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Large Volume Injection with Solvent Venting - Application to Trace Detection of Analytes in Water

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Introduction

Modern capillary GC injection generally uses the universal dual mode split/splitless injection port or inlet in which the pneumatic mode is selected at the outset. The inlet is maintained at a sufficiently high set temperature to allow instant vaporization of the sample after deposition in the inlet liner (usually 4mm i.d.) Of course this explosive vaporization is associated with the well documented undesirable phenomenon of needle discrimination. If analytes are in aqueous solution another effect is induced by the very high saturated vapor volume of water compared to other solvents. A 1 µL injection of water in hot splitless mode will give a vapor volume greater than the retaining capacity of the liner and can inhibit the subsequent vaporization of analytes.

There is another injection technique, which avoids many of the above problems and also offers the attractive possibility of actually removing solvents such as water before transfer of analytes to the column. This is temperature programmed sample introduction or PTV injection, proposed by Abel [1] and developed by Vogt et al [2]. The Programmable Temperature Vaporization (PTV) type inlet offers 3 modes of sample introduction: The previously described split and splitless modes and an additional “solvent vent” split mode. In all modes a major advantage is that the sample can be deposited in the liner at ambient temperature and only then are analytes transferred to the column by rapid heating of the inlet. For either a PTV or hot split/splitless inlet in initial split or splitless mode the pneumatic condition for transfer of analytes is predetermined by the choice of mode. However the PTV type inlet in solvent vent mode offers the

additional possibility to inject at low temperature with the split valve open and then to revert to standard split or splitless mode for the heating step. It is intuitively clear that this approach can be used to initially remove solvents or even low boiling analytes. In the remainder of this Application Note, we will attempt to explain the interesting interplay between the properties of the analytes and the chosen solvent, as well as the PTV and pneumatic method parameters chosen, in order to fully exploit this technology for routine trace analysis.

Experimental

Standards. A 1000 ppm solution in HPLC grade methanol of three semi-volatile esters (Ethyl Vanillate, Ethyl Homovanillate and Ethyl Syringeate) was prepared and serially diluted to working 1 ppm solutions in HPLC grade methanol, ethyl acetate and water.

Instrumentation. Analysis was performed on a GC-MS system consisting of a 7890 GC and a 5975B mass spectrometer (both Agilent Technologies). A GERSTEL CIS 4, PTV-type inlet, with Universal Peltier Cooling (UPC) was used as injection port and injections were performed by a MultiPurpose Sampler equipped with a 100 µL syringe (MPS, GERSTEL).

Analysis conditions.

MPS:	100 µL injections with speed programming optimized for each solvent type.
PTV:	liner packed with silanized glass wool conditions discussed in next section
Column:	25 m CP-Sil 5CB (Agilent), d_i = 0.15 mm d_f = 2.0 µm
Pneumatics:	He, constant flow (0.5 mL/min)
Oven:	60°C (2 min), 10°C/min, 150°C, 5°C/min, 320°C
MSD:	Full scan, 32-350 amu

Solvent venting. The simplest way to approach an understanding of solvent venting injection is to directly compare a 1 µL injection of analytes in water by both standard splitless, and solvent vent followed by splitless. The solvent venting step represents a pre-run operation and must be followed by standard split or splitless transfer of analytes. This can be more easily explained by comparing inlet- and pneumatic conditions for both modes:

Table 1. Comparison of injector and pneumatic conditions.

Hot splitless	Solvent venting + splitless	Solvent venting + split	
Injector 300°C *Pneumatics go to splitless*	PTV Temp 40°C PTV initial Time 0.2 min Vent flow 500 mL/min		*Injection Pre-Operation*
GC-Start			
splitless transfer Ramp to 300°C at 10°C/s Purge time 2.0 min Purge flow 50 mL/min	Vent end time 0.01 min		*Pneumatics go to splitless*
	splitless transfer	*split transfer*	
	Ramp to 300°C at 10°C/s Purge time 2.0 min Purge flow 50 mL/min	Ramp to 300°C at 10°C/s Purge time 0.02 min Purge flow 50 mL/min	

