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Project Summary

Evaluation of Capillary Systems for the Analysis of Environmental Extracts

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The quantitative and qualitative aspects of splitless and on-column capillary column injectors for the analysis of environmental extracts for priority pollutants are investigated. Precision data are presented for a wide variety of compounds introduced into a splitless injector by manual and totally automated injection practices. Similar studies are performed using manual injections into an on-column injector. Other studies include capillary system reactivity toward labile analytes, column selection, and the identification of the limitations of current capillary column systems with respect to the analysis of complex extracts.

Sufficient experimental evidence is documented to support the need for a unique quality assurance program, not associated with packed column systems.

This Project Summary was developed by EPA's Environmental Monitoring and Support Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Background

The report describes a laboratory study of the application of capillary column gas chromatographic systems to the analysis of environmental extracts for a variety of synthetic organic compounds.

The purpose was to determine if the accuracy, precision, and sensitivity of analytical data generated by capillary systems is equivalent to that obtained from a properly operated packed column gas chromatograph, and finally, as deficiencies are identified to eliminate them through hardware modifications or by adding unique capillary column quality control and procedural practices to packed column methodology that will guarantee equivalent superior analytical data.

As part of this study, several commercial capillary columns were evaluated to identify those column parameters best suited for the analysis of environmental extracts containing organic compounds of current interest to the Agency.

Equipment Used

A Hewlett-Packard 5730 gas chromatograph equipped with dual flame ionization detectors (FID), linear electron capture detectors (ECD), and a model 18740B capillary column control was used for column evaluations. A Hewlett-Packard 5880 gas chromatograph equipped with a Hewlett-Packard 7672 autosampler, dual FID, dual ECD, a Hewlett-Packard Level 4 data system, dual splitless injectors, and a Model 19320D on-column injection assembly, was used to determine the quantitative aspects of splitless and on-column injections. Although several different capillary columns were used early in this study, all of the qualitative and quantitative data listed were obtained from a fused silica 30M, 0.32 mm ID, 0.25 μm film thickness polymethyl (5% phenyl) siloxane bonded phase capillary column.

Experimental

The gas chromatograph and all of its peripheral devices was installed by factory personnel. Each unit was found to meet all of the manufacturer's specifications and was operated for this evaluation strictly in accor-

dance with the operators manual or recommendations obtained through personal communications with factory personnel. Sample introduction techniques and system optimization were concurrent with commonly used capillary column practices.

The performance of the injectors was determined by repetitive injections of standard solutions containing pesticides in different solvents. The pesticides and the concentrations studied are listed in Table 1. All of the area measurements and retention data were determined by the automatic integration system; less than 1% of the data were rejected as outliers.

Column Selection

Several commercially available fused silica capillary columns were evaluated. Based upon observations of resolution, inertness toward labile compounds, column life, acceptable peak geometry for acids and bases, reasonable analysis times and minimal column bleed over the temperature extremes required for the analysis, a 30m, 0.32 mm ID, polymethyl (5% phenyl) siloxane bonded phase column with a 0.25 μm film thickness was selected as the best column availabe for analyzing compounds of current interest to the Agency and was used to gather all of the data presented in the report.

Capillary System Sensitivity Evaluation

After a reasonable column and system conditioning period, hexane reagent blanks were analyzed using both FID and ECD at their most sensitive settings. An evaluation of the resulting chromatograms ws used to determine low level system noise, signals from column substrate bleed, ghost peaks, or interference originating in the sampling system which could preclude the use of the capillary system for trace level analyses. It was found that over the temperature program extremes studied, the detector noise level remained constant (<1% of full scale) and the baseline did not change more than 20% of full scale, establishing that the ECD and single column FID temperature programmed analyses are feasible using capillary columns for the measurement of trace level environmental extracts normally associated with carefully optimized isothermally operated packed columns. This observation assumes that the detector retains the same linearity and sensitivity characteristics operated in the capillary column mode as it exhibits during packed column operation. Future studies will determine actual linearity characteristics and system detection limits for diverse analytes.

