), d_i = 0.25 mm d_f = 0.25 µm
Pneumatics:	He, constant flow (1 mL/min)
Oven:	60°C (1 min), 10°C/min, 310°C (5 min)

Standard preparation. A matrix matched composite standard of organochlorine and organophosphorus pesticides was prepared at 50 ng/mL by adding a standard solution of the pesticides in acetonitrile to the final extract (post cleanup) of a control sample. Analyte recoveries were calculated by comparing area counts from the samples to this standard.

Sample preparation. Processed fruit and vegetable extracts were provided by the FDA. The extracts were spiked to obtain a 50 ng/mL concentration of each OC/OP pesticide. 1 mL of the extract was pipetted into a 2 mL autosampler vial containing the sorbent blend from Agilent's SampliQ QuECHERS dispersive SPE kit for fatty samples, AOAC (p/n: 5982-5122). The sample was placed onto the MPS autosampler tray for automated dSPE cleanup. Figure 2 shows the MAESTRO prep sequence for this procedure.

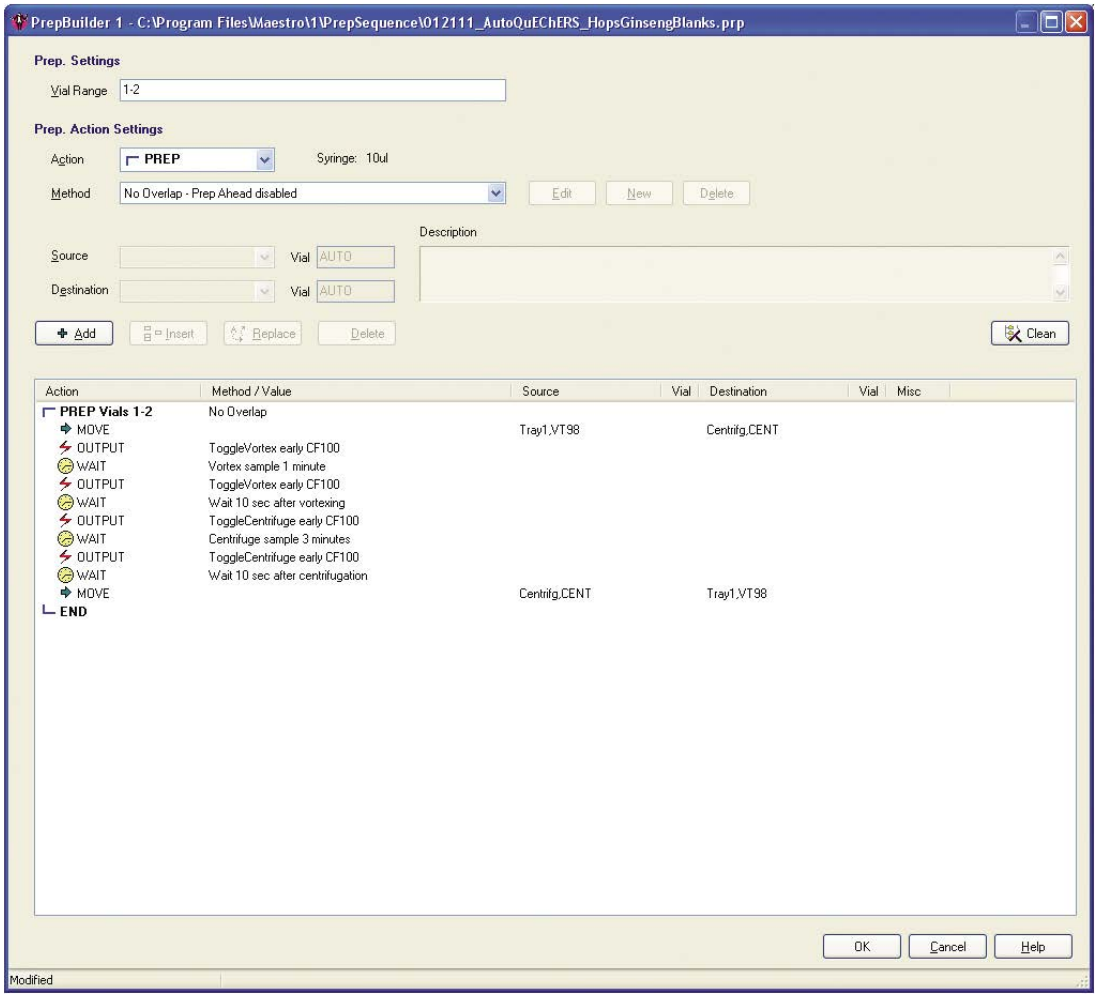

Figure 2. MAESTRO Prep Sequence for automated QuECHERS clean-up.

