Stability of Two Antifungal Agents, Fluconazole and Miconazole, Compounded in HUMCO RECURA Topical Cream to Determine Beyond-use Date

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ABSTRACT

A novel compounding vehicle (RECURA) has previously been proven to penetrate the nail bed when compounded with the antifungal agent miconazole or fluconazole, providing for an effective treatment for onychomycosis. In this study, miconazole and fluconazole were compounded separately in RECURA compounding cream, and they were tested at different time points (0, 7, 14, 28, 45, 60, 90, and 180 days), determining the beyond-use date of those formulations. The beyond-use date testing of both formulations (10% miconazole in RECURA and 10% fluconazole in RECURA) proved them to be physically, chemically, and microbiologically stable under International Conference of Harmonisation controlled room temperature (25°C ± 2°C/60% RH ±5%) for at least 180 days from the date of compounding. Stability-indicating analytical method validation was completed for the simultaneous determination of miconazole and fluconazole in RECURA base using high-performance liquid chromatography coupled with photodiode array detector prior to the study.

INTRODUCTION

Onychomycosis is a fungal infection of the nail bed in the fingers, or more commonly the toes, which affects an estimated 10% of the world's population.[1] *Trichophyton* is the typical fungal genus that causes these infections in Western countries, while those living tropical regions experience *Candida, Aspergillus,* or *Scytaldium* infection,[2] but the symptoms of these infections are similar across the board. Minor infection causes a yellow or black thickening of the nail bed, while further progression can result in the nail chipping away and leaving an open sore, leading to secondary infection. Without treatment, these infections can cause problems beyond the cosmetic, with infection spreading onto the whole digit, which might lead to difficulty walking or doing manual tasks.[1]

Treatment of onychomycosis is notoriously difficult. While safe and effective anti-fungal medications have been developed, the challenge is to deliver these medications effectively to the site of action—underneath a nail. Oral treatment with an anti-fungal can deliver the medication to the site of action. However, to achieve high enough concentration under the nail bed to kill the fungus, a patient must ingest large amounts of these drugs, which can have side effects including liver and kidney toxicity.[3] Terbinafine oral treatment has been shown to be effective (76% cure rate versus itraconazole's 60% cure rate and fluconazole's 48% cure rate),[1] yet concern about long-term dosing and severe side-effects due to oral administration exists. Topical treatment, and the accompanying reduction in serious side-effects, is preferred, but topical treatment requires patient compliance to apply the medication daily for up to a year for complete treatment.

Topical treatments for onychomycosis vary in cost and efficacy. Nail paints and lacquers (e.g., ciclopirox) have been used, mostly with low degrees of efficacy. Topical creams containing azole type antifungals have proven to show better efficacy, but those treatments don't have success in all cases.[4] Azole antifungal drugs are powerful in destroying fungus, as they inhibit the enzyme lanosterol 14 α-demethylase; the enzyme necessary to convert lanosterol to ergosterol. Depletion of ergosterol of the fungal membrane disrupts the structure and many functions in fungal membrane leading to inhibition of fungal growth. These medications must be in direct contact with the fungus in order to kill it.[5] The FDA-approved newer azole antifungals have been shown to be only slightly more effective, specifically for onychomycosis, than other treatments. Topical efinaconazole (Jublia), while having a cure rate of 15% to 17% and being two to three times more effective than ciclopirox, still does not cure over half of the fungal infections of this type.[6]

In order to deliver the azole medication to the site of action, the carrier vehicle is very important, penetrating through keratinized skin and thick nail plates that form the nail bed in order to be delivered effectively. A compounded prescription, formulated in such a vehicle, would be appropriate for the treatment of onychomycosis, tailored with the specific anti-fungal that treats the individual dermatophyte. RECURA is a compounding base designed to allow for penetration of anti-fungal medications to subungual tissues.[7]

Prescribed compounding drugs are expected to be efficacious and safe at the same time, and compounding pharmacies have the responsibility to assure their patients that their compounded preparations are stable over a given time frame.[8] Beyond-use date (BUD) is the estimation of time interval or the date after which a compounded prescribed preparation should not be used, and it is determined from the date the compounder or pharmacist compounded the prescription drug.[8,9] Pharmacists are responsible for assigning a BUD based on the guidelines described in *United States Pharmacopeia (USP)* Chapter <795> Pharmaceutical Compounding—Non-Sterile Preparations. The maximum BUD of any compounding preparation is up to six months, however, a shorter BUD is generally recommended, especially for those drugs that are known to be labile to decomposition.[9] Stability studies have been published to show the stability of certain actives in various compounding vehicles over time,[10,11] so the aim of the work presented here is to show the BUD stability for the two antifungal agents miconazole and fluconazole in RECURA topical compounding vehicle.

