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The role of ointment base on stability of dexketoprofen trometamol in ointments

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ABSTRACT: The stability of the active pharmaceutical ingredients (API) and the final product is one of the most important factors in the design and development of drug forms. The storage conditions stated on the product label arise from the evaluation of the scientific data extracted from stability studies. In this study, the effect of ointment base excipients on stability was conducted, besides the effect of freeze-thaw tests on product stability. Dexketoprofen trometamol which is a highly soluble active substance in the water was selected as a model drug within the scope of this study. It was formulated as a semi-solid dosage form with different types of bases (an oil-based, a water-based, and emulsion-based ointment excipients). The stability tests in long-term and accelerated conditions were performed on each product at certain periods. The commercial product of dexketoprofen trometamol (Dexalgin gel) was also evaluated. The loss of API in the ointment with high oil content was higher than the others in both long term and accelerated stability test conditions. The active ingredient loss was higher than 5% even after the first freeze-thaw process for all formulation including commercial product in gel form. After two cycle-freeze-thaw processes, the amount of API was dramatically changed (from 18% of degradation in even commercial gel products, which were the most stable formulation in this study). It was presented that freeze-thaw processes were much more crucial than the two-month storage in high temperatures (i.e. 40°C).

KEYWORDS: Stability; dexketoprofen trometamol; semi-solid products; topical formulations; freeze-thaw.

1. INTRODUCTION

Stability is defined as retaining the properties and characteristics of pharmaceutical product within the specified limits at the time of its manufacturing, throughout its period of production, transportation, storage, and use [1]. The stability of the active pharmaceutical ingredients (API) and the product is one of the most important factors in the design and development of drug forms that provides insight into the product quality. The loss of potency of the drug, modifying efficacy, loss of content uniformity, contamination, loss of pharmaceutical elegance or patient acceptability may occur in product, so the drug substance and/or product may become instable. A variety of reasons which causes instability can be sorted: i. environmental factors (e.g. temperature, humidity and light), ii. product related factors (e.g. physical and chemical properties of API, pharmaceutical excipients, the dosage form and its composition, the potential interaction between active and inactive ingredients, manufacturing process, the nature of the container closure system and properties of packaging material, and so forth) iii. chemical factors and catalysts (e.g. oxidation, reduction, hydrolysis, or racemization), iv. other factors (e.g. the duration between manufacture and usage, other conditions during shipment, storage, and handling) [2–4].

Stability testing assesses the role of environmental factors in the quality of a drug substance or a final product. Thus, designing final formulation, proper packaging, appropriate storage conditions, and the shelf life of product can be determined, besides, the regulatory requirements can be met [5]. According to the The International Council for Harmonization (ICH) Q1A (R2) Stability Testing of new Drug Substances and Products Guidelines, the stability studies include the long term testing (min. 12 months) and accelerated stability studies (min. 6 months) [6]. The ICH guideline also states the stress testing which identifies the degradation products, although details about the practical approach are not provided. This may because the degradation condition such as hydrolytic, oxidation, photolytic or thermal are specific in each case [7]. For

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instance, freeze-thaw studies for liquid products in general, and/or cyclic studies for semi-solid dosage forms are used as additional stress testing [8].

Dexketoprofen trometamol (DT) is the effective enantiomer of ketoprofen which is potent *in vitro* inhibitors of prostaglandin synthesis [9]. DT is used for the treatment of osteo-arthritis, low back pain, post-operative pain, toothache and dysmenorrhea. The tromethamine salt improves the drug absorption and thus promotes rapidity of action. DT rapidly dissolves and it is highly permeable [10, 11]. DT is formulated in various drug forms such as tablet, gel, solution, sachet or granule.

In this study, we aimed to investigate the potential effect of ointment base on product stability. We produced five different ointments based on four general classes of ointment bases to be used therapeutically that recognized by the United States Pharmacopeia (USP). The long term testing and accelerated stability studies were performed, after we dispersed DT into these vesicles. In the meantime, the pH changes were recorded. We evaluated the freeze-thaw effect on formulation stability. All studies were also performed on the DT included commercial gel products for comparison.