Sample Introduction. A 10 µL GERSTEL Automated TDU-Liner Exchange (ATEX) syringe was used for sample introduction. The ATEX syringe has a gripping mechanism, which allows it to move thermal desorption tubes into and out of the thermal desorption unit. Clean, empty TDU tubes with micro-vials are placed in the VT-98t tray of the autosampler. A clean tube is placed in the TDU. The syringe then aspirates a 10 µL aliquot of a sample from a 2 mL autosampler vial in a VT-98 tray and injects it into the micro-vial in the TDU through a septum in the TDU transport adapter. The acetonitrile is evaporated and vented through the TDU split vent, leaving the analytes and non-volatile matrix components in the micro-vial. After the solvent is evaporated, the TDU split vent is closed, and the TDU is heated to transfer the analytes into the GC inlet. The non-volatile sample components are left in the TDU micro-vial, which is removed after the injection and replaced with a clean tube and micro-vial, which are used for the next sample.

Results and Discussion

Figure 3 shows a photograph of spinach extracts before and after dSPE cleanup. Most of the green color is effectively removed from the sample. Figure 3A shows a clean micro-vial next to one used for injection of a spinach extract.

Figure 3a. Clean micro-vial and micro-vial used for spinach extract.

Figure 3. Photo of spinach extracts before and after dSPE clean-up.

Even after dSPE cleanup, there is a significant amount of chlorophyll left in the extract that would build up and contaminate the GC inlet liner if injected directly, resulting in an increased maintenance work-load and a deterioration in performance. The ATEX sample introduction technique helps to reduce inlet maintenance by keeping non-volatile sample components from reaching the inlet. ATEX also enables lower analyte detection limits by providing a means to inject samples of 10 µL or greater.

Figure 4 illustrates the effectiveness of dSPE cleanup of a ginseng extract. A significant reduction in the size of interfering peaks eluting in the 17-21 min region of the total ion chromatogram (TIC) can be seen. These peaks are mainly fatty acids from the sample.

