Application Note No. 032

A Novel Large Volume At-Column Concentrating Technique and its Applicability to Labile Pesticide Analysis

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Introduction

Rapid large volume injection using an Optic programmable injector has been widely used in recent years, when analyzing a large variety of trace compounds. This combines the advantages of the ability to inject greater than 100 times more sample [1,2], therefore reducing the amount of sample preparation, as well as its use as a method of hyphenation [3,4,5] and automation. There are, however a couple of disadvantages when using the RLVI packed liner. Decomposition of some analytes may occur due to the catalytic effects of the liner packing [7,8], whereas some analytes adsorb to the packing and are too strongly retained. The disadvantages with the conventional large volume on-column injection method, [9,10,11], is that a long pre-column is necessary and the auto-sampler injection speed must be strictly controlled [12,13]. The new large volume at-column concentrating technique has been devised to overcome these problems.

The Large Volume At-Column Concentrating Technique

The standard Optic injector is used, although the use of a Merlin Microseal with a modified injector top is recommended. A special at-column liner, containing no packing material, is employed for this technique, greatly reducing the probability of target compounds being decomposed or irreversibly retained. The sample solvent is held and evaporated in the liner and the target compounds are concentrated at the front end of the analytical capillary column, this therefore eliminates the need for a long pre-column, solvent vent line and the precise control of the injection speed.

This method is consequently ideal for the analysis of labile compounds and has been compared with other PTV injection techniques for the analysis of various heat labile pesticides including DEP, Bendiocarb, Carbaryl, Methiocarb, Endrin, p,p'-DDT, Iprodione and EPN.

The At-Column Theory

Figure 1 shows the theory behind the at-column concentrating technique. If a tube is heated with the temperature around the boiling point of a solvent, when the solvent is injected it stops flowing at the point where the carrier gas pressure and the solvent vapour pressure are equilibrated. This theory is used for the at-column liner.

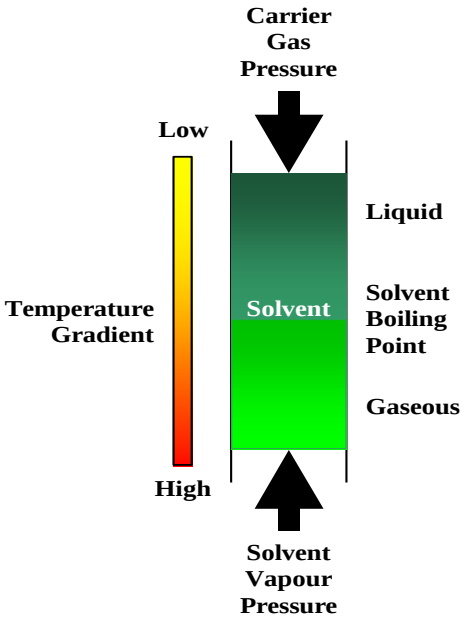

Figure 1: *At-column theory in a tube*

The at-column liner tapers to a very narrow tube, at the entrance of which is positioned a small glass bead, approximately 1 mm in diameter, which prevents the rapid flow of the sample solvent into the column by increasing the flow resistance. The outlet is used as a press-fit connector for the short deactivated silica capillary retention gap. The retention gap is then connected to the capillary column using a mini-union or a press-fit connector. There is a small diameter hole at the side of the upper liner, at a sufficient height for the injected sample volume, through which the evaporated solvent is vented.

The temperature of the injector is set slightly below the corrected boiling point of the sample solvent and the GC oven temperature is set slightly higher than it. Therefore, when the sample is injected a small quantity infuses down the temperature gradient becoming gaseous and producing a solvent vapour pressure. See Figure 2. The majority of the sample then remains in the liner and the evaporated solvent flows out of the side hole and through the split line. The target analytes are concentrated into the inlet of the capillary column through the pre-column. The oven temperature programme is then started to separate the analytes.

