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A RAPID GAS CHROMATOGRAPHY- MASS SPECTROSCOPY METHOD FOR SIMULTANEOUS QUANTIFICATION OF ORNIDAZOLE AND MICONAZOLEFROM CREAM FORMULATIONS: DEVELOPMENT, VALIDATION AND APPLICATION

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Keywords:

Ornidazole, Miconazole, GC MS, Hyphenation, Formulation analysis

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ABSTRACT: Combination drug formulations are better in terms of effectiveness and hence are used many times for treatment of diseases. The physico-chemical properties of the different API used in a formulation have a significant impact in the development of a single method for the analysis of such drugs. In the current research a rapid analytical method employing GC with Mass Spectroscopy detection has been developed and validated for simultaneous quantification of the active ingredients Ornidazole and Miconazole from the cream formulation. The analytes were extracted from cream base and filtered. A 95% dimethyl polysiloxane column with 5% phenyl polysiloxaneis used for the separation of the analytes. The method involves simple temperature gradient and MS detection. Validation of the method showed response was a linear function of concentration in the range 50-150 µg/mL for both Ornidazole and Miconazole. The method was suitably validated and was found to be precise and robust, with recoveries for both the analytes being consistent and complete. The method has been successfully applied for the analysis of samples from marketed cream formulations.

INTRODUCTION: Drug resistance development is on the rise due to the rampant and uncontrolled use of antibiotics and other drugs used in the treatment of infections. Combination drug treatments and therapies are being developed for many drugs that were originally used as standalone therapies for a faster and better control of the infections. For the analytical chemist this presents with a new set of challenges. Many times the components of this combination therapy have significantly differing physical (solubility, melting point etc.) and chemical (pKa, UV absorption maxima, stability in solvent etc.) properties.

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In addition to this, there are the excipients and preservatives which also need to be separated from the analytes of interest for the quantification of the active drugs in the formulation. If separate methods are employed for the determination of the active ingredients then efficiency of the QC lab is affected as more time and efforts are needed from the chemist and less output is delivered in terms of number of samples analysed. Efforts are now being put in developing a common analytical method for multicomponent formulation analysis.

Ornidazole (**Fig. 1**) is an antifungal agent of the 5-nitro imidazole class of compounds. Ornidazole has a molecular formula $C_7H_{10}ClN_3O_3$ and its molecular weight is 219.625. It is soluble in chloroform and methanol. It is available commercially in the form of tablets, creams etc. Various analytical methods have been developed for the pharmaceutical analysis of Ornidazole alone

or in combination with other drugs using HPLC [1-3], HPTLC [4-6], GC [7], Derivative spectroscopy [8-11] method of analysis etc.

Miconazole (**Fig. 2**) is an imidazole antifungal agent. Miconazole has a molecular formula $C_{18}H_{14}Cl_4N_2O$ and its molecular weight is 416.127. It is soluble in ethanol, methanol, acetone and chloroform. It is marketed as injection, tablet, cream etc. Various analytical methods have been developed for the pharmaceutical analysis of Miconazole alone or in combination with other drugs using HPLC [12-15], HPTLC [15-17], GC[18], Derivative spectroscopy [19] method of analysis etc.

