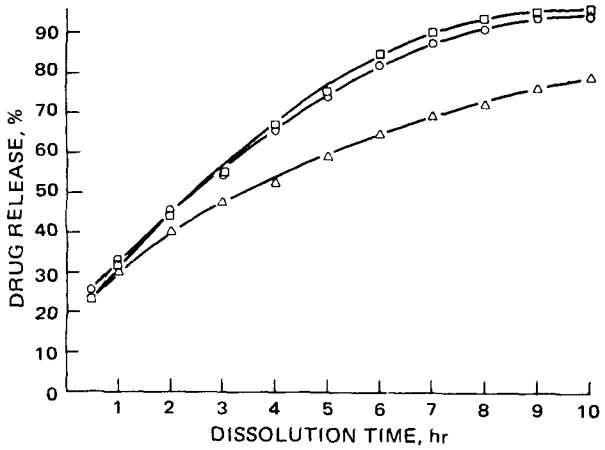


**Figure 4**—*Effect of I (mol. wt. 40,000) on tripelennamine hydrochloride release in modified intestinal fluid from fused cores. Key (polymer concentration):* △, *5%;* □, *10%; and* ○, *20%.*

the polymer, then the same effect should be expected when I is included in the medium. This was not the case, because there was a decrease in the drug release rate (Table V). The decrease in dissolution became more pronounced as the polymer concentration in the medium was increased. The apparent decrease was probably due to the increasing viscosity of the medium as the polymer concentration increased.

This conclusion was further substantiated by an IR study. Tripelennamine and low and high molecular weight I were dissolved in ethanol, the solvent was evaporated on a boiling water bath, and the mixture was dried under vacuum. The high frequency region of the IR spectrum[9] of the drug–polymer system was simply the summation of the spectra of the two components; there was no evidence of complexation between the two compounds. This result was not unexpected, because the tripelennamine molecule cannot hydrogen bond with the carbonyl group of I. Such bonding is possible with, for example, tolbutamide (6).

The optimum situation for the tripelennamine core would be the release of its entire content in 10 hr. Therefore, an average of 6.67 mg (66.7 mg/200-mg core) of drug should be released each hour. Table VI shows the slope and correlation coefficient for the zero-order component of the release profile of cores having various levels of I (mol. wt. 10,000) and prepared by fusion. At the 10–20% I level, the drug was released in a fashion approaching the optimum situation, at least in the 0.5–8-hr interval, with a total release of 89% for the 10% level and 94% for the 20% level over 10 hr.

Table III shows the effect of the method of preparation, concentration, and type of I on the core disintegration time. There was a rank correlation between the disintegration time and polymer concentration for both types. It appears that I acts as a disintegrant through channel formation. The data also clearly indicate the lack of cohesiveness in the cores prepared by double compression in comparison with the fused cores. While in the dissolution apparatus, the fused polymer-free and 5% I cores remained intact with a smooth surface at the end of 10 hr. On the other hand, cores containing higher concentrations of I appeared to have fissures as dissolution proceeded. The occurrence of the latter phenomenon was probably due to the I reducing the interparticle bonding of the drug–wax granules during compression.

The higher release of drug initially (0.5–8 hr) suggests that it may be possible to incorporate 100 mg of tripelennamine hydrochloride into a sustained-release matrix and to design the formulation in such a manner that 30–40% of the drug would be released in the first 2 hr in the stomach, with the remaining 60–70% released over the following 8 hr in the intestinal tract.

While it might be possible to design a sustained-release form as described, a certain percentage of the drug, about 10%, probably will always be coated very effectively with a wax film that is impermeable to GI fluids. Consequently, total drug release may be difficult to achieve.

[9] Perkin-Elmer model 567 grating IR spectrophotometer.

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# Analysis of Betamethasone and Its Organic Esters in Pharmaceutical Products

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Received March 28, 1977, from the **Food and Drug Administration, Dallas District, Dallas, TX 75204,* and the ‡*Department of Chemistry, Southern Methodist University, Dallas, TX 75275.* Accepted for publication July 1, 1977.

