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Direct Injection of Distilled Spirits with PTV Matrix Removal: The Perfect Splitless Injection ?

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KEYWORDS
Large Volume Injection, PTV, Distilled Spirits, Water Removal, Solvent Venting.

INTRODUCTION
Distilled spirits are complex mixtures of flavour compounds in an ethanol/water matrix [1]. These compounds originate from the raw materials, and the fermentation, distillation and ageing stages of the production process. These sources are reflected in the range and concentration of compounds present, and their large volatility spread.

Different distilled spirits have many of these compounds in common, and chromatographic authentication procedures tend to rely on establishing unique patterns and ratios based on similar compounds. These patterns are contributed by different traditional and production processes, and geographic factors.

The aqueous ethanol base of distilled spirits represents a relatively clean matrix, even allowing for the contribution of semi-volatile compounds from wood ageing. Direct split capillary injection is therefore an effective technique for profiling some of the most abundant flavour compounds in spirits [2, 3]. The split ratio is adjusted so that the amount of ethanol/water matrix transferred to the column does not interfere with resolution of important compounds eluting just before and after the ethanol peak. This immediate data set has been used extensively in authenticity investigations [4, 5]. However the price to be paid for this success is that later eluting compounds, which are naturally present in lower amounts, can now be at their limit of detection because of the intentional losses in split injection.

Direct splitless injection of the sample offers a solution for detection of these compounds as it involves transfer of the total injection volume to the column. The much greater amount of matrix solvent on the column effectively destroys the resolution of early eluting compounds, but this information is already available from the split injection. This scheme of split and splitless injections then constitutes a complimentary approach in which each part contributes its own information [6]. Full recovery of the compounds of interest in the splitless mode is never achieved as now the high water content of the sample plays a dominant role. Due to its relatively high vapour volume water vapour will quikkly fill the injection liner and inhibit the vaporisation of other compounds. Conditions must also be carefully chosen to optimise retention of solutes at the head of the column and avoid peak splitting. Water can also attack chromatographic stationary phases and cause unwanted peak retention time shifting [7]. Problems associated with introduction of water into chromatographic systems have been summarised [8].

To avoid the problems associated with water it is necessary to selectively remove it, but if possible without expensive sample preparation. One approach has been use of a zeolite membrane under vacuum which selectively removes only water [9]. However the commercial offering of this technology uses a membrane bed design which is too slow for rapid processing of samples for GC. Second generation membrane designs are expected to offer much higher water flux rates. For wine aroma compounds an approach is described in which 10 mls of wine is rapidly extracted with 100 µl of Kaltron, followed by injection of 25 µl of the aroma extract with solvent removal in a programmed temperature injector (Gerstel CIS-3). The most simple extraction procedure is used and the required detection limits are achieved by large volume injection with solvent venting and enrichment of solutes of interest in the liner [10]. This technique can be used for rapid determination of both fermentation and varietal compounds.

A logical extension of this approach is direct injection of actual distilled spirits with the possibility of ethanol and water removal in the PTV liner. Initial work has been done in this area together with an optimisation study involving the different variables effecting the efficiency of the process. Successful solvent removal together with enrichment of solutes of interest in the liner requires that the solvent be vented as its vapour. Injection speed is critical, together with type of liner packing, purge gas flow during solvent removal, and ability to control pneumatics to minimise transfer of solvent to the column. This technique replaces classical splitless injection for normal injection volumes while avoiding the problems caused by water. It also extends the range of compounds that can be detected because much larger volumes can be injected with simultaneous elimination of solvent in the PTV liner. It is the purpose of this contribution to demonstrate how injection volumes up to 50 µl of distilled spirits can be automatically and routinely injected with PTV matrix removal and subsequent splitless transfer of compounds of interest. Injection volumes can be suited to the detection limits required for different classes of compounds. In addition to complete instrumental automation an on-line software calculator for optimum venting of solvents of interest is available.

EXPERIMENTAL

Instrumental. The system consisted of a Hewlett-Packard 5890 Series II gas chromatograph equipped with a Multi Purpose Sampler (Gerstel GmbH) with a 50 µl syringe, a HP 7673 autosampler tray for 100 2 ml standard vials, and a programmed temperature injector (CIS-3, Gerstel GmbH) which acts as interface for solvent venting and subsequent venting and analysis.