Time

In the solvent vent pre-run operation the split vent is kept open with a very high split flow of 500 mL/min going through it (this is related to the water matrix and will be discussed later). The CIS (PTV) temperature is set to a value of 40°C, close to ambient temperature. These parameters are the same whether the analytes are subsequently transferred by standard split or splitless mode. The vent end time means an end to this period of initial high split flow together with an instruction to the pneumatics to establish standard splitless operation. The transition usually takes about 0.1 min and this is the reason why the CIS initial time (here at 0.2 min) must always be longer than the vent end time. In effect this difference gives the splitless pneumatics time to reestablish before the rapid heating takes place resulting in analyte transfer to the column. The heated splitless transfer time for analytes in this example is 1.8 min as the purge time is 2.0 min. The objective is to remove as much solvent as possible during the venting step while retaining the maximum amount of analytes on interest for subsequent separation and detection.

The right hand column in Table 1 also illustrates the simple change needed in the purge time in order to change to solvent vent injection with split transfer of analytes to the column. Split mode transfer could be useful, for example, to check for peak coelution since a simpler chromatogram is generated. Everything remains the same as it was for solvent vent with splitless transfer except that now the purge time begins at 0.02 min. This

means that the system still changes from solvent vent to splitless mode at the 0.01 min vent end time, but then reverts to standard split mode at 0.02 min before the CIS (PTV) starts to heat at 0.2 min. Now the purge flow is also the split flow for transfer of analytes and can be adjusted to any desired value.

If the standard 1 µL injection was always being used then the advantage of solvent vent injection would seem limited (except perhaps when it was necessary to exclude water from sensitive chromatographic phases). The logical extension of this observation is to extend the application to injection of much larger volumes than 1 µL. This technique is called Large Volume Injection (LVI) and it represents the real potential benefit of the solvent vent technique. The only difference now is that injections greater than 10-20 µL must be performed using speed programmed injection, instead of the normal "at once" procedure typically used to deposit smaller volumes into the liner using standard injection technology. For larger volumes progressive introduction into the liner must be used as the solvent must be removed or vented in the gas phase. If the injection speed is too high, liquid solvent with analytes will simply channel through the liner packing and exit through the split vent. In this scenario an injection of 100 µL could take up to 10 min, depending on the solvent. The actual GC-MS run and pneumatic and the CIS (PTV) parameter changes described in Table 1 do not start until the pre-operation of injection is over and the syringe has been withdrawn from the liner.

Equal volumes of different solvents result in widely different vapor volumes, requiring very different introduction rates for successful venting of different solvents. Staniewski et al developed the basic calculations in this area with an equation relating the maximum injection speed for a solvent with its various physical properties and the PTV (CIS) temperature and solvent vent split flow [3] This calculation is offered as a user friendly software routine in the GERSTEL LVI Calculator. The calculation predictions work best for apolar solvents and there are some additional considerations unique to polar solvents which must be taken into account. These aspects will be explored further in the next section. Further theoretical background and practical advice on large volume injection can be found in publications from the Eindhoven [4] and Leipzig Groups [5, 6]

Results and Discussion

Fig 1 shows a TIC trace resulting from a 100 µL injection of a 1.0 ppm solution of the three esters in water. The conditions were solvent vent split followed by splitless transfer (Column B in Table 1) and an injection speed of 12 µL/min. Greater than 80 % recovery of analytes is achieved when compared to a pro-rata 1 µL standard split injection of the more concentrated stock solution. However these conditions were developed in an empirical way, i.e. determining which combination of CIS (PTV) temperature, solvent vent split flow and injection speed actually works in practice to achieve acceptable recoveries and good peak shapes. For water the most sensitive parameters are vent split flow and injection speed while the CIS (PTV) temperature can be set to 40°C, a temperature which is easily achievable.

