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Determination of Iodochlorhydroxyquin and Corticosteroids in Pharmaceutical Formulations

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Abstract □ Iodochlorhydroxyquin was separated from various corticosteroids using an acetonitrile–diatomaceous earth column. Iodochlorhydroxyquin was eluted with cyclohexane, and the corticosteroid was eluted with chloroform. Iodochlorhydroxyquin was determined by both a UV absorbance method and a new compleximetric method using the nickel chelate of iodochlorhydroxyquin. The corticosteroid was determined by the blue tetrazolium and isoniazid procedures. The average percent recovery for these four methods was 100.8, 99.4, 100.7, and 99.9, respectively, for 10 known mixtures. The standard deviation for the absorbance for 10 determinations of the nickel complex was 0.002 absorbance unit (0.31%). Various characteristics of the nickel and other complexes were evaluated, including the sensitivity, solubility, and wavelength of maximum absorbance in 14 different solvents. The analyses of 23 typical products are reported, for which the standard deviation, expressed as a percentage of the amount declared, was 1.31% for the UV, 1.34% for the compleximetric, 1.49% for the blue tetrazolium, and 1.22% for the isoniazid procedures. Methods of determination in the presence of interferences are discussed.

Keyphrases □ Iodochlorhydroxyquin and corticosteroid formulations—partition chromatographic separation, compleximetric analysis of iodochlorhydroxyquin, blue tetrazolium analysis of corticosteroid □ Corticosteroid and iodochlorhydroxyquin formulations—partition chromatographic separation, compleximetric analysis of iodochlorhydroxyquin, blue tetrazolium analysis of corticosteroid □ Partition chromatography—separation, iodochlorhydroxyquin and corticosteroid formulations □ Nickel complex formation—analysis, iodochlorhydroxyquin after separation from iodochlorhydroxyquin and corticosteroid formulations

The "American Drug Index 1973" (1) lists 36 manufacturers of pharmaceutical formulations that contain iodochlorhydroxyquin (5-chloro-7-iodo-8-quinolinol) (I). Five of these manufacturers prepare products containing only I, while 33 manufacture products containing I plus a corticosteroid such as hydrocortisone, hydrocortisone acetate, or prednisolone.

USP XVIII (2) specifies an IR procedure that is specific for I in creams, ointments, and suppositories. NF XIII (3) requires the same IR technique to determine I in creams, lotions, and ointments containing I plus hydrocortisone and uses the blue tetrazolium method for the corticosteroid. IR methods specific for I in pharmaceutical products were described (4, 5), but these procedures are difficult to use due to the volatility and odor of the carbon disulfide and the small volumes required in the extraction step.

Other reported methods include UV absorption (3), gravimetry (3), combustion in an oxygen flask followed by titration (6), polarography (7), fluorescence (8), GLC (9), and spectrophotometry (10–12). Spectrophotometric methods depend upon the formation of a colored metallic complex with I. The iron (III) complex (10, 11) and the copper (II) complex (12) have been utilized for the determination of I in certain pharmaceutical products.

This paper reports a simple, rapid, quantitative method for the separation of I from corticosteroids by partition chromatography using the diatomaceous earth–acetonitrile column described previously (13, 14). After separation, I is determined by conversion to the nickel complex and measurement of the absorption at 406 nm while the corticosteroid is analyzed by the blue tetrazolium procedure of USP XVIII (15). Twenty-one different pharmaceutical formulations of creams, lotions, ointments, and suspensions were analyzed by the proposed method. When necessary, the method may be supplemented by the use of IR (3), GLC (9), or TLC (16) to detect the presence of impurities such as 8-hydroxyquinoline, 5-chloro-8-hydroxyquinoline, 5-iodo-8-hydroxyquinoline, 5,7-diiodo-8-hydroxyquinoline, and 5,7-dichloro-8-hydroxyquinoline.

