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≪Note≫ A Procedure for the Determination of D. D. T. and γ-B. H. C. in Organ by an Activation Analysis Using a Thin Layer Chromatographic Technique

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During the course of performing a project on the determination of a pesticide residue in a fish, a rapid and sensitive method of analysing a trace quantity of organic chlorine compounds in an organ was required, and consequently a separate study for the procedure was attmepted with views to minimize the amount of the sample and to increase the sentivity of the procedure. Existing procedures for the determination of D. D. T. and γ-B. H. C.[1], perchloro biphenyl[2], and various contaminants in marine products[3] are all of a gas chromatographic separation of the compounds with an electron capture detector, which require a complicate set of the charging metrials of the column to achieve a well defind, resolved peak of that component, and an efficient programming system both for temperature and scanning devices to facilitate the elution of a high boiling compound of this nature from a column. Furthermore, various resinous or high boiling impurities other than the compound may cause a serious clogging of the column when many numbers of samples are subjected to be analyzed, as this kind of works are usually supposed to be.

On the contrary, an activation analytical method for the determination of an organic compound is usually of very sensitive for the trace quantity of the sample and requires a small quantity of a sample within the error of the counting devices, if the complicate components of an activated organic compound are well separated and identified[4]. In a separate study carried in this laboratory aromatic chloro derivatives of a solid state, when they are irraidated in a research reactor, were found to afford a steady organic yield and a high yield of the recombined product which are easily separated by a thin layer chromatography[5]. These results prompt us to attempt the proposed work by means of an activation analytical procedure. It may be able to eliminate the complicacies of the exsiting instrumental method and to afford a simple and sensitive way of the determination of trace quantity of organic chloro compound in an organ, provided that the detection limit within the permissible range of counting error is small enough to merit analytical viewpoints. Among many types of organic contaminants of an organ, D. D. T. and γ-B. H. C. were at first subjected to be studied, since the chemical structures of these compounds are of aromatic nature.

The mode of the separation of the cold γ-B. H. C. and D. D. T. by means of a thin layer chromatography was proved to be very efficient when a mixture of silica gel and alumina coated on the plate and a slovent system of cyclohexane and silicon oil (92: 8v/v) was used as the developing solvent. Rf

values of two compouds developed on a plate were 0.60 for γ-B.H.C. and 0.85 for D.D.T. which were identified by means of a coloring reagent of 0.5% solution in methanol of o-tolidine. The separation of activated compounds was also effective when the reactor irradiated compounds were developed after the organic portions of the sample were dissolved in benzene. In the table 1 and 2 radio activity of the separated component of the irradiated D.D.T. and γ-B.H.C. were listed and these values were plotted against the amount of the sample irradiated in the Fig. 1. A linear relationship was obtained after checking the relationship by a least square method. This result revealed that the recombined yield of the recoiled ^{38}Cl atoms is consistent enough to assure an analytical application thereof.

Generally, the processing time consumed for the thin layer chromatography was around 2 hours and hence a considerable radioactivity was lost during the course of the processing, which may cut down the final activity of the separated product to afford a poor detection limit for trace analysis and to give a large error in the quantitative work. Furthermore, the part of the solution of the irradiated sample was spotted on the plate and hence the amount of the solution spotted may have an influence on the final radioactivity of the separated product. In order to ascertain these points of analytical aspects, the exsisting criteria of evaluating the consistensy of a quantitative radioactivity counting[6] (280 counts/min) was considered as a detection limit.

Table 1. γ-Activity* of various amounts of standard D.D.T. irradiated.

Concentration of D.D.T. (ml./cc.)	Quantity of D.D.T. irradiated.	Counts/min of the separated portion on the plate.
0.05	10μg	36,440
0.25	50μg	185,590
0.50	0.10mg	371,900
1.00	0.20mg	759,410
1.60	0.32mg	1,174,540
3.10	0.62mg	2,460,420
5.00	1.00mg	3,740,590
10.1	2.05mg	7,650,410
13.4	2.68mg	10,076,000

Table 2. γ-activity* of various amounts of standard γ-B.H.C. irradiated.

