Separation of DDT and related compounds on a silicic acid column using gradient elution

Metabolites of ^{14}C-labelled DDT extracted from the cattle tick *Boophilus microplus* (Canes.) were required in a high degree of purity for spectroscopic examination. Although various paper chromatographic methods[1,2] had been used successfully in other related studies of DDT metabolism in tick larvae, difficulties of contamination arise in the preparation of microgram quantities of metabolites by such means owing to the presence of solvent-extractable materials in the chromatographic paper and to contamination by stationary phase. These difficulties have been avoided by using solvent-washed, activated silicic acid for all preliminary separations and subsequent clean up of metabolites. Column chromatography was selected in preference to thin-layer methods because of better control of water in the adsorbent and uncomplicated elution of compounds. Gradients were employed to elute compounds of unknown adsorptivity. Miniature columns were employed in later stages of purification to minimise losses due to irreversible adsorption and co-distillation of solutes when evaporating solvents. A column chromatographic method similar to that used for initial separations of metabolites is presented here. This method has been used successfully for model separations of DDT and related compounds and it should prove useful in studies of the metabolism of this type of pesticide.

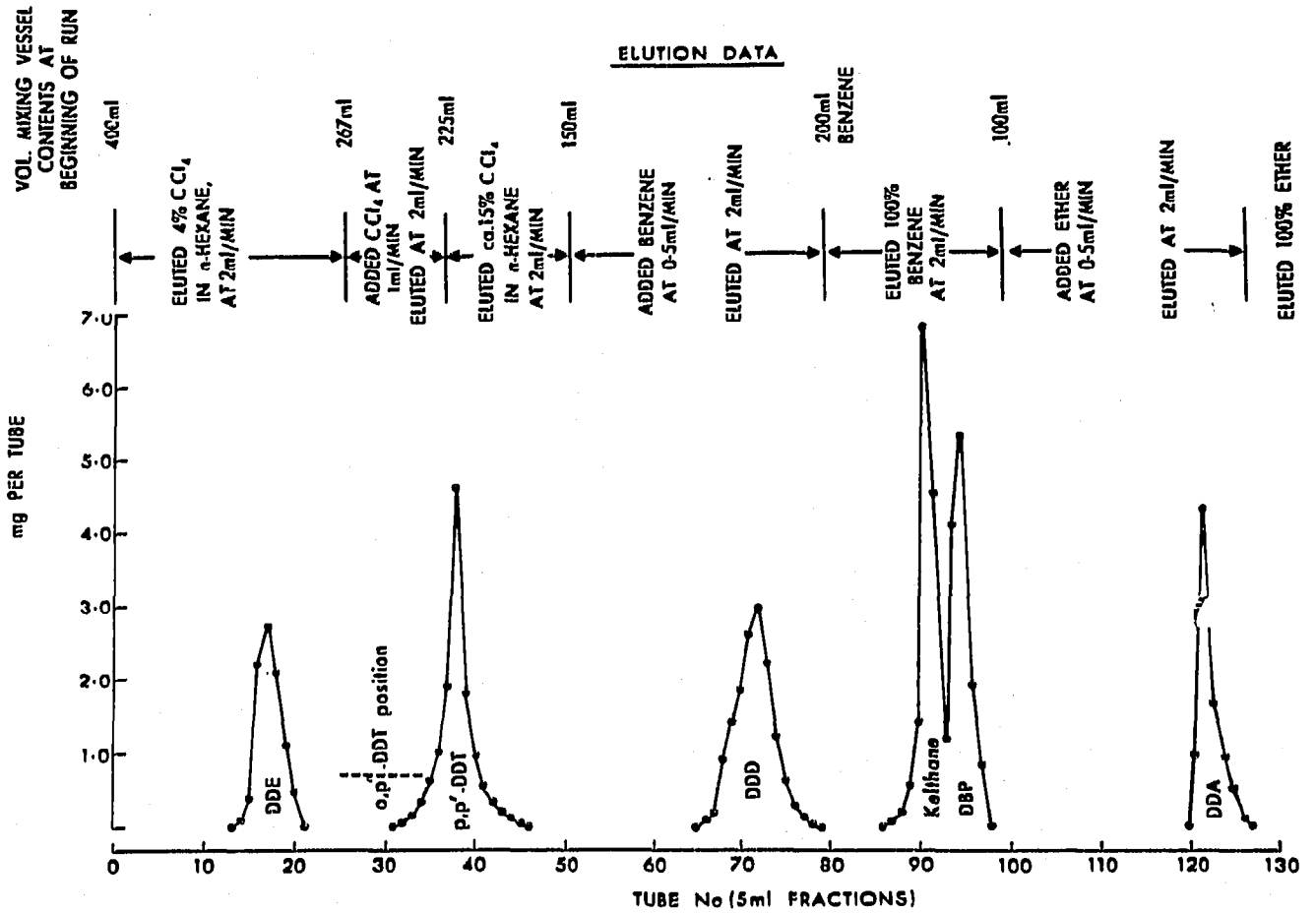

Fig. 1. Chromatogram and conditions for the elution of a mixture of DDE, DDT, DDD, Kelthane, DBP and DDA.

