THE VOLUMETRIC DETERMINATION OF THIOURACIL AND CERTAIN HOMOLOGUES

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Although several volumetric methods for the determination of thiouracil and its methyl and propyl homologues have been published, none of these is entirely satisfactory. The best-known method is probably that adopted by the British and United States pharmacopœias, which is based on the addition of excess of silver nitrate followed by titration of the liberated nitric acid with standard alkali. The drawbacks to this method are (*a*) the end-point of the titration is not sharp, and (*b*) there is a negative bias of about 1 per cent. The latter was compensated in the B.P. 1948 by a modified equivalence factor, but the most recent direction (B.P. Addendum 1951) adopts the theoretical equivalence factor for methyl and propylthiouracil with consequent lowering of the minimum standard by 1 per cent. In an attempt to provide a more accurate process for propylthiouracil, Smith[1] investigated several volumetric methods, including the B.P. method, and concluded that direct titration of an ethanolic solution with standard alkali was the most effective, but even this did not give a sharp end-point. Other alternatives which have been studied include a spectrographic method (Smith[2]) and a colorimetric method (McAllister and Howells[3]) neither of which appear to offer advantages over the method of the Pharmacopœia. In reviewing this problem in these laboratories, it seemed that the reaction of thiouracils with solutions of mercuric salts might be worthy of investigation, since the marked insolubility of the mercury derivatives of the thiols is well known. As a result, it has been found possible to obtain satisfactory results by titrating aqueous solutions of the thiouracils with 0·05M mercuric acetate solution, using diphenylcarbazone as internal indicator.

Experimental

(*a*) *Materials*

These were purified as follows:

Thiouracil. B.P. material was first recrystallised from 50 per cent. aqueous pyridine, and then from ethanol, and dried at 100° C. Micro analysis, C, 37·8; H, 3·3; N, 21·8 per cent., $C_4H_4ON_2S$ requires C, 37·47; H, 3·15; N, 21·86 per cent.

Methylthiouracil. B.P. material was first recrystallised from 50 per cent. aqueous pyridine, and then from water, and dried at 100° C. Micro analysis, C, 42·5; H, 3·9; N, 19·8 per cent.; $C_5H_6ON_2S$ requires C, 42·24 H, 4·25; N, 19·71 per cent.

Propylthiouracil. B.P. material was first recrystallised from 50 per cent. aqueous pyridine, and then from ethanol, and dried at 100° C.

M.pt. 219° C. Micro-analysis C, 49·3; H, 5·8; N, 16·3 per cent.: $C_7H_{10}ON_2S$ requires C, 49·41; H, 5·92; N, 16·46 per cent.

Further recrystallisations did not increase the purity, and it was concluded that these materials were sufficiently pure to be used as standards for the purpose of the investigation.

(*b*) *Reagents*

12 g. of red mercuric oxide was boiled with 10 g. of glacial acetic acid in 200 ml. of water for 10 minutes. The solution was cooled and filtered through a double thickness of No. 1 Whatman filter paper, and made up to 1000 ml. Alternatively, 16 g. of reagent grade mercuric acetate were dissolved in a mixture of 600 ml. of water and 5 ml. of glacial acetic acid. The solution was filtered and made up to 1000 ml. To standardise the solution, 25 ml. was measured accurately into a 500 ml. flask, and 200 ml. of water, 50 ml. of dilute nitric acid and 5 ml. of ferric alum solution added. The solution was titrated with 0·1N ammonium thiocyanate until the first red tinge was observed. 1 ml. of 0·1N $NH_4CNS \equiv$ 1 ml. of 0·05M $(CH_3COO)_2Hg$.

The ammonium thiocyanate solution was standardised by adding a known excess of standard silver nitrate solution to a known weight of sodium chloride and titrating the excess of silver nitrate with the ammonium thiocyanate, as described in Cumming and Kay.[4] The solution is stable for several weeks.

