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An Ultraviolet Detector for Automated Size Exclusion Chromatographic Cleanup of Lipid-Containing Residue Samples

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The use of automated size-exclusion chromatography as a rapid cleanup method for fatty samples, has become widely accepted in most pesticide residue laboratories. The Instrument, a GPC Autoprep-1001 of Analytical Biochemistry Laboratories, is routinely used in this laboratory for cleanup of samples containing animal and vegetable lipids. It's main advantage is that it can be used to perform cleanups unattended, however, there is no direct control over the separation efficiency when the instrument is used unattended. It would thus be advantageous to have a detector which could keep track of the separation when the instrument works unattended.

A further drawback of the instrument is that the calibration of the run parameters takes considerable time because a sample has to be fractioned and the fractions tested gravimetrically to determine when the lipid will elute. A detector coupled to the outlet and giving a direct measurement would thus shorten this calibration exercise.

In this paper the use of a secondhand ultraviolet detector normally used for liquid chromatography is described. This type of detector is found in most laboratories and should thus be readily available to users of the GPC-Autoprep.

PROCEDURE

A LKB 8300A Uvicord II ultraviolet detector and control unit was coupled to the GPC Autoprep-1001 as shown in figure 1.

The operating wavelengths of the detector are 254 and 280 µm. The latter vavelength was used because of the relatively high ultraviolet cutoff point of the toluene: ethyl acetate (1:3) solvent used. The light source of the detector is a low pressure mercury lamp which emmits a wavelength of 254 µm. By inserting a fluorescence rod in the lightpath a wavelength of 280 µm can be obtained. The measuring cell of the detector is of a flow type, manufactured of quartz and with a optical path length of 3 mm and a volume of 0,1 ml.

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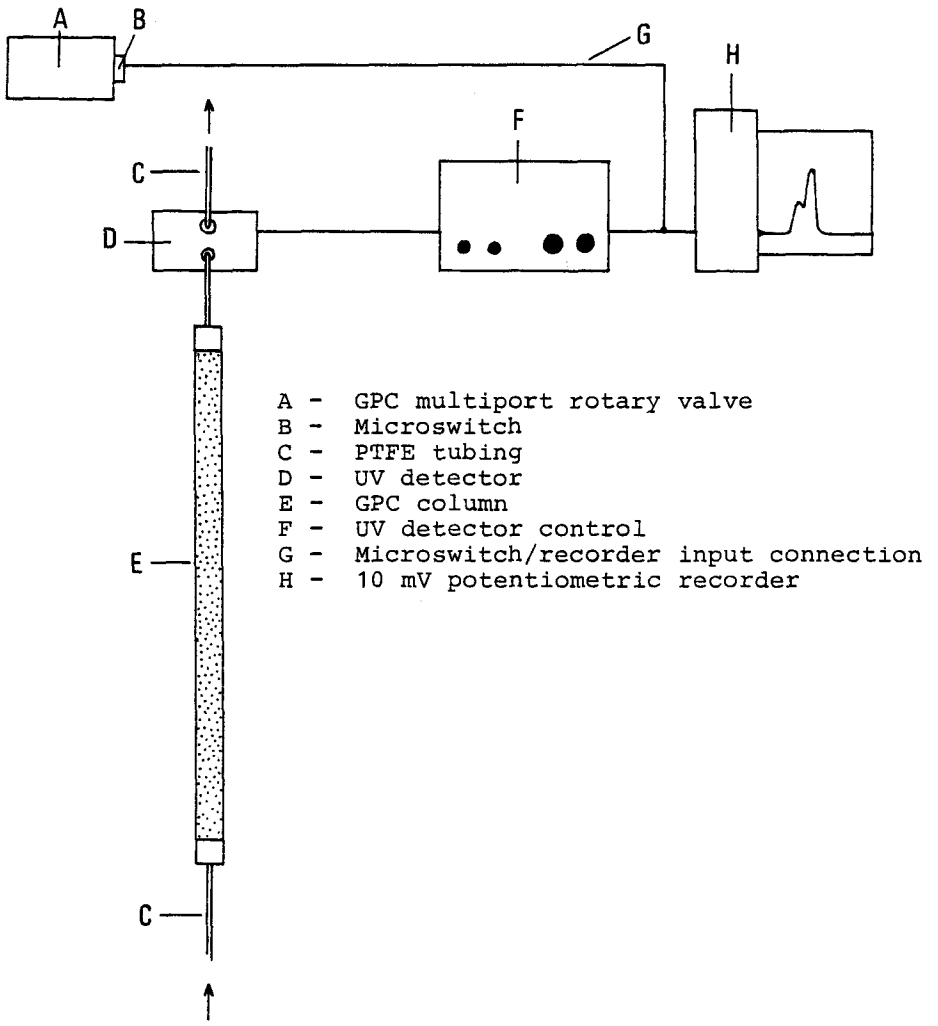

