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Optimisation of a Large Volume Injection (LVI) Method for Organic Contaminants in Water Using Design of Experiments (DoE)

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Introduction

Large Volume Injection (LVI) is a very powerful technique that allows you to overcome the struggles of low limits of detections in gas chromatography trace analysis. In fact, LVI allows significantly larger injection volumes (typically 10 µL to 100 µL), depending on the injection solvent and application.

It can be performed using a Programmable Temperature Vaporisation (PTV) inlet working in Solvent Vent Mode. In Solvent Vent Mode the inlet is kept at low temperature to allow focusing of the target analytes while the solvent is vented via the split valve at a certain flow and pressure optimised by the operator. In order to guarantee successful LVI the boiling point of the injection solvent should be at least 20 ^{o}C below the boiling point of the most volatile analyte. Only when all solvent has been vented, analytes can be conveyed from the inlet to the column by means of a fast temperature ramp.

Large Volume Injection is particularly useful in water analysis where very low limit of detections are required to meet regulation requirements. Selection of the right setting for all the parameters involved in the LVI method is crucial to achieving good and robust chromatography.

Whenever analytical challenges require the optimisation of several parameters which might be interacting with each other, the best approach to method development is a multivariable-at-a-time approach (MVAT).

Statistically designed experiments which can vary several variables simultaneously are often a more efficient way to explore a complex experimental space. Design of experiment (DoE) is a controlled set of tests designed to model and explore the relationship between experimental variables and one or more responses.

Definitive Screening Designs (DSDs) are very useful for factor screening and they have the ability to detect and identify nonlinear effects on the response requiring a relatively small number or experiments.

This application note describes the use of DSD to optimise the LVI settings for the detection of a mixture of organic contaminants in water when extracted using a dispersive liquid-liquid micro extraction technique. This involves a dual solvent extraction which makes the development of a suitable LVI method all the more technically difficult.

Instrumentation

Autosampler: GERSTEL MPS xt Dual Rail, Left MPS 100 uL syringe

Modules: Automated Liner Exchange (ALEX), Cooled Injection System (CIS). If you are interested to see the ALEX module in action click on the link to the video:
https://youtu.be/0nY3SlgcwHA

GC-MS: Agilent GC 7890-MSD 5977, High Efficiency Source (HES)

Software packages:

- Statistical analysis software for Design of Experiment data processing
- MassHunter Quantitative Data Analysis for peak integration

Figure 1: GERSTEL MPS equipped with Automated Liner Exchange (ALEX) for LVI method optimisation

Methods

For the purpose of these experiments, a 100 ng/L standard was prepared in extraction solvent which involves an 80:20 ratio mix of DCM to pentane. The standard solution contained a mix of organochlorine pesticides and polychlorinated biphenyls which were that targets of the analysis.
To prepare, 20 µL of 50 µg/L standard solution was spiked into 10 mL of DCM:pentane solvent. Aliquots were then pipetted into 2 mL autosampler vials ready for analysis.

Design of Experiment Factors and Ranges for LVI Optimisation

Table 1 lists factors, abbreviations and ranges selected for the optimisation of the LVI method using Definitive Screening Design.

Factors	Abrv.	Lowest	Highest
Injection Volume [µL]	Vol(Inj)	10	100
Injection Speed [µL/s]	Speed(Inj)	0.25	5
Injection Temperature [°C]	T(Inj)	-30	30
Injection Depth [mm]	Depth(Inj)	20	40
Vent Flow [ml/min]	Flow(Vent)	10	150
Vent Pressure [psi]	P(Vent)	0	9
Vent time [min]	t(Vent)	0.1	1
Liner type	Liner	Type 1*	Type2**

Table 1: Factors and ranges for the DSD

* Type 1: Glass beads
**Type 2: PDMS foam

The use of the ALEX module allowed the investigation of the liner type. ALEX could change the liner within the sequence without intervention of the operator. This kept the process seamlessly integrated, limiting variability to the system performances.

GC-MS analysis

CIS: Solvent Vent Mode, Splitless

GC: Column: HP-5MS Ultra inert 30 m x 0.25 mm x 0.25 µm
Flow: 1 mL/min
GC ramp: 40 °C held for 2 min, 10 °C/min to 220 °C, 2°C/min to 231 °C, 40 °C/min to 3000 °C held for 2 min
Runtime: 31 min

MSD: Auxiliary temperature: 300 °C
EI mode at 230 °C, Quadrupole 150 °C, Mass range 30-800 m/z

Results and Discussion

The optimisation of the LVI method using DoE was carried out focusing on two responses: peak area and peak symmetry. Both values were generated by Mass Hunter Quantitative Analysis when processing the data. Peak symmetry represents the balance between the back and the front of the chromatographic peak. Symmetry of 1.0 means the peak is balanced.
Peak area allowed to optimise the sensitivity of the method whilst peak symmetry provided an insight on the quality of the results. In fact, peak shape is crucial for quantitation purposes since good peak shape guarantees more reliable integration and therefore lower relative standard deviations.

Figure 2 shows the experimental matrix generated by the Definitive Screening Design for the investigated factors.

