THE UNRELIABILITY OF THE OFFICIAL ASSAY FOR TABLETS OF GLYCERYL TRINITRATE

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The monograph of the current Pharmacopœia on Tablets of Glyceryl Trinitrate prescribes limits of 81–121 per cent. for the declared glyceryl trinitrate content, that a tablet containing 1/130 grain (approximately 0·5 mg.) shall be dispensed or supplied when no strength is specified, and an assay reading as follows:—"Weigh and powder 20 tablets. Mix an accurately weighed quantity of the powder, equivalent to about 1 mg. of glyceryl trinitrate, with 5 ml. of glacial acetic acid, shake continuously for one hour, and filter. Mix 1 ml. of the filtrate with 2 ml. of phenoldisulphonic acid, stir well, and allow to stand for 15 minutes. Add about 8 ml. of water, make alkaline with strong solution of ammonia, cool to about 20° C., dilute to 20 ml. with water, and filter if necessary. Compare, under similar conditions in a suitable colorimeter, the colour of this solution with the colours of solutions containing known quantities of potassium nitrate which have been treated in an exactly similar manner. Each gram of potassium nitrate is equivalent to 0·7487 g. of $C_3H_5O_9N_3$. Calculate the weight of glyceryl trinitrate in each tablet of average weight." The following directions are given in the B.P. for the preparation of phenoldisulphonic acid—"Heat 3 g. of phenol with 20 ml. of sulphuric acid on a water-bath for 6 hours; transfer the resulting liquid to a stoppered vessel."

The above method is basically that published by Meek[1] in 1935, which was a development of an original suggestion made by Scoville[2] in 1911. The main differences are the use of potassium nitrate as standard in place of the silver salt and glyceryl trinitrate itself, and the comparison of the colours of the test and standards in a "suitable colorimeter" in preference to Meek's use of Lovibond yellow units. It has been our experience that the results given by the official assay do not always agree with those given by the U.S.P. assay so we decided to subject both methods to a detailed study. An additional object was to discover if it was possible to use a spectrophotometric standard in the official assay. There appeared to be two main obstacles to this objective, firstly a possible variation in the colour produced by different batches of phenoldisulphonic acid reagent and secondly, the importance attached by Meek to the temperature at which the colour was measured; he reported that values obtained by him at 25° C. were 10 per cent. higher than they were at 15° C. We found that if we made the reagent exactly as the B.P. specifies, i.e., 20 ml. at a time, there was no significant difference in the results obtained when experiments were repeated using different batches of reagent, and secondly that there was no variation between the values obtained at different temperatures. We were unable to reproduce Meek's 10 per cent. difference even over the range of 10–30° C.

Two minor variations were made in the official assay; we used the equivalent of four tablets with 10 ml. of glacial acetic acid which enabled us conveniently to perform a triplicate assay from each extraction and we made up the final volume of the solution immediately before the determination of the absorption to 25 ml. instead of 20 ml. When the intensity of the colour was plotted against the logarithm of the concentration the relation appeared to fit the Beer-Lambert equation.

THE SPECTROPHOTOMETRIC CHARACTERISTICS OF THE NITRATE STANDARD

The direction in the B.P. assay that the standard and test are to be treated in an exactly similar manner is very important; we stress this point as we have seen it stated in text-books[3,4] that the standard may be prepared by taking silver nitrate solution to dryness with the subsequent addition of phenoldisulphonic acid reagent. It is imperative that the colour of the standard be developed in the presence of glacial acetic acid otherwise it will be far too intense. Our examination of the standard comprised a determination of the values obtained by the use of potassium and silver nitrates in both the presence and absence of glacial acetic acid and also by the employment of a standard acetic acid solution of glyceryl trinitrate itself. In addition to the magnitude of the absorption at $\lambda_{max.}$ we were interested in the reproducibility and the spectrophotometric characteristics of the curves obtained from the different standards used; for comparison purposes the curve having an E value of 1·000 at $\lambda_{max.}$ was calculated for each standard.

