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Automated Disposable Pipette Extraction of Pesticides from Fruits and Vegetables

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Abstract
Disposable Pipette Extraction (DPX) has been shown to be a rapid, efficient and reproducible method for performing solid-phase extraction (SPE) of pesticides from fruits and vegetables. DPX is a unique SPE method because the solid phase sorbent is contained inside a disposable pipette tip and is mixed with sample solutions. This mixing allows for the use of less solid phase sorbent material and results in faster extractions. Elution can be performed using small amounts of solvent, effectively providing a concentration step. Hence, solvent evaporation is not required for many applications such as pesticide analyses.

Without the need for centrifugation or solvent evaporation, DPX methods can be readily automated and the resultant eluents directly injected into a gas or liquid chromatograph. The analyst is only required to initially process the samples to be analyzed and place the sample solutions into corresponding vials. The rest of the sample preparation can be automated, including the injection of the eluent into the analytical instrument.

In this study, samples of fruits and vegetables (spiked with various pesticides) are blended with organic solvent, and the samples are then filtered and placed into sample

vials. A GERSTEL MultiPurpose Sampler (MPS 2) is used to perform the DPX extractions and inject into the chromatographic instrument. Various types of fruits and vegetables are included in this study, and numerous pesticides are analyzed by this method including organophosphates, organochlorines, pyrethroids, and fungicides.

Introduction

The determination of pesticide levels in fruits and vegetables is very important in the field of food safety. To ensure that levels of toxic pesticides are below tolerance levels and are safe to ingest, routine and comprehensive testing must be performed. The most time-consuming part of this analysis is sample preparation.

Recently, there has been a great amount of interest in the QuEChERS method [1-2]. QuEChERS is an acronym for quick, easy, cheap, effective, rugged, and safe. The method concentrates on removing fatty acid components rather than extracting and isolating the pesticides. The main advantage of this method is that it is comprehensive, providing very high recoveries for most pesticides. The main disadvantage of the method is that the resultant extracts are relatively „dirty“. As a result there have been numerous modifications and variations of the original method. Some of the different methods include the use of dispersive tubes or cartridges with various amounts of $MgSO_4$ and PSA sorbent that may contain graphite. Another challenge is that the method requires the evaporation of over 15 mL of organic solvent for concentration and solvent exchange for every sample processed.

In order to provide high throughput testing and ensure safe food products, it is advantageous to automate sample preparation. In this paper, we present data for the analysis of a diverse group of pesticides using Disposable Pipette Extraction (DPX), fully automated using the GERSTEL MPS 2 (Figure 1). DPX is a dispersive solid phase extraction (SPE) method which is readily automated. It requires only small solvent volumes, and the process is very fast. Compared to the QuEChERS method, DPX is much faster, provides as high recoveries for most pesticides, and requires no solvent evaporation.

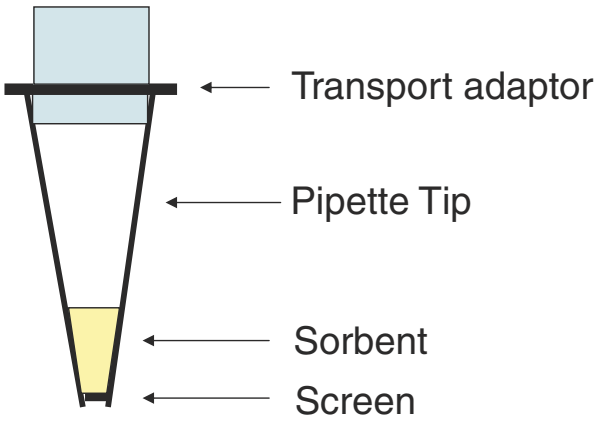

Figure 1. A schematic diagram of a DPX tip.

Experimental

Instrumentation. The data was collected based on automated DPX extractions using an MPS 2 autosampler (GERSTEL) in combination with a 6890N GC and a 5975 MSD (Inert XL) (Agilent).

Analysis conditions.

