Table V—Force Needed to Detach the Commercially Available Potassium Chloride Formulations from the Isolated Porcine Esophagus

Drug Product	Amount of Potassium Chloride, g	Coating Material	Detaching Force[a], N	Mean Force per Unit Area, $mN \cdot mm^{-2}$
A	1.0	Polyvinyl chloride; sucrose crystals	0.38 ± 0.03	0.9
B	0.6	Sugarcoating	0.39 ± 0.01	0.9
C	1.0	Hydroxypropyl methyl cellulose; silicic acid	0.54 ± 0.04	1.1
D	0.5	Hydroxypropyl methyl cellulose	0.78 ± 0.06	2.4
E	0.75	Hydroxypropyl cellulose	0.93 ± 0.09	2.6
F	0.75	Hydroxypropyl methyl cellulose	0.94 ± 0.12	2.2
G	1.0	Hydroxypropyl methyl cellulose	0.98 ± 0.13	2.1
H	0.75	Special wax; talc	1.00 ± 0.11	2.8
I	1.0	Special wax; talc	1.10 ± 0.13	2.4
J	0.75	Ethyl cellulose	1.20 ± 0.10	2.2

[a] Mean ± *SD*; $n = 18$.

with low adherence. In contrast, sparingly water-soluble ingredients (talc, titanium dioxide, and ethyl cellulose) and, in addition, lactose, mostly increase adherence.

On the basis of this research it seems possible to develop pharmaceutical products having a very low tendency to adhere to the esophageal mucosa. It seems desirable to develop such products in the case of drugs known to cause esophageal strictures or ulcerations, in particular.

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High-Performance Liquid Chromatographic Analysis of Iodochlorohydroxyquin and Hydrocortisone in Ointments and Creams

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Abstract □ A simple isocratic, high-performance liquid chromatographic (HPLC) assay procedure was developed for the simultaneous determination of iodochlorhydroxyquin and hydrocortisone in ointments and creams using phenyl salicylate as an internal standard. Ointment samples were extracted by direct dissolution in ether. Homogeneous suspensions of the creams were prepared in the mobile phase. The samples were spiked by the addition of standard iodochlorhydroxyquin, standard hydrocortisone, and the internal standard and subsequently extracted with the mobile phase. HPLC was performed using a reverse-phase microparticulate C-18 column, a precolumn, and a UV detector set at 256 nm. A mobile phase containing methanol and 0.05 *M* phosphoric acid (70:30) was employed at a flow rate of 1 ml/min. The percent iodochlorhydroxyquin and hydrocortisone found to be present in eight commercial products is reported.

Keyphrases □ Iodochlorhydroxyquin—simultaneous determination with hydrocortisone, ointment and cream, high-performance liquid chromatography □ Hydrocortisone—simultaneous determination with iodochlorhydroxyquin, ointment and cream, high-performance liquid chromatography □ High-performance liquid chromatography—simultaneous determination of iodochlorhydroxyquin and hydrocortisone, ointment and cream

Iodochlorhydroxyquin (I) is used alone or in combination with hydrocortisone (II) as an antimycotic, antibacterial, and anti-inflammatory agent in topical preparations. Because of its toxicity, several analytical procedures have been developed for the determination of I in biological fluids (1–7). The present pharmacopeial assay method for I in creams or ointments (8, 9) is time consuming, requires heating during the extraction procedures (which can lead to decomposition of I), and involves the use of an IR spectrophotometric assay procedure. Other IR spectrophotometeric procedures specific for I in pharmaceutical products have also been described (10, 11). However, these methods are cumbersome, require a large sample, and involve the use of carbon disulfide in the assay procedure. The pharmacopeial methods for assaying II in creams and ointments (12) depend on the oxidation of the α-ketol side chain by either triphenyltetrazolin chloride or blue tetrazolin. Base concentration, water, air, solvent, and light are known to interfere with color formation in the tetrazolin reaction (13). Excipients that interfere with color formation include sorbitan monooleate, lanolin, and stearic acid (14).

