SHOOT-AND-DILUTE GAS CHROMATOGRAPHY – A POWERFUL TECHNIQUE FOR ANALYSIS OF ENVIRONMENTAL SAMPLES WHEN USING ULTRA-SENSITIVE DETECTORS

Cochran J, Kowalski J, Rattray C, Misselwitz M

Restek Corporation, 110 Benner Circle, Bellefonte, Pennsylvania, USA

Introduction

The so called "dilute-and-shoot" method is an approach to avoiding "detector" issues such as matrix-suppressed/enhanced compound response and quantification bias in LC-MS and LC-MS/MS because of charge competition in electrospray ionization. Essentially, the sample is diluted in mobile phase prior to injection to the point where matrix no longer negatively influences the LC-MS/MS analysis of trace level components. In GC-MS, most of the problems occur on the front end, at the GC inlet, where compounds can degrade during hot splitless injection, active compounds can be irreversibly adsorbed to inlet liner surfaces, and nonvolatile material from dirty samples can compromise the transfer of less volatile compounds of interest from the inlet to the GC column. These issues are magnified due to the very slow inlet flow during splitless injection, which is typically less than 2 mL/min. A way to mitigate them is to instead use split injection, what we term "shoot-and-dilute", where the much higher flow rate through the inlet results in a substantially reduced residence time and a proportionately higher transfer for difficult compounds of interest. This technique is especially appropriate with ultra-sensitive detectors like the electron capture detector (ECD) and newly introduced electron ionization (EI) and atmospheric pressure ionization (API) GC-MS/MS systems. This paper will demonstrate the use of split injection GC-ECD for trace analysis of organochlorine pesticides in EPA Method 8081B while illustrating benefits such as increased system uptime and shorter overall analysis time and higher sample throughput.

Materials and methods

For the organochlorine pesticides calibration work, mixed standards were prepared in hexane at concentrations ranging from 2 to 200 pg/µL for each compound. An Agilent 6890 gas chromatograph with a µ-electron capture detector (ECD) was used with a 30m x 0.25mm x 0.25µm Rtx®-CLPesticides column from Restek. Hydrogen carrier flow was constant at 2.5 mL/min. One microliter injections were made into a 250°C Restek Sky® 4.0 mm ID Precision® inlet liner with deactivated quartz wool at a 50:1 split ratio. The GC oven program was 180°C (0.1 min), 28.3°C/min to 320°C (0.95 min). The µ-ECD was at 335°C with column + nitrogen makeup gas flow set to 50 mL/min. Ruggedness experiments were conducted with Restek used motor oil composite standards at various concentrations and an Ultra Scientific Pesticide Degradation Check Solution containing Endrin and DDT at 1 and 2 µg/mL, respectively, analyzed using the same injection and inlet and detector conditions, but with slightly different GC column conditions: helium carrier constant at 1.9 mL/min; oven program180°C (0.1 min), 18.3°C/min to 320°C (1.2 min).

Results and discussion

Shorter overall GC analysis time is possible with split injection because the narrow injection band allows a higher oven temperature start without compromising early eluting peak shapes that would happen in splitless injection. **Figure 1** demonstrates this for the relatively volatile surrogate compound used in organochlorine pesticides (OCPs) analysis, tetrachloro-m-xylene (TCMX). Even at the 180°C GC oven temperature start, the TCMX peak is very narrow because of the split ratio 50 used for sample introduction. Later eluting OCP peaks are equally narrow, which preserves the fast, selective separation achieved in less than 4.5 min. Instrumental throughput is doubly improved since less time is spent waiting on the GC oven to cool to the much lower temperature that would be necessary in splitless injection to achieve proper peak focusing for early eluting compounds.

Split injection is only viable in environmental sample analysis when limits of detection and quantification (LOD and LOQ) allow it, either through higher levels of analytes present or when sensitive and selective detectors, such as an ECD, are used. At low pg levels injected, ultimately resulting in low fg levels on column, a split injection of organochlorine pesticides yields an acceptable and useful calibration curve with easily seen ECD peaks, even at the lowest level for the calibration (**Figure 2**).