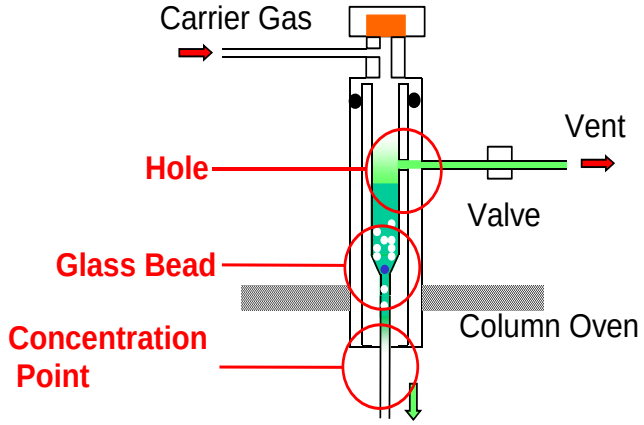

Figure 2: *Scheme of solvent evaporation and concentration of target compounds in the liner*

Optimisation of Parameters

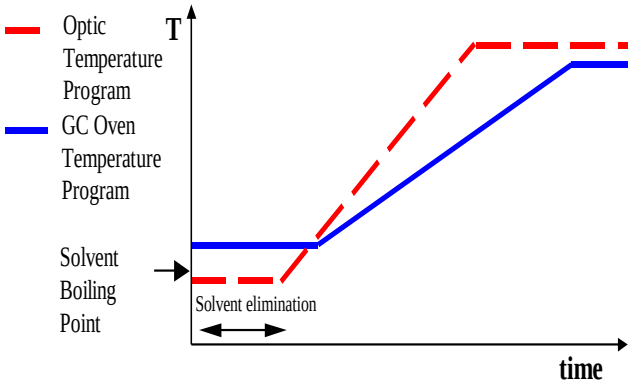

Figure 3: *Temperature program of the injector and oven*

Figure 3 shows the temperature program of the Optic and the GC oven in relation to the boiling point of the solvent. In order to achieve equilibrium to prevent the liquid sample from flowing into the capillary column, the injector temperature must be set below the solvent boiling point and the GC oven above it. The boiling point is dependent on the carrier gas pressure and therefore the corrected solvent boiling point must be used. The optimisation of these two parameters is critical for obtaining good recoveries and reproducibility.

Instrumentation

Experimental work was carried out using an HP5890 gas chromatograph equipped with an HP5971 mass selective detector. An ATAS Optic 2-200 programmable injector was used with a special at-column liner designed and manufactured by GL Sciences Inc., Japan. The capillary pre-column was a GL Sciences Inc. 0.53 mm I.D., 0.3 m length deactivated fused silica. The analytical GC capillary column was NB-5, 0.25 mm I.D., 30 m length and 0.25 µm film thickness by GL Sciences Inc. A capillary column of 0.1 µm film thickness was used to separate the labile pesticide compounds. A press-fit connector was used to connect the pre-column to the analytical column. Manual injections were carried out within two seconds.

Chemicals and Reagents

Hydrocarbon standard reagents C_{10} to C_{30} (even carbon numbers), DEP, Bendiocarb, Carbaryl, Methiocarb, Endrin, p,p'-DDT, Iprodione and EPN supplied by GL Sciences Inc. Solvents acetone, dichloromethane, hexane, ethylacetate and cyclohexane purchased from Kishida Chemical Co. pesticide residue analysis grade. Stock standard solutions were prepared by dissolving 500 mg in 50 ml acetone.

Optimisation of Injector Temperature

Using a carrier gas inlet pressure of 25 kPa gave the boiling point of acetone as 62.8°C. Variation of the injector temperature at 58, 60, 62 and 68°C during solvent elimination, gave evaporation times of 210, 180, 140 and 90 seconds respectively for an injected volume of 50 µL. The results are shown in Figure 4. Less response was obtained when a temperature higher than the solvent boiling point was used, thought to be due to the fact that the solvent boiled in the liner splashing away some analytes. A high temperature, still lower than the BP of the solvent is better for quick solvent evaporation, therefore the optimized injector temperature was 60°C. Hence, the optimized injector temperature is 3°C below the boiling point of the solvent.