MATERIALS AND METHODS

MATERIALS AND CHEMICALS

Miconazole, USP grade (Lot 2EG0292) (Figure 1), and high-performance liquid chromatography (HPLC)-grade water (Lot 4506B48) were purchased from Spectrum Chemicals (New Brunswick, New Jersey) and Fluconazole, USP grade (Lot X4YGD) (Figure 2), was purchased from Tokyo Chemical Industry Co. Ltd. (Portland, Oregon). Potassium phosphate monobasic monohydrate salt (Lot 201017511) and 1N NaOH (Lot HC56320819) were purchased from EMD Chemicals (Gibbstown, New Jersey). Acetonitrile (Lot 158054), HPLC grade, was acquired from Fisher Scientific (Fairlawn, New Jersey). RECURA antifungal compounding cream (Lot A12628) as a placebo was supplied by HUMCO Compounding (Texarkana, Texas). A Waters HPLC Model 2695 coupled with a photodiode array detector (PAD) (Model 2996) was used for the analytical tests for potency. The analytical column (P/N 00F-4435-E0; Gemini, C18 5 µm 150 mm) was purchased from Phenomenex (Torrance, California), a Mettler-Toledo pH meter (Model SevenMulti) was used for the pH determination. Additionally, a Brookfield DV-E Viscometer (Model LVDVE115; Middleboro, Massachusetts) and analytical balance (AL-201S; American Weight Scale, Cumming, Georgia) were also used for sample testing during the stability study.

FIGURE 1. CHEMICAL STRUCTURE OF MICONAZOLE.

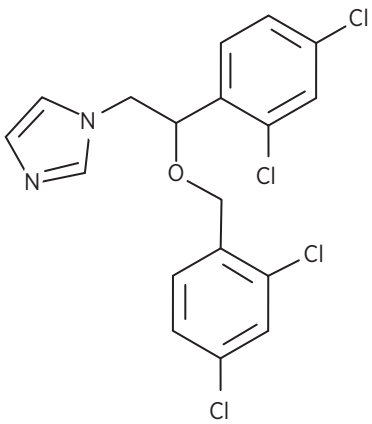
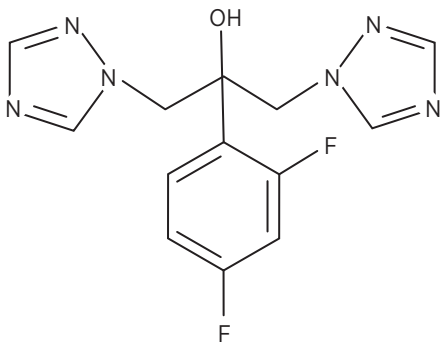


FIGURE 2. CHEMICAL STRUCTURE OF FLUCONAZOLE.



PREPARATION OF MICONAZOLE AND FLUCONAZOLE CREAMS

A bulk amount (approximately 250 g) of each formulation (miconazole 10% in RECURA and fluconazole 10% in RECURA) was prepared and divided into individual samples contained in polypropylene ointment jars (50 mL) with foiled lined caps. Samples from each bulk batch were also stored in unguator jars (30 mL) to assess the air permeability of the container.

SAMPLE TESTING AND TESTING INTERVALS

All BUD samples were placed in a stability chamber equilibrated to International Conference of Harmonisation (ICH) controlled room temperature 25°C ± 2°C/60% RH ±5%. Samples for BUD analysis were fully tested for all the listed requirements (Table 1) at the following time points: 0, 7, 14, 21, 28, 28, 45, 60, 90, and 180 days. At each time point, samples were removed from the stability chamber and weighed to determine the weight loss (evaporation) due to container permeability. Samples in the jars were evaluated for organoleptic properties and visually for separation prior to mixing the sample with a glass rod. Additionally, the potency of both formulations was also carried out for the sample in the unguator jar to determine if the container permeation had an effect on the preparation over time (at day 14 and day 28).

For microbiological stability, jars of both formulations were analyzed at day 0 and day 180 for different microbiological tests including: Total Aerobic Microbial Count (TAMC) (as per *USP* <61>), Total Combined Yeast and Mold (TCYM) (as per *USP* <61>), *Staphylococcus aureus (S. aureus)*, and *Pseudomonas aeruginosa (P. aeruginosa)* (both as per *USP* <62>).

