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Method Development and Validation of High Performance Liquid Chromatography Method for Methylparaben and Butylparaben in Ointment Form

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Abstract

The main aim of this study was to investigate the performance of development method for methylparaben and butylparaben determination in skin ointment product by High Performance Liquid Chromatography (HPLC). The method validation parameters were evaluated as per International Conference on Harmonisation (ICH) Guidelines. The method shows the specificity has retention time of methylparaben and butylparaben at 2.10 minutes and 6.64 minutes, respectively. The mobile phase had acetonitrile, methanol and phosphate buffer with the ratio of 10:40:50 v/v. The flow rate was set as 1 ml/minute and the maximum absorption was observed at 270 nm using Shimadzu SPD-20A Prominence UV-Visible detector. Correlation coefficient values are 0.9998 and 0.9996, respectively. The specificity, linearity, accuracy (established by % recovery), precision (repeatability and intermediate precision), the sensitivity (limit of detection and limit of quantitation), and robustness were validated for methylparaben and butylparaben in skin ointment product. The validation results fulfilled the acceptance criteria of validation method based on ICH Guideline.

Keywords: HPLC, UV, Methylparaben (MEPA), Butylparaben (BUPA) and Skin Ointment.

Introduction

One of the very popular pharmaceutical products is cream and ointment, which is used to cure the skin disease, such as allergies, dermatitis, and skin rashes. Cream or ointment is a semi solid form containing one or many dissolved/dispersed medicine (USP, 2017). Prednisolone, crotamiton, dibucaine, chlorhexidine hydrochloride, and glycyrrhetinic acid had been evaluated as active ingredients in the ointment because that matrix can be used for skin infections (Labat et al., 2000). Based on the analysis, the products contain at least parabens, fragrances, formaldehyde releaser, lanolin alcohol, methyl-isothiazolinone, and methyl-chloroisothiazolinone. One additional ingredient in the cream and ointment is preservative that is used for preventing microbial contamination influenced by high water content in the cream (Soni, Carabin and Burdock, 2005; Mahuzier, Altria and Clark, 2001). The preservatives commonly used in the cream and ointment products are combination of both methylparaben and butylparaben (Uysal and Güray, 2008). Paraben is one of preservatives that is commonly used due to its low toxicity on human, but it has high antimicrobial activity, especially for mold and yeast (Uysal and Güray, 2008). Maximum content of methylparaben and butylparaben is 0.3% and 0.5%, respectively (Uysal and Güray, 2008; Shabir, 2004). Determination of both compounds as raw materials was done by HPLC, which refers to United States of Pharmacopeia (USP) 40 National Formulary (NF) 35 on 2017 (USP, 2017; Labat et al., 2000). Whereas the standard method for determination of both compounds in the products is not available yet, so the method needs to be validated.

Recently, the development of analytical method for determination of active pharmaceutical ingredients and preservative compounds in pharmaceutical products is needed to maintain the quality in order to be in the range of product standard (Xiao et al., 2007; ICH Guideline, 1995). Development of analytical method for betamethasone-17-valerate, hydrocortisone, fusidic acid, potassium sorbate, methylparaben, and butylparaben determination in a tropical cream preparation by stability-indicating reversed phase HPLC in single running analysis has been studied (Saad et al., 2005; Mathkar et al., 2009). Based on the validation referring to current International Conference on Harmonisation guidelines, that method was selective, linear, precise, accurate and robust within the validated range. The RP-HPLC method for the estimation of methylparaben, and butylparaben is a new method, which will fulfil all ICH guidelines requirements of validation. Increasing the importance of speed, time and reliability of analysis in pharmaceutical analytical laboratories, a new method for methylparaben, and butylparaben determination in an ointment formulation with a

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very short analysis time is described in this method.

Materials and Methods

Chemicals and Reagents

Skin ointment product purchased from local market. Jazan, KSA, pure methylparaben, butylparaben Acetonitrile, methanol HPLC-grade (Sigma), potassium dihydrogen phosphate and HPLC grade purified water (Merck) were purchased.

RP-HPLC instrumentation

High Performance Liquid Chromatography used for analysis was Shimadzu LC2030C 3D equipped with oktadesil silica (ODS) C18 column (4.6 mm × 25 cm, 5 µm) and UV-Vis detector by 270 nm. The sample injection volume was 10 µl and the wavelength was set as 270 nm, the HPLC run time was set for 10 minutes.