**Abstract** □ A rapid, quantitative analysis for betamethasone and its organic esters at room temperature is described. The method is similar to the official blue tetrazolium reaction for corticosteroids, except that methylene chloride is used as the solvent. The reaction is complete in 27–69 min, and the formazans produced are stable for at least 90 min after the addition of tetramethylammonium hydroxide. The results of the analysis of 13 different pharmaceutical formulations by the proposed method are reported. The degradation of betamethasone and its esters caused by strong bases is a pseudo-first-order reaction in methylene chloride. The average half-life of the corticosteroids studied is 56 min under the basic conditions described.

**Keyphrases** □ Betamethasone and various esters—spectrophotometric analyses in pharmaceutical products □ Spectrophotometry—analyses, betamethasone and various esters in pharmaceutical products □ Glucocorticoids—betamethasone and various esters, spectrophotometric analyses in pharmaceutical products

Betamethasone and its esters are synthetic corticosteroids which are included in several types of pharmaceutical preparations. Chafetz *et al.* (1) reported a colorimetric procedure for betamethasone benzoate in topical gel preparations that utilized preliminary oxidation of the 17-keto function, followed by reaction with phenylhy-

Table I—Absorbance [a] Replication of Proposed Procedure

| Number | Betamethasone | Betamethasone Acetate | Betamethasone Valerate | Betamethasone Benzoate | Betamethasone 21-Benzoate |
|---|---|---|---|---|---|
| 1 | 0.510 | 0.592 | 0.535 | 0.484 | 0.496 |
| 2 | 0.530 | 0.594 | 0.533 | 0.488 | 0.490 |
| 3 | 0.515 | 0.595 | 0.537 | 0.489 | 0.491 |
| 4 | 0.518 | 0.589 | 0.533 | 0.509 | 0.492 |
| 5 | 0.511 | 0.590 | 0.531 | 0.487 | 0.494 |
| 6 | 0.510 | 0.591 | 0.530 | 0.489 | 0.492 |
| 7 | 0.507 | 0.586 | 0.528 | 0.482 | 0.496 |
| 8 | 0.519 | 0.580 | 0.544 | 0.486 | 0.490 |
| 9 | 0.516 | 0.587 | 0.530 | 0.484 | 0.499 |
| 10 | 0.505 | 0.596 | 0.528 | 0.481 | 0.494 |
| Average | 0.514 | 0.590 | 0.533 | 0.488 | 0.493 |
| *SD* [b] | 0.008 | 0.005 | 0.005 | 0.009 | 0.003 |
| *RSD*, % | 1.6 | 0.9 | 0.9 | 0.8 | 0.6 |

[a] Solutions were kept in the dark except when being measured. [b] Calculated from the range by the method of Dean and Dixon (8).

drazine. NF XIV (2) includes monographs for betamethasone, betamethasone acetate, and betamethasone valerate in 11 different formulations.

The final determinative step for all of these products is the blue tetrazolium reaction at elevated temperatures (45 or 50°) for either 30 or 90 min, followed by quenching with acetic acid. The elevated temperatures help offset the steric interference of the C-16 $\beta$-methyl group (3) of the betamethasone molecule in the blue tetrazolium reaction. Possible problems in these methods arise from evaporative concentration, incomplete color development, and less specificity of the oxidation–reduction reaction for the C-17 $\alpha$-keto function. The blue tetrazolium reaction with betamethasone and certain of its organic esters is quantitative when run at room temperature in methylene chloride (4).

This paper reports a quantitative analytical procedure for betamethasone and its organic esters utilizing the blue tetrazolium reaction at room temperature in methylene chloride. The results of the analyses of 13 different pharmaceutical formulations by the proposed method are given.

## EXPERIMENTAL

**Apparatus**—The following were used: UV–visible recording spectrophotometers[1] with 1-cm stoppered quartz cells, glass chromatographic columns for partition chromatography (2.2 × 25 cm constricted at one end to 0.4 × 5 cm), an aluminum tamping rod, an electrobalance[2], and TLC equipment[3].