This work describes a high-performance liquid chro-

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matographic (HPLC) method that can be used routinely to assay I and II in ointments and creams. The method is simple and combines specificity and sensitivity not attainable by previously described methods.

EXPERIMENTAL

Apparatus—The HPLC system consisted of a reciprocating minipump[1]; a stainless steel tube, 6.35-mm o.d. and 4.76-mm i.d. × 1 m as a pulsation damper; a 34.5-MPa (5000 psi) pressure gauge[2]; a fixed-volume sample injector[3] with a 20-μl loop; and a variable-wavelength UV detector[4]. A multivoltage 25.4-cm strip chart recorder[5] was connected to the UV detector. A microparticulate reverse-phase chromatographic column (250 × 2.6 mm, packed with ODS-HC-SIL-X-1[6]) and a 5 × 40-mm guard column (RP-18-MPLC[7]) were connected to the HPLC system. The following chemicals were used: iodochlorhydroxyquin[8]; anhydrous ether (analytical reagent grade)[9]; methanol distilled in glass, residue free[10]; phenyl salicylate[11]; hydrocortisone[12]; and water, which was deionized, demineralized, and glass-distilled in our laboratory. No impurities were detected by HPLC in the iodochlorhydroxyquin (I) or hydrocortisone (II) reference standards or in the phenyl salicylate internal standard.

Ointments and Creams—The following products used were reported by the manufacturers to contain the indicated percentages of each drug: product A[13], 3% I; product B[14], 3% I; product C[15], 3% I and 1% II; product D[16], 3% I and 1% II; product E[17], 3% I, 1% II, and 0.5% pramoxine hydrochloride; product F[18], 3% I and 0.5% II; product G[19], 3% I and 0.5% II; and product H[20], 3% I and 1% II.

Standards—Stock solutions of I were prepared in methanol at 1.0 mg/ml. Solutions of II and phenyl salicylate were each prepared in the mobile phase (methanol–0.05 *M* phosphoric acid, 70:30) and contained 1.0 mg/ml. These solutions could be stored in the refrigerator for at least 2 weeks without deterioration. Working standards were prepared daily, using methanol–0.05 *M* phosphoric acid (70:30) to make the desired dilutions.

Chromatographic Conditions—The parameters used for this investigation included a reverse-phase column and precolumn, as previously described, and a 20-μl injector loop, with the recorder chart speed set at 0.254 mm/min. A mobile phase containing methanol–0.05 *M* phosphoric acid (70:30) was used at a flow rate of 1 ml/min. The mobile phase was filtered using a 0.20-μm filter[21], deaerated under vacuum, and maintained at 40° during chromatography. The UV detector was set at 256 nm. The column was flushed at the end of each day with methanol. Not more than 30 min was required for column equilibration prior to use each day.

Extraction Procedures—*Ointments*—Fifty milligrams of each ointment was dissolved in 10 ml of ether by vortexing. Aliquots (200 μl) were transferred to 20-ml screw-cap vials and spiked with varying amounts of I working standard. A fixed amount of the internal standard (phenyl salicylate) was added to each vial. In cases where the ointments contained II, the ointment solutions were spiked with standard II solution in addition to I and the internal standard. These solutions were evaporated to dryness at 40° under a stream of nitrogen. The contents of each vial were dissolved in 10.0 ml of mobile phase by warming for 1 min on a steam bath followed by vortexing for 1 min. After cooling to room temperature, aliquots of each solution were removed, diluted with mobile phase, and injected onto the column.

Creams—Fifty milligrams of each cream was uniformly suspended in 10 ml of mobile phase by vortexing. Aliquots were removed, spiked with the standard solutions and internal standard, and evaporated to dryness as described above for ointments. The contents of each vial were warmed, suspended in 10.0 ml of the mobile phase by warming on a steam bath followed by vortexing. Aliquots were subsequently diluted with mobile phase and assayed by HPLC.