Preparation of Standard Solution

The standard solution containing methylparaben and butylparaben solution with 1 mg/mL concentration was made by mixing each of 10 mg methylparaben and butylparaben with 50 mL solvent in 100 mL volumetric flask, then it was sonicated for 15 minutes. After dissolved, solvent was added until the boundary of volumetric flask and was shaken until getting homogenous. The solution was passed thorough 0.45 µm filter membrane.

Preparation of Mobile Phases

Accurately weighed 6.75 g of KH_2PO_4 was transferred to 1-liter volumetric flask and dissolved by 500 ml of HPLC grade water and the pH was adjusted to 6 by gradual addition of phosphoric acid. The resulting solution was filtered with 0.45 µ membrane filter. The final mobile phase was prepared by adding the ratio of 10:40:50 v/v acetonitrile, methanol and phosphate buffer.

Preparation of stock solution

Standard methylparaben solution

Accurately 2 mg methylparaben reference standard was added to 100 ml volumetric flask and mixed with 75 ml of mobile phase solution, and the resulting solution was kept in the sonicator for 10 minutes. The concentration of 100-500 µg/ml was achieved by diluting the standard stock solution with mobile phase.

Standard butylparaben solution

Accurately 2 mg butylparaben reference standard was added to 100 ml volumetric flask and mixed with 75 ml of mobile phase solution, and the resulting solution was kept in the sonicator for 10 minutes. The concentration of 25-125 µg/ml was achieved by diluting the standard stock solution with mobile phase.

Preparation of Sample Solution

Sample preparation was done by weighing of 100 mg ointment product, which is equal to 3 mg methylparaben and 8mg for butylparaben. The sample was added to 100 ml volumetric flask and mixed with 75 ml of mobile phase solution, and the resulting solution were kept in the sonicator for 10 minutes. Solvent was, then, added until the boundary of volumetric flask and passed through 0.45 µm filter membrane. Further dilutions were made based on the required concentrations.

Solution stability

The prepared drug solution stability was analyzed during the time of analysis and also repeated the same analysis method on same day with different time intervals. The same analysis was repeated after 24 hrs by keeping the drug solution under laboratory temperature (37 ± 1°C) and in refrigeration (5 ± 1°C).

Method validation of methylparaben and butylparaben analysis in skin ointment product

In this study referring to ICH guidelines for validation of analytical procedures by European Medicines Agency 1995, full validation of methylparaben and butylparaben analysis in skin ointment product was conducted including specificity, linearity, accuracy (established by % recovery), precision (repeatability and intermediate precision), sensitivity (limit of detection and limit of quantitation), and robustness were determined.

Result and Discussion

Method Development

Analytical method was developed and validated according to ICH Guideline for Validation of Analytical Procedures Table 1.

Table 1. Acceptance requirements and test result for validation of method development on HPLC method for methylparaben and butylparaben in skin ointment product

No	Parameters	Test Result	
		Methylparaben	Butylparaben
1.	Specificity	Retention time at 2.10 minutes	Retention time at 6.64 minutes
2.	Linearity	r = 0.9970	r = 0.9963
3.	Accuracy	Max: 101.76% Min: 99.17%	Max: 100.33% Min: 99.16%
4.	Repeatability	0.88% < 3.15%	0.70% < 2.96
5.	Intermediate Precision	-1.11 < 2.27	-0.85 < 1.97
6.	Robustness	0.00056 < 6.36	0.00059 < 6.36
7.	Limit Detection	0.00137 mg/mL	0.00124 mg/mL

8.	Quantitation Limit	0.00437 mg/mL	0.00324 mg/mL

Specificity

According to ICH guideline for validation of analytical procedures, specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. For the ointment formulation no excipient interference was detected, which shows the specificity of the method. The proposed method shows the ability to determine the analyte in presence of excipients. Results of specificity are shown in Table 2.

Table 2. Retention time of methylparaben standard, butylparaben standard, sample, mobile phase, methylparaben and butylparaben solvent.

