all samples indicated that molecular absorbance was not interfering with the analysis of manganese in this procedure. The possibility of molecular absorption occurring at the analytical wavelength of copper seems small since the copper analytical wavelength is not far from the manganese line. No calculations are reported for the two-line method since the results would not differ from the results of the direct method.

A typical standard curve for copper (Fig. 1) reveals good linearity up to 6 p.p.m. The slope of the line gives a sensitivity of 0.107 mcg./ml., in agreement with reported (7) values, and the standard deviation of the slope indicated suitable accuracy. The results of the analysis of the tablets for copper are shown in Table II. Both the direct method (3% average *SD*) and the method of additions (5% average *SD*) show reasonable precision. Probably some error is produced by difficulties in obtaining homogeneous samples of the vitamin tablets, and this is supported by the differences in precision among the different tablets. The ability to obtain a homogeneous sample would depend upon the particular formulation of the tablet. Some tablets were extremely difficult to reduce to a fine powder while others were powdered with comparative ease. This method of sampling in the analysis of iron has been reported to give a higher variation than an ashing procedure (4). The increased error in the method of additions can be anticipated because of the larger number of pipetings and the extrapolation procedure required. Statistical comparisons of the two methods indicate that Tablet 5 showed a significant difference in the two analytical methods. Inspection of Table II shows that the significance was produced by the unusually small standard deviations associated with this tablet. Other workers (2) found a greater variance produced by the method of additions. The slightly higher results from the method of additions for the analysis of copper cannot be presently explained.

A typical standard curve for manganese (Fig. 2) was linear up to 3.5 p.p.m., and the sensitivity (0.061 mcg./ml.) agrees well with reported (7) values. The standard deviation of the slope is within acceptable limits. The results of the analysis of manganese in multiple-vitamin–mineral tablets (Table III) are more precise than the copper analysis. The average errors in the direct determination and in the method of additions (2 and 3% average *SD*, respectively), are extremely satisfactory. Unlike the copper analysis, there seems no direction in the differences in the two methods (Tablets 2 and 5 are lower by the method of additions and Tablets 1, 3, and 4 give higher results by the method of additions). The existence of the small, but significant, differences in the results of the two methods of determination might indicate a small matrix effect. Since the differences between the methods are small, the more convenient direct method can be used for all but the most crucial applications.

At the beginning of the project, the decision was made to produce the analytical solution by grinding the tablet and filtering the resulting suspension, thus avoiding ashing or digesting of the sample. This procedure obviously introduced some variation into the results; but from the results of Tables II and III, this effect cannot exceed 3% and is probably much less. By avoiding digestion or ashing, a solution is produced that can be utilized in the analysis of the vitamins soluble in 1% acid media, minimizing the number of solutions needed for the quality control of the multiple vitamins. The results (Table IV) indicate acceptable recovery for both manganese and copper and again demonstrate the smaller variability of the manganese analysis. The determination of manganese and, to a slightly lesser degree, copper by direct atomic absorption spectrophotometry is a simple and precise method of analyzing these metals in multiple-vitamin–mineral tablets.

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Determination of Chloramphenicol Palmitate in Pharmaceutical Suspensions

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Abstract □ Three simple procedures for the quantitative determination of chloramphenicol palmitate in commercially available chloramphenicol palmitate oral suspensions are presented. One method is a modification of the USP method in which the suspension residue is washed with water prior to the USP specified steps of dissolving the residue in chloroform and determining the quantity spectrophotometrically. The second method takes advantage of the presence of two chlorine atoms in the side chain of chloramphenicol which can be converted into alkali chloride to be estimated potentiometrically with a standard silver nitrate solution. The third method is based on the liberation of chloramphenicol from chloramphenicol palmitate by treatment with dilute alkali at room temperature and microbiological assay of chloramphenicol using *Escherichia coli* (ATCC 10536). The potentiometric and microbiological assay methods were equally suitable for determination of chloramphenicol palmitate in all of the different suspensions tested. The microbiological assay method was more specific for the active antibiotic, was less cumbersome, and took less time.

