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GC Application Note

Highly Precise GC Injection with the PAL System

Recommended parameters for the Injection of QuEChERS acetonitrile pesticide extracts for superior reproducibility using the Liquid Band and Hot Needle injection technique

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Highlights

The PAL RTC and RSI provide outstanding quantitative precision for GC injections:

• Capable to perform *liquid band* injection and *hot needle* injection using the "Fast" and "Normal" injection modes.

• For *liquid band* injection, only a minimum syringe needle penetration into GC inlet is necessary. For optimized conditions with the used injector only 36mm penetration depth is applied.

• For *hot needle* injection, it is mandatory prior injection that the syringe needle penetrates the hot liner zone at an optimized injection depth of 45 mm and stayed for 5 seconds. This ensures efficient and good thermal spray during injection.

• Syringe needle penetration depth for the GC inlet in use and the pre-injection delay times should be optimized according to employed GC injector type and injection mode.

• SSL and PTV type injectors share the same PAL parameters for the "Fast" and the "Normal" injection mode.

• Analyte protectants provide narrower peak shapes and improved compound response. Thus, method sensitivity and ruggedness are enhanced.

• The standard 10 µL injection syringes with steel plunger were used to achieve precise results.

Introduction

Liquid injection onto gas chromatography systems (GC) or with its hyphenation to mass spectrometry (GC-MS) is the most frequently used GC injection technique. PAL Systems for robotic sample preparation deliver superior quality results for high precision analysis.

In general, modern GC systems allow two different liquid injection modes for the hot split/splitless injector, known as the *'hot needle'* and the *'liquid band'* injection, terms coined by Koni Grob and Maurus Biedermann from the Official Food Control Authority of the Canton of Zurich, Switzerland, already two decades ago [1]. The *hot needle* injection mode resembles the manual injection very much, but the *liquid band* mode is reserved for autosamplers. Each method delivers distinct advantages for applications. The slower *hot needle* injection with its evaporation from aerosols droplets formed in the hot syringe needle is the gentlest injection technique for sensitive and absorptive compounds, and achieves the best reproducibility results. But, *hot needle* injections tend to discriminate high boilers. Highly linear responses for samples with analytes over a wide boiling range from volatile to very high boiling analytes are achieved using the *liquid band* injection mode with fast autosamplers. The unaltered liquid sample is deposited within about hundred milliseconds only into a plug of deactivated glass wool into the hot injector liner. Here the solvent evaporation is cooling the glass wool. The analyte evaporation starts from the wool surface after the solvent is evaporated and leaving non-evaporative matrix behind.

While the analytical results of well experienced manual and the optimized automated *hot needle* injection can be very similar, the *liquid band* injection mode requires fast autosamplers and cannot be performed manual or with the regular slow autosamplers.

The PAL Systems provides optimized GC injection for both GC injection modes. The so-called "Normal" mode refers to the *hot needle* injection with best quantitative performance. The “Fast” mode uses the *liquid band* injection for the low discrimination of high boiling analytes.

This technical note describes the recommended PAL System parameters for the normal *hot needle* and fast *liquid band* injection. Important parameters for the optimized injection modes are the syringe needle penetration depth into the injector liner, the speed of penetration and liquid injection, and the dwell time before the syringe plunger injects the sample plug. The GC temperature programming remains unaltered for both modes, just making sure that the initial isothermal for a splitless injection with one to two minutes allows the complete transfer of the sample vapor into the GC column.

Especially for pesticides analysis with the popular QuEChERS extraction technique combined with the automated µSPE clean-up, the GC injection of acetonitrile extracts comes into the center of discussion. The direct injection of acetonitrile extracts into hot split/splitless injectors was reported to be problematic in GC-MS as the polar solvent causes poor peak focusing on an unpolar GC column, and in addition, poses limitations on injection volumes due to the high expansion volume. Under standard injection conditions at 250 °C and 100 kPa (14 psi) column head pressure one microliter of acetonitrile generates 416 µL of solvent vapor, just fitting a standard GC inlet liner of 78.5 mm length and 4 mm ID[2]. Using inlet liners with glass wool and fast liquid band injection of the PAL System solves the reported behavior of acetonitrile, and improves the GC performance significantly. The current report documents the optimized GC injection with the PAL System for excellent performance data.

