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Eliminate Invisible High Boiling Matrix in GC and GC/MS by Using PTV Backflush Injection Technique for Increased Productivity and Reliability

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Key Words

- Backflush
- GC Column Lifetime
- GC Injection
- High Boilers
- Matrix Load
- Method Robustness
- Multiresidue Methods
- Pesticide Analysis
- PTV

Introduction

High laboratory sample throughput with short sample preparation and extract clean-up requires special care for the chromatographic system in GC and GC/MS. Extraction methods using low polar solvents e.g. ethyl acetate, cyclohexane, acetone or any blend of these, are typically used for pesticide extraction in low fat food samples.[1-6] While there is a high recovery reported for a large number of pesticide components, medium and less polar compounds of high molecular weight also get into the extracts. Fruits and vegetables from fresh as well as from processed food give rise to high concentrations of a large variety of lipoid components as a matrix of high boiling compounds in the extracts. Although these compounds are present in the final extracts, the sample vials look clear and appear almost colorless. Consequently, no visual quality control is possible.

Once injected into the chromatographic system high boiling substances persist in the insert liner of the GC and on the analytical column. While insert liners typically are exchanged after a certain number of injections, the analytical column gets increasingly contaminated. The elution of high boiling compounds at temperature programs used for pesticides analysis does not occur sufficiently. Matrix compounds accumulate and deliver an increasingly high background level with routine analysis of a large number of samples. Bake-out procedures are used, but add additional time between samples, do not work efficiently and finally lead to reduced column lifetime. The situation is even worse with more polar columns with limited temperature range that cannot be baked out efficiently without destroying the column film.

An optimum solution would be the separation of the analytes directly after injection from all high boiling matrix material. The more volatile analytes, e.g. pesticides, can travel quickly into the analytical column while high boilers are kept in the insert liner and a pre-column that is swept backwards during the analysis run, and can be replaced easily like the injector insert liner.

Pears and other fruit cuticula carry longchain lipoids as high boiling matrix into analytical extracts.

A routine solution using the temperature programmable PTV injector, equipped with pre-column and carrier gas backflush option for pesticide analysis, is described in this application note. Among the injection systems available for Capillary GC, the Programmable Temperature Vaporizing (PTV) inlet is the most flexible one, offering different operating modes and setup. This flexibility can be advantageous for the analysis of various samples with different analytical needs.

The PTV injection technique shows significant advantages over conventional hot split-splitless or on-column injection. First, by avoiding evaporation from the syringe needle, it eliminates an important source of discrimination of higher boiling components. Additionally, non-volatile sample by-products are retained in the injector insert liner. Finally, in the solvent split mode, the PTV allows splitless injection, even of larger sample volumes than only 1 or 2 µL, with a minimum transfer of solvent to the column and to the detection system.

The PTV can also use a carrier gas backflush of the heavier components of the extract when they are not of interest, preventing their entrance in the analytical column. This greatly increases the robustness of the whole chromatographic system for routine applications.

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Backflush Operation

The backflush (BKF) system consists of four elements that can be installed in the Thermo Scientific TRACE GC Ultra: a 3-way solenoid valve (backflush valve) in the carrier gas line, a wide-bore pre-column, a T connector in the GC oven, and a flow restrictor (see Figures 1 and 2). The flow restriction connects the carrier gas line of the injector with the backflush line to the T-piece. The flow of the restriction is factory set.