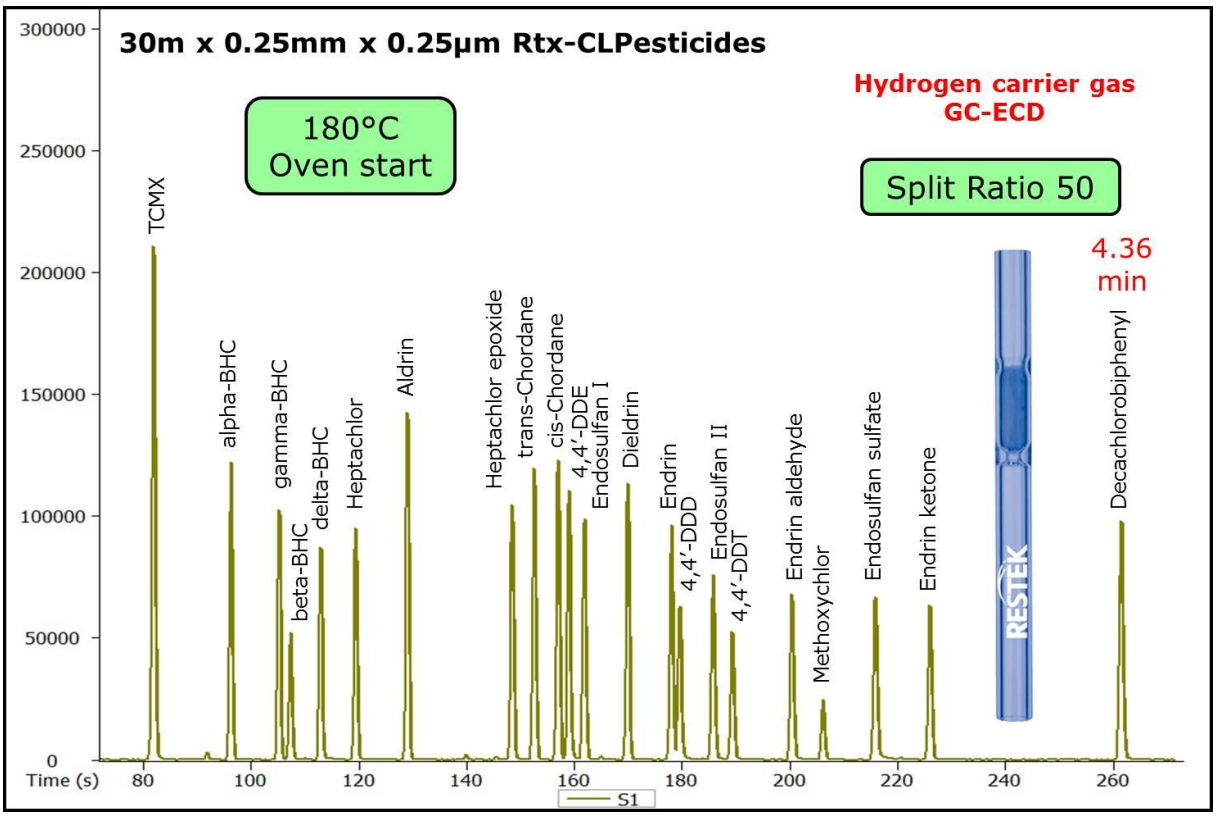

Figure 1. A split injection with a split ratio of 50 allows a high GC oven start temperature without compromising early eluting peak shapes, including for the tetrachloro-m-xylene (TCMX) surrogate compound used in organochlorine pesticides analysis.

The 40 fg OCP peaks shown in the chromatogram inset of **Figure 2** are representative of what was seen for the range of OCPs for this work as regards signal-to-noise for GC-ECD. If a conservative 50 fg for each analyte is used for estimation of instrumental LOQs, based on liquid-liquid extraction of one liter of water, concentration of a solvent extract to a final volume of 10 mL, injection of one microliter at split ratio 50, and analysis by GC-ECD, aqueous sample estimated LOQs for OCPs would be 5 ppt. This same logic for 10 grams of soil prepared using pressurized fluid extraction and extract concentration to 10 mL would put OCPs LOQs at 0.5 ppb. Examples like this can be calculated for any ultra-sensitive detector reported to detect in the fg range, including GC with high resolution MS, and EI and API GC-MS/MS systems, to determine if "shoot-and-dilute" (split injection) is an appropriate GC sample introduction technique.