EXPERIMENTAL

Equipment—The following were used: a UV–visible recording

Table I—Recovery (Percent) of Prepared Standards

Sample Number[a]	Iodochlorhydroxyquin: Complexi-metric Method	Iodochlorhydroxyquin: UV Method	Hydrocortisone: Blue Tetra-zolium Method	Hydrocortisone: Isoniazid Method
1	99.8	101.1	100.7	100.6
2	99.0	100.6	100.8	99.2
3	99.2	100.8	100.8	100.4
4	99.8	101.3	100.3	100.4
5	99.5	100.5	100.6	99.7
6	99.2	100.8	100.2	99.6
7	99.4	100.5	100.3	100.0
8	99.5	101.1	100.4	99.7
9	99.6	100.6	101.6	100.8
10	99.4	101.1	99.3	98.6
Average	99.4	100.8	100.5	99.9
SD[b]	0.3	0.3	1.0	0.7

[a] Each standard mixture contained approximately 6 mg of iodochlorhydroxyquin and 1 mg of hydrocortisone. [b] Calculated from the range by the method of Dean and Dixon (18).

spectrophotometer[1] with 1-cm quartz cells, a glass chromatographic column (2.2 × 25 cm constricted at one end to 0.4 × 5 cm) plus aluminum tamping rod, and an electrobalance[2].

Reagents—*Solvents*—All solvents were spectrograde, analytical reagent grade, pesticide grade, USP, distilled-in-glass, or nanograde. Absolute ethanol, acetone, acetonitrile, alcohol USP, carbon tetrachloride, chloroform, cyclohexane, dimethyl sulfoxide, dioxane, acetic acid, *n*-heptane, *n*-hexane, methanol, methylene chloride, and 2,2,4-trimethylpentane were used.

Acetonitrile–Cyclohexane (Mutually Saturated)—Mix 20 ml of acetonitrile and 270 ml of cyclohexane (sufficient for two determinations plus a reagent blank) in a separator, agitate vigorously for 2 min, and allow to stand with occasional swirling until both layers are clear. These mutually saturated solutions must be used whenever acetonitrile or cyclohexane is called for in these directions.

Chloroform (Water Saturated)—Add 50 ml of water to 250 ml of chloroform in a separator, agitate for 2 min, and allow both layers to clarify. This water-saturated reagent is required when aqueous trap layers are inserted but should not be used otherwise.

Diatomaceous Earth[3]—Acid washed was used.

Nickel Reagent (*0.010* M)—Dissolve 248.8 mg of nickel (II) acetate tetrahydrate in absolute ethanol with heating on a steam bath. Cool and dilute to 100.0 ml with absolute ethanol. This solution is stable for at least 6 months. Other metal-ion reagents (0.050 *M*) were prepared for method development studies using the acetates in ethanol or methanol.

Standards—All solid standards were USP or NF reference standards. Prepare corticosteroid standards (0.0100 mg/ml) in alcohol USP and I standards (0.0600 mg/ml) in cyclohexane. Individually weighed samples of the standards were used in some studies.

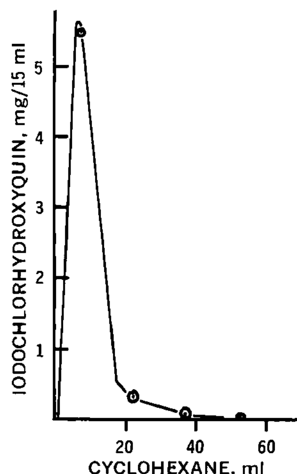

Figure 1—*Elution curve for iodochlorhydroxyquin.*

[1] Cary model 15.
[2] Cahn model G2.
[3] Celite 545, Johns-Manville Product Corp., New York, NY 10016

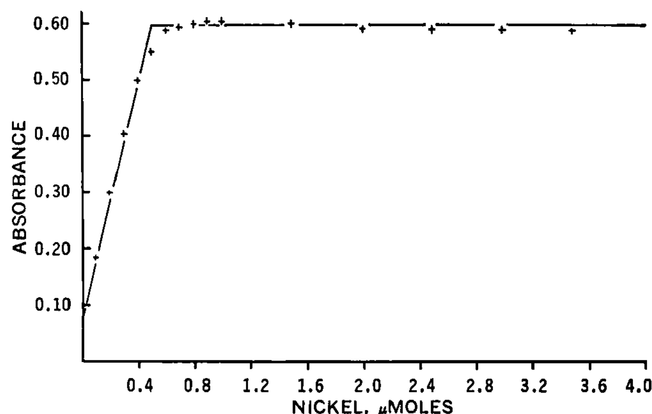

Figure 2—*Mole ratio study for nickel–iodochlorhydroxyquin complex.*

Sample Preparation—*Creams, Ointments, Lotions, Jellies, and Suspensions*—Prepare a composite sample by mixing thoroughly the contents of several containers and accurately weigh a sample equivalent to about 6 mg of I into a 100-ml beaker. Dissolve in 1.5 ml of acetonitrile plus 1.5 ml of cyclohexane with slight warming on a steam bath (1.5 ml of methanol plus 1.5 ml of water may be substituted for these solvents when necessary for complete solution of the formulation). Continue as directed under *Sample Layer*.

