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# NEW VALIDATED STABILITY INDICATING GRADIENT RP-HPLC METHOD FOR THE ASSAY &RELATED SUBSTANCES OF MICONAZOLE NITRATE AND ASSAY OF BENZOIC ACID IN MICONAZOLE NITRATE 2.0% W/W GEL

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## ABSTRACT

An innovative, simple, economic, selective and sensitive of the stability indicating gradient reverse phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for the analysis of assay of Miconazole nitrate and Benzoic acid and related substances of Miconazole nitrate in gel dosage form with better precision and accuracy. Miconazole nitrate & Benzioc acid are found to be degraded together under different set of conditions as followed according to ICH guidelines and the degradants so formed along with Miconazole nitrate organic impurities were separated by using INERTSIL ODS C18(150mm x 4.6, 3.5µ) column in gradient mode with mobile phase A consisting of 0.1% v/v solution of phosphoric acid in water and mobile phase B consisting of water : acetonitrile (22:78) with a flow rate of 1.0 mL/min and throughout the gradient program with an UV detection wavelength of 227nm for both the compounds with an injection volume of 10µL.Its chromatographic run time was 15.0 min. The method was validated according to ICH guidelines with respect to all validation parameters such as selectivity, specificity, linearity, precision, accuracy, robustness and limits of detection and quantification. All parameters examined were found to be well within the stated guidelines. The results were indicating the method is selective and specific in analysis of both Miconazole nitrate & Benzioc acid in the presence of degradation products formed under various stress conditions. The Proposed Gradient RP-HPLC method is utilized for the simultaneous estimation of Assay of Miconazole nitrate and Benzoic acid and related substance determination in Miconazole Nitrate Gel.

**KEYWORDS:** Miconazole nitrate, Benzoic acid, Gradient RP-HPLC, INERTSIL ODS C18, Orthophosphoric acid, Acetonitrile, ICH Guidelines.

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# INTRODUCTION

Miconazole nitrate is 2-(2,4-Dichlorophenyl)-2-(2,4-dichlorobenzyloxy)-1-(1*H*-imidazol-1-yl)-ethane and usually available in different forms such as a 2% cream, lotion, or powder in the treatment of fungal infections of skin including candidiasis, dermatophytosis, and pityriasis versicolor[1-5]. Literature review reveals that the methods for Miconazole nitrate alone or in combined dosage forms are Massaccesi reported the development of a two-phase titration method for the analysis of miconazole and other imidazole derivatives in pure form and in pharmaceutical formulation[6]. Szabolcs determined the active principle in preparations based on miconazole and clotrimazole[7]. The two drugs were determined in ointments by extraction with chloroform, evaporation of the solvent, dissolution of the residue in acetic acid, and titration with 0.1 N perchloric acid in the presence of Gentian Violet. Mapsi et al developed a potentiometric method for the determination of the stability constants of miconazole complexes with iron(II), iron(III), cobalt(II), nickel(II), copper(II) and zinc(II) ions[8]. Shamsipur and Jalali described a simple and accurate pH metric method for the determination of two sparingly soluble in water antifungal agents; miconazole and ketoconazole in Micellar medium[9]. Bonazzi et al reported the determination of miconazole and other imidazole antimycotics in creams by supercritical-fluid extraction and derivative ultraviolet spectroscopic method[10]. Goger developed a quantitative method for the determination of miconazole in cream formulations that benzoic acid as preservative by second order derivative spectrophotometry[11]. Erk described a spectrophotometric method for the simultaneous determination of metronidazole and miconazole nitrate in ovules[12]. El-Shabouri et al used a charge-transfer complexation method for the spectrophotometric assay of miconazole nitrate and other imidazole antifungal drugs[13]. Chen used a second-derivative spectrophotometric method for the determination of miconazole nitrate in Pikangshuang[14]. Erk N, and Altum ML used a ratio spectra derivative spectrophotometric method and a high performance liquid chromatographic method for the analysis of miconazole nitrate and metronidazole in ovules[15]. Neill et al described an automated screening procedure using gas chromatography-mass spectrometry for identification miconazole and other drugs after their extraction from biological sample[16]. Aboul-Enein and Ali used a high performance liquid chromatography method by using chiral colums for the analysis of miconazole and other imidazole antifungal agents in commercial dosage forms [17-19]. Zhang and Nunes used a high performance liquid chromatographic-coupled with electrospray ionization mass spectrometry (HPLC-ESI-MS) method to study the structure and generation mechanism of a novel degradation product formed by oxidatively induced coupling of miconazole nitrate with butylated hydroxytoluene in a topical ointment [20].