Table 1. Organochlorine Pesticides

Compound	Concentration[a] ng/μL
α-BHC	0.01
β-BHC	0.01
γ-BHC	0.01
%-BHC	0.01
Heptachlor	0.005
Aldrin	0.008
Heptachlor epoxide	0.008
γ-Chlordane	0.01
Endosulfan I	0.01
P,P'-DDE	0.01
Dieldrin	0.01
Endrin	0.02
Endosulfan II	0.01
P,P'-DDD	0.01
Endrin aldehyde	0.02
Endosulfan sulfate	0.04
P,P'-DDT	0.04
Endrin ketone	Not present
Methoxychlor	0.06
Mirex	0.01

[a]Concentration of analyte in pentane, hexane, 2,2,4-Trimethylpentane and "Samples."

Splitless Injector Study

To determine the accuracy and precision of splitless injections and what effect various solvents may have upon splitless injections, repetitive splitless injections were performed manually and with an autosampler using standards contained in pentane BP 35-37°C, mixed hexanes BP 68-69°C and isooctane BP 99°C. The analytes and their concentrations are listed in Table 1. Using the autosampler, six 1.8 μL splitless injections of each standard solution were randomly injected into the gas chromatograph. The samples were analyzed under the conditions stated under Retention Data Studies. The chromatograms, peak areas, relative standard deviations of peak areas, peak heights, peak widths, and retention data of the resulting injections were then evaluated. The peak widths and retention data were the same for each of the three mixtures, however, severe differences were noted between the areas for each analyte as the solvent was changed. From the results of this test, it is apparent that if the sample and standard are not contained in exactly the same solvent and if external standard calibrations are used, then serious analytical errors will result. The precision of the hexane and isooctane autosampler injections are comparable to those normally experienced with manual and automated packed column injections while that obtained from pentane appears somewhat higher.

For packed column gas chromatography, the quality of the quantitative data resulting from imprecise injection practices can usually be improved by incorporating internal standard calibrations. The area variances produced were injector related and appeared to be uniformly predictable, and it was decided to reexamine the data using internal standard techniques. Ideally, internal standards should be chemically and physically similar to the analytes and elute in an adjacent interference-free area of the chromatogram. In lieu of selecting internal standards and reanalyzing the samples, endosulfan I was selected as an internal standard because it is a mid-range eluter and consistently has a low area relative standard deviation for replicate injections.

Modifications were performed upon the injection system in an effort to minimize the problem associated with changing solvents. The injector liner was packed with long filament, silane treated glass wool to form a continuous plug extending 3.5 cm down the injector sleeve. Measurements indicated that the syringe needle of the autoinjector penetrated the glass wool and exposed about 1 mm of the tip below the glass wool plug during injection. Excellent agreement was achieved between the average area values for hexane and isooctane injections. The average area value for the pentane injections appears to agree well with the results obtained from the other two solvents. However, the relative standard deviation between pentane injections is excessive and totally unsuited for quantitative analyses. As in the case of previous study area ratios for hexane and isooctane show excellent agreement.

As these data were being collected with the automatic injector, manual injections were also being performed using the unpacked injector sleeve and the glass wool packed injector sleeve. The results are similar to those obtained from automated injections with one noteworthy exception. The precision between replicate injections is totally unacceptable. All efforts to improve the precision for manual splitless injections failed. Additionally, the precision of area ratio data where sufficient samples were analyzed is excessively high indicating that the problem is associated with the compounds being injected or the injector but not the injection technique.

Retention Data Studies

The primary reason for applying capillary column systems to the analysis of environmental extracts is to utilize their unique ability to rapidly resolve complex, wide-boiling range mixtures of organic compounds. For many capillary operations, it is not unusual to observe well resolved peaks eluting from the column within seconds of one another. In reviewing data system programs routinely used to reduce packed col-

umn chromatograms, retention time windows between ±1 and 5% of the average retention time of the analyte are normally recommended by data system manufacturers to make qualitative identifications. The application of packed column criteria is not suitable for capillary column work, since the ±5% retention window for a compound eluting after 20 minutes is a window 60 seconds wide. To determine reasonable retention windows for capillary column work, the retention data for the chromatograms and other related studies were examined. For these studies, the following multip-ramp temperature program was selected which resolved all of the priority pollutant pesticides. The 30m × 0.32 mm ID, 0.24 μm df polymethyl (5% phenyl) siloxane bonded phase column was maintained at 90°C for five minutes then rapidly programmed at 12°/minute to 230°C. At 230°C, the program rate was changed to 5°/minute until the column temperature reached 250°C. The column was maintained at 250°C for 21 minutes, then recycled to 90°C for the next analysis. It is evident from the data on average retention time, relative standard deviation, and the upper and lower limits (window) at the 95% confidence level, that properly designed capillary column gas chromatographic systems can generate precise retention data from both automatic and manual splitless injections. Secondly, if the column is capable of resolving adjacent peaks by three seconds, a totally automated data system can be used to reproducibly identify complex mixtures of compounds with a high degree of reliability.

