Attempt for an On-Line Size Exclusion Chromatography–Gas Chromatography Method for Analyzing Pesticide Residues in Foods

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Advantages and limitations of an on-line SEC–GC method for the analysis of pesticide residues in foods are discussed; results are shown for olive oil, fat extracts of chicken and fish, and lettuce. The method allows automated integration of sample preparation into the GC analysis and eliminates corresponding manual work. It is well suited as a multimethod for analyzing many strongly differing components at the same time, and, as high molecular weight materials are removed, it allows on-column introduction into GC without contaminating the column inlet. On the other hand, SEC is poorly suited to "thin out" gas chromatograms, i.e., to eliminate interfering peaks. Detection limits of chlorinated pesticides related to the amount of fat injected were 10–50 μg/kg.

INTRODUCTION

Adding up the worldwide costs for the analysis of pesticide residues in foods would result in an enormous sum, and the largest portion of this sum would concern wages of those performing the sample extraction and cleanup operations. It is no surprise that there is a corresponding interest in an automated sample workup process integrated with the final GC analysis—a pesticide analyzer. Of course, we are still far away from an analyzer digesting whole fishes, but we are near the machine capable of analyzing raw extracts. This paper does not yet describe an approved routine method but presents a concept together with some results showing how far we have already got and where the limitations are.

The presently used cleanup methods preseparate the food extracts either by polarity (usually using Florisil), by molecular size [gel permeation chromatography (GPC), e.g., on Bio-Beads SX-3], or by a combination of both. The two types of preseparations could be termed "horizontal" and "vertical", as shown in Figure 1.

Preseparation by Polarity. Vertical preseparation by polarity efficiently "empties" the gas chromatogram: Substances eluted within a certain range of oven temperatures are of similar molecular weight. Among these substances, LC by adsorption just selects components of similar polarity and, hence, efficiently cleans samples as far as coextractives form interfering peaks. On the other hand, the fraction is unlimited in molecular size. If the fraction happens to contain, e.g., fat or wax esters, often present in very large amounts, this does not produce interfering peaks but rapidly ruins the GC system: Peaks tail and become broad and are reduced in size; quantitation turns poor. In fact, vertical preseparation is an efficient cleanup method if components do not fall into the fraction windows of the major high molecular weight components, such as fat and wax esters.

Preseparation by Molecular Size. Horizontal preseparation separates by the same dimension as GC, i.e., by molecular size. Characteristics are the opposite of what was said above: Polar and apolar components are left together, with the advantage that groups of components with broadly varying polarity can be analyzed together, and the drawback that gas chromatograms remain "full" of peaks. On the other hand, high molecular weight material is removed, resulting in the kind of cleanup rendering the sample GC compatible. Removal of involatile material allows the use of on-column injection or transfer (the widely used LC–GC transfer techniques are on-column), which renders quantitative results accurate and reliable.

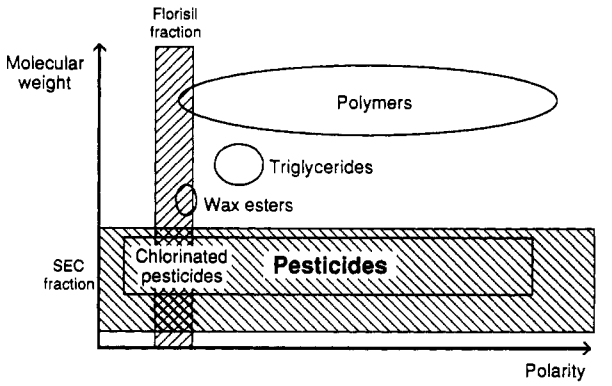

Figure 1. Two methods of sample preparation: selectivity by polarity or by molecular size. Fractionation by polarity, e.g., using Florisil (vertical fraction) selects a limited range of the pesticides but does not remove high molecular weight materials of similar polarity. SEC (horizontal fraction) primarily removes material of high molecular weight, leaving all pesticides (and other compounds of similar molecular weight) in the fraction.