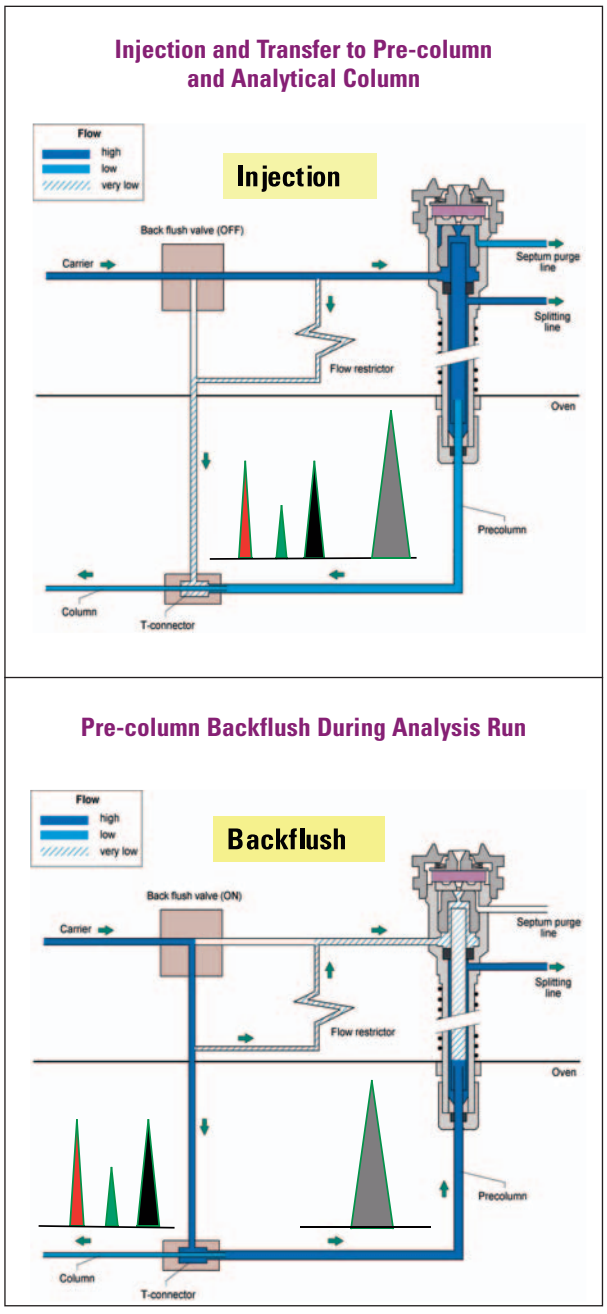


Figure 1: PTV injector with backflush installed on the Thermo Scientific TRACE GC Ultra.

Schematic flow diagram during the injection (left) and backflush (right) phase. While the analytes (red, green yellow peak) are transferred to the analytical column the slowly travelling high boilers (large grey peak) are still in the pre-column when the BKF is activated. Those matrix compounds are eliminated through the split line of the injector during the run time of the analysis. The analytes that have been transferred to the analytical column continue the regular chromatography.

During injection the backflush valve is off. The carrier gas flows in the normal direction to the GC injector. Just a small flow provided by the restrictor grants sufficient purging of the T-connection. In the standard configuration the pre-column consists of 2 m × 0.53 mm ID uncoated fused silica tubing. The flow restrictor is designed to provide a purge flow of about 5% of the main flow when the split valve is closed.

When the backflush valve is activated by the TRACE™ GC Ultra, the carrier gas flow to the inlet is reversed. The backflush valve diverts the carrier gas directly to the T-connection to provide a reverse flow through the pre-column into the injector. The majority of the carrier gas now enters the inlet from the bottom and is vented through the split line, cleaning the pre-column. A small carrier gas stream is provided by the restrictor to the top of the injector purging the insert liner during the PTV cleaning phase (see Figure 2).

The timing of switching the backflush valve is set such that all analytes pass through the pre-column to the analytical column. High boilers remain in the pre-column and get backflushed through the split exit of the injector.

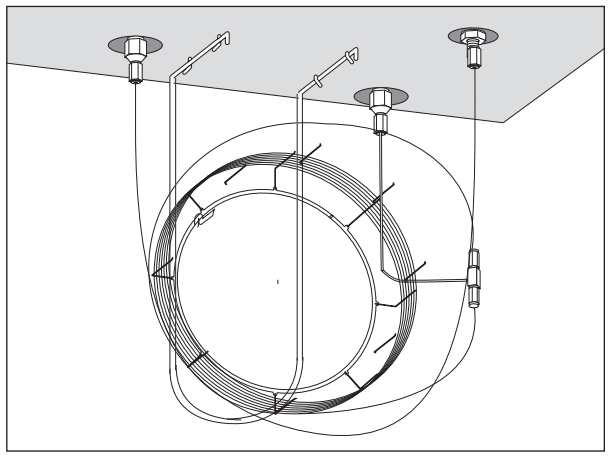


Figure 2: Pre-column and column set-up in the GC oven for backflush operation on PTV inlet.