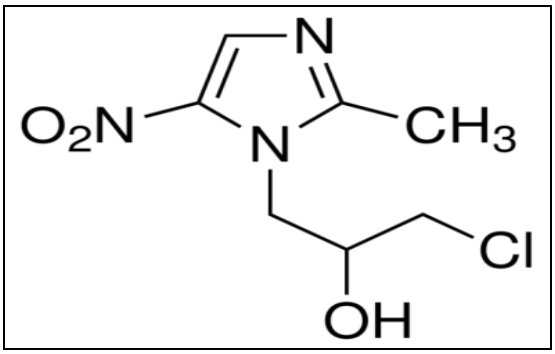

FIG.1: CHEMICAL STRUCTURE OF ORNIDAZOLE

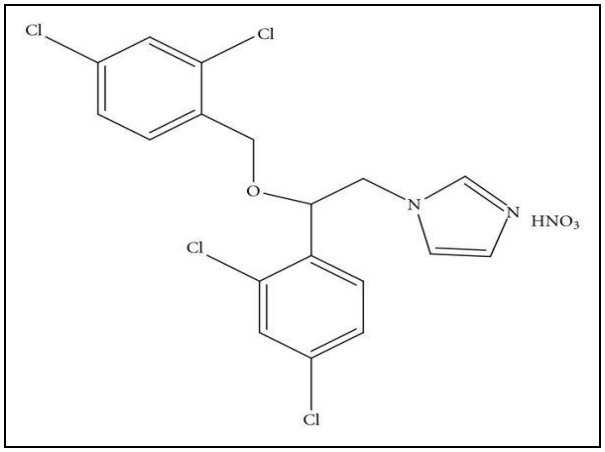

FIG.2: CHEMICAL STRUCTURE OF MICONAZOLE

There are methods available in public domain for the estimation of Ornidazole and Miconazole using HPLC [20] and HPTLC [21]. However, HPTLC technique is affected by various atmospheric factors such as humidity and temperature. Furthermore, the solvents used in the HPTLC analysis are Class 2 solvents such as Chloroform and Toluene; which presents additional challenges in the disposition of the analysis waste. For the HPLC method, the column performance and mobile phase can affect the results. In case of stationary phase degradation over time, the retention times can shift creating overlapping of peaks. Additionally the time required for the saturation and equilibration of an HPLC column is significantly higher and also consumes the costly solvents. The GC analysis method on the other hand uses only small quantity of Class 3 solvents and the components required for chromatography such as air, nitrogen, oxygen and hydrogen can be generated easily from the atmosphere and pure water. Furthermore the mass spectroscopic detection provides unparalleled specificity for the compound under investigation.

There is hence a need for developing an analytical method better suited for quantification of Ornidazole and Miconazole for routine quality control analysis. The current research involves development and validation of a new GC-MS method to quantify the drugs from marketed cream formulations as per the ICH Q2 (R1) guidelines [22].

MATERIALS AND METHODS:

Chemical and reagents:

The working standards of Ornidazole (99.85%) were provided by Endoc Lifecare Pvt. Ltd., India and Miconazolenitrate (99.70%) were obtained from Cipla Ltd., India. Analytical standards of methyl paraben and propyl paraben were provide by Cipla Ltd., India. HPLC grade methanol was used from Ranchem.

Preparation of solutions:

Two separate stock solutions each of Ornidazole (OZ) and Miconazole (MZ)nitrate were prepared for the calibration curve and precision and accuracy experiment for the method validation exercise. The stock solutions of OZ and MZ were prepared in Methanol and stored at 2-8°C. The stock concentration for OZ and MZ were 1000µg/ ml respectively by dissolving about 50 mg of each standard in 50 ml of methanol. Subsequent dilutions of the stock solutions were prepared from stock solutions by dilution with Methanol. For identification purpose, solutions of 5 and 0.5 µg/ ml respectively of methyl paraben and propyl paraben were prepared. The working standard solutions thus prepared were used to prepare the solutions used in the validation experiment.

For Ornidazole and Miconazole asevenpoint standard curve was prepared. The calibration curve ranged from 50 - 150µg/mL with concentration levels as 50, 60, 80, 100, 120, 140 and 150µg/mL for both OZ and MZ.

Sample preparation procedure:

The cream samples of about 1 gm was weighed in a clean dried 100 ml volumetric flask. Care was taken so that the cream sample does not stick to the neck of the flask. 30 ml of methanol was added to the flasks and sonicated for 60 seconds to disperse the cream in the methanol. The flask was further heated at 70°C for about 5 minutes to aid in the cream dispersion. The flasks were allowed to cool to come to the room temperature and then diluted to volume with methanol. The samples were further filtered with syringe filters into GC vials for analysis and crimped with Teflon septa caps to avoid any solvent evaporation.

Gas chromatography conditions:

Chromatographic separation was carried out using a Shimadzu QP2010 Ultra GCMS with a95% dimethyl polysiloxane 5% phenyl polysiloxane ((Restek CorpRtx5MS; 60m x 0.25mm ID, 0.1 µm film thickness) column. For each of the solutions 1 µl was injected in the chromatography system; in split mode with spilt ratio of 1:5. Nitrogen gas was used as carrier gas at a flow rate of 1.0 ml/ min. The injector port was maintained at 280ºCThe column oven is maintained initially at 180 ºC for 2 min and ramped to 280 ºC at the rate of 25ºC and held at 280ºC for 5 mins. The total run time for each sample analysis was 11 min. For the Mass spectroscopy detector, the following settings were maintained in **Table 1**.