There is no use in confronting the two types of preseparations in general terms, as both have their fields of applications. For many applications it might be difficult to circumvent the use of a combination of both cleanup steps. In fact, an on-line SEC–LC–GC method would provide an extremely powerful trace analyzer: It provides removal of the high-boiling and involatile material by SEC and then fractionates by LC according to polarity and analyzes with the high separation efficiency of capillary GC. Such an analyzer would be useful not only for pesticide residues but also for veterinary drug residues, mycotoxins, and pharmaceuticals in biological fluids. While such a goal should be kept in mind, we are just able to present some results of an on-line SEC–GC method.

GPC–SEC for Sample Preparation. GPC has been used for sample cleanup in pesticide analysis since the early 1970s. Ault et al. (1979) have already described automated GPC. Specht and Tillkes (1980, 1985) developed GPC to a method which became part of the official German methods for cleanup of pesticide samples. Similar GPC methods were described by Roos et al. (1987) and Chamberlain (1990). Large GPC columns were involved, packed with Bio-Beads SX-3 (Bio-Rad). Lunardini and

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Passini (1989) described cleanup with SEC, utilizing a Waters Ultrastyrogel 50-nm column with toluene.

For on-line coupling to GC, GPC or SEC columns must be smaller than those currently used. First, the complete transfer of the pesticide fraction to GC cannot make use of the large capacity of wide-bore columns (off-line SEC–GC involves injection into GC of less than 1% of the eluate). Second, fraction volumes of wide-bore GPC columns are excessively large to be completely transferred to GC. Ghijs et al. (1989) went to the other extreme, packing 0.32 mm i.d. fused silica capillaries of 1–2 m in length with RoGel 5 μm/3 nm. They showed some preliminary results on on-line SEC–GC, but the capacity of such columns is insufficient.

Recently, Tuinstra et al. (1990) described the packing of 2 mm i.d. columns with Bio-Beads SX-3. The volume of the pesticide fraction eluted from such columns is in the range 200–300 μL, i.e., well in the range of volumes that are easily transferred to GC. Samples of some 30 μL can be injected without noticeable loss in separation efficiency, allowing the introduction of raw extracts without reconcentration by solvent evaporation.

We did not find problems in packing 2–3 mm i.d. columns of up to 1 m in length with Bio-Beads. However, the compressibility of the packing material and the strong influence of compression on the retention times were severe problems. Therefore, we preferred a commercially available column with an essentially solid packing material, which allowed changes of pressure and eluents without much precaution.

Tailing Triglyceride Peak. Removal of large amounts of triglycerides has to deal with an additional problem: The triglycerides are eluted first; the pesticide fraction starts being eluted 2.5–3 min later. However, the triglyceride peak (of enormous size!) tails into the pesticide fraction. It was shown that the amount of triglycerides transferred easily severely disturbs GC (Grob and Kaelin, 1991): If, e.g., 1 mg of fat was injected into SEC, easily more than 1 μg of it entered the GC, which caused peak tailing and inaccurate quantitative results. The 1 μg of triglycerides might appear to be a surprisingly large amount. However, the triglyceride concentration in the pesticide fraction was at least 10 000 times lower than at the maximum of the triglyceride peak—all chromatographic peaks tail to some extent, but, of course, such a small increase of the baseline is not observed in the liquid chromatogram.

At present, the tailing triglyceride peak is a factor limiting sensitivity of SEC–GC for pesticides in edible oils/fats or samples rich in fat (milk products, meat, eggs). The largest contribution to this tail was found to be from the injection valve (rotating switching valve). In fact, the tail could be reduced by a factor of around 10 by bypassing the injection valve shortly after the injection. The remaining tail of the triglyceride peak is due to similar contributions by the various connections, the LC–GC transfer valve, and the LC column; it will be more difficult to eliminate them. This remaining tail limits the amount of fat/oil that can be injected in SEC to a few milligrams.