Standards—Accurately weigh (or pipet volumes that contain) about 1 mg of corticosteroid and 6 mg of I into a 100-ml beaker. Proceed as directed in *Creams, Ointments, Lotions, Jellies, and Suspensions*, beginning with "Dissolve in 1.5 ml of acetonitrile plus. . . ."

Column Preparation—*Acetonitrile Layer*—Insert a glass wool plug in the bottom of a chromatographic column. Thoroughly mix 4.0 g of diatomaceous earth with 4.0 ml of acetonitrile, transfer to the column, and pack firmly.

Trap Layer—Prepare and insert a neutral aqueous trap layer, as needed, according to Graham *et al.* (14).

Sample Layer—Thoroughly mix the dissolved sample or standard, prepared as directed under *Sample Preparation*, with 3.0 g of diatomaceous earth. Transfer to the column above the acetonitrile or trap layer and pack firmly. Dry wash the sample beaker, tamping rod, spatula, and funnel with 1 g of diatomaceous earth and transfer to the column. Dry wash the same equipment with

Table II—Characteristics of Complexes in Various Solvents

Solvent	Nickel Complex A	Nickel Complex λ_{max}	Zinc Complex A	Zinc Complex λ_{max}	Iron Complex A	Iron Complex λ_{max}
Acetone	0.565	412	0.474	405	0.132	620
Acetic acid	0.574	378	—[a]	—[a]	0.133	630
Methylene chloride	0.574	401	0.509	395	0.229	620
Chloroform	0.592	402	0.468	398	0.239	620
Alcohol USP	0.592	400	0.507	400	0.301	645
Methanol	0.593	404	0.523	400	0.323	670
Dioxane	0.608	413	0.360	405	0.120	620
Ethanol (100%)	0.626	403	0.507	401	0.327	660
2,2,4-Trimethylpentane	0.636	405	0.463	405	0.146	605
Carbon tetrachloride	0.637	405	0.435	402	ND[b]	ND
Hexane	0.638	405	0.483	403	0.204	615
Acetonitrile	0.640	414	0.494	402	0.174	625
Cyclohexane	0.655	406	0.498	403	0.204	615
Dimethyl sulfoxide	0.666	418	0.531	407	0.065	645

[a] Complex does not form at this low pH. [b] ND = not determined.

Table III—Analysis of Typical Products

Sample Number[a]	Type of Product	Iodochlorhydroxyquin UV Method	Iodochlorhydroxyquin Compleximetric Method	Corticosteroid Blue Tetrazolium Method	Corticosteroid Isoniazid Method
		Average Amount Found, % of Declared[b]			
1	Suspension	104.2 (2)	102.2 (2)	116.3 (2)	113.0 (2)
2	Cream	104.1 (6)	102.5 (6)	107.1 (4)	105.8 (4)
3	Cream	107.4 (6)	106.0 (6)	98.7 (2)	103.6 (2)
4	Cream	121.9 (6)	119.4 (6)	114.1 (2)	110.9 (2)
5	Cream	107.2 (2)	106.8 (2)	99.6 (2)	103.6 (2)
6	Lotion	83.5 (6)	83.1 (5)	75.1 (6)	92.9 (6)
7	Jelly	101.7 (6)	101.0 (6)	101.2 (2)	101.3 (2)
8	Cream	83.3 (8)	86.3 (10)	107.5 (5)	116.6 (4)
9	Ointment	103.7 (4)	101.0 (4)	102.6 (4)	104.6 (4)
10	Cream	119.4 (2)	118.2 (2)	111.0 (2)	112.8 (2)
11	Cream	103.6 (2)	102.6 (2)	104.4 (2)	104.4 (2)
12	Ointment	101.6 (2)	98.2 (2)	102.5 (2)	99.8 (2)
13	Cream	119.7 (3)	119.0 (3)	111.5 (3)	113.3 (3)
14	Cream	106.8 (2)	104.2 (2)	102.5 (2)	101.1 (2)
15	Cream	105.1 (2)	102.6 (2)	102.1 (2)	104.8 (2)
16	Cream	107.9 (4)	106.4 (6)	102.8 (4)	103.7 (4)
17	Ointment	ND[c]	118.9 (4)	109.8 (4)	109.6 (2)
18	Cream	110.0 (4)	109.0 (4)	101.0 (4)	106.2 (2)
19	Cream				
	Tube 1	ND[c]	97.4 (4)	109.2 (4)	133.4 (2)
	Tube 2	ND[c]	153.0 (2)	129.9 (2)	163.2 (2)
20	Cream	105.7 (4)	105.9 (4)	108.9 (4)	110.4 (2)
21	Cream	99.2 (2)	99.2 (2)	94.6 (2)	98.6 (2)
22	Cream				
	Tube 1	104.0 (4)	103.5 (4)	52.0 (4)	53.6 (4)
	Tube 2	103.0 (2)	102.9 (2)	100.8 (2)	105.5 (2)
23	Cream				
	Tube 1	ND[c]	325.4 (4)	113.1 (4)	117.5 (4)
	Tube 2	ND[c]	324.9 (2)	116.0 (2)	118.9 (2)
SD[d]		1.31	1.34	1.49	1.22