This application demonstrates the excellent analytical performance with optimized injection parameters for the "Normal" and "Fast" injection modes available with the PAL System. A regular commercial pesticide standard was

[1] Grob, K. et al. (2002) ‘The two options for sample evaporation in hot GC injectors: Thermospray and band formation. Optimization of conditions and injector design’, Anal. Chem., 74(1), pp. 10–16. doi: 10.1021/ac0107554.

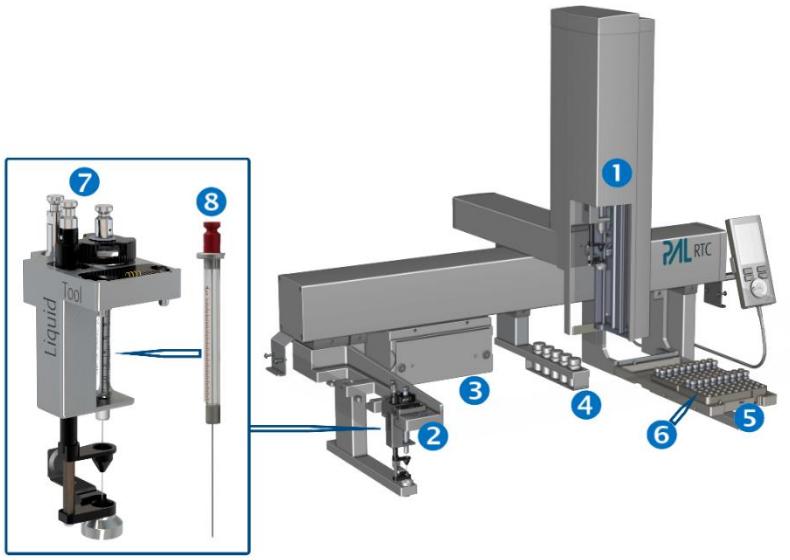
[2] Restek Solvent Expansion Calculator, https://www.restek.com/en/tools-and-calculators/tools/solvent-expansion-calculator/

used for the optimization experiments. Two types of injectors, a regular split/splitless injector type (SSL) and a programmable temperature injector type (PTV) was used to evaluate potential differences in the parameter setting for both types of injection modes "Normal" and "Fast". Also, the use of analyte protectants is demonstrated.

Instruments

PAL RTC System

A PAL RTC configuration as shown in Figure 1 was used. A Peltier stack can be used for keeping sample vials at controlled temperature, otherwise and in these experiments, sample vials are stored in a regular tray holder at room temperature. A D7/57 Liquid Tool equipped with a 10 µL syringe with metal plunger, CTC part number SF10-57-M-23S-CO), was used for GC injections.



No	Item
❶	PAL RTC
❷	Park Station
❸	Cooled Stack
❹	Standard Wash Module
❺	Tray Holder
❻	Rack VT54 for 2 mL vials
❼	Liquid Tool, D7/57
❽	Liquid Syringe, 10 µL

Figure 1: PAL RTC System for liquid injections, typically GC top mounted.

GC-MS/MS System

The GC used was an Agilent 7890B coupled with 7000D triple quadrupole mass spectrometer. The GC was equipped with split/splitless (SSL, back inlet)) and PTV type injectors (MMI inlet, front inlet). Deactivated splitless liners with single taper containing a deactivated glass wool plug as of manufacturer standard were used for both injector types.

Experimental

Standard & Chemicals

- Pesticide standard
  - GC Multiresidue Pesticide Standard #9 (Restek Corporation catalog number 32571), containing the following analytes at 100 µg/mL each in toluene:
  - Disulfoton (CAS: 298-04-4)
  - Fonofos (CAS: 944-22-9)
  - Methyl parathion (CAS: 298-00-0)
  - Mevinphos (CAS: 7786-34-7)

  - Parathion (ethyl parathion) (CAS: 56-38-2)
  - Phorate (CAS: 298-02-2)
  - Piperonyl butoxide (CAS: 51-03-6)
  - Triazophos (CAS: 24017-47-8)
- Acetonitrile HPLC grade (Sigma-Aldrich)
- Analyte protectants
  - D(+)Gluconic acid s-lactone (CAS: 90-80-2) >99% (Sigma-Aldrich)
  - D-Sorbitol (CAS: 50-70-4) >98% (Sigma-Aldrich)

Liquid Injection Workflow

PAL Injection Parameters

A standard GC liquid injection script was employed. In this experiment the script GC-LIQ-STD-V4.2 for Agilent GC was used. Table 1 shows the PAL operation parameters. The table shows the method default values, as well as the optimized parameters for the normal *hot needle* and the fast *liquid band* injection.