When the above LOQs are fit for purpose, there are several benefits to performing "shoot-and-dilute" GC-ECD for organochlorine pesticide analysis. The first concerns quality control associated with GC system performance, specifically as regards Endrin and DDT breakdown in the GC inlet. According to US EPA Method 8081B, corrective action must be performed on the GC system when degradation of either Endrin or 4,4'-DDT exceeds 15% as determined by monitoring for degradation components endrin aldehyde, endrin ketone, DDE, and DDD. Corrective action includes changing the GC inlet liner and bottom seal, and trimming the front of the GC column. Corrective action for the GC inlet, while necessary, results in instrument downtime and increased consumable parts costs. GC column trimming maintenance eventually leads to loss of resolution between critical OCP peaks and then the need to replace the column. Split injection, again because of decreased residence time for analytes in the hot inlet, not only encourages lower Endrin and DDT breakdowns at the start of an analysis queue, but also allows the system to stay up longer, even after injections of many extremely dirty environmental sample extracts (**Figure 3**). In this case, a standard of used motor oil, which contains high molecular weight involatile compounds and metals, was used as a surrogate "dirty" sample to test split inlet liner ruggedness.

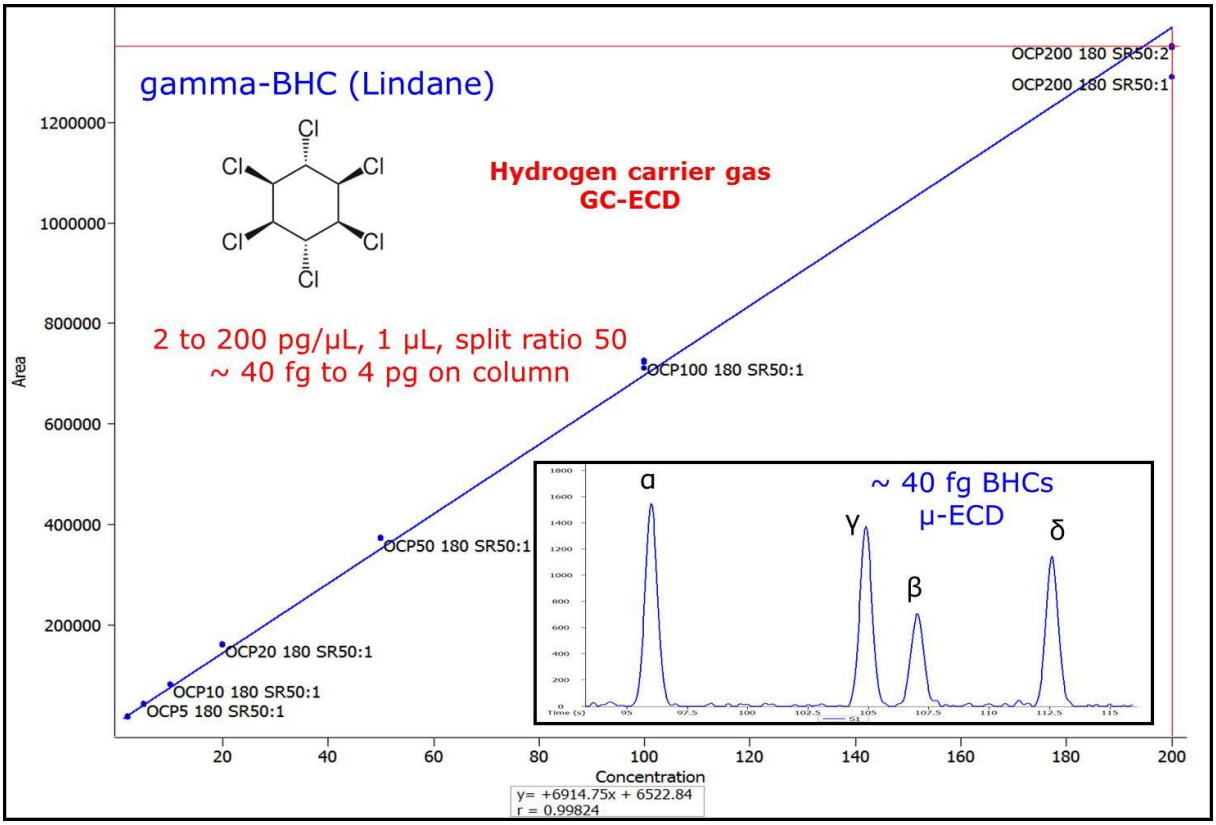

Figure 2. An excellent GC-ECD calibration curve is produced for Lindane ranging from 2 to 200 pg/µL, even with a split ratio 50 injection. Triplicate injections are plotted, for each calibration point. Even at 40 fg on column, organochlorine pesticide peaks with high signal-to-noise were generated, giving the possibility to determine even lower levels.