In the official assay the equivalent of two tablets corresponding to approximately 1 mg. of glyceryl trinitrate, is extracted with 5 ml. of glacial acetic acid; one ml. of the filtrate is used for colour development, hence the standard required is the colour given by 0·2 mg. of glyceryl trinitrate in 1 ml. of glacial acetic acid. The solution of glyceryl trinitrate used for standard determinations was assayed by both the official method of the B.P.C. for solution of glyceryl trinitrate and the U.S.P. assay for tablets after suitable adjustment; the solution used by us had a strength of 0·95 per cent., both methods of assay agreeing with this figure. All figures given in this paper concerning this solution however, are recalculated to those expected from an exactly 1·0 per cent. solution. When potassium nitrate is used as standard it can be calculated from theoretical considerations that a solution containing 0·2672 mg. of potassium nitrate per ml. is required; for silver nitrate the concentration should be 0·4500 mg. per ml. The values obtained for these standard solutions are given in Table I, and it will be seen that standards prepared by the evaporation of potassium or silver nitrates to dryness with the subsequent addition of glacial acetic acid before colour development, agreed very well with that obtained from glyceryl trinitrate itself. The value for glyceryl trinitrate was 0·646, for silver nitrate 0·649 and for potassium nitrate a little lower at 0·638. If glacial acetic acid was omitted during colour development the values obtained were significantly higher at 0·727 for potassium nitrate and 0·738 for silver nitrate. The characteristics of the absorption curves originating from the use of these different standards are given in Table II; it is evident

TABLE I

STANDARD NITRATE E VALUES AT $\lambda_{max.}$ (405 mμ) OBTAINED FOR GLYCERYL, POTASSIUM AND SODIUM NITRATES

Glyceryl trinitrate						
	0·651	0·623	0·642	0·637	0·645	0·645
	0·647	0·657	0·660	0·647	0·649	0·645
	0·631	0·642	0·645	0·639	0·669	0·651
	Mean value 0·646 ± 0·005 (P = 0·95)					
Potassium nitrate						
(*a*) Glacial acetic acid present						
	0·642	0·639	0·642	0·639	0·637	0·640
	0·650	0·633	0·631	0·648	0·633	0·623
	Mean value 0·638 ± 0·005 (P = 0·95)					
(*b*) Glacial acetic acid absent						
	0·730	0·726	0·737	0·728	0·735	0·707
	0·737	0·732	0·722	0·735	0·714	0·726
	Mean value 0·727 ± 0·006 (P = 0·95)					
Silver nitrate						
(*a*) Glacial acetic acid present						
	0·638	0·639	0·658	0·656	0·656	0·644
	0·650	0·638	0·654	0·640	0·658	0·654
	Mean value 0·649 ± 0·005 (P = 0·95)					
(*b*) Glacial acetic acid absent						
	0·758	0·745	0·747	0·742	0·738	0·756
	0·728	0·717	0·738	0·725	0·733	0·747
	Mean value 0·738 ± 0·008 (P = 0·95)					

that the characteristics are practically identical for all three sources of nitrate whether acetic acid is present or not; the effect of acetic acid in the case of both inorganic nitrates is to depress the value of the absorption obtained without alteration of its characteristics. The wavelength of maximum absorption is well defined at 405 mμ in each case. It is suggested that, using the data in Table I, we can fix a standard for the official assay; if the equivalent of two tablets containing in each 0·5 mg., i.e., a total glyceryl trinitrate content of 1 mg. is subjected to the B.P. assay and the final dilution is to 25 ml. instead of 20 ml. then an E value of 0·646 at 405 mμ should be obtained, if the glyceryl trinitrate content is exactly 0·5 mg. per tablet. A close examination of the data in Table I reveals a