Keyphrases □ Chloramphenicol palmitate—three methods of analysis in pharmaceutical suspensions □ Suspensions, chloramphenicol palmitate—three methods of analysis □ Antibiotics—analysis of chloramphenicol palmitate in pharmaceutical suspensions

In chloramphenicol palmitate oral suspensions containing 0.5% vanillin as the flavoring agent, the potency of chloramphenicol palmitate, as determined by the USP spectrophotometric method (1, 2), was always high. The results varied between 116 and 133% of the labeled content. However, the USP method was suitable

for suspensions identical in other respects but containing flavoring agents such as soluble essence raspberry, essence apricot, or essence American ice cream. Vanillin is a commonly used and highly acceptable flavoring agent, and the use of a flavoring agent in the chloramphenicol palmitate oral suspension is permissible according to USP (3). It was, therefore, necessary to modify the USP method to make it useful for the assay of chloramphenicol palmitate suspensions containing vanillin.

Chloramphenicol palmitate cannot be estimated by the official method (1, 2) in combination with other drugs such as streptomycin, tetracycline, nitrofurantoin, sulfa drugs, or B vitamins. Several other methods have been suggested for the estimation of chloramphenicol palmitate in pharmaceutical formulations, including colorimetry (4, 5), reverse phase TLC (6), and column chromatography (7), but these methods are not always suitable for chloramphenicol palmitate in combination with other drugs. The polarographic method for chloramphenicol palmitate in commercial preparations cannot be used because of its solubility characteristics (8). A potentiometric method based on the chloride determination has been reported (9). Chloramphenicol contains two chlorine atoms in the side chain which can be converted into alkali chloride when refluxed with alkali and can be potentiometrically titrated with 0.1 *N* silver nitrate. This method can be extended to different suspensions of chloramphenicol palmitate in combination with the drugs mentioned previously.

Chloramphenicol palmitate has no antimicrobial activity and cannot be estimated by microbial assay. However, the drug can be hydrolyzed by alkaline hydrolysis and the liberated chloramphenicol can be determined microbiologically by measuring the zones of inhibition using *Bacillus subtilis* (10). Enzymatic hydrolysis of chloramphenicol palmitate by pancreatin (11–13) and the estimation of chloramphenicol by analyzing for total amyl nitro compounds by colorimetry has been reported (11). Although total hydrolysis by pancreatin (13) and by lipase (13, 14) has been described, the complete hydrolysis of chloramphenicol palmitate with pancreatin could not be obtained in the present experiments. Certain crystalline forms of chloramphenicol palmitate are not hydrolyzed or are only incompletely hydrolyzed (15, 16).

Amorphous chloramphenicol palmitate was used in these studies, and the purity of the substance was determined by IR spectrophotometry. Therefore, incomplete hydrolysis was not due to the nature of the chloramphenicol palmitate used. However, it might be due to the pancreatin used because enzyme activity is likely to vary from sample to sample. Because microbial methods are more sensitive than chemical assays and more specific for the active antibiotic, a microbiological method of assay of chloramphenicol palmitate in suspensions with or without other drugs or flavoring agents would be desirable. Therefore, a microbiological assay method of chloramphenicol palmitate in different formulations after alkaline hydrolysis was developed.

Table I—Results[a] of Spectrophotometric Assay of Chloramphenicol Palmitate in Chloramphenicol Palmitate Oral Suspension USP[b]

	Samples Containing Vanillin, mg./ml.		Identical Samples without Vanillin, mg./ml.	
Sample	USP Procedure	Modified USP Procedure	USP Procedure	Modified USP Procedure
1	37.1	30.7	30.4	30.4
2	40.0	32.6	31.7	31.9
3	38.6	32.8	30.4	31.2
4	37.0	30.4	31.5	31.4
5	36.8	30.7	34.6	34.6
6	39.0	31.6		
7	38.5	30.0		
8	41.5	32.5		
9	39.0	32.5		
10	38.5	31.2		

[a] Amount of chloramphenicol base contained in chloramphenicol palmitate, milligrams per milliliter. [b] One milliliter suspension contained chloramphenicol palmitate equivalent to 31.25 mg. chloramphenicol base.

EXPERIMENTAL

Determination of Chloramphenicol Palmitate by Modified USP Method—Four milliliters chloramphenicol palmitate oral suspension, equivalent to 125 mg. chloramphenicol, containing 20 mg. vanillin was quantitatively transferred to a sintered-glass crucible No. 4; 10 ml. water was added and the contents were quickly filtered by suction. The residue on the filter was washed twice with 10 ml. water by suction. The sintered crucible containing the residue was fitted into a dry 250-ml. filtering flask. The residue was dissolved in chloroform and the solution was collected in the filtering flask by suction, quantitatively transferred to a 250-ml. volumetric flask, and diluted to volume with chloroform. The remainder of the procedure was as described in the *Code of Federal Regulations* (2) commencing with: "Pipette 4 milliliters of the clear chloroform solution into a 100 milliliter volumetric flask . . ." The results of analysis of 10 samples by the USP method (1, 2) and the modified USP method are given in Table I.