Inlet/Inj.:	S/SL; 250°C; 2 µL
Column:	30 m DB-17MS (J&W)
	d_i = 0.25 mm d_f = 0.25 µm
Pneumatics:	He, constant flow = 1 mL/min
Oven:	80°C (1 min); 20°C/min;
	280°C (8 min)

SIM analysis for organochlorine pesticides.

group	time [min]	ions [m/z]	dwell time [ms]
1	7.8	181, 219	100
2	9.0	100, 272, 286, 288	50
3	9.45	66, 263	100
4	9.8	81, 353	100
5	10.2	79, 195, 241, 246, 263, 318	30
6	10.75	81, 165, 235, 263	50
7	11.05	67, 165, 229, 235, 272, 345	30
8	11.5	227, 228	100

SIM analysis for organophosphorous pesticides.

group	time [min]	ions [m/z]	dwell time [ms]
1	4.8	109, 185	100
2	6.0	127, 192	100
3	7.5	158, 242	100
4	7.9	61, 75, 88, 260	50
5	8.4	88, 179, 274, 304	50
6	9.0	109, 125, 263, 285, 286, 288	30
7	9.4	109, 125, 197, 270, 278, 297	30
8	10.0	329, 331	100
9	10.4	113, 209, 267, 298	50
10	10.7	156, 293, 308, 322	50
11	12.6	226, 322	100

Initial Sample Preparation.

- Add 15 g blended sample (carrots) and 15 mL acetonitrile into 50 mL centrifuge tube
- Add 1.5 g NaCl and 6.0 g $MgSO_4$
- Cap and shake tube vigorously for a few minutes; let stand app. 10 minutes
- Centrifuge at 3,000 rpm for 10 minutes
- Transfer 1 mL ACN for the manual DPX method and 0.5 mL ACN for the automated DPX method into a clean labeled test tube

Manual DPX extraction steps.

1. Add 2.4 mL deionized water and 0.8 mL saturated NaCl to 1 mL of the sample solution and vortex mix
2. Using the syringe device, aspirate the entire sample solution into the 5 mL DPX-RP tip and app. 5 mL of air to mix the solution with air bubbles
3. Wait approximately 30 seconds
4. Dispense back into the test tube
5. Aspirate 0.5 mL of DI water (from a test tube) and app. 3 mL of air to mix the solution
6. Wait 10 seconds and dispense to waste
7. Remove the syringe device and add 0.7 mL of 50/50 hexane-ethyl acetate
8. Re-attach the syringe device and dispense the eluent into a small labeled test tube
9. Remove the bottom layer (app. 100 µL) of water using a disposable Pasteur pipette (or pass the solution through a small amount of Na_2SO_4 using a Pasteur pipette with a plug of glass wool to remove the water)
10. Add 50 µL of external standard (methyl chlorpyrifos) into the eluent
11. Transfer the final eluent (app. final volume of 0.5 mL) into a GC vial insert and place into a GC vial
12. Cap, place on the autosampler and inject into the GC

Automated DPX extraction.

1. Add 1.2 mL DI water and 0.4 mL saturated NaCl to 0.5 mL sample solution in a small test tube and vortex mix (total volume of 2.1 mL)
2. Place sample(s) on sample vial rack of GERSTEL MPS 2
3. Run DPX extraction method for pesticides (using 3 multiple extractions--see GERSTEL automated procedure below)
4. Collect vials with final eluents

GERSTEL Automated Procedure.

1. Draw in 0.5 mL of 30% acetonitrile in DI water
2. Pick up 1 mL DPX-RP tip with attached transport adaptor (patent pending)
3. Dispense solution onto top of DPX-RP tip to wet the hydrophobic sorbent
4. Go to sample tube and aspirate 0.8 mL of sample solution
5. Lift DPX tip out of solution, slowly aspirate 1.5 mL of air to mix the sorbent and the solution
6. Wait 20 seconds, then dispense sample solution to another vial (or waste container)
7. Repeat the extraction of an additional 0.8 mL of sample solution repeating steps 4-6.
8. Complete the extraction of the sample solution by repeating steps 4-6 again.
9. Remove DPX tip from syringe, aspirate 0.25 mL of water into syringe and 1.5 mL of air
10. Pick up DPX tip and dispense water and air through the top of DPX tip into waste
11. Remove DPX tip, aspirate 0.35 mL of 50/50 hexane-ethyl acetate and 1.5 mL of air into syringe.
12. Dispense elution solvent through top of DPX tip into vial providing a final volume of app. 0.25 mL of organic solvent (after removing immiscible water layer)

Sample loading for GC analysis (off-line).

1. Collect vials with eluents and remove the bottom layer (app. 50 µL) of water using a disposable Pasteur pipette (or pass the solution through a small amount of Na_2SO_4 using a Pasteur pipette with a plug of glass wool to remove the water)
2. Add 25 µL of external standard (methyl chlorpyrifos) into the eluent
3. Transfer the final eluent (app. final volume of 0.5 mL) into a GC vial insert and place into a GC autosampler vial
4. Cap the vial and place it in the autosampler for injection into the GC

Results and Discussion

The method used for the initial sample preparation was the same as outlined in the QuEChERS method. In this method, the sample is „shaken“ in a ratio of 1: 1 with acetonitrile in order to achieve good sensitivity. The addition of salt is used to separate the acetonitrile layer from the water layer. The concept of QuEChERS is to then remove the sample matrix components. The DPX method presented here is based on reversed phase mechanisms (similar to common HPLC methods), and the goal is to extract and isolate the analytes of interest onto the sorbent of the DPX tip. This is facilitated by adding water and salt to reduce the amount of organic solvent needed.

By incorporating „mixing“ of the sorbent with the extract, very high extraction efficiencies can be achieved using only a small amount of sorbent. Performing the extraction with a small amount of sorbent in return means that much less solvent is required for elution, eliminating the need for solvent evaporation. Without solvent evaporation, the extractions can be performed much faster and are more environmentally friendly.

Table 1 shows results from the analysis of a diverse group of pesticides (including organochlorine, organophosphate and fungicides) manually extracted using 5 mL DPX-RP tips. The results are exceptional for these pesticides, approaching around 100% recovery for most of them. The hexane-ethyl acetate used for elution is immiscible with water, which is therefore separated out. The solvent is an excellent „keeper“ solvent (little to no sample degradation) that is ideally suited for GC analysis with various detectors. This means that both the solvent exchange and the concentration steps are accomplished in one automated step.

Table 1. Statistical results for the analysis of a diverse group of pesticides spiked in carrot matrices and extracted manually using 5 mL DPX-RP tips.

Pesticide	% Recovery	RSD (4)
HCB	91.7	4.5
Chlorothalonil	111.9	4.9
Chloropyrifos	103.2	3.5
Endosulfan-I	99.2	4.4
p,p‘-DDE	89	1
Endosulfan-II	99.9	2.9
Ethion	98.7	3.6
Endosulfan Sulfate	103.8	1.7
Phosmet	108	0.2
Azinphos-Methyl	107.1	1.5
trans-Permethrin	81	8.3

Another noteworthy observation is that the described DPX method provides efficient and reproducible recovery of chlorothalonil (Figure 2). This analyte is apparently difficult to determine using the QuEChERS method, making the DPX method a viable alternative for this analyte in particular.

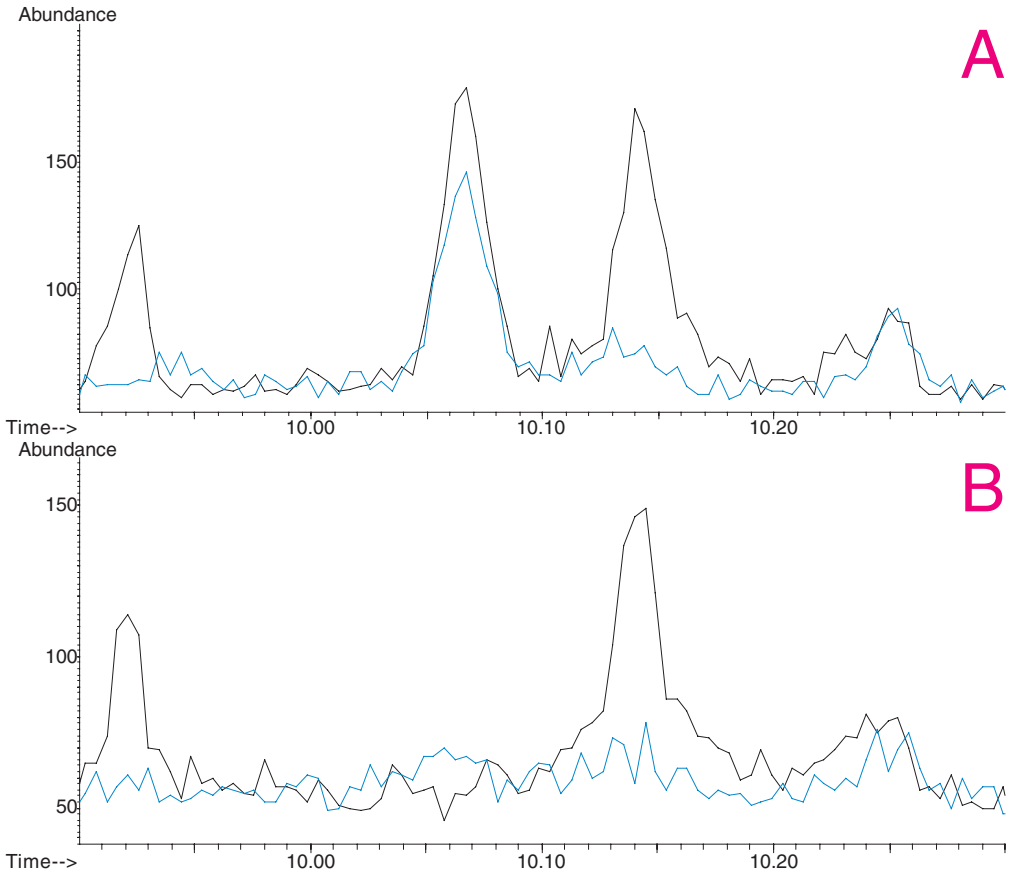

Figure 2. Extracted ion chromatograms (ions m/z = 266 and 268) of 10 ppb chlorothalonil spiked in carrots (A) and blank carrot matrix (B).

Table 2. Linear calibration and regression data for organophosphate and organochlorine pesticides. The calibrations are based on 6 standards at 0.1 ppm; 2 standards at 0.2, 0.5 and 1.0 ppm; and 6 standards at 2.0 ppm. Methyl chlorpyrifos was used as external standard.

Pesticide	Linear Regression [R^2]	
	Carrot	Orange
Aldrin	0.9979	0.9984
α-BHC	0.9988	0.9988
β-BHC	0.9991	0.9986
δ-BHC	0.999	0.9983
γ-BHC	0.9991	0.9987
4,4'-DDD	0.9986	0.9968
4,4'-DDE	0.9955	0.9981
4,4'-DDT	0.9974	0.9957
Dieldrin	0.9991	0.999
Endosulfan I	0.999	0.9987
Endosulfan II	0.9974	0.9984
Endosulfan sulfate	0.999	0.9954
Endrin	0.9992	0.9986
Endrin aldehyde	0.998	0.9987
Heptachlor	0.9984	0.9984
Heptachlor epoxide	0.9978	0.9986
Methoxychlor	0.9982	0.9965
Bolstar	0.9977	0.9983

Pesticide	Linear Regression [R^2]	
	Carrot	Orange
Chlorpyrifos	0.999	0.9996
Coumaphos	0.9976	0.9984
Demeton-S	0.9972	0.9995
Diazinon	0.9996	0.9997
Dichlorvos	0.9984	0.9967
Disulfoton	0.9995	0.9978
Ethoprophos	0.9997	0.9992
Fenthion	0.999	0.9995
Fensulfothion	0.9977	0.999
Merphos	0.9991	0.9959
Methyl parathion	0.9991	0.9978
Mevinphos	0.9945	0.9989
Phorate	0.9996	0.999
Ronnel	0.9993	0.9995
Stirofos	0.9992	0.9956
Tokuthion	0.9988	0.9983
Trichloronat	0.9994	0.9993

To automate the described DPX method using the GERSTEL MPS 2 autosampler, the method had to be adapted to 1 mL DPX tips. Starting from 0.5 mL of acetonitrile extract, water and salt had to be added to the acetonitrile sample solution resulting in a final volume greater than 1mL. In order to perform this extraction with 1 mL DPX tips, multiple extractions were performed. In Figure 3, the MAESTRO software method for the GERSTEL MPS 2 is shown. One advantage of DPX is that it does not require conditioning steps. The first step in the DPX method is to aspirate „wetting solution" into the syringe and dispense it onto the top of the DPX tip to facilitate mixing of sorbent and sample. Mixing is performed by simply aspirating air bubbles. The DPX tip is then transported to the sample vial and a user defined volume of the sample solution is aspirated into the DPX tip, followed by aspiration of air to facilitate mixing. This type of mixing is much simpler to automate than shaking or vortex mixing. By mixing the sample solution with the sorbent, the extraction efficiency is based on the equilibration time which is easily controlled. This is another advantage of DPX compared to other SPE methods in which the flow rate of the solution through the sorbent dictates the efficiency, which is usually not easy to control. After a user defined equilibration time, the solution is dispensed either into another vial or to waste. The sample solution can be readily retained for further analysis if necessary.

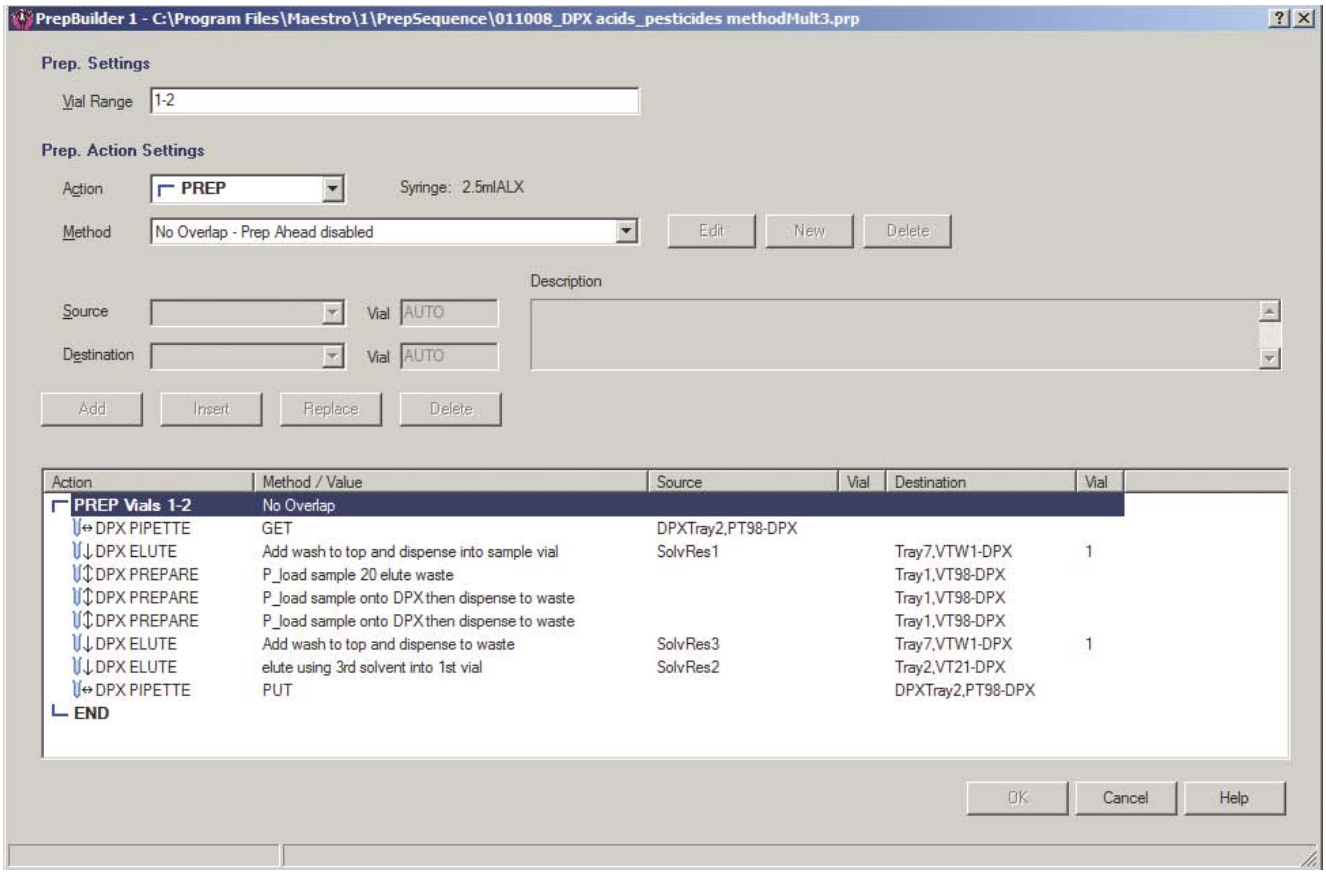

Figure 3. MAESTRO software PrepBuilder page for the DPX method.

The extraction steps were repeated twice in order to extract a total of 2.1 mL sample solution. The sorbent was washed by adding water to the top of the DPX tip and dispensing it to waste. The final automated DPX method required just a small volume of elution solvent to give a final volume of approx. 0.25 mL. External standard was added to compensate for variations in eluent volumes and provide accurate quantification.

Table 3 shows results for the analysis of 0.5 ppm organochlorine pesticides. The data shows exceptional recoveries, close to 100% for most compounds. These results are very similar to those obtained using the manual extraction methods. Detection limits for most of these pesticides were found to be lower than 50 ppb.

Table 3. Statistical results for the analysis of organochlorine pesticides spiked in carrot matrices and extracted using GERSTEL automation, based on 1 mL DPX-RP tips.

OC Pesticides	ret. time [min]	Ion [amu]	Recovery [%]
α-BHC	8.185	181	102.5
β-BHC	8.48	181	111.1
γ-BHC	8.547	181	103.1
δ-BHC	8.812	181	100.6
Heptachlor	9.297	100	96.5
Aldrin	9.667	66	92.7
Heptachlor epoxide	10.056	353	101.9
Endosulfan I	10.42	195	100.5
p,p'-DDE	10.581	246	89.0
Dieldrin	10.667	79	100.6
Endrin	10.891	263	100.3
p,p'-DDD	10.985	235	95.0
Endrin aldehyde	11.149	67	88.6
Endosulfan sulfate	11.369	272	101.5
p,p'-DDT	11.335	235	92.1
Methoxychlor	11.831	227	102.5

Table 4 shows preliminary results for the analysis of 0.5 ppm organophosphate pesticides. The data is very good for most of the pesticides. Unfortunately, low recoveries were achieved for the most polar pesticides, dichlorvos and mevinphos. Nevertheless, the DPX-RP method provides about 90% recovery for more than 80% of the organophosphate pesticides in the test. To improve recovery of polar compounds, it may be necessary to incorporate a normal phase mechanism using a polar sorbent or an anion exchange mechanism using an anion exchange resin. Another option is to utilize a DPX-QuEChERS tip which focuses on removing the sample matrix components rather than isolating these most polar compounds.

Table 4. Statistical results for the analysis of organophosphate pesticides spiked in carrot matrices and extracted using GERSTEL automation, based on 1 mL DPX-RP tips.

OP Pesticides	ret. time [min]	Ion [amu]	Recovery [%]
Dichlorphos	5.23	109	39.2
Mevinphos	6.474	127	3.3
Ethoprophos	7.73	158	83.1
Phorate	8.094	75	92.0
Demeton-S	8.262	88	62.1
Diazinone	8.632	179	91.4
Disulfoton	8.722	88	89.2
Parathion-methyl	9.188	109	105.5
Ronnel	9.328	285	90.9
Fenthion	9.637	278	90.8
Chlorpyrifos	9.647	197	88.0
Trichloronat	9.795	109	87.7
Stirofos	10.324	329	94.5
Tokuthion	10.51	267	84.3
Merphos	10.544	209	84.3
Fensulfothion	10.932	293	64.9
Bolstar	11.119	322	92.0

Figure 4 shows typical chromatograms of a DPX carrot extract blank spiked with 0.5 ppm organochlorine (A) and organophosphate (B) pesticides. Identified compounds are listed in the corresponding Tables 3 and 4.

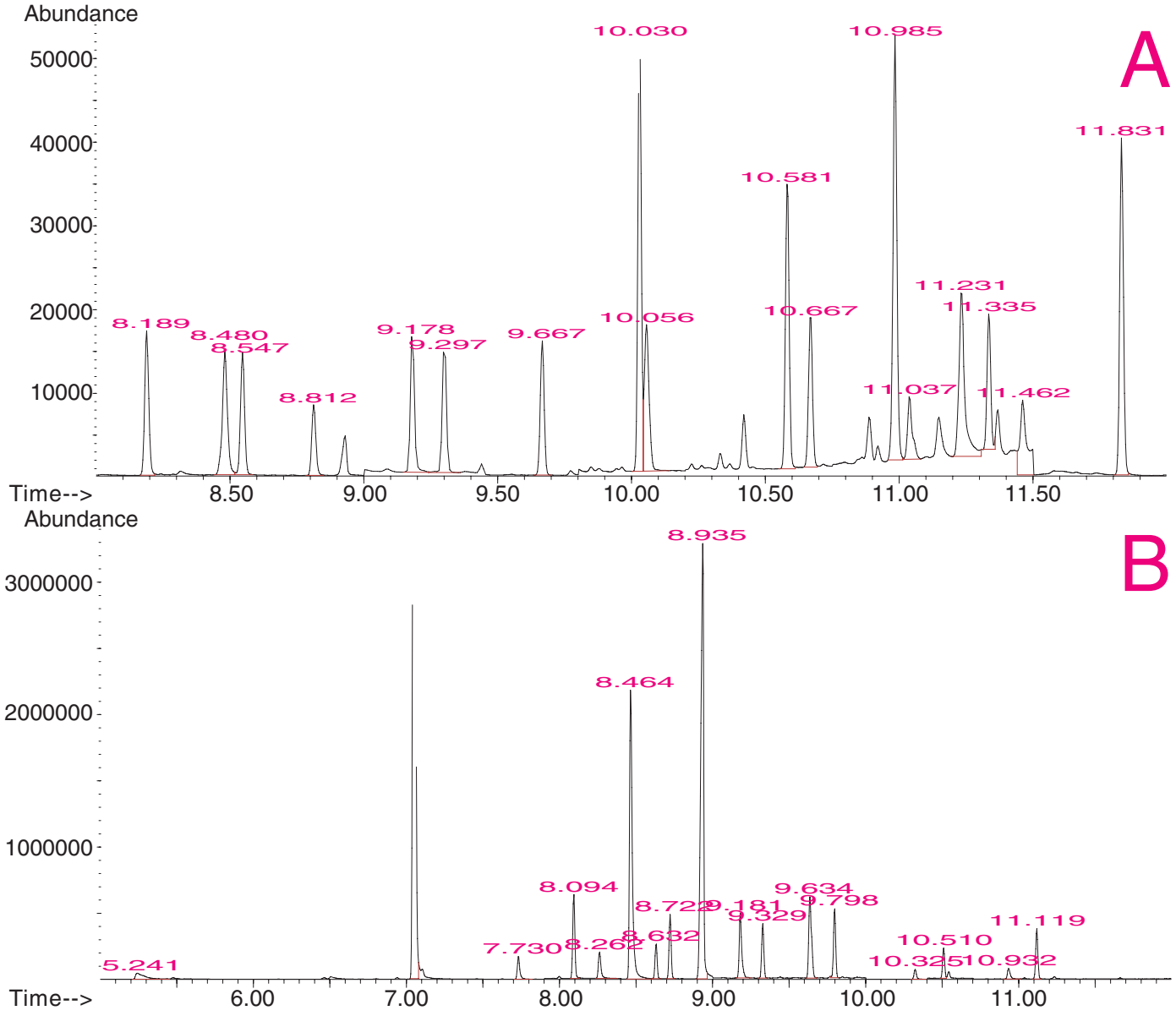

Figure 4. GC/MS chromatograms (HP-5 column) for carrot extracts analyzed by automated DPX spiked with 0.5 ppm organochlorine pesticides (A) and spiked with 0.5 ppm organophosphate pesticides (B).

Conclusions

- DPX is a rapid sample preparation technique wich is readily automated using the GERSTEL MPS 2; the MAESTRO software enables easy set up of DPX methods.
- High recoveries are achieved for most pesticides, including organochlorine and organophosphate pesticides, as well as for many fungicides such as chlorothalonil.
- The DPX technique requires only small volumes of solvent for elution. Further concentration steps such as solvent evaporation are normally not required. The DPX method is „environmentally friendly“.
- Future work will focus on using the GERSTEL CIS inlet incorporating large volume injections, and thereby enabling the analyst to reach the required limits of detection based on smaller sample volumes. In combination with DPX, smaller sample volumes can be extracted even faster.
- Automated DPX extractions enable high-throughput analysis and screening of food samples for the presence of pesticides.

References

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