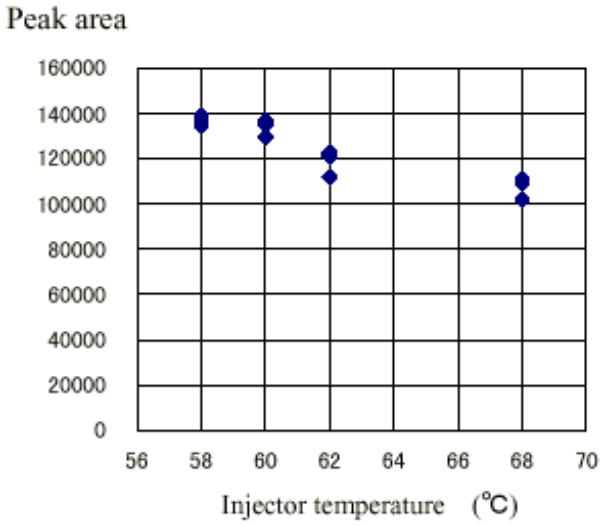

Figure 4: *Effect of injector temperature on recovery of C_{16} in acetone*

Optimisation of Oven Temperature

The oven temperature was set at 78, 73, 70 and 65°C with the injector temperature at 60°C, the results are shown in Figure 5. Peak distortion occurred at a temperature close to the boiling point of the solvent, thought to be due to heat fluctuations at the inlet of the capillary column caused by the heat of solvent evaporation. Consequently, the optimized oven temperature was 73°C, which is 10°C above the boiling point of the solvent.

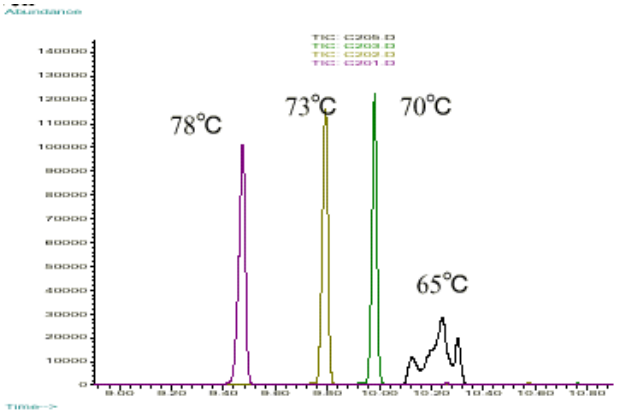

Figure 5: *Effect of GC oven temperature on peak shape*

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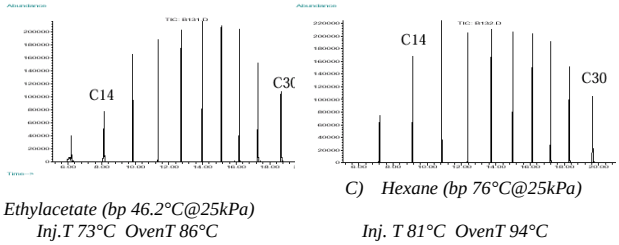
Investigation of Other Solvents

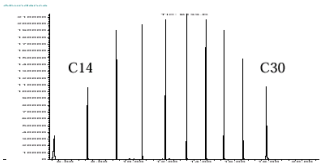
Using the injector and oven optimization theories developed above, various solvents were used for the hydrocarbon standard, including acetone, dichloromethane, hexane, ethylacetate and a mixtures of ethylacetate:cyclohexane 50:50 and 30:70. The results and conditions are shown in Figures 6 and 7. This work concludes that various solvents and solvent mixture can be used with this technique.