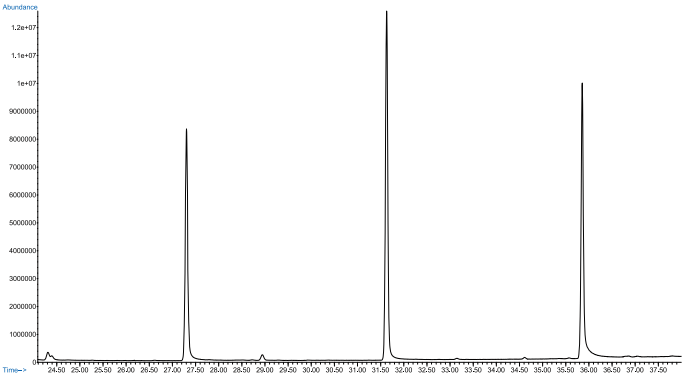

Figure 1. TIC of 100 µL of a 1.0 ppm solution of three esters in water.

Figures 2 and 3 summarize peak area changes seen when either the vent flow or injection speed is moved away from the optimum value (lower vent flow or higher injection speed). It is clearly seen that injection speed is the most critical factor for analyte recovery.

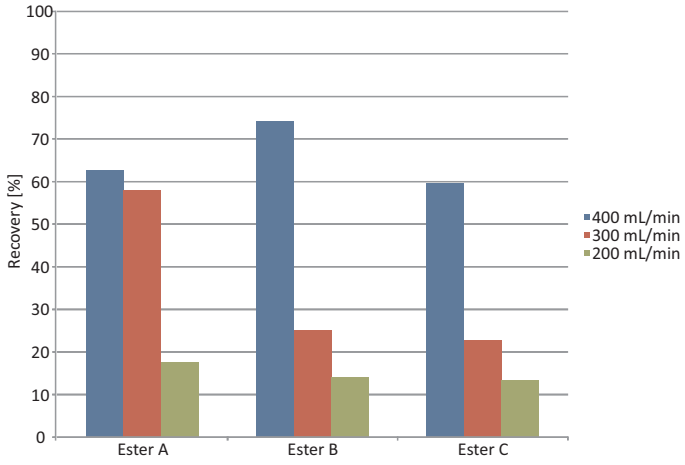

Figure 2. Effect of decreasing vent flow.

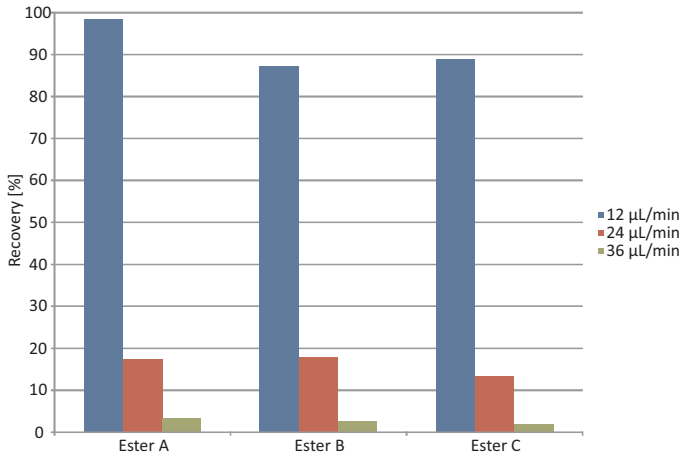

Figure 3. Effect of increasing injection speed.

LVI Optimization. As already mentioned, LVI in combination with a CIS (PTV type) inlet in solvent vent mode can be used to increase the injected sample volume. The technique is applicable to a wide variety of solvents. A basic equation has been developed [3] relating the maximum injection speed for a specific solvent with its physical properties, the CIS (PTV) initial temperature and the split vent flow. When this speed is correctly optimized the solvent will be selectively removed and analytes of interest concentrated in the liner for transfer to the column and detection system. This equation is used in the GERSTEL MAESTRO software, shown in Figure 4. The software enables rapid method development by calculating the optimum injection speed from the above-mentioned parameters. A too low injection speed may lead to loss of low boiling analytes, while a too high injection speed leads to general loss of analytes as they will exit the inlet liner with unvaporized solvent. In general the more apolar solvents closely follow the equation used in the calculator software resulting in injection speeds compatible with good analyte recoveries. A safety margin correction is available in the software and this is usually set to 0.7 – 0.8.