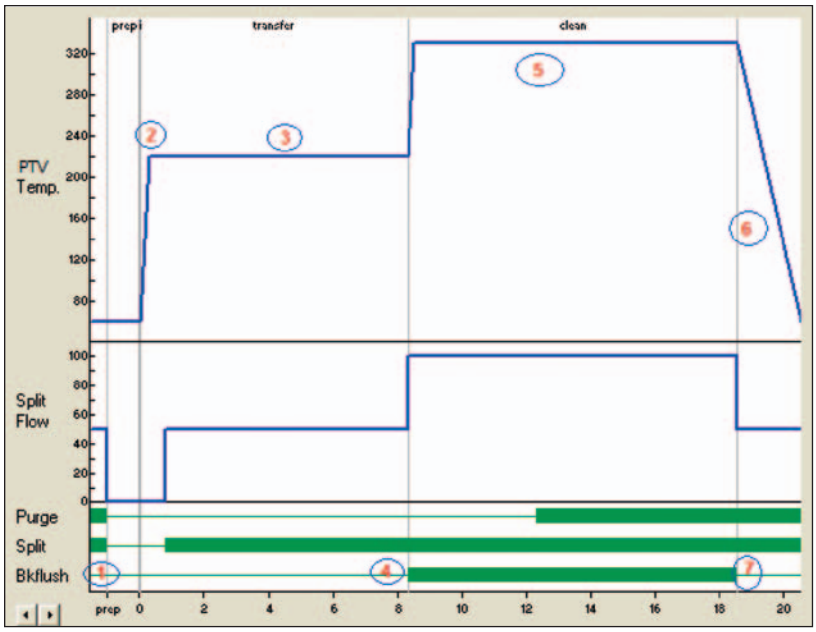
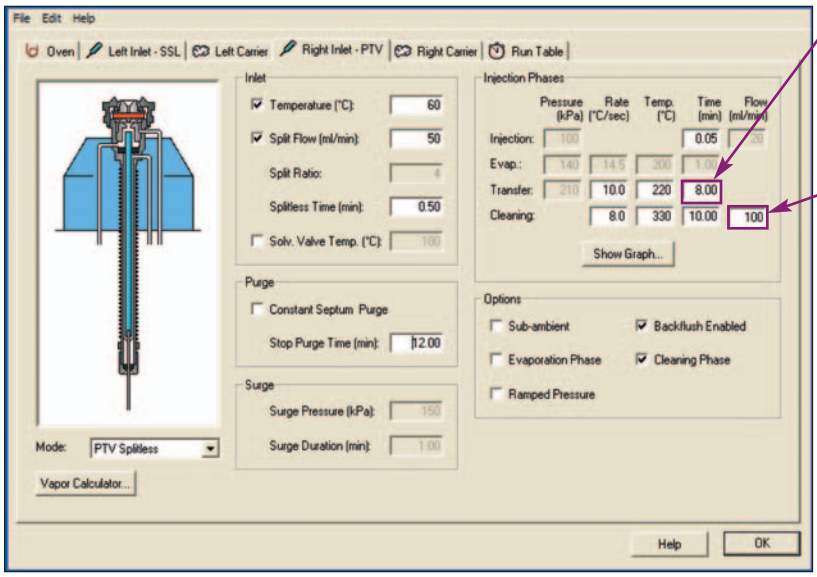


Figure 3: PTV operation phases using backflush and insert liner cleaning step

1. Injection, BKF off
2. Start PTV
3. PTV hold, analyte transfer to column
4. BKF activated, clean pre-column and injector liner
5. Cleaning phase PTV at higher temperature
6. PTV cool down
7. BKF off



Easily programmable transfer time activates BKF according to the elution time of the analytes.

Flows are programmed according to the volatility of the components of the matrix that can be transferred and backflushed from the pre-column.

Figure 4: The BKF time is programmed through the PTV inlet method

Experimental Conditions

Initially, a standard chromatogram using the optimized column temperature program without the use of BKF is acquired as reference. In the following the BKF operation is optimized with standards starting with a long transfer time.

