Rapid Analysis of Chlorinated Hydantoin by Gas Chromatography

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Gas chromatographic separation of a number of chlorinated hydantoins has been carried out using a split column. A quantitative method for the determination of 3-trichloromethylthio-5-(1-ethylamyl)hydantoin (chlordantoin) in suppositories has been developed. The accuracy of the method and results of the assay are reported.

A NUMBER OF trichloromethylthio derivatives of the phthalimide and hydantoin series have been investigated (1, 2) for their microbiological activity. Kupferberg and Doscher (2) found one of these hydantoin compounds, 3-trichloromethylthio-5-(1-ethylamyl)hydantoin (chlordantoin) to be the most active topical agent against *C. albicans* infections. A simple and rapid method of chemical analysis was required for this compound formulated in suppositories.

Hydantoins have been quantitated volumetrically in tablets by either compleximetric or precipitation techniques (3, 4). Borkowski and Dluzniewska (5) have used spectrophotometric procedures in biological fluids. These methods are sensitive but they lack the specificity required in the presence of other hydantoins.

Gas–liquid chromatography was considered as a desirable technique for estimating these halogenated hydantoins because it offered quantitation with concurrent fractionation. Chlorinated compounds used in pharmaceutical, pesticide, and fumigant areas have successfully been subjected to gas–liquid chromatography using either flame ionization or electron capture detectors (6–15).

The work reported here describes an analytical method utilizing a preliminary solvent extraction followed by a direct gas chromatographic determination using a flame ionization detector.

EXPERIMENTAL

Apparatus—A modified F and M model 1609 gas chromatograph equipped with flame ionization detector was used throughout this study. (The injection port was modified by F and M Scientific to eliminate the potential for inlet sample splitting.) The column used was a 12 in. long and 1/4 in. o.d. copper tube, half of which (injection port end) was packed with 4% Carbowax 20M on Gas Chrom Z, 100/120 mesh, and the other half with 2% phosphoric acid on Diatoport S, 60/80 mesh. The injection port and detector temperatures were approximately 250° and 235°, respectively. The column was conditioned at 210° and operated at 130° and 190°. Nitrogen was used as a carrier gas with a flow rate of 45 ml. per minute. Hydrogen and oxygen flow were maintained at approximately 45 and 400 ml. per minute, respectively. The attenuation and range throughout the determinations were at 32 and 10, respectively. The chart speed was maintained at 15 in. per hour.

Analytical Procedure—Two 100-ml. standard solutions containing 25 mg./ml. of chlordantoin and 3 mg./ml. of 9-anthraldehyde, respectively, were prepared in chloroform. A 2.0-ml. aliquot of the standard chlordantoin solution was evaporated in a 10 dr. vial and 2.95 Gm. of suppository base was added. To this and another vial containing two suppositories (equivalent to 50 mg. chlordantoin) were added 5.0 ml. of distilled pentane and 10.0 ml. of nitromethane. The mixture was shaken to effect complete solution followed by centrifugation for 2 min. A 5.0-ml. aliquot of the nitromethane solution from each vial was transferred to two other vials and evaporated. One milliliter of standard 9-anthraldehyde solution (internal standard) was added to each vial, mixed, and a 3-μl. injection was made into the gas chromatograph. The retention times for chlordantoin and 9-anthraldehyde were 1 and 2 min., respectively. The concentration of chlordantoin in each suppository was obtained as follows:

$$\text{mg. of chlordantoin/suppository} = \frac{A_s}{B_s} \times \frac{S_b}{S_a} \times 25$$

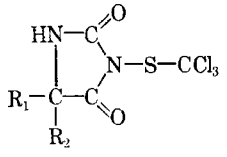
where

A_s, peak height of chlordantoin in sample,
B_s, peak height of 9-anthraldehyde in sample,
S_a, peak height of chlordantoin in standard,
S_b, peak height of 9-anthraldehyde in standard,
25, milligram of chlordantoin in standard.

