TABLE I.—ANALYSIS OF PHENMETRAZINE AND PHENDIMETRAZINE AND THEIR PHARMACEUTICAL DOSAGE FORMS

Product	Code No.	Assay, %	S. D.[a]
Pure Drugs			
Phenmetrazine hydrochloride		100.0[b]	0.29
Phendimetrazine bitartrate		99.5[b]	0.20
Phenmetrazine hydrochloride		100.1[c]	0.36[c]
Phendimetrazine bitartrate		99.7[c]	0.10[c]
Tablets			
Phenmetrazine hydrochloride	A	100.6	0.02
	B	102.8	0.57
	C	100.2	0.45
	D	100.5	0.79
Phendimetrazine bitartrate	E	95.9	1.09
	F	96.7	0.73

[a] Based on six assays for each product. [b] By direct titration in acetic acid. [c] Four assays by the method described for tablet assay.

acid to form the acetate of the base before the solution is concentrated. Both phenmetrazine and phendimetrazine are somewhat volatile, and losses occur during evaporation of the solvent if the drugs are not first converted to their salts. Evaporation of the solution is necessary to remove water from the extract by azeotropic distillation as well as to obtain a smaller volume for titrimetric analysis.

Good recoveries of the pure drugs were realized by this procedure and satisfactory results obtained for commercial products marketed in Canada, as shown in Table I. The two lots of phendimetrazine tablets (code No. E and F) assayed 95.9 and 96.7%, respectively. Commercially available phendimetrazine bitartrate was also of similar purity and had to be recrystallized several times to obtain an analytical specimen which could be used as reference standard (99.5% purity). The results show that the pure drug is recovered quantitatively by the method described for the assay of tablets. The low values obtained with the commercial samples are therefore probably due to the use of impure bulk drug.

REFERENCES

(1) Sandri, G. C., *Boll. Chim. Farm.*, **100**, 162(1961).
(2) Fulton, C. C., *J. Assoc. Offic. Agr. Chemists*, **43**, 262 (1960).
(3) Thies, H., and Ozbilici, Z., *Arch. Pharm.*, **295**, 194 (1962).
(4) Floderer, I., Kekesy, T., and Toth, Z., *Acta Pharm. Hung.*, **29**, 161(1959).
(5) Smith, E., Worrell, L. F., and Sinsheimer, J. E., *Anal. Chem.*, **35**, 58(1963).
(6) Clarke, F. H., *J. Org. Chem.*, **27**, 3251(1962).
(7) Drefahl, G., Hartmann, M., and Skurk, A., *Ber.*, **96**, 1011(1963).

Quantitative Reaction for Pentylenetetrazole

By LEWIS J. THROOP*

The published procedures for pentylenetetetrazole include either a gravimetric determination or a filtration step. The method described allows direct titration with hydrochloric acid following precipitation of a silver–pentylenetetrazole phosphotungstate salt. The precision of the method is established, and the composition of the phosphotungstate salt is characterized.

THE PROCEDURES described in the literature for the determination of pentylenetetrazole include complexation with mercuric chloride (1, 2), cuprous chloride (3), and cadmium chloride (4). The official compendia, the "United States Pharmacopeia" (5) and the "National Formulary" (6), use an extraction procedure for the analysis of pentylenetetrazole injection. All of these procedures involve a gravimetric determination or a filtration step.

In looking for a more suitable method of analysis, it was found that silver ion forms a complex with pentylenetetrazole; however, it was not sufficiently insoluble to be used as a basis for analysis. The phosphotungstate salt of the silver complex, however, has a low solubility in water. This reaction was made the basis of an analytical procedure for pentylenetetrazole tablets. The stoichiometry of the reaction requires 4 moles of pentylenetetrazole for each 3 moles of silver ion in the formation of the phosphotungstate precipitate. This ratio was found to be constant in the presence of an excess of either pentylenetetrazole or silver ion.

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EXPERIMENTAL

Composition of the Precipitate.—A quantity of the complex phosphotungstate salt was collected and dried *in vacuo* at room temperature. The

TABLE I.—MOLE RATIOS OF TWO SALT COMPONENTS

Component	Phosphotungstate		Phosphomolybdate	
	% Present	Mole Ratio[b]	% Present	Mole Ratio[b]
Ag	8.75	3.00	11.7	3.00
Pentylenetetrazole	14.8	4.03	20.3	4.11
H_2O	2.0	4.11	2.66	4.11
$PW_{12}O_{40}$ (calcd.)[a]	74.45	0.96		
$PMo_{12}O_{40}$ (calcd.)[a]			65.34	0.99

[a] Obtained by difference. [b] Assuming Ag = 3.00.