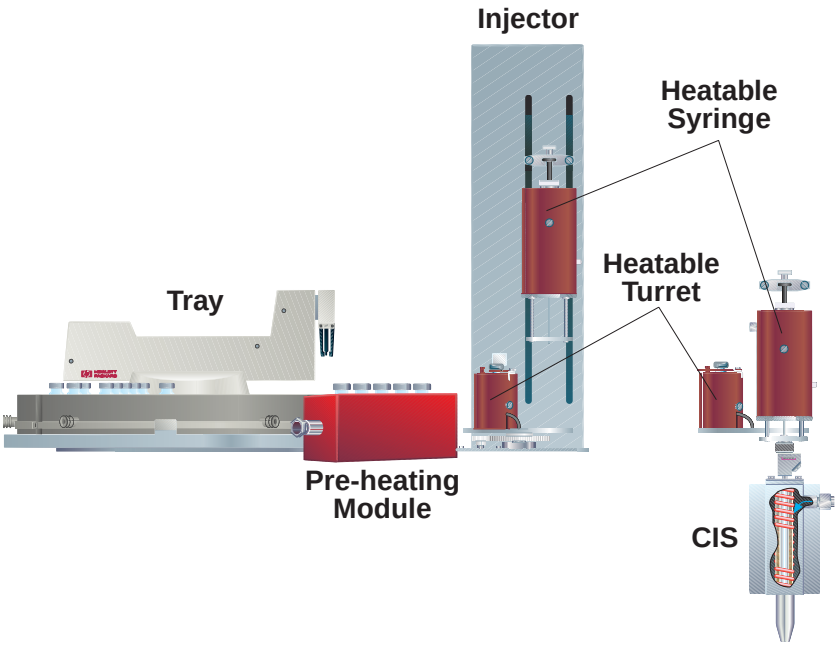

Figure 1. Gerstel MultiPurpose Sampler, here used as Large Volume Injector. Heated zones can be used optionally or for headspace mode.

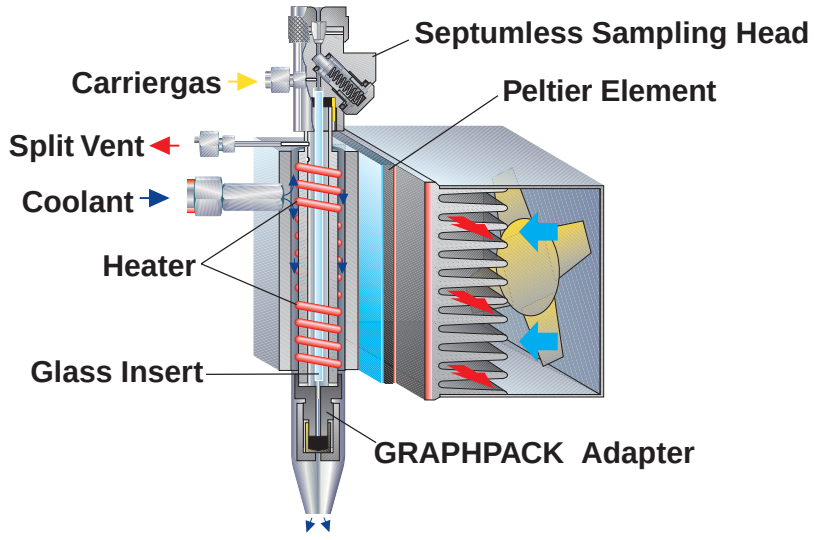

Figure 2. Gerstel Cooled Injection System CIS 3.

Large Volume Injection. The Multi Purpose sampler fills the syringe with sample and with the split vent open and PTV cooled down to a suitable temperature the sample is injected with a programmed speed into the glass insert packed with a suitable adsorbent. The depth of penetration into both the sample vial and the injection liner can be controlled. The split flow through the liner at the selected temperature preferentially removes the solvent, and solutes remain in the liner. After this stage the split is closed and the PTV is ramped to the desired temperature for splitless transfer of the analytes to the column.

Large volume injection parameters for optimum removal of solvent are calculated from [12]

$$V_{inj} = \frac{M * p_{solv}}{r * R * T_0} * \frac{p_0}{p_{inj}} * F_{split} \qquad [12]$$

where V_{inj} is the calculated maximum rate of injection, F_{split} is the gas flow via the split line, P_{solv} the vapour pressure of the solvent, ρ the density of the liquid, P_0 the outlet pressure, P_{inj} the pressure in the insert, and T is the temperature of the PTV inlet.