# MATERIALS AND METHODS

The reference standard was supplied by M/s Microlabs limited, Bangalore, India. Acetonitrile (HPLC grade), methanol (HPLC grade), Orthophosphoric acid (HPLC grade), calcium chloride (AR grade) were purchased from Merck (Mumbai, India). Milli-Q water was used as HPLC grade water. Chromatographic Conditions: The determination was carried out on Waters HPLC equipped with Photo diode array as detector using data handling system – waters empower software. The column used in the development for the determination is INERTSIL ODS C18 (150 x 4.6, 3.5µ). The mobile phase composition was used as per gradient Program indicated in Table 1. The detector wavelength was set at 227nm. A flow rate of 1.0mL/min was used for the determination. The samples and standards were dissolved in diluent (water: ACN, 30: 70) and 10µL sample were injected into HPLC system at the column at a temperature of 35ºC.

***Mobile phase***

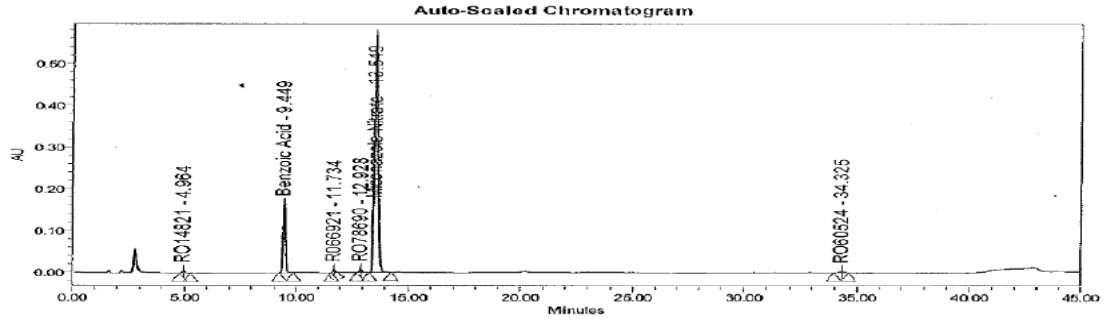
1.0 mL of orthophosphoric acid was mixed with 1000mL of milli Q water used as a mobile phase A. Water: Acetonitrile mixture (22:78) used as a mobile phase B. The mobile phases were filtered through 0.45µm Membrane filter and degassed by sonication.

# RESULTS AND DISCUSSION

All the system suitability parameters according to ICH guidelines was achieved by using INERTSIL ODS C18 (150 x 4.6, 3.5µ) column with a flow rate of 1.0 mL/min and an UV detection wavelength of 227nm with a injection volume of 10µL.

**Table 1**
**Gradient Program**

| Time(minutes) | Flow Rate (ml/min) | %Mobile Phase A | %Mobile Phase B |
|---|---|---|---|
| 0 | 1.0 | 80 | 20 |
| 2 | 1.0 | 80 | 20 |
| 8 | 1.0 | 53 | 47 |
| 21 | 1.0 | 50 | 50 |
| 25 | 1.0 | 20 | 80 |
| 38 | 1.0 | 20 | 80 |
| 40 | 1.0 | 80 | 20 |
| 46 | 1.0 | 80 | 20 |




Peak Results

| | Name | RT | Area | % Area | Purity1 Angle | Purity1 Threshold | Purity1 Flag | Int Type |
|---|---|---|---|---|---|---|---|---|
| 1 | RO14821 | 4.964 | 35951 | 0.48 | 0.517 | 3.635 | No | BB |
| 2 | Benzoic Acid | 9.449 | 1180084 | 15.61 | 0.199 | 0.490 | No | BB |
| 3 | RO66921 | 11.734 | 39030 | 0.52 | 0.608 | 2.250 | No | BV |
| 4 | RO78690 | 12.928 | 43079 | 0.57 | 0.338 | 3.764 | No | BB |
| 5 | Miconazole Nitrate | 13.549 | 6221957 | 82.32 | 0.509 | 1.309 | No | BB |
| 6 | RO60524 | 34.325 | 38299 | 0.51 | 0.997 | 5.059 | No | BB |

**Figure 1**
***Spiked sample chromatogram***

To validate the RP-HPLC method (Figure 1). The detection limit determined for the API and each validation impurity. Detection limit value will be set at 0.05% w/w. The quantitation limit (LOQ) determined for the API and each validation impurity. Quantitation limit value is set equal to reporting threshold value (0.10% w/w) and shall be supported by the accuracy at reporting threshold level. Limit of Detection (LOD) is confirmed by preparation of solution containing Miconazole Nitrate,IMP-A, IMP-B and IMP-C injected one injection of this solution in the proposed methodology. Linearity solutions prepared from LOQ to 200% of IMP-A, IMP-B and IMP-C of the specification limit concentration and for Miconazole Nitrate from LOQ to 120% of test concentration and the correlation coefficient was found to be 0.9999.