CHROMATOGRAPHIC CONDITIONS

Assay of miconazole and fluconazole in their respective formulations for BUD study was determined using a validated stability-indicating HPLC method. The HPLC system was a Waters 2695 Series coupled with a quaternary pump, degasser, thermostated column compartment, auto-sampler, and PAD. For data acquisition and processing, the Empower-Pro Software was used. The Gemini C18 Column was used for the assay analysis. 0.03M potassium

phosphate monobasic buffer at pH 5.5 was prepared as a mobile phase solution A, and HPLC-grade acetonitrile was used as a solution B. Buffer was filtered through vacuum filtration through 0.45-µm pore size membrane filters. Column temperature was maintained at 40°C and an injection volume of 10 µL was used. A solvent gradient elution was programmed in the HPLC at the flow rate of 1.0 mL/min and is shown in Table 2. The data was processed and analyzed for miconazole and fluconazole assay at the wavelengths 230 nm and 260 nm, respectively. Diluent was prepared by mixing 30:70 Solution A: Acetonitrile. Samples were analyzed at 15°C for a 15-minute run time.

TABLE 1. TESTS CONDUCTED FOR BEYOND-USE DATE STUDY.

TEST	METHOD	SPECIFICATION	TESTING INTERVAL
Description/Physical Form/Odor	Organoleptic	Must match initial description No evident separation or stratification	All Time Points
Feel	Tactile	No grittiness or crystallization	All Time Points
Assay	HPLC	90% to 110% of Label Claim	All Time Points
pH (neat)	*USP* <791>	Report pH	All Time Points
Viscosity	Viscometer	Report viscosity	All Time Points
Weight Loss	Initial weight minus final weight	Report weight loss	All Time Points
Density	Pycnometer	Report	All Time Points
Total Aerobic Microbial Count	*USP* <61>	≤200 cfu/mL	Time 0 and 180 days
Total Combined Yeast & Mold	*USP* <61>	≤100 cfu/mL	Time 0 and 180 days
Staphylococcus aureus	*USP* <62>	Absent	Time 0 and 180 days
Pseudomonas aeruginosa	*USP* <62>	Absent	Time 0 and 180 days

HPLC = high-performance liquid chromatography; *USP = United States Pharmacopeia*

TABLE 2. MOBILE PHASE GRADIENT PROGRAMMING IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY.

TIME (MIN)	FLOW RATE (ML/MIN)	% SOLUTION A (BUFFER)	% SOLUTION B (ACETONITRILE)
0.00	1.00	70.0	30.0
3.50	1.00	70.0	30.0
3.51	1.00	25.0	75.0
13.00	1.00	70.0	30.0
15.00	1.00	70.0	30.0

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY STANDARD, SAMPLE, AND PLACEBO PREPARATION

Stock standards (1.0 mg/mL nominal concentration) of miconazole and fluconazole were prepared by weighing and diluting (by diluent) both standards in the volumetric flask. Stock solutions were diluted to make a 0.2-mg/mL concentration of both analyte as a working standard. Placebo (topical cream RECURA without actives) and two RECURA formulations were prepared by weighing 1.0 g of sample into a 100-mL volumetric flask. The aliquots were sonicated and further diluted to maintain the final analyte concentration of 0.2 mg/mL. All the working sample solutions were filtered through a Phenomenex 0.45-µm polytetrafluoroethylene syringe filter prior to the analysis.

CHROMATOGRAPHIC ACCEPTANCE CRITERIA

Acceptance criteria were set for the acceptance of the chromatographic results. For example, the independently prepared duplicate standard must have a comparison of 98% to 102% recovery. The relative standard deviation (RSD) of the peak area responses of miconazole and fluconazole for five consecutive injections at the beginning of the run for the working standard solution must be ≤2%. The overall RSD of the peak area responses of fluconazole and miconazole in all of the working standard solution injections must be ≤2%. The theoretical plates for miconazole and fluconazole in the working standard solution must be Symbol 2000. The resolution between any active peak and any other peak of interest should be ≥2. The tailing factor for miconazole and fluconazole in the working standard solution must be Symbol 2. No interference should be observed at the retention time of miconazole and fluconazole in the blank and placebo solutions. Expected retention times of fluconazole and miconazole are 2.9 minutes and 10.7 minutes, respectively. Example chromatograms of miconazole in RECURA base and fluconazole in RECURA base are shown in Figures 3 and 4.