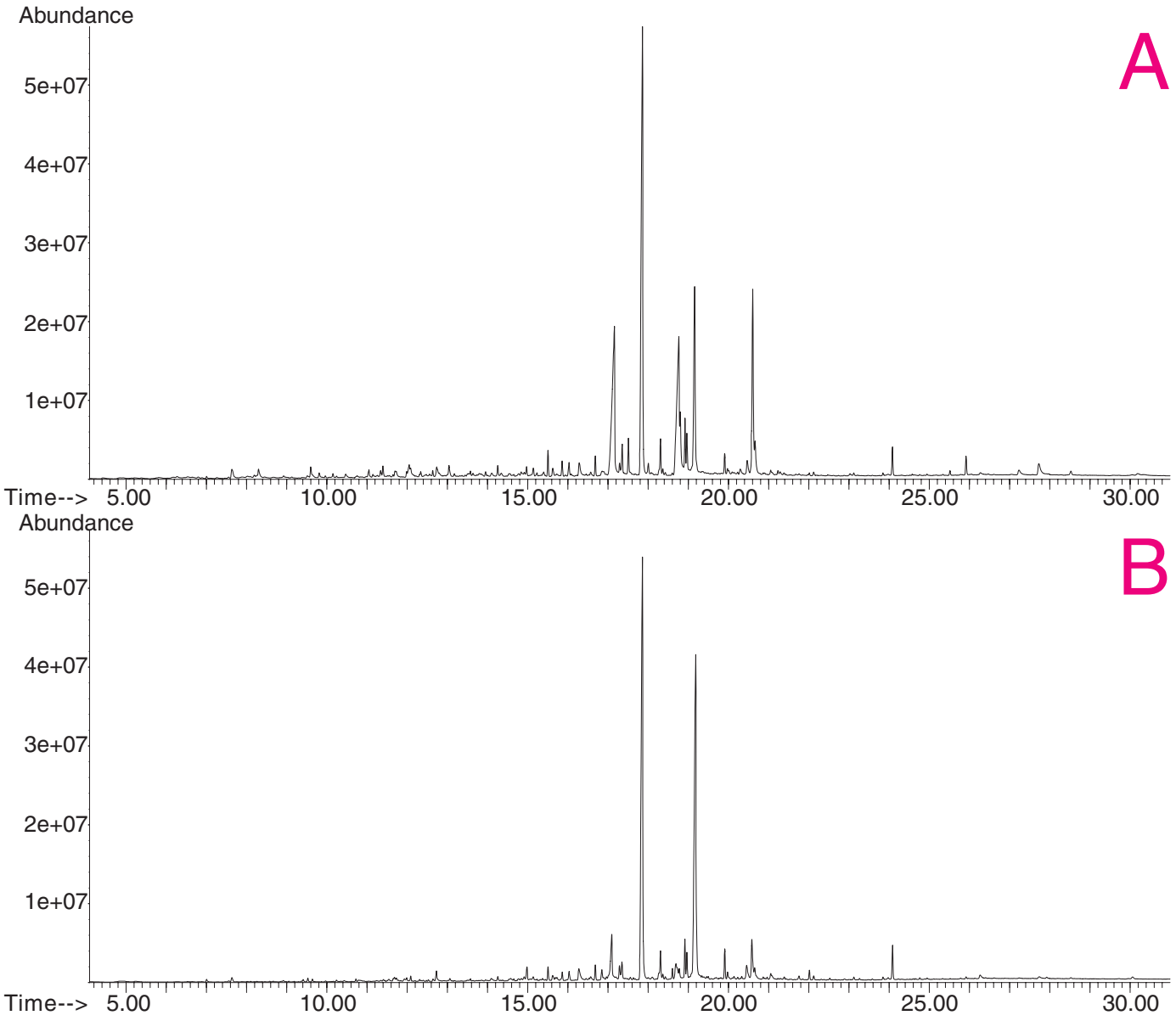

Figure 4. Chromatograms showing a blank ginseng extract before (A) and after (B) clean-up.

Figure 5 shows overlay TICs (SIM data) of a blank hazelnut extract and one spiked with pesticides at 50 ppb. The peak numbering in the figure corresponds with the pesticide numbering in Table 1. Fifteen pesticides were determined in this study.