**Materials**—Alcohol USP, analytical reagent grade absolute ethanol, and distilled-in-glass grade acetonitrile, chloroform, *n*-heptane, absolute methanol, and methylene chloride were used. Blue tetrazolium[4], 10% aqueous tetramethylammonium hydroxide[5], and acid-washed diatomaceous earth[6] were also used. NF reference standard betamethasone, betamethasone acetate, and betamethasone valerate were used as well as betamethasone benzoate[7] and betamethasone 21-benzoate[7].

**Reagents**—A 1% solution of tetramethylammonium hydroxide was prepared by diluting 5.00 ml of the 10% aqueous solution to 50.0 ml with absolute ethanol. Blue tetrazolium, 5 mg/ml, was prepared by dissolving 50.0 mg in 10.0 ml of absolute methanol. Standard corticosteroid solutions were prepared to contain 0.012 mg of the steroid/ml in methylene chloride unless otherwise indicated.

*Acetonitrile–n-Heptane (Mutually Saturated)*—Acetonitrile, 25 ml, was mixed with 300 ml of *n*-heptane (sufficient for two determinations) in a separator, agitated vigorously for 2 min, and allowed to stand until both layers were clear. These mutually saturated solutions were used whenever acetonitrile or *n*-heptane was called for in these directions.

*Chloroform (Water Saturated)*—Water, 50 ml, was added to 250 ml of chloroform in a separator. The separator was agitated vigorously for 2 min, and both layers were allowed to clarify before use. The water-saturated reagent was used only when aqueous trap layers were inserted in the column.

**Proposed Procedure: Sample Preparation**—*Tablets*—Twenty tablets were weighed, ground to pass a 60-mesh sieve, and mixed thoroughly. Then a sample containing approximately 1.2 mg of the steroid was weighed accurately into a 100-ml beaker. Absolute methanol, 3 ml, was added and allowed to set for about 5 min, and the entire contents were washed into a separator with 1 ml of dilute hydrochloric acid USP, 75 ml of water, and 25 ml of methylene chloride.

After agitation, the methylene chloride extract was passed through cotton (previously washed with methylene chloride and dried) into a beaker, and the extraction procedure was repeated five times. The volume of the combined extracts was reduced to about 75 ml under air on the steam bath. The solution was cooled to room temperature, transferred to a 100-ml volumetric flask, adjusted to volume with methylene chloride, and mixed thoroughly for determination as described under *Determinative Procedures*.

*Creams, Lotions, Gels, and Ointments*—Water-soluble interferences found in several samples necessitated the use of a preliminary aqueous acid extraction. For this type of sample, a composite of several containers was prepared, and a sample containing approximately 1.2 mg of the steroid (0.6 mg for the more dilute creams) was weighed accurately. If the product showed interference, the sample was dissolved in 150 ml of water plus 5 ml of dilute hydrochloric acid USP and transferred to a separator.

Anhydrous sodium sulfate was added when necessary to break emulsions, and the sample was extracted six times with 25-ml portions of chloroform. The extracts were filtered through cotton (previously washed with chloroform and dried), and the combined extracts were taken just to dryness under air on the steam bath. The residue (or the unextracted sample for products that did not show interferences) was dissolved in 1.5 ml of acetonitrile plus 1.5 ml of *n*-heptane with slight warming on the steam bath. The procedure was continued as directed under *Column Preparation*.

*Aerosols*—The container was placed in dry ice and acetone for about 10 min, the top was removed carefully, and the container was allowed to sit overnight at room temperature. The nonvolatile residue was washed into a 100-ml volumetric flask and made to volume with chloroform. Aliquots containing approximately 1.0 mg of the steroid were evaporated under air on the steam bath just to dryness. The residue was dissolved in 1.5 ml of acetonitrile plus 1.5 ml of *n*-heptane, and the procedure was continued as directed under *Column Preparation*.