Contrasting inlet flows for split and splitless injections helps with understanding the decreased GC inlet residence time benefit for OCP analysis, which ultimately translates into a system ruggedness advantage. In an Agilent GC, the total flow through the GC inlet is approximately a sum of the split flow and the GC column flow. For the split injection example shown in **Figure 3** the GC column flow was 1.9 mL/min and the inlet split flow was 95 mL/min (95/1.9 = split ratio 50), so the total inlet flow is 95+ mL/min. This fast inlet flow rate minimizes the opportunity for interaction of sensitive pesticides like Endrin and DDT with inlet surfaces that could cause degradation, such as the liner, wool, and bottom seal. In fact, after dirty sample injections, another catalytic surface of concern for degradation is involatile sample matrix that never leaves the liner.

In splitless injection, since the split valve is closed for a minute or more during the injection process prior to opening it to flush out residual solvent and sample, the total GC inlet flow is defined by the column flow, which is slightly less in the inlet (versus the outlet, which is the value used to define "column flow") due to gas compressibility. **Figure 4** defines the detrimental effect of this slow inlet flow during splitless injection as regards Endrin and DDT degradation when the inlet liner is dirty from used motor oil injections. The splitless injection breakdown percentages are extremely high, approximately 50% for each compound, which is completely unacceptable for analyzing OCPs via EPA Method 8081B. It is obvious that very frequent GC inlet maintenance would be required to analyze these types of samples via splitless injection.

Other expected benefits from "shoot-and-dilute" GC include mitigation of matrix-enhanced response effects for active compounds (e.g. pesticides) and less volatile analytes (e.g. PAHs), and less frequent GC column maintenance since less "dirt" from the sample extract makes it to the column.