TABLE II

CHARACTERISTICS OF THE VARIOUS ABSORPTION CURVES OBTAINED

Wavelength, mμ	Glyceryl trinitrate	Potassium nitrate	Potassium nitrate + acetic acid	Silver nitrate	Silver nitrate + acetic acid
355	0·715	0·671	0·650	0·661	0·675
360	0·529	0·515	0·506	0·510	0·512
365	0·544	0·532	0·530	0·531	0·532
370	0·612	0·603	0·602	0·601	0·600
375	0·693	0·679	0·682	0·677	0·679
380	0·773	0·763	0·768	0·761	0·764
385	0·850	0·840	0·848	0·840	0·841
390	0·916	0·908	0·912	0·902	0·909
395	0·962	0·960	0·961	0·955	0·960
400	0·991	0·993	0·989	0·990	0·991
405	1·000	1·000	1·000	1·000	1·000
410	0·982	0·988	0·983	0·984	0·987
415	0·945	0·947	0·947	0·949	0·954
420	0·884	0·890	0·886	0·892	0·895
425	0·813	0·818	0·813	0·818	0·824
430	0·725	0·733	0·726	0·730	0·741
435	0·640	0·647	0·641	0·643	0·655
440	0·550	0·550	0·546	0·549	0·562
445	0·463	0·460	0·459	0·461	0·476
450	0·384	0·379	0·399	0·377	0·392
455	0·313	0·307	0·306	0·306	0·319
460	0·251	0·243	0·245	0·241	0·258
465	0·201	0·192	0·195	0·192	0·207
470	0·158	0·148	0·152	0·148	0·161
475	0·126	0·114	0·120	0·114	0·131

certain variation between replicates and although this variation is small, it is as well to realise that it exists; as normal routine we took the mean of triplicate determinations.

The U.S.P. Assay

It is very useful in an investigation of this type to have available an entirely independent method of assay and we have used the official assay of the U.S.P. for checking our results. In this method 100 tablets are used for each test; after ethereal extraction of the glyceryl trinitrate and subsequent hydrolysis the nitrate is reduced to ammonia which is estimated by the usual distillation into standard acid—a blank on the reagents being subtracted. We have carried out tests using this assay and obtained practically 100 per cent. recoveries.

The B.P. Assay

It was the study of the curves obtained on various samples of trinitrin tablets and the comparison of their characteristics with those of the curves obtained from the different standards that led us to examine the assay in detail. We observed that the characteristics of the curves obtained from the great majority of tablets were very different from those exhibited by the standards and it was an explanation of these differences that we sought. It is always useful in spectrophotometry to keep a check on the shape of a curve by calculating two ratios one on either side of the maximum absorption; in this case we made much use of the ratios of the absorptions at 375 mμ and 430 mμ to that at the maximum at 405 mμ. Upon investigation we found that the absorption curve obtained on a typical sample of tablets could be influenced by a number of factors. One of the more obvious ones was the effect of incomplete filtration; we found that the fat derived from the cocoa contained in the base was the main cause of cloudy solutions, the difficulty being eliminated when experiments were carried out on tablets made with a fat free base. In the official assay, unless great care was taken with the filtration, solutions were obtained which although clear, were not as bright as the standard solutions. The absorption due to the suspended fat was uniform in character (i.e., its magnitude was constant at different wavelengths) as distinct from the more usual general absorption encountered in spectrophotometry. This fact was illustrated by further filtration of such cloudy solutions, resulting in a constant decrease in the absorptions at three wavelengths 375, 405, and 430 mμ. It was a common cause of gross disagreements between replicates. We found that the use of a Whatman No. 42 filter paper, the rejection of the first 5 ml. of the initial filtrate, followed by refiltration of the remainder through the same filter into a clean beaker, gave solutions which were both bright and clear. It was however apparent that even when this source of error was eliminated, the characteristics of the absorption curve were still not the same as those of the standard. The next step was the investigation of any possible blank given by the reagents and also by the base in the absence of glyceryl trinitrate. The blank given by the reagents was approximately 0·018 at 405 mμ and it is

of interest that the same value was obtained for the B.P.C. base when the cocoa was omitted from it. The different values obtained for what may be termed the various "background" absorptions are given in Table III. In the B.P. assay the proportion of solid matter to acetic acid in the initial extraction, assuming the weight of an average trinitrin tablet to be 0·3 g., is 0·6 g. to 5 ml., or in our case 1·2 g. to 10 ml. of acid. It will be seen that where the B.P.C. base was used without granulation, there was little difference in background absorption over the range 0·6 to 2·4 g.; one set of figures refers to a sample of fat extracted base and was not significantly different from the remainder, showing that an efficient filtration procedure was being employed. In the case of the granulated base the doubling of the normal weight did seem to result in somewhat higher absorptions.

