Application Note: 10110

30X Increased Sensitivity in the Analysis of Pesticides in Food by GC - Large Volume Splitless Technique

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Key Words

- Food Analysis
- High recovery of labile pesticides
- Large Volume Injection
- Pesticides
- Splitless Injection
- 30X increased sensitivity

TRACE GC Ultra™ with TriPlus™ AS Autosampler.

Introduction

The stringent legislations proposed by US EPA Code of Federal Regulations and by European Commission Directives [2,3] aim at a minimization of pesticide residues in food products. Maximum Residue Levels (MRL) of 0.01 mg/kg or less are frequently decreed. This represents a great challenge for analytical chemists who thus need enhancement of detectability in trace analysis. For this purpose, large sample volume techniques [1 and reference therein] emerged as fundamental tools in capillary gas chromatography.

Among the different techniques used for large volume injection, the recently introduced Large Volume Splitless (LV-SL) [1] has the following advantages:

(1) it is simpler because it allows injections of up to 50 µL in a conventional split/splitless injector without any special tuning of operating parameters;

(2) it is robust versus sample by-products or contaminants and extremely suitable for food matrices.

This application note reports the principles of this patented technology and provides some examples of pesticides analysis in food matrices achieved through this technique.

Principles of Large Volume Splitless injection technique

Classical splitless injection is based on the concept that the sample is rapidly vaporized in the liner chamber, and the vapors must be stored in this chamber until they are transferred into the column by the carrier gas [5]. Since vaporization is much faster than the transfer into the column, the vaporizing chamber must be sufficiently large to house almost the whole vapor volume. This limits the injection volume to a few microliters since the vaporizing chamber cannot be enlarged beyond a given size. LV-SL injection overcomes the limitation of the maximum sample volume to 1-2 µL of classical splitless injection by exploiting the Concurrent Solvent Recondensation technique (CSR).

CSR technique allows injection of large volumes by combining a restricted evaporation rate with an accelerated sample transfer granted by the pressure surge generated by solvent evaporation and by the quick solvent recondensation in a precolumn.

Concurrent Solvent Recondensation: Steps of the Process

CSR LV-SL injection requires a split/splitless injector with low dead volume and an uncoated precolumn with a capacity for retaining liquid at least corresponding to the volume of sample to be injected (e.g. 5 m x 0.32 mm i.d. or 3 m x 0.53 mm i.d. for 30 µL volumes). Figure 1 shows the five crucial steps of the CSR-LV-SL process: (1) fast, automated injection, minimizing contact between syringe and injector, and exploiting liquid band formation; (2) auto pressure surge strongly accelerating transfer of vapors in the precolumn; (3) recondensation of the solvent vapors in the precolumn; (4) transfer of solutes into the precolumn; (5) solvent evaporation in the precolumn.

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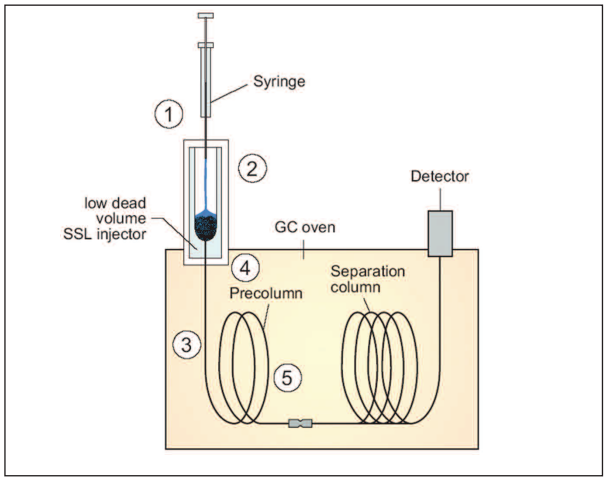


Figure 1: CSR Large Volume Splitless Injection schematic diagram. Number corresponds to the steps of the process (see text).

Injection with Liquid Band Formation

Band formation is achieved by a fast injection combined with a cool injector head suppressing solvent evaporation in the needle. The liquid band is stopped, and the liquid is kept in place generally by means of a packing of low thermal mass, such as deactivated glass wool. Unfortunately, the use of high-surface packing material as a means of stopping is well known to catalyze thermo-degradation of labile pesticides [6]. Alternatively, the liquid can be trapped between obstacles preventing escape from a small chamber. This latter case had been accomplished for this application note (see Experimental section).