2. RESULTS AND DISCUSSION

2.1. Preparation of ointments

Ointments are defined as semi-solid preparations intended for external application to the skin or mucous membranes. Each therapeutic ointments possesses its base as a vesicle of API. Many factors like action desired, bioavailability, stability, desired shelf life of the final product determine the choice of an ointment base. Traditionally, ointment bases categorize four general classes; the hydrocarbon bases, the absorption bases, the water-removable bases, and the water-soluble bases [12]. White Ointment (USP 27 – the National Formulary 22 (NF22)) is classified as hydrocarbon bases which are highly oleaginous and have occlusive properties. Simple Ointment (BP 1999) is a member of the absorption bases which are oleaginous but capable of liquid absorption and has good emollient properties. Cold Cream (USP 21 – NF 16) is another member of the absorption bases. It has a soap-type of emulsifiers and is categorized as a water-in-oil emulsion sub-group. Hydrophilic Ointment (USP 27 – NF 22) is classified as a water-removable base. It is oil-in-water emulsion (it is also classified as a cream) and non-greasy. Polyethylene Glycol Ointment typifies the water-soluble bases that are greaseless [13]. To investigate the effect of ointment base on stability, we prepared five different ointments as a representative of each classes so that the effect of the oil or water content in ointments on stability can be demonstrated. DT, which is highly water-soluble salt, was homogenously dispersed into these ointments at the last stage of classical fusion method. The amount of DT was constant to 1.25% of each formulation to compare to the commercial gel formulation.

After preparation of ointments, some preliminary studies were performed to eliminate the potential interaction between product and primer packaging. Firstly, the commercial product was put into a plastic containers and was kept at 40±2°C for 10 days. The drug content was determined using dialysis bag method (see section 4.7). The percentage of DT in commercial product decreased to 68.4±1.3% within 10 days. Products in plastic containers may suffer from the rate of moisture vapor loss, tightness of closures, the extraction or release of materials from the polymer, potential interaction between polymers within the plastic container and API, incapability of the protection of the product from external factors [14]. It was revealed with this study that plastic container was not appropriate as a primer packaging. Apart from the plastic containers, glass containers will not react with their content and impermeable to water and air [15]. Glass containers were used as primer packaging for all prepared samples. The commercial product was kept its original packaging.

2.2. Physical controls

The presence of discoloration, water loss and viscosity, appearance and, homogeneity were visually evaluated for prepared ointments and commercial product. There were no prominently differences in samples as a matter of color and water content or viscosity with regard to visual evaluations, for the first trimester in terms of long-term stability. For the first three months the absence of conspicuous discoloration, water loss and heterogeneity in samples kept in accelerated stability conditions seemed as an indicator of good stability, however some signs of losing homogeneity were obtained in oily samples, in particularly two phase systems like simple ointment or cold cream. Solid oily droplets were partly seen in those samples. The changes of appearance and homogeneity in samples at 6th months were summarized at Table 1.

In general, single phase systems like white ointment with fully oily nature or polyethylene glycol ointment and the commercial product with fully water-based excipients protected their appearance and homogeneity. Although no physical separation was observed in polyethylene glycol ointment or the

commercial product during stability evaluation at real time studies as well as accelerated stability studies, other samples with two phase noticeably suffered the preservation of physical properties.

Table 1. Physical evaluation of ointment stability at 6th month.

	Description			
	Appearance		Homogeneity	
	Long Term	Accelerated	Long Term	Accelerated
White Ointment	Oily and dry	Oily	Homogenous	Homogenous
Simple Ointment	Oily	Oily	Heterogeneous	Separation
Cold Cream	Watered	Dry	Separation	Solid droplets
Hydrophilic Ointment	Foamy	Foamy	Solid droplets	Solid droplets
Polyethylene Glycol Ointment	Foamy	Foamy	Homogenous	Homogenous
Commercial product	Transparent	Transparent	Homogenous	Homogenous

2.3. Stability studies

As a routine procedure, stability testing on samples was performed, according to the regulations in ICH Q1C (Stability Testing: Requirements for New Dosage Forms) which is mainly based on ICH Q1A (R2). The long-term stability studies and the accelerated stability studies were performed. The former reflects real-time stability for longer duration of the test period, while the latter indicates the product behavior and its potential failure at high temperature to predict shelf life or compare the relative stability of alternative formulations [16]. Six samples (five various ointments and the commercial gel product) were kept in two different climatic chambers and the drug content in samples was measured. Time-dependent changes of the amount of DT in samples stored at room temperature were given in Figure 1.