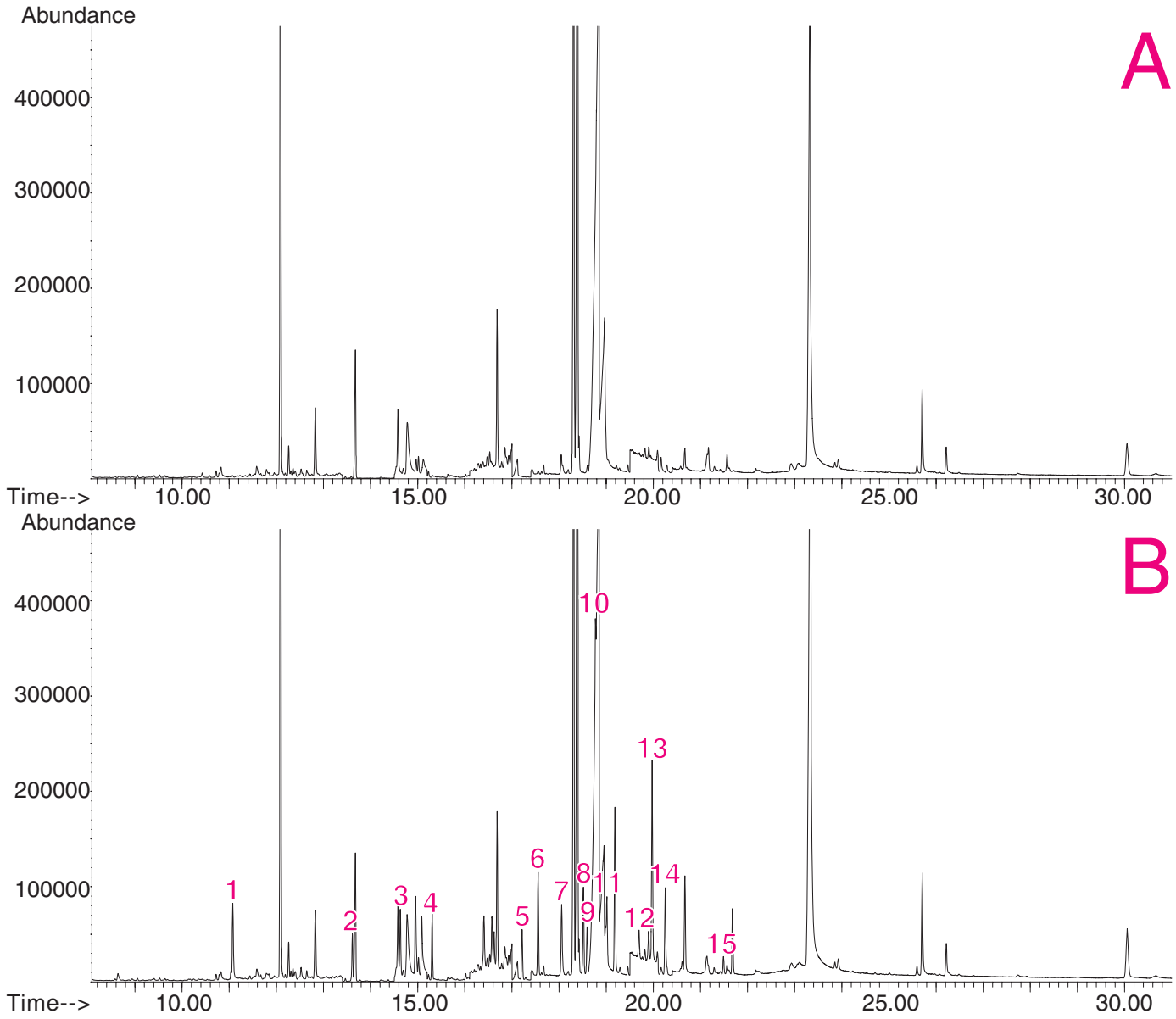

Figure 5. Chromatograms showing blank (A) and spiked hazelnut (B) extracts.

Table 1. Ginseng precision results.

	RT [min]	Compound	RSD [%]
1	11.08	Mevinophos	16.1
2	13.61	Ethoprofos	15.8
3	14.64	Demeton S	2.21
4	15.32	Diazinon	14.7
5	17.22	Chlorpyrifos	9.79
6	17.56	Trichloronate	7.74
7	18.06	Heptachlor Epoxide	11.2
8	18.52	Trans-Chlordane	8.87
9	18.58	Tetrachlorvinphos	2.20
10	18.78	cis-Chlordane	8.27
11	19.01	Prothiofos	4.49
12	19.69	Endrin	4.23
13	19.98	4,4‘ DDD	2.23
14	20.26	Sulprofos	2.60
15	21.49	Endrin Ketone	3.85

Figures 6 and 7 show the TICs (SIM data) for spiked orange and spinach extract, respectively. The target compounds are easily detected in both sample types.