Experimental

Sample Preparation

A series of organophosphorous (OPP), organochlorinated (OCP), nitrogen-containing and carbamate pesticides were selected according to two criteria: 1) pesticides reported as fragile in the literature [4-6]; 2) widely used pesticides for protection of fruits and vegetables and subjected to most severe legislation [2-3]. Standard solutions for Large Volume Splitless (LV-SL) injection were prepared at different levels of concentration from 0.8 to 100 ppb. Standard solutions for conventional Splitless (SL) and Cold On-column (COC) were prepared in Ethyl Acetate (EtAc) in the 240/430 ppb (ng/mL) range. Test samples were prepared by spiking (at levels of 10-50 ppb) negative-to-pesticides food extracts, GPC purified and reconcentrated at about 0.3 g/mL. 1-Bromo-2-Nitrobenzene was selected as internal standard (I.S.) due to its detectability with all detectors (NPD, ECD, FID) and its thermal stability.

Instrumentation

Analyses were performed with a Thermo Scientific TRACE GC Ultra™ equipped with a low dead volume split/splitless injector and a Digital Pressure and Flow Controller (DPFC) for the carrier gas supply, as well as NPD and ECD detectors (See instrument image on page 1). The injection system was designed to minimize the internal volumes in order to achieve an efficient auto pressure surge.

Injections of 1.0 µL (SL and COC) and 30 µL (LV-SL) were performed with a Thermo Scientific TriPlus™ Autosampler. Cold Needle technique was used (100 µL/sec as injection speed). A LV-SL deactivated laminar liner newly designed for this analysis was used. The split outlet and the septum purge were closed for 0.8 min.

The LV-SL oven method was determined with the help of LV-SL Assistant software shown in Figure 2.

The injector was thermostated at 250 °C.
Oven program: 80 °C (4 minutes) to 300 °C (1 minute) @ 10 °C/min.
Column flow: 1.5 mL/min. Splitless time: 0.8 minutes.
Detector Temperatures: NPD 300 °C, ECD 280 °C.
An uncoated deactivated guard column 5 m x 0.32 mm i.d. was connected to a OV-5 15 m, 0.25 mm i.d., 0.25 µm f.t. separation column through a deactivated press-fit connector.

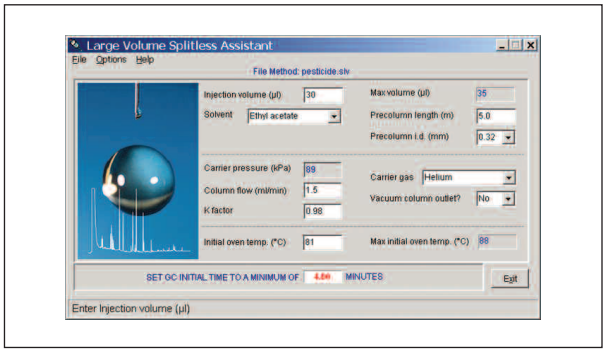


Figure 2: First screen of the LV-SL Assistant with determination of oven method parameters suitable for the injection of a 30 µL pesticides solution in ethyl acetate. This software provides the maximum initial oven temperature for a solvent at given conditions of carrier gas pressure and calculates the minimum initial oven time.

Results and discussion

Evaluation of Pesticides Recoveries

Results obtained by injecting 30 µL of the low concentrated standard solution were compared to those obtained with the injection of 1 µL of the 30 times more concentrated solution in COC and SL (Table 1). NPD response for almost every compound is the same with SL and LV-SL, provided no packing material is used in the injector. In several cases, as for Acephate or Azinphos, relative peak areas of LV-SL (empty liner) are even better than SL and more similar to COC. This can be explained considering that, by applying LV-SL technique, analytes are shielded due to the local drop of temperature caused by fast evaporation of large amounts of solvent.

Evaluation of Calibration Linearity

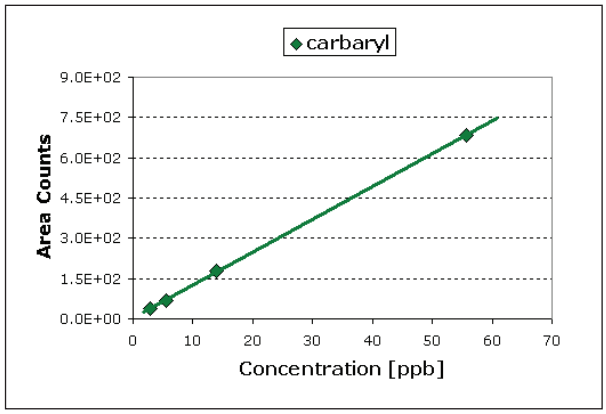
A high level of linearity (Table 2) was obtained with NPD by injecting 30 µL of standard solution at different concentrations. Similar results were obtained with ECD. Linearity is respected even at the sub ppb level and also with compounds known as labile (i.e. carbamates) from literature [4].

