In Vitro Release of Hydrocortisone from Topical Preparations and Automated Procedure

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The *in vitro* drug release profile of hydrocortisone (HC) from creams, ointments, and lotions has been determined using an automated procedure. A diffusion cell system and commercially available synthetic membranes were utilized for the studies. The use of a synthetic membrane obviates the problems associated with using skin membranes. Uniform creams and ointment samples for determining release rate profile were prepared by using the teflon mask. Automated sampling avoids operator artifacts. The automated technique developed for determining the *in vitro* release rate profile of the drug from creams, ointments, and lotions using a diffusion-cell system appears to be a reasonable and practical procedure for assuring batch-to-batch uniformity of topical drug products.

KEY WORDS: *in vitro* release for creams, ointments, lotions; diffusion cell; synthetic membranes; automation; hydrocortisone.

INTRODUCTION

In vitro dissolution testing for tablets and capsules has been extensively used to assure batch-to-batch uniformity and bioavailability. Several automated procedures, including use of robotics, are available for dissolution testing of oral drug products (1,2). Until recently *in vitro* drug release methods to determine batch-to-batch uniformity did not exist for topical products such as creams, ointments, and lotions. Shah *et al.* (3) have developed an *in vitro* release method to determine hydrocortisone (HC) from the cream product using a diffusion cell and a synthetic membrane. In this procedure, the commercially available synthetic membrane is saturated with the receptor phase, and the drug release from the cream formulation into the receptor phase is determined. Using the release rate data, the diffusion (partition) coefficient between the vehicle of the cream formulation and the receptor phase was shown to be independent of the source of the hydrophilic synthetic membrane studied but dependent on the formulation. It was also shown that the release characteristics determined using a synthetic membrane can be used as a quality-control procedure, similar to dissolution studies (drug release of a solid oral dosage form in a dissolution medium), for assuring batch-to-batch uniformity of the cream products (3,4). The use of synthetic membranes minimizes the variability associated with animal or human skin. The application of this *in vitro* release method for creams to other topical dosage forms such as ointments and lotions is presented here. Automation of the method, which allows for ease of operation, sample acquisition, and analysis, is also described.

MATERIALS AND METHODS

Products

The following marketed products were used in the study: hydrocortisone 2.5% cream, Synacort (Syntex), lot no. 24796 (Other strengths of creams, ointments, and lotions from this manufacturer were not available at the time of this study.); hydrocortisone 2.5% cream, Hytone (Dermik), lot no. 67514; hydrocortisone 1% cream, Hytone (Dermik), lot no. 77520; hydrocortisone 0.5% cream, Hytone (Dermik), lot no. 74366; hydrocortisone ointment, Hytone (Dermik), lot no. 73794; and hydrocortisone lotion, Hytone (Dermik), lot no. 68759.

Synthetic Membranes and Reagents

The following synthetic membranes were studied: cellulose acetate membrane with wetting agent, 0.45-μm pore size, 150 μm thick (Gelman Sciences, Inc., Ann Arbor, MI); cellulose acetate membrane, 0.45-μm pore size, 150 μm thick (MSI, Honeoyo Falls, NY); Cellulastic Hydrophobic Membrane, HVHP, 0.45-μm pore size (Millipore, Corp., Bedford, MA); isopropyl myristate (IPM) (Eastman Kodak Co., Rochester, NY); and Ethomeen S 12 (*N,N*-bishydroxyethyl ollylamine, ET S12) (Armak Chemicals, McCook, IL).

Equipment

Diffusion-Cell System. A standard Franz diffusion cell, open cap, flat ground glass with 15-mm-diameter orifice (1.767-cm^2 area; total diameter of cell, 25 mm) was used. A six-unit system and a three-unit system were utilized (Crown Glass Co., NJ). The receptor phase was stirred by means of a constant spinning bar magnet.

Receptor Phase. Three media—representing the pH of the blood, 0.05 *M*, pH 7.4, phosphate buffer; representing the pH of the skin surface, pH 5.0 phosphate buffer; and isotonic solution, normal saline—were utilized as the receptor media. The medium was degassed by vacuum before using. Samples were removed from the middle area of the receptor phase at intervals of 30, 60, 120, 240, and 360 min for analysis.