A calculator in the Gerstel software gives V_{inj} for different solvents at different PTV temperatures and venting flows. The injection rate above was calculated in this way for the mixture 40% ethanol/ 60% water, representing the matrix of distilled spirits.

Sample Preparation. Samples were injected directly with the option of Decanol-3 as internal standard.

Analysis Conditions.

MPS:	10, 25, 50 µl, at 5 µl/min
CIS:	4 mg Tenax TA, 20/40 mesh 1.8 min solvent vent (150 mL/min), splitless (3 min) 30°C (2 min), 10°C/s, 300°C (10 min)
Column:	30m HP-InnoWax (HP), d_i= 0.25 mm, d_f= 0.25 µm
Pneumatics:	He, P_i= 80 kPa (5 kPa during large volume injection)
Oven:	40°C (2 min), 3°C/min, 240°C (10 min)

Results and Discussion.

Figure 3 shows the FID trace of a test mix in 40% ethanol/60% water after a 50 µl injection. The compounds were at 1-2 ppm and Table I gives peak identities and area reproducibility data after six successive injections. The automation features of the large volume sampler and precise pneumatic and temperature control in the PTV has given excellent reproducibility. The compounds represent a series of secondary flavour species in distilled spirits and at a level difficult to analyse and quantify without some form of enrichment. This usually involves some form of solvent extraction and while this can be a simple extract with large volume injection, there is still the problem of different extractabilities for different compounds. The increased coefficient of variation for the less volatile compounds correlates with increased retention of these compounds on the adsorbent and greater desorption difficulties.

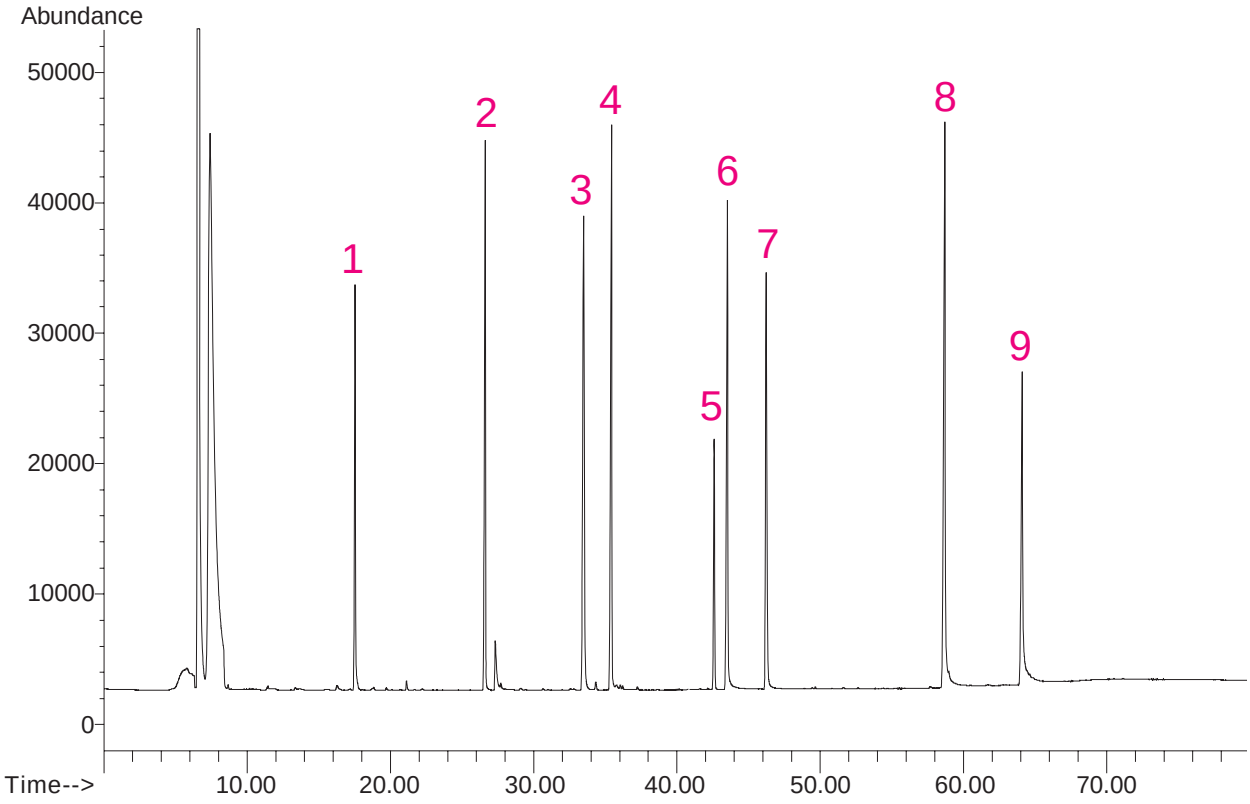

Figure 3. FID-chromatogram of a 50 µl injection of a test-mix, compound identification see table I.