Diphenylcarbazone indicator. A 0·5 per cent. w/v. solution of diphenylcarbazone in ethanol. This solution should be freshly prepared daily.

(Note—One sample of diphenylcarbazone gave a deep red-brown solution in ethanol and therefore was recrystallised from benzene before use. Although diphenylcarbazone is also soluble in acetone, the latter is unsuitable for titrations involving mercuric acetate since it reacts with mercury salts and hence causes fading of the end-point.)

All other reagents used were those of the British Pharmacopœia, 1948.

(*c*) *General Method of Assay*

0·3 to 0·4 g. of thiouracil or methylthiouracil, or 0·35 to 0·45 g. of propylthiouracil was dissolved in 50 ml. of dilute sodium hydroxide solution (approximately 0·4 per cent. w/v.) and 200 ml. of water, warming to complete the solution and then cooling. 10 g. of sodium acetate was added, the solution was made acid to litmus with acetic acid, 1 ml. of diphenylcarbazone indicator was added, and the mixture titrated with 0·05M mercuric acetate solution to the appearance of the first rose-violet colour which persisted for 2 to 3 minutes, the mixture being well shaken near the end-point. The addition of sodium acetate is not essential, but was found to increase the sharpness of the end-point. 1 ml. of 0·05M mercuric acetate is equivalent to 0·01282 g. of thiouracil, 0·01422 g. of methylthiouracil, or 0·01702 g. of propylthiouracil.

(*d*) *Effect of Common Tablet Excipients*

The general method of assay was carried out on suitable amounts of the different thiouracils, to each of which was added approximately 15 per

cent. w/w. of the excipient under examination, the following tablet excipients being investigated in turn:—starch, sucrose, lactose, acacia, kaolin, calcium carbonate, stearic acid and magnesium stearate. The only modification adopted was to boil the sodium hydroxide-water-thiouracil mixture for at least 2 minutes to ensure that the excipient, and in particular the starch, was fully dispersed, and all the thiouracil was in solution. Of these excipients, only lactose was found to interfere, producing a yellow colour on boiling with the sodium hydroxide solution, which subsequently interfered with the end-point. This interference was overcome by boiling the tablet mixture with 200 ml. of water for 2 to 3 minutes to dissolve the thiouracil compound, and then cooling to 70° C. before adding the 50 ml. of 0·4 per cent. w/v. sodium hydroxide solution. The mixture was allowed to stand for 5 minutes with occasional shaking, 10 g. of sodium acetate was added and the solution made acid to litmus with acetic acid. After further cooling to room temperature the solution was titrated in the usual manner.

(N.B. If stearic acid is not present together with the lactose the addition of the sodium hydroxide solution may be omitted.)

(*e*) *Statistical Investigation*

A statistical comparison was made of the accuracy and precision of the general method outlined above with that of the Addendum 1951 to the British Pharmacopœia, 1948. The statistical plan of comparison was for 2 operators to carry out daily 2 determinations by each method on the pure material alone and also (to simulate a simple tablet mixture) in the presence of starch. Single determinations were carried out alternately by each method, the method of first choice being selected at random. The determinations were repeated for a further 3 days, making a total of 16 determinations by each method for each operator. Attempts were made to extend the nature of the tablet mixture by adding other common excipients, but as most of these were found to interfere with the B.P. method, for the purposes of the statistical investigation the determinations were carried out in the presence of starch only, adding approximately 30 per cent. of the weight of thiouracil taken for the determination.

(*f*) *Results*

The results, expressed as percentages for thiouracil, methylthiouracil and propylthiouracil, are shown in Tables I, II and III respectively. In the proposed method, there is no appreciable operator bias, and consequently the results of the 2 operators have been combined to calculate the standard deviation and mean of the individual results. In the B.P. method, there is some slight indication of operator bias, but this is included in the mean and standard deviation to give an overall comparison of the two methods. Each standard deviation is calculated on the basis of 15 degrees of freedom, and the ± figure after each mean represents the standard deviation of the mean.