Sample Acquisition and Data Analysis. A Microette automated sampler (Hanson Research Corporation, Chatsworth, CA); a WISP autoinjector and an HPLC system equipped with a μBondapak C18 (3.9-mm i.d. × 30 cm) reverse-phase column and a 254-nm fixed-wavelength detector (Waters Associates, Milford, MA); and Heath PC (PC Electronics) and Lotus 1, 2, 3 software (Lotus Development corporation) were utilized.

Sample Preparation

A 2-mm-thick × 17-mm-diameter Teflon "mask" with a 15-mm hole in the center was centered over the synthetic membrane (25-mm diameter). This 15-mm hole corresponded to the circumference of the membrane holder on the Franz cell. For determining the *in vitro* release from ointments, a synthetic membrane impregnated with IPM/amine

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was used. This was achieved by adding a mixture of 15% ethoxylated aliphatic amine (Ethomeen S12) in IPM dropwise to the synthetic membrane until fully saturated. After 1 hr, the excess was removed using absorbent tissue (Kleenex), and the prepared membrane was used immediately to study *in vitro* release of HC from ointments. No such treatment for the membrane was essential for studying *in vitro* release from creams and lotion. The membrane was saturated with the receptor phase before adding cream or ointment. The sample (HC cream or ointment) was placed in the hole and spread uniformly with a Teflon squeegee by pulling across the Teflon mask. The product surface was checked for holes, channels, and other irregularities. This procedure resulted in a smooth even film of the sample, uniform in thickness and diameter. The mask was removed using tweezers, and the prepared membrane was transferred to the diffusion cell, cream/ointment side facing up.

Experimental

The lower part of the diffusion cell was filled with deaerated receptor phase medium. The top part of the cell, holding the membrane and the sample, was placed membrane-side-down on the bottom of the cell and checked for air bubbles. If air bubbles were present, the sample preparation and procedure were repeated. If no air bubbles were present, the cells were clamped in place. In the case of the lotion product, the membrane was saturated with the receptor phase, then placed in position with clamps, and lotion was added on top of the membrane. It is very important to degas the medium to avoid outgassing; otherwise bubbles appear after several hours. The medium was equilibrated to 32°C. The sample size was usually 200 mg; however, only the surface area affects the release. Initial experiments using 0.20–1.0 g of cream resulted in no detectable difference in drug release profile. A Hanson Microette automated sampler was interfaced with the diffusion cell, and a 150-μl aliquot was withdrawn from the middle part of the cell over a 6-hr period at programmed time intervals and transferred into microvial inserts in the auto injector carousel.

The HPLC analysis of HC was carried out using a μBondapak C18 column, 3.9-mm i.d. × 30 cm long, and a 254-nm UV detector. Samples were eluted using an isocratic mobile phase consisting of tetrahydrofuran:acetonitrile:water (1:3:6) at ambient temperature and a flow of 60 ml/hr. Under these conditions, the retention time for HC was 4.9 min, and the detection limits were 8 ng per 20 μl injection, with a 5–10% coefficient of variation. The concentration (μg/ml) of HC in the sample (receptor phase) was calculated using a standard of known concentration. The total amount of HC released (conc. as μg/ml × volume of receptor phase) and amount of HC released per unit area (μg/cm^2) were calculated. The percentage coefficient of variation of the *in vitro* release profile of HC from the dosage forms, in most cases, was less than 10% at any time interval.

RESULTS AND DISCUSSION

Using the diffusion-cell system, a synthetic membrane, and HC cream, it was shown that there was no statistically significant difference in the HC concentration in the upper, middle, and lower part of the receptor phase at all time intervals over a 2-hr period (3), thus assuring mixing and homogeneity in the receptor phase. In the experiments reported here, all samples were collected from the middle area of the receptor chamber. The possibility of HC binding to the synthetic membrane was checked by filtering a standard solution through the synthetic membrane and determining the concentration of HC in the filtered and unfiltered solutions. Both solutions gave the same HPLC readings (concentrations), thus eliminating the possibility of any binding of HC to the membrane. In addition, in order to eliminate the possibility that the synthetic membrane may contribute to the diffusion (partition) coefficient measurement of HC, a 2.5% solution of HC was subjected to the release rate profile experiment, substituting the solution for the cream or lotion. The HC levels in the receptor phase were seven- to eightfold higher than those achieved from the HC cream product (Fig. 1), suggesting that the permeation across the synthetic membrane is not the rate-limiting step in the course of drug release profile studies. The HC release characteristics from the cream were not influenced by the sample size (amount of the cream/thickness) used in the diffusion cell (200 mg–1g).