	Vol(Inj)	Speed(Inj)	T(Inj)	Depth(Inj)	Flow(Vent)	P(Vent)	t(Vent)	Liner
1	100	2.625	30	20	150	9	1	Type1
2	10	0.25	30	30	10	9	0.1	Type1
3	10	0.25	-30	20	150	9	1	Type1
4	100	0.25	30	40	150	0	0.1	Type1
5	100	5	-30	40	150	9	0.1	Type1
6	55	2.625	0	30	80	4.5	0.55	Type1
7	55	5	30	40	150	9	1	Type2
8	100	5	-30	30	150	0	1	Type2
9	100	0.25	30	20	80	9	0.1	Type2
10	100	5	-30	20	10	9	0.1	Type2
11	10	0.25	-30	40	150	9	0.1	Type2
12	100	5	30	40	10	0	0.1	Type2
13	10	5	-30	20	10	9	1	Type2
14	55	2.625	0	30	80	4.5	0.55	Type2
15	55	0.25	-30	20	10	0	0.1	Type1
16	10	5	0	20	150	0	0.1	Type1
17	10	5	-30	40	80	0	1	Type1
18	100	0.25	-30	40	10	4.5	1	Type1
19	10	5	30	40	10	9	0.55	Type1
20	10	0.25	30	40	150	0	1	Type1
21	100	5	30	20	10	0	1	Type1
22	55	2.625	0	30	80	4.5	0.55	Type1
23	10	2.625	-30	40	10	0	0.1	Type2
24	100	0.25	0	40	10	9	1	Type2
25	10	5	30	20	150	4.5	0.1	Type2
26	100	0.25	-30	20	150	0	0.55	Type2
27	10	0.25	30	20	10	0	1	Type2
28	55	2.625	0	30	80	4.5	0.55	Type2

Figure 2: Definitive Screening Design (DSDs) experimental matrix for the optimisation of LVI method

One of the main advantages of using a MVAT approach in contrast to the more traditional one-variable-at-a-time approach (OVAT) is the capability to explore the whole possible experimental space with a relative limited number of experiments. Exploration of the whole experimental space pushes the boundaries of the investigated process to fully understand the relationship between the factors and the response.

As an example of the power of DoE in effectively exploring the experimental space, Figure 3 shows the results obtained for one of the investigated compounds in experiment 1, 5 and 13,

respectively. A quite drastic change in both peak area and peak symmetry can be observed, suggesting the design is looking at a significant portion of the experimental space.

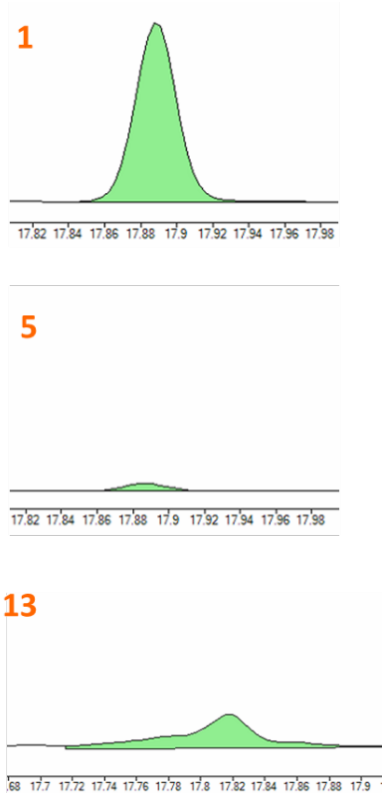

Figure 3: Results obtained for DSD experiments 1 (top), 5 (middle) and 13 (bottom) for one of the investigated peaks

A strong correlation was observed in terms of peak areas for all the 18 investigated compounds. The best settings for all targets were found using the sum of the peak areas as response. Correlation however was not very strong in the case of peak symmetry. Figure 4 shows the correlation maps obtained for both peak areas and peak symmetry. Correlation is shown in red (positive) and in blue (negative). Grey areas suggest no correlation present.

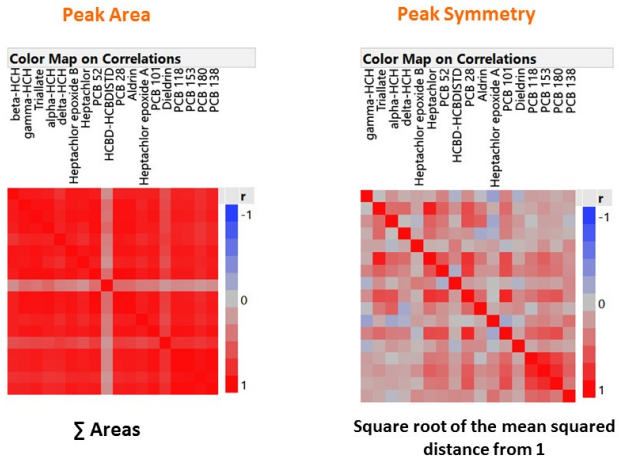

Figure 4: Correlation maps for peak areas (left) and peak symmetry (right).

