A CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF PHENOTHIAZINE

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A chromatographic assay of phenothiazine is described employing an acetonitrile-hexane partition system supported on Celite. Chromatographic separation is followed by ultra-violet measurement of the eluate fractions at 253 mμ. The system enables a determination of phenothiazine to be made in the presence of diphenylamine, carbazole, phenothiazone and phenothiazine sulphoxide, in addition to ingredients used as excipients in dispersible powders. The method has been tested on a range of samples and the results compared with those obtained by alternative procedures.

ALTHOUGH used mainly in veterinary medicine, phenothiazine is described in several national pharmacopoeias in most of which a Kjeldahl nitrogen determination is used as a method of assay.

Several more specific procedures have been proposed for example, the chromatographic assays of Gunew (1960) and Brierley and Langbridge (1961). For reasons described later, none of these can be considered entirely satisfactory for routine application, and an attempt has been made to devise a selective and accurate procedure suitable for routine use by a works analytical laboratory.

EXPERIMENTAL

Previous experience with partition chromatography on columns supported on Celite had shown that sharp and quantitative separation of structurally similar compounds can be achieved and led us to believe that this technique could be adapted for assaying phenothiazine. Of several solvent systems tried, the following was selected. Acetonitrile (1 vol.) was shaken with hexane (10 vols.), the lower layer was employed as stationary phase, and the upper layer was used to develop the chromatogram. The progress of the separation was followed by measuring the extinction at 253 mμ* of successive fractions of the column eluate. The relationship between extinction and the volume of eluate, using a repeatedly sublimed sample of phenothiazine, is illustrated in Fig. 1.

METHOD

Celite 545 prepared as described by Holbrook, Bailey and Bailey (1963) was used in a column 70 × 2·2 cm.

Preparation of sample and standard. Dissolve an accurately weighed quantity of about 100 mg. of pure phenothiazine in methanol and dilute to 100 ml. with methanol in a volumetric flask. Transfer a 10·0 ml.

* This represents the wavelength of maximum absorption of phenothiazine in the eluant phase.

aliquot of the above solution to a second 100 ml. volumetric flask and dilute to volume with methanol. Transfer 1·0 ml. of the latter solution (≡ 0·1 mg. phenothiazine) to a 50 ml. beaker, evaporate to dryness in a current of air, and set aside until required. Prepare an extract of the sample in a similar manner.

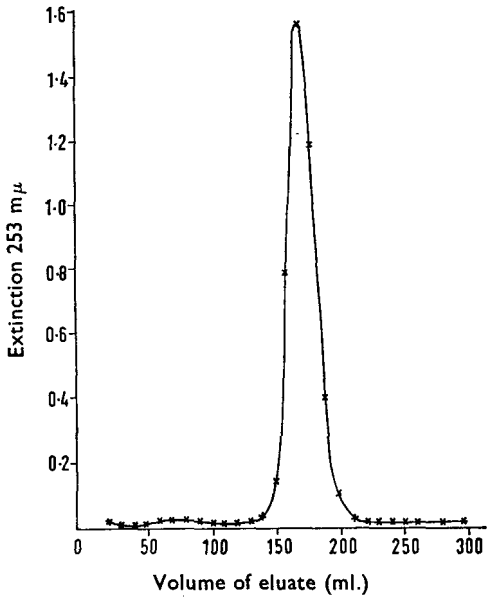

FIG. 1. Plot of extinction of eluate at 253 mμ against eluate volume.

Preparation of chromatographic column and subsequent treatment. Mix prepared Celite (25 g.) with stationary phase (12·5 ml.) in a 250 ml. beaker and transfer the mixture to the chromatographic column in portions of about 3 g., packing down firmly with a tamper between each successive addition. Dissolve the standard phenothiazine extract contained in the 50 ml. beaker in 1·0 ml. of stationary phase, add prepared Celite (2 g.), mix well and transfer quantitatively to the top of the stationary phase in the column. Carefully add eluent phase until the stationary phase is covered to a depth of about 40 cm. and adjust the flow of eluate from the column to about 5 ml./min. Maintain a constant flow rate by adding eluent phase to the top of the column and collect 35 successive 10 ml. fractions of eluate in 6″ × 1″ stoppered test tubes. Measure the extinction of each fraction in 1 cm. silica cells at 253 mμ against eluent phase in the reference cell. Repeat the entire chromatogram using the extract of the sample in place of the standard. Summate the extinction readings obtained from the chromatogram band of the standard and those of the corresponding band of the sample.