[a] Components present in each sample are listed in Table IV. [b] Number in parentheses indicates number of determinations. [c] Not determined due to interference in UV spectra caused by a cyclohexane-soluble complex or an ingredient other than I. [d] Expressed as percent of amount declared calculated from the difference in duplicates (20).

glass wool, transfer to the column, and pack firmly. Retain the beaker for washing with cyclohexane and chloroform during the *Column Elution* step.

Column Elution—*Compound I*—Wash the beaker successively with six 20-ml portions of cyclohexane and pour into the column, maintaining the liquid head between 8 and 12 cm above the column bed and catching the effluent in a 100-ml volumetric flask since the column holds up approximately 23 ml. Allow the last wash to drain completely, wash the tip of the column with cyclohexane, make the effluent to volume, and retain for determination as described under *Determinative Procedures*.

Corticosteroids—Rinse the sample beaker with 125 ml of chloroform in several portions and pour each through the column, maintaining the liquid head as close to the top of the column as possible and catching the effluent in a 150-ml beaker. Allow the last portion to drain completely from the column and rinse the tip with alcohol USP. Carefully evaporate the effluent just to dryness on a steam bath in a hood with a current of air to ensure complete removal of the acetonitrile. Dissolve in alcohol USP and dilute accurately to a volume containing approximately 10 μg/ml of corticosteroid. Retain for determination as directed under *Determinative Procedures*.

Determinative Procedures for I—*Ultraviolet Method*—Mix the cyclohexane effluent thoroughly and scan from 500 to 280 nm *versus* cyclohexane as the reference. Measure net absorbance from the baseline to the absorbance maximum at 326 nm. Calculate by comparison with standards run concurrently.

Compleximetric Method—Pipet 5.00-ml aliquots of the cyclohexane effluents for each sample and standard into small glass-stoppered flasks. Prepare a reagent blank using 5.00 ml of cyclohexane. To each flask, add 0.1 ml of nickel reagent, swirl, let stand for at least 1 min, and then scan from 500 to 340 nm *versus* the reagent blank. Measure the net absorbance of the maximum at 406 nm and calculate by comparison with the standards run concurrently.

Determination Procedures for Corticosteroids—*Isoniazid Method*—The procedure of Umberger (17) was used, except that the concentration of hydrochloric acid was doubled to increase the sensitivity.

Blue Tetrazolium Method—The procedure given in USP XVIII (15) was followed without modification.

METHOD DEVELOPMENT

Column Characteristics—*Elution*—A column was prepared as directed under *Column Preparation*, using 6.050 mg of standard I and fitting the column with a separator to allow the maintenance of a 12-cm liquid head which ensured a constant flow rate of 7 ml/min. A total of 285 ml of cyclohexane was collected in 15-ml fractions. Each fraction was taken to dryness carefully under air on a steam bath, dissolved in and diluted to an appropriate volume with acetic acid, and scanned from 500 to 280 nm.