TABLE II.—ANALYTICAL PRECISION

Anal. No.	Composite No. 1	Composite No. 2
1	0.993	0.987
2	0.985	0.990
3	0.989	0.995
4	0.987	0.993
5	0.994	0.995
6	0.990	0.989
7	0.991	0.985
8	0.980	0.995
9	0.987	0.994
10	0.992	0.988
Av.	0.989	0.991
S. D.	0.0043	0.0036

silver content was determined by refluxing the precipitate with an excess of 10% hydrochloric acid and weighing the precipitated silver chloride. The percentage of pentylenetetrazole was obtained by determining the Kjeldahl nitrogen content. The water content was obtained by Karl Fischer titration, and the $PW_{12}O_{40}$ content was obtained by difference. The phosphomolybdate salt also was prepared and analyzed by the above procedures. Table I shows the mole ratios of the components of the two salts. The calculations are based on the silver content analysis, since it was felt that this was the most accurate of the analytical procedures used. The mole ratios of the components were found to fit the following formulas: Ag_3(pentylenetetrazole)$_4$ $PW_{12}O_{40}\cdot 4H_2O$ and Ag_3(pentylenetetrazole)$_4PMo_{12}O_{40}\cdot 4H_2O$.

Solubility of the Precipitate.—Two-hundred milliliters of boiled distilled water was equilibrated with an excess of pentylenetetrazole silver phosphotungstate for 3 days in the dark at room temperature. This solution was analyzed by a porous-cup technique on a Bausch & Lomb dual grating two meter spectrograph using a SpecPower source unit No. 110-2 and National Carbon L-3927 electrodes. The silver line at 3280.68 Å. was used as the analytical line and was compared with standard silver nitrate solutions. The silver concentration in solution was found to be $2.5 \pm 0.5 \times 10^{-5}$ moles/L. With three silver atoms per mole of precipitate, the solubility of the complex would be $8.3 \pm 0.6 \times 10^{-6}$ moles/L. at room temperature.

Equipment and Reagents.—A Beckman model 76 pH meter, equipped with calomel and silver electrodes, was used in conjunction with a potassium nitrate–agar salt bridge for the potentiometric titrations. The phosphotungstic acid was reagent grade (Merck and Co.).

Recommended Procedure.—Grind 20 tablets and weigh a quantity of powder equivalent to 1 Gm. of pentylenetetrazole. Transfer the powder to a 100-ml. volumetric flask, add about 50 ml. of distilled water, and shake until the pentylenetetrazole is dissolved. Dilute the solution to volume with distilled water, mix, and allow to stand until most of the insoluble tablet excipients settle.

Add 1.5 Gm. of phosphotungstic acid, 10 ml. of distilled water, 10 ml. of 0.1 *N* sulfuric acid, and exactly 20 ml. of 0.100 *N* standard silver nitrate solution to a 125-ml. conical flask in the order mentioned. Add a 10-ml. aliquot of the sample preparation to the flask and heat to boiling until the curdy precipitate which forms changes to a fine granular form (about 2 min.). Cool the flask in running water and transfer the contents to a 150-ml. beaker with distilled water. Titrate the solution potentiometrically with standardized 0.100 *N* hydrochloric acid.

Perform a blank titration to correct for any halide ion in the sample. Each milliliter of silver nitrate solution consumed is equivalent to 18.40 mg. of pentylenetetrazole.

This procedure is capable of the same degree of precision as that of other argentimetric analyses.

Table II lists the values and precision obtained by replicate analyses of two sample composites.

DISCUSSION

The following compounds formed insoluble phosphotungstates under the conditions used for analysis but caused no interference: aniline, 4-amino-1,3,4-triazole, 3-amino-1,2,4-triazole, triethylamine, triethanolamine, pyridine, and strychnine. Two compounds, 2,5-dimethylpyrrole and *N*-ethyl-2,5-dimethylpyrrole, did cause interference due to their reducing action on the silver ion. The absence of interference from the above compounds may indicate that the reaction involves the precipitation of a complex triple-charged cation containing three silver ions coordinated with four pentylenetetrazole molecules, rather than precipitation of the double salt. The solubility of the complex, which is within an order of magnitude of that of silver chloride, would also favor the silver complex type of salt.

REFERENCES

(1) Berman, S. M., *J. Assoc. Offic. Agr. Chemists*, **25**, 790 (1942).
(2) Horsley, T. E. V., *Analyst*, **71**, 308(1946).
(3) Sharp, L. K., *J. Pharm. Pharmacol.*, **4**, 52(1952).
(4) Paulsen, A., *Medd. Norsk Farm. Selskap*, **18**, 139 (1956); through *Chem. Abstr.*, **51**, 18470c(1957).
(5) "United States Pharmacopeia," 15th rev., Mack Publishing Co., Easton, Pa., 1955, p. 517.
(6) "National Formulary," 11th ed., J. B. Lippincott Co., Philadelphia, Pa., 1960, p. 267.