Starch does not interfere with either method, and combining the 2 series of results it will be seen that the bias of the proposed method is −0·15 per

cent., and of the B.P. method −1·05 per cent. The proposed method is therefore more accurate (i.e., it gives results nearer the theoretical 100 per cent.), and also more precise as evidenced by the lower standard deviation, these differences being statisticaly significant.

The B.P. method in this case appears to be more variable in the presence of starch, but combining the 2 series of results, the bias of the proposed

TABLE I

THIOURACIL

Day	Thiouracil alone				Thiouracil in presence of Starch			
	Proposed method		B.P. method		Proposed method		B.P. method	
	Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2
1	99·91 100·04	100·02 100·04	98·95 99·04	99·24 99·31	100·04 99·79	99·83 99·84	99·16 98·83	98·93 99·00
2	99·77 99·64	100·23 99·68	99·10 98·42	99·19 98·74	99·68 99·86	99·70 100·00	99·36 98·81	98·60 98·91
3	99·82 99·65	99·93 99·84	98·93 99·10	98·95 99·04	99·65 99·63	100·16 99·77	98·93 98·58	98·88 98·88
4	99·77 100·05	99·91 99·63	98·91 99·10	98·72 98·63	99·84 99·68	99·88 99·86	98·45 99·13	99·22 99·31
Mean	99·87 ± 0·044		98·96 ± 0·059		99·83 ± 0·037		98·94 ± 0·065	
Standard Deviation	0·176		0·236		0·147		0·258	

method is +0·20 per cent. and of the B.P. method −1·15 per cent. The proposed method is therefore more accurate and also more precise, again with statistical significance.

Starch does not appear to affect the results and combining the two series of results the bias of the proposed method is −0·11 per cent. and of the B.P. method −1·61 per cent. The proposed method is therefore more accurate and also more precise.

TABLE II

METHYLTHIOURACIL

Day	Methylthiouracil alone				Methylthiouracil in presence of Starch			
	Proposed method		B.P. method		Proposed method		B.P. method	
	Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2
1	100·39 100·18	100·35 100·09	98·83 98·42	98·68 98·79	100·05 100·25	100·09 100·18	99·15 98·91	98·86 99·19
2	100·05 100·35	99·99 100·09	98·47 98·40	98·95 99·02	100·21 100·21	100·51 100·44	99·04 98·13	98·06 98·63
3	100·32 100·16	100·14 100·44	98·93 98·88	98·95 99·17	100·00 100·16	100·09 100·37	99·00 99·17	99·13 98·91
4	100·32 100·12	99·93 100·12	98·65 98·51	98·81 98·95	100·00 100·21	100·30 100·23	99·19 98·97	99·00 99·13
Mean	100·19 ± 0·038		98·78 ± 0·058		100·21 ± 0·037		98·91 ± 0·087	
Standard Deviation	0·152		0·231		0·147		0·349	

TABLE III

PROPYLTHIOURACIL

Day	Propylthiouracil alone				Propylthiouracil in presence of Starch			
	Proposed method		B.P. method		Proposed method		B.P. method	
	Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2
1	99·91 100·02	100·09 99·86	98·93 98·65	97·86 98·24	99·74 99·82	100·09 100·16	98·74 98·60	98·15 97·68
2	99·70 99·97	99·97 99·97	98·60 98·68	97·90 98·00	99·65 99·79	100·10 100·00	98·86 98·77	98·74 98·77
3	99·84 99·95	99·74 99·88	98·65 98·45	98·33 98·37	99·72 99·77	99·82 99·61	98·86 98·74	98·25 98·45
4	100·02 99·79	100·02 100·00	98·42 98·15	97·77 97·79	99·84 99·88	99·65 100·07	98·70 98·51	97·95 97·79
Mean	99·92 ± 0·028		98·30 ± 0·090		99·86 ± 0·044		98·47 ± 0·098	
Standard Deviation	0·110		0·359		0·176		0·390	

With regard to tablet excipients other than starch, Table IV gives the results obtained on adding to the thiouracil compound an equal weight of a combined tablet excipient mixture consisting of equal parts of sucrose, acacia, kaolin, calcium carbonate, stearic acid and magnesium stearate.