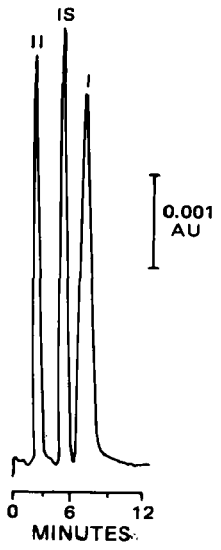

Figure 1—*Chromatogram of extracted product C to which the internal standard (IS), phenyl salicylate (4 μg/ml), had been added. The amounts of iodochlorhydroxyquin (I) and hydrocortisone (II) that were present in the product were 2.8 and 0.93%, respectively. The detector was set at 256 nm and 0.02 AUFS.*

RESULTS AND DISCUSSION

Iodochlorhydroxyquin (I) standard was scanned in methanol–0.05 *M* phosphoric acid (70:30) and exhibited maxima at 256 (α = 0.15) and 204 (α = 0.11) nm. For optimum sensitivity the detector was set at 256 nm.

The use of 4,7-dichloroquinoline[22], 10-chloro-9-anthracenemethanol[22], 2,4-quinolinediol[22], 3-quinolinecarbonitrile[22], 5,7-diiodo-8-hydroxyquinoline[23], and 5,7-dichloro-8-hydroxyquinoline[22] as internal standard was attempted without satisfactory results. 4,7-Dichloroquinoline, 5,7-diiodo-8-hydroxyquinoline, 10-chloro-9-anthracenemethanol, and 5,7-dichloro-8-hydroxyquinoline had retention times that overlapped with I, while 2,4-quinolinediol and 3-quinolinecarbonitrile had retention times that interfered with II. Phenyl salicylate was found to be suitable as an internal standard. It was easily separated from I, absorbed adequately at 256 nm, and produced consistent peak heights when extracted in conjunction with ointments or creams. Suitable retentions for I, II, and phenyl salicylate were obtained when a mobile phase containing methanol–0.05 *M* phosphoric acid (70:30) was used.

To determine whether the ointment and cream bases interfered with the HPLC of I, II, or the internal standard, the peak height ratios of the absorbances at two different wavelengths (256 and 246 nm) were determined on extracts from each product. These results were compared with the same ratios for the standard I, II, and internal standard. No differences were found when comparing the respective ratios, indicating that constituents in the bases that may have been extracted did not interfere with the analyses of the desired compounds.

Figure 1 is a chromatogram of an extracted ointment (product C) which contained I and II and was spiked with the internal standard. The capacity factor (k') for I was 2.94 and for the internal standard was 1.76,

[1] Milton Roy Model 326.
[2] Laboratory Data Control, Riviera Beach, FL 33404.
[3] Rheodyne Inc., Berkeley, CA 94710.
[4] Model Spectro-Monitor III; Laboratory Data Control, Riviera Beach, FL 33404.
[5] Beckman Instruments, Palo Alto, CA 94304.
[6] Serial No. 1303; Perkin-Elmer, Norwalk, CT 06858.
[7] Manufactured by Brownlee Lab and obtained from Rheodyne Inc., Berkeley, CA 94710.
[8] 5-Chloro-7-iodo-8-hydroxyquinoline, Vioform; Ciba Pharmaceutical, Summit, NJ 07901.
[9] Mallinckrodt Inc., St. Louis, MO 63147.
[10] Burdick and Jackson Labs Inc., Muskegan, MI 49442.
[11] Merck and Co., Rahway, NJ 07065.
[12] Sigma Chemical Co., St. Louis, MO 63178.
[13] Vioform Cream, Exp. date Aug. 82, Batch No. 13312; Ciba Pharmaceutical, Summit, NJ 07901.
[14] Vioform Ointment, Exp. date July 84, Batch No. 13642; Ciba Pharmaceutical, Summit, NJ, 07901.
[15] Vioform with Hydrocortisone Ointment, Exp. date July 83, Batch No. 16171; Ciba Pharmaceutical, Summit, NJ 07901.
[16] Vioform with Hydrocortisone Cream, Exp. date Sept 83, Batch No. 15503; Ciba Pharmaceutical, Summit NJ 07901.
[17] F-E-P Cream, Exp. date May 83, Batch No. 3967; Boots Pharmaceuticals, Inc., Shreveport, LA 71106.
[18] Racet LCD Cream, Exp. date May 83, Batch No. 6080; Lemmon Co., Sellersville, PA 18690.
[19] Racet Cream, Exp. date Jan 85, Batch No. 6626; Lemmon Co., Sellersville, PA 18690.
[20] Racet 1% Cream, Exp. date July 81, Batch No. 6084; Lemmon Co., Sellersville, PA 18690.
[21] Catalogue No. EGWP-04700; Millipore Corp., Bedford, MA 07130.
[22] Aldrich Chemical Co., Inc., Milwaukee, WI 53233.
[23] K and K Laboratories, Plainview, NY 11803.