Injected Sample	Number of Peak	Retention Time (minute)
Methylparaben Standard	1	2.106
Butylparaben Standard	1	6.643
Sample	2	2.23 and 6.54
Mobile Phase	no peak	no retention time
Methylparaben and Butylparaben solvent	no peak	no retention time

Based on Table 2, it informs that retention time of 2.107 minutes has peak of methylparaben standard and retention time of 6.643 minutes has peak of butylparaben standard. The HPLC chromatogram of methylparaben standard and butylparaben standards are presented in figures 1 and 2. Sample running has double retention time at 2.23 and 6.54 minutes which refer to methylparaben and butylparaben, respectively. Chromatogram of methylparaben and butylparaben ointment formulation is shown in the figure 3. Whereas the running of mobile phase, methylparaben and butylparaben solvent, and placebo do not show any peak. It can be interpreted that no peak disturbs at retention time of methylparaben and butylparaben. This phenomenon shows that HPLC instrument and the method used can analyze methylparaben and butylparaben specifically.

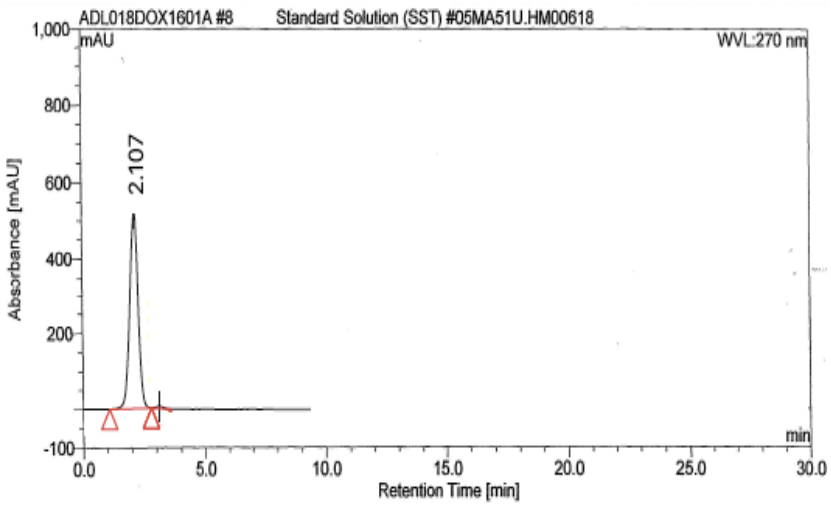

Fig. 1: A Chromatogram of methylparaben by RP-HPLC method

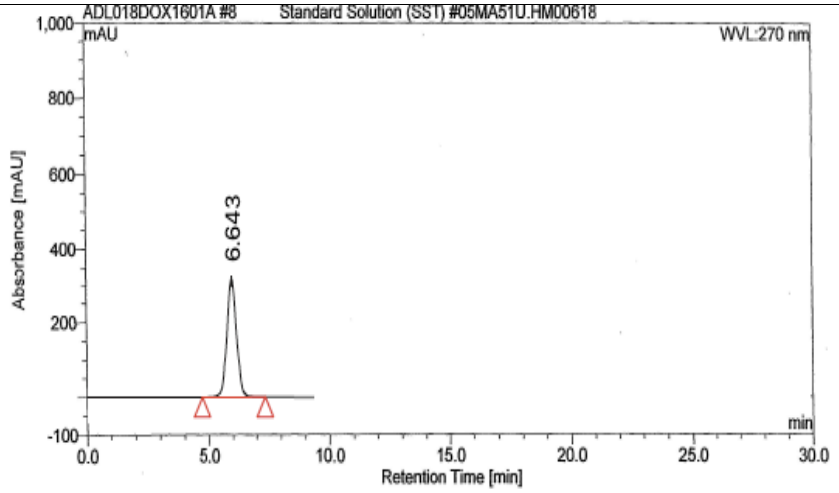

Fig. 2: A Chromatogram of butylparaben by RP-HPLC method

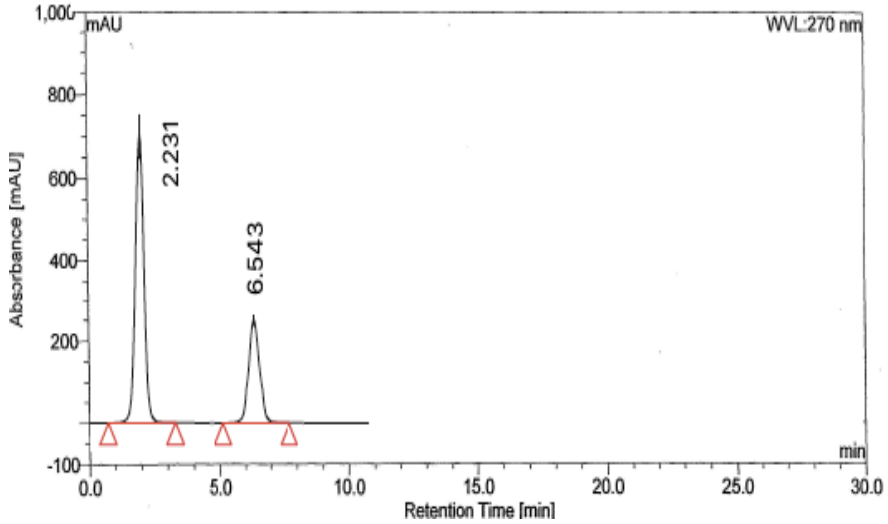

Fig. 3: A Chromatogram of methylparaben and butylparaben ointment formulation by RP-HPLC method

Linearity

As per ICH guideline for validation of analytical procedures, linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. Results of linearity are shown in Figure 4. The correlation coefficient value for methylparaben is 0.9998 and for butylparaben is 0.9996. This is indicated that there is linear and proportional relation between methylparaben and butylparaben