The *in vitro* drug release from the HC cream product was determined in the receptor fluid over a 6-hr time period using the diffusion cell and a synthetic membrane. The amount of drug released per unit area (μg/cm^2) was plotted against the square root of time. The HC release from the cream follows Higuchi's diffusion controlled model (5). The cumulative amount of drug released was linear and directly proportional to the square root of time. The slope, which represents the release rate, steady-state flux, was calculated by linear regression (Fig. 2). The correlation coefficient was greater than 0.99 in most cases.

The release rate characteristics of two HC cream products were studied using five different sources of synthetic hydrophilic membranes and three receptor media (3). The results indicated that the drug release (flux) from a given cream was not influenced by the type of the synthetic membrane used with the exception of the retardation seen with the glass-fiber filter (3). The release rate, flux, was 9.9 and 14.3 μg/cm^2/min$^{0.5}$ for Synacort and Hytone 2.5% HC cream, respectively. The difference in the flux between the products is attributed to the difference in formulation of the two products. Thus, release rate determinations can be utilized to compare formulations. Since the synthetic membranes are wetted and saturated with the receptor phase, the flux and the diffusion (partition) coefficient for HC are pri-

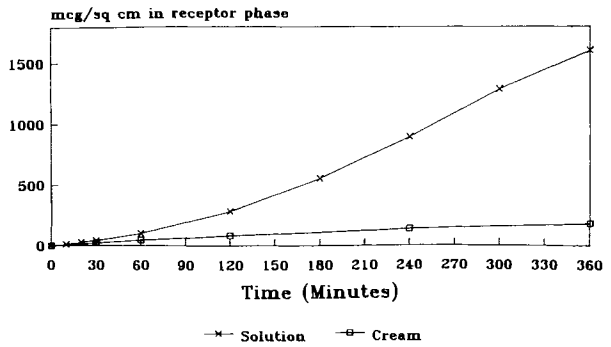

Fig. 1. Comparison of hydrocortisone concentrations in the receptor phase (7.4 buffer) from the solution and from the cream through synthetic membrane.

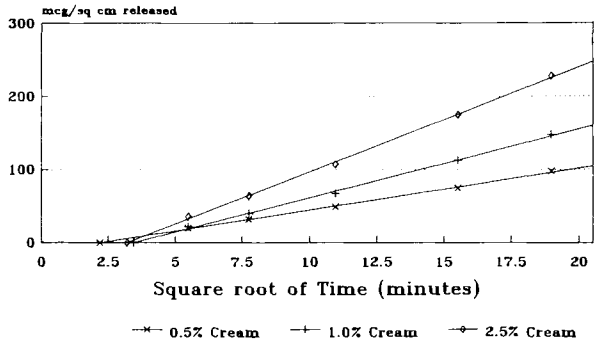

Fig. 2. *In vitro* release of hydrocortisone from 0.5, 1.0, and 2.5% cream.

marily between the vehicle of the formulation and the receptor phase. Drug release profiles of different batches from a given manufacturer were not significantly different (P = 0.05) (3). The drug release from different brands and strengths of HC cream using cellulose acetate membrane and different receptor media is shown in Table I. Using the diffusion cell with a synthetic membrane, the method can be utilized to determine the drug release characteristics from the cream formulation and, thus, can be used as a quality-control procedure to assure batch-to-batch uniformity.

The methodology developed for determining the *in vitro* release of HC creams was applied to the *in vitro* release determination of HC from lotions and ointments. The HC release data ($\mu g/cm^2$) from the lotion, using a cellulose acetate membrane, is given in Table I. No problems were encountered while studying the HC release from creams and lotions using the hydrophilic membranes. However, under the same conditions, no drug could be detected from the ointment in the receptor phase for up to 24 hr. Several different hydrophilic, hydrophobic, and neutral (glass-fiber) membranes were tried, but without any success. At this time it seemed logical to use a membrane which has both hydrophilic and lypophilic properties. The isopropyl myristate (IPM)-treated cellulose acetate hydrophilic synthetic membrane seems to acquire this mixed characteristic. IPM has been recognized as a good model compound representing skin lipids (6). Its use in studying diffusion coefficients for compounds and comparing them to *in vivo* percutaneous absorption has been well documented (7). Hadgraft and coworkers simulated epidermal barrier by using cellulose nitrate membrane impregnated with IPM and a carrier, Ethomeen S12, an ethoxylated aliphatic amine (amine), to study percutaneous absorption of caffeine and salicylate ions (8). Treating the cellulose acetate membrane with IPM enhanced the release of HC from the ointment. Various combinations of amine in IPM (0 to 50% amine in IPM) were studied to determine the optimum concentration of the carrier to