Then the percentage of phenothiazine in sample $= \frac{\epsilon_a \cdot W_s \cdot 100}{\epsilon_s \cdot W_a}$ where ϵ_a

and ϵ_s are the sums of extinction values under the sample and standard chromatogram peaks respectively. w_a = weight of sample (mg.) w_s = weight of standard phenothiazine (mg.)

Samples of likely impurities in crude phenothiazine were assayed as above. The curves relating volume of eluate to extinction at 253 mμ for phenothiazone, carbazole and diphenylamine, (Fig. 2) show that none of these compounds interferes with the determination of phenothiazine by the proposed method. Phenothiazine sulphoxide, remained on the column even after 400 ml. of eluate had been collected.

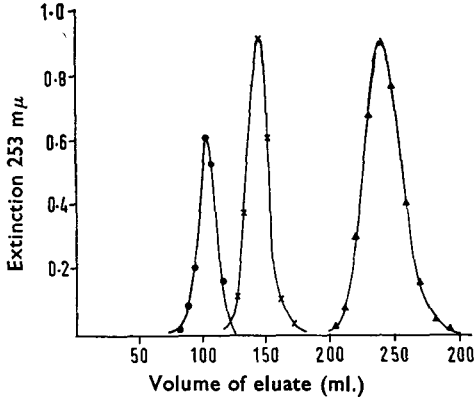

FIG. 2. Phenothiazine impurities. Plot of extinction at 253 mμ against eluate volume. ●——● Diphenylamine. ×——× Phenothiazone. ▲——▲ Carbazole.

RESULTS AND DISCUSSION

The high order of reproducibility by the proposed method is shown by the following results: pure phenothiazine 100, 99·9, 99·8 per cent: commercial sample I, 81·3, 80·6, 81·7 per cent: commercial sample II 88·2, 88·3, 87·8 per cent.

TABLE I

ANALYSIS OF ARTIFICIAL MIXTURE OF PHENOTHIAZINE AND IMPURITIES

Phenothiazine mg.	Phenothiazone mg.	Diphenylamine mg.	Carbazole mg.	Phenothiazine	
				per cent added	per cent recovered
9·04	1·41	1·17	1·15	70·7	69·8
9·32	8·34	8·46	8·69	26·8	27·4

Table I gives results on artificial mixtures of pure phenothiazine containing varying proportions of postulated impurities.

Of the alternative chromatographic methods, the Gunew procedure is extremely tedious to operate and in our opinion unsuitable for routine application, whilst in that of Brierley and Langbridge the phenothiazine passes through the column unabsorbed and is not separated from carbazole.

A comparison of the results obtained by the proposed method with those by the Kjeldahl nitrogen determination is given in Table II.

TABLE II

COMPARISON OF RESULTS BY PARTITION COLUMN CHROMATOGRAPHY WITH THOSE OBTAINED BY NITROGEN DETERMINATION

Sample	Nitrogen calc. to mol. wt. 199	Partition Chromatography
Phenothiazine pure		
1	100·0	100·0
2	99·3	98·8
Phenothiazine technical		
3	98·2	95·7
4	98·5	97·2
5	98·4	92·8
6	98·4	95·3
7	98·0	91·1 91·1
8	98·5	95·9
9	98·5	97·7
10	98·6	92·7
Phenothiazine dispersible powder		
11	93·0	92·5
12	93·8	89·9
13	93·9	87·0
14	98·0	85·5 86·0

The results on phenothiazine-dispersible powders from eight manufacturers fell within the range 81·7–92·5 per cent.

The Kjeldahl method gives results from 1–12 per cent higher than those obtained by the proposed method.

REFERENCES

Brierley, A. and Langbridge, D. M. (1961). *Analyst*, **86**, 709–713.
Gunew, D. (1960). *Ibid.*, **85**, 360–364.

The paper was presented by MR. HOLBROOK.