TABLE 1: CHROMATOGRAPHIC SYSTEM PARAMETERS

Gas Chromatograph	Shimadzu 2010 GC
Detector	QP 2010 Ultra MS detector
Column	RTX-5 (60 m, 0.25 mm id, 0.1 µm FT)
Injection volume	1 µL (Split mode 1:5)
Carrier gas	Nitrogen
Carrier gas flow	1.0 ml/ min (in constant flow mode)
Injector temperature	280 ºC
Temperature programing	180 ºC hold for 2 mins 180 ºC to 280 ºC @ 25 ºC/min Hold @ 280 ºC for 5 mins
Interface temperature	250 ºC
Ion source temperature	200 ºC
Detector voltage	0.7 KV
Solvent cut off	0 mins
Acquisition mode	Scan
Acquisition speed	1111 scans/ sec
Scan range (m/z)	20 to 550

Method Validation:

The analytical method for quantification of OZ and MZ from cream formulation has been validated for selectivity, linearity, precision, accuracy, solution stability, ruggedness and robustness following appropriate recommendations of the ICH Q2(R1)regulatory guidelines recommendations [22].

RESULTS AND DISCUSSION:

Specificity:

Specificity was performed by chromatographing the individual working level solutions of Ornidazole, Miconazole, methyl paraben and propyl paraben. OZ and MZ were solutions of 100 ppm each and 5 ppm solution of methyl paraben and 0.5 ppm solution of propyl paraben were injected in the chromatographic system. No interfering peak of endogenous compounds was observed at the retention time of the analytes. The theoretical plates, tailing factor observed for peaks of OZ and MZ are 25286 &1.17and 93824&1.22 respectively. The resolution between the peaks of OZ and MZ was 57.27. Representative chromatograms of Ornidazole, miconazole, methyl parabenand propyl paraben are presented in **Fig. 3, 4**, **5** and **6** respectively. The representative chromatogram for sample solution is presented in **Fig. 7**.