It is important to note, with the "Fast" injection mode several mandatory operation parameters are already preset. The inlet penetration speed, pre-injection time delay, injection flow rate and post-injection time delay are set by the standard injection script used. Such parameter values are required to ensure the fast injection procedure is completed within 100 milliseconds, as shown in Figure 2.

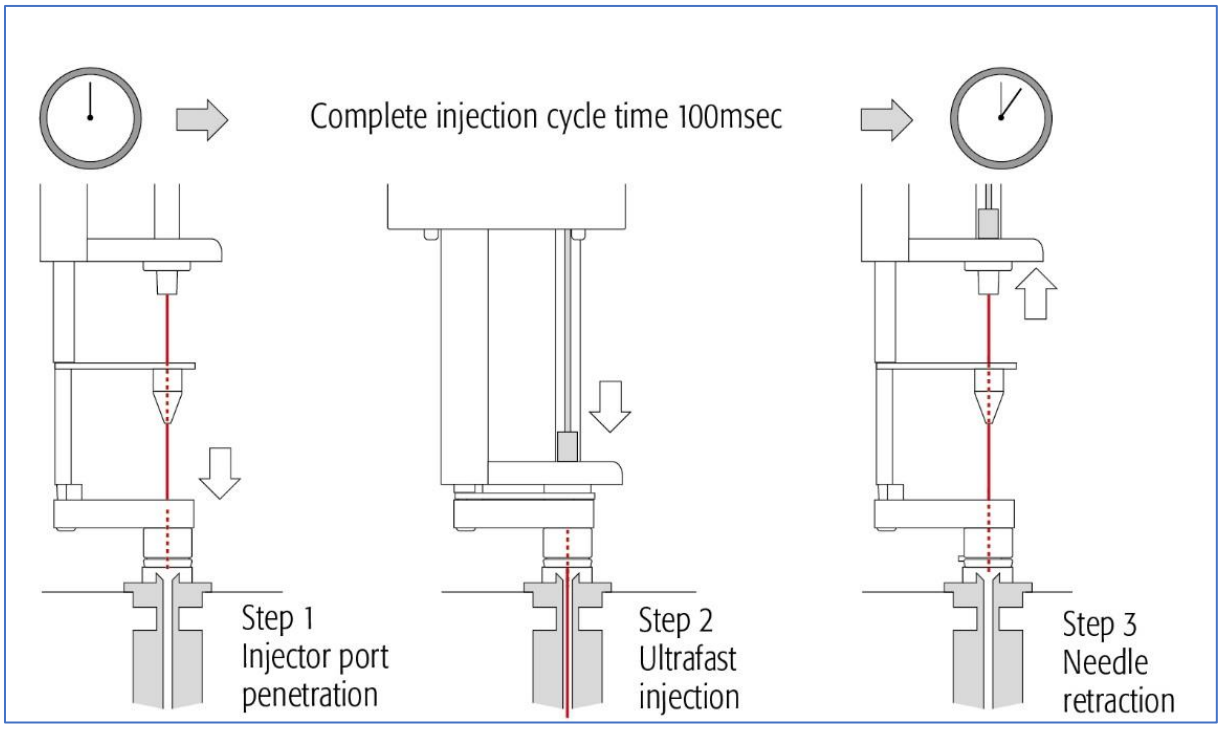


Figure 2: Fast Injection: A complete injection cycle in only 100 milliseconds.

Table 1: PAL GC injection parameters with the script default and in bold the optimized "Fast" *liquid band* injection and "Normal" *hot needle* injection for the particular GC injector type use.