Setting up a BKF method with the PTV Injector:

1. First deactivate the BKF mode by select “Disable BKF” through the GC keyboard or data system.
2. Inject a standard with the optimized oven temperature program required for the complete elution of the analytes of interest.
3. Set “Enable BKF” and program a long BKF time (called “Transfer Time” on the PTV programming page). As a reference use the elution time of the last component of interest (for pesticides analysis this is usually Deltametrin). Enable the “Cleaning Phase” and set the cleaning time almost equal to the analysis time (e.g. 20-30 min) and flow to approximately 50-100 mL/min.
4. Inject the sample and compare the chromatogram with the reference obtained at point 2.
5. If the last component of interest is present in the chromatogram, this will be the correct time to set for the BKF. If the peak corresponding to this component has a lower area or is not present increase the transfer time in 1 min increments.

The BKF will then be activated reproducibly at the same time for each subsequent run. If the temperature program and carrier flow conditions are modified the optimum performance need to be checked with the injection of a standard solution.

Sample Measurements

Example of Analytical Setup for BKF

A typical example on the BKF operation is the analysis of a mixture of hydrocarbons (C10 to C40 in n-hexane) using a column of 15 m × 0.25 mm ID × 0.25 µm silicon film and FID detector. In Figure 5, three chromatograms show the effect of a different BKF time programming.

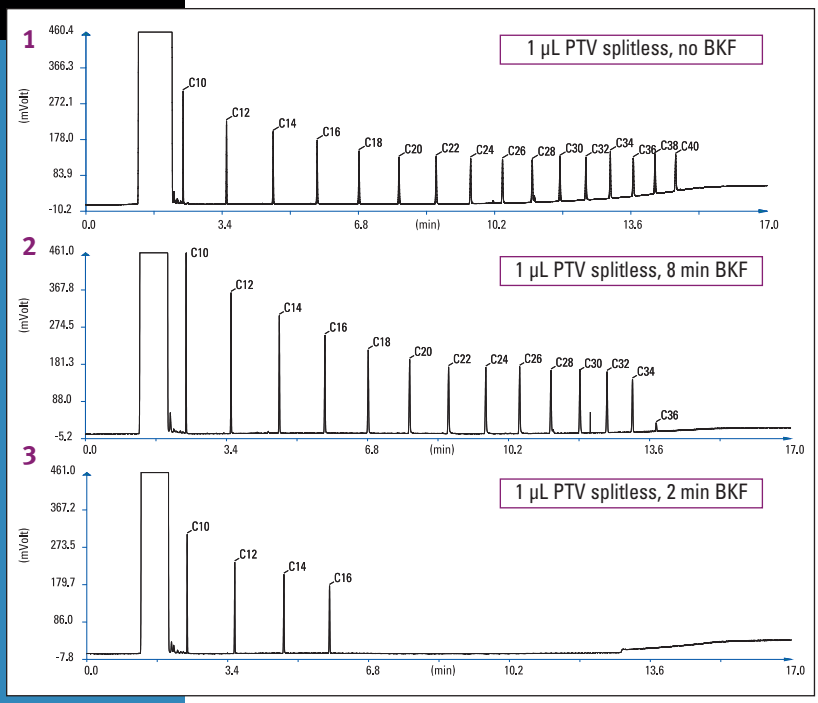


Figure 5: Typical example of BKF operation for a mixture of hydrocarbons from C10 to C40 using a pre-column of 5 m × 0.53 mm, sample is 1 µL C10-C40, 20 ng/µL in n-Hexane.

1. "No BKF" activated, all the hydrocarbons are eluted
2. With a longer BKF time (transfer time) of 8 min from injection, only the last 3 hydrocarbons (C36,C38,C40) are eliminated
3. Using a short BKF time (transfer time) of 2 min, the hydrocarbons with more than 16 carbon number are completely eliminated

Results

Pesticides Analysis in QuEChERS Extracts

QuEChERS is a quick and simple extraction technique used very often for extracting of pesticides and other contaminants from low fat food or other type of sample matrix.[2,7-9] This sample preparation is very fast but there is no significant concentration step in the procedure. This can lead to a diluted solution that may not have a suitable concentration of the components to be easily determined with a common MS detector. This sample procedure is also not highly selective for pesticides, so the extracts can contain components that exhibit long retention characteristics with the capillary columns used for the separation. This may lead to difficulties with detection or may contribute to robustness issues with the entire chromatographic system.