Silicic acid (Mallinckrodt 100 mesh A.R.) was washed twice each with carbon tetrachloride, benzene and *n*-hexane, freed of excess solvent by centrifugation between washings, spread on shallow plates to dry and activated at 120° for 16 h. The activated material was desiccated over silica gel until required. All solvents used were A.R. grade, double distilled. Washing of adsorbent and double distillation of solvents were essential for minimising residues which interfered with spectra. It was noted that solvent-washed adsorbent did not become as active as did untreated material when similarly heated.

A water jacketed column 22 cm long × 1.85 cm I.D. was packed with silicic acid as follows. Twenty g of activated adsorbent were mixed thoroughly with 0.6 ml of distilled water in a stoppered jar, then converted to a slurry in a mortar and pestle with 4 % carbon tetrachloride in *n*-hexane and transferred to the column.

After the column was equilibrated at 14° using cold water from a reservoir, a mixture of weighed amounts of *p,p'*-DDE, *p,p'*-DDT, *p,p'*,-DDD, Kelthane, *p,p'*-dichlorobenzophenone (DBP) and *p,p'*-DDA was dissolved as far as possible in 5 ml of solvent and applied to the column. Some undissolved material (DDA) was applied as a suspension. Elution was commenced using 4 % carbon tetrachloride in *n*-hexane at a flow rate of 2 ml per min. Fractions of 5 ml volume were collected and solvent evaporated to allow weighing of eluted compounds and their identification by U.V. spectral scans. Solvent gradients were prepared using a stainless steel and

TABLE I

COLUMN CONDITIONS FOR THE SEPARATION AND PURIFICATION OF SOME DDT TYPE COMPOUNDS AND METABOLITES

% Water added to silicic acid	*Solvent system*	*Compound or metabolite chromatographed*
(*a*) *Adsorption*		
3, 5, 10	Eluted *n*-hexane	DDE, *o,p'*-DDT, *p,p'*-DDT
0, 2, 3, 5, 10	Benzene gradient on *n*-hexane	DBP, phenol*, ketone*
3, 10	Carbon tetrachloride gradient on *n*-hexane	DDE, *o,p'*-DDT, *p,p'*-DDT
3	Benzene gradient on carbon tetrachloride–*n*-hexane mixture	Kelthane, ketone*, DBP, DDD
5, 10	Diethyl ether gradient on *n*-hexane	DBP, DDA, phenol*, carboxylic acid*
(*b*) *Partition and mixed*		
5	Acetonitrile stationary phase 50 % v/w of silicic acid. Eluted *n*-hexane	Kelthane, DBP
10	Acetonitrile stationary phase 65 % v/w of silicic acid. Eluted *n*-hexane	Phenol*
5	Acetonitrile stationary phase 50 % v/w of silicic acid. Eluted benzene gradient on *n*-hexane	Kelthane, phenol*
3, 5	Acetonitrile stationary phase 50 % v/w of silicic acid. Eluted acetonitrile gradient on benzene, starting with 2 % acetonitrile in benzene	Kelthane, ketone*
0	Dioxane stationary phase 50 % v/w of silicic acid. Eluted benzene gradient on *n*-hexane	DBP, phenol*

* Unidentified metabolite of DDT with this group indicated.

teflon pump with two independently adjustable cylinders, one pumping the gradient solvent to a magnetically stirred mixing vessel and the other pumping the resulting mixture into the column. After tube 100 the column temperature was allowed to rise to ambient room temperature (20°) which facilitated elution of DDA.

DDE was eluted with the starting solvent, then gradients were used for the remaining elutions as in Fig. 1. There was a clear separation of DDE, DDT, DDD and DDA. Although Kelthane and DBP were not completely separated here they should be resolvable by continuing the benzene gradient instead of using pure benzene elution. In the original metabolite work *o,p'*-DDT was present as a minor impurity and separated satisfactorily, eluting ahead of the *p,p'*-DDT in the position of the dotted line in Fig. 1. Recoveries in the present experiment ranged from 95% to 100% by gravimetry and these could no doubt be improved by more precise assay.

Silicic acid columns with various percentages of water in the adsorbent, a range of solvents, gradients, systems and temperatures gave a very flexible technique for separations and clean up of metabolic products, although reproducibility of results depended on standardisation of each batch of adsorbent. A table of systems utilised for this work (Table I), some of which were used in the production of the chromatogram represented in Fig. 1, gives some idea of the versatility which may be exploited for resolutions of organic solvent soluble compounds with very similar chromatographic properties.

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Permanent records of thin-layer chromatograms on transparent paper

Several methods are described in the literature for the documentation of thin-layer chromatograms[1,2]. A widely accepted approach is the spraying of the chromatograms with a polymer dispersion (Neatan, Brinkman) and the subsequent mounting of the plastic film on transparent acetate tape. Other methods are reported in which the chromatogram is photographed or copied as a blueprint[3–5] or reproduced on a sheet of transparent paper[1]. Advantages and disadvantages are inherent with each of these methods and in general the nature of the work in which thin-layer chromatography is used determines which kind of documentation will be most suitable.

During the course of preparative and analytical work on urinary constituents we found that the recording of chromatograms on transparent paper was the best way to process rapidly a large number of chromatograms without interference with further preparative or analytical procedures. This communication will describe our procedures