Chloramphenicol palmitate oral suspensions identical in all respects to these samples but not containing vanillin were analyzed by both the USP method and the modified method (Table I). Recovery experiments were performed for the modified method to show that chloramphenicol palmitate was not dissolved by the additional washing procedure (Table II).

Determination of Chloramphenicol Palmitate in Suspensions Containing Chloramphenicol Palmitate in Combination with Other Drugs by Potentiometric Method—Ten to 20 ml. of the suspension, equivalent to about 300 mg. chloramphenicol, was quantitatively transferred to a 500-ml. round-bottom flask to which 20 g. sodium hydroxide and 80 ml. water were subsequently added. The mixture was refluxed on an open flame for 4 hr., cooled, acidified with nitric acid, quantitatively transferred to a 500-ml. volumetric flask, and diluted to volume with water. Two hundred milliliters of the solution was transferred to a 500-ml. beaker, and the alkali chloride solution was titrated with 0.1 *N* silver nitrate potentiometrically using silver–silver chloride and calomel electrodes. The total volume of 0.1 *N* silver nitrate required for 500 ml. was calculated. For a blank titration, the same volume of the sample as taken for alkaline hydrolysis was transferred to a 500-ml. beaker to which 5 ml. nitric acid and 80 ml. water were added. The mixture

Table II—Recovery of Chloramphenicol Palmitate by the Modified USP Method

Amount of Chloramphenicol Base Contained in Added Chloramphenicol Palmitate, mg.	Chloramphenicol Estimated, mg.	Recovery, %
123.5	120.2	97.3
98.56	96.8	98.2
122.0	119.0	97.5
124.0	124.0	100.0
101.0	100.0	99.0

Table III—Determination of Chloramphenicol Palmitate in Suspensions of Differing Compositions[a]

Dosage Form	Microbiological Assay after Alkaline Hydrolysis, mg./ml.	Potentiometric Titration, mg./ml.	t
Chloramphenicol palmitate (equivalent to 31.25 mg. chloramphenicol/ml.) and streptomycin sulfate (equivalent to 31.25 mg. streptomycin/ml.)	31.7 ± 0.905	32.0 ± 0.638	0.27
Chloramphenicol palmitate (equivalent to 15.625 mg. chloramphenicol/ml.) and tetracycline (equivalent to 15.625 mg. tetracycline hydrochloride/ml.)	15.3 ± 0.501	15.4 ± 0.228	0.18
Chloramphenicol palmitate (equivalent to 31.25 mg. chloramphenicol/ml.) and nitrofurantoin (6.25 mg./ml.)	30.5 ± 0.308	31.0 ● 0.412	0.99
Chloramphenicol palmitate (equivalent to 15.625 mg. chloramphenicol/ml.), sulfadiazine (20 mg.), and phthalylsulfathiazole (15 mg./ml.)	15.6 ± 0.233	15.3 ± 0.241	0.90
Chloramphenicol palmitate (equivalent to 31.25 mg. chloramphenicol/ml.), thiamine hydrochloride (0.1 mg.), riboflavin (0.05 mg.), pyridoxine hydrochloride (0.05 mg.), niacinamide (1.5 mg.), cyanocobalamin (1.5 mcg.), and dexpanthenol (0.1 mg./ml.)	33.4 ± 0.659	32.2 ± 0.247	1.70

[a] Amount of chloramphenicol base contained in chloramphenicol palmitate, milligrams per milliliter. Values are mean of 10 observations ± standard error.

was titrated potentiometrically with 0.1 *N* silver nitrate. The difference between the two titrations represented the amount of chloride liberated from chloramphenicol. Each milliliter of 0.1 *N* silver nitrate is equivalent to 0.01616 g. of chloramphenicol. The results of analysis of different suspensions of chloramphenicol palmitate in combination with other drugs are given in Table III.

