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DETERMINATION OF GAMMA ISOMER IN HEXACHLOROCYCLOHEXANE BY ISOTOPE DILUTION

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SUMÁRIO

O método de análise por diluição isotópica é aplicado para a determinação do isômero gamma de BHC usando-se cloro-36 como radioisótopo marcador. A separação do isômero gamma marcado é levada a efeito por cromatografia em coluna de ácido silícico.

A reprodutibilidade do método é excelente dando um desvio padrão da média da ordem de 0,1% para amostras contendo cêrca de 10% de isômero gamma.

RESUMÉ

On employe la méthode d'analyse par dilution isotopique pour la determination de l'isomére gamma de BHC en utilisant le chlore-36 comme traceur. La separation de l'isomére gamma est faite par chromatographie sur colonne de acide silicic.

On a constaté une trés bonne reproductibilité en la méthode et on obtient un écart-type de l'ordre de 0,1% pour des echantillons contenant environ 10% de l'isomer gamma.

SUMMARY

Isotope dilution method, associated with chromatographic separation, was applied to analysis of gamma isomer of BHC.

Precision of the method is very good giving an standard deviation of average of less than 0.1% for samples with about 10% of gamma isomer.

INTRODUCTION

Analysis of the pesticide BHC (hexachlorocyclohexane benzene hexachloride) for the biological active gamma isomer is usually carried out by polarography, infra-red spectrophotometry and chromatography in silicic acid columns[1]. However, discrepant results have been found when those methods are applied by different laboratories [2]. An "absolute" method of analysis that would not depend on analysts or laboratories would be most desirable.

Biological methods such as paralysis of mosquito larvae of "Ades aegypti", or methods based upon solubility determination or lowering of melting point, have their reliability dependent upon the nature and amount of nonbenzene hexachloride material present in any given sample being assayed. It has been reported (3) that some commercial crudes give

erroneous results when assayed by direct infrared spectrophotometry. Also it has been observed that the accompanying material exerts an effect upon the solubility behaviour of the gamma isomer [3].

To obviate all those restrictions of existing methods, Trenner "et al" [3] developed a method, that might be considered "absolute", of analysis of BHC by isotopic dilution using deuterium as labeling isotope. The ratio of $C_6H_6Cl_6$ to $C_6D_6Cl_6$ in the isolated material is determined by direct infrared spectrophotometry.

To avoid the use of deuterium as labeling isotope, Craig and Tryon [2], applied the method of isotopic dilution analysis using chlorine-36 as the radioisotope to label the gamma isomer of BHC. In this method the final separation of the gamma isomer is carried out by fractional crystallisation which is repeated, at least, three times. By having to repeat so many times the crystallisation procedure a very small amount of material is left for the final weighing and counting and this will introduce chance for errors in the determination of the specific activity of the recovered material specially on the case of samples with low content of gamma isomer.

The method would be of larger possibility of application and less subjected to error, if it could be applied without the necessity of separation, by fractional crystallisation, of the final material to be counted and weighed.

With this aim in mind, the method of isotope dilution

associated with the separation technique of chromatography, was applied. As it will be shown the proposed method in which isotope dilution is associated with chromatographic separation gave a higher recovery of pure gamma isomer with a very good accuracy and precision for the analytical results.

PREPARATION OF LABELED STANDARD

Labeled gamma isomer of BHC was prepared by the method described by Craig and Tryon[2]. Radioactive Chlorination of benzene is based on rapid establishement of exchange equilibrium between chlorine and chloride-ion in aqueous solution. Inactive chlorine passed through hydrochloric acid tagged with chlorine-36 becomes active by exchange in accordance with the reaction.

$$Cl_2 + 2\,HCl^* \rightleftharpoons Cl_2^* + 2\,HCl$$

The reaction is practically quantitative and almost all of Cl-36 is transferred to the gaseous phase.

Gaseous chlorine, labeled with Cl-36 by means of the reaction above, is bubbled through benzene in which it becomes dissolved. The solution is then iluminated with a 150 watt lamp until the yellow-green color disappears, when the reaction is completed and $C_6H_6Cl_6$ is formed. The excess of benzene is distilled and pure gamma isomer is added to act as carrier for the labeled isomer.