concentration by tool response namely analytic signal in the range of concentration. The proposed method linearity was examined for five concentrations. The concentration ranges from 100-500 µg/ml for methylparaben, to 25-125 µg/ml for butylparaben. The methylparaben and butylparaben standard linearity was determined by the plotting graph concentration vs absorbance. By absorbance as a functional of analyte concentration linearity was evaluated for methylparaben and butylparaben. The linearity graph is presented in figure 4. The system suitability is demonstrated by the linearity analysis.

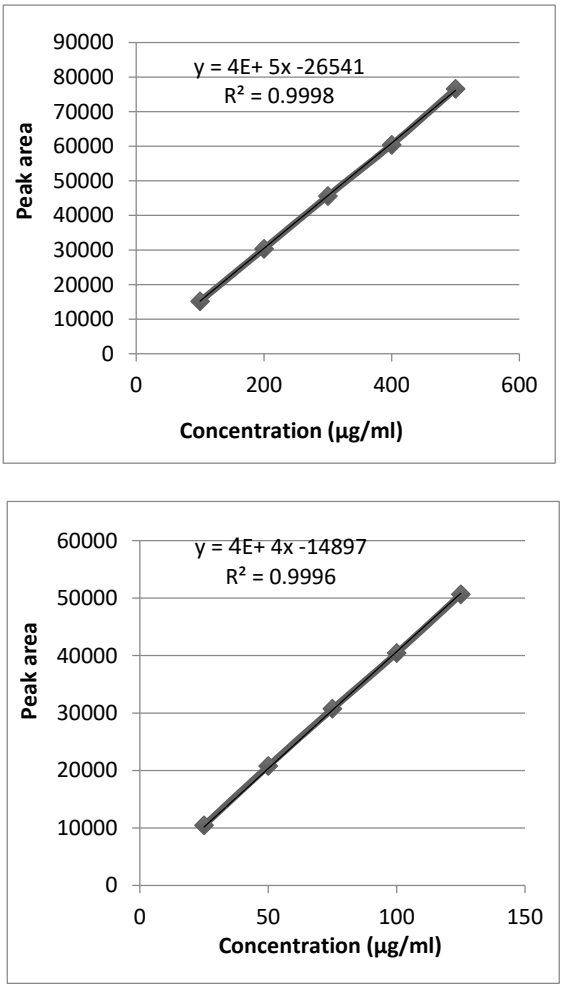

Fig. 4: Standard curve of correlation between concentration and area of methylparaben and butylparaben

Accuracy

Based on ICH guidelines for validation of analytical procedures, accuracy is conducted to evaluate the proximity between test result from the measurement and the real analyte content, which is avowed in % recovery. The result of accuracy can be seen in Table 3. The result shows some failures namely the test result is less than the real value. This systematic failure can be caused from the incompletely dissolved sample. That sample is 80% butylparaben. The other failure is the test result that is more than the real value. It is caused by the impurity of compounds in the sample matrix that is also measured because of having the similar character with analyte in the sample. This failure is in the 80% and 100% concentration of methylparaben and 100% and 120% concentration of butylparaben. On the other hand, it has random error that shows variation of test result causing unstable HPLC machine. This failure is in the 120% of methylparaben. Although there are some failures, but the result is in the range of 95-105%.