Determination of Chloramphenicol Palmitate in Suspensions by Microbiological Assay after Alkaline Hydrolysis—One milliliter of chloramphenicol palmitate oral suspension, equivalent to 31.25 mg. chloramphenicol, was transferred to a 25-ml. volumetric flask (the pipet was washed three times with water) and diluted to volume with ethanol. One milliliter of the ethanolic solution was transferred to a 200-ml. volumetric flask, treated with 10 ml. 0.01 *N* sodium hydroxide in ethanol, and kept at room temperature for 15 min. The solution was neutralized with 0.01 *N* hydrochloric acid and diluted to volume with 0.2 *M* phosphate buffer, pH 6.0. The liberated chloramphenicol was estimated microbiologically using *Escherichia coli* (ATCC 10536) as described in the *Code of Federal Regulations* (18) (Table IV), and recovery experiments were performed to determine the accuracy of the method (Table V).

Determination of Chloramphenicol Palmitate in Suspensions by Microbiological Assay after Enzymatic Hydrolysis—One milliliter chloramphenicol palmitate oral suspension, equivalent to 31.25 mg. chloramphenicol, was diluted to 100 ml. with water. One milliliter of the diluted suspension was transferred to a 100-ml. volumetric flask containing a suspension of 100 mg. pancreatin in 9 ml. of either tromethamine buffer, pH 9.0, or simulated intestinal fluid, pH 7.5. The mixture was incubated under toluene at 37° for 16 hr. and diluted to volume with 0.2 *M* phosphate buffer, pH 6.0; chloramphenicol was estimated microbiologically with *E. coli* (Table V).

Determination of Chloramphenicol Palmitate in Suspensions Containing Chloramphenicol Palmitate in Combination with Other Drugs by Microbiological Assay—The different dosage forms used are described in Table III. With the exception of the preparation containing tetracycline, all of the other preparations were hydrolyzed with 0.01 *N* sodium hydroxide in ethanol and chloramphenicol was estimated microbiologically as described earlier. For suspensions containing tetracycline, 2 ml. of the suspension, equivalent to 31.25 mg. chloramphenicol, was transferred to a 100-ml. centrifuge tube, mixed with 50 ml. of 0.1 *N* hydrochloric acid, and centrifuged at 10,000 r.p.m. for 15 min. The supernate was decanted and the residue was washed twice with 50 ml. 0.1 *N* hydrochloric acid by centrifugation. The residue contained chloramphenicol palmitate free from tetracycline as determined by microbiological assay. The residue was dissolved in ethanol and diluted to 25 ml. One milliliter of the ethanolic solution was hydrolyzed with 0.01 *N* sodium hydroxide in ethanol, and chloramphenicol was estimated microbiologically as described earlier (Table III).

Table IV—Results[a] of Microbiological Assay of Chloramphenicol Palmitate in Chloramphenicol Palmitate Oral Suspension USP[b]

Sample	After Alkaline Hydrolysis, mg./ml.	After Enzymatic Hydrolysis with Pancreatin, mg./ml. In Simulated Intestinal Fluid, pH 7.5	After Enzymatic Hydrolysis with Pancreatin, mg./ml. In Tromethamine Buffer, pH 9.0
1	30.2	24.1	21.2
2	32.6	24.1	25.0
3	32.5	24.1	25.0
4	30.0	25.0	27.0
5	31.0	24.1	27.0
6	30.0	24.0	21.2
7	31.0	27.0	28.0
8	35.0	26.0	28.0
9	32.5	26.0	28.0
10	31.0	26.0	26.2

[a] Amount of chloramphenicol base contained in chloramphenicol palmitate, milligrams per milliliter. [b] One milliliter suspension contained chloramphenicol palmitate equivalent to 31.25 mg. chloramphenicol base.

RESULTS AND DISCUSSION

Table I shows that the estimation of chloramphenicol palmitate by the USP method gave results 16–33% higher when the suspen-

Table V—Recovery of Chloramphenicol Palmitate by the Microbiological Assay Method after Alkaline Hydrolysis

Amount of Chloramphenicol Base Contained in Added Chloramphenicol Palmitate, mg.	Chloramphenicol Estimated, mg.	Recovery, %
16.20	18.0	111
14.40	15.0	104
15.25	14.6	96
32.10	31.8	99
29.13	32.0	110
31.24	28.1	90