Transfer to GC. On-line transfer from LC to GC is commonly carried out either by concurrent eluent evaporation (and the loop-type interface) or by partially concurrent evaporation utilizing the on-column interface (Grob, 1991). The application of (fully) concurrent evaporation is limited by coevaporation of volatile components with the solvent. As a rough rule of thumb, it is applicable if the first solutes of interest are eluted above some 140 °C and the mobile phase is volatile (e.g., consisting of pentane and ether). As this is a problem for the most volatile pesticides [e.g., hexachlorobenzene (HCB) and the hexachlorocyclohexanes (HCHs)], partially concurrent evaporation was applied. However, for the large majority of pesticides, concurrent evaporation is applicable and would be of first choice because of its simplicity.

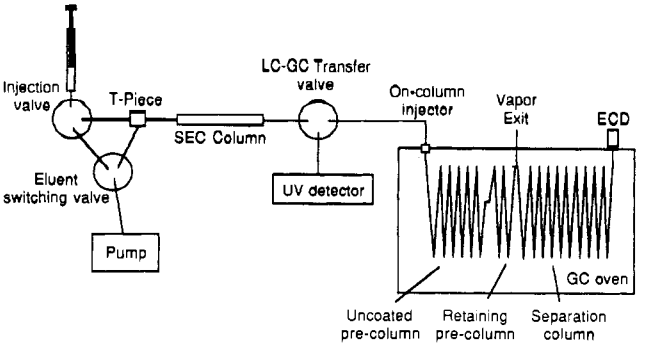

Figure 2. On-line system for SEC–GC of pesticides.

Partially concurrent evaporation is a retention gap technique making use of the solvent effects for retaining volatile components. Some eluent from LC is allowed to flow into the uncoated GC precolumn to form a film on the capillary wall. This film retains all except the extremely volatile sample components up to the end of eluent evaporation, to release them then at once. However, as the liquid spreads far into the precolumn, higher boiling material remains spread throughout the flooded zone, forming correspondingly long initial bands. These higher boiling components are reconcentrated at the beginning of the coated column by the retention gap effect.

Partially concurrent eluent evaporation reduces the volume of liquid spreading into the uncoated precolumn. A standard 10 m × 0.53 mm i.d. uncoated precolumn has the capacity for retaining 80–100 μL of sample liquid. If, as in our case, 400 μL of LC eluent is transferred, at least 300 μL must be evaporated during transfer ("concurrently") to prevent overfilling of the uncoated precolumn. For this purpose, the LC flow rate and the rate of eluent evaporation in GC must be adjusted to each other such that slightly more enters the precolumn than is evaporated at the same time. This is done by starting out from predetermined evaporation rates in GC (Schmarr et al., 1989) and fine corrections after measurement of the actual evaporation rate.

EXPERIMENTAL PROCEDURES

Size Exclusion Chromatography. A 25 cm × 3 mm i.d. SEC column packed with a cross-linked polystyrol, PSS SDV, of 5-μm particle size was used (Polymer Standards Service, Mainz, Germany). Pore sizes were indicated as 10 nm. Cyclohexane/ethyl acetate (1:1) was the mobile phase, delivered by a Phoenix 20 syringe pump (Carlo Erba) at 80 μL/min.

Sample Extraction. Chicken and fish were Soxhlet-extracted with redistilled hexane; 25 g of lettuce was blended and extracted with 50 mL of ethyl acetate for 30 min on a shaking machine.

On-Line SEC–GC System. The system utilized is shown in Figure 2. The SEC mobile phase passed through the eluent switching valve, a Valco rotating switching valve, either to the injection valve (a six-port rotating switching valve) or to the T-piece. The injection valve incorporated a 15-μL loop and a Valco fill port. The T-piece was of press-fit type, with short pieces of 0.32 mm i.d. capillaries attached to it. The transfer capillary to the SEC column was a 0.17 mm o.d./0.12 mm i.d. fused silica capillary. Connections of fused silica capillaries to the valves and the LC column were made with short pieces of PTFE tubing and metal ferrules (de Jong, 1989). From the LC–GC transfer valve, a 0.17 mm o.d./0.12 mm i.d. fused silica capillary passed through the on-column injector into the uncoated precolumn, consisting of a 10 m × 0.53 mm i.d. fused silica capillary silylated with diphenyltetramethyldisilazane (DPT-MDS) by MEGA, Legnano, Italy. The 3 m × 0.32 mm i.d. retaining precolumn was taken from the separation column, a 18 m × 0.32 mm i.d. column coated with immobilized PS-255, a me-