The labeled gamma isomer is separated and purified from the material obtained, by extraction with n-hexane satu-

rated with nitromethane, evaporation of the solvent and fractional crystallization in ethyl alcohol solution. Purity is checked by melting point determination of the dried crystals, which should be 112.0-112.8ºC. The labeled gamma isomer BHC is then ready for use. Figure I shows the apparatus for labeling the BHC.

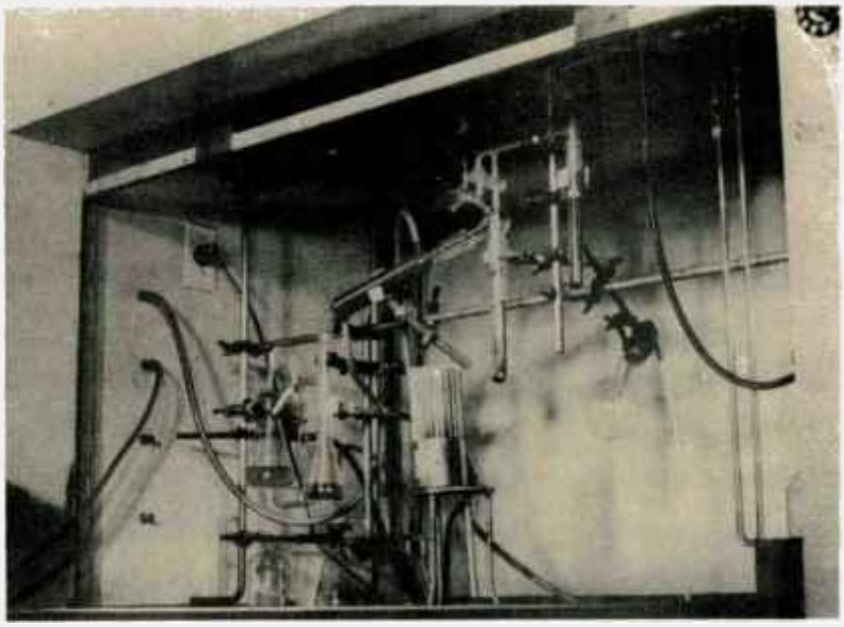

FIGURE I

Apparatus used for labeling the BHC

ANALYTICAL PROCEDURE

Reagents

Silicic acid, 100 mesh, Mallinckrodt Chemical Works.
Petroleum benzin (naphta) Technical grade.
Nitromethane, Analytical grade.
Acetone, technical grade.
Ethyl Alcohol 99.8% queel.

Mobile solvent - Saturate one liter of naphta with 20 milliliters of nitromethane. Decant and separate excess of nitromethane.

Chromatographic column - The column is made of Pyrex glass with 80 cm length and 2.5 cm diameter. At the bottom of the column a coarse porosity fritted glass disk is sealed. The top of the column is closed with ground glass stopper provided with relieve valve and entrance to apply pressure, Figure II. 50 gr of silicic acid are stirred with 150 ml of mobile solvent and 27 ml of nitromethane. After mixing the mixture is poured into the chromatographic column through a glass funnel. The slurry is stirred with a long glass rod to displace air bubbles. The sides of the column are washed down with mobile solvent and pressure is applied to pack the column and eliminate excess solvent; the solvent level should be 3 mm above the adsorbent. The column, prepared as described, is ready for use and can be used for about 10 to 15 operations

Dye solution - Made up by dissolving 25 mg D C violet n.2 (1-Hydroxy-4-p-toluino-anthraquinone) in 50 ml of mobile solvent.