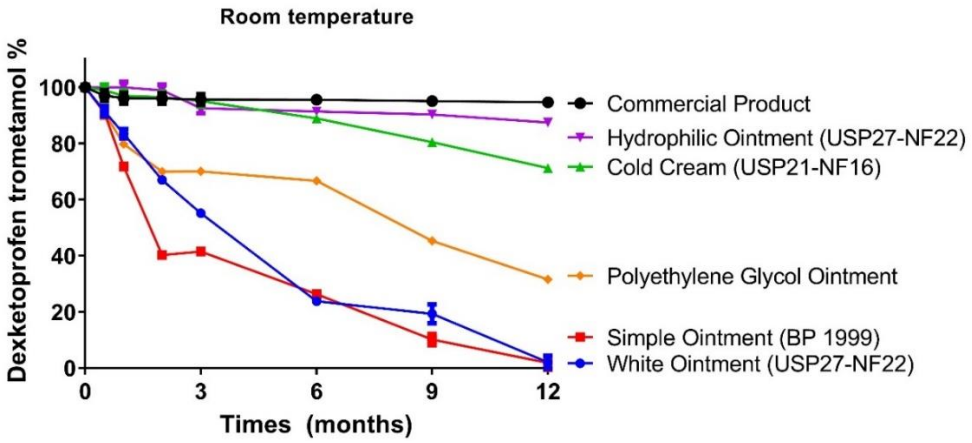

Figure 1. Long term stability test results pointed the changes in the amount of dexketoprofen trometamol in samples

The commercial gel product was the most stable preparation. Other ointments were in order: hydrophilic ointment, cold cream, polyethylene glycol ointment, simple ointment and white ointment. There were no significant differences between commercial product and hydrophilic ointment ($p>0.05$), and between simple ointment and white ointment. Other comparisons were found to be significant ($p<0.05$). It was seen that the degradation rate of drug was high in oily samples. However, the amount of DT in cold cream was not very low, although it was classified as the absorption bases which are oleaginous. The reason of that may be related to inclusion of soap-type emulsifiers. Beeswax and sodium borate forms anionic emulsifier, which is also called in-situ emulsifier. This interaction resulted cream more stable. The other ointments in two phase system (i.e. hydrophilic ointments or cold cream) showed better stability than others. However, none of prepared ointments could represent improved stability than the commercials. The commercial products include various polymers such as Carbopol 974P, Macrogol. The high-chain polymers may help to protect the API.

The accelerated studies are used to make the product stress at several high temperatures and the degradation in early stages evaluates to determine the recommended long-term shelf life and expiration dates [16]. The accelerated stability test results were given in Figure 2.

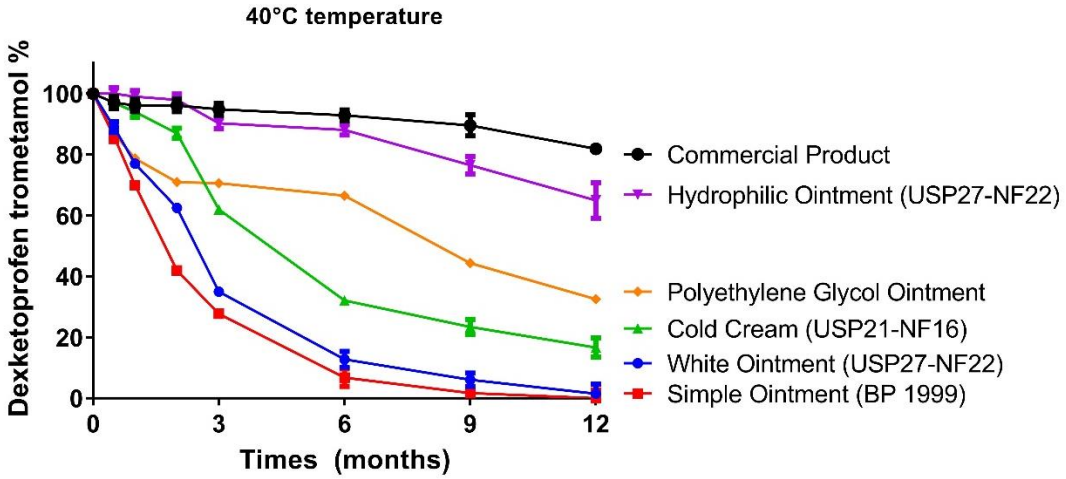

Figure 2. Accelerated stability test results pointed the changes in the amount of dexketoprofen trometamol in samples.