RESULTS AND DISCUSSION

Table I gives the retention times of a number of hydantoins at 130°. It can be seen that the increase in bulkiness of the alkyl groups at C_5 does not correspondingly increase the retention time. Theoretically, any hydantoin in Table I can serve as an internal standard for chlordantoin (No. 4). However, because of the unavailability of these hydantoins and the time it would take to carry out each determination (130°), we chose to use an internal standard from another chemical series, namely 9-anthraldehyde, and carry out the determination at 190°. This speeds up the determination by a factor of four and is quite useful for control purposes.

TABLE I—RETENTION TIME OF HYDANTOINS AT 130°



No.	R_1	R_2	Time, min.
1	CH_3	C_2H_5	3.05
2	CH_3	i-C_3H_7	2.95
3	CH_3	i-C_4H_9	3.80
4	H	$CH(C_2H_5)(C_4H_9)$	14.00
5	H	$CH_2CH(CH_3)(CH_2)_3CH(CH_3)_2$	3.05

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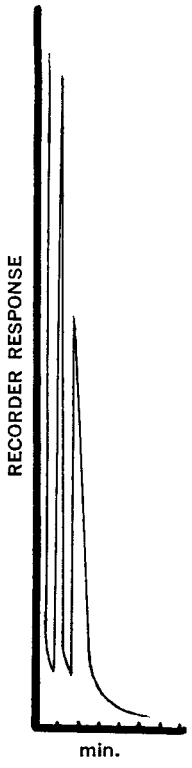

Fig. 1—A typical gas chromatogram for a mixture of chlordantoin and 9-anthraldehyde.

The analytical procedure utilizes extraction by two organic solvents, namely pentane and nitromethane. Thus, a modified extraction p-value determination (fractional amount of a solute partitioning into the nonpolar phase of an equal volume two-phase system) for chlordantoin was carried out. According to the procedure of Bowman and Beroza (16), p-values are determined by analysis of the nonpolar solution of the solute before and after extraction. However, we determined the p-value by analyzing both the polar and nonpolar phase and found it to be 1.0 in the nitromethane. Next the "binding" of chlordantoin to the suppository base was investigated in the same manner as the determination of p-value with different proportions of the suppository base in the pentane. It was found by analyzing the polar phase that there was a definite amount of retention in the pentane layer containing the base. This observation necessitated the addition of a fixed amount of base to the standard solution for the sake of relative comparison of available chlordantoin in samples.

Figure 1 illustrates a typical gas chromatogram of chlordantoin containing 9-anthraldehyde. The ratio of peak heights obtained for various concentrations of hydantoin in the standard solutions is given in Table II. A plot of these data is a straight line. The means for a series of injections of extracted samples containing 25 mg. of chlordantoin were ±1.5% (Table III) with a standard deviation of ±0.26 mg. (Table IV). This procedure affords a rapid means of comparative assay for chlordantoin.

TABLE II—CONCENTRATION *Vs.* RATIO OF PEAK HEIGHT

No.	Chlordantoin, mg.	Ratio of Peak Ht.
1	20	0.854
2	25	1.069
3	30	1.320

TABLE III—DETERMINATION OF PER CENT RECOVERY

Sample	mg. Added	mg. Found	% Recovery (X)
1	25.0	24.4	97.6
2	25.0	25.2	100.8
3	25.0	25.0	100.0
4	25.0	25.4	101.6
5	25.0	25.0	100.0

$\bar{X} = 100.0$

$\sigma = \pm 1.5\%$

TABLE IV—DETERMINATION OF CHLORDANTOIN IN SUPPOSITORIES[a]

Sample	Found (x)	$(\bar{X} - x)$	$(\bar{X} - x)^2$
1	25.1	0.1	0.01
2	24.9	0.1	0.01
3	25.2	0.2	0.04
4	24.7	0.3	0.09
5	25.2	0.2	0.04
6	24.6	0.4	0.16

$\bar{X} = 25.0$

$$\sigma = \sqrt{\frac{\Sigma (X - x)^2}{n - 1}}$$

$= \pm 0.26$ mg.

[a] Each suppository contains theoretical amount of 25 mg.

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