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RESEARCH ARTICLE

Simultaneous Determination and Validation of Miconazole Nitrate with the Anitmicrobial Preservatives using RP-HPLC Method: for Benzoic Acid, Sodium Propyl Hydroxyl Benzoate, and Sodium Methyl Hydroxy Benzoate in Pharmaceutical Cream

Asghar S*, Sheikh S, Ahmad S

Unijules Life Sciences Ltd., B - 35, 36 MIDC Area Kalmeshwar Nagpur – 441501, Maharashtra, India.

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ABSTRACT

A new HPLC-RP method has been developed and validated for the simultaneous determination of Benzoic acid, sodium methyl hydroxyl benzoate, sodium propyl hydroxyl benzoate and miconazole nitrate in combined dosage form. The method uses a Qualisil, C18 column, 250 mm x 4.6 mm, 5µm in isocratic elution. The mobile phase consisted of a mixture of 0.6% Ammonium acetate: ACN: Methanol (380: 300: 320); which pumped at a flow rate of 2.0ml/min. Tetrahydrofuran: methanol in 1:1 ratio was used as diluent. The detector was set at 235nm. The retention time of the Benzoic acid, sodium methyl hydroxyl benzoate, sodium propyl hydroxyl benzoate and miconazole nitrate was found to 1.280, 1.698, 2.275 and 3.500 respectively. The validation study was carried out fulfilling the ICH guidelines in order to prove that the new analytical method, meets the reliability characteristics, and these characteristics showed the capacity of analytical method to keep, throughout the time, the fundamental criteria for validation: selectivity, linearity, precision, accuracy and sensitivity. The method was applied during the quality control of miconazole cream in order to quantify the drug (miconazole nitrate) and preservatives; and proved to be suitable for rapid and reliable quality control method. The detector response was linear in the range of 25 to 150 µg/ml, 20.0-160.0µg/ml, 4-14 µg/ml and 150-1500 µg/ml, for Benzoic acid, sodium methyl hydroxyl benzoate, sodium propyl hydroxyl benzoate and miconazole nitrate respectively.

KEYWORDS

RP-HPLC, Benzoic acid, Sodium Propyl Hydroxyl Benzoate, Sodium Methyl Hydroxyl Benzoate and Miconazole Nitrate, Validation, Antibacterial Formulation and Method Development

INTRODUCTION

The pharmaceutical cream contains miconazole nitrate 2% w/w which is indicated in various fungal infection of skin. Miconazole Nitrate (MCN), or 1-[2, 4-dichloro-β-[(2, 4-dichlorobenzyl) oxy] phenethyl] imidazole, is an antifungal of the class of Imidazole.

***Address for Correspondence:**
Suhail Asghar
Unijules Life Sciences Ltd.
B - 35, 36 MIDC Area, Kalmeshwar
Nagpur – 441501, Maharashtra, India.
E-Mail Id: suhailasghar@unijules.com

Miconazole Nitrate (Figure 1) is indicated in the treatment for various fungal infections such as infection of skin and nails, vulvo-vaginal candidosis, oral and esophageal candidosis and fungal colonization of GIT.[1,2]

Both the British Pharmacopoeia (BP) and United States Pharmacopoeia (USP) describe titremetric procedure for the assay of MCN bulk form and HPLC methods for the assay of various MCN formulations.[3-5]

Various methods are available for the quantitative determination of Miconazole Nitrate such as HPLC, Gas chromatography, spectrophotometric methods such as UV spectrophotometry, Colorimetry and other methods such as Voltammetry.[6-10] These reported methods have been used for the determination of Miconazole Nitrate individually or in combination with other drugs in different dosage forms such as tablets, creams, gels etc.

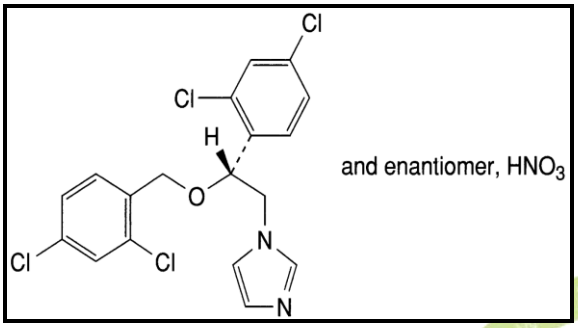

Figure 1: Miconazole Nitrate structure

However, there is no HPLC method available for the estimation of Miconazole Nitrate in cream along with its antimicrobial preservatives simultaneously.