Concentration of γ-B.H.C. (ml./cc.)	Quantity of γ-B.H.C. irradiated	Counts/min. of the separated portion on the plate.
0.04	8.9μg	22,516
0.32	64.0μg	164,540
0.80	0.16mg	449,350
1.40	0.28mg	762,280
1.9	0.38mg	1,040,100
4.00	0.8 mg	2,314,000
8.30	1.66mg	4,695,100
15.1	3.02mg	8,305,000

*Total 2 hrs were required for the separation and counting of the irradiated sample after the reactor irradiation.

In this porocedure one fourth (250 λ) of irradiated sample (1.0 cc) was spotted and counted. Then the detection limit was 0.08 μg for D.D.T. and 0.12 μg for γ-B.H.C. If total amount of the sample were spotted, the detection limit would be 0.02 μg. for D.D.T. and 0.03 μg for γ-B.H.C., respestively.

These mean that down to 0.02 p.p.m. of D.D.T. and 0.03 p.p.m. of γ-B.H.C. contained in 1g. of an organ could be determined within the permissible range of a quantitative error. These values may further be leveled down a half (0.01 p.p.m. for D.D.T. and 0.015 p.p.m. for γ-B.H.C.) as the of amount the sample is increased two times or further. These results discussed hereto revealed that the present procedure would flexibly be ap-

plicable for an analytical work, the detection limit of which may easily be selected as required.

In a practical operation of this procedure, a fish could be analyzed for it's D. D. T. and γ-B. H. C. contents as summarized in the table 3. The reproducibility of of the analytical values (ca. 0. 03 p. p. m.) was of constistent and the procedure itseif was very simple and convenient as described in the experimental. Problems were, however, encountered to eliminate bromide, sodium, and other nucleides in the irradiated sample, which were spread all over the thin layer plate with a considerable radio activity to make the component of the resolved peak on the plate contaminated. This

Tabl 3. The Determination of γ-B. H. C. and D. D. T. Contaminants in a fish.

Contaminant	Weight of sample	Counts/min. of the separated portion on the plate.	Quantity equivalent	
			μg	p. p. m.
D. D. T.	1. 0410gr	928	1. 24±0. 04	1. 23±0. 04
	1. 1960gr	995	1. 32±0. 04	1. 12±0. 04
γ-B. H. C.	1. 0410gr	203	0. 40±0. 03	0. 36±0. 03
	1. 1960gr	248	0. 44±0. 03	0. 37±0. 03

difficulty was eliminated by washing the extracts of the sample with an aqueous solution of sodium sulfite, which could give a clean γ-spectrum of the main peak for ^{38}Cl.

In conclusion the present technique was proved to be efficient enough to be used as a procedure of the determination of γ-B. H. C. and D. D. T. within the permissible error. Though the detection limit of the procedure is adjustable as the amount of the sample is varied, the process of the sample for the analytical works are more simple and convenient than those of a gas chromatographical method as decribed hitherto. The procedure may further be extented to the determination of per chloro biphenyl or other aromatic chloro contaminats of an organ, provided that the recombination yield of the irradiated sample is consistent enough to assure an analytical application as stated.

Experimental

1. A thin layer plate:

Each 2g. portion of silica gel G (Merck, for thin layer chromatography) and aluminum oxide G(Merck, for thin layer chromatography) were mixed together and ground in a mortar to form a paste adding 10cc of the double distilled water. This paste was equally coated on four plates of an aluminum strip of 2. 5 cm in width and 18 cm in length. The coated plates were then dried at the room temperature and activated further at 105°C for an hour.

2. Standard Sample:

Commercial products of D. D. T. and γ-B. H. C. were repeatedly recrystallized in a solvent until they attain a constant melting point. (108°C for D. D. T. and 112°C for γ-B. H. C.) The purified products were irradiated and processed as follows.

3. Preparation of a calibration curve for the determinaton.

(1) Irradiation of the sample: A weighed amount of the sample was sealed in a polyethylene tube(I. D. 1cm. , Height 2cm.) and the tube was irradiated at the pneumatic tube system of TRIGA Mark-II research reactor(Neutron Flux, 3.0×10^{12} n/cm^2 sec.) for 30 minutes. A flux monitor* was attached to the tube during the course of the irradiation and the flux was calibrated by checking the γ-activity of the monitor through it's γ-spectrum.

* 15mg. of Fe wire which contain a Mn impurity was used as the monitor. The γ-photo peak of Mn at 0.845MeV was measured by a multichannel analyzer.