The PTV with BKF device and the Large Volume injection technique offers the possibility to overcome some of these problems.

As it is possible to notice in Figure 6 and 7, the QuEChERS extract from pears for example contains compounds with high molecular weight (Sitosterol and other compounds) that are eluted in the last part of the chromatogram. This leads to an increase of the analysis time and the need to overheat the column (Oven temperature: 320 °C) for the complete elution of these compounds. In fact the pesticides are eluted earlier (see upper chromatogram of Figure 6), not requiring high temperature of the oven.

Name	RT	Name	RT
Methamidophos	7.29	Cyproconazole	15.23
Dichlorvos	7.36	Ethion	15.48
3,5-Dichloroaniline	8.86	Triazophos	15.68
Ethiofencarb	8.98	Benalaxil	15.83
Mevinphos	9.09	Propiconazole	15.95
Acephate	9.33	Tebuconazole	16.15
Heptenophos	10.41	Nuarimol	16.17
Omethoate	10.64	Iprodione	16.43
Sulfotep	11.26	Tetramethrin	16.57
Dicloran	11.67	Bromopropylate	16.58
Carbofuran	11.77	Dicofol	16.72
Pirimicarb	12.57	Tetradifon	16.98
Vinclozolin	12.90	Fipronil	17.07
Tolclofos-methyl	12.97	Phosalone	17.09
Pirimiphos-methyl	13.28	Acrinathrin	17.15
Fenitrothion	13.30	Pyrazophos	17.45
Fenthion	13.57	Azinphos-ethyl	17.56
Chlorpyrifos	13.58	Fenarimol	17.56
Penconazole	14.07	Azinphos-methyl	17.56
Isofenphos	14.13	Bitertanol	17.77
Tetrachlorvinphos	14.53	Cypermethrin	18.38
Vamidothion	14.55	Flucythrinate	18.54
Hexaconazole	14.76	Fluvalinate	19.15
Profenofos	14.82	Tralomethrin	19.42
Miclobutanil	15.00	Deltamethrin	19.56

Table 1: Compound list for peak identification for Figures 6 and 7.

Figure 6: Comparison between 2 µL QuEChERS extract from pears in Ethyl Acetate/Cyclohexane (1:1) with BKF at 10 min (lower chromatogram), with BKF at 8 min from injection (middle chromatogram) and 2 µL of Pesticides Mix at 400 ppb with BKF at 8 min (upper chromatogram).