*Suspensions*—After the suspension was mixed well, duplicate samples were taken immediately with 1-ml (T.C.) pipets and drained into separate 100-ml beakers. The pipets were washed three times with 0.5-ml portions of acetonitrile and three times with 0.5-ml portions of *n*-heptane into the

Table II—Reaction Time and Stability of Formazan in Methylene Chloride

| Steroid | Time to Attain Maximum Absorbance, min | Absorbance | | | Absorbance [a] per Micromole |
|---|---|---|---|---|---|
| | | Original [b] Maximum | After 70 min | After 90 min | |
| Betamethasone | 27 | 0.518 | 0.519 | 0.520 | 1.017 |
| Betamethasone acetate | 27 | 0.489 | 0.490 | 0.476 | 1.036 |
| Betamethasone valerate | 69 | 0.519 | 0.519 | 0.520 | 1.043 |
| Betamethasone benzoate | 57 | 0.499 | 0.500 | 0.501 | 1.022 |
| Betamethasone 21-benzoate | 44 | 0.491 | 0.493 | 0.491 | 1.004 |

[a] See Ref. 3 for definition. The average is 1.024. [b] The absorbance noted at the time specified in the preceding column.

[1] Cary models 15 and 17.
[2] Cahn model G-2.
[3] Eastman No. 6060 silica gel with fluorescent indicator.
[4] Dajac Laboratories.
[5] Eastman Organic Chemicals.
[6] Celite 545, Johns-Manville Product Corp., New York, NY 10016.
[7] Warner-Lambert, Morris Plains, NJ 07950.

**Table III—Analyses of Typical Pharmaceutical Formulations**

| Number and Type of Sample | Steroid[a] | Amount Declared, | Amount Found, % of Declared | | | |
|---|---|---|---|---|---|---|
| | | | By Proposed Method | | By Isoniazid Method | |
| 1 Tablets[b] | I | 0.6 mg/tablet | 106.2 | 106.9 | 106.3 | 105.6 |
| 2 Cream[c] | I | 0.2% | 114.3 | 113.4 | 113.6 | 112.3 |
| 3 Cream[c] | II | 0.1% | 131.6 | 132.5 | 116.8 | 116.9 |
| 4 Cream[d] | III | 0.025% | 93.2 | 91.5 | 93.0 | 93.5 |
| 5 Cream[c] | III | 0.025% | 108.6 | NR[e] | 99.5 | NR[e] |
| 6 Cream[d] | II | 0.01% | 112.2 | 113.8 | 108.8 | 109.7 |
| 7 Lotion[c] | II | 0.1% | 117.8 | 115.9 | 117.8 | 118.6 |
| 8 Lotion[f] | III | 0.025% | 96.7 | NR[e] | 96.9 | NR[e] |
| 9 Gel[f] | III | 0.025% | 94.6 | 90.6 | 89.5 | 93.7 |
| 10 Gel[b] | III | 0.025% | 106.1 | 109.6 | 106.8 | 107.4 |
| 11 Ointment[c] | II | 0.1% | 111.7 | 110.8 | 113.6 | 114.4 |
| 12 Aerosol[c] | II | 0.15% | 96.7 | 95.6 | 96.9 | 95.3 |
| 13 Suspension[c] | IV | 3 mg/ml | 107.8 | 108.4 | 109.3 | 109.3 |
| | V | 3 mg/ml | ND[g] | ND[g] | 95.2 | 97.3 |

[a] Compound I is betamethasone, II is betamethasone valerate, III is betamethasone benzoate, IV is betamethasone acetate, and V is betamethasone sodium phosphate. [b] These samples required aqueous acid extraction but no column separation. [c] These samples required only the column separation. [d] These samples required an aqueous acid extraction plus the column separation. [e] NR means that these samples were not run in duplicate. [f] These samples required sodium sulfate saturated aqueous acid extraction but no column separation. [g] ND means not determined by this method.

beakers, and the procedure was continued as directed under *Column Preparation*.

*Standards*—A sample of about 1.2 mg of the desired corticosteroid standard was weighed accurately into a 100-ml beaker and dissolved in 1.5 ml of acetonitrile plus 1.5 ml of *n*-heptane. The procedure was then continued as directed under *Column Preparation*.