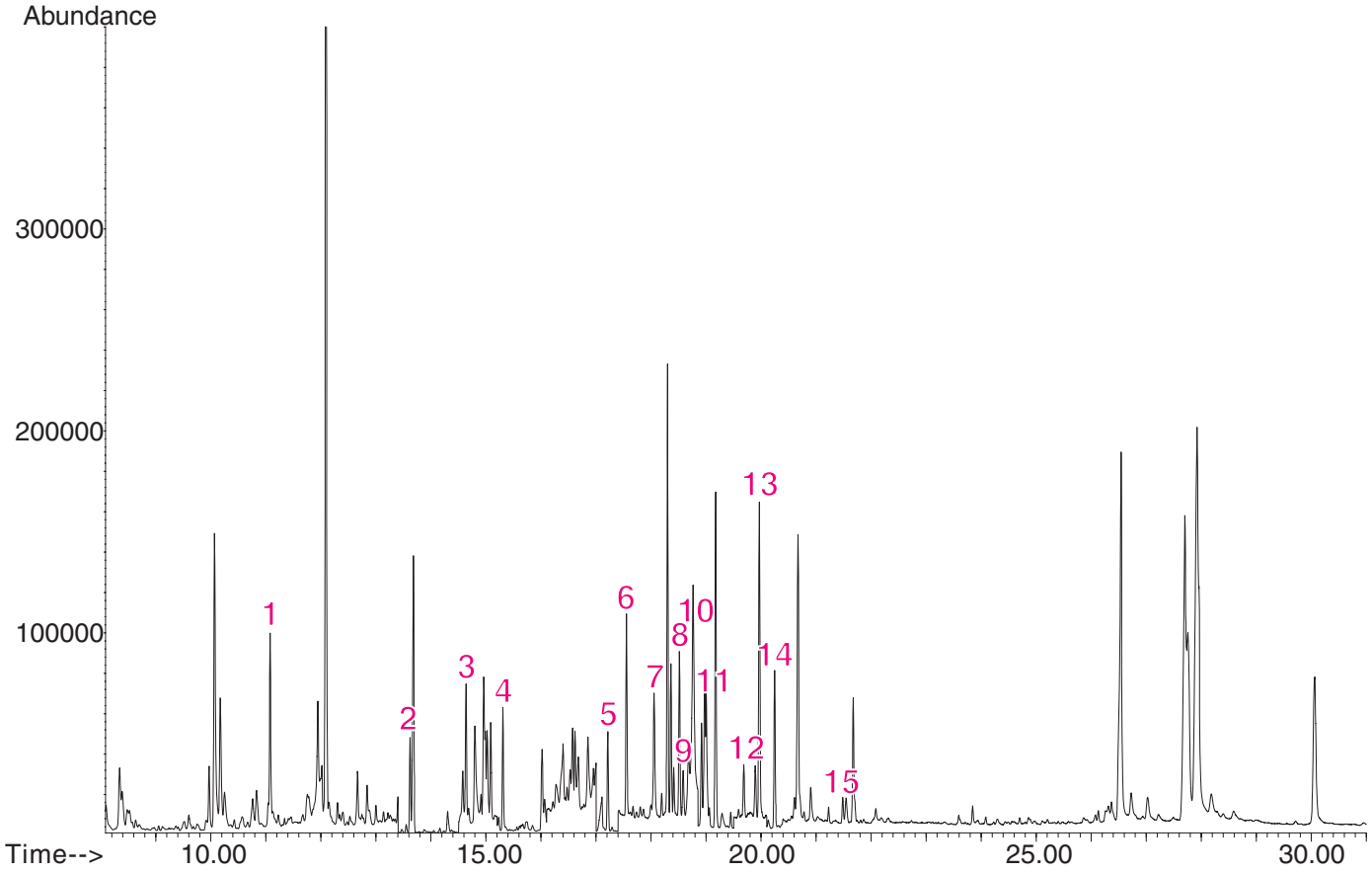

Figure 6. Chromatogram of a spiked orange extract after cleanup.