VALIDATION OF ANALYTICAL METHOD

The analytical method for the simultaneous determination of miconazole and fluconazole in the novel compounding cream RECURA was validated, with all method validation parameters (i.e., linearity, accuracy, specificity, robustness, precision, repeatability, reproducibility) successfully meeting established acceptance criteria prior to analysis of the compounded preparation for the BUD

FIGURE 3. EXAMPLE CHROMATOGRAM OF FLUCONAZOLE IN RECURA TOPICAL CREAM SAMPLE.

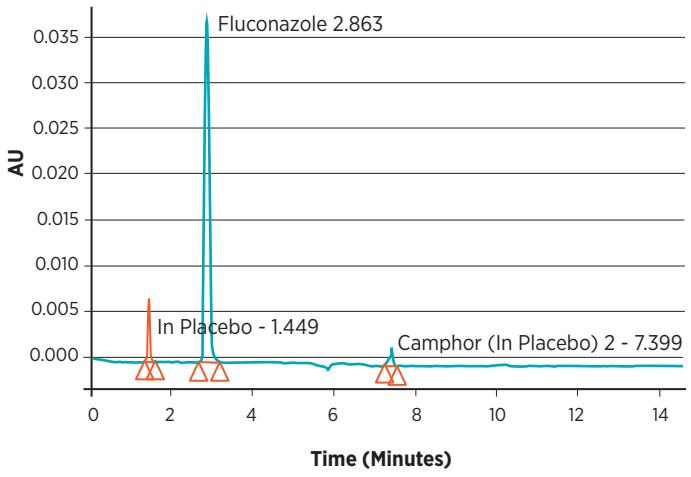
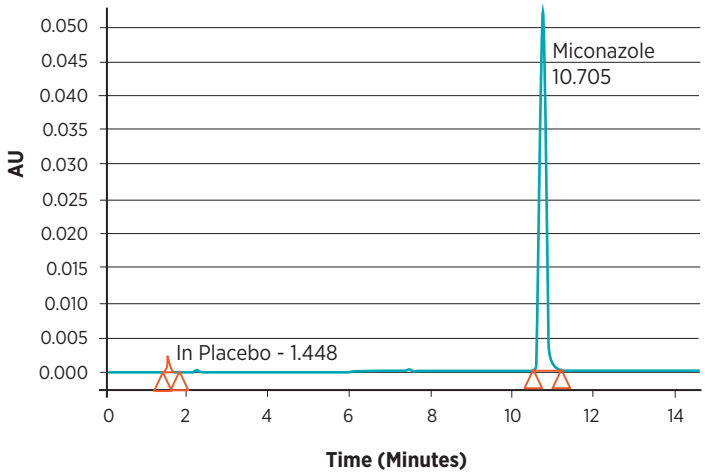


FIGURE 4. EXAMPLE CHROMATOGRAM OF MICONAZOLE IN RECURA TOPICAL CREAM SAMPLE.



study. The method specificity included evaluation of the blank and placebo preparations for interference with the analytes' peaks of interest, as well as evaluation of the forced degradation of the active, active-containing formulation, and excipient matrix under stressed conditions. The method validation elements met the acceptance criteria set forth in *USP* <1225> Validation of Compendial Procedures and ICH guidelines in *ICH Publication Q2 (R2) Validation of Analytical Procedures*.

The forced degradation study was conducted to assure that there is no interference of any possible degradation peaks with each analyte's peak of interest. The individual pure actives, active-containing compounds, and placebo matrix were subjected to stress conditions of hydrolysis (acid and base), oxidative, and thermal stress degradation. The stress conditions for the degradation study comprised of heat (90°C), acid hydrolysis (1N hydrochloric acid), base hydrolysis (1N sodium hydroxide), and oxidation (0.3% hydrogen peroxide). For each forced degradation condition, the pure actives, active-containing compounds, and placebo matrix were stressed for a period of 24 hours. After analyzing the samples produced at each stress condition using the HPLC method, the percent recovery of each analyte was identified for each condition.