Parameters	Default Value	Liquid Band Injection (Fast)	Hot Needle Injection (Normal)
Basic			
Tool	Liquid Tool with 10 µL syringe	Liquid Tool with 10 µL syringe	Liquid Tool with 10 µL syringe
Sample Volume	1 µL	**2 µL**	**2 µL**
Wait for System Ready	At Start	At start	At start
Wash Vial Depth	40 mm	40 mm	40 mm
Waste Port Depth	10 mm	15 mm	15 mm
Bottom Sense	Off	Off	Off
Height from Bottom of Sample Vial	0.5 mm	0.5 mm	0.5 mm
Cooled Stack	none	none	none
Cooled Stack Temperature	20 °C	20°C	20°C
GC Cycle Time	60 min	**25 min**	**25 min**
Pre Injection			
Syringe Fill For Wash	80 %	**40 %**	**40 %**
Wash Aspirate Flow Rate	5 µL/s	**5 µL/s**	**5 µL/s**
Wash cycles with Solvent 1	1	**3**	**3**
Wash cycles with Solvent 2	0	0	0
Wash cycles with Solvent 3	0	0	0
Wash cycles with Solvent 4	0	0	0
Sample			
Sample Vial Penetration Depth	30 mm	30 mm	30 mm
Sample Vial Penetration Speed	50 mm/s	50 mm/s	50 mm/s
Sample Wash Cycles	1	1	1
Sample Wash Volume	1 µL	**2 µL**	**3 µL**
Filling Strokes	4	4	4
Filling Strokes Volume	3 µL	3 µL	3 µL
Filling Strokes Aspirate Flow Rate	5 µL/s	5 µL/s	5 µL/s
Delay After Filling Strokes	0.5 s	0.5 s	0.5 s
Sample Aspirate Flow Rate	1 µL/s	1 µL/s	1 µL/s
Sample Post Aspirate Delay	2 s	2 s	2 s
Air Volume	1 µL	**0.2 µL**	1 µL
Injection Mode	Normal	**Fast**	Normal

Injection Signal Mode	Plunger Up	Plunger Up	Plunger Up
Inlet Penetration Depth	45 mm	**36 mm**	45 mm
Inlet Penetration Speed	100 mm/s	**100 mm/s*	100 mm/s
Pre-Inject Time Delay	0 s	**0 s*	**5 s**
Injection Flow Rate	100 µL/s	**100 µL/s*	25 µL/s
Post Inject Time Delay	0 s	**0 s*	0 s
Post Injection			
Syringe Fill For Wash	80 %	**40 %**	**40 %**
Wash Aspirate Flow Rate	5 µL/s	**5 µL/s**	**5 µL/s**
Wash cycles with Solvent 1	1	**5**	**5**
Wash cycles with Solvent 2	0	0	0
Wash cycles with Solvent 3	0	0	0
Wash cycles with Solvent 4	0	0	0

**Pre-set parameters by the liquid injection script.*

Bold parameter *denotes the GC injector optimized values.*

GC-MS/MS Parameters

- Analytical column: Rxi-5Sil MS (30 m x 250 µm x 0.25 µm)
- SSL and MMI injectors were equipped with deactivated splitless single taper liner containing deactivated glass wool.
    - Pulsed splitless mode at 280 °C
    - Injection pulse pressure: 100 kPa for 0.75 min
    - Purge flow to split vent: 50 mL/min at 1 min
- Carrier gas:
    - Helium gas
    - Constant flow at 1.2 mL/min
    - Post run flow at 1.5 mL/min
- GC oven program:
    - Initial temperature at 60 °C, hold for 1 minute,
    - Ramp at 40 °C/min to 170 °C,
    - Ramp at 10 °C/min to 270 °C,
    - Post run at 300 °C for 3 minutes.
- Transfer line temperature: 280 °C
- Ionization mode: EI
- Ion source temperature: 230 °C
- Quadrupole temperature: 150 °C
- Quench gas: Helium at 2.25 mL/min, collision gas: Nitrogen at 1.5 mL/min
- Detection mode: MRM monitoring mode, see Table 2 for the MRM transitions used.

Analyte Protectants for Injection

2 µL of the pesticide solution (each analyte at 10 ng/mL concentration) in acetonitrile containing 2 mg/mL D-(+)-gluconic acid δ-lactone and 1 mg/mL D-sorbitol was injected onto the GC-MS/MS system.

Table 2: Pesticides compounds, retention time, MRM transitions and collision energy.

Compound	Retention Time [min]	Quantitation [m/z, eV]	Qualifier [m/z, eV]
Mevinphos	5.247	127.0 > 109.0, 10	192.0 > 127.0, 10
Phorate	7.038	260.0 > 75.0, 5	121.0 > 65.0, 10
Fonofos	7.764	245.9 > 109.0, 15	136.9 > 109.0, 5
Disulfoton	7.932	88 .0> 60.0, 5	153 > 96.9, 10
Methyl parathion	8.634	262.9 > 109.0, 10	125.0 > 47.0, 10
Parathion (ethyl parathion)	9.430	290.9 > 109.0, 10	138.9 > 109.0, 5
Triazophos	12.048	161.2 > 134.2, 5	161.2 > 91.0, 15
Piperonyl butoxide	12.843	176.1 > 103.1, 25	176.1 > 131.1, 15

Results

The PAL injection parameters optimized of the particular GC injectors used, both, for the "Fast" *liquid band* and "Normal" *hot needle* injection, as stated Table 1, were applied to MMI and SSL, respectively. There was no internal standard correction applied to all results shown from this experiment to provide the genuine response data. Two batches of analysis with ten replicate injections of each batch were carried out and evaluated.