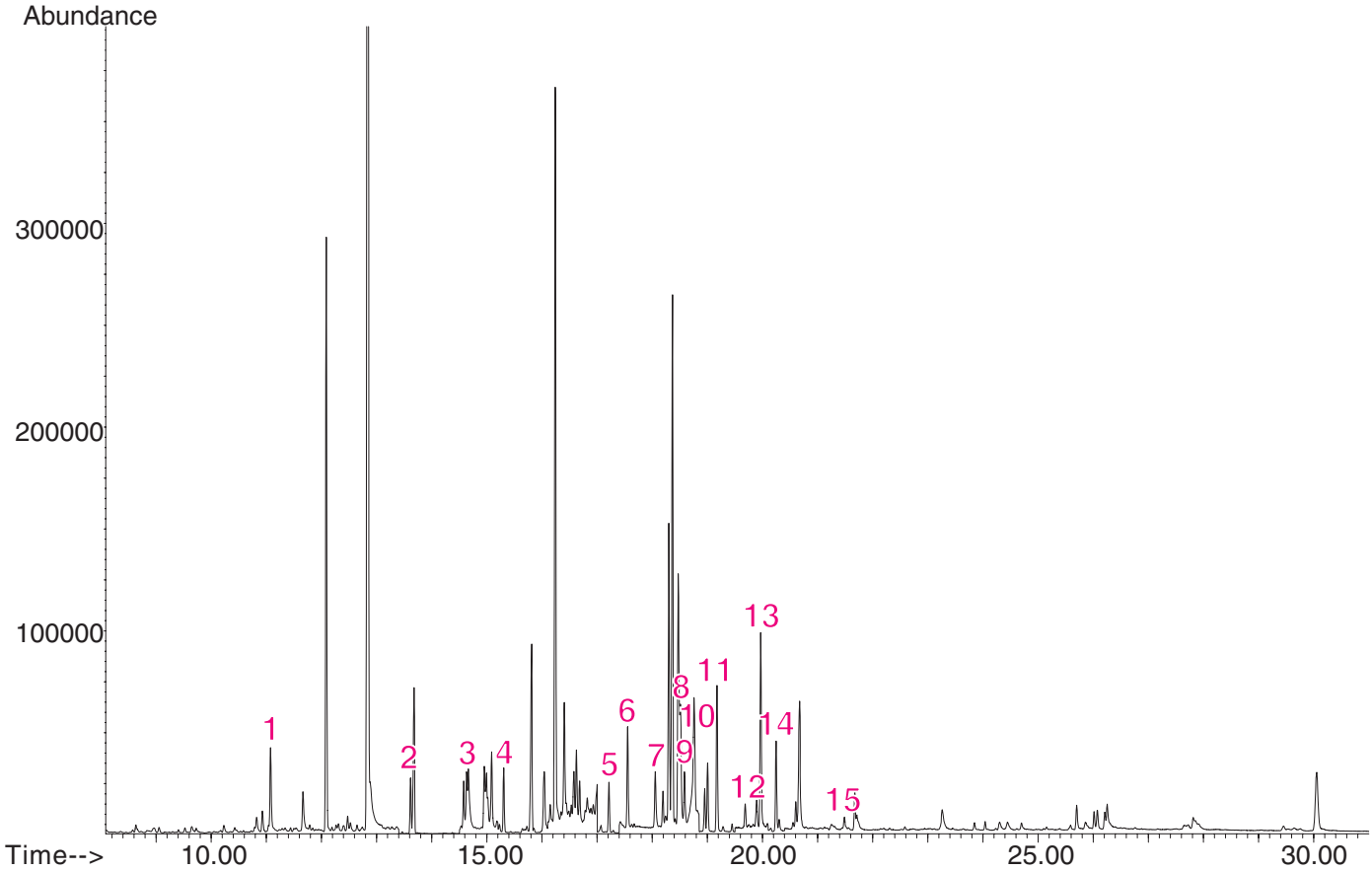

Figure 7. Chromatogram of a spiked spinach extract after cleanup.

The recoveries for the pesticides in the four extract types are listed in Table 2. The recoveries are good showing that the combination of automation and dSPE does not adversely affect detection and quantitation of these analytes.

Table 2. Pesticide recoveries, spike level = 50 ppb.

	Compound	Ginseng	Orange	Spinach	Hazlenut
1	Mevinphos	58.4	79.1	105	69.3
2	Ethoprofos	59.7	82.6	124	98.7
3	Demeton O & S	91.7	92.0	80.5	93.8
4	Diazinon	63.2	81.0	92.9	63.8
5	Chlorpyrifos	60.8	86.6	92.3	99.6
6	Trichloronate	55.6	83.3	92.7	127
7	Heptachlor Epoxide	52.3	93.2	91.0	100
8	trans-Chlordane	60.1	93.4	90.7	99.4
9	Tetrachlorvinphos	129	38.3	76.8	68.2
10	cis-Chlordane	62.2	93.5	90.3	102
11	Prothiofos	85.0	162	90.4	101
12	Endrin	79.7	105	86.0	110
13	4,4‘ DDD	100	78.4	89.8	90.1
14	Sulprofos	110	87.5	87.9	98.8
15	Endrin Ketone	185	134	96.4	166

Figure 8 shows overlay TICs (SIM data) of three replicate injections of the ginseng extract. The precision of the analysis was good. The results are listed in Table 1. The % RSDs ranged from 2-16 %.