Both analytes, miconazole and fluconazole, appeared to be stable under the forced degradation conditions that were applied, and both actives did not appear to be reactive under acidic, basic, oxidative, and elevated temperature conditions. For the pure actives, the miconazole active percent recoveries in acidic, basic, oxidative, and heat conditions were 98%, 100%, 103%, and 104%, respectively, and the fluconazole active percent recoveries in such conditions were 100%, 95%, 102%, and 101%, respectively. No additional degradant peaks were observed when stressing the individual pure actives. The characteristic peroxide peak was observed around the retention time of 1.8 minutes (close to solvent front) in the peroxide degradation. Similarly, two antifungal formulations (miconazole compounded in RECURA and fluconazole compounded in RECURA) also appeared to be stable, showing no additional degradant peaks over the HPLC method's analysis time. Miconazole in RECURA formulation showed percent recoveries in acid, base, oxidation, and heat conditions of 90%, 101%, 101%, and 69%, respectively. There is no explanation made for the low percent recovery of miconazole in acidic and heat stress conditions, as there were no apparent degradation peaks which appeared in the chromatogram. In contrast, fluconazole in RECURA formulation showed complete recovery under the stress conditions: 103% (acid), 102% (base), 104% (oxidation), and 100% (heat). Following forced degradation of the placebo matrix, no interference peaks were observed during analysis. Like the other method validation elements, the specificity (no degradant peaks interfered with the analytes' peaks of interest) met acceptance criteria for the method validation. This stability-indicating HPLC method was, therefore, validated and shown to be appropriate for testing samples for BUD.

RESULTS AND DISCUSSIONS

PHYSICAL TESTING

All the samples from two RECURA formulations (10% miconazole and 10% fluconazole) were evaluated for their physical attributes including their visual look, odor, feel, pH, viscosity, and density for all time points as described in the BUD study design. Detailed information is shown in Table 3.

Both formulations of antifungal RECURA topical cream (10% miconazole and 10% fluconazole) were off-white, and non-flowable at the time of compounding (day 0). No changes were observed in

overall physical appearance, odor, and feel of those creams in their respective jars in every analyzed time point. No evident separation, stratification, grittiness, or crystallization occurred over time. These properties met specifications set forth in the BUD protocol. Both formulations had a similar pH trend over time with the average pH 8.92 ± 0.3 (miconazole 10%) and pH 8.83 ± 0.3 (fluconazole 10%). Average viscosity, measured by a Brookfield Viscometer spindle 'F' at 2.5 rpm, for miconazole 10% and fluconazole 10% was 167,529 ± 28,481 cps and 186,778 ± 20,036 cps, respectively. An aluminum-alloy pycnometer was used to determine the density of the cream at all time points. The density of both miconazole 10% and fluconazole 10% creams was measured to be 1.08 ± 0.01 g/mL. Up to 0.6 g (approximately 4% of actual sample) of each analyzed cream sample was lost in both formulations throughout the BUD analysis. The average weight loss in 10% miconazole formulation was 0.26 ± 0.2 g and in fluconazole formulation was 0.25 ± 0.32 g throughout the 180-day study period. These tests were reported for each time point, and no specification was given for these evaluations.

TABLE 3. PHYSICAL TESTING RESULTS OF 10% MICONAZOLE AND 10% FLUCONAZOLE FORMULATIONS.

TESTS	MICONAZOLE 10% IN RECURA	FLUCONAZOLE 10% IN RECURA
Visual and Odor[a]	Met Criteria	Met Criteria
Feel[a]	Met Criteria	Met Criteria
pH (neat)[b]	pH 8.92 ± 0.3	pH 8.83 ± 0.3
Viscosity[b]	167,529 ± 28,481 cps	186,778 ± 20,036 cps
Weight Loss[b]	0.26 ± 0.2 g	0.25 ± 0.32 g
Density[b]	1.08 ± 0.01 g/mL	1.08 ± 0.01 g/mL

[a]Overall testing results of all time-points

[b]Average data of all time-points ± standard deviation (n = 9)

TABLE 4. MICROBIOLOGICAL TESTING RESULTS OF BOTH RECURA FORMULATIONS.

MICROBIOLOGICAL TESTS	MICONAZOLE 10%		FLUCONAZOLE 10%	
	Day 0	Day 180	Day 0	Day 180
Total Aerobic Microbial Count	0 cfu/mL	0 cfu/mL	0 cfu/mL	0 cfu/mL
Total Combined Yeast & Mold	0 cfu/mL	0 cfu/mL	0 cfu/mL	0 cfu/mL
Staphylococcus aureus	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent	Absent