The aim of the present work is to develop an Accurate, Specific, Repeatable, and Stability - Indicating HPLC method for the simultaneous determination of Miconazole Nitrate in the presence of its antimicrobial preservatives in developed cream.

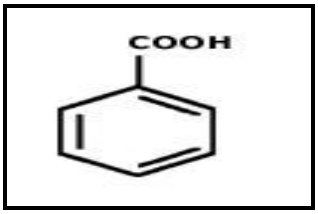

Figure 2: Chemical Structure of Benzoic acid.

Benzoic Acid is Antifungal agent; pharmaceutical aid (anti- microbial preservative) having molecular formula and molecular weight as $C_7H_6O_2$ and 122.12. It is Colourless, light crystals, scales or needles; odour, slight and characteristic.

Benzoic acid[15] is freely soluble in ethanol (95%), in chloroform and in ether; slightly soluble in *water* but soluble in boiling wate*r*. It is soluble in fixed oils.

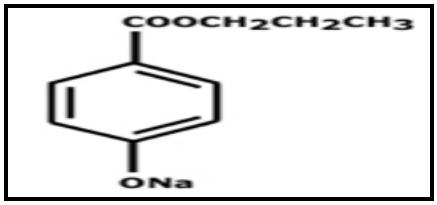

Figure 3: Chemical Structure of Sodium propylparaben

Sodium propyl paraben[15] is also called as Sodium Propyl Hydroxybenzoate is the sodium salt of propyl 4- hydroxybenzoate. It is also a (antimicrobial preservative). It has molecular formula $C_{10}H_{11}NaO_3$ and molecular weight 202.18 respectively. It is White, crystalline powder; odourless or almost odourless; hygroscopic.

Sodium propyl paraben is freely soluble in water and in ethanol (50%); sparingly soluble in ethanol (95%); practically insoluble in fixed oils.

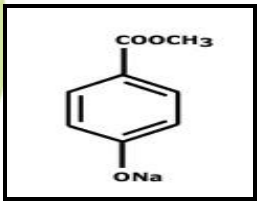

Figure 4: Chemical Structure of Sodium methyl paraben

Sodium methyl paraben[15] also called as Sodium Methyl Hydroxybenzoate is the sodium salt of methyl 4-hydroxybenzoate. Molecular formula and molecular weight $C_8H_7NaO_3$, 174.13. It is White, crystalline powder; odorless or almost odorless; hygroscopic.

Benzoic acid is freely soluble in water; sparingly soluble in ethanol (95%); practically insoluble in fixed oils.

MATERIALS AND METHOD

Benzoic acid (BA), sodium propyl hydroxyl benzoate (SPHB), sodium methyl hydroxyl benzoate (SMHB) and miconazole nitrate (MCN) were of USP grade. The entire reagents used were of analytical grade. Water was deionised and double distilled. Pharmaceutical

formulation containing BA, SPHB, SMHB and MN was of our in house formulation. The Ammonium acetate, methanol, acetonitrile, tetrahydrofuran, was of high purity HPLC grade and nylon membrane filters of 0.2µ and 0.45µ also purchase from Merck.

HPLC Instrument

A Shimadzu LC-2010 $_{CHT}$ consisting of a quaternary pump and UV-spectrophotometric detector and system controlling module as LC solutions with an auto sampler was used for the development.

Chromatographic Conditions

Mobile phase contains 0.6% ammonium acetate: Acetonitrile: Methanol (38: 30: 32) with a stainless steel Qualisil C-18 column (250mm x 4.6 mm, 5µ), having column temperature as 60º C and the flow rate was 2.0ml/min.

A mixture of methanol: tetrahydrofuran in the ratio of 1:1 was used as diluents. The mobile phase was filtered through 0.45 µm Millipore membrane filter and degassed. The detection performed as 235nm using deuterium lamp.

Sample Preparation

Standard Stock Solution for Benzoic Acid

About 25mg of the standard BA was accurately weighed and transferred to 25ml of volumetric flask, dissolved in 10ml of the diluent and diluted up to the mark with mobile phase. (Solution A). Take 1ml of the stock solution and dilute it up to 10ml with the same diluents. Filter the solution with 0.2µ nylon membrane filter and allow injecting 20µl.