Ethylacetate (bp 46.2°C@25kPa)
Inj.T 73°C OvenT 86°C

C) Hexane (bp 76°C@25kPa)
Inj. T 81°C OvenT 94°C

A) *Acetone (bp 62.8°C@25kPa)*
Inj.T 60°C OvenT 73°C



B) *DCM (bp 46.2°C@25kPa)*
Inj. T 43°C OvenT 56°C

D)

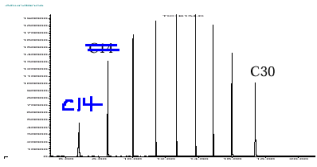
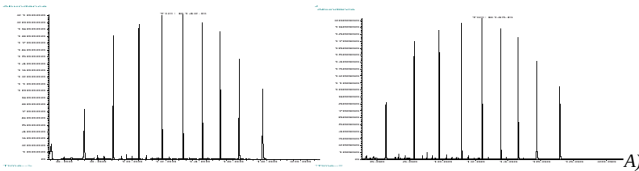


Figure 6: *Chromatograms from injecting 50 µl of various solvents*



Inj.T 75°C OvenT 88°C

Inj. T 85°C OvenT 98°C

A) Ethylacetate/cyclohexane 5/5

B) Ethylacetate/cyclohexane 5/5

Figure 7: *Chromatograms from injecting 50 µl of mixed solvents*

Linearity and Reproducibility

The relationship between the peak areas and the injected volumes of a sample at constant concentration were investigated, the results are shown in Figure 8. It was found to be linear in the experimented range of 10 to 100 µl. Therefore, one hundred times higher sensitivity can be obtained by injecting 100 µl of a sample compared to a 1 µl injection, and so this method is effective for obtaining high sensitivity.

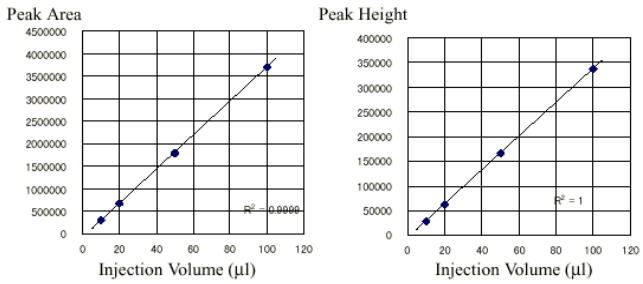


Figure 8: *Relationship between injection volume and peak area and peak height*

A 50 µl injection of a standard sample of 0.01 ng/µl concentration was repeated 9 times employing the at-column technique. The peak areas and the R.S.D. (relative standard deviation) are shown in Table 3. The R.S.D. in this experiment was within 5%. The reproducibility was satisfactory.

	1	2	3	4	5	6	7	8	9	Average	%RSD
C12	1059549	1124095	1105928	1132251	1173256	1110468	1138186	1068430	1181246	1121490	3.69
C14	1113853	1186166	1187112	1197177	1236483	1210877	1220751	1158818	1268749	1197776	3.74
C16	1281403	1362959	1360981	1360981	1403837	1405533	1416523	1342136	1455447	1377336	3.64
C18	1120442	1184975	1176412	1183874	1201856	1233702	1249041	1194455	1276524	1202365	3.81
C20	1117889	1165477	1167401	1175801	1172800	1244518	1257592	1204548	1273963	1197777	4.28
C22	1105673	1118605	1139791	1146009	1131088	1212008	1219210	1178475	1234343	1165022	4.07
C24	1081617	1079668	1105763	1114805	1088347	1171424	1180011	1151225	1199245	1130234	4.07
C26	1019542	1012698	1040743	1049667	1031075	1099408	1108152	1084038	1137452	1064753	4.13
C28	997298	991976	1021932	1039429	1017204	1083405	1094263	1071149	1121600	1048695	4.36
C30	968764	966875	996795	1017242	997102	1061379	1069361	1049885	1096275	1024853	4.52

Figure 9: *Peak areas of the n-alkanes and their RSDs*

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Comparison of Large Volume Methods for Labile Pesticides

Employing conventional large volume injection methods, target compounds are likely to be decomposed during the desorption stage due to the effects of the packing material in the liner. However, using the at-column technique for heat labile pesticides this is expected not to occur, since there is no packing material. Various injection methods were compared for the determination of heat labile pesticides. A mixture of pesticides was used, including DEP, Bendiocarb, Carbaryl, Methiocarb, Endrin, p,p'-DDT, Iprodione and EPN in acetone. The internal standard used was C_{20}. Five injection methods were compared: hot splitless (1 µl); on-column (1 µl); large volume PTV with Tenax TA 60-80 mesh (48 mg) packing material (50 µl) and Dexsil 300 GC Chromosorb 750 12 % 60-80 mesh (40 mg) packing material (50 µl); and the at-column concentrating large volume technique (50 µl). The results are shown in Figure 10.