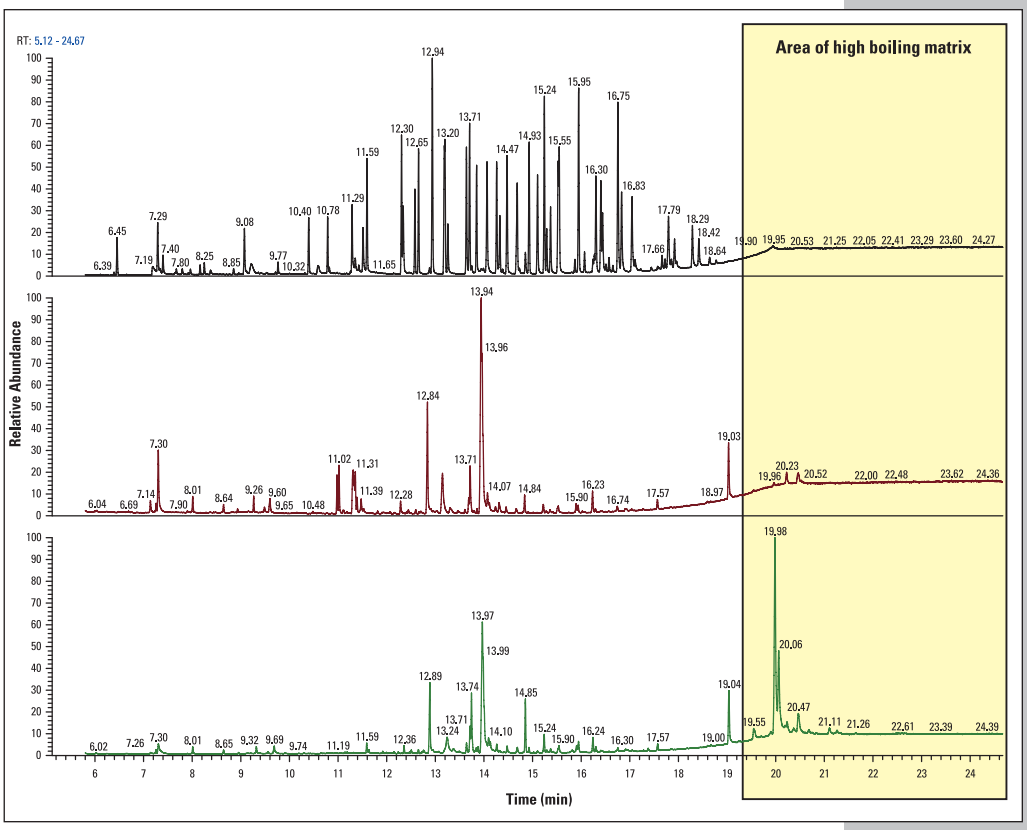
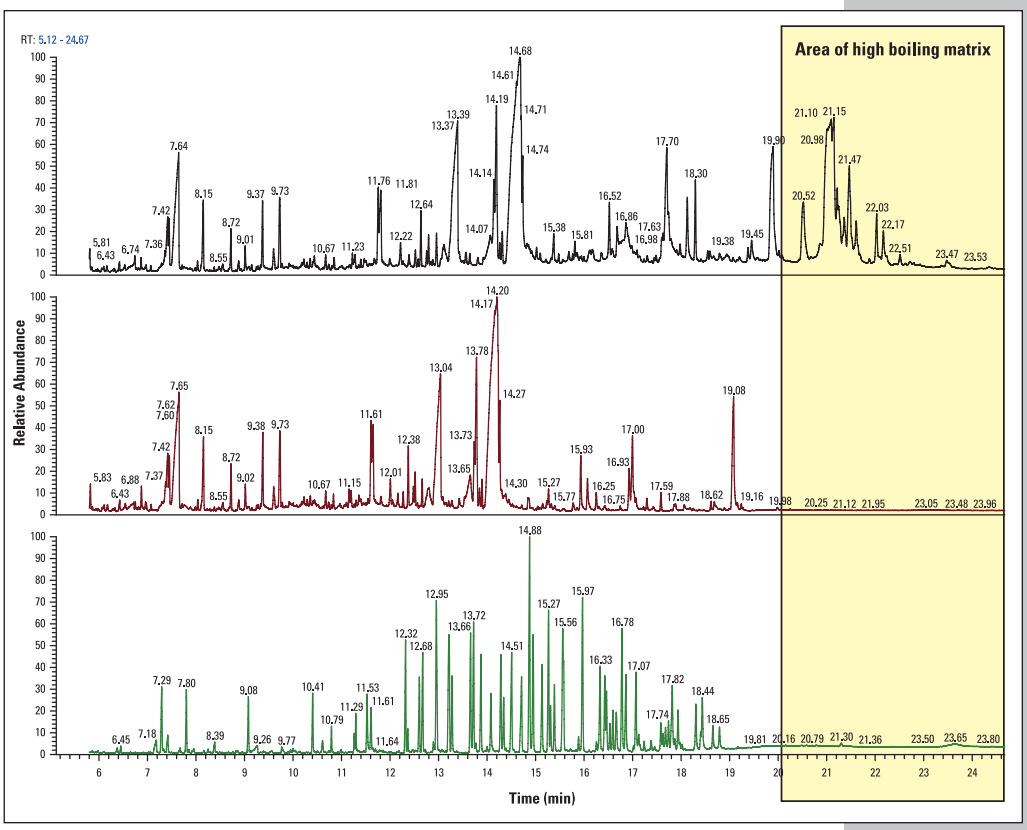


Figure 7: The top chromatogram corresponds to the injection of 120 µL of QuEChERS pear extract in Ethylacetate/Cyclohexane without BKF. The middle shows the same injection volume and sample type, but with a BKF at 10 min. The third chromatogram corresponds to the injection of 120 µL of Pesticides Standard Mixture at 400 ppb concentration with BKF at 10 min.