Table I. Timetable for SEC-GC Analysis

00.00	15-μL injection of sample containing up to 10% fat, with the eluent switching valve feeding eluent to the injection valve
00.30	eluent switching valve switched to T-piece
17.15	(3 min after maximum of triglyceride peak) transfer valve switched to transfer to GC; vapor exit opened
22.15	transfer valve returned to standby
23.05	vapor exit closed; GC analysis started, 4 °/min to 270 °C, then at 8 °C/min to 350 °C (5 min isothermal)

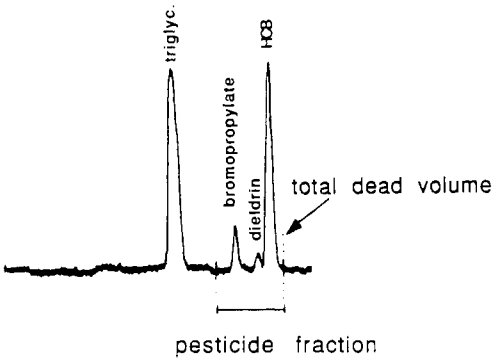

Figure 3. SEC-UV chromatogram (run from left to right) of olive oil (triglyc. = triglycerides) spiked with bromopropylate, dieldrin, and hexachlorobenzene (HCB).

thylsilicone, of 0.15-μm film thickness. The connection between the precolumns and the vapor exit T-piece were of press-fit type.

The UV detector was installed in the outlet line from the transfer line (instead of between the column and the transfer valve as usual) to rule out contributions to the tailing triglyceride peak. It consisted of a Kontron 433 UV detector, equipped with a Z-shaped capillary UV cell (75 μm i.d. fused silica). Triglycerides were detected at 270 nm.

Transfer Conditions. Using hydrogen as carrier gas, the GC column required an inlet pressure of 0.5 bar. The resulting evaporation rate seemed to fit the flow rate suiting SEC. Fine tuning involved the determination of the evaporation rate. Cyclohexane/ethyl acetate (1:1) was fed at 100 μL/min into the GC system thermostated at 70 °C. The duration of the eluent evaporation was determined by lighting the gas/vapor mixture leaving the vapor exit (the flame turning yellow upon elution of solvent). The evaporation rate was determined as 69–72 μL/min (depending on transfer volume). At the chosen flow rate for SEC, 80 μL/min, this left some 10 μL/min of eluent in the flooded zone of the uncoated precolumn. As a 10 m × 0.53 mm i.d. uncoated column has a capacity of retaining about 100 μL of liquid, this would have allowed transfer of eluent during 10 min, corresponding to a volume of 800 μL; the transfer of a 400-μL fraction exploited only half of this capacity. The timetable of LC-GC transfer is given in Table I.

GC Analysis. GC analysis was started at the end of eluent evaporation. Temperature was programmed from the transfer temperature (70 °C) at 4 °C/min to 270 °C. To heat out the triglycerides, temperature programming was continued at 8 °C/min to 350 °C. Pesticides were detected by ECD.

After maybe 50 transfers, peaks started to show slight broadening, which was attributed to polymerized triglycerides. The precolumn system was cleaned in place: the mobile phase was pumped through the precolumn at ambient temperature (switching the LC–GC transfer valve to transfer), which largely suppresses evaporation and causes the solvent to flow through the precolumns. The vapor exit was opened; the exit of the ECD was closed, which caused the makeup gas (introduced at 2 bar) to flow backward through the separation column. After some 5 min, the eluent flow was stopped and the GC oven heated to 70 °C until the remaining solvent was evaporated. The ECD outlet was opened again and the next analysis started immediately.