The elution curve shown in Fig. 1 indicates that all of I is completely eluted in the first 60 ml of cyclohexane. In the proposed procedure, the column holds up approximately 23 ml of the 120 ml of cyclohexane used so that the retention of 97 ml provides an adequate safety factor. In several cases, a second 100 ml of cyclohexane was poured through the column. No I was found in any of these effluents. Tests on several alcoholic extracts remaining from the corticosteroid determinations also were negative for I.

Separation and Recovery—Ten separate standard mixtures containing approximately 6 mg of I and 1 mg of hydrocortisone were analyzed by the proposed procedure using all four determinative methods (Table I). The overall average recovery was 100.2%, and the overall average standard deviation was less than 0.6%. Portions of each sample effluent were evaporated, dissolved in small volumes, spotted on TLC plates, and chromatographed. In each case, a single spot appeared, showing complete separation.

Characteristics of the Nickel-I Complex—*Beer's Law*—During the investigation, samples containing 0.030–0.83 mg of I/5.1 ml showed a linear relationship between absorbance and concentration.

Table IV—Sample Ingredients

Sample Number[a]	Ingredient Numbers[b]
1	(1), (7), (14), (15), (18), (19), (26), (36), (38), (39), (52)
2	(10), (17), (26), (28), (29), (31), (36), (37), (43), (52)
3	(5), (8), (14), (17), (24), (26), (41), (45), (48), (52), (53)
4	(15), (17), (26), (31), (32), (33), (37), (46), (47), (52)
5	(13), (17), (35), (49), (52)
6	(6), (10), (17), (23), (25), (26), (36), (52)
7	(9), (17)
8	(2), (3), (17), (22), (26), (31), (36), (37), (39), (42), (48), (52)
9	(8), (17), (37), (41), (52), (53)
10	(17), (26), (31), (36), (44), (48), (51), (52)
11	(4), (12), (17)
12	(4), (12), (17)
13	(17), (26), (31), (36), (44), (48), (51), (52)
14	(8), (16), (17), (26), (31), (36), (37), (39), (43), (47), (52)
15	(17), (26), (27), (36), (37), (39), (48), (52), (53)
16	(17), (26), (27), (36), (37), (39), (48), (52), (53)
17	(17), (27), (53)
18	(8), (15), (17), (20), (21), (26), (30), (36), (37), (45), (52)
19	(17), (26), (27), (36), (40), (52), (54)
20	(10), (17), (26), (28), (29), (31), (36), (37), (43), (52)
21	(4), (12), (17)
22	(13), (17), (35), (49), (52)
23	(10), (11), (14), (17), (26), (36), (41), (47), (48), (50), (52)

[a] Number listed in Table III. [b] Ingredient list: (1) acetic acid, (2) acetylated lanolin, (3) aluminum acetate, (4) hydrophilic ointment base [Aquaphor (Duke)], (5) beeswax, (6) carboxymethylcellulose, (7) carboxymethylene disodium ethylenediaminetetraacetic acid, (8) cetyl alcohol, (9) chlorobutanol, (10) citric acid, (11) coal tar solution, (12) cold cream, (13) free fatty alcohols, (14) glycerin, (15) glyceryl monostearate, (16) heather aroma, (17) hydrocortisone, (18) hydrocortisone acetate, (19) isopropyl myristate, (20) isopropyl palmitate, (21) lanolin, (22) lanolin alcohols extract, (23) lidocaine, (24) liquid petrolatum, (25) magnesium aluminum silicate, (26) methylparaben, (27) mineral oil, (28) mineral wax, (29) mono- and diglycerides of fat-forming fatty acids, (30) neomycin base, (31) petrolatum, (32) polyoxyethylene oxypropylene stearate, (33) polyoxyethylene stearate, (34) polyoxyl 40 stearate, (35) pramoxine hydrochloride, (36) propylparaben, (37) propylene glycol, (38) sodium acetate, (39) sodium bisulfite, (40) sodium borate, (41) sodium lauryl sulfate, (42) sorbitan sesquioleate, (43) sorbitol, (44) sorbitan fatty acid esters (Span 60), (45) spermaceti, (46) squalene, (47) stearic acid, (48) stearyl alcohol, (49) sulfonated and polyoxylated fatty alcohols, (50) triethanolamine, (51) hydrophilic ether esters (Tween 80), (52) water, (53) white petrolatum, and (54) white wax.

Mole Ratio—The mole ratio study (Fig. 2) showed that the complex contained 2 moles of I for each mole of nickel.