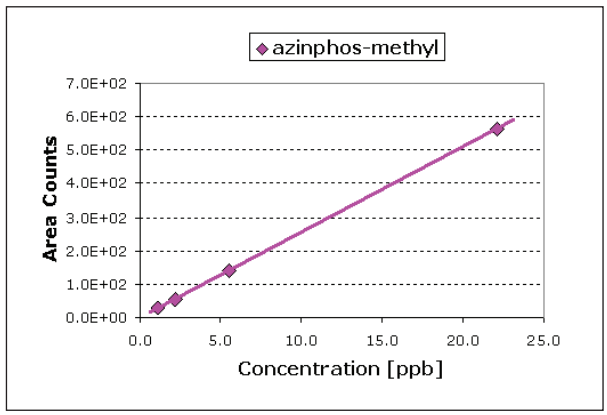
	PEAK AREAS			
INJECTOR	**ON-COLUMN**	**SPLITLESS**	**LARGE VOLUME SPLITLESS**	
INJECTION VOLUME [µL]	**1**	**1**	**30**	**30**
LINER	**-**	**EMPTY**	**PACKED WITH GLASS WOOL**	**EMPTY-LAMINAR**
CONCENTRATION RANGE [PPB]	**240-430**	**240-430**	**8-14**	**8-14**
metamidophos	25.8	17.6	-	18.4
dichlorvos	8.4	10.6	-	13.4
acephate	24.6	12.1	1.6	21.8
mevinphos (trans)	6.6	6.0	0.5	8.3
omethoate	11.1	9.1	9.8	19.1
dimethoate	31.0	28.2	1.9	31.8
atrazin	4.8	6.4	-	3.9
aminocarb	4.3	6.5	5.6	4.4
ethiofencarb	12.5	10.3	9.6	7.5
carbaryl	8.5	3.4	4.5	5.5
pirimiphos-ethyl	24.6	26.7	32.2	30.3
captan	1.6	0.7	0.3	0.8
folpet	1.4	0.6	0.4	0.6
bromophos-ethyl	19.1	19.1	24.8	29.9
vamidothion	13.1	7.1	7.2	11.3
jodfenphos	12.5	16.0	16.7	16.1
azinphos-methyl	5.2	3.8	3.8	5.5

Table 1: Peak Areas (relative to I.S.) in COC, SL and LVSL.

COMPOUND	**R^2**
Metamidophos	0.9998
Dichlorvos	0.9999
Acephate	0.9989
Mevinphos (trans)	0.9991
Omethoate	0.9999
Dimethoate	0.9998
Atrazin	0.9999
Carbaryl	0.9998
Pirimiphos-ethyl	0.9998
Captan	1.0000
Folpet	0.9998
Bromophos-ethyl	0.9998
Vamidothion	0.9997
Captafol	0.9999
Azinphos-methyl	1.0000

Table 2: LV-SL Linearity between 0.5 and 100 ppb (NPD) with examples.





Analysis of food extracts

Figure 3 shows the NPD chromatogram obtained through LV-SL injection of mid-range calibration (around 10 ng/µL in tomato extract).

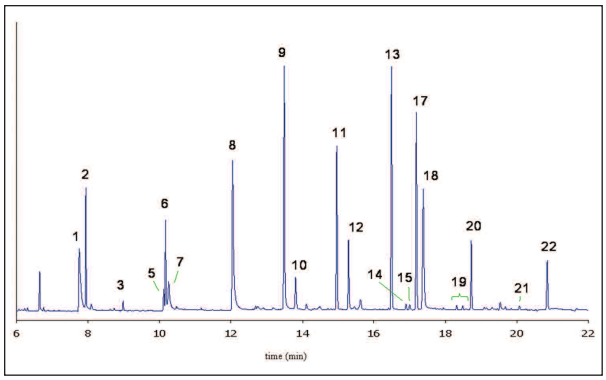


Figure 3: 30 µL injection of a tomato extract spiked with pesticides (NPD response). Compounds: 1 Methamidophos, 2 Dichlorvos, 3 IS, 4 Chlormefos, 5 isomer of Mevinphos, 6 Mevinphos, 7 Acephate, 8 Omethoate, 9 Dimethoate, 10 Atrazin, 11 Dichlorfenthion, 12 Carbaryl, 13 Pirimiphos-ethyl, 14 Captan, 15 Folpet, 16 Profenofos, 17 Bromophos-ethyl, 18 Vamidothion, 19 isomers of Celathion, 20 Celathion, 21 Captafol, 22 Azinphos-methyl.