TABLE III

"BACKGROUND" ABSORPTION

	E value at		
	375 mμ	405 mμ	430 mμ
(*a*) Reagents	0·021	0·018	0·015
(*b*) Ungranulated B.P.C. chocolate base; 10 ml. of glacial acetic acid plus the stated weight of base			
0·6 g.	0·032	0·025	0·023
1·2 g.	0·034	0·021	0·018
1·2 g. (defatted)	0·038	0·023	0·020
2·4 g.	0·038	0·022	0·020
(*c*) Granulated B.P.C. chocolate base; 10 ml. of glacial acetic acid plus the stated weight of base			
0·6 g.	0·045	0·031	0·024
1·2 g.	0·052	0·030	0·029
1·2 g. (defatted)	0·041	0·028	0·024
2·4 g.	0·079	0·045	0·040
(*d*) B.P.C. base with cocoa omitted	0·022	0·018	0·017
(*e*) Base composed entirely of cocoa	0·049	0·032	0·027

The absorptions obtained when the base was composed entirely of cocoa were similar to the others. It is to be emphasised that even though the figures given are the mean values obtained from six replicates, they are not exact figures capable of showing fine shades of difference; the broad conclusion that we have reached is that there exists a background irrelevant absorption of the order of 0·030 at 405 mμ. As the blank on the reagents (which is unaltered for a lactose-sucrose base) is 0·018 and is of course present in the standard, the net irrelevant absorption due to the cocoa of the base is of the order of 0·012, which corresponds to an over-estimate of approximately 2 per cent. and for all practical purposes can be ignored.

Another factor we encountered was the presence of vanillin in some tablets. This is quite permissible in the official base; in the concentrations normally employed its absorption commenced at around 390 mμ and rose steeply into the ultra-violet region, thus having the effect of greatly lifting the absorption on the shortwave side of the maximum. It had however no effect upon the absorption value at the maximum itself. The net result of the presence of vanillin was that after all the normal corrections for the usual background absorptions had been made the ratio of the absorption at 375 mμ to that at 405 mμ was still very much above the normal.

To sum up we may say that the normal absorption curve is composed of at least two components and possibly four. The two main components are the absorption due to the glyceryl trinitrate colour complex, and the background general absorption due to the base and the reagents, estimated quantitatively at approximately 0·030 at 405 mμ. The other two possible components are the avoidable uniform absorption due to the effects of cocoa fat, which can be removed by efficient filtration, and lastly the frequently encountered "lift" in the absorption on the short-wave side of the maximum due to the presence of vanillin. Although the vanillin if present has no effect on the value of the absorption at 405 mμ in a spectrophotometric assay, the possibility is to be borne in mind that if an absorptiometer is used for measurement which uses a filter with a comparatively wide band width then the presence of vanillin might influence the assay.

Recovery Tests

The data obtained by us on recovery tests are summarised in Table IV. In part I of the Table the percentage recoveries at different glyceryl trinitrate levels are given. It will be remembered that if in the assay the

TABLE IV

RECOVERY TESTS

I. Effect of different bases upon the percentage recoveries at different potency levels.

(*a*) Ungranulated B.P.C. chocolate base.
In each case 1·2 g. of base plus 10 ml. of glacial acetic acid containing:—

1·0 mg. trinitrin	Recovery 86·9 per cent.
1·5 mg. ,,	,, 90·2 ,, ,,
2·0 mg. ,,	,, 88·9 ,, ,,
3·0 mg. ,,	,, 90·7 ,, ,,