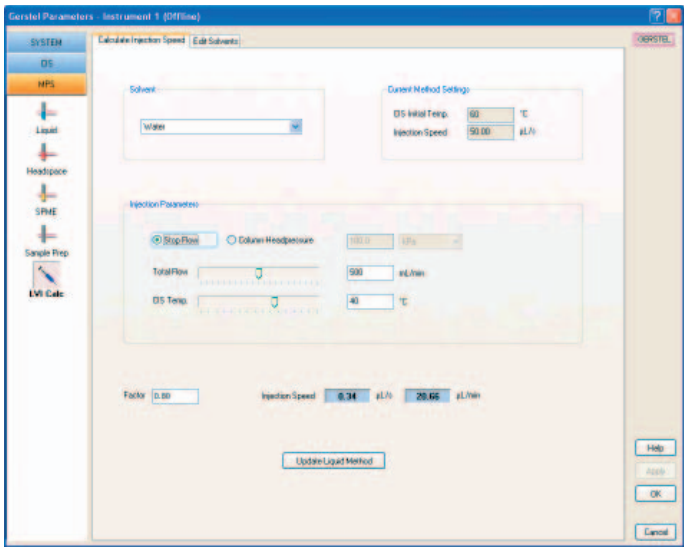

Figure 4. Solvent vent calculator in GERSTEL MAESTRO software.

Calculator predictions for water and samples containing large amounts of water, such as distilled spirits, are not successful however, not even when the recommended correction factor is used. The reason for this is that water has an abnormally high latent heat of vaporization which causes an additional cooling effect in the liner during its removal. A second reason is related to the maximum saturation value of water in the helium venting gas, which results in the need for very high split vent flows. Not taking these considerations into account has led many practitioners to conclude that water cannot be successfully vented. Using a vent flow of 500 mL/min, a CIS (PTV) temperature of 40°C and a correction factor of 0.8, the calculator predicts an injection speed of 21 µL/min for water. However, experimental optimization gave best results when the injection speed was lowered to 12 µL/min for the same conditions using the same factor. More than 80 % more analytes were recovered at the lower injection speed. A correction factor of 0.43 would have been necessary in order to arrive at the optimum injection speed.

We can now compare results from the above successful working conditions for water with predictions from the GERSTEL Calculator with a correction factor of 0.4 applied. This will also be compared with predictions for similar 1 ppm solutions in methanol and ethyl acetate respectively, both with a correction factor of 0.8 (figure 5).

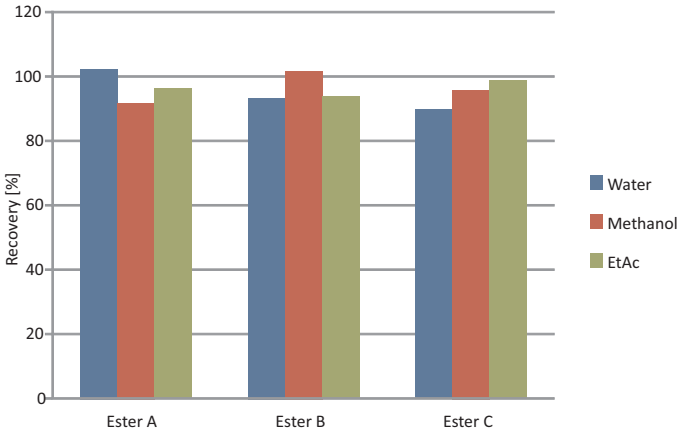

Figure 5. Comparison of analyte recoveries from large volume injections using different solvents.

The comparison of analyte recoveries from 100 µL large volume injections using different solvents correlate well with each other, showing that the calculated injection parameters can successfully be translated into working sample introduction conditions.

Additional large volume approaches. This concept of Large Volume Injection with Solvent Venting can be further extended to include various headspace injection modes and desorption from fibres (SPME) and stir bars coated with sorbent (SBSE). The only difference now is that a simple solventless extraction step has been performed on the sample; incubation for static headspace, incubation and trapping for dynamic headspace, and partitioning for fibres and stir bars. Otherwise the exact same logic and conditions as outlined in Table 1 applies. Now a gas containing the analytes is the "large volume" and these analytes must be separated from the carrier gas and concentrated in the CIS (PTV) liner.