Absorbance Replication—Ten separate 5.00-ml aliquots of standard I in cyclohexane were pipetted into small glass-stoppered flasks, 0.1 ml of nickel reagent was added, and the resulting solutions were scanned from 500 to 340 nm. The average net maximum absorbance at 406 nm was 0.645 with a standard deviation calculated from the range (18) of 0.002.

Stability—The absorbance of a sample of I in cyclohexane was measured at 1-min intervals after the addition of the nickel reagent until the absorbance became constant and was then monitored periodically for 24 hr. The procedure was repeated several times and was also repeated using I in acetic acid. In all cases, the absorbance reached a maximum within 1 min and remained constant for 24 hr.

Volatility—Samples of the nickel complex with I were prepared in methanol and carefully evaporated under air on the steam bath just to dryness. Two of the dry samples were left on the bath for 40 min and two were left for 90 min, after which they were dissolved and the absorbance was measured. There was no loss due to volatility in either case.

Volatility of I—Twelve separate 5.00-ml aliquots of standard I were pipetted into small glass-stoppered flasks and placed under air on the steam bath. Eight aliquots were removed as soon as they reached dryness, two remained on the bath 30 min after dryness, and the last two remained on the bath 60 min after dryness. Each sample was dissolved in 5.00 ml of acetic acid, 0.10 ml of nickel reagent was added, and the solution was scanned from 500 to 280 nm *versus* a reagent bank. The recoveries for the first eight samples averaged 100.1% (range 98.6–101.2%) while the second two averaged 64% and the last two 49%. As a consequence, the cyclohexane effluent from the column should not be evaporated to dryness before the determination of I.

Metal Complexes with I (Solvent Effect)—Standard solutions of I were prepared in 14 different solvents. Separate 5.00-ml aliquots were treated with 0.1 ml of nickel reagent and scanned as in the proposed procedure against the reagent blank. The study was repeated with an iron (III) reagent (0.050 *M* in ethanol) and with a zinc (II) reagent (0.050 *M* in ethanol). The nickel and zinc solutions were scanned from 500 to 340 nm, and the iron solutions were scanned from 720 to 380 nm.

The net absorbance and the wavelength of maximum absorbance of each complex in each solvent are shown in Table II. The nickel complex with I was the most sensitive in all solvents, and the iron (III) complex was the least sensitive. The net absorbances for the nickel and the zinc complexes were at a maximum in dimethyl sulfoxide, but the iron complex showed minimum absorbance in this solvent and maximum absorbance in absolute ethanol. Cyclohexane appears to be a satisfactory solvent for all three complexes. The wavelength of maximum absorbance depended in part upon the solvent and varied from 378 to 418 nm for the nickel complex, from 395 to 407 nm for the zinc, and from 605 to 670 nm for the iron.

This experiment was repeated using only acetic acid as the solvent but using different metal reagents (0.050 *M* in ethanol or methanol). Of the metal ions tested, magnesium (II) and manganese (II) did not react, zinc (II) reacted partially, and copper (II), cobalt (II), and nickel (II) reacted completely. The reagent blanks were then scanned against acetic acid. There was a significant absorbance at the wavelength of maximum absorbance only in the case of the copper reagent blank.

RESULTS AND DISCUSSION

The analyses of 23 typical formulations containing both I and corticosteroids are shown in Table III. The standard deviation, expressed as the percentage of the amount declared, was 1.31% for the UV, 1.34% for the compleximetric, 1.49% for the blue tetrazolium, and 1.22% for the isoniazid procedures. Samples 6 and 22 were low in hydrocortisone while Samples 1, 19, and 23 were above the recommended limits set by USP. Samples 6 and 8 were low in I while Samples 4, 10, 13, 17, 19, and 23 were high. For Samples 3, 5, 6, 8, 18, 19, 21, and 22, comparison of the corticosteroid values by the blue tetrazolium and isoniazid methods indicates from 4 to 34% decomposition of the corticosteroid according to the test of Graham *et al.* (19). Application of the variation of absorbance with time test suggested in the same reference indicates interferences in the blue tetrazolium procedure with Samples 1, 4, and 5.