(*b*) Powdered granulated B.P.C. chocolate base.
In each case 1·2 g. of base plus 10 ml. of glacial acetic acid containing:—

1·0 mg. trinitrin	Recovery 92·8 per cent.
1·5 mg. ,,	,, 91·3 ,, ,,
2·0 mg. ,,	,, 91·8 ,, ,,
3·0 mg. ,,	,, 92·2 ,, ,,

(*c*) Fat extracted powdered granulated B.P.C. chocolate base.
In each case 1·2 g. of base plus 10 ml. of glacial acetic acid containing:—

1·0 mg. trinitrin	Recovery 98·4 per cent.
1·5 mg. ,,	,, 94·8 ,, ,,
2·0 mg. ,,	,, 96·2 ,, ,,
3·0 mg. ,,	,, 94·3 ,, ,,

II. Effect of variations in the weight of base upon the percentage recovery when the potency level is kept constant.

(*a*) 1 mg. potency level.
1 mg. of trinitrin in 10 ml. of glacial acetic acid plus a weight of base as follows.

0·6 g.	Recovery 96·1 per cent.
1·2 g.	,, 92·8 ,, ,,
2·4 g.	,, 76·8 ,, ,,

(*b*) 2 mg. potency level.
2 mg. of trinitrin in 10 ml. of glacial acetic acid plus a weight of base as follows.

0·6 g.	Recovery 91·0 per cent.
1·2 g.	,, 91·8 ,, ,,
2·4 g.	,, 77·3 ,, ,,

III. Effect of the presence of cocoa upon the percentage recovery.

(*a*) Base without cocoa.
1·2 g. of base plus 10 ml. of glacial acetic acid containing:—

1 mg. trinitrin	Recovery 99·0 per cent.
2 mg. ,,	,, 98·9 ,, ,,

(*b*) Base composed entirely of cocoa.
1·2 g. of cocoa plus 10 ml. of glacial acetic acid containing:—

1 mg. trinitrin	Recovery 50·0 per cent.
2 mg. ,,	,, 50·2 ,, ,,

equivalent weight of four tablets was taken then 10 ml. of glacial acetic acid were used for extraction and this is the normal 2 mg. level for the usual 0·5 mg. tablets: the weight of base taken in this case was 1·2 g. In experiments tabulated in part I of Table IV we took in each case 1·2 g. of base but varied the amount of trinitrin included in the 10 ml. of glacial acetic acid added. It will be seen that the recoveries obtained with the ungranulated base are slightly lower than those given by the granulated base; this suggests that the degree of subdivision of the powder may affect the recovery. There was a significantly lower recovery with the normal base than with one which had been freed of its fat content; this seems to indicate that the fat plays some part in the percentage recovery obtained. The recovery for the B.P.C. granulated base used remained constant at about 92 per cent. which means that the official method underestimates the true glyceryl trinitrate content by approximately 8 per cent. Furthermore if the filtrates obtained are not bright and clear a further error is introduced which helps to compensate this underestimation. This explains why it is possible for a whole series of assays to be done in which the B.P. and U.S.P. methods are compared and a somewhat fortuitous agreement obtained—normally the U.S.P. assay should always be higher than the B.P. figure. In experiments summarised in part II of Table IV we studied the effect of the weight of base present, i.e., the size of the tablet. It appeared that if the tablets were one half normal size there was not a great difference in the percentage recovery, but if they were twice the normal size there was a significant drop in the percentage recovery. Finally in part III of Table IV the effect of the percentage of cocoa in the base is illustrated. The easiest way to do this was to prepare a base containing no cocoa and also to use cocoa itself as base. It will be obvious from the data obtained that the cocoa is a major cause of the failure to obtain good recoveries; if there was no cocoa present the recovery was almost complete, but if the base was composed solely of cocoa this fell to somewhere in the region of 50 per cent.

Application of the Method to Official Tablets

We carried out analyses of a number of tablets using the data obtained and we summarise the results below.