Figures 6 to 9 show GC-MS TIC traces obtained from a premium commercial gin using static headspace, dynamic headspace, Stir Bar Sorptive Extraction (SBSE) using the PDMS-based GERSTEL Twister and Large Volume Injection, respectively.

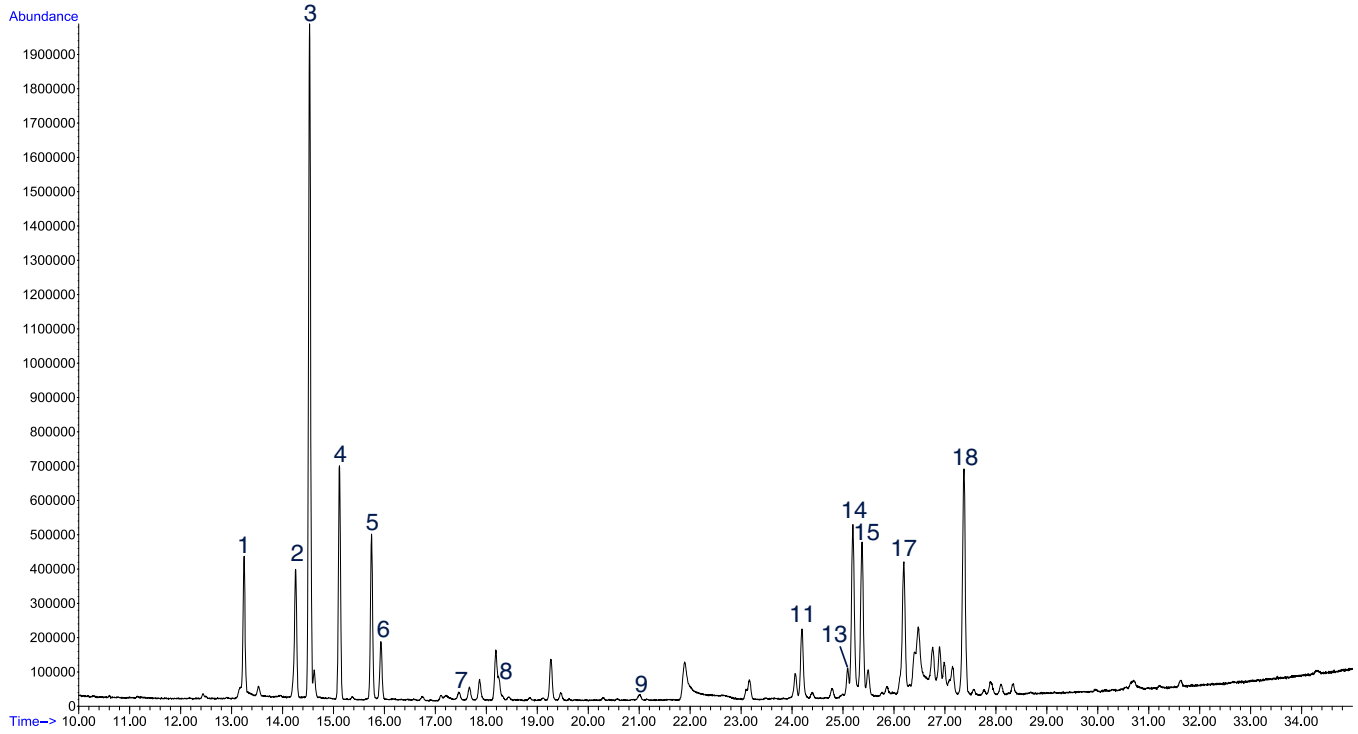

Figure 6. Static headspace TIC of a premium commercial gin.