Samples 3, 4, 5, 7, 8, 10, 13, 14, 15, 17, 19, and 23 were varying shades of yellow, which was taken to indicate varying amounts of decomposition of I caused by the reaction with metal ions present in the ingredients or containers to form metal chelates. The presence of metal chelates of I in samples containing I often can be determined quantitatively by slight modifications of the proposed compleximetric procedure since all of the metal chelates studied are converted to the nickel complex upon addition of the nickel reagent. Two possibilities exist: *(a)* the metal chelate is not removed from the column with cyclohexane but is removed by the chloroform and *(b)* the metal chelate is removed from the column with the cyclohexane.

Sample 8, which contains aluminum acetate, is an example of the first type. When a sample from one tube was run by the proposed procedure, only 91.0% of the amount declared was found to be present. A portion of the chloroform eluate was concentrated by evaporation, the nickel reagent was added, and the solution was scanned against a blank of the nickel reagent in chloroform. Calculations showed that 16.0% of the amount of I declared was present in the chloroform eluate. A second sample from the same tube composite was dissolved directly in chloroform; total I was determined by adding the nickel reagent and was found to be 105.9%,

which agrees satisfactorily with the sum of 107.0% found separately. The presence of a metal–I complex of this type is indicated whenever the residue resulting from the evaporation of the chloroform fraction does not completely dissolve in the alcohol solvent used for the determination of the corticosteroid.

The presence of a cyclohexane-soluble metal complex of I is indicated when the amount of I cannot be determined by the UV procedure due to interference in the absorbance at 326 nm and when there is an absorbance peak close to 406 nm in the UV spectra. Sample 19 is an example of a formulation that contains both types of metal–I complexes. Analysis of a single tube composite by the proposed procedure indicated the presence of 88.1% by the UV method and 98.1% by the compleximetric method. The chloroform fraction was treated in the same manner as described for Sample 8 and was found to contain 46.1% of the amount of I declared. The total complexed and free I was determined by dissolving a second sample of the composite in chloroform, adding the nickel reagent, and scanning against a blank of the nickel reagent in chloroform. Total I was determined to be 149.8%, which compares satisfactorily with the 144.2% found by summing the values obtained separately.

Chloroform is not the solvent of choice for the determination of I by the compleximetric method since the nickel complex can exist in two different forms in chloroform; one absorbs at 402 nm and one absorbs at 470 nm. The equilibrium between these forms is affected greatly by the water concentration and causes the measurement at 402 nm to be lower than it should be whenever any water is present. The chloroform fraction is used to estimate the amount of the cyclohexane–insoluble metal–I complex since the solid metal–I complex remaining from the evaporation of the chloroform eluate was not sufficiently soluble in the 13 solvents listed in Table II. In the early phases of the investigation, the cyclohexane fraction was evaporated on a steam bath and the residue was dissolved in chloroform for the compleximetric determination. The trouble with the equilibrium between the two complex forms and the volatility of I led to the development of the direct measurement in the cyclohexane eluate.

The results for Samples 4, 7, 8, 19, 22, and 23 that were run on different days and/or from different containers did not agree satisfactorily, even though duplicates run at the same time were in close agreement for both I and the corticosteroid. This finding indicates nonuniformity in mixing during preparation and/or packaging.

Several products tested contained other pharmaceutically active ingredients such as lidocaine, neomycin sulfate, and pramoxine hydrochloride. No attempt was made to determine these components quantitatively.

Sample preparations and column elution for two samples require approximately 1.5 hr, and complete determination by all four determinative procedures can be completed in approximately 6 hr.

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Assay of Sulfacetamide Sodium Ophthalmic Solutions by High-Pressure Liquid Chromatography

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Abstract □ A high-pressure liquid chromatographic method, using an adsorption column and sulfabenzamide as the internal standard, is proposed for the determination of sulfacetamide sodium and its principal hydrolysis product, sulfanilamide, in eye drops. It affords an average recovery of 100.9% of added sodium sulfacetamide with a relative standard deviation of 1.9%.

Keyphrases □ Sulfacetamide sodium—ophthalmic solutions, assay, high-pressure liquid chromatography □ Sulfanilamide—assay as hydrolysis product of sulfacetamide sodium in ophthalmic solutions, high-pressure liquid chromatography □ High-pressure liquid chromatography—assay of sulfacetamide ophthalmic solutions

Sulfacetamide sodium solutions have been shown to undergo hydrolysis to sulfanilamide and sodium acetate and oxidative discoloration (1–5). The nitrite titration method used for sulfacetamide sodium ophthalmic solution in USP XVIII (6) does not distinguish between sulfacetamide and sulfanilamide