**Table 2**
**Limit of Quantitation**

| S.No | Component | LOQ | |
|---|---|---|---|
| | | Conc.(µg/mL) | Conc.(% w/w) |
| 1 | Miconazole | 0.202 | 0.081 |
| 2 | Miconazole IMP-A | 0.234 | 0.094 |
| 3 | Miconazole IMP-B | 0.214 | 0.086 |
| 4 | Miconazole IMP-C | 0.238 | 0.095 |

**Table 3**
**Linearity Studies**

| Sample | Miconazole Nitrate | | Benzoic acid | |
|---|---|---|---|---|
| | Concentration (µg/mL) | Area | Concentration (µg/mL) | Area |
| **80.0 %** | 199.4320 | 5305644 | 17.6323 | 863637 |
| **90.0 %** | 224.3610 | 5980038 | 21.4106 | 1051773 |
| **100.0 %** | 249.2900 | 6624970 | 25.1889 | 1234269 |
| **110.0 %** | 274.2190 | 7252894 | 28.9673 | 1415689 |
| **120.0 %** | 299.1480 | 7943777 | 32.7456 | 1601912 |
| **Correlation coefficient (r)** | 0.999992 | | 0.999981 | |
| **Slope** | 26546.0858 | | 48711.0962 | |
| **Intercept** | 2875.8539 | | 6475.1201 | |
| **Residual sum of squares** | 1570093447.6066 | | 12660869.5301 | |

**Table 4**
**Precision studies**

| Parameters | Method Precision | | | | | Intermediate Precision | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample No. | Assay | | Purity (% w/w) | | | Assay | | Purity (% w/w) | | |
| | Benzoic acid | Miconazole Nitrate | IMP-A | IMP-B | IMP-C | Benzoic acid | Miconazole Nitrate | IMP-A | IMP-B | IMP-C |
| 1 | 99.3 | 99.1 | 0.517 | 0.524 | 0.491 | 101.5 | 99.5 | 0.504 | 0.527 | 0.510 |
| 2 | 98.9 | 98.6 | 0.522 | 0.530 | 0.493 | 101.7 | 99.4 | 0.513 | 0.527 | 0.515 |
| 3 | 99.1 | 98.7 | 0.516 | 0.521 | 0.491 | 102.0 | 99.9 | 0.504 | 0.527 | 0.491 |
| 4 | 99.2 | 98.9 | 0.528 | 0.538 | 0.503 | 101.1 | 98.8 | 0.506 | 0.523 | 0.503 |
| 5 | 98.1 | 97.8 | 0.522 | 0.522 | 0.491 | 101.6 | 99.4 | 0.504 | 0.523 | 0.491 |
| 6 | 98.6 | 98.3 | 0.535 | 0.540 | 0.501 | 101.4 | 99.5 | 0.505 | 0.512 | 0.501 |
| **Mean** | 98.9 | 98.6 | 0.523 | 0.529 | 0.495 | 101.6 | 99.4 | 0.506 | 0.523 | 0.495 |
| **%RSD** | 0.5 | 0.5 | 1.3 | 1.5 | 1.2 | 0.3 | 0.4 | 0.8 | 1.1 | 0.4 |

The precision studies (expressed as the relative standard deviation (RSD) were determined for miconazole nitrate and benzoic acid and the values are presented in Table 4.

**Table 5**
**Recovery studies**

| Drug | Levels | % recovery | % RSD |
|---|---|---|---|
| **Miconazole Nitrate** | 80% | 99.0 | 0.2 |
| | 100% | 99.1 | 0.2 |
| | 120% | 98.9 | 0.3 |
| **Benzoic acid** | 70% | 98.5 | 0.3 |
| | 100% | 98.6 | 0.0 |
| | 130% | 98.5 | 0.1 |
| **Miconazole Impurity-A** | LOQ | 96.0 | 1.3 |
| | 0.5% | 97.4 | 0.5 |
| | 1.0% | 96.9 | 1.0 |
| **Miconazole Impurity-B** | LOQ | 101.5 | 1.3 |
| | 0.5% | 100.4 | 0.7 |
| | 1.0% | 99.7 | 0.7 |
| **Miconazole Impurity-C** | LOQ | 94.0 | 0.6 |
| | 0.5% | 97.3 | 0.6 |
| | 1.0% | 97.4 | 0.7 |