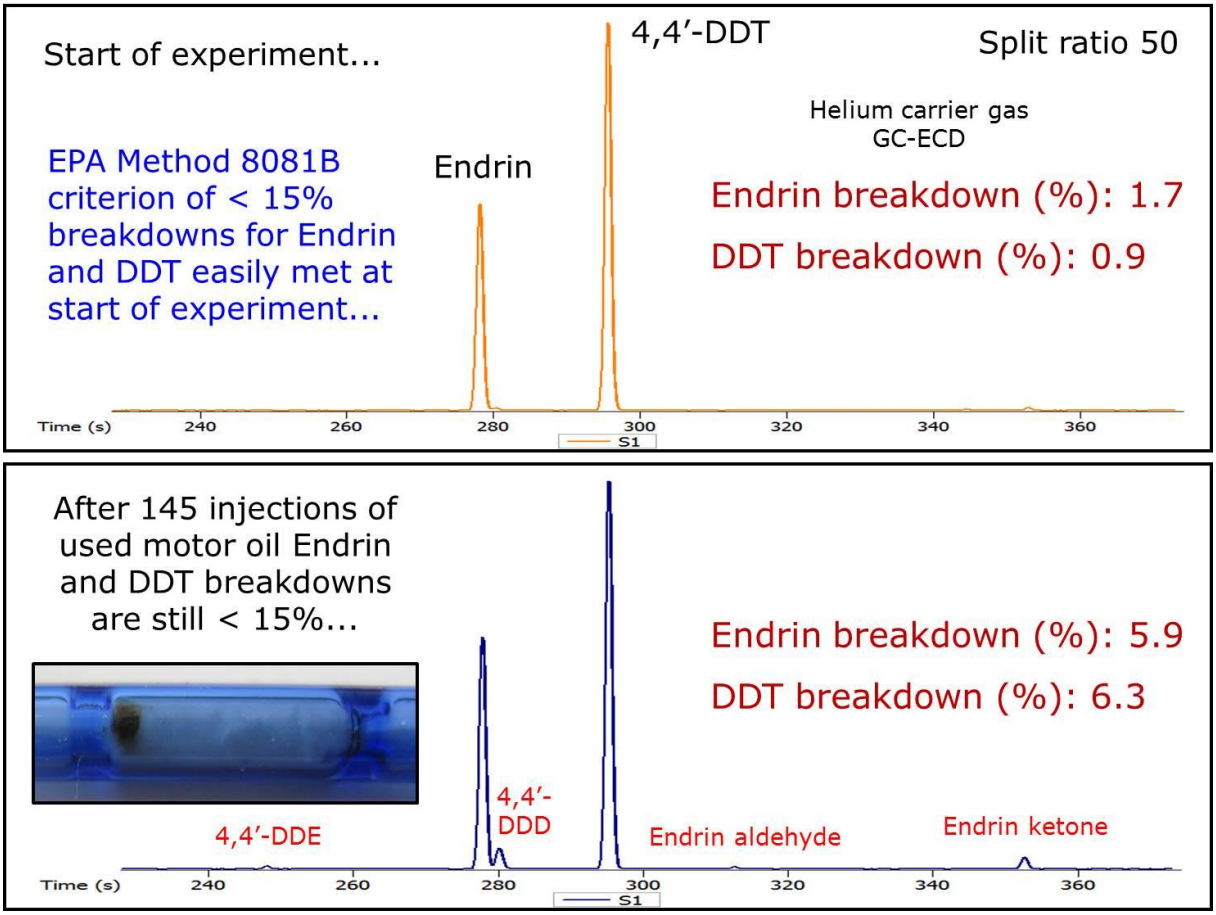

Figure 3. Initial GC inlet performance for organochlorine pesticide analysis as determined by Endrin and DDT breakdown percentages was very good based on using a split ratio of 50, which increases inlet flow. Less residence time of these sensitive compounds in the hot inlet improves their survival, even after many injections of used motor oil results in extreme inlet liner contamination.

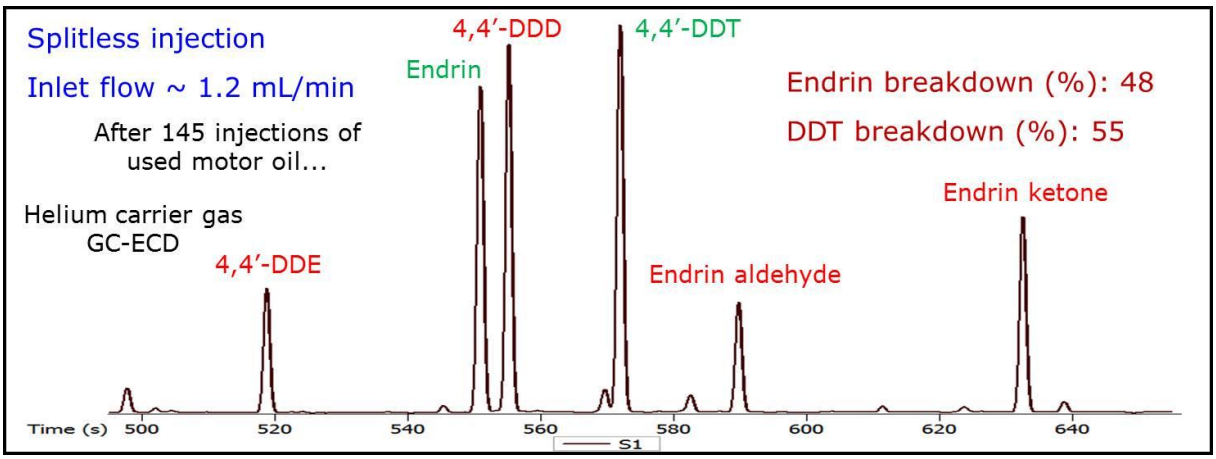

Figure 4. A hot splitless injection after 145 injections of used motor oil indicates the GC system is out of control, with very high Endrin and DDT breakdown percentages. Corrective action (i.e. GC inlet maintenance) would have been required after very few analyses using splitless injection.