Coefficient of variation (sample standard deviation as a percentage of the mean) for peak areas from 6 replicate injections.

Table I. Test mixture reproducibility for 50 µl injections.

No.	Compound	Coefficient of Variation
1	Ethyl Caproate (C6)	1.2 %
2	Ethyl Caprylate (C8)	1.0 %
3	Decanol-3 (ISTD)	1.1 %
4	Ethyl Caprate (C10)	1.2 %
5	Phenyl Ethyl Acetate	1.1 %
6	Ethyl Laurate (C12)	1.9 %
7	2-Phenyl Ethanol	1.8 %
8	Ethyl Palmitoleate (C16:1)	3.9 %
9	Ethyl Stearate (C18)	6.8 %

Figures 4 to 6 in turn show FID traces for a whiskey for a 10, 25 and 50 µl injections. Peak shape and resolution are good and very fine detail is evident. The injection volume can be used as a variable to enhance detection limits in various elution areas of the chromatogram. Later eluting higher fatty acids and esters are more clearly profiled with the 50 µl injection.

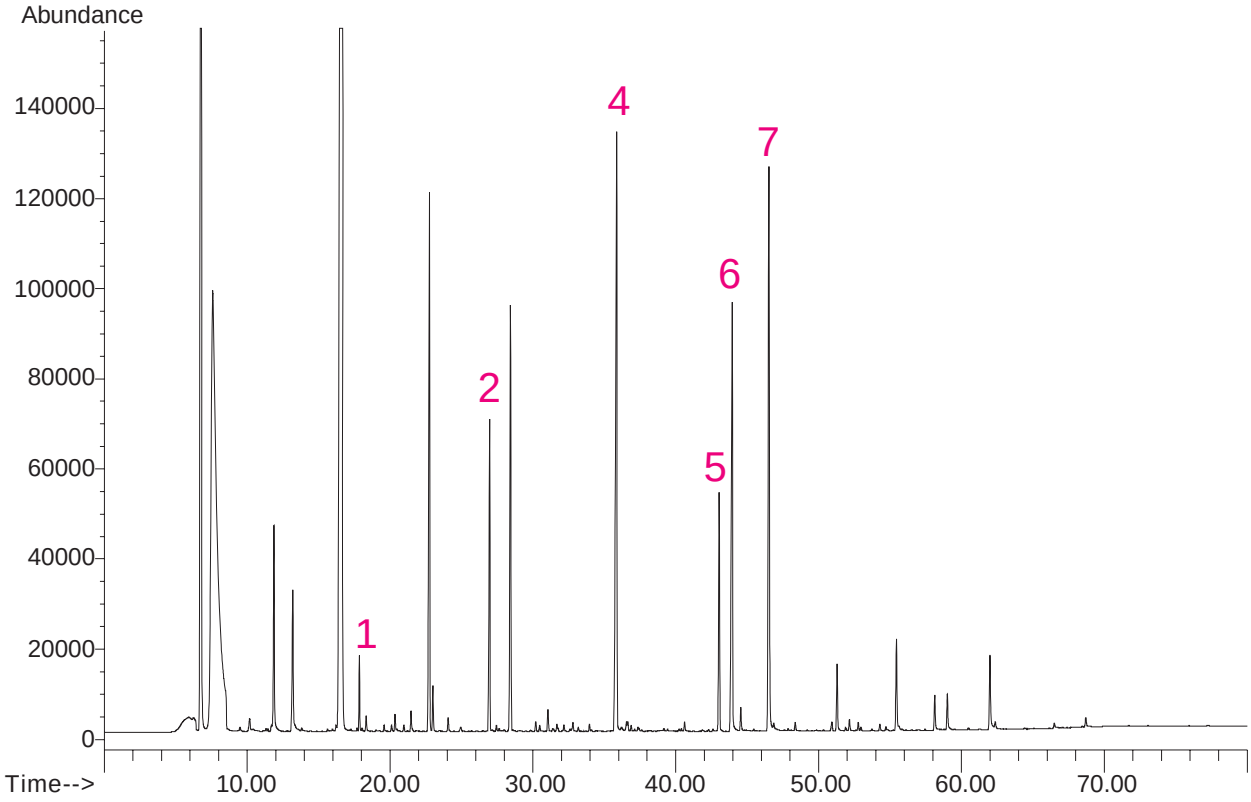

Figure 4. FID chromatogram of a 10 µl injection of a whiskey, compound identification see table I.

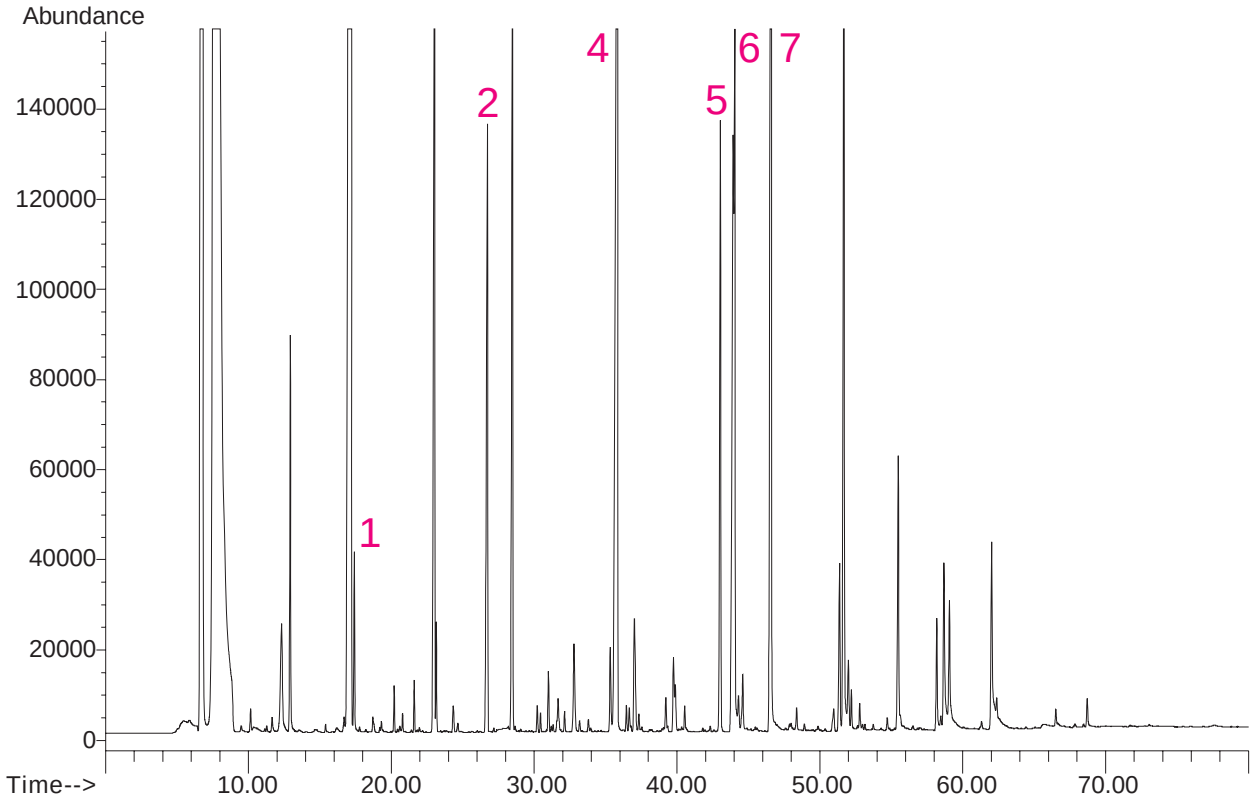

Figure 5. FID chromatogram of a 25 µl injection of a whiskey, compound identification see table I.