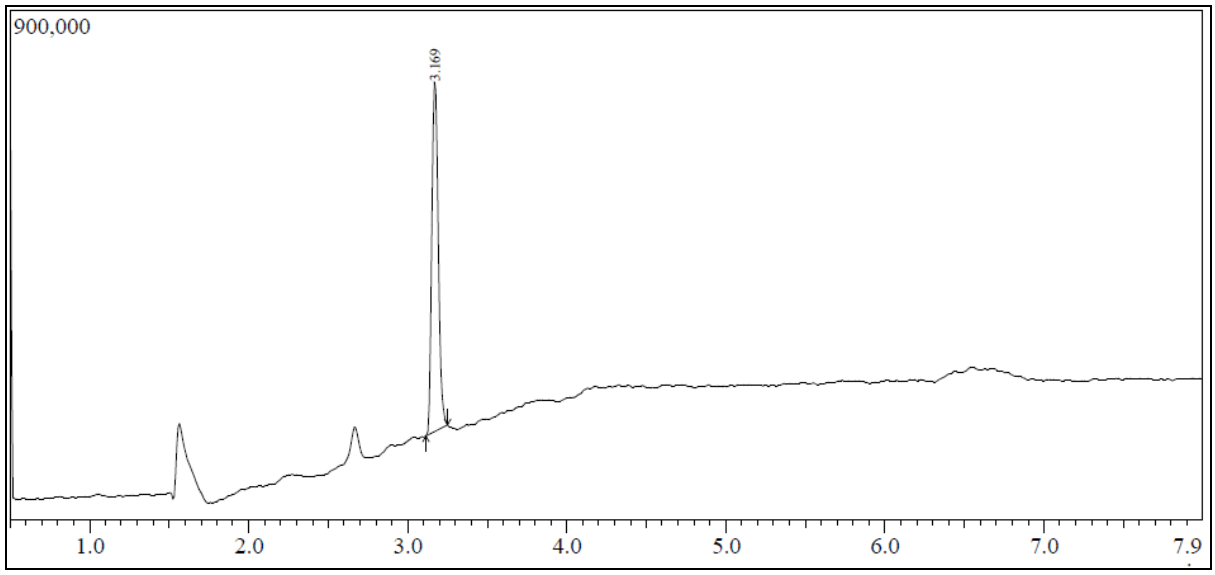

FIG.3: REPRESENTATIVE CHROMATOGRAM OF ORNIDAZOLE

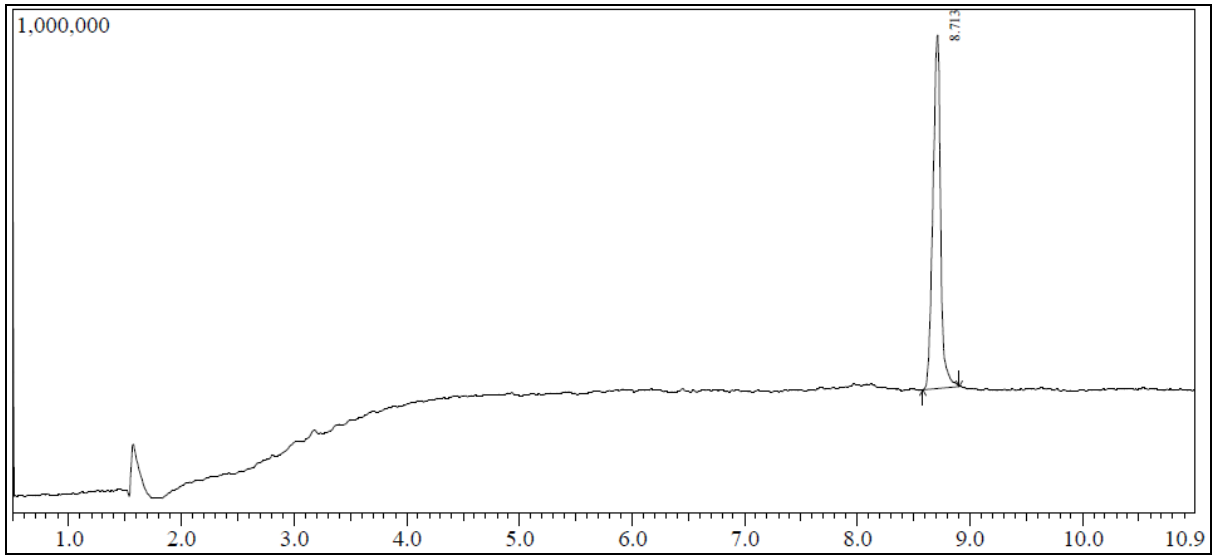

FIG. 4: REPRESENTATIVE CHROMATOGRAM OF MICONAZOLE

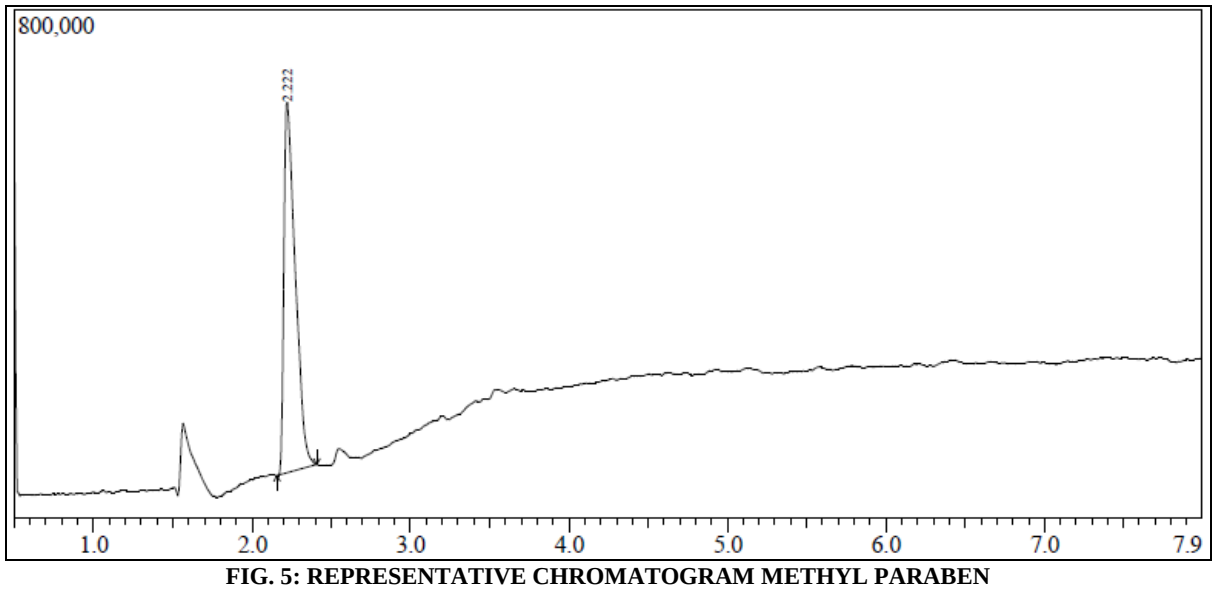

FIG. 5: REPRESENTATIVE CHROMATOGRAM METHYL PARABEN