Table 3. Recovery for determination of methylparaben and butylparaben content in ointment skin

Concentration	Recovery (%)		Concentration	Recovery (%)		Concentration	Recovery (%)	
	MEPA	BUPA		MEPA	BUPA		MEPA	BUPA
80%	100.60	98.76	100%	101.30	101.13	120%	100.13	100.79
	101.10	99.04		101.86	101.22		99.25	100.34
	101.23	99.15		100.18	100.17		99.72	99.89
Average	101.08	99.24	Average	101.21	101.43	Average	99.90	100.34
RSD	0.04	0.06	RSD	0.37	0.11	RSD	0.56	0.45

Precision: Repeatability and Intermediate Precision

According to ICH guideline for validation of analytical procedures, repeatability is conducted to show the proximity of test result from sample using similar laboratory, instrument, and analyst. The results are shown in Table 4. This test was done at six concentrations of sample with six times replication. Repeatability test result has 0.58 and 0.62 of RSD value of methylparaben and butylparaben, respectively. It means that the repeatability is valid. It indicates that the testing method used has good performance to give the similar test result for some similar contents in the sample. Intermediate precision is done to evaluate the performance of method toward the sample analyzing by different analysts, instruments and time, but in the same laboratory.

Table 4: Repeatability test for determination of methylparaben and butylparaben content in ointment

No	Methylparaben	Butylparaben
	Content (µg/ml)	Content (µg/ml)
1	100.16	99.62
2	99.13	100.26
3	100.90	100.16

4	100.14	100.11
5	100.72	100.08
6	100.12	99.11
Average	100.05	100.19
SD	0.61	0.44
%RSD	0.58	0.62

Robustness

Referring to ICH guideline for validation of analytical procedures, this test is needed to know the performance of method toward the effect of small variety in parameter of analysis method. The result of robustness can be seen in Table 5. The variety is in injection volume for ± 20%. Table 6 informs that F_{count} of methylparaben and butylparaben are 0.77 and 1.25, whereas F_{table} has 3.48 for both of methylparaben and butylparaben. The result indicates that $F_{count} < F_{table}$. It means that variation of injection volume for ± 20%, do not have significant difference.

Table 5. Robustness test for determination of methylparaben and butylparaben content in ointment skin

Compounds	**F_{count}**	**F_{table}**
Methylparaben	0.77	3.18
Butylparaben	1.25	3.48

Sensitivity: Detection and Quantitation Limit

Based on ICH guideline for validation of analytical procedures, sensitivity is consisted of detection and quantitation limit tests. The result is shown in Table 6. Detection and quantitation limit tests are done by evaluating cream product solution starting from the lowest concentration of methylparaben and butylparaben at standard series. The limit of detection and quantification for Aciclovir is presented in table 6. Limit of detection (LOD) and limit of quantification (LOQ) were examined by minimum detectable peak area by injecting known concentration of drug solution. As per the International Conference on Harmonization guidelines the results are multiplied thrice to get LOD and 10 times to get LOQ. LOD and LOQ were found at concentrations of 0.87µg/mL and 1.75 µg/mL for methylparaben and 0.20µg/mL and 0.95 µg/mL for butylparaben, respectively. The limit of detection and quantification for methylparaben and butylparaben is presented in table 6.

Table 6. Detection limit test for determination of methylparaben and butylparaben content in ointment skin

Parameters	**Methylparaben**	**Butylparaben**
LOD (µg/ml)	0.87	0.20
LOQ (µg/ml)	1.25	0.95

Conclusion

In conclusion we have developed and validated a method that was found to be cost-effective. Determination method of methylparaben and butylparaben content in the ointment product by HPLC is valid and appropriate for analysis with good performance.

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Conflict of Interest

The authors declare no conflict of interest.

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