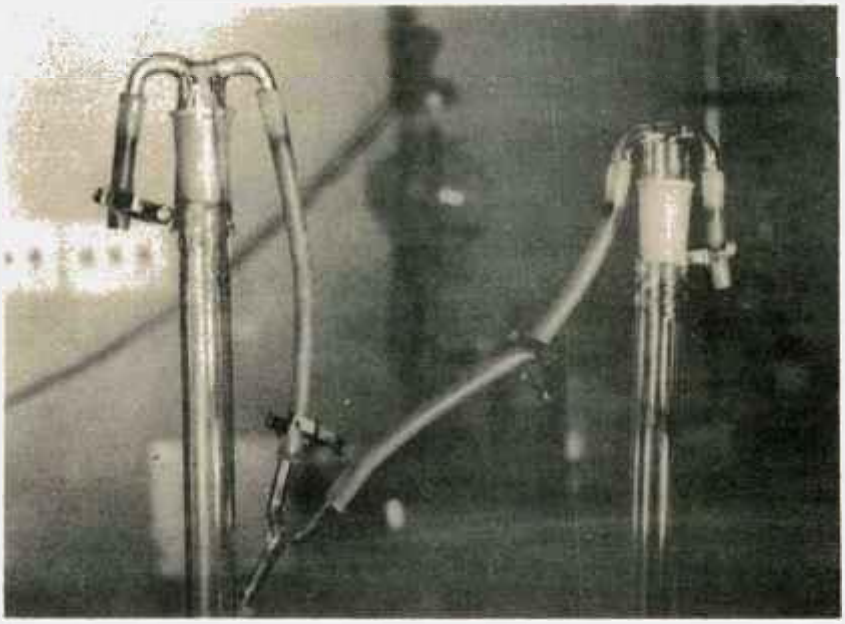

FIGURE II

Top of chromatographic column with relieve and pressure valves

Procedure

Weigh from 4 to 5 g of BHC to be analysed, for samples having from 1 to 15% of gamma isomer. Place the samples in a beaker of 50 ml. Add about 100 mg of labeled gamma isomer with specific activity of about 3×10^{-4} microcuries per milligram.

Add from 15 to 20 ml of acetone and heat in a water bath with constant stirring. After complete dissolution of the material distill the acetone by heating with an infra-red lamp.

Add 10 ml of mobile solvent. Heat in a water bath with constant stirring and breaking the lumps of solid mate-

rial with a glass rod.

Let it cool and filter through sintered glass of medium porosity. Dissolve the residue with acetone, in the same beaker of the first dissolution and repeat the treatment with 10 ml of mobile solvent. Transfer both filtrates to the same beaker and add 1 ml of dye solution. Transfer the filtrates , colored by the dye, to the chromatographic column.

Apply pressure to the column until the surface of the liquid is at the same level with the silicic acid. Relieve pressure and add 10 ml of mobile solvent, washing, at same time, the sides of the column. Apply pressure until the surface of liquid is at same level with the silicic acid, as before. Add 180 ml of naphta saturated with nitromethane and apply pressure. The blue spot of dye will be displaced through the silicic acid column. When the spot reaches the bottom of column start the collection of the efluent in fractions of 5 ml. Gamma isomer will elute in these fractions, the concentration being higher in the last fractions.

Distill the solvent in each fraction by heating in a water bath. Transfer the residue that appears in the last fractions to a 10 ml test tube, using 2 to 3 ml of acetone. Place the test tube in a vacuum-dessicator and distill the acetone by heating gently with a infra-red lamp.

In order to purify the crystals that are left at the bottom of the test tube, add 0.3 ml of ethyl alcohol at 95%, heat in a water bath while stirring with a glass rod. If the volume of ethyl alcohol is not enough to dissolve the

crystals completelly, add some drops more of alcohol and heat again.

Let the suspension cool to room temperature and apply suction through a glass tube provided with a plate of sintered glass. In this way the alcohol is removed.

Repeat the purifying procedure twice and dry the crystals for two hours in a vacuum dessicator by heating gently with an infra-red lamp.

About 50 mg of gamma isomer crystals will be obtained for BHC samples with 1% of isomer and using about 5 g of samples for analysis.

Determination of Specific Activity

Take from 20 to 40 mg of crystals obtained as before and weigh. Transfer the crystals to counting planchets of 3 cm diameter and 0.5 cm height to the bottom of which a circular piece of filter paper, imbibed in modeling varnish , is placed. Dissolve the crystals with acetone and evaporate the solvent by gently heating with infra-red lamp. The dried material will distribute uniformly in the filter paper and, in such a way, errors in counting will be avoided. Count the material for a length of time that will give the desired statistical counting deviation.

APPLICATION

In order to test the method described above various

samples of BHC, containing known amounts of gamma isomer, were prepared.

100 g of BHC with a content of gamma isomer of about 10% were disolved in a beaker of 500 ml, placed in a water-bath, with 150 ml of mobile solvent. The suspension was let to cool to room temperature and filtered through a sintered glass plate of medium porosity. The solid residue was returned to the dissolution beaker and the treatment was repeated twice. The solid was dried under infra-red heat and homogenized with a glass rod. With this treatment practically all of the gamma isomer was removed.

From this BHC, from which most of the gamma isomer was removed, nine samples (in duplicate) were prepared by adding increasing amounts of gamma isomer to each pair of samples and analysed. Each sample of BHC had a weight of about 4 to 5 g.