Standard Stock Solution for Sodium Propyl Hydroxyl Benzoate

About 25mg of the standard SPHB was accurately weighed and transferred to 25ml of volumetric flask, dissolved in 10ml of the diluent and diluted up to the mark with mobile phase. Take 1ml of the stock preparation to 10ml of the volumetric flask and make up the volume with the same solvent. (Solution B). Further take another 1ml form the above and dilute it to 10ml with the diluents. Filter the solution with 0.2µ nylon membrane filter and allow injecting 20µl.

Standard Stock Solution for Sodium Methyl Hydroxy Benzoate

About 25mg of the standard SPHB was accurately weighed and transferred to 25ml of volumetric flask, dissolved in 10ml of the diluent and diluted up to the mark with mobile phase. (Solution C) Take 1ml of the stock preparation to 10ml of the volumetric flask and make up the volume with the same solvent. Filter the solution with 0.2µ nylon membrane filter and allow injecting 20µl.

Standard Stock Solution for Miconazole Nitrate

About 25mg of the standard MCN was accurately weighed and transferred to 25ml of volumetric flask, dissolved in 15ml of the diluent and diluted up to the mark with mobile phase (Solution A). Filter the solution with 0.2µ nylon membrane filter and allow injecting 20µl.

Combine Standard

Transferred an accurately weighed 25mg quantity of miconazole nitrate and measured volumes of Solution A, B and C: 1ml, each respectively to 10ml volumetric flask and diluted up to the mark with the mobile phase. (Figure 5)

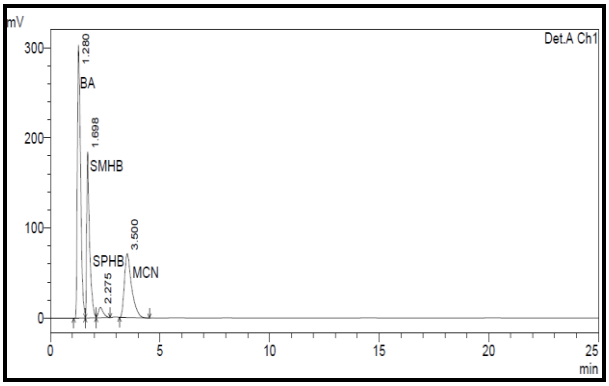

Figure 5: Shows the HPLC Graphs of BA, SMHB, SPHB and MCN in Combine Standard

Sample Solution

For the determination of the content of BA, SPHB, SMHB and MCN; the formulations equivalent to 5.0mg, 0.5mg, 5.0mg and 50mg

was transferred to a 50ml volumetric flask and the content was allowed to dissolve in 30 ml of the mobile phase and make up the volume to 50 ml volumetric flask with the mobile phase. (Figure 6)

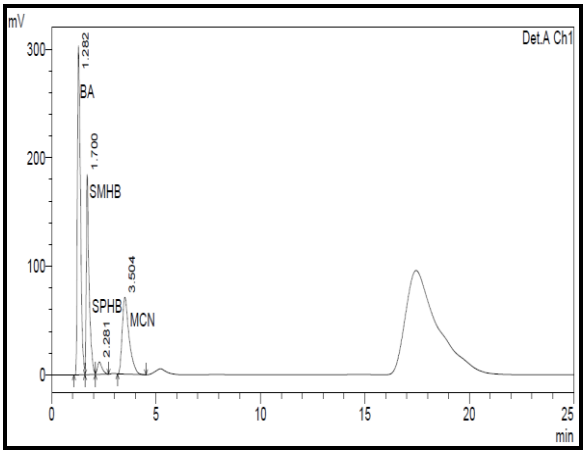

Figure 6: Shows the HPLC Graphs of BA, SMHB, SPHB and MCN in Pharmaceutical Cough Preparation

Stability Studies

For stability studies the commercially available suspension samples were placed at accelerated conditions of temperature that is at 40°C with 75% relative humidity and at ambient conditions of 30°C with 75% relative humidity in environmental chamber for six months. The stability protocol showed elsewhere was followed for six months and assays were made as described in method development.