Table I. *In Vitro* Release Profile of Hydrocortisone from Creams and Lotion[a]

Product	Receptor medium	$\mu g/cm^2$ released at time (min) 30 (5.48)[b]	60 (7.75)	120 (10.95)	240 (15.49)	360 (18.97)	Slope[c]
2.5% cream (Hytone)	Normal saline	38.3	68.0	112.8	184.0	241.0	15.05
		(8.8)	(6.1)	(6.5)	(5.7)	(5.9)	
	pH 7.4 buffer	23.3	48.3	91.5	156.3	217.5	14.40
		(13.1)	(5.7)	(5.2)	(5.2)	(4.3)	
	pH 5.0 buffer	36.0	64.0	107.3	175.3	227.8	14.30
		(9.7)	(7.6)	(6.2)	(5.3)	(6.6)	
1.0% cream (Hytone)	pH 7.4 buffer	21.2	34.5	55.9	87.9	112.8	6.8
		(3.5)	(5.2)	(8.5)	(6.4)	(5.0)	
	pH 5.0 buffer	21.8	39.5	66.7	112.5	148.0	9.40
		(9.6)	(8.1)	(5.2)	(3.4)	(5.5)	
0.5% cream (Hytone)	pH 7.4 buffer	14.1	22.2	35.9	57.1	73.0	4.4
		(6.8)	(6.2)	(4.8)	(7.2)	(7.3)	
	pH 5.0 buffer	19.7	31.6	48.7	75.2	97.8	5.74
		(7.4)	(4.1)	(4.7)	(5.2)	(3.9)	
2.5% lotion (Hytone)	pH 7.4 buffer	50.0	92.0	170.0	268.0	320.0	20.6
		(7.3)	(10.2)	(3.2)	(4.4)	(3.5)	
	pH 5.0 buffer	22.0	55.0	110.0	198.0	269.0	18.40
		(13.9)	(7.0)	(5.9)	(4.8)	(5.5)	
2.5% cream (Synacort)	Normal saline	34.8	58.8	93.3	145.5	187.5	11.30
		(4.9)	(4.6)	(5.0)	(5.0)	(5.8)	
	pH 7.4 buffer	25.5	48.0	84.3	139.0	182.5	11.70
		(14.3)	(5.6)	(3.2)	(3.5)	(2.4)	
	pH 5.0 buffer	30.0	51.8	83.8	129.8	162.8	9.90
		(12.7)	(11.8)	(10.7)	(10.1)	(10.6)	

[a] Using diffusion cell, cellulose acetate membrane. The data in parentheses represent the percentage coefficient of variation; N = 6.
[b] Time as $min^{0.5}$.
[c] Slope as $\mu g/cm^2/min^{0.5}$.

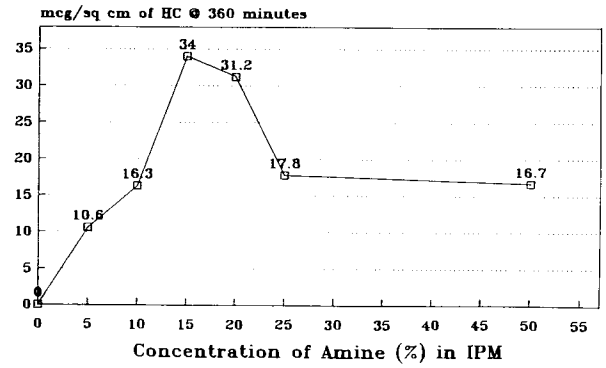

Fig. 3. The *in vitro* release profile of hydrocortisone from ointment using different amounts of amine in IPM/amine-treated membrane.