sion contained 0.5% vanillin, a commonly used flavoring agent. If the method of extraction of the suspension is modified by washing the sample with water on a sintered crucible, dissolving the residue in chloroform, and determining the absorbance of the chloroform solution diluted with ethanol at 271 nm. according to the official method, the results obtained are very close to the labeled amount of chloramphenicol palmitate. According to the USP (3), chloramphenicol palmitate is insoluble in water, but Weiss *et al.* (17) reported that chloramphenicol palmitate is very slightly soluble in water, 1.05 mg./ml. water at 28°. According to them, to test the solubility of the drug, it must be shaken thoroughly for 2 min. The present authors observed that when the suspension was washed with three 10-ml. aliquots of water in quick succession in a sintered crucible No. 4 by suction, there was practically no loss of chloramphenicol palmitate (Table II). Washing on a sintered crucible is less cumbersome and more effective in removing substances likely to interfere with the spectrophotometric estimation of chloramphenicol palmitate than extracting with chloroform in a separator, which did not remove vanillin. When chloramphenicol palmitate suspension identical in all respects but without vanillin was estimated by both the USP method and the suggested modification, similar results were obtained (Table I). This further indicates that chloramphenicol palmitate is not eliminated by the water-washing procedure.

Suspensions of chloramphenicol palmitate were prepared with flavoring agents other than vanillin such as essence raspberry, essence American ice cream, or essence apricot. These essences did not interfere with the estimation of chloramphenicol palmitate by the USP method. Chloramphenicol palmitate oral suspension USP marketed in the United States may not contain vanillin. The preparation[1] marketed in India does not contain vanillin. Furthermore, the USP gives only a lower limit of the requirement of chloramphenicol palmitate in the suspension without specifying the higher limit. These reasons may explain the lack of this particular problem in the United States. In the light of these observations, the USP method of estimation of chloramphenicol palmitate may be suitably modified.

However, the modified USP method is not suitable for estimation of chloramphenicol palmitate in suspension when it is in combination with other drugs such as tetracycline, streptomycin, nitrofurantoin, sulfa drugs, and B vitamins. Drastic treatment of the suspension with sodium hydroxide to convert the chloride of chloramphenicol molecule to sodium chloride and determination of chloride with silver nitrate by potentiometric titration was found suitable for the estimation of chloramphenicol palmitate in the different dosage forms studied (Table III). The usefulness of this method, however, is severely limited because it is nonspecific for chloramphenicol and more complicated than other methods.

Chloramphenicol palmitate in suspensions, with or without other drugs, could be easily hydrolyzed with dilute alkali at room temperature to make chloramphenicol available for microbial assay. The presence of streptomycin, nitrofurantoin, sulfa drugs, or B vitamins in the concentrations present (Table III) had no effect on the test organism. In chloramphenicol palmitate suspension containing tetracycline, the latter must be removed because of its interference in the microbiological assay of chloramphenicol. The removal was carried out by washing with dilute hydrochloric acid, which did not remove chloramphenicol palmitate. The results of the microbiological assay with *E. coli* (Tables III and IV) and the recovery of added chloramphenicol palmitate (Table V) indicate the suitability of the method for the routine assay of chloramphenicol palmitate in the different forms of suspensions tested. The flavoring and coloring agents or the presence of streptomycin, nitrofurantoin, tetracyclines, sulfa drugs, or B vitamins did not interfere in the microbiological assay of chloramphenicol.

Since chloramphenicol palmitate is dissolved in ethanol prior to alkaline hydrolysis, polymorphism of the drug disappears. Therefore, the method of assay would not distinguish the different polymorphic forms, if present. This, however, is not a disadvantage as compared to the USP method which also does not take into consideration the polymorphic forms of chloramphenicol palmitate.

Enzymatic hydrolysis of chloramphenicol palmitate in suspension with pancreatin, either in simulated intestinal fluid (pH 7.5) or in tromethamine buffer (pH 9.0), released only about 80% of chloramphenicol from chloramphenicol palmitate in the present experiments (Table IV). Earlier reports, however, indicated complete enzymatic hydrolysis of chloramphenicol palmitate (13, 14). Our failure to obtain complete hydrolysis might be due to the pancreatin used whose enzyme activity is likely to vary from sample to sample. The data in Table IV show variations in hydrolysis, 21–28 mg./ml., indicating nonuniformity of enzyme action. Enzymatic hydrolysis followed by microbiological assay of chloramphenicol, therefore, may not always be suitable for the routine determination of chloramphenicol palmitate. In any case, hydrolysis of chloramphenicol palmitate with dilute alkali is much simpler than the enzymatic hydrolysis. Enzymatic hydrolysis, however, can indicate the presence of polymorph A crystals of chloramphenicol palmitate which is only hydrolyzed 0–5% (15, 16).

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