**Column Preparation**—*Acetonitrile Layer*—A glass wool plug was inserted into the bottom of a chromatographic column, and a 4.0-g portion of diatomaceous earth was mixed with 4.0 ml of acetonitrile, transferred to the column, and packed firmly with a tamping rod.

*Trap Layer*—When needed, a neutral aqueous trap layer was prepared and inserted in the column as directed by Graham *et al.* (5).

*Sample Layer*—The dissolved sample, standard, or residue, prepared as directed under *Sample Preparation*, was mixed thoroughly with 3–4 g of diatomaceous earth to yield a light fluffy mixture. This mixture was transferred to the column above the trap or acetonitrile layer and packed firmly. The sample beaker, tamping rod, spatula, and funnel were dry washed with about 1 g of diatomaceous earth, which was added to the column and packed firmly. The same equipment was dry washed with glass wool, which was placed on top of the sample layer plus washings and packed firmly. The beaker was retained and washed with the *n*-heptane and chloroform used during the column elution step.

**Column Elution**—The beaker was washed with 150 ml of *n*-heptane in small portions and transferred to the column to maintain a liquid head approximately 12 cm above the column bed. The last wash was allowed to drain completely from the column, the tip was rinsed with alcohol USP, and the entire effluent was discarded. A new beaker was placed under the column, and the sample beaker was washed with 125 ml of chloroform in small portions, which were added to the column to maintain the liquid level close to the top of the column. The last portion was allowed to drain completely, and the tip was rinsed with alcohol USP.

The effluent was evaporated carefully just to dryness on a steam bath under a hood to ensure complete removal of the acetonitrile. The residue was dissolved in methylene chloride and diluted accurately with methylene chloride to a volume that contained approximately 0.012 mg of corticosteroid/ml.

Betamethasone sodium phosphate, if present, was eluted from the neutral aqueous trap layer following the chloroform elution with 125 ml of alcohol USP. The effluent was diluted accurately to a volume that contained approximately 0.015 mg of corticosteroid salt/ml and retained for determination by the isoniazid method as directed under *Determinative Procedures*.

**Determinative Procedures**—*Isoniazid Method*—The procedure of Umberger (6) was used, except that the concentration of hydrochloric acid was doubled to increase the sensitivity.

*Blue Tetrazolium Method*—Aliquots of 20 ml were run by the procedure given in USP XIX (7), except that: (*a*) the tetramethylammonium hydroxide reagent was diluted with absolute ethanol instead of alcohol USP, (*b*) the blue tetrazolium reagent was prepared with absolute methanol in place of alcohol USP, (*c*) the photometric scans from 720 to 490 nm were made against methylene chloride as the reference instead of the reagent blank, and (*d*) the scans were started 70 min after the addition of the tetramethylammonium hydroxide. The scans were always made in the order of reagent blank, standard, samples, and reagent blank; the reagent blank was kept in the dark between the original and final scans. The absorbance was read from the absorbance maximum at 525 nm.

Each measured absorbance value had to be corrected for the continuously increasing reagent blank absorbance. The correction for each reading was obtained by dividing the increase in absorbance of the reagent blank by the number of scans (omitting the original reagent blank scan) to obtain an average increase per scan. This average was then multiplied by the scan number and added to the original reagent blank absorbance. The net absorbance was then calculated by subtracting the corrected reagent blank absorbance from the measured absorbance for that scan.

**Base Degradation of Betamethasone**—Approximately 2.4 mg of each of the five reference standards was weighed accurately, dissolved in methylene chloride, and made to volume in a 200-ml volumetric flask with methylene chloride. Two milliliters of 1% tetramethylammonium hydroxide was added to each of five 20-ml aliquots taken from each steroid solution, 2.00 ml of blue tetrazolium reagent was immediately added to one aliquot of each set, and the absorbance was determined 70 min after the addition of the blue tetrazolium. At specified successive time intervals covering 430 min, 2.00 ml of blue tetrazolium reagent was added to the remaining aliquots of each set and the absorbance produced during 70 min was determined.