Therefore, instead of maximising the peak symmetry value for every peak, we focused on finding conditions which would get peak symmetry close to 1 for every peak. An estimate of the deviation from the ideal value of 1 was generated as a response using the square root of the mean squared distance from 1.

The DSD generated a model which highlighted the presence of significant factors and interactions between the factors for both the sum of peak areas and the distance from 1 for peak symmetry.

For the sum of the peak areas Injection Volume, Injection Speed, Injection Temperature and Vent Pressure were significant factors. Interactions between Injection Volume and Injection Temperature and Injection Volume and Vent pressure respectively were found, together with quadratic behaviour for Injection Speed and Injection Temperature. Figure 5 shows the predicted profiles for the significant factors for the peak areas.

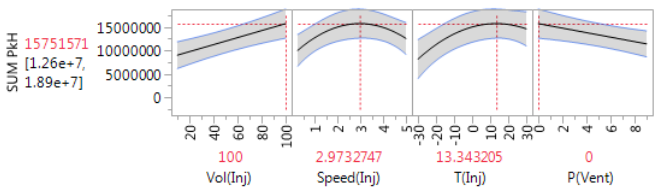

Figure 5: Prediction profiler graphs for the significant factors found for the sum of the peak areas

For the optimisation of peak symmetry, the model suggested Injection Depth, Vent Pressure and Liner Type as significant

factors. Liner Type 1 (Glass beads) gave the best performances in terms of peak shape. Figure 6 shows the prediction profiler graphs for the significant factors for peak symmetry.

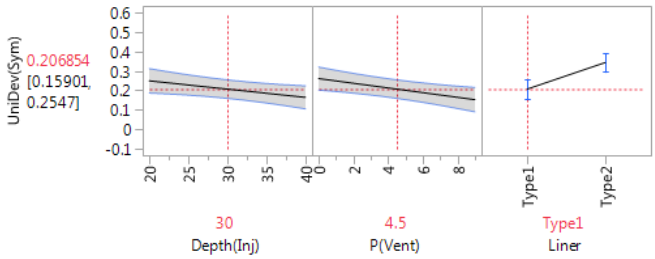

Figure 6: Prediction profiler graphs for the significant factors found for the distance from the value of 1 for peak symmetry

Combining the profile graphs for both peak area and peak symmetry allowed to identify the best settings to maximise performances on both fronts. Figure 7 shows the stacked prediction profilers for peak areas and peak symmetry and the selected optimal values.

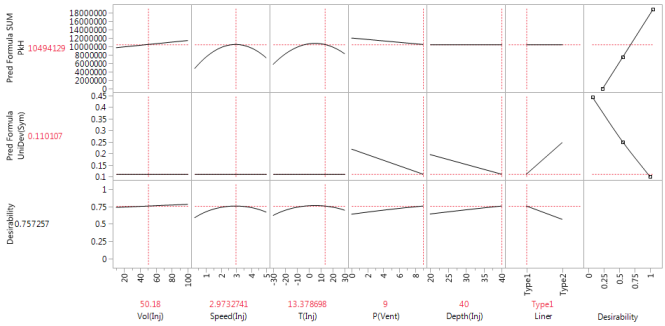

Figure 7: Prediction profiler graphs for both peak area and peak symmetry

It's noteworthy that the design suggested maximising the Injection Volume in order to maximise peak areas, as you would expect. However when working with sample preparation involving liquid-liquid extraction, sample volume can be limited due to the need of enriching analyte concentration to boost sensitivity. For this reason the optimal conditions for Injection Volume were set at lower values. The design will suggest the optimal conditions but the user has got the power to customise the answer to find the best conditions relevant to their analytical question.

Figure 8 shows the SIM chromatogram obtained for the 18 target analytes at 100 ng/L using the optimised LVI conditions. Peaks are mostly sharp and tall, providing very good signal-to-noise ratio and chromatography.

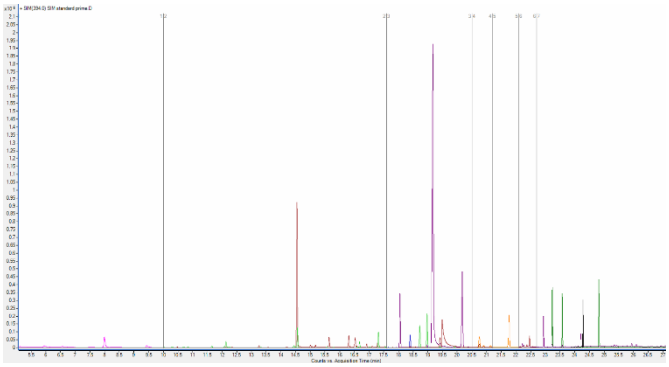

Figure 8: SIM Chromatogram for the 18 target analytes using the optimised LVI conditions

Conclusions

Synergism between analytical tools is a very powerful approach to method development. The use of design of experiment to optimize the LVI method provided a very good understanding of the process and the factors affecting it. The optimal conditions were found for the best analytical performance for this application.

We would like to thank Phil Kay for his contribution on the DOE software.