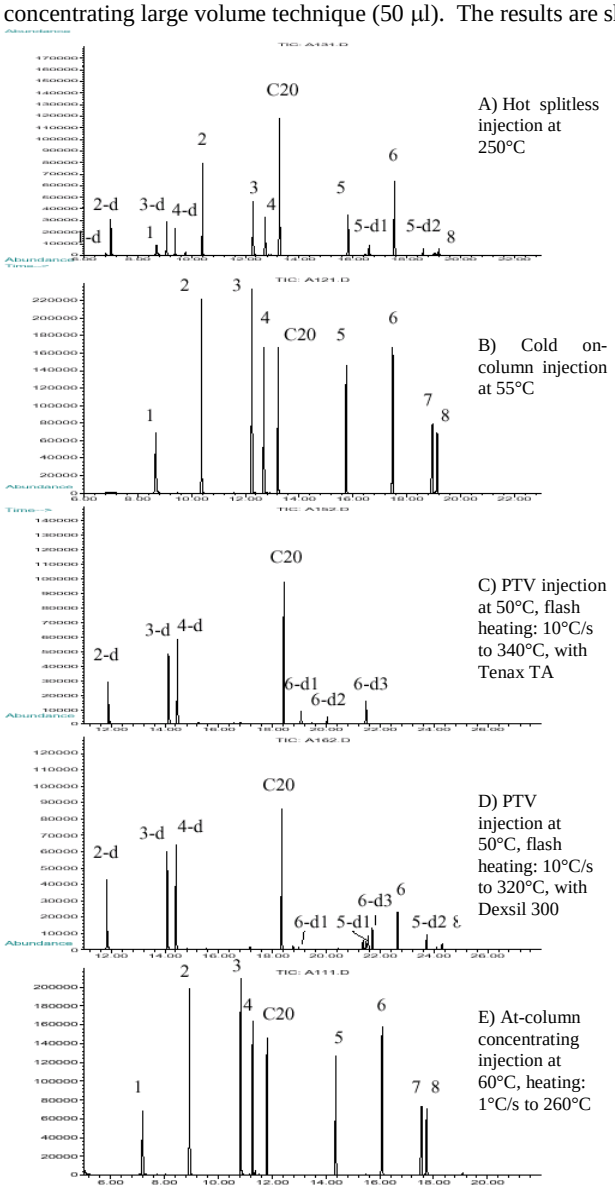

Figure10: *Comparison of pesticide thermal degradation using different injection methods: C20 (IS), (1) DEP, (2) Bendiocarb, (3) Carbaryl, (4) Methiocarb, (5) Endrin, (6) p,p'-DDT, (7) Iprodione, (8) EPN; d = degradation*

Figure 10-A illustrates that heat degradation occurs when a high injector temperature is used. The on-column injection method, Figure 10-B, provides a good reference chromatogram as no degradation occurs using a low injector temperature. However, the maximum injection volume is only 1 to 2 µl. Employing the conventional PTV large volume injection method using two types of packing materials, all kinds of heat labile pesticides are degraded as shown in Figures 10-C and 10-D. However, no compound degradation is visible when employing the at-column concentrating technique where a large volume of a sample of heat labile pesticides was injected, as shown in Figure 10-E. Therefore, this method is useful for the determination of heat labile pesticides with high sensitivity.

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Conclusions

The novel at-column concentrating large volume technique has been shown to give linear and reproducible results and can be used with a range of solvents and solvent mixtures. As packing materials are not necessary, this is a useful technique for the analysis of compounds which decompose on or are irreversibly adsorbed to the liner packing, and also for labile pesticides and other heat sensitive compounds which are prone to decomposition in hot injectors.

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