The similar results to long-term stability were obtained in accelerated test conditions. The differences between ointments were significantly important (p:0.0168 between hydrophilic ointments and commercial product; p<0.0001 for all other comparisons). The degradation of DT was quite attention-grabbing for all oleaginous ointments. The main differences were observed in cold cream. The amount of DT significantly decreased to 34.5±1.0% in sixth month and reached to 16.7±3.1% in a year. After two months, its degradation accelerated. The possible reason of that might be the distinctly negative impact on oily base of cold cream because of high temperature. Oily bases are prone to deteriorate by reason of rancidity [17, 18]. It was obvious that the degradation behavior of white ointment, simple ointment and cold cream for DT followed first-order kinetics. On the other hand, the samples with high hydrophilic nature showed higher stability. Outer water phase in hydrophilic ointment which are o/w emulsion might block the rancidification. Surprisingly, the amount of DT in polyethylene glycol (PEG) ointments which have a fully aqueous composition was lower than hydrophilic ointments. The explanation of it might be the length of polymer chain in PEG. The commercial products comprise of PEG 8000 and other long-chain polymers, whereas the prepared PEG ointment includes PEG4000 and PEG400.

2.4. The pH measurements

Under normal conditions, the skin pH varies between 5 to 6. Semi-solid formulations should be preferred with pH close to skin pH to avoid irritability [19]. The pH values were variable in the 7.35 to 4.42 range in samples exposed to long-term stability conditions (Figure 3). The pH changes in commercial product were constant at 5.15±0.5 pH during six months.

On the other side, the pH variations in prepared ointments at 6th months were significant, though their pH changes were not considerable for the first two months. There was a consistently decrease at pH of white ointment and simple ointment. This might be associated with a type of physical instability because of their oleaginous constitutions. The instability at pH was also observed at 6th months in hydrophilic ointment. The presence of water in outer phase of hydrophilic ointment may lead to microbial augmentation. This explanation may also cover the pH changes in PEG ointment.

The pH changes in samples stored at accelerated stability conditions were also followed (Figure 4). Small changes at pH in commercial product was not statistically important. The fluctuations at pH in hydrophilic ointment and PEG ointment were observed, however they were not significant. The dramatically changes at pH were seen in white ointment and simple ointment. The two units at pH values decreased in 15th day, they kept it for other 6 months. The pH changes in cold cream were important for only 15th day comparing to initial (p<0.01).

2.5. The stress freeze-thaw studies

The various factor may affect the stability of ointments. The main purpose of stress tests is to force the degradation process of preparations. The freeze-thaw, one of the stress tests, triggers the degradation as a function of temperature. In this study, the loss of DT was evaluated after one and two cycle of freeze-thaw process. The results were given in Figure 5.

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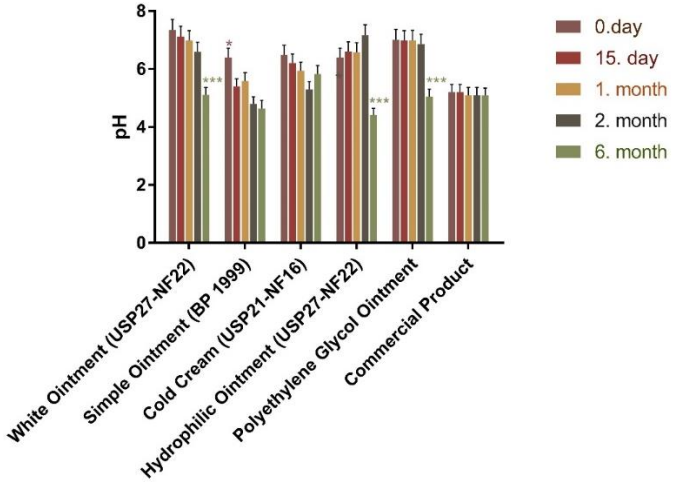

Figure 3. The results of pH measurements in samples stored at room temperature (*: p<0.05, and ***: p<0.001).