## METHOD DEVELOPMENT

**Recovery Studies**—The five standards of betamethasone and its esters were put through the column cleanup and the determinative procedures of the proposed method. Comparison of the absorbances obtained from the steroid standards run through the column separation procedures with those obtained by direct determination on the identical standard solutions without the column procedure showed recoveries of 100.8% for betamethasone, 99.5% for betamethasone acetate, 100.5% for betamethasone valerate, 101.5% for betamethasone benzoate, and 101.8% for betamethasone 21-benzoate. The overall average recovery was 100.8%.

**Replication Studies of Absorbances Produced by Proposed Blue Tetrazolium Method**—Accurately weighed portions of each of the five corticosteroid standards were dissolved and diluted to 500 ml with methylene chloride to yield solutions containing approximately 0.012 mg/ml. Ten 20-ml aliquots of each were developed by the proposed blue tetrazolium determinative procedure, and the absorbances were determined (Table I). All solutions were kept in the dark during the color development period. The relative standard deviation, calculated from the range (8), varied from 0.6 to 1.8% with an overall average of 1.2%.

This experiment was repeated, except that the solutions were exposed to laboratory light during the color development period. The absorbance of the reagent blank increased an average of 0.161 absorbance unit compared to the reagent blank kept in the dark. The average relative standard deviation in this study was 2.6%.

**Beer's Law Study**—More concentrated solutions (up to 3.2 mg/100 ml) were prepared for each of the five standards in methylene chloride. Aliquots of 2.00, 5.00, 10.0, 15.0, and 20.0 ml of each of these solutions were taken, diluted to 20.0 ml with methylene chloride, and put through the proposed blue tetrazolium determinative procedure. Absorbances ranged from 0.070 to 1.200, and correlation coefficients of 0.9999 or better were obtained for each steroid using a least-squares fitting program.

**Table IV—Decrease of Absorbance with Time due to Degradation by Base in Methylene Chloride**

| Drug | | Absorbance ($A$)[a] and Time ($T$, min)[b] | | | | | Rate of Decrease of Absorbance with Time, $A$/min |
|---|---|---|---|---|---|---|---|
| Betamethasone | $A$ | 0.631 | 0.218 | 0.113 | 0.030 | 0.011 | $4.7 \times 10^{-3}$ |
| | $T$ | 70 | 130 | 190 | 310 | 370 | |
| Betamethasone acetate | $A$ | 0.558 | 0.292 | 0.143 | 0.043 | 0.017 | $3.0 \times 10^{-3}$ |
| | $T$ | 70 | 120 | 180 | 300 | 420 | |
| Betamethasone valerate | $A$ | 0.528 | 0.318 | 0.109 | 0.020 | 0.010 | $5.0 \times 10^{-3}$ |
| | $T$ | 70 | 130 | 190 | 310 | 430 | |
| Betamethasone benzoate | $A$ | 0.630 | 0.349 | 0.155 | 0.054 | 0.028 | $3.4 \times 10^{-3}$ |
| | $T$ | 70 | 120 | 180 | 300 | 360 | |
| Betamethasone 21-benzoate | $A$ | 0.605 | 0.360 | 0.140 | 0.009 | ND[c] | $3.7 \times 10^{-3}$ |
| | $T$ | 70 | 130 | 190 | 310 | | |

[a] Absorbance was always measured 70 min after the addition of the blue tetrazolium reagent. [b] These time periods were measured from the addition of the tetramethylammonium hydroxide and include the final 70 min after the addition of the blue tetrazolium reagent. [c] Not determined.

**Color Development Time and Stability of Formazan Produced**—A 20.00-ml aliquot of each of the five standards was treated according to the proposed blue tetrazolium determinative procedure, except that the solution was transferred to a cell immediately after the addition of the tetramethylammonium hydroxide. Readings were taken every minute *versus* a reagent blank for 90 min, and the time necessary to reach maximum absorption was noted for each steroid. The results (Table II) show that the completion of the reaction required from 27 min for betamethasone to 69 min for betamethasone valerate and that the formazan was stable for at least 90 min after the addition of the reagents.