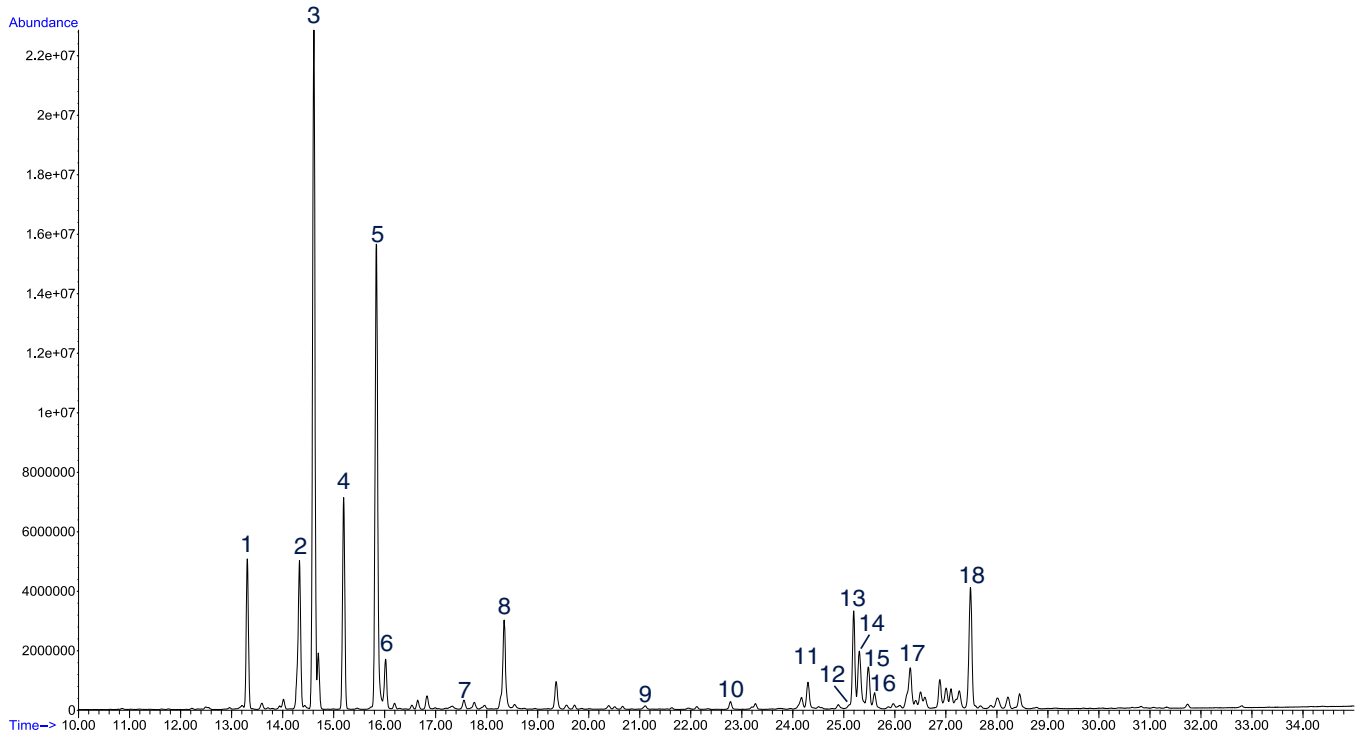

Figure 7. Dynamic headspace TIC of a premium commercial gin.