(*a*) Freshly made tablets using the actual base previously studied; manufactured to contain approximately 1/100 grain (0·65 mg.) of trinitrin. U.S.P. assay 0·67 mg. found: B.P. assay uncorrected *E* value 0·806, equivalent to a trinitrin content of 0·62 mg. per tablet which on correction by multiplying by 100/92 gives 0·68 mg. per tablet.

Another batch of tablets* with the same base containing approximately 1/130 grain (0·50 mg.) trinitrin. U.S.P. assay 0·49 mg.: B.P. assay 0·45 mg. uncorrected and 0·49 mg. corrected.

A third batch of tablets declared to contain 0·50 mg. with an unknown base. U.S.P. assay 0·34 mg.; B.P. assay 0·31 mg. and 0·34 mg. corrected.

* This batch of tablets analysed again 4 months later gave U.S.P. assay 0·44 mg.; B.P. assay 0·39 mg. uncorrected, and 0·42 corrected. A further correction (see later) for "bound" trinitrin gave a final figure of 0·44 mg.

The problem seemed to be solved but the following examples showed that this was not the case.

(*b*) Tablets with unknown base declared to contain 1/130 grain (0·50 mg.). U.S.P. assay 0·39 mg.; B.P. 0·29 mg. and 0·32 mg. corrected. A recovery test on this particular sample gave 93·4 per cent. of the amount added, i.e., the normal expected recovery. We investigated this sample further by attempting a soxhlet extraction with ether, followed by careful evaporation of the ether and determination of trinitrin by the B.P. assay; we expected a somewhat lower figure by this method because of the possible loss of trinitrin when evaporating the ether; the result obtained was approximately 10 per cent. below that obtained by the B.P. method and it appeared that ethereal extraction still failed to remove sufficient trinitrin to account for the U.S.P. figure. Another experiment which we tried on this particular sample was to grind the powdered tablets in a mortar with the glacial acetic acid before shaking. This failed to increase the previous figure obtained by the B.P. assay. There appeared therefore to be a portion of the trinitrin that was not removed in the B.P. assay but was recovered in the U.S.P. assay.

In none of our experiments on the B.P. method so far had we tried the addition of water followed by ether extraction which is part of the U.S.P. process. We therefore modified the B.P. assay by washing the residue on the filter paper after the acetic acid filtration with about 200 ml. of glacial acetic acid, suspended it in water, extracted with ether and then found that the "missing" trinitrin could be recovered in this way. We calculated the total trinitrin content as follows: the "free" trinitrin gave an *E* value of 0·376. This we multiplied by 100/92 to compensate for its distribution on extraction between the base and the acetic acid. We assumed that washing the residue with 200 ml. of acetic acid would remove the remaining adsorbed "free" trinitrin; the base then suspended in water was extracted with ether and after careful evaporation of the latter the residue was subjected to the B.P. assay and gave an *E* value of 0·090. From this we subtracted the reagent blank of approximately 0·020, so that our final *E* value was 0·376 × 100/92 (i.e., 409), plus 0·070 = 0·479. This corresponded to 0·37 mg. per tablet.

(*c*) Another sample assayed at 0·47 mg. per tablet by the U.S.P. method and at 0·31 mg. per tablet by the B.P. method, increased by correction to 0·36 mg. per tablet. The original *E* value was 0·400 and 0·435 corrected; the ethereal extract from aqueous suspension gave a further *E* value of 0·136. Subtraction of the reagent blank decreased this to 0·116 and this figure added to the original *E* value gave a total of 0·551, corresponding to 0·43 mg. per tablet. It is interesting that in this last example a portion of the U.S.P. extract which gave 0·47 mg. per tablet gave only 0·42 mg. per tablet when subjected to the B.P. assay, showing that there is some loss when the trinitrin in ethereal solution is freed from ether by evaporation. In Table V we have selected 13 samples at random from some 50 samples of commercial trinitrin tablets on retail sale recently analysed by us[5] and it gives a good illustration of the magnitude of error involved in the use of the B.P. assay without modification. The mean trinitrin content of the

samples in the table is according to the B.P. assay about one half what it should be whereas on correction this rises to approximately two thirds. It is of interest that the mean value for the trinitrin content of the 50 samples analysed by a modified B.P. assay was approximately 65 per cent. while a U.S.P. assay carried out on 100 tablets made up of two tablets taken from each of the 50 samples gave a trinitrin content of 67 per cent.