TABLE IV

Thiouracil recovered	Methylthiouracil recovered	Propylthiouracil recovered
Per cent.	Per cent.	Per cent.
100·01	100·18	99·82
99·88	99·96	100·02
100·00	100·00	99·96
100·05	100·11	99·91

In the case of the B.P. method, it is obvious that any substance that will react with nitric acid or sodium hydroxide will interfere, and this was confirmed with such excipients as calcium carbonate and stearic acid. Sucrose and lactose were also found to interfere due to reduction of the silver nitrate obscuring the end-point.

DISCUSSION

The proposed method is free from the faults inherent in the B.P. method. The end-point is easy to detect, i.e., the sharp appearance of a rose-violet colour superimposed on the white suspended precipitate present almost throughout the titration; the colour change occurs with the addition of 0·05 ml. of titrant. With thiouracil the end-point tends to fade on standing for a time and hence the direction to titrate "to the appearance of the first rose-violet colour which persists for 2 to 3 minutes." With propylthiouracil a pale blue colouration is produced during the *initial* stages of the titration, but on allowing to stand for a few minutes with occasional shaking this disappears, and in any case cannot be mistaken for the rose-violet colour of the normal end-point.

When applied to thiouracil, methylthiouracil and propylthiouracil, the method gives results which are very close to the true value, the bias calculated from a total of 32 determinations being at its greatest at +0·2 per cent. for methylthiouracil and at its lowest at −0·1 per cent. for propylthiouracil at the 100 per cent. level, as opposed to the much larger values of from −1·05 per cent. to −1·6 per cent. for the B.P. method. In addition, the proposed method is more precise than the B.P. method, as shown by the standard deviation of individual results.

The method is applicable to tablet analysis, for, of the common tablet excipients tested, only lactose was found to interfere and this can be overcome by a slight modification of the general procedure. This is a distinct advantage over the B.P. method where several common excipients were found to vitiate the results.

The choice of mercuric salt was limited by two requirements, both linked to the use of diphenylcarbazone; (*a*) it must be highly ionised, (*b*) it must not require a high concentration of mineral acid to ensure stability, since mineral acid prevents colour development. For this reason, mercuric acetate seemed the obvious choice.

The chemical reaction involved is presumably:

$$2R{\cdot}SH + (CH_3{\cdot}COO)_2Hg = (R{\cdot}S)_2Hg + 2CH_3COOH \quad \ldots\ (1)$$

whence 1 litre of 0·05M mercuric acetate ≡ Mol. wt. of thiouracil/10. At an early stage of the experimental work attempts were made to devise a method based on addition of a known excess of mercuric acetate followed by back titration with standard thiocyanate. It was found that the results varied according to the standing time of the reaction mixture, but, in general, they were double the values anticipated from the above equation. It seems probable, therefore, that in the presence of an excess of mercuric acetate the reaction is—

$$R{\cdot}SH + (CH_3COO)_2Hg = \begin{matrix} RS \\ CH_3COO \end{matrix}\!\!>\!Hg + CH_3COOH \quad \ldots\ldots\ (2)$$

In view of the considerable variation in results, this investigation was not pursued further. The possibility of interaction between the precipitate obtained in equation (1) with a further quantity of mercuric acetate does not appear to interfere in any way with the use of diphenyl carbazone, as indicator.

Summary

1. A method is described for the determination of thiouracil and its homologues by titration with mercuric acetate solution.

2. The method is more accurate and more precise than that at present adopted by the British Pharmacopœia (1951 Addendum), as shown by statistical analysis.

3. The method is applicable in the presence of common tablet excipients.

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