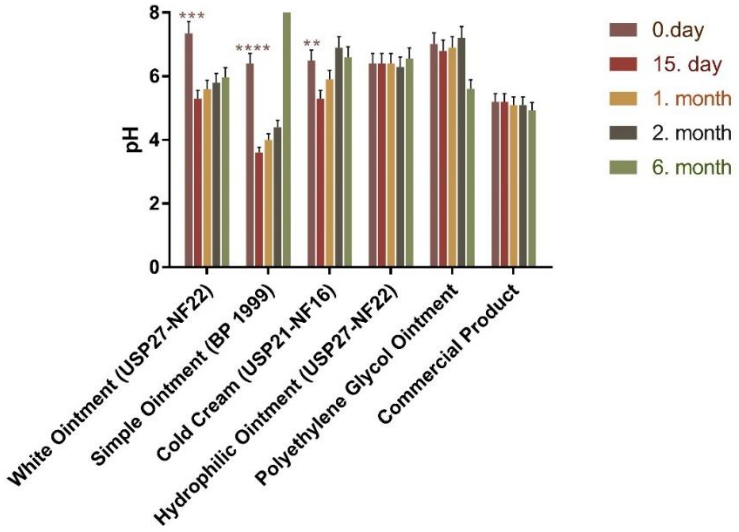

Figure 4. The changes at pH in time in samples stored 40°C (**: p<0.01, ***: p<0.001, ****:p<0.0001).

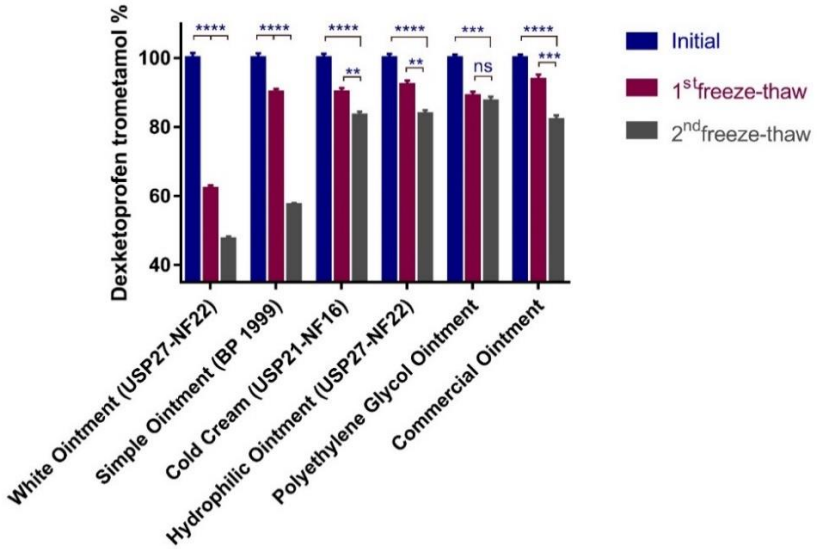

Figure 5. The amount of dexketoprofen trometamol in various ointments exposed to freeze-thaw processes (ns: non-significant, **: p<0.01, ***: p<0.001, ****:p<0.0001).

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The freeze-thaw studies revealed that the temperature changes caused loss of API in preparations, as seen in Figure 5. The most dramatic loss of DT was seen in white ointment. Approximately 40% of drug degraded even after the first cycle. The degradation reached to 50% after the second cycle. The similar results were seen in simple ointment. Although the amount of drug loss was 10% after the first cycle, the abrupt decrease to 57.4±0.6% in DT amount was observed in the second cycle. These results pointed that oleaginous bases were particularly affected by temperature changes. On the other hand, cold cream which is classified into absorption bases, the same class with simple ointment, showed almost 10% degradation in each cycle. The emulsifier in cold cream, w/o type emulsion, might aid to keep drugs in inner phase and protect drugs from degradation. There were no differences between the findings of cold cream and hydrophilic ointments. The amount of DT dispersed in PEG ointment decreased to 89.0±1.3% after the first cycle and significant changes were not observed in second cycle. In commercial ointment, the DT amount was 93.7±1.6% and 82.0±1.4% after the first and second cycle, respectively. Having regard to the degradation limit of products which is 10% as a general estimate to determine the shelf-life, all samples including commercial product were under that limit value in two cycle freeze-thaw. These findings underlined that dexketoprofen trometamol in semi-solid preparations could be delicate to temperature changes, contrary to general tendency to store drug products in the refrigerator although the dosage forms have the warning "stored at controlled room temperature". The refrigerator may increase its stability duration in terms of providing a cooler environment, but a continuous freeze-thaw process may drag products into degradation ensuing from the temperature changing. This study demonstrated that the temperature change might turn into a sore point during storage and/or use, even it did not completely represent in-use stability.