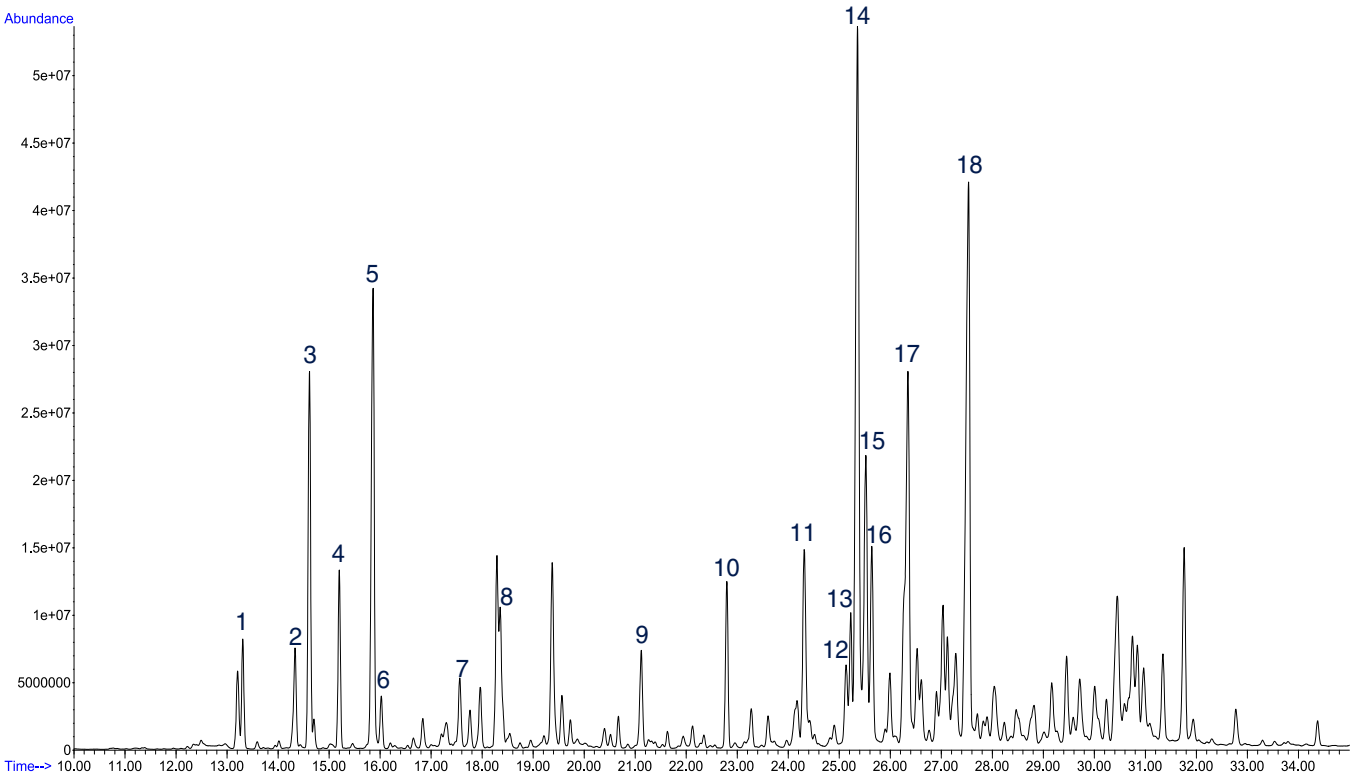

Figure 8. SBSE TIC of a premium commercial gin.