Figure 3 shows quantitation ion chromatogram for all targeted analytes. At 10 ng/mL concentration all peaks were sharp and well resolved from baseline. Identical chromatograms were obtained using both MMI and SSL injectors.

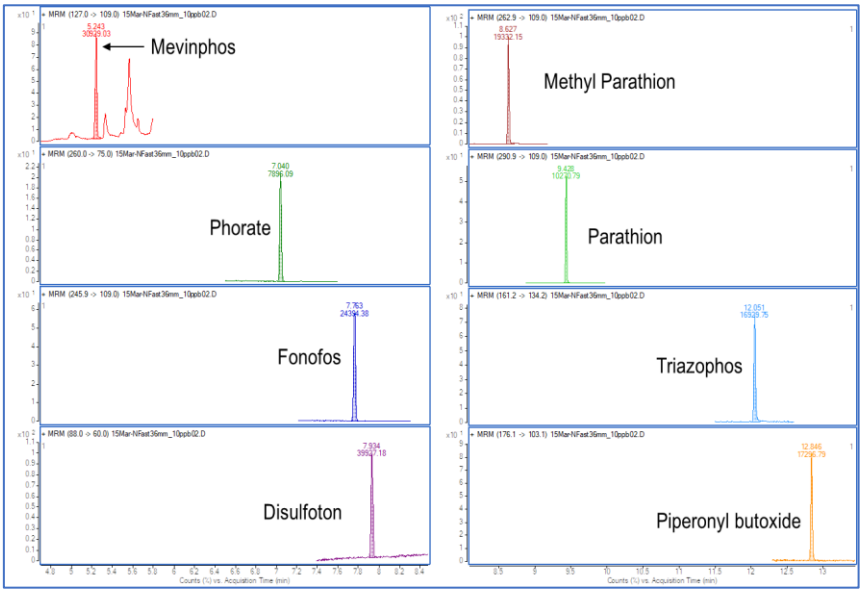


Figure 3: Quantitation ion chromatogram for all targeted analytes (each analyte at 10 ng/mL concentration).

Multi-Mode Inlet (MMI)

Table 3 shows the average peak area, standard deviation, and precision in %RSD of the peak area of all target analytes obtained from "Fast" and "Normal" injection mode using MMI. The injection precision denoted by peak area %RSD was at or better than 5 % for all analytes.

For all analytes the "Normal" injection showed significantly better precision data compared to the "Fast" injection regardless of the lower peak area achieved.

Table 3: Average area, standard deviation, and precision of the peak area in %RSD for all target analytes obtained using the MMI inlet.

Multi-Mode Inlet		Peak Area							
		Mevinphos	Phorate	Fonofos	Disulfoton	Parathion Methyl	Parathion	Triazophos	Piperonyl Butoxide
Liquid Band Injection									
Batch 1	Average	31614.00	9955.60	26731.10	44575.80	18633.80	8024.30	7479.00	16805.50
n = 10	Std Dev	1548.58	408.67	969.14	1059.33	381.99	166.58	211.62	476.97
	%RSD	4.90	4.10	3.63	2.38	2.05	2.08	2.83	2.84
Batch 2	Average	33192.30	10311.60	27372.90	44639.50	18997.90	8119.80	7468.60	16598.40
n = 10	Std Dev	1687.75	422.12	725.90	958.89	483.55	244.18	330.17	707.74
	%RSD	5.08	4.09	2.65	2.15	2.55	3.01	4.42	4.26
Hot Needle Injection									
Batch 1	Average	22889.80	7774.20	22439.60	37842.10	15618.30	6423.80	5423.70	12400.10
n = 10	Std Dev	689.11	198.12	350.56	569.43	172.70	124.46	238.00	303.83
	%RSD	3.01	2.55	1.56	1.50	1.11	1.94	4.39	2.45
Batch 2	Average	23385.50	7909.80	22213.00	37104.30	15418.90	6393.50	5463.10	12072.00
n = 10	Std Dev	834.63	239.19	206.79	396.74	243.19	144.69	175.87	341.42
	%RSD	3.57	3.02	0.93	1.07	1.58	2.26	3.22	2.83

Split-Splitless Inlet (SSL)

Table 4 shows the average peak area, standard deviation, and precision in %RSD of the peak area of all target analytes obtained from the "Fast" and "Normal" injection modes using SSL. The achieved injection precision denoted by peak area %RSD was better than 8.0% for all analytes. The injection precision increased with chromatographic elution order, especially when using the "Normal" injection technique.