RESULTS

Figure 3 shows an SEC chromatogram of pesticide standards and 1.5 mg of triglycerides. The pesticide fraction transferred to GC is marked. It reached up to the estimated total dead volume of the column and involved a volume of 400 μL.

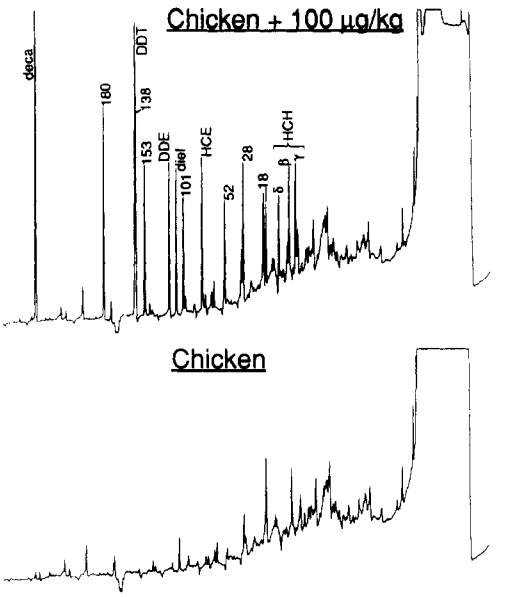

Figure 4. SEC-GC-ECD chromatograms of the fat extract of a chicken with and without an addition of some standards: 50 μg/kg of the hexachlorocyclohexanes (HCH), 100 μg/kg of the PCBs (labeled by numbers), hexachloroepoxide (HCE), dieldrin (diel), and DDE, as well as 200 μg/kg of decachlorobiphenyl (usually used as internal standard).

The chromatogram shows considerable separation among the pesticides. For SEC–GC analysis of a single component, such preseparation is of advantage as it allows more efficient cleanup. However, for a multimethod including all pesticides, preseparation merely increases the volume of the fraction to be transferred to GC and thus also the amount of fat from the tail of the triglyceride peak reaching GC.

SEC preseparation of the pesticides is influenced by the distribution of the pore sizes in the polystyrene: A broad distribution within the range of sizes fitting the pesticide molecules causes preseparation. For a multimethod, the ideal SEC column packing material would have pores of uniform size, allowing the pesticides to enter but excluding the triglycerides.

Figure 4 shows the SEC–GC–ECD chromatograms of a raw fat extract of a chicken with and without an addition of 50–100 μg/kg of the labeled chlorinated pesticides and PCBs. Concentrations refer to the fat; they are correspondingly lower for the chicken. The chromatograms indicate a detection limit of 10–20 μg/kg related to the fat.

Figure 5 shows three SEC–GC–ECD chromatograms of various foodstuffs. The extract from a fish of a local lake (a 10% fat solution was injected) shows PCBs with concentrations of up to 80 μg/kg related to the fat (some 4 μg/kg related to the fish). In the olive oils analyzed, no chlorinated pesticides were detected (10% dilution of the oil in the mobile phase, sensitivity corresponding to Figure 4). The lettuce contained 5.5 mg/kg iprodione, a fungicide, and some 30 μg/kg vinclozolin.

DISCUSSION

The results show advantages as well as limitations of on-line SEC–GC for pesticide analysis at the present stage of development. It is an important step ahead that raw food extracts can be analyzed by an automated technique integrating SEC (GPC) sample preparation into the analysis; it eliminates a considerable amount of manual