(2) The separation of the recombined product from the irradiated sample by a thin layer chromatography: The irradiated tube was cooled for minutes and opened in a hume hood. The contents of the tube were then dissolved in 1.0cc. of benzene and each 50 λ portion of the solution was spotted on the thin layer plate prepared as above. (Size of the spot; 0.8-0.5cm in the diameter). The spotted plate was dried and developed in a chamber by an ascending way with a solvent system of cyclohexane and silicon oil (92: 8v/v). The plate was taken out from the chamber when the solvent front reached around 12cm from the spotting line. Separately, a soution of o-tolidine in methanol (0.5%) was prepared and the solution was sprayed over the dried plate to give a blue coloring at R_f value of 0.60 and 0.85 when the plate was irradiated under an U.V. light source. The colored spots were identified by comparing them with those of the cold standard samples, and each colored band was scrapped into a test tube. The radioactivity of the contents of the tube was then counted at a defined geometryat a γ-well scintillation counter (SA-250, Fujitzu). Total time of the processing of the irraidated sample was recorded from the end of the reactor irradiation. (Usually, 2 hours), and the counts obtained were calibrated for the fluctuation of the neutron flux. The results were summarized in the table 1 and 2. A plot between the count per minute of the separated product above and the amount of the original sample irradiated was examined for it's linearity by a least square calculation and a calibration curve was plotted as shown in the Fig. 1.

(2) Determination of the detection limit: The standard deviation of the background

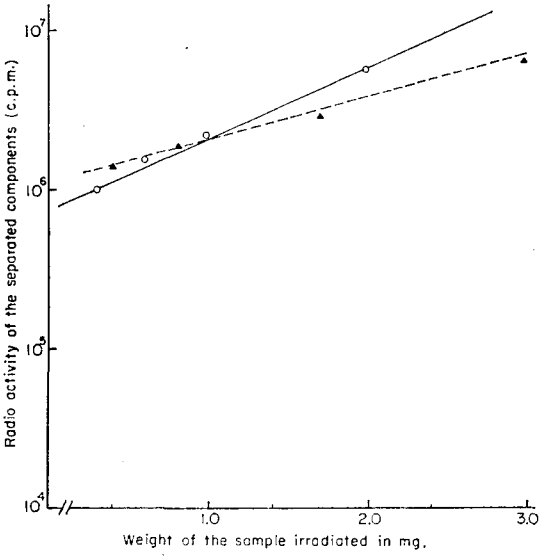

Fig. 1. 1. Radioactivity of the serparated components *v.s.* the amount of the sample irradiated. (A part of the curve was shown)
(The full line denoted the line for D.D.T., whereas the dotted line denotes for the line for γ-B.H.C.)

of the counter was calculated. The amount of the background was 510 counts/min. and the standard deviation was 22.6 counts/min. By definition of detection limit of Curie[6], 280 counts/min* is defined as the lowest counts of the sample irradiated which can be determined quantitively.

The lowest counting value was calculated in μg. to give 0.08μg. for D.D.T. and 0.12μg of γ-B.H.C as the quantitative detcetion limit, respectively. According to these values, the lowest determinable p.p.m. of the sample can be estimated proportional to the sample to be taken.

4. The determination of D.D.T. and γ-B.H.C. contaminats in a fish.

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* 280 counts/min$=K_\alpha\sigma_\alpha=50\left(1+\frac{\mu_b}{25}\right)^{\frac{1}{2}}$ ($\alpha=\beta=0.05$, α=first kind error; β=second kind error, μ_B; mean value of background counting.
K_Q; constant depending on the confidence level
σ_Q; standard deviation of sample counting.

(1) Extraction of the organic portion: A fish sample was processed to remove it's digestive organs and washed with a distilled water. The washed sample was further dried under a reduced pressure at a low temperature. (A freeze drying.) The dried sample was weighed in a 1g. portion and extracted for 7 hours with n-hexance by means of a Soxleht extractor. The extracts were washed twice with an equal volume of 5% solution of Na_2SO_3 and washed again with distilled water. The organic portion was dried with anhydrous magnesium sulfate. The organic portion was filtered and it was dried under a reduced pressure to give a solid matrix at the room temperature.

(2) Analystical procedure. : The solid matrix obtained above was irradiated in the reactor, thin layer chromatographed, and counted for the radioactivity as described above. The results were evaluated on basis of the calibration curve for it's D.D.T. and γ-B.H.C. contents as summarized in the table. 3.

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