**Methylene Chloride Volatility Study**—Five 100-ml aliquots of methylene chloride were pipetted into five separate 500-ml glass-stoppered volumetric flasks, the stoppers were inserted, and the flasks were weighed immediately and reweighed each day for 7 days. The average weight loss per day was equivalent to a decrease of 0.12% in volume. The experiment was repeated using four 100-ml flasks for 5 days with the stoppers sealed with masking tape. The average weight loss per day in this experiment was equivalent to a volume decrease of 0.06%/day.

**Water Effect Study**—An accurately weighed sample of approximately 1.2 mg of betamethasone acetate was dissolved and diluted to 100 ml with water-saturated methylene chloride, which was prepared by vigorous agitation with water in a separator for 2 min and then filtered through chloroform-washed cotton. A second similar sample was prepared with dry methylene chloride, and three 20.0-ml aliquots of each were run by the proposed blue tetrazolium determinative procedure. The average absorbance of the samples prepared with the water-saturated solvent was 100.6% of the average absorbance of the samples prepared with the dry solvent.

## RESULTS AND DISCUSSION

The results of the analyses of 13 different pharmaceutical preparations containing either betamethasone or an organic ester of betamethasone by the proposed procedure are shown in Table III. The isoniazid method (6), which measures the amount of conjugated ketone in the A ring, gave comparable results and showed that there was essentially no decomposition in the samples examined. Interference with the blue tetrazolium reaction was noted with Samples 3 and 5 and was confirmed by the method of variation of absorbance with time previously reported (9). TLC of the betamethasone acetate fraction of the suspension (Sample 13) did not reveal the presence of betamethasone.

The data in Table IV show that base degradation of betamethasone and its organic esters proceeded rapidly in methylene chloride. The decomposition was a pseudo-first-order reaction, which showed decreases of from $3.0 \times 10^{-3}$ to $5.0 \times 10^{-3}$ (overall average $4.0 \times 10^{-3}$) absorbance unit/min. The half-life of the five corticosteroids under the conditions used varied from 51 min for betamethasone to 62.5 min for betamethasone benzoate with an overall average of 56 min.

Due to the low solubility of water in methylene chloride, it is necessary to dilute the tetramethylammonium hydroxide and to prepare the blue tetrazolium reagent with water-free solvents that are miscible with methylene chloride. As an example of the effect of water, the substitution of alcohol USP for both solvents increased the water content from 0.9 to 1.2% and changed the time necessary to reach maximum absorbance from 13 to 90 min for hydrocortisone (4).

Beer's law was obeyed for concentrations between 0.05 and 0.50 mg/24 ml for all five corticosteroids.

Methylene chloride underwent evaporative concentration in glass-stoppered volumetric flasks at a daily average rate of approximately 0.1% (v/v). This finding indicates that the same standard could be used for several days without significant change. In this study, however, the standards were used only on the day of preparation.

The rate studies listed in Table II show that the reaction of blue tetrazolium with betamethasone and its organic esters in methylene chloride was complete at room temperature in relatively short periods—from 27 to 69 min. Once the reaction was complete, the formazan was stable for at least 90 min from the time of the addition of the tetramethylammonium hydroxide. The calculated values of the absorbance per micromole (3) shown in Table II indicate that the reaction had gone to completion in the specified time. The average absorbance per micromole for the five standards was 1.024, which agrees favorably with the value of 1.031 previously reported (3) for 21 different corticosteroids not including betamethasone.

While the blue tetrazolium reaction with the steroids was complete in the specified times and the formazans produced were stable, the absorption of the blank continued to increase. However, the rate of increase of absorbance in the light-protected blank was slower than the rate of increase of absorbance of the blank in the reference cell when a long series of scans was necessary. As a consequence, the procedure described in the USP XIX (7), which requires that the samples be scanned *versus* the reagent blank, will cause an error of approximately 0.001 absorbance unit/min when a long series of scans is made. The correct net absorbance for the samples can only be obtained by scanning against the solvent and calculating the corrected net absorbance as described under *Determinative Procedures*.

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