Method Validation

The method validation was performed in following ICH guidelines according to which the assay validation was performed via various procedures including specificity, linearity, range, accuracy, intra-day, and inter-day precision etc.

To study the linearity of standard solutions, different dilutions were prepared from stock solution to give standard solutions in the range of the drugs content. The standard calibration curve was generated using regression analysis. For specificity commonly used excipients in cream preparation were spiked in a pre-weighed quantity of drugs and then peak areas measured and calculations done to determine the quantity of the drugs recovered.

The precision was calculated by analyzing corresponding standard daily for a period of three days i.e. inter day precision, and three times a day with an interval of 8 hours (Intraday precision) against freshly prepared standard solutions. For determining accuracy the reference standards were accurately weighed and added to the cream sample, to get three different concentration levels i.e. 80% 100% and 120% of the ingredients. At each level, samples were prepared in triplicate and the recovery percentage was determined.

Limit of detection and quantification (LOD and LOQ) for the method were established by sequential diluting the standard solutions a decreasing concentration in the range and injected onto the chromatographic system. The limit of detection was defined as the concentration for which a signal to noise ratio of 3.3 was obtained and for quantification limit; a signal-to-noise ratio of 10 was consider.

The robustness was studied by analyzing the same samples of cream by deliberate variation in the method parameters, such as in the chromatographic conditions, like mobile phase, pH, flow rate, temperature etc. System suitability of the method was evaluated by analyzing the symmetry of the standard peaks, resolution and theoretical plates of the column.

RESULTS AND DISCUSSION

The HPLC method development and its validation are the utmost requirements for any drug available in the market to have high quality products. A few methods are available for determination of the MCN as described earlier, but many of them are used only for certain definite objectives and none can be generalized for its simultaneous determination with preservatives in form of pharmaceutical products. Similarly none of them is as much sensitive as ours is; in terms of its Precision, accuracy, %recovery, limit of detection (LOD) and limit of quantification (LOQ).

Method Development & Optimization

For developing an efficient method for analysis, parameters, such as detection wavelength, mobile phase composition, optimum pH and concentration of the standard solutions were comprehensively studied. All the ingredients were diluted in dilution solvent and then run through UV spectrophotometer in UV range of 190 nm-400 nm to get maximal wavelengths, where maximum absorbance was gained i.e. 220 nm, 254nm, and 240 nm BA, SMHB, SPHB and for MCN respectively.

However considering the difference of concentration of the ingredients and intensity of their absorbance a single wavelength method was adopted, that is 235 nm at which all the molecules gave a satisfactory absorbance. The chromatographic parameters were evaluated using a Merck® C18 column. Mobile phase of an already developed method by our research team was selected in terms of its components and their proportions and was modified. The mobile phase composed of 0.6% ammonium acetate : Acetonitrile : Methanol (380 : 300 : 320)of previously mentioned proportion promoted a short run time (20 min) as shown in (Figure 5), so this condition was adopted in subsequent analysis.

Validation Studies

The linearity was determined in the range of 15%-160% for all ingredients. The assay was judged to be linear as the correlation coefficient was greater than 0.995 in all cases. A linear correlation was found between the peak areas and the concentrations, in the assayed range. The regression analysis data are presented in Table 1.

Chromatogram shown in Figure 6 proves specificity or selectivity of the assayed method, as the chromatograms in samples were found identical with standard chromatogram and no interference peak was observed. Peak purities higher than 98.0% were obtained in the chromatograms of sample solutions, demonstrating that other compounds did not co-elute with peaks of interest. The chromatogram obtained with the mixture of the cream excipients proves that there is no interference from excipients and peak of interest fulfill all the requirements of symmetrical peak, and hence the specificity is confirmed.

The precision of an analytical method is the degree of coherence among individual test results when the method is applied repeatedly to multiple sampling of homogeneous bulk. Intra-day precision of the method was evaluated at three different independent concentrations i.e. 80%, 100%, and 120% for the drugs (n=3) by determining their assays. Inter-day precision of the method was tested for 3 days at the same concentration levels. Solutions for calibration curves were prepared every day on freshly basis. Since the inter-day and intra-day precision obtained %RSD was less than 2% it assures that the proposed method is quite precise and reproducible as shown in Table 2.