Figure 1. LKB Uvicord II ultraviolet detector coupled to a GPC Autoprep-1001.

The output of the detector was coupled to a 10 mV potentiometric recorder via the Uvicord control unit. The recorder has a chart speed of 5 mm/min which was determined to be constant.

It was found necessary to provide a mark on the chromatogram which would coincide with the automatic sample injection. This was done by the installation of a microswitch on the actuator of the GPC Autoprep multiport rotary valve. The microswitch is connected via two wires to the recorder input. When the multiport valve is activated, the microswitch closes momentarily causing a short circuit over the recorder inputs

resulting in a mark on the chromatogram. This mark then signifies the time of injection.

These modifications were evaluated using the same solvents and column packing materials as described by JOHNSON et al. (1976).

RESULTS AND DISCUSSION

Figure 2 shows typical chromatograms obtained with various lipid samples. In each case the concentration of the lipid solution injected was 10% m/v (1 g/10 ml) in a toluene/ethyl acetate mixture.

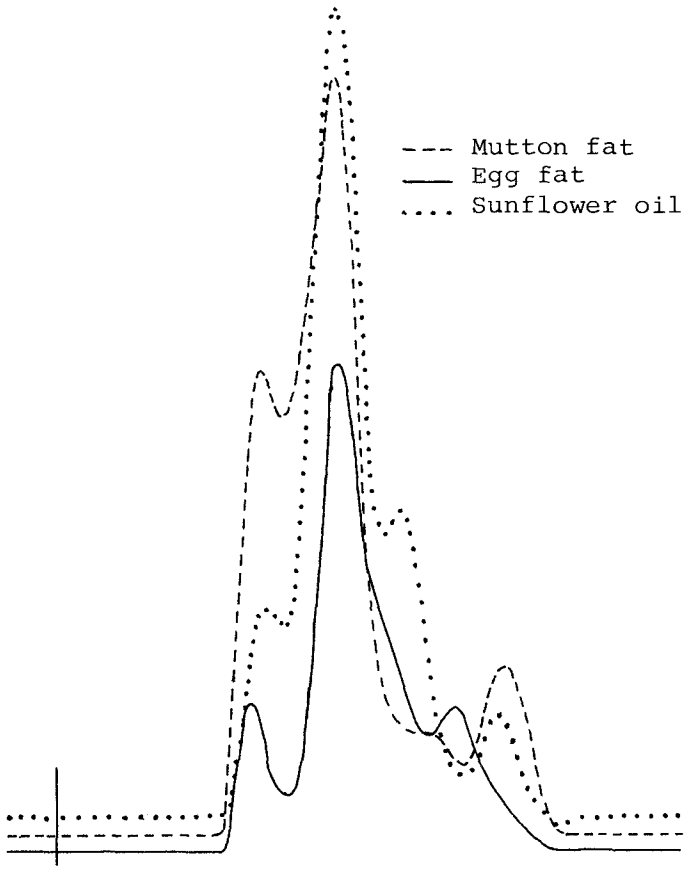

Figure 2. Typical chromatograms obtained with various lipid samples

Near full scale deflection was obtained with 0,5 g of lipid (only 50% of the lipid is actually processed). This indicates the excellent sensitivity of the system. The shape of the different lipid peaks as well as their elution rates are illustrated.

When a cleanup programme for a specific type of sample is needed, the sample is injected and a chromatogram is obtained. From the chromatogram the program can be calculated as illustrated in figure 3.