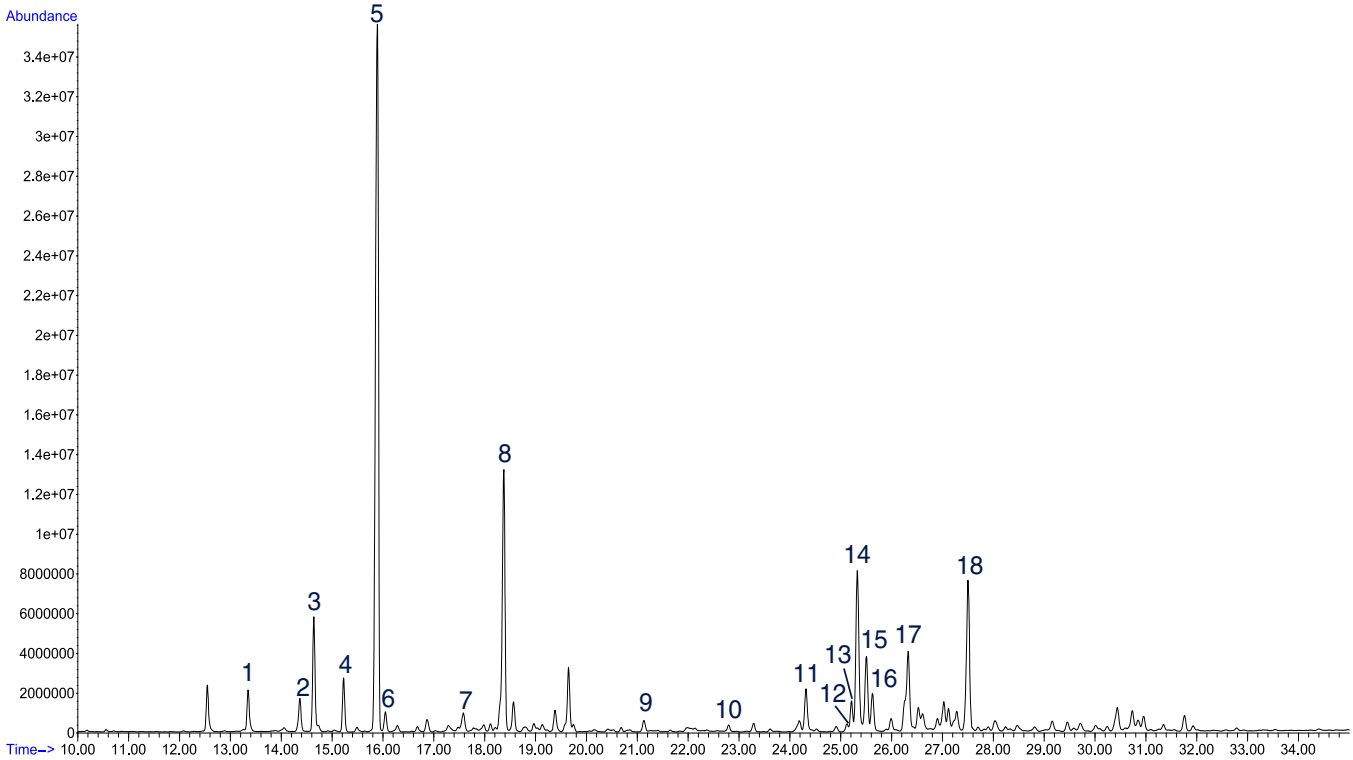

Figure 9. LVI TIC of a premium commercial gin.

Table 2 details some important compounds from the sample. All injections were performed using solvent vent split followed by splitless transfer of the isolated analytes. The chromatograms are different both in their profiles and with respect to their relative peak abundances. Each chromatogram must be interpreted as a function of the technique. The headspace techniques may reflect more the sensory properties of the sample, with dynamic headspace extending both compound recovery and abundance. SBSE on apolar PDMS is very sensitive but recovery of very polar compounds will be low (a new Ethylene Glycol Silicone stir bar is now available, which is well suited for phenols and similar compounds). Finally recovery by liquid injection is related more to compound boiling point and will be therefore be applicable to both polar and apolar compounds.

Table 2. List of compounds identified in premium commercial gin.

No.	Compound	No.	Compound
1	Myrcene	10	Geranyl acetate
2	p-Cymene	11	β-Elemene
3	Limonene	12	γ-Elemene
4	γ-Terpinene	13	β-Farnesene
5	Linalool	14	Germacrene
6	α-Terpinolene	15	β-Caryophyllene
7	Camphor	16	β-Cubebene
8	Terpinen-4-ol	17	α-Humulene
9	Bornyl acetate	18	δ-Cadinene

Conclusions

This Application Note has attempted to explain the concept and procedures for successful application of combined in-liner PTV extraction and analysis of trace analytes from liquid matrices. In this way the off-line classical solvent extraction approach can be avoided and is replaced by a solventless step which is an integral part of a fully automated procedure, and in which any number of samples can be run in automated sequence batches. Each different solvent vent injection mode adds a unique dimension of information to the total profile of a sample and could be further usefully combined with sensory data from an Olfactory Detection Port (ODP).

References

[1] K. Abel. J. Chromatogr., 13 (1964) 14-21

[2] W. Vogt et al., J. Chromatogr., 186 (1979) 197-205

[3] Staniewski et al., J. Chromatogr., 623 (1992) 105-113

[4] H. Mol et al., Trends in Anal Chem., 15 (1996) 206-214

[5] S. Müller et al., Chromatographia, 38 (1994) 694-700

[6] J. Teske et al., Chromatographia, 46 (1997) 580-586

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