achieve the fastest release rate from the ointment (Fig. 3). The release rate of HC from the ointment increased with increasing concentrations of amine from 0 to 15% but then decreased with further increases in concentration of the amine. Thus, impregnating the cellulose acetate membrane with a solution containing 15% ethoxylated aliphatic amine in IPM significantly improved the release rate of HC from the ointment. The release rate of HC from the ointment improved when two other membranes, cellulose acetate membrane with wetting agent and hydrophobic membrane, were impregnated with amine in IPM (Table II). However, because of low release measurements, the percentage coefficient of variation was relatively high. The release rate from the two HC creams using cellulose acetate membranes with and without IPM/amine treatment in normal saline were found to be 11.3 and 10.8 $\mu g/cm^2/min^{0.5}$ for Synacort and 15.5 and 14.8 $\mu g/cm^2/min^{0.5}$ for Hytone cream, respectively. Thus, the IPM/amine-impregnated cellulose acetate membrane had no significant effect ($P = 0.05$) on the release rate of HC from the creams. The results for HC lotion were similar.

The release rate of HC was approximately the same from both the cream and the lotion but was significantly lower from the ointment (Tables I and II). This observation is consistent with the reported release rate for chlorpheniramine maleate from cream and ointment using Sartorius absorption simulator technique (9). In general, the release rate is influenced by the viscosity of the medium and should be faster from solution than from suspension, cream, or ointment (10). As discussed earlier, the release rate reflects the diffusion coefficient of HC between the vehicle and the receptor phase. The observed comparative release profile, lotion > cream > ointment, is probably in reverse order of the expected pharmacological response. In general, for the same concentration, the pharmacological response is in the order of ointment > cream > lotion (11). The application of the ointment on the skin surface prevents evaporation of water and develops a state of hydration of the skin. Hydration results in increased drug penetration. Application of creams and lotions under normal conditions (nonoccluded) allows free exchange of air and water, and the skin does not achieve the state of hydration. Thus, different formulations may result in differing amounts of drug penetration into the skin and may, thus, exhibit different intensities of activity. This suggests that the release characteristics should not be compared across types of formulations, such as creams, ointments, and lotions. All comparisons should be done only with similar formulations, e.g., among creams or among ointments or among lotions. This is similar to the dissolution data comparison, where the dissolution profile of a conventional (immediate)-release product is compared with another conventional-release product, but not against a sustained- or a controlled-release product.

The amount released vs square root of time plot showed that the release rates were 14.3, 9.4, and 5.7 $\mu g/cm^2/min^{0.5}$ from the 2.5, 1.0, and 0.5% HC creams, respectively. The release was faster from 2.5% compared to 1% creams, which in turn was faster than from 0.5% HC cream (Fig. 2).

In most cases, the release rate of the drug from the topical vehicle to the skin surface is the rate-limiting step in the overall drug diffusion process. Once the drug is presented to the skin surface, it penetrates through the stratum

Table II. *In Vitro* Release of Hydrocortisone from 2.5% Ointment (Hytone)[a]

Membrane	Receptor medium	$\mu g/cm^2$ released at time (min) 30 (5.48)[b]	60 (7.75)	120 (10.95)	240 (15.49)	360 (18.97)	Slope[c]
Cellulose acetate with wetting agent	pH 7.4	5.0	6.9	8.3	10.6	12.0	
		(19.3)	(9.9)	(14.5)	(17.9)	(19.1)	0.51
	pH 5.0	11.8	18.3	22.7	28.3	28.7	
		(13.7)	(4.5)	(4.5)	(2.9)	(5.4)	1.23
Cellulose acetate	pH 7.4	7.6	10.3	13.6	16.6	18.2	
		(4.6)	(4.6)	(8.8)	(8.3)	(9.4)	0.78
	pH 5.0	10.0	20.4	26.4	30.9	33.1	
		(15.3)	(12.4)	(8.4)	(8.0)	(8.5)	1.57
Hydrophobic	pH 7.4	3.5	4.4	5.4	6.3	6.8	
		(18.5)	(18.0)	(16.8)	(14.8)	(15.8)	0.24

[a] Diffusion-cell system with synthetic membrane impregnated with IPM and 15% amine. Data in parentheses represent the percentage coefficient of variation; $N = 6$.

[b] Time as $min^{0.5}$.

[c] Slope as $\mu g/cm^2/min^{0.5}$.