MICROBIOLOGICAL TESTING

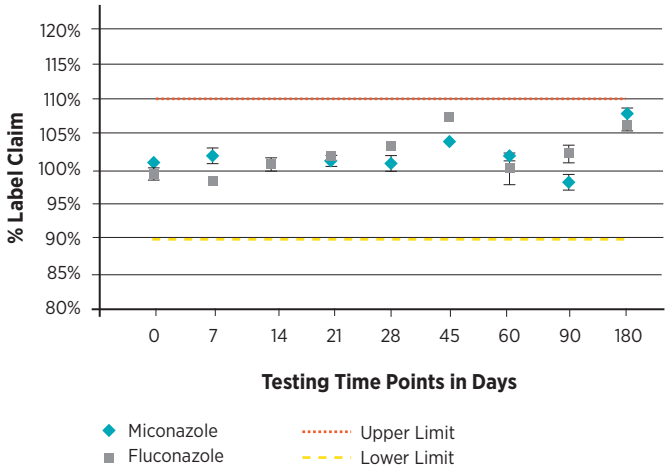
As listed in Table 1, four microbiological tests (TAMC, TCYM, *S. aureus*, and *P. aeruginosa*) were detailed in the BUD protocol. Results of both RECURA formulations at day 0 and day 180 showed no microbiological growth (Table 4), meeting the specification.

ASSAY TESTING

USP-grade miconazole (free base) and fluconazole (free base) standards were used for the calibration and quantification purpose. Standard solutions, including duplicate standard, were prepared in each time-point prior to preparation of the samples for assay analysis. Before the quantification of actives in the samples for the determination of their assay, all the system suitability criteria was checked to ensure the data quality in the HPLC. Compounded samples were analyzed with a HPLC-PAD using a stability-indicating HPLC method validated prior to the stability study. Blank and placebo samples were also run in the instrument while analyzing assay of both actives in all time points. This was done to ensure that no peaks are present at the retention time of the miconazole and fluconazole in the blank and placebo run. In other words, there should be no interference from blank and placebo peaks with any analyte peaks. In each stability time point, duplicate samples were prepared with duplicate injections and acquired the assay (% w/w) of both actives in the creams. Average percent label claim along with overall standard deviation of each actives were reported for the final assay.

The assay (potency) of miconazole and fluconazole in RECURA formulations appeared to be stable (average % label claim (mean ± standard deviation) 101.6% ± 2.9% and 102.2% ± 3.0%, respectively) over the six-month stability study under normal conditions. At time 0, the percent label claim of the assay was 100.8% ± 0.3% (miconazole) and 99.30% ± 0.6 % (fluconazole). After 180 days of the formulation, the percent label claim of the assay of miconazole 10% formulation was 108.0% ± 1.0%, and fluconazole was 106.2% ± 0.2%. No degradation of any actives in both RECURA formulations was observed. The assay results of both actives over the stability study period were within the specification (90% to 110% label claim) (Figure 5). The higher concentrations of actives obtained were probably due to the slow evaporation of the cream in the sample jars while sitting in the stability chamber at 25°C, which is reflected in the sample weight loss over the course of the stability study. The percent label claim of the assay results from the unguator samples that were tested at day 14 and 28 were miconazole 102.1% and 102.4%, respectively. Similarly, the percent label claim of the assay results of fluconazole samples in unguator jars at day 14 and 28 were 101.9% and 103.4%, respectively. This concludes that the container permeation of the unguator jar did not have significant

FIGURE 5. MICONAZOLE AND FLUCONAZOLE ASSAY PERCENT LABEL CLAIM VS TESTING TIME INTERVALS.



effect on the potency of the two actives in RECURA topical cream over time (at day 14 and day 28). This study confirms that the two antifungal agents, miconazole and fluconazole are chemically stable for at least six months while compounded in the RECURA cream.

CONCLUSION

Two anti-fungal agents, miconazole 10% and fluconazole 10%, compounded in HUMCO RECURA anti-fungal topical cream were analyzed at different time points (0, 7, 14, 21, 28, 45, 60, 90, and 180 days) for the BUD study. No significant changes in appearance, odor, or feel were observed during the BUD study period. Both formulation's pH, viscosity, and density values were reported according to the BUD protocol. Miconazole and fluconazole in RECURA topical cream formulations are therefore considered physically stable over the BUD period of 180 days. Analytical quantification of miconazole and fluconazole in RECURA cream by HPLC showed that the preparations are within the acceptable specification (label claim: 90% to 110%). Therefore, these two formulations are chemically stable for a 180-day period. Additionally, microbiological testing of TAMC, TCYM, *S. aereus* and *P. aeruginosa* during the 180-day study period under ICH controlled room temperature showed no microbiological growth, meeting the specifications. Therefore, the two antifungal agents studied (miconazole and fluconazole) are physically, chemically, and microbiologically stable in the novel compounding vehicle RECURA for at least 180 days.

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