The accuracy was investigated by means of addition of reference standards to a mixture of the cream excipients. Recovery studies of the drug were carried out for the accuracy parameter at three different concentration levels i.e. multiple level recovery studies. A known amount of API's standards were added into pre-analyzed sample and subjected to the proposed HPLC method. The mean recovery (n=9) was 99.67%-100.30% for BA, 99.23%-100.20% for SMHB, 99.50%-99.58% for SPHB, and for MCN 99.35%-100.30%, demonstrating the accuracy of the method. Percentage recoveries for marketed products were found to be within the limits.

The statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of some parameters were introduced. The parameters used in system suitability test were symmetry of peaks, tailing factor, resolution and RSD of peak area for replicate cream. Thus, the method showed to be robust for changes in mobile phase acetonitrile proportion, mobile phase pH, flow rate, and column temperature (Table 3).

Table 1: Data of calibration curve, LOD and LOQ

Parameter	BA	SMHB	SPHB	MCN
Linearity range (mcg/ml)	25 to 150	20 to 160	4 to 14	150 to 1500
Correlation coefficient (r^2)	0.998	0.9995	0.9990	0.999
Limit of detection(ng/ml)	25	8	5	500
Limit of Quantification(ng/ml)	100	50	20	1500

Table 2: Data of Inter day and Intra-day precision

Drugs	Inter day Precision	%RSD	Intraday Precision	%RSD
BA	99.67	0.62	100.29	0.4563
SMHB	100.20	1.32	100.07	1.351
SPHB	99.58	0.54	99.95	0.162
MCN	100.30	0.859	98.98	1.201

Table 3: Data of Robustness of method

Chromatographic conditions	Variations	Retention time			
		BA	SMHB	SPHB	MCN
Temperature (°C)	57	1.308	2.278	1.716	3.499
	63	1.300	2.344	1.758	3.532
Flow rate (ml/min)	1.8	1.562	2.455	1.806	3.611
	2.2	1.377	2.156	1.678	3.330
% Acetonitrile	28	1.356	2.298	1.778	3.546
	32	1.220	2.101	1.561	3.106

Table-4: Data of stability studies

Results of Accelerated Studies (40°C ± 75% RH)									
Drugs	**Initial**	**1M**	**2M**	**3M**	**4M**	**5M**	**6M**	**MEAN**	**%RSD**
BA	99.45	98.88	100.02	99.87	99.82	99.6	100.05	99.70667	0.4096
SMHB	99.23	99.66	98.95	98.97	99.56	98.53	99.91	99.26333	0.4807
SPHB	100.11	98.9	98.56	98.45	100.06	98.41	99.31	98.94833	0.7083
MCN	100.05	98.96	98.55	99.72	100.11	98.8	100.01	99.35833	0.538
Results of Long Term Studies (30°C ±75% RH)									
Drugs	**Initial**	**1M**	**2M**	**3M**	**4M**	**5M**	**6M**	**MEAN**	**%RSD**
BA	99.45	99.2	100.22	99.98	99.89	99.86	100.56	99.95167	0.4538
SMHB	99.23	99.98	99.87	100	100.02	99.77	100	99.94	0.2831
SPHB	100.11	100.07	99.25	98.95	100.2	99.45	99.87	99.63167	0.485
Drugs	100.05	99.76	99.6	100.01	100.21	99.5	100.52	99.93333	0.3575

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light and enables recommendation of storage conditions, retest periods, and shelf life to be established. The two main aspects of drug product that play an important role in shelf-life determination are assay of the active drug and the degradation products generated during stability studies. The proposed assay method was applied to stability study of commercially available cream, for which the samples were placed at 30°C with relative humidity of 75% and at 40°C with relative humidity of 75%. Stability study was performed according to stability protocol as described in previous section (Table 4). Samples were analyzed and percentage of contents was measured. According to the results obtained MCN with all the three preservatives were found to be stable at applied conditions of temperature and relative humidity, and were accurately analyzed with the proposed method.

CONCLUSION

The proposed new HPLC method described in this paper provides a simple, convenient and reproducible approach for the simultaneous identification and quantification of Benzoic acid, sodium propyl hydroxyl benzoate, sodium methyl hydroxyl benzoate and miconazole nitrate in bulk, and also in pharmaceutical formulations with good separation and resolution. Analytical results are accurate and precise with good recovery study and lowest detection limit values which is reconfirmed by statistical parameters.

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