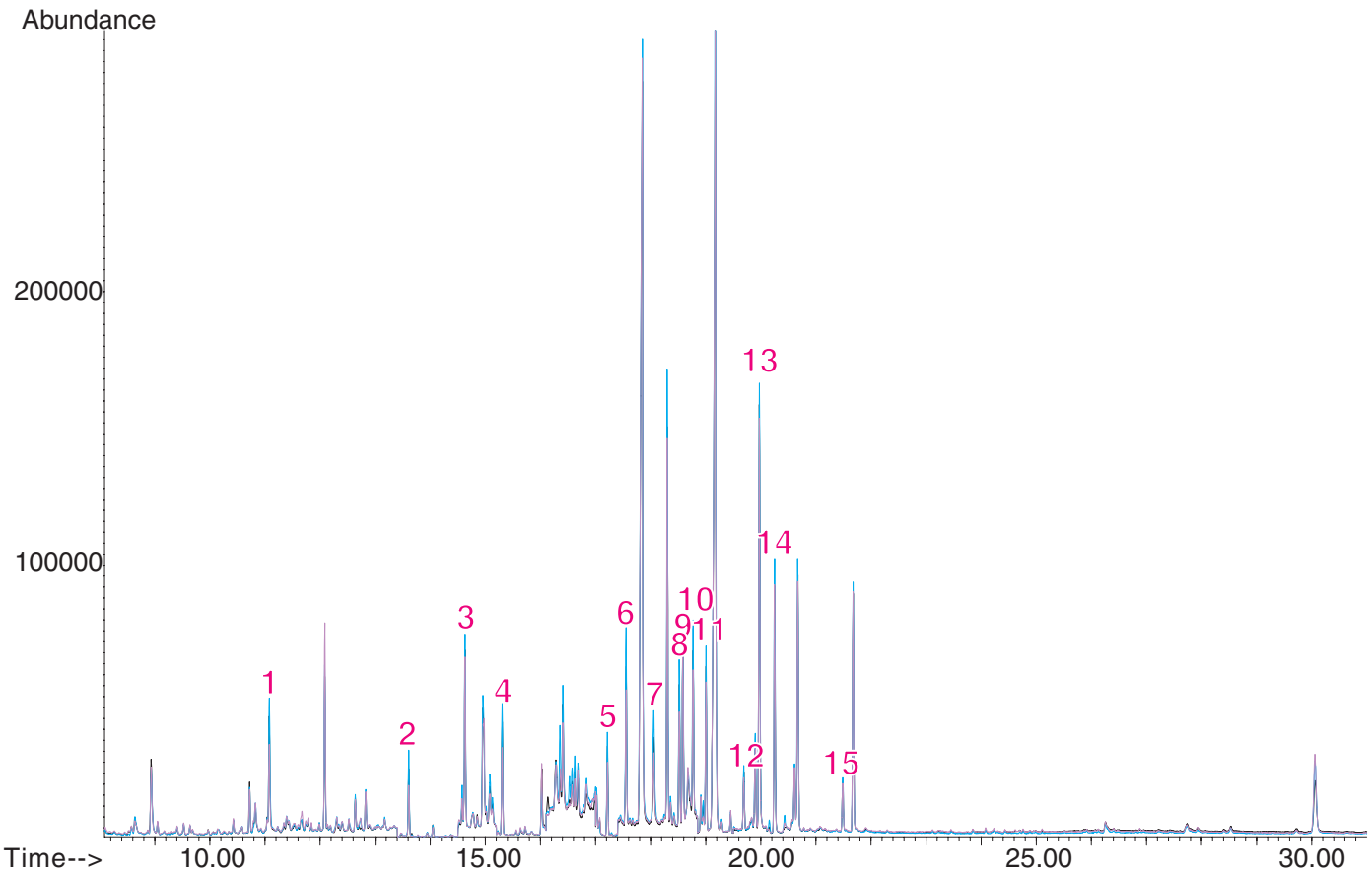

Figure 8. Overlay of ginseng replicates.

References

The authors would like to thank Jon Wong, of the US FDA, for providing QuEChERS extracts used in this study.

Conclusions

This study demonstrates the feasibility of automating dSPE cleanup and centrifugation of QuEChERS type extracts using the GERSTEL MultiPurpose Sampler (MPS) equipped with an Anatune CF-100 centrifuge. Combining this clean-up procedure with matrix elimination using ATEX injection results in improved detection limits for pesticides while reducing the amount of inlet maintenance required. Automation of this part of the process improves sample throughput while reducing both the number of manual steps required and analyst exposure to potentially hazardous solvent. GERSTEL MAESTRO software PrepAhead helps to ensure that all samples are handled in exactly the same way for consistent and uniform sample analysis and that they are ready “just in time” for introduction to the GC/MS system when it becomes ready for the next analysis. Optimized synchronization helps ensure maximum throughput and best possible system utilization.

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