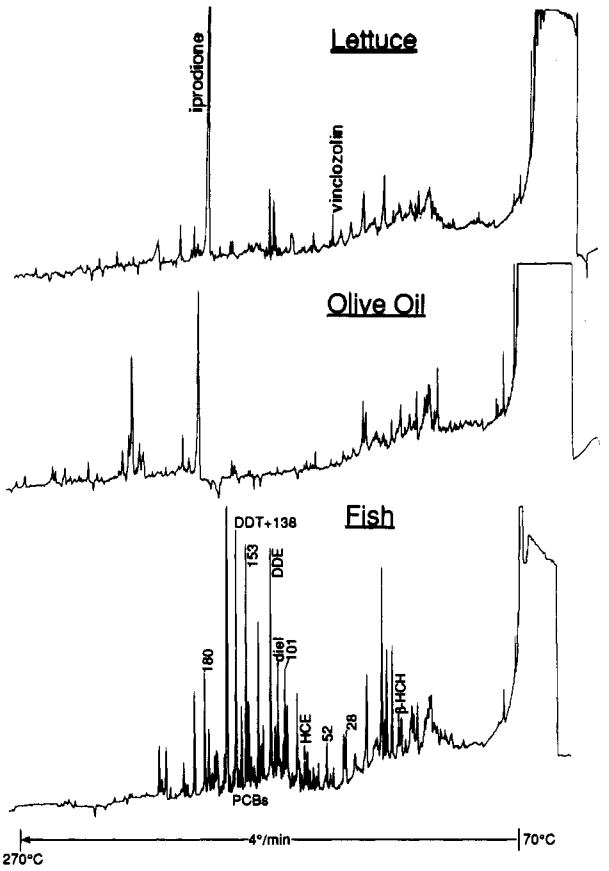

Figure 5. SEC-GC-ECD chromatograms of extracts from fish and lettuce, as well as of an olive oil.

sample preparation work and renders analyses more reliable.

For the samples tested, the method provided a sensitivity fully satisfying the needs for the routine control of pesticide residues—in fact, at least some 5 times less fat could be injected to reach this goal (ECD attenuation could be somewhat reduced then). As the method runs on available instrumentation (Carlo Erba Dualchrom 3000), there is no obstacle against a broad testing in practice.

Limitations to Sensitivity. Sensitivity was sufficient for pesticide analysis, where modest requirements on detection limits are combined with the availability of sensitive and selective detectors. However, lower detection limits would be desirable for many other purposes. Achievable sensitivity depends on two factors: the amount of triglycerides injectable without overloading the GC system and the SEC column and, second, peaks interfering with the components of interest in the gas chromatogram.

The chromatograms show that interfering peaks do not allow a substantial decrease of detection limits—if more sample material is injected, chromatograms are rapidly overcrowded. Hence, additional cleanup would be required. This conclusion agrees with the experience from conventional off-line GPC–GC, which is explained by the fact that on-line SEC–GC does not provide more efficient cleanup than conventional GPC–GC: the same principle of preseparation is applied.

The 25 cm × 3 mm i.d. SEC column used does not allow a strong increase of the amount of fat injected. There are two reasons for this: the amounts of triglycerides reaching GC due to the tailing fat peak were near the tolerable maximum when 1.5 mg of fat was injected under the conditions used. Triglyceride concentrations in the transferred pesticide fraction are approximately proportional to the amount (concentration) introduced, and when 5 mg of fat was injected, triglycerides started disturbing GC (visible by slightly broadened peaks). Second, when the amount of fat injected was increased from 1.5 to 5 mg, the triglyceride peak started overloading. Overloading of the SEC column causes broadening of the triglyceride peak toward the pesticide fraction.

Larger Bore SEC Columns? The capacity of the SEC column could be increased by using larger bore columns. As the increased eluent flow rate going along with larger bore columns more efficiently purges the system, the triglyceride concentration in the pesticide fraction would decrease at the same time—or would at least remain constant when larger amounts of fat were injected. However, the larger column bore also enlarges the volume of the pesticide fraction: in SEC, there is no possibility of compensating by a stronger eluent. Transfer to GC becomes more difficult when the fraction volume approaches 1 mL. This means that the volume of the SEC column could be at most doubled when the whole fraction is transferred and increased by a factor of 5 when more narrow SEC fractions are analyzed.

Larger SEC columns would also complicate reconcentration of an SEC fraction on a following silica gel LC column. This could be important, because SEC–LC–GC might finally be the most successful principle for a trace analyzer.

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