The large volume injection of the extract did not compromise the performance of the chromatographic system or the MS, because the material transferred to the column and consequently to the MS was only that which could be easily eluted. The high molecular weight compounds, even if present in at a high concentration, were prevented from entering into the column and consequently into the MS source. A large number of injections of the extract (more then 100) could be done without the need of maintenance for the column or MS.[10]



Oven Method	
Initial Temperature (C):	70
Initial Time (min):	3.00
Rate #1 (deg/min):	15.0
Final Temperature #1 (C):	320
Hold Time #1 (min):	5.00

Right PTV Method	
Base Temperature:	On
Base Temperature (C):	85
Mode:	PTV Large Volume
Split Flow:	On
Split Flow Flow (mL/min):	60
Splitless Time (min):	0.80
Solvent Valve Temperature:	On
Solvent Valve Temperature (C):	150
Surge Pressure (kPa):	150.00
Surge Duration (min):	1.00
Constant Purge:	Off
Stop Purge At: (min):	0.80
Evaporation Phase:	Off
Cleaning Phase:	On
Ramped Pressure:	On
Backflush:	On
Inject Pressure (kPa):	150.00
Inject Time (min):	0.20
Vent Flow (mL/min):	100
Transfer Pressure (kPa):	180.00
Transfer Rate (deg/sec):	8.0
Transfer Temperature (C):	260
Transfer Time (min):	10.00
Transfer Time (min) (NO BKF):	20.00

Right Carrier Method	
Clean Rate (deg/sec):	10.0
Clean Temperature (C):	320
Clean Time (min):	26.00
Clean Flow (mL/min):	60
Mode:	Programmed Pressure
Initial Value:	On
Initial Value (kPa):	150.00
Initial Time:	3.00
Rate #1 (kPa/min):	10.0
Final Value #1 (kPa):	287.00
Hold Time #1 (min):	3.00
Vacuum Compensation:	On

Thermo Scientific TriPlus Autosampler	
Injection Mode:	Basic
Injector Port:	Injector A [PTV LV]
Start Sync Mode:	Delayed
Sample Volume (µL):	120.0
Plunger Strokes:	1
Air Volume (µL):	2.0
Filling Volume (µL):	80.0
Vial Depth:	99
Pre-injection Dwell Time (s):	0.1
Post-injection Dwell Time (s):	0.1
Injection Dept (mm):	33
Injection Speed (µL/s):	1.0

Table 2: TRACE GC Ultra conditions used for the analysis of pesticides with PTV backflush operation. The liner for the injector was a deactivated sintered glass liner, suitable for low concentration of pesticides and for large volume injection with programmed injection speed.

Conclusions

It has been shown how to use the PTV injector, equipped with the backflush system, for the analysis of pesticides from matrix loaded QuEChERS extract with MS detection.

The PTV-GC/MS system can be used for normal injection volumes as well as for larger volume injections. In both cases there are numerous advantages including increased sensitivity, easier maintenance and higher robustness of the entire analytical system.

The analytical benefits provided by the use of the backflush system are multiple, each contributing to increased productivity of a GC and GC/MS system:

- Chromatographic integrity maintained even with difficult matrix samples
- Time saved by avoiding additional bake-out phases of the column
- Short run times which stop after last eluting analyte
- Lower final oven temperatures expedite cool down to next analysis
- Shorter analysis times by backflushing heavy components from the pre-column
- Better column selection for the separation of the lighter components
- Injection of high matrix loaded extracts for trace analysis, as only low level and relatively volatile analytes are transferred to the column since the amount of matrix in the extract would exceed the column loading capacity
- Less-cleaned extracts can be injected with high boiling matrix load
- Increased column lifespan – no cuts from beginning, no high bake-out temperatures
- Thinner column films possible for less matrix with faster separation
- The PTV inlet can be upgraded to BKF capability at any time
- Ability to perform large volume PTV injections, venting the excess of solvent and isolating the column from the injector
- Easy maintenance of the injection system with BKF active
- Pre-column maintenance without venting the MS detector

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