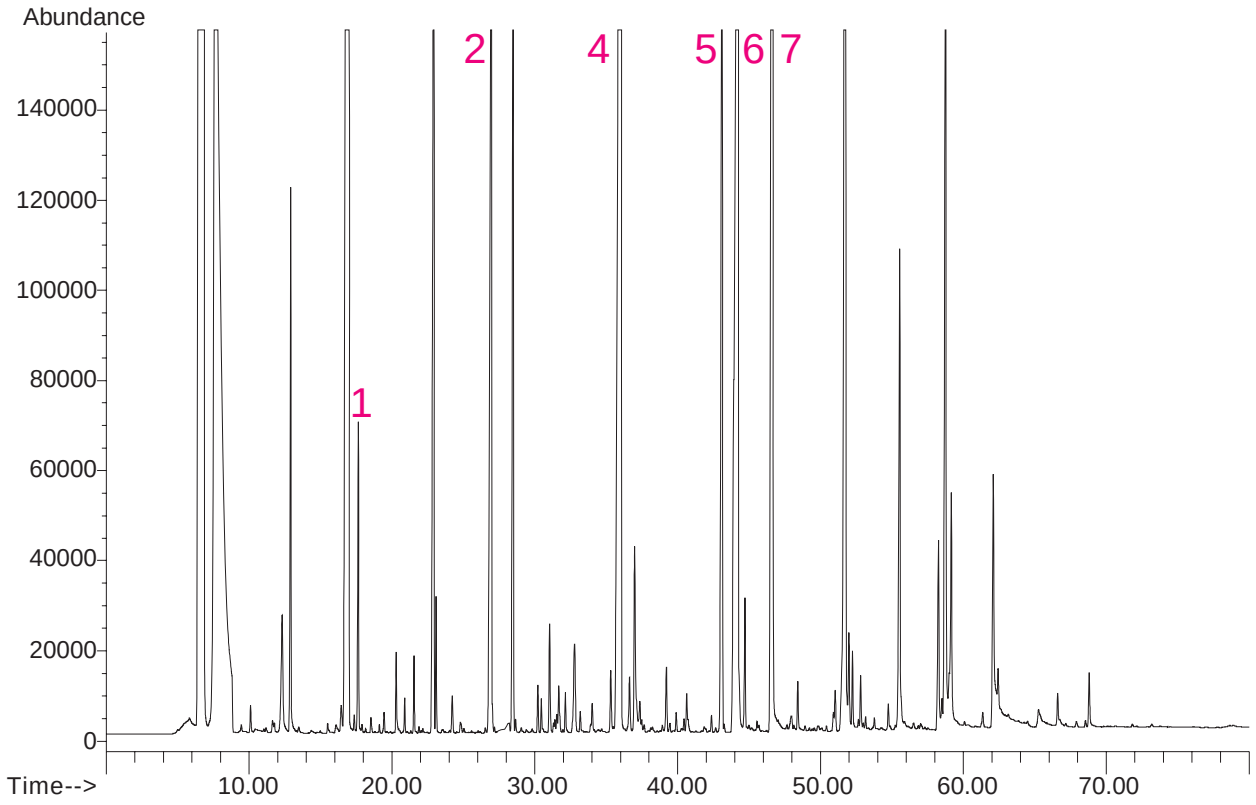

Figure 6. FID chromatogram of a 50 µl injection of a whiskey, compound identification see table I.

This particular application was performed using Tenax TA as the liner packing. Tenax TA weakly retains water and does not need unnecessarily high temperatures for desorptive release of compounds in a certain volatility range. In an earlier application a two column arrangement was used in which the first column packed with Tenax TA was used to remove water [13]. Large volume sampling in conjunction with a PTV injector for solvent removal simplifies this by allowing both solvent removal and solute enrichment on the packing in the injection liner.

Use of an adsorptive packing still necessitates efficient desorption after enrichment. In the case of aged distilled spirits we have found that compounds such as vanillin would require unattainably high desorption temperatures after enrichment on Tenax. However the boiling point difference between vanillin and water is such that enrichment after large volume injection succeeds for these compounds when the liner contains only glass wool. In this case most of the compounds profiled in figure 3 would be lost with the solvent, and so the exact analytical conditions need to be tailored to the compounds of interest. Again the idea of complimentary analyses with instrumental conditions for different classes of compounds is an attractive proposition. Recent trials with a new packing material which is a pure chromatographic phase, and therefore only involves partitioning of solutes from solvent of interest in the injection liner, have proved very interesting [14, 15]. This offers the possibility of a universal packing material in which gas phase separation of solvent from solutes to be enriched can be achieved from predicted chromatographic behaviour.

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