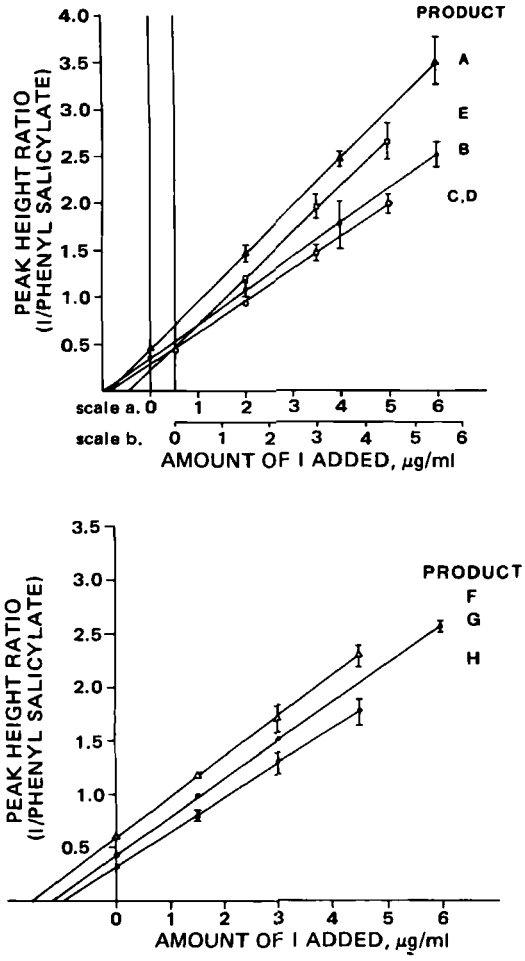

Figure 2—*Plots of I/phenyl salicylate peak height ratio* versus *amount of I added to products A–H. Extrapolation of the regression lines to the x-axis and multiplication by the appropriate dilution factor permits determination of I in each ointment or cream. The concentration of phenyl salicylate added to extracts from the products was 4.0 μg/ml. Each assay was performed in triplicate; standard deviations are shown only when >0.10.*

where the retention times for I (Rt_I) and phenyl salicylate were 7.50 and 5.25 min, respectively. The elution time of the solvent front (Rt_0) was 1.90 min and was determined by injecting methanol onto the column. In cases of ointments or creams which contained II, it was necessary to use the mobile phase in the extraction procedure and preparation of the standards to elminate the solvent front which interfered with the chromatographic peak of II (Rt_{II}, 2.50 min). As can be seen in Fig. 1, the peaks for I, II, and internal standard are well separated. Optimum extraction of I was obtained with the mobile phase (pH 2.8). Extraction of I from ointments and creams at other pH values was less efficient.

Figure 2 shows the regression lines of peak height ratio of iodochlorhydroxyquin (I)/phenyl salicylate *versus* concentration of I for the eight different products examined. Each product was spiked with varying concentrations of I, and the determinations were performed in triplicate. The mean values are presented with the standard deviations. Correlation coefficients for each of the lines for the eight products were >0.96. The regression lines for I in products C and D are identical and are presented as a single line in Fig. 2; only the largest standard deviations are given.

In Fig. 3 is plotted the peak height ratios of hydrocortisone (II)/phenyl salicylate against II concentration for five products that contained II. Determinations were performed in triplicate, and each value represents the mean with the standard deviation. The correlation coefficients for each of the plots are again >0.96. The regression lines for II in products F, G, and H are identical and are presented as a single line in the figure.