The recovery experiment values in Table 5 obtained were performed by adding known amount of IMP-A, IMP-B and IMP-C impurities spiked into nine different flasks containing placebo with Miconazole nitrate drug substance and for Miconazole nitrate, drug substance stock solution spiked into nine different flasks containing placebo in three different levels, each in triplicate viz., LOQ level, 0.5% & 1.0% impurity level (Figure 2). Miconazole Nitrate at high concentration levels accuracy performed at 80.0%, 100.0% and 120.0%, w/w of sample concentration. Each concentration will be prepared 3 times by separate weighing and contains placebo (without Active) at 100%, w/w level. Accuracy for Benzoic Acid performed at 70.0%, 100.0% and 130.0%, w/w levels. Each concentration prepared 3 times by separate weighing and contains placebo (without Benzoic Acid) at 100%, w/w level. The stability of sample was checked by forced degradation in different conditions and % of degradation was calculated. The peak purity of the analytes was passed in all conditions (purity angle should be less than the threshold value). The following values in Table 6 indicate that any other impurity is not merging with the main peak.

**Table 6**
**Robustness studies**

| S.NO | Condition | % RSD | |
|---|---|---|---|
| | | Miconazole Nitrate | Benzoic acid |
| 1 | **Flow (+20%)** | 1.0 | 1.0 |
| 2 | **Flow (-20%)** | 1.0 | 1.0 |
| 3 | **Temperature (35ºc)** | 0.2 | 0.2 |
| 4 | **Temperature (40ºc)** | 0.0 | 0.1 |
| 5 | **Wavelength (+2nm)** | 0.2 | 0.2 |
| 6 | **Wavelength (-2nm)** | 0.1 | 0.2 |
| 7 | **Sonication time (+2%)** | 0.5 | 0.5 |
| 8 | **Sonication time (-2%)** | 0.5 | 0.5 |
| 9 | **Column lot-1** | 0.2 | 0.3 |
| 10 | **Column lot-2** | 0.3 | 0.3 |

The reliability of the method was determined by made small deliberate variations in method parameters and the RSD values (Table 7) obtained, an indication of its reliability on normal usage. Selectivity solution and reference solution was injected on different days during the validation study. Using the system suitability software, USP resolution between Imp- B and Miconazole Nitrate was calculated from selectivity solution and USP tailing, USP plate count for Benzoic peak and Miconazole Nitrate were calculated from Reference solution-1. % RSD for five replicate injections for Miconazole nitrate peak and Benzoic acid peak were calculated from reference solution-1. % Recovery for Miconazole nitrate peak and Benzoic acid peak was calculated from reference solution-2 against reference solution-1

**Table 7**
**System suitability studies**

| Experiment Name | Resolution | USP Tailing | | USP Plate count | | % RSD | | % Recovery | |
|---|---|---|---|---|---|---|---|---|---|
| | | Peak-1 | Peak-2 | Peak-1 | Peak-2 | Peak-1 | Peak-2 | Peak-1 | Peak-2 |
| System Suitability | 2.3 | 1.1 | 1.7 | 50172 | 32938 | 0.2 | 0.1 | 101.0 | 100.6 |
| Method Precision, Filter study & Robustness | 2.2 | 1.1 | 1.7 | 45529 | 27239 | 0.3 | 0.3 | 101.0 | 100.4 |
| Solution stability | 1.7 | 1.1 | 1.5 | 52168 | 19448 | 0.2 | 0.1 | 101.0 | 99.9 |
| Specificity | 2.4 | 1.1 | 1.4 | 37500 | 31568 | 1.0 | 1.0 | 100.0 | 100.4 |
| Linearity | 2.3 | 1.0 | 1.5 | 45654 | 28981 | 0.1 | 0.1 | 99.4 | 99.3 |
| Accuracy | 2.4 | 1.0 | 1.4 | 44603 | 32699 | 0.1 | 0.2 | 98.7 | 99.5 |
| Stress study & Robustness | 2.3 | 1.0 | 1.4 | 36589 | 29110 | 0.2 | 0.3 | 100.1 | 100.1 |

## CONCLUSION

The validated stability indicating HPLC method for Assay, Chromatographic Purity of Miconazole Nitrate and Assay of Benzoic Acid in Daktarin Gel (Miconazole Nitrate 2.0% w/w) is specific, linear, accurate, precise and robust. Analytical data of method precision study (performed by reference/sending laboratory, Lab-1) and intermediate precision study (performed by receiving laboratory, Lab-2) were considered for the analytical method transfer activity. Hence, the analytical methodology for the assay of Miconazole nitrate, assay of Benzoic acid and degradation products of Miconazole nitrate in Daktarin gel is suitable for its intended use.

## CONFLICT OF INTEREST

Conflict of interest declared none.

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