Peak areas for all analytes were significantly higher using the "Fast" *liquid band* injection mode, except piperonyl butoxide, the last eluted analyte among all target analytes.

Table 4: Average area, standard deviation, and precision of the peak area in %RSD for all target analytes obtained using the SSL inlet.

Split-Splitless Inlet		Mevinphos	Phorate	Fonofos	Disulfoton	Parathion Methyl	Parathion	Triazophos	Piperonyl Butoxide
Liquid Band Injection									
Batch 1	Average	28575.40	7952.10	24775.60	40635.20	17687.90	9905.20	16981.90	18101.20
n = 10	Std Dev	2114.14	532.84	1421.82	2311.95	797.53	385.96	555.30	763.57
	%RSD	7.40	6.70	5.74	5.69	4.51	3.90	3.27	4.22
Batch 2	Average	27734.80	7980.80	24500.50	39749.20	18071.40	9957.50	16963.10	16957.20
n = 10	Std Dev	965.46	179.78	546.33	612.87	566.84	312.23	403.23	947.83
	%RSD	3.48	2.25	2.23	1.54	3.14	3.14	2.38	5.59
Hot Needle Injection									
Batch 1	Average	21359.50	7085.80	20875.50	34244.30	13647.90	8314.70	11382.40	28503.00
n = 10	Std Dev	1369.85	204.39	643.80	846.99	282.99	163.49	183.97	566.91
	%RSD	6.41	2.88	3.08	2.47	2.07	1.97	1.62	1.99
Batch 2	Average	19852.30	6770.00	20205.30	33445.00	13234.20	7925.00	10783.20	25930.40
n = 10	Std Dev	1588.42	379.16	982.66	1407.62	591.58	235.02	268.47	753.33
	%RSD	8.00	5.60	4.86	4.21	4.47	2.97	2.49	2.91

Conclusion

In the described experiments the precise injection of a pesticide solution in acetonitrile at 10 ng/mL, as it is the typical situation after a QuEChERS extraction and clean-up, was demonstrated with both the "Fast" *liquid band* injection and the "Normal" *hot needle* injection. Analyte protectants were used as described in the literature. The same PAL parameters, optimized for the particular GC injectors in use, managed to get good reproducibility results on both GC inlet types, namely PTV (MMI) and SSL.

With the temperature programmable PTV type (MMI) injector a precision of or better than 5% RSD on peak areas was achieved with the "Fast" *liquid band* injection mode. The "Normal" *hot needle* injection delivered for all compounds a better quantitative precision but at significantly lower peak areas.

Using the hot SSL injector, the injection precision was better than 8.0% RSD on peak areas for all analytes. Also, with the SSL injector the registered peak areas were significantly higher using the "Fast" *liquid band* injection mode.

Table 5: A comparison of the "Fast" *liquid band* vs. the "Normal" *hot needle* injection highlights the important different parameter settings for the "Fast" *liquid band* and the "Normal" *hot needle* injection employed in this experiment.

"Fast" *Liquid Band* Injection	**"Normal" *Hot Needle* Injection**
Pull 0.2 µL of air plug into injection syringe.	Pull 1 µL of air plug into injection syringe.
Syringe needle penetrates 36 mm into GC inlet.	Syringe needle penetrates 45 mm into GC inlet.
Inject sample immediately after syringe needle insertion into GC inlet.	Inject sample after 5 s delay after syringe needle insertion into GC inlet.
Fast injection with high flow rate at 100 µL/s.	Slow injection flow rate at 25 µL/s.

Summary:

- The optimized PAL GC injection parameters for the used GC injector types were recommended for GC injection with mid-polar or polar solvents, for example for pesticides analysis in acetonitrile after the QuEChERS extraction, using either *liquid band* or *hot needle* injection technique.
- Identical parameter settings can be applied for extract injections onto the GC via PTV type (MMI) and Split-Splitless Inlet (SSL).
- The "Fast" *liquid band* injection of the PAL System delivers significantly higher peak areas with the PTV type (MMI) and SSL injector.
- Analyte Protectants (AP) protect analytes from active sites in the GC flow path for improved peak shapes and reproducibility.

Imprint

Date of print: 08.2021

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