The concentrations of I and II found in the eight products, as determined by HPLC, are presented in Table I and were derived by using the regression lines presented in Figs. 2 and 3. These results are compared with the percentages of I and II claimed to be present in the products by

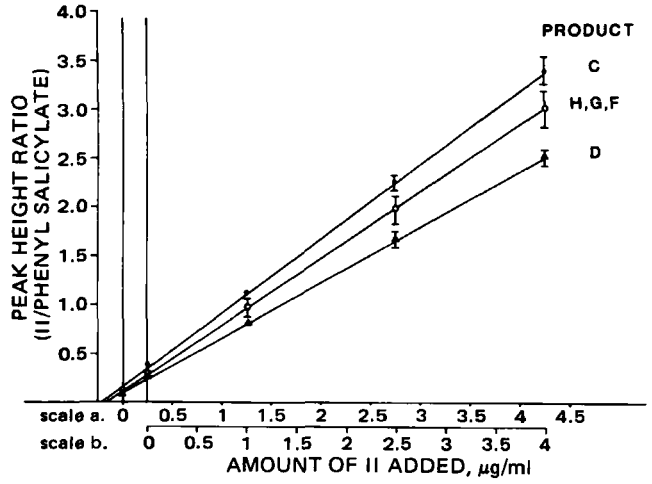

Figure 3—*Regression lines of II/phenylsalicylate peak height ratio* versus *amount of II added to products C, D, F, G, and H. The x-intercept of each regression line when multiplied by the appropriate dilution factor yields the concentration of II in the ointment or cream. The concentration of phenyl salicylate added to each product extract was 4.0 μg/ml. Each assay was performed in triplicate; standard deviations are shown only when >0.10.*

the manufacturers. As can be seen, the concentrations of I and II do not agree with the manufacturers' claims in all cases.

Products E and H were reported to contain 3% I, but were experimentally determined to contain only 2.0%; product G was purported to contain 3% I, but was experimentally found by HPLC to contain only 2.4%. These discrepancies may in part be due to the decomposition of I to products which were not extracted by the methods employed. No unidentified components were detected by HPLC (Fig. 1). The formation of unextracted complexes between I and constituents in the ointment bases is not likely based on the recovery data presented in Table II. The results obtained for products E, G, and H were reproducible on repeated analysis. As previously stated, for all other products containing I good agreement between the concentrations of I claimed by the manufacturers and the amounts determined by HPLC was obtained.

Compound II was present in six products; the concentration of II could be determined in five of the products. The concentrations of II determined experimentally by HPLC for two of the products contained 20% less than that claimed by the manufacturer. Better agreement was ob-

Table I—Results of HPLC Assay of Iodochlorhydroxyquin and Hydrocortisone in Ointments and Creams

Product	Claimed by Manufacturer, %		Determined by HPLC, %	
	Iodochlor-hydroxyquin	Hydro-cortisone	Iodochlor-hydroxyqin	Hydro-cortisone
A	3	—	2.8	—
B	3	—	3.0	—
C	3	1	2.8	0.93
D	3	1	2.8	0.88
E	3	1	2.0	ND[a]
F	3	0.5	3.1	0.40
G	3	0.5	2.4	0.40
H	3	1	2.0	0.88

[a] Not determined

Table II—Percent Recovery of Iodochlorhydroxyquin and Hydrocortisone from Extracted Ointments and Creams [a]

Product	Iodochlorhydroxyquin	Hydrocortisone
A	87.1 ± 4.6	—
B	95.3 ± 1.5	—
C	97.9 ± 0.9	91.2 ± 1.9
D	92.3 ± 0.0	63.4 ± 3.9
E	57.9 ● 3.4	ND[b]
F	94.0 ± 3.9	77.3 ± 5.7
G	90.0 ± 0.0	83.2 ± 5.5
H	79.9 ± 1.4	71.4 ± 0.0

[a] Each value is the mean with the standard deviation for three determinations. [b] Not determined.

tained for the other three products between our results and the purported concentrations of II. Product D contains pramoxine hydrochloride, which had a retention time on HPLC that overlapped with II, precluding the determination of II in this product.