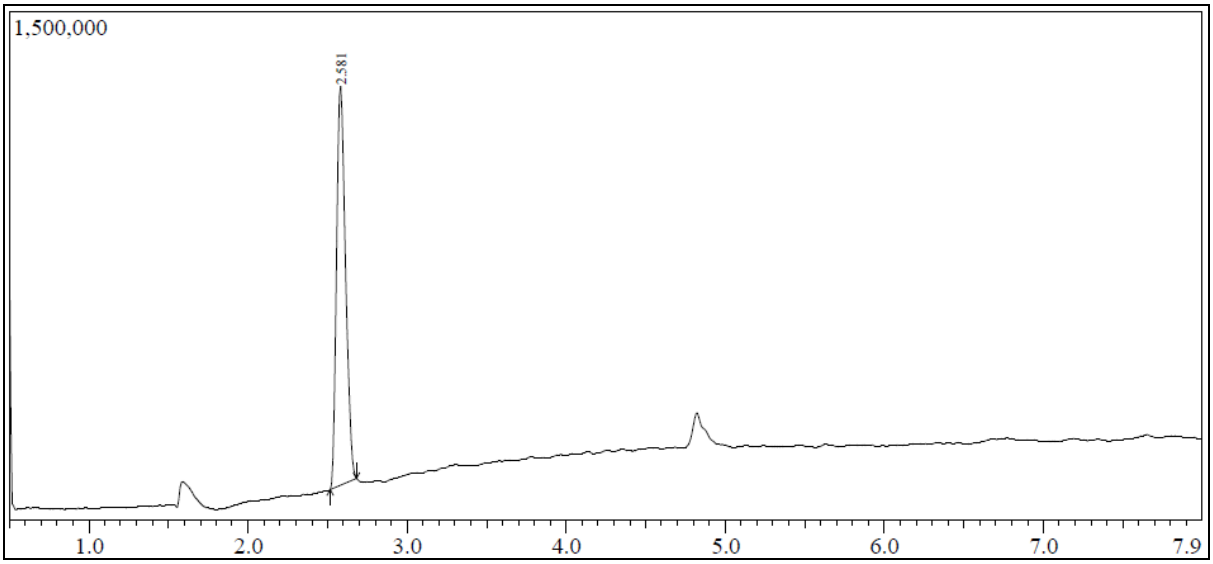

FIG.6: REPRESENTATIVE CHROMATOGRAM PROPYL PARABEN

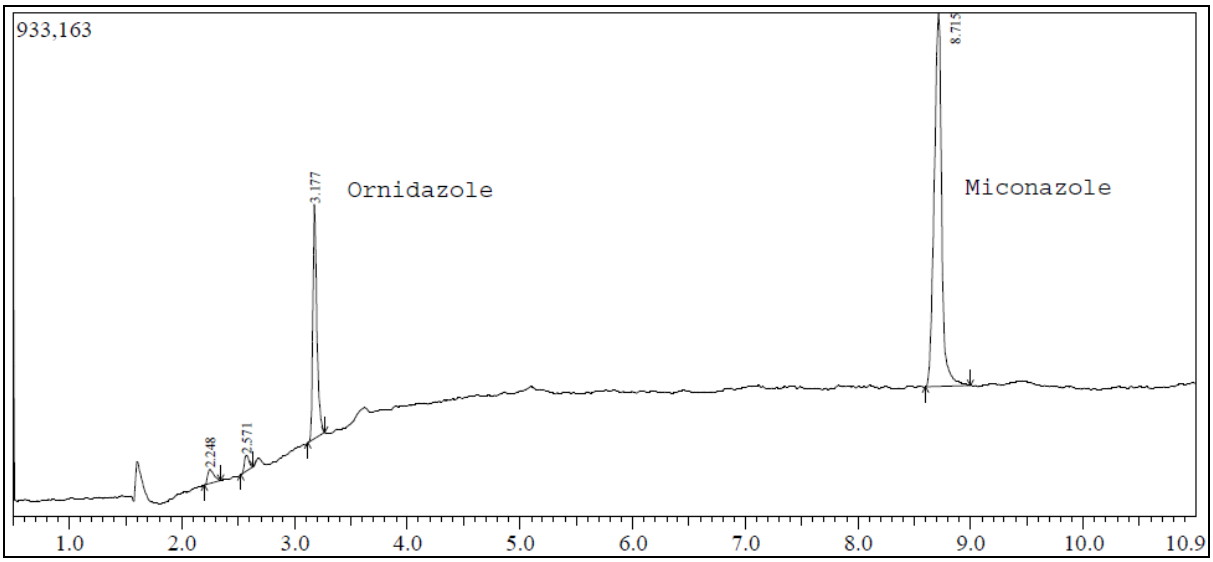

FIG.7: REPRESENTATIVE CHROMATOGRAM OF SAMPLE

Precision

System precision

System suitability was evaