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Univariate Optimization of Automated Evaporation and Concentration Combined with Large Volume Injection to Increase the Sensitivity of Pesticide Analysis

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Keywords

Pesticides, Large Volume Injection, Automated Evaporation and Concentration, Gas Chromatography-Mass Spectrometry, PTV Inlet, Cooled Injection System.

Abstract

Pesticides are frequently determined in various food and beverage types and in environmental samples. A common sample preparation method used is known as QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe). The method involves salting-out liquid-liquid extraction with acetonitrile and subsequent clean-up of the extract using dispersive solid-phase extraction. Usually, 1 – 2 µL of the extract is then injected into the analytical instrument for analysis. To further improve the sensitivity, automated evaporative concentration of the extract using the GERSTEL ^{m}VAP can be combined with Large Volume Injection in the GERSTEL CIS 4 inlet and GC-MS analysis. In this work, various parameters of automated evaporation and concentration using ^{m}VAP and Large Volume Injection with CIS 4 were studied and optimized on pesticides-spiked acetonitrile in a univariate approach.

Introduction

In many applications, trace amounts of analytes may hardly be detected when using a standard 1 – 2 µL splitless injection and conventional gas chromatography (GC)-mass spectrometry (MS) analysis. One such application is the analysis of pesticide residues in food, beverages, and environmental samples. Some users may turn to detectors with better detection capability such as triple-quadrupole MS or quadrupole Time-of-Flight (qTOF) MS. These options, however, can be very costly and sometimes still inadequate even though pesticide residue analysis is streamlined by well-established QuEChERS methods. In those situations, users can consider improving sample preparation techniques to further enhance sensitivity instead. One straightforward technique is to inject a greater sample size into the GC system, known as Large Volume Injection (LVI). LVI-GC-MS is readily performed given the right modern analysis systems.

Routine LVI-based analysis is achievable when using the GERSTEL Cooled Injection System (CIS 4) inlet, a programmable temperature vaporizer (PTV)-type inlet, as shown in Figure 1. The large-volume sample (i.e., 2 – 1000 µL) is introduced slowly into a cold inlet in solvent-venting mode. A continuous flow of inert gas facilitates the evaporation of the solvent and its removal through the split vent to prevent the liner from being overloaded, which can be detrimental to both the GC column and detector. Various parameters, such as initial inlet temperature, vent flow, injection speed and vent time, can be optimized to minimize the amount of solvent in the inlet while the analytes of interest are being concentrated.

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Figure 1: GERSTEL CIS 4 (a PTV-type inlet).

An additional evaporation and reconstitution step prior to LVI can be included to further concentrate the extract and/or to change to a more suitable solvent. Evaporation and concentration steps are often carried out manually off-line. Using the GERSTEL MultiPosition Evaporation Station (mVAP, as shown in Figure 2), the evaporation process can be automated and controlled through the Maestro software. GERSTEL mVAP evaporates the solvent in the sample by means of vacuum. In a previous work, GERSTEL mVAP performance was shown to be comparable with manual evaporation using a commercial station [1]. After evaporation to dryness, redissolution in a smaller volume of a user defined solvent can be specified in the method to obtain a more concentrated extract in the most suitable solvent for the analysis. These steps can improve the sensitivity of the overall analysis.

Figure 2: GERSTEL mVAP Station.

Both techniques aim to produce a higher concentration of analytes in the extract injected into the GC-MS and thus more analyte mass on column. They are relatively simple to carry out. The concentration techniques can be applied individually or as a combination. This preliminary study describes how automated evaporation and concentration using mVAP can complement LVI for enhanced sensitivity in GC-MS analysis. A series of experiments using pesticide-spiked acetonitrile were carried out to evaluate and optimize the various method parameters in a univariate approach.

Experimental

Reagents and Materials

High-performance liquid chromatography-grade acetonitrile was obtained from Merck (Darmstadt, Germany). A 31152 QuEChERS Performance Standard Kit, consisting of three ampoules of Standards A, B and C, was purchased from Restek (Bellefonte, PA, USA). Each standard is 300 µg/mL in acetonitrile:acetic acid (99.9:0.1). The standards contain a combined total of 40 pesticide compounds.

Sample Preparation

A standard solution, with a 1:1:1 composition of Standards A, B and C, was prepared. Aliquots were diluted with acetonitrile to obtain concentrations of 10 mg/L, 1 mg/L, 0.5 mg/L and 0.2 mg/L for this project. The 10 mg/L sample was used as reference standard for calculation of recoveries based on a cold splitless 1 µL injection.

Settings for LVI Optimization

A deactivated glass-wool liner was chosen for carrying out LVI. Other than providing a larger surface area for condensates, the inert packing material does not interfere with solvent-venting. In addition, the column head pressure was reduced to a low value to minimize the amount of solvent entering the column while venting.

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Using the built-in LVI calculator (Figure 3) in the Maestro software, the respective injection speeds were determined according to specific values of total flow and CIS temperature. In this experiment, the sample injected was 10 µL of a 1 mg/L standard.

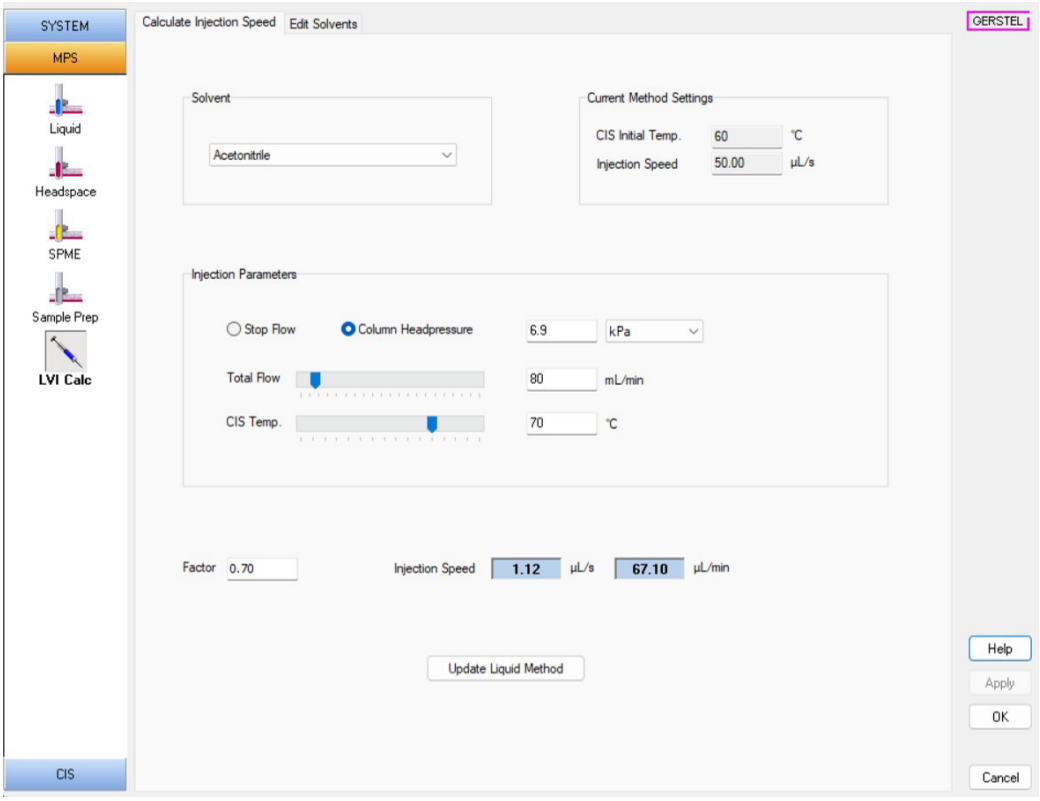

Figure 3: Screenshot of LVI Calculator in MAESTRO software.

Settings for ᵐVAP Optimization

The volume of solvent evaporated in the ᵐVAP is proportional to the elapsed time at a specific pressure and temperature. Time needed to obtain dryness under different pressures and temperatures was estimated from volume-against-time linear profiles that were separately obtained prior to experimentation (data not shown).

Each 2 mL vial contained 1000 µL of 0.2 mg/L sample. The sample was subjected to a reduced pressure of 60 mbar and maintained at a temperature of 30 °C for 20.46 min without agitation (optimized evaporation settings as shown in Figure 4). After the solvent had completely evaporated, the remaining analytes were redissolved in 200 µL of acetonitrile solvent and agitated at 500 rpm for one minute.

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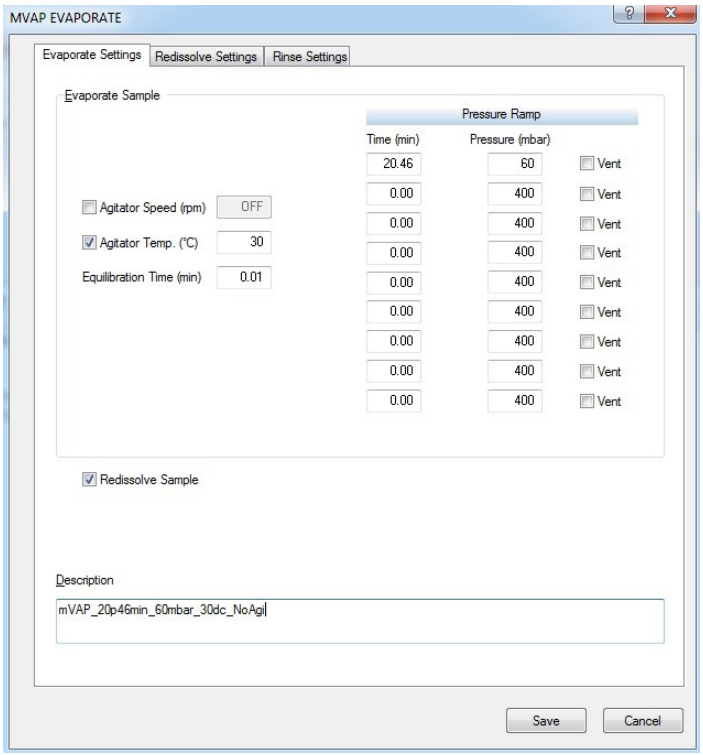

Figure 4: mVAP method parameter window. Optimized settings as shown.

Instrumentation

GERSTEL MPS robotic pro autosampler, GERSTEL MultiPosition Evaporation Station (ᵐVAP), Cooled Injection System (CIS 4) with Cryostatic Cooling Device (CCD2) and Agilent® 7890 GC/5977B MSD.

Analysis Conditions

MPS

Injection volume	10 µL
Injection speed	1.12 µL/s

CIS 4

Liner	deactivated glass wool
Pneumatics mode	solvent venting
Vent flow	80 mL/min
Vent pressure	0 kPa until 0.5 min
Splitflow	25 mL/min @ 2.3 min
Temperature	70 °C (0.5 min), 12 °C/sec to 260 °C (10 min)

GC Agilent 7890

Column	30 m DB-5MS (Agilent), d_i=0.25 mm, d_f=0.25 µm
Pneumatics	He; P_i = 68.63 kPa constant flow, 1.0 mL/min
Temperature	90 °C (5 min), 10 °C/min to 150 °C, 3 °C/min to 200 °C, 8 °C/min to 280 °C (10 min)

MSD Agilent 5977B

Scan	29 to 400 amu

Results and Discussion

Four compounds (Table 1), namely 2-phenylphenol, vinclozoline, cyprodinil and trans-permethrin, were selected as representatives to ensure coverage of the entire chromatogram and to showcase the trends observed at each stage of optimization.

Table 1: Analyte information.

No.	Analyte	B.P.[a] [°C]	Ret. Time [min]	Frag. Ions[b] [m/z]
1	Methamidophos	208.7	8.19	94, 141, 95
2	Dichlorvos	176.8	8.59	109, 185, 79
3	Mevinphos	288.7	11.66	127, 192, 109
4	Acephate	340.4	11.75	136, 94, 42
5	2-Phenylphenol	282.0	13.22	170, 169, 141
6	Omethoate	364.2	14.54	156, 110, 109
7	Dimethoate	310.3	17.59	87, 125, 93
8	γ-BHC (Lindane)	287.8	18.45	181, 219, 183
9	Chlorothalonil	350.0	19.22	266, 264, 268
10	Diazinone	315.9	19.41	179, 137, 152
11	Vinclozoline	369.9	21.82	212, 198, 285
12	Carbaryl (Sevin)	366.5	22.11	144, 115, 116
13	Metalaxyl	295.9	22.43	206, 160, 132
14	Pirimiphos Methyl	386.6	23.31	290, 276, 305
15	Methiocarb	341.3	23.35	168, 153, 225
16	Dichlofluanid	336.8	23.51	123, 167, 224
17	Malathion	385.1	23.99	125, 173, 127
18	Chlorpyrifos	*160* [c]	24.16	199, 197, 314
19	Fenthion	330.0	24.39	278, 125, 109
20	Dicofol (Kelthane)	225.0	24.81	139, 250, 111
21	Cyprodinil	405.9	25.99	224, 225, 210
22	Thiabendazole	446.0	26.59	201, 174, 202

Table 1 (cont'd): Analyte information.

No.	Analyte	B.P.[a] [°C]	Ret. Time [min]	Frag. Ions[b] [m/z]
23	Captan	314.2	26.69	79, 149, 107
24	Folpet	333.8	26.98	104, 260, 76
25	Imazalil	347.0	29.04	41, 215, 173
26	Myclobutanil	465.2	29.73	179, 150, 82
27	Endrin	416.2	30.23	263, 81, 265
28	Endosulfan sulfate	480.7	32.12	272, 387, 274
29	Fenhexamid	320.0	32.34	97, 177, 179
30	4,4'-DDT	416.2	32.42	235, 165, 237
31	Propargite	450.7	33.12	135, 173, 150
32	Iprodione	481.1	33.79	314, 187, 316
33	Bifenthrin	453.2	34.14	181, 165, 166
34	Fenpropathrin	448.2	34.37	97, 181, 125
35	Phosalone	446.7	34.91	182, 121, 184
36	Azinphos Methyl	421.3	34.96	160, 132, 77
37	cis-Permethrin	437.6	36.65	183, 163, 184
38	Coumaphos	449.9	36.74	362, 226, 109
39	trans-Permethrin	465.9	36.83	183,163, 184
40	Deltamethrin	535.8	40.52	181, 253, 255

[a] at 760 mmHg, Data source: ChemSrc (https://www.chemsrc.com/en)
[b] The underlined fragment ion was used as quantifier
[c] Decomposition temperature

LVI Optimization

LVI is a suitable technique for this study due to the great differences in boiling point between the solvent and the pesticides of interest. By optimizing several key factors of LVI, such as the initial CIS temperature, vent flow, injection speed and vent time, the pesticides can be concentrated within the liner in the cooled inlet while excess solvent is vented. The resulting sample is then transferred to the GC column for separation, followed by MS determination.

Firstly, different starting temperatures of the inlet were evaluated to determine the effect on the concentration factor achieved for the analytes in the liner. An initial quick comparison was done between a low temperature at 40 °C and a high temperature at 70 °C. Temperatures higher than 70 °C were not considered as the boiling point of acetonitrile is 82 °C. Basically, a higher inlet temperature results in a faster solvent evaporation. This shortens the injection time of the sample, and the loss of analytes is thus minimized. This phenomenon is illustrated in Figure 5 with better recovery achieved for most pesticide compounds at the higher inlet temperature. However, the higher inlet temperature led to greater loss of a few earlier-eluting compounds (as represented by 2-phenylphenol). This could be attributed to their higher volatility as well as to their boiling points being relatively closer to that of acetonitrile. Nonetheless, based on the overall results of all 40 compounds, 70 °C was selected as the optimized condition for initial CIS temperature.

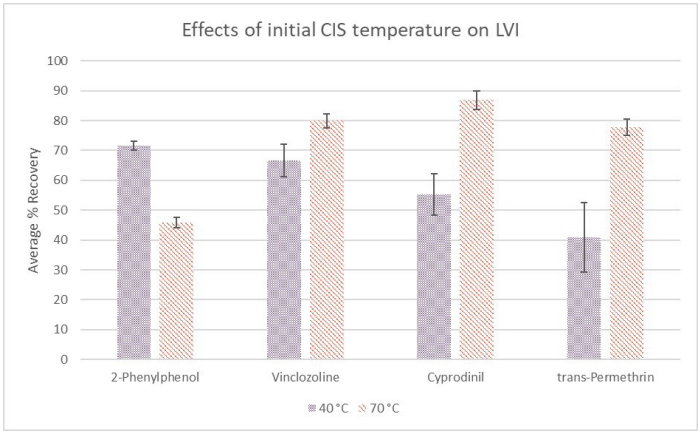

Figure 5: Comparison of % recovery of selected analytes among different initial CIS temperatures. Conditions: vent flow, 50 mL/min; correction factor, 0.8; vent time/initial CIS time, 0.5 min. Error bars show the standard deviations (n=3).

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Another method parameter that affects the efficiency of the LVI technique is the vent flow. Generally, a higher vent flow increases the rate of evaporation of the solvent. Different vent flows were applied in this stage of optimization. As seen in Figure 6, for compounds with lower boiling points (as represented by 2-phenylphenol), a better recovery was achieved at lower vent flow. Higher vent flows, on the other hand, resulted in better recovery of the later-eluting compounds. Increasing the vent flow resulted in a greater extent of vaporization of the more volatile compounds, which were then lost during solvent-venting. However, this was not observed for the later-eluting compounds. Instead, the higher vent flows enabled solvent to be removed more efficiently and thus, allowing a higher sensitivity to be obtained for these high boilers. The recovery for the middle-group compounds, which included vinclozoline and cyprodinil, peaked between vent flows of 50 and 100 mL/min before experiencing loss to evaporation at much higher vent flows. To reach a suitable compromise for all compounds, a vent flow of 80 mL/min was selected for subsequent experimentations.

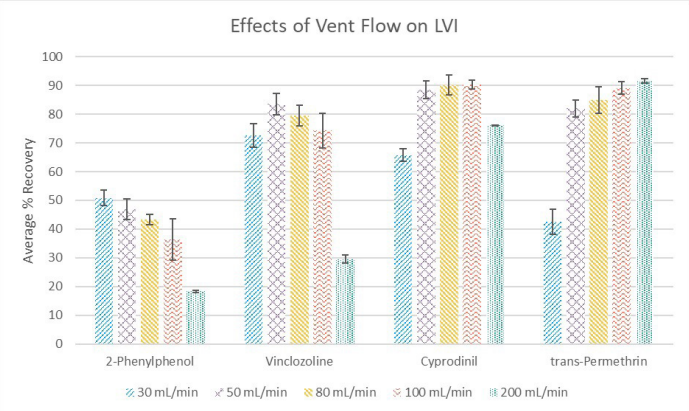

Figure 6: Comparison of % recovery of selected analytes among different vent flows. Conditions: initial CIS temp, 70 °C; correction factor, 0.8; vent time/initial CIS time, 0.5 min. Error bars show the standard deviations (n=3).

Thirdly, the effects of injection speed in LVI were evaluated by altering the correction factors for acetonitrile in the LVI calculator. A safety margin correction factor for initial optimization studies can be set at 0.7 to 0.8 [2]. If a factor of 1 was applied in the beginning, i.e., injecting at higher speed, the solvent might not evaporate as fast due to the presence of matrix and the injected sample could exit the liner leading to a general loss of analytes across the whole volatility range as seen in Figure 7. Poor general analyte recovery was observed when large-volume injections were performed at higher injection speed. Furthermore, the ensuing flooding of the liner and split vent can cause carry-over in subsequent runs. If, on the other hand, the injection speed was too low, the liner might run dry, causing significant loss of more volatile analytes. Ultimately, the injection speed should be similar to the evaporation speed to achieve good injection performance. The corrected injection speed at 0.7 was determined to be the most favorable setting, resulting in better recovery for most of the compounds.

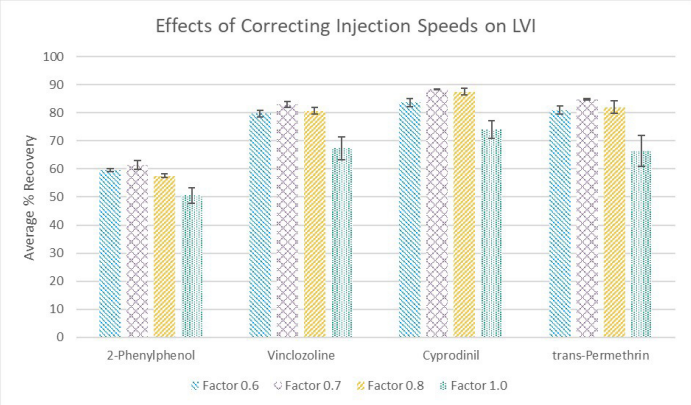

Figure 7: Comparison of % recovery of selected analytes among different correction factors for injection speed. Conditions: initial CIS temp., 70 °C; vent flow, 80 mL/min; vent time/initial CIS time, 0.5 min. Error bars show standard deviations (n=3).

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Lastly, different additional vent times were applied to determine the effect on the analyte amount retained in the liner. The vent time refers to the time span over which the split vent remains open after the GC and the CIS programs are started (at t=0). A longer vent time allows more solvent to be removed. While solvent-venting is still ongoing, the CIS remains at its initial temperature for the same duration to retain the analytes in the cooled liner.

As represented by 2-phenylphenol and vinclozoline in Figure 8, shorter vent times resulted in better recovery for some of the first 20 eluting compounds. Prolonged opening of the split vent, as well as the continuous high vent flow, caused more analytes of higher volatility to evaporate and be removed together with the solvent. On the other hand, recovery of later-eluting compounds (as represented by cyprodinil and trans-permethrin) peaked at 0.5 min but deteriorated when the vent time was further extended to 0.8 min. A slightly longer vent time (more than 0.3 min) allowed more solvent to be removed while a high percentage of the less volatile compounds were retained. However, further extension of vent time would cause increased analyte loss. Overall, a vent time of 0.5 min was determined to be the optimal value.

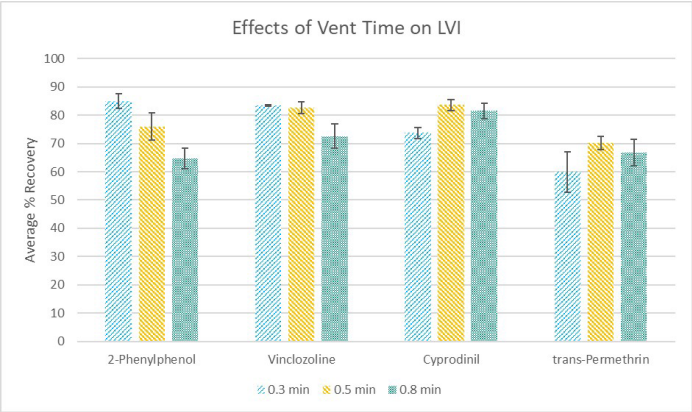

Figure 8: Comparison of % recovery of selected analytes among different vent times. Conditions: initial CIS temp, 70 °C; vent flow, 80 mL/min; correction factor (Injection speed), 0.7 (1.12 µL/s). Error bars show the standard deviations (n=3).

In summary, LVI can be a suitable technique which allows a greater mass per injection to be analyzed in the GC-MS system. When comparing with the results from a standard cold splitless injection of 1 µL, LVI produces bigger and taller peaks as seen in Figure 9. In fact, some compounds could only be detected with LVI since lower limits of detection were reached. Proper optimization of the method parameters can greatly improve the sensitivity of the overall analytical method without altering the sample preparation or changing to a more sensitive analytical detector.

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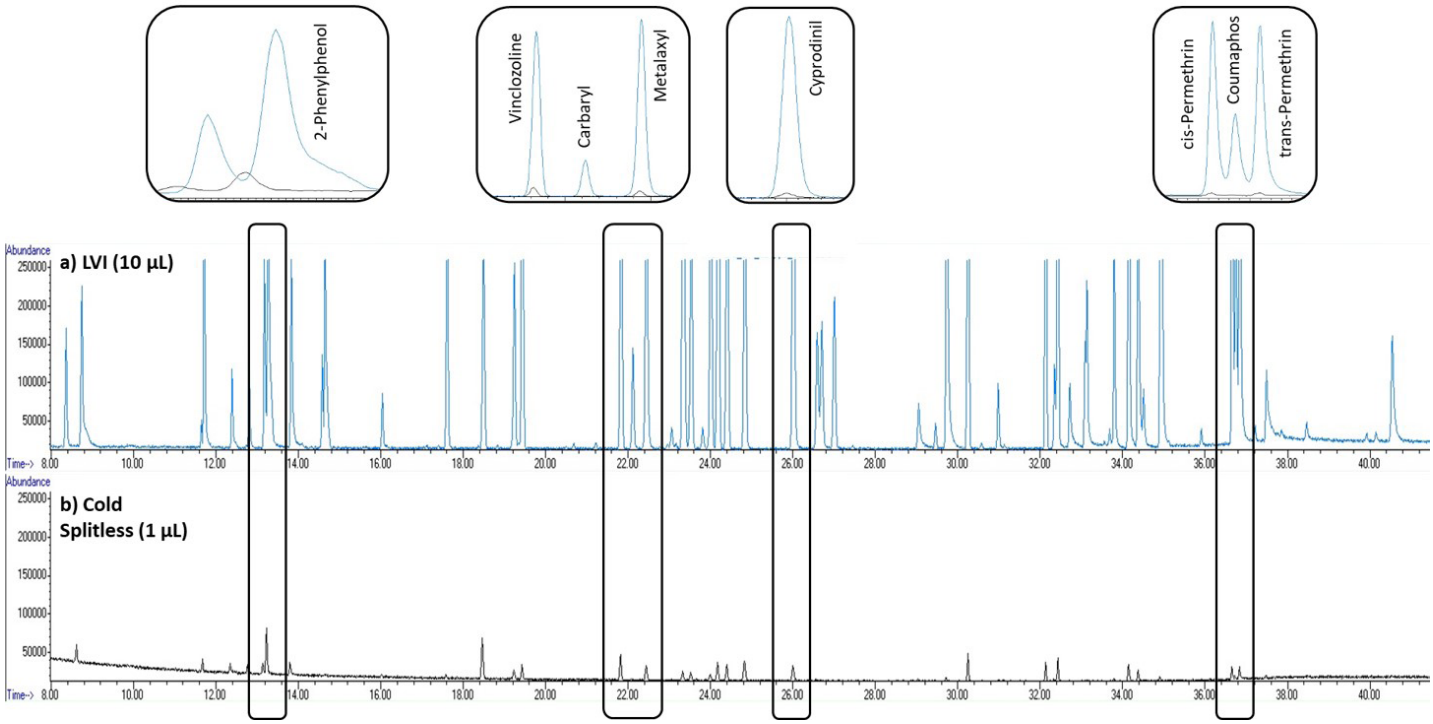

Figure 9: TICs of a) LVI, and b) standard cold splitless 1 µL injection using 0.5 mg/L ABC-pesticide standard.

*m*VAP *Optimization*

To further increase the mass on column per injection, the sample extract can be concentrated by solvent evaporation and reconstitution in a smaller volume of the same or another suitable solvent. This evaporation and concentration step is easily automated with the GERSTEL *m*VAP and can be included prior to LVI. Several factors, which affect evaporation in the *m*VAP, such as level of subambient pressure and sample temperature, are studied in this work.

Different subambient pressures (60 – 300 mbar) were applied to dry the samples and to consequently find out how the analyte concentration would be affected. Generally, a lower pressure speeds up the rate of evaporation of solvent. Pressures above 300 mbar were not evaluated as the duration of the sample preparation would far exceed the GC run time and would therefore lead to reduced system throughput. The GERSTEL <Prep Ahead> function ensures that samples are prepared during the GC run of the preceding sample making the GC cycle time the determining factor. This is shown in Figure 10 in which the automated *m*VAP procedure of the sample next in line is performed during GC-MS analysis of the current sample.

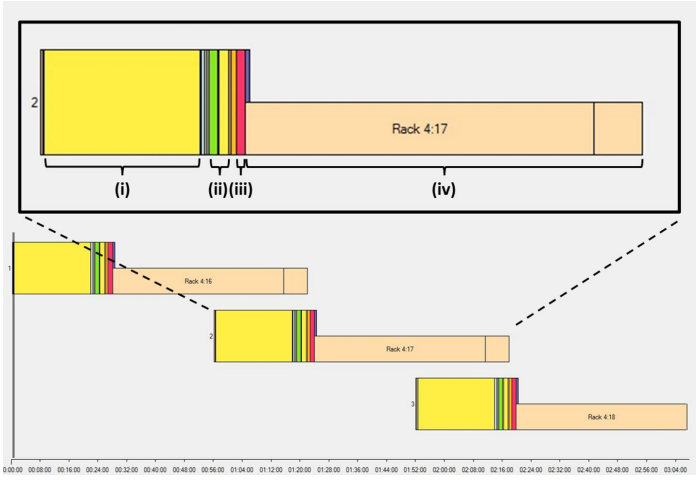

Figure 10: Sequence scheduler of three consecutive runs. (i) Evaporation to dryness (yellow), (ii) redissolution and mixing (green and yellow), (iii) slow injection of large-volume sample (red), (iv) GC-MS analysis (beige).

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As shown in Figure 11, a drop in recovery was observed when pressure was raised. At higher (subambient) pressure, a longer duration was needed to evaporate a volume of sample to dryness likely contributing to the greater loss of analytes to evaporation. Based on the results achieved for most of the compounds, 60 mbar was determined to be the most favorable setting.

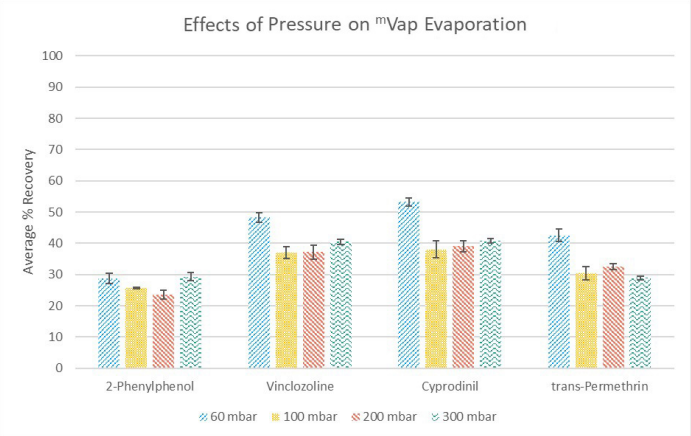

Figure 11: Comparison of % recovery of selected analytes at different pressures applied in the mVAP. Conditions: mVAP temperature, 60 °C; no agitation; LVI optimized settings. Error bars show the standard deviations (n=3).

The samples were heated at different temperatures (30 °C to 70 °C) and the effect on the concentration of compounds was evaluated. As shown in Figure 12, recovery of most analytes decreased as temperature rose. The rate of evaporation of these volatile analytes was higher at higher temperatures, leading to more being removed together with the solvent. On the other hand, this trend was not observed in later-eluting compounds such as trans-permethrin. For those, slightly better recovery was achieved at temperatures of around 50 °C to 60 °C. The loss of high boilers to evaporation was reduced as the time required to dry the sample at higher temperatures was shorter. Increasing the temperature further would, however, lead to a greater loss of analytes to evaporation. Nevertheless, 30 °C was decided to be the most favorable temperature for removing acetonitrile while still achieving satisfactory analyte recovery in the mVAP.

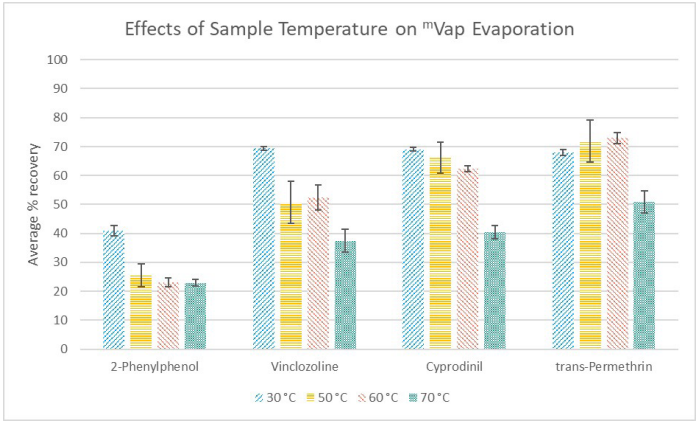

Figure 12: Comparison of % recovery of selected analytes among different sample temperatures in mVAP. Conditions: pressure, 60 mbar; no agitation; LVI optimized settings. Error bars show the standard deviations (n=3).

In summary, mVAP offers a simple approach to concentrating the sample by automating evaporation and redissolution prior to injection. Optimization of the evaporation method parameters resulted in improved GC-MS analysis results. As seen in the total ion chromatograms (TICs) in Figure 13, taller and bigger peaks result from combining the mVAP and LVI techniques compared with using only LVI. Signals of the analytes were found to have increased by a factor of 4.5 on average, which was highly satisfactory. This highlights how the use of mVAP can complement LVI to introduce even more mass on column from your sample and further lower the limits of detection. In fact, lower concentration samples could be analyzed as fronting peaks were observed in the chromatogram (Figure 13a). In addition, while extracted ion chromatograms (EICs) could provide better analyte peak resolution for quantitation, the observed peak resolution of compounds like 2-phenylphenol in the TICs would need to be further improved by optimizing the GC oven program.

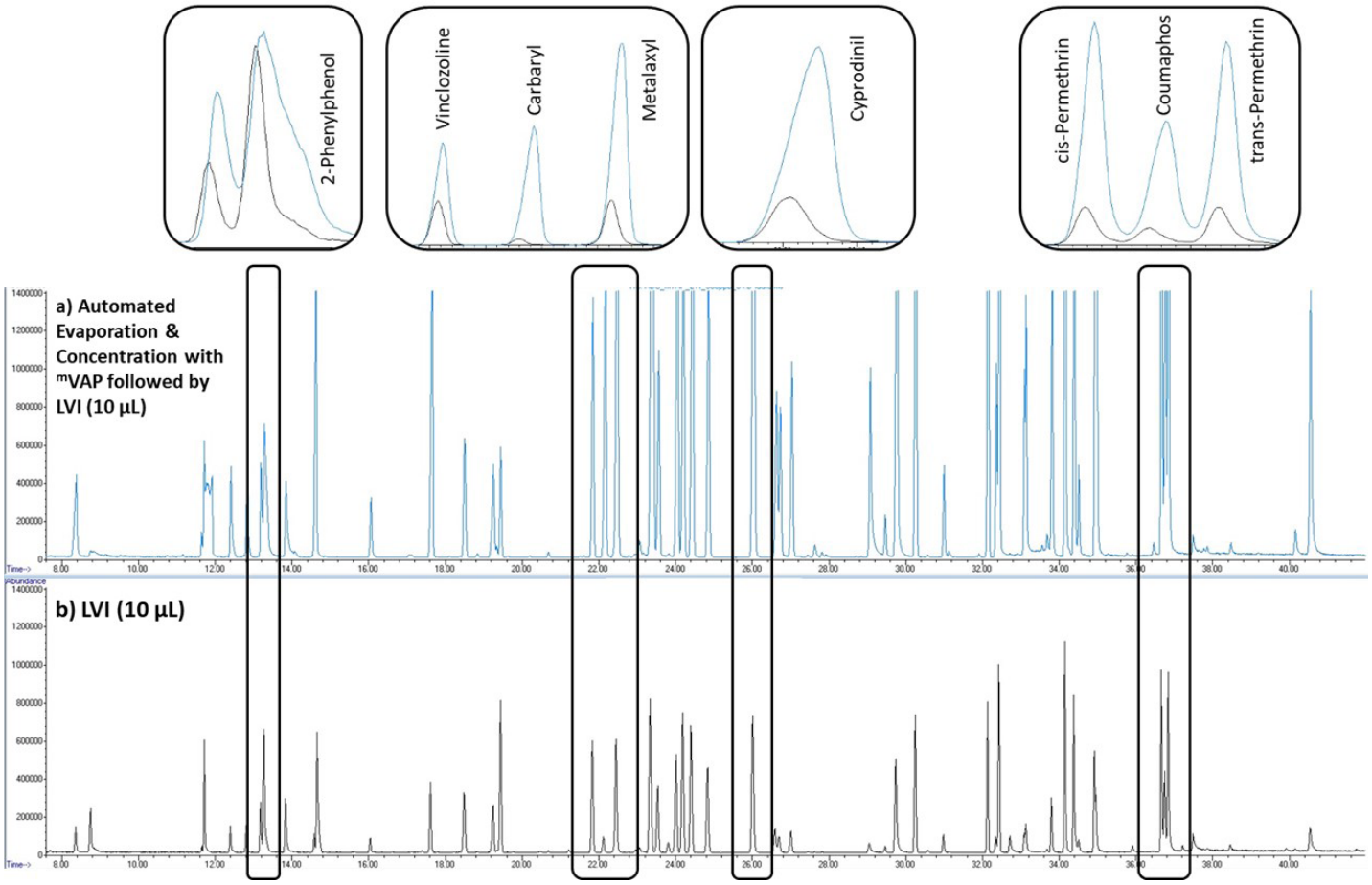

Figure 13: TICs of a) combined automated evaporation and concentration with mVAP and LVI technique, and b) LVI technique only, using 0.5 mg/L ABC-pesticide standard

Within-day and day-to-day repeatability were determined with 7 replicates and 3 replicates respectively to evaluate the precision of the optimized methodology. The results are presented in Table 2. On average, for most of the analytes, the relative standard deviation (% RSD) was 11.0 % for within-day repeatability and 13.9 % for day-to-day repeatability. For the more sensitive compounds such as dichlorvos and chlorothalonil, the addition of deuterated internal standards could improve repeatability. Moreover, when applying the combined mVAP-LVI technique to real samples, matrix residue accumulation in the CIS liner could affect repeatability as well. Therefore, an automated liner exchange system should be considered in such cases [3].

Table 2: List of analytes with % RSD achieved for within-day (n=7) and day-to-day (n=3) repeatability.

No.	Analyte	Intra-day % RSD (n=7)	Inter-day % RSD (n=3)
1	Methamidophos	17.8	10.2
2	Dichlorvos	31.6	39.9
3	Mevinphos	13.0	16.0
4	Acephate	16.4	12.2
5	2-Phenylphenol	9.7	19.5
6	Omethoate	16.1	9.5
7	Dimethoate	10.6	9.6
8	γ-BHC (Lindane)	11.3	21.7
9	Chlorothalonil	21.5	88.7
10	Diazinone	11.8	23.5
11	Vinclozoline	9.7	10.3
12	Carbaryl (Sevin)	13.6	11.8

Table 2 (cont'd): List of analytes with % RSD achieved for within-day (n=7) and day-to-day (n=3) repeatability.

No.	Analyte	Intra-day % RSD (n=7)	Inter-day % RSD (n=3)
13	Metalaxyl	9.8	10.5
14	Pirimiphos Methyl	10.0	10.2
15	Methiocarb	11.1	9.5
16	Dichlofluanid	9.2	17.7
17	Malathion	9.8	7.5
18	Chlorpyrifos	9.7	10.5
19	Fenthion	9.7	7.2
20	Dicofol (Kelthane)	9.4	8.0
21	Cyprodinil	9.7	7.6
22	Thiabendazole	10.6	23.9
23	Captan	11.8	15.7
24	Folpet	10.6	14.4
25	Imazalil	12.7	16.4
26	Myclobutanil	10.4	13.0
27	Endrin	10.5	9.9
28	Endosulfan sulfate	9.9	14.5
29	Fenhexamid	11.8	33.2
30	4,4'-DDT	9.6	10.0
31	Propargite	9.4	15.4
32	Iprodione	11.5	13.9
33	Bifenthrin	9.7	10.2
34	Fenpropathrin	9.7	12.2
35	Phosalone	10.3	14.3
36	Azinphos Methyl	8.1	15.3
37	cis-Permethrin	10.3	11.7
38	Coumaphos	10.7	15.6
39	trans-Permethrin	9.8	11.3
40	Deltamethrin	12.2	23.2

Conclusions

Concentration techniques like LVI and evaporation using ^{m}VAP are easily automated. These two techniques can be applied individually or as a complementary pair. The potential of combining both techniques has been demonstrated in this application note. Sample preparation optimization can be performed in a univariate approach to further enhance the sensitivity of the analytical methods. This work is a preliminary study, and more can be done to further develop and improve the procedure, such as addition of internal standards and experimentation on real samples. Apart from pesticide residue analysis, this combined approach to concentrating samples prior to analysis can also be extended to other suitable applications such as food contaminants, environmental sample extracts, and flavors and fragrances analysis.

References

[1] S. Sperling, *Evaporative Concentration of Substances Listed in the European Water Framework Directive (2000/60/EC and 2008/105/EC). A Performance Comparison Between an Automated System and a Manual System*, GERSTEL Application Note 152

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