Analysis of Product Mixtures

Included in the list of priority pollutants are several commercial products containing mixtures of closely related chlorinated compounds. These include: Toxaphene: Aroclors 1016, 1221, 1232, 1242, 1248, 1254, and 1260; and chlordane. Capillary column separations of these mixtures provided a significant improvement in the ability to resolve the individual components over packed column gas chromatography. Close examination of the data, however, shows that for routine analyses, the capillary column provided too much information as lengthy complex chromatograms were obtained. Further complicating the capillary column analyses, significant differences were noted between various production batches of each product resulting in a moderate degree of uncertainty in qualitative identifications and potentially serious quantitative errors if calculations are based upon single peaks. For such mixtures, assuming no interferences, packed column gas chromatography affords an advantage over capillary columns, since sufficient resolution is obtained to identify the parent product through the recognition of a series of peaks with correct relative intensities at known retention times. For packed column analysis, the multitude of coeluting compounds tend to "average out" small variations in product batches resulting in accurate qualitative and quantitative analyses.

Conclusions and Recommendations

The results of this evaluation show that the application of capillary column techniques to the analysis of priority pollutants can afford a distinct advantage over most conventional packed column technology. Dilute complex mixtures of coextractables can be simultaneously analyzed using temperature programmed capillary columns and most popular ionization detectors. To obtain a similar degree of separation and conformation the packed column analytical protocol currently described in the 600 series methods requires sample clean-up by column chromatography followed by one or more injections into packed columns operated under isothermal or limited temperature programmed conditions especially when the analytes are near the instrument limit of detection. For the analysis of individual analytes, the capillary systems clearly provide these advantages; however, for complex formulations or technical mixtures such as toxaphene and Aroclors packed columns greatly simplify the analysis.

Even though the glass and fused silica capillary column surfaces were shown to be inert through the use of polarity test mixes, the splitless injector caused endrin to degrade unless a continuous extraordinary deactivation program was undertaken. For the National Interim Primary Drinking Water Regulations methods and methods 608 and 625 the quality control, calibration and interference sections should be revised so that the analyst will be alerted to the possibility of false negative results for endrin and false positive data for endrin aldehyde.

The priority pollutants include compounds with neutral, acetic and basic chemical properties. Of all the columns tested, a 30m, 0.32 mm ID, 0.25 μm df fused silica column with a polarity similar to polymethyl (5% phenyl) siloxane afforded the best separations of such mixtures within a reasonable period of time. The life expectancy of bonded phase columns significantly outperformed nonbonded phase columns. The substrate bleed signal for bonded phase columns generated during temperature programs was lower than that of nonbonded phase columns with similar polarities providing the advantage of lower instrument sensitivity limits when bonded phase columns are used.

If splitless injections are to be used for quantitative analyses, then injections must be made using autosamplers in order to generate precision data comparable to that of manual packed column techniques. The precision of autosampler data appears to be adversely affected as the boiling point of the sample solvent decreases. From the limited studies performed herein, the best performance is obtained using solvents that exceed the boiling points of pentane and methylene chloride. For accurate analyses the sample and the standard must be contained in exactly the same solvent if external calibration techniques are to be used. When samples and standards are contained in dissimilar solvents then internal standard calibrations must be used to compensate for variable amounts of analytes entering the column during the splitless injection routine. If splitless capillary systems are to be used in place of normal packed column operations, then it is imperative that the calibration and procedure sections of the methods be updated to reflect these observations.

On-column injection techniques appear to resolve all the problems identified in this study associated with splitless injections. On-column injections consistently provided precise qualitative and quantitative data for organohalide pesticides and demonstrated no signs of analyte decomposition or variable amounts analyte entering the column as solvents are varied.

This is a continuing study and an effort will be made to determine if the problems encountered with splitless injections are inherent to the injector design chosen for this study or if they are associated with the mechanism involved in sample delivery.

As the opportunity arises, other automated splitless injectors and on-column injections will be evaluated.