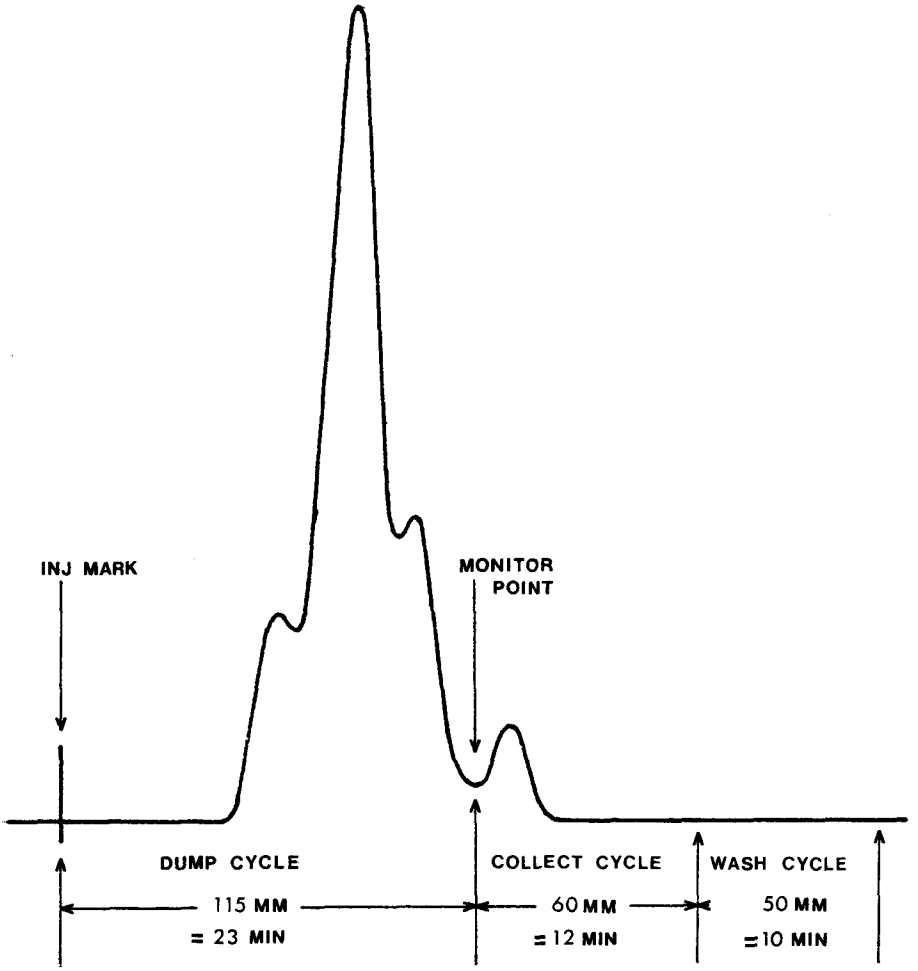

Figure 3. Example of a cleanup program for sunflower oil.

From the sample injection mark, the distance is measured to the point indicated in the figure. This distance is converted into time (chartspeed 5 mm/min) and this gives the duration for the dump cycle. All the chlorinated pesticides elute for a period of approximately 8 minutes after the lipid peak. This period may be lengthened to 12 minutes to include slow eluting pesticides (VAN DYK et al. 1978). A typical program for the lipid as illustrated in the figure thus would be: dump cycle 23 minutes, collect cycle 12 minutes and wash cycle 10 minutes.

According to JOHNSON et al.(1976) a certain amount of overlapping does occur between the lipid peak and some of the organo chlorine pesticides. This is substantiated by experience gained in this laboratory, thus the end of the dump cycle is usually not calculated to the end point of the lipid peak and a small amount of lipid is present in each sample. Tests indicated that most of the organochlorine pesticides showed a recovery of over 95%. The small amount of lipid however, does not normally interfere with the analysis of the pesticides.

When a series of samples are cleaned up over an extended period each lipid peak will be registered on the chromatogram together with its injection mark. To monitor the performance of the instrument and to check if anything went wrong during a run, the rate of elution of each peak is measured from the injection mark as indicated in figure 3.

If the instrument did not function correctly for some reason, the elution rate of a part of a series of samples would differ from the original calibration peak and it might be neccessary to do a re-run on these samples after the fault is rectified.

The use of the old liquid chromatography ultraviolet detector coupled to an ABC Autoprep was thus demonstrated to be feasible. The use of this system greatly simplifies calibration of run parameters and gives complete control over the performance of the separation parameters.

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