Table II presents the percent recovery of I and II from each of the products as determined without the addition of the internal standard, phenyl salicylate. Approximately 58–98% of I was recovered, while 63–91% of II was recovered.

In summary a simple, precise, and accurate HPLC method has been developed that can be used for routine analysis and quality control for iodochlorhydroxyquin and hydrocortisone in creams and ointments.

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Urinary Excretion of Methylparaben and Its Metabolites in Preterm Infants

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Abstract □ A high-performance liquid chromatographic (HPLC) assay to quantitate methylparaben in urine was developed. Standard curves were linear and recovery of the paraben from urine averaged 82.6%. The urinary excretion of methylparaben in six preterm infants (≤31 weeks gestational age), who were receiving intramuscular injections of a paraben-containing gentamicin formulation, ranged from 13.2 to 88.1%. Small quantities of the metabolite, *p*-hydroxybenzoic acid, were detected by GC–MS.

Keyphrases □ Methylparaben—determination in human urine, preterm infants, high-performance liquid chromatography □ High-performance liquid chromatography—methylparaben in human urine, preterm infants, gentamicin formulations □ Preservatives—methylparaben, determination in human urine, preterm infants, high-performance liquid chromatography

Methylparaben (methyl *p*-hydroxybenzoate) and propylparaben (propyl *p*-hydroxybenzoate) are used in combination (in some medications) as preservatives. Reports that methylparaben can displace bilirubin from its binding sites on albumin *in vitro* has produced some concern regarding its use in medicinal formulations administered to the newborn and, in particular, the preterm infant (1–3). Displacement of bilirubin from its binding sites in infants with neonatal jaundice could lead to brain damage (kernicterus).

Gentamicin, an effective antibiotic for treatment of a neonatal Gram-negative sepsis, was also suspected of being a bilirubin displacer (4). Subsequent experiments have proven that gentamicin does not displace bilirubin (2, 5). However, some gentamicin formulations do contain methylparaben as a preservative. In an *in vivo* study a group of infants received a single intramuscular injection of a paraben-containing gentamicin formulation. No reduction in the albumin binding capacity for bilirubin was detected (3). The differences seen between this *in vivo* study and the *in vitro* studies may have been due to *in vivo* preservative catabolism or low paraben serum levels achieved after intramuscular injection. The concentrations of methylparaben used *in vitro* were relatively high, *e.g.*, 167 μg/ml of serum (2).

In this study, the excretion and metabolism of methylparaben was monitored in preterm infants after they had received multiple doses of a gentamicin formulation containing paraben preservatives.

EXPERIMENTAL

Materials—Methyl-, propyl-, and *n*-butylparabens[1], phosphoric acid[2], glacial acetic acid[3], and sodium hydroxide[4] were obtained commercially, and appropriate aqueous or methanolic solutions were prepared as required. Urine samples were extracted with freshly glass-distilled ether[5]. An acetate buffer (100 ml, pH 5.0) was prepared by mixing 0.2 *M* acetic acid (14.8 ml), 0.2 *M* sodium acetate[2] (35.2 ml), and water. A 1 *M* carbonate buffer (pH 9.5) was prepared by adding sodium carbonate[2] (5.3 g) and sodium bicarbonate[2] (4.2 g) to water (100 ml). Gentamicin[6] was obtained commercially; each vial (2 ml) contained gentamicin (80 mg), methylparaben (3.6 mg), and propylparaben (0.4 mg).

Patients—Six preterm babies (4 males, 2 females), with estimated gestatinal ages of 26–31 weeks and birth weights of 0.69–1.50 kg, were studied. Each infant was admitted to the neonatal intensive care unit and

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Ajax Chemicals Ltd., Sydney, Australia.
[3] B.D.H. Chemicals (Aust) Pty. Ltd., Fairy, Vic., Australia.
[4] E. Merck, Darmstadt, West Germany.
[5] Anesthetic ether B.P.; Hoechst Australia Ltd., Melbourne, Vic., Australia.
[6] Gentamicin Injection, B.P.; David Bull Lab. Pty. Ltd., Mulgrave, Vic., Australia.

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