Table III. Comparison of Release Profile ($\mu g/cm^2$) of Hydrocortisone Between Manual and Automated Procedure

Time (min)	Manual mean (±RSD)	Automated mean (±RSD)	*t* statistic
30	35.1 (2.4)	36.8 (5.1)	2.03*
60	57.0 (1.8)	58.4 (4.8)	1.13
120	88.4 (1.5)	93.2 (3.3)	3.48*
240	135.0 (2.5)	144.2 (2.3)	4.76*
360	171.0 (3.1)	183.1 (2.5)	4.22*

* Not different, $P = 0.05$.

corneum for its pharmacological action. Thus, the release rate determination is an important quality-control parameter. The *in vitro* release rate of the drug determined using synthetic membranes can be utilized as a tool to assure lot-to-lot uniformity for each type of the product. Data from *in vivo* and *in vitro* studies indicate that the *in vitro* release data from the two 2.5% HC cream formulations maintain a rank order (correlation) when compared with both the pharmacokinetic and the pharmacodynamic parameters (12). During *in vitro* release experiments, the drug (HC) partitions between the vehicle and the receptor phase. When the vehicle is an oily base, nonmiscible with the receptor media, it is necessary to have the synthetic membrane impregnated with IPM/amine, which incorporates lypophilicity to the membrane. On the other hand, creams and lotions are already in an aqueous base, and IPM/amine treatment to the membrane had no effect in the partitioning of HC between the vehicle and the receptor phase.

Several commercial sources of cellulose acetate membranes were studied to explore the possible use and interchange of such membranes in routine drug release profile testing of creams. The membrane commonly referred to as cellulose acetate membrane contains mixed esters of cellulose acetate and cellulose nitrate, unlike pure cellulose acetate membrane, which contains only cellulose acetate. Unfortunately, pure cellulose acetate membranes are no longer available, due to an EPA ban on one of the chemicals needed in the manufacturing process of such membranes. Experiments with several of the marketed mixed ester membranes, as is and treated with IPM/amine, showed no significant difference in the release of HC from creams. On the other hand, for the ointment, it was necessary to treat the membrane with IPM/amine. Therefore, to simplify the procedure cellulose acetate/cellulose nitrate mixed ester membrane impregnated with IPM and 15% amine can be used for all HC topical products with pH 5.0 phosphate buffer as the receptor medium.

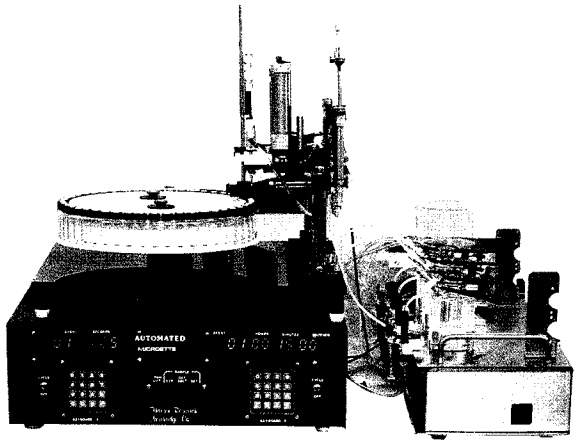

Fig. 4. A Microette autosampler interfaced to the Franz diffusion-cell system.

Automated Sample Acquisition and Analysis

In order to simplify the procedure, to avoid the artifacts due to the operator, and to reproduce the experimental results, it is desirable to automate the procedure. In addition to automating the experimental procedure, the sample analysis and data analysis can also be automated. The *in vitro* release profiles obtained with both the manual and the automated procedure did not differ significantly at most time points (Table III). The diffusion-cell system was interfaced with the Microette automated sampling system for sample collection, analysis, and data management (Fig. 4). Using this system, it was possible to remove the samples (100 to 500 µl) as frequently as needed and, at programmed time intervals, refill the receptor medium automatically after sample withdrawal (this is necessary because the membrane must be in contact with the receptor medium for diffusion to take place), and transfer the aliquot into microvial inserts in the auto injector carousel. The samples are automatically injected into the HPLC using the WISP for automatic HPLC analysis.

In this report, the release characteristics of HC from creams, ointment, and lotion have been presented. The use of synthetic membranes minimizes the variability observed with animal or human skin membrane. The method developed shows promise as a tool for comparison of *in vitro* release profiles of creams, ointments, and lotions, with possible future applications to ophthalmic and other products. The method can be employed as a quality-control procedure for assuring batch-to-batch uniformity of topical products.

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