The same solvent used for the food extraction and clean-up step, ethyl acetate, was also used for the GC analysis.

Results obtained by 30 µL injections of different real sample extracts spiked with a known amount of pesticides are shown in Table 3. The detector used was the NPD. The response is similar to that obtained with the standard solutions. Matrix effect was in most cases weak with limited response variations.

LV-SL repeatability and stability were evaluated by six consecutive 30 µL injections of a strawberry extract (Table 4). NPD absolute peak areas show deviations below 5 % for 13 pesticides out of 17. Slightly higher deviations were found for those compounds featuring low NPD response (e.g. captan). Standard deviations of retention times were around 0.005 minutes. Results were confirmed after carrying out 50 matrix injections: the same LV-SL liner can effectively be used for at least 80-100 large volume injections without maintenance.

compound	added amount [ppb]	concentration found [ppb]			
		beet	tomato	strawberry	pineapple
metamidophos	52.0	39.8	22.0	37.8	12.1
dichlorvos	20.0	16.7	15.6	14.2	14.5
acephate	10.1	10.9	11.0	8.8	8.1
mevinphos	20.0	14.3	15.6	13.4	12.4
omethoate	40.4	39.2	41.5	35.0	35.9
dimethoate	36.0	31.6	34.3	30.9	30.4
atrazin	8.1	7.4	8.3	7.3	7.2
carbaryl	27.9	31.0	33.4	32.0	31.8
pirimiphos-ethyl	33.7	30.2	32.4	28.6	29.0
captan	26.1	26.2	28.8	17.2	30.3
folpet	16.5	17.4	19.6	8.3	19.2
bromophos-ethyl	38.4	36.1	38.7	33.1	34.6
vamidothion	34.9	37.4	39.6	35.0	35.7
azinphos-methyl	11.1	11.5	11.3	10.2	9.9

Table 3: Determination of pesticides in different food matrices.

compound	Retention times		Absolute peak areas	
	mean [min]	SD [min]	mean [counts]	RSD%
metamidophos	7.820	0.013	666306	3.7%
dichlorvos	7.914	0.001	208138	3.5%
Br-NO2-benzene	8.944	0.002	19920	4.5%
acephate	10.401	0.010	129199	6.7%
mevinphos (trans)	10.136	0.001	180446	3.9%
omethoate	11.881	0.019	555006	3.7%
dimethoate	13.460	0.005	627220	3.1%
atrazin	13.829	0.004	102705	2.5%
ipofenthion	14.893	0.001	295997	1.9%
carbaryl	15.234	0.002	110905	2.1%
pirimiphos-ethyl	16.429	0.002	528071	2.3%
captan	16.822	0.001	8272	13.1%
folpet	16.923	0.001	6141	9.6%
bromophos-ethyl	17.106	0.002	408103	2.4%
celathion	18.639	0.001	58969	2.3%
vamidothion	17.376	0.013	401545	5.8%
azinphos-methyl	20.757	0.002	121300	3.8%

Table 4: Repeatability of Retention Times and Peak Areas.

Conclusions

LV-SL technique allows the operator to analyze residual pesticides in food with a 30X sensitivity increase. The recovery of labile pesticides is equivalent or better than in classical splitless analysis: degradation due to glass wool in the hot injector is overcome by using empty LV liners with optimized design and deactivation.

LV-SL is the simplest large volume technique since it does not require any special hardware (so no re-validation of splitless methods!) or tedious tuning of operating parameters. In addition, it features unique robustness when faced with "dirty" samples, such as food matrices.

The possibility of injecting larger sample volumes allows the operator to simplify the sample preparation procedure by: 1) shortening the re-concentration step and saving extraction solvent; 2) purifying more quickly and more efficiently (GPC clean-up column lifetime extended).

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