3. CONCLUSION

Excipients' role in designing different dosage forms has critical importance not only for imparting desired properties but also for protecting these desired properties during the shelf life. In this study, we investigated the stability of various DT included topical semi-solid products comparing to commercial products in terms of physical assessment, long term stability, accelerated stability, pH and freeze-thaw studies. The results demonstrated that the choice of excipients had a significant influence on stability, especially on stress conditions. Among samples, the freeze-thaw process caused 7% of degradation after the first cycle and 18% of degradation after the second cycle in even commercial gel products, which were the most stable formulation in this study.

4. MATERIALS AND METHODS

4.1. Materials

Dexketoprofen trometamol was a kind gift from Nobel Pharmaceuticals, Turkey. The commercial gel product included dexketoprofen trometamol was obtained from local pharmacy in Turkey (batch number: XB20020A). The cellulose acetate membrane (MWCO: 12 kDa) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

4.2. Preparation of ointments

Five different ointments (White Ointment (USP 27 – NF 22); Simple Ointment (BP 1999); Cold Cream (USP 21 – NF 16); Hydrophilic Ointment (USP 27 – NF 22); Polyethylene Glycol Ointment) were prepared according to pharmacopeia. Polyethylene Glycol Ointment as a water-soluble base was prepared according to NF 11 with some modifications. PEG4000 was used in formulation instead of PEG3350 with the same amount. 1.25% of DT (the exact amount in the commercial gel formulation) was dispersed in the ointments just before the cooling process completed.

4.3. Physical controls

The prepared ointments and commercial gel product of DT was investigated for visual observation in terms of appearance, homogeneity and color during the stability test period.

4.4. Stability studies

Stability studies were carried out according to the ICH guidelines which addresses climatic zones I and II. The long term stability studies were performed at 25±2°C and 60±5 %RH, while the accelerated stability studies were performed at 40±2°C and 75±5 %RH (*Nüve* ID300, England) . The sampling period was adjusted

15th day and 1st, 2nd, 3rd and 6th months for both stability tests that meets the criteria of regulations in ICH Q1C Stability Testing: Requirements for New Dosage Forms.

4.5. The pH measurements

Weighed amount of samples (both ointments and commercial gel product) was melted at 40±2°C. pH of these samples was measured using the pH probe (Isolab, Germany). The measurements were done at each stability intervals.

4.6. The stress freeze-thaw studies

The ointments and commercial gel formulation were subjected to the freeze-thaw cycles. Each cycle consists of the freezing step (keeping the samples at –20°C for 24h) followed by the thawing step (keeping the samples at room temperature for 24h) [20]. The certain amount of samples was taken and the amount of DT inside the samples was measured by dialysis method.

4.7. The dialysis bag method

The quantitation of DT in samples was determined through the dialysis method. The certain amount of samples was put into the dialysis bag (MWCO: 12kDa). The study conditions were set at a stirring speed of 1200 rpm, dissolution medium volume 500 mL distilled water at room temperature. After 24 hours, the samples were taken and analyzed. The DT content in samples was estimated by UV spectrophotometer (PharmaSpec6100 UV-Visible spectrophotometer, MAPADA Instruments) at λmax 260 nm [21]. A set of DT in distilled water was analyzed and calibration curve was obtained in the range of 0.78 – 25 µg/mL with 0.999 of determination coefficient value (r^2).

4.8. Statistical analysis

The presented results were reported as mean ± standard deviation (n:3). For statistical comparison of the findings, pairwise comparisons of the ointments were performed using the Students' t-test within the ANOVA. Tukey's test was used for multiple comparisons. P values below 0.05 are considered significant.

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