Table I sumarizes the data and results.

Samples obtained from factory-runs of BHC , with amounts of gamma isomer running from 10 to 12%, were also analysed by the proposed method. To samples, taken from the same batch, known amounts of gamma isomer were added and the new samples so obtained were analysed. Data and results are presented in Table II.

TABLE -I-

Analysis of samples of BHC containing increasing and known amounts of gamma isomer.

Sample	a* (mg)	B* (cpm/mg)	Gamma isomer found (%)		Gamma isomer in sample (%)
				average	
1	211.4	45.44	0.65	0.6	0.6
1a	211.4	45.94	0.59		
2	203.5	29.09	3.04	3.0	2.7
2a	203.5	29.08	3.04		
3	216.1	25.71	4.30	4.2	4.4
3a	216.1	26.44	4.08		
4	202.8	18.43	6.34	6.3	5.8
4a	202.8	18.62	6.25		
5	209.2	16.66	8.30	8.2	8.1
5a	209.2	16.85	8.15		
6	208.1	12.57	11.70	11.7	11.3
6a	208.1	12.59	11.68		
7	203.8	8.51	17.17	17.5	17.1
7a	203.8	8.23	17.85		
8	201.4	6.15	23.31	23.5	23.5
8a	201.4	6.09	23.59		
9	202.1	4.65	33.50	33.5	33.2
9a	202.1	4.66	33.50		

A = 52.45 cpm/mg

* a, A, B, are the amount of labeled gamma isomer added, the specific activity of isomer added, and the specific activity of isomer recovered, respectively. The amount of isomer is given by the known formula $x = a(A/B - 1)$; see reference (4).

TABLE -II-

Analysis of two samples of BHC to which known amounts of gamma isomer were added.

Sample	a (mg)	B (cpm/mg)	Gamma isomer Added (%)	Total gamma isomer in sample (%)	Gamma isomer found (%)	Difference
1	222.5	16.68	---	11.77	11.87	-0.10
1a	222.5	16.89	---	11.77	11.66	+0.10
1b	201.5	12.95	2.90	14.67	14.69	-0.02
1c	201.5	12.96	2.90	14.67	14.67	0.00
1d	201.5	13.06	2.90	14.67	14.53	+0.14
1e	243.2	20.77	6.10	17.87	17.86	+0.01
1f	243.2	20.68	6.10	17.87	17.98	-0.11
2	204.2	16.69	---	10.70	10.76	-0.06
2a	204.2	16.82	---	10.70	10.74	-0.04
2b	200.9	13.87	3.23	13.93	13.81	+0.12
2c	200.9	13.63	3.23	13.93	14.15	-0.22
2d	200.3	11.40	5.51	16.21	16.52	-0.31
2e	200.3	11.66	5.51	16.21	16.06	+0.15
A = 35.78 cpm/mg						

DISCUSSION

One of the main advantages of the proposed method in which the chromatographic separation method is associated

to the isotopic dilution method is that a larger amount of labeled gamma isomer is recovered at the end of the analytical procedure. In this way weighing and counting of samples are less subjected to errors. Purification of gamma isomer, prior to obtaining the data for the calculation of the specific activity B, by fractional crystallization, will permit recovering of only a very small fraction of mass and of added activity. This will require longer counting time and weighing in microbalances. Reproducibility of results will thus be easily influenced by operation techniques and analysts, than when weighing in ordinary analytical balances and counting of samples with higher specific activities.

Counting of samples is made, in this work, with micawindow Geiger counters, instead of liquid counters. In this way the decontamination of counters in between counting of different samples is avoided. For the amount of material to be counted it is not necessary to mount infinite thick samples, the activity being proportional to the amount of material in the samples for the range of recovered and added weight of labeled gamma isomer.

Reproducibility of results, i.e., precision, in terms of standard deviation, is very good. Standard deviation of the average is less than 0.1% (quadruplicate set of analysis) for samples containing around 10% of gamma isomer. For samples with larger content of gamma isomer standard deviation of average is even better than the one reported.

Accuracy of results can be checked by the figures mentioned in Table I. It can be seen from the data of Table I

that the results of analysis of samples with known content of gamma isomer have an average deviation of ± 0.11% for thirteen determinations and on the range from 11 to 16% of gamma isomer.

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