TABLE V

COMPARISON OF THE RESULTS OBTAINED BY THE OFFICIAL AND MODIFIED ASSAYS WHEN APPLIED TO COMMERCIAL SAMPLES OF TRINITRIN TABLETS STATED TO CONTAIN 1/130 GRAIN OF MEDICAMENT

Sample No.	Gross *E* value at 405 mμ (*a*)	Percentage of trinitrin calculated from (*a*)	Gross *E* value × 100/92	Residual *E* value less 0·020 allowance for reagent blank (*c*)	Corrected *E* value (*b*) plus (*c*)	Percentage trinitrin calculated from corrected *E*
1	0·529	82	0·575	0·009	0·583	90
2	0·241	37	0·262	0·121	0·383	59
3	0·338	52	0·367	0·007	0·374	58
4	0·341	53	0·371	0·091	0·462	72
5	0·245	38	0·266	0·126	0·392	61
6	0·291	45	0·316	0·076	0·392	61
7	0·343	53	0·373	0·067	0·440	68
8	0·242	37	0·263	0·165	0·428	65
9	0·310	48	0·337	0·053	0·390	60
10	0·370	57	0·400	0·030	0·430	67
11	0·329	57	0·358	0·038	0·396	61
12	0·341	51	0·371	0·069	0·420	65
13	0·434	67	0·472	0·071	0·533	81
		Mean value 52				Mean value 67

CONCLUSIONS

A comparison of the assay procedures of the B.P. and the U.S.P. has shown that there is always the danger with the former method of a serious underestimate owing to its failure to extract the "bound" trinitrin: whether or not the U.S.P. assay itself estimates the total trinitrin is a question we are unable to answer. There are a number of unsatisfactory aspects of the assay of the trinitrin content of the official tablet. It has been reported by Stephenson and Humphreys-Jones[6] that after storage for many years there is always some 30 per cent. of the original trinitrin content remaining in the tablets—this is most unusual as one would expect the trinitrin content to fall eventually to zero. . The fact that from a medical standpoint there is no recorded report that we can trace of trinitrin tablets in practice failing to perform the function for which they were given makes the whole subject of the chemical assay suspect.

The following conclusions are based upon the work reported in this paper but we do feel that there is need for much more work to be done before a truly satisfactory method can be established.

1. The use of glacial acetic acid to remove the trinitrin in one extraction from the powdered tablets results in an approximate recovery of 92 per cent. of the "free" trinitrin. This is only a very general statement and the figure is greatly influenced by the size of the tablet and the percentage of cocoa in the base employed.

2. Even if we assume that a suitable correction is possible for the above there is still the problem of the "bound" trinitrin, the amount of

which may vary considerably. We have little information on this subject but it may be that the age of the tablet, the formulation of the base and probably other unknown factors are involved. It may well be that the B.P. assay can be modified as regards the extraction to provide a satisfactory assay; if so the use of a spectrophotometric standard will increase its accuracy.

3. We feel that for the present the use of the U.S.P. assay or some modification thereof is far more satisfactory than the present B.P. assay which has been shown to be unreliable.

SUMMARY

1. A comparison of the assay procedures of the B.P. and U.S.P. has shown that there is a danger of low results with the former owing to its failure to extract "bound" trinitrin.

2. Use of glacial acetic acid to remove the trinitrin in one extraction from powdered tablets results in an approximate recovery of 92 per cent. of the "free" trinitrin. The figure is influenced by the tablet size and percentage of cocoa in the base.

3. The use of a suitable correction factor is affected by the unknown and very variable amount of "bound" trinitrin.

4. The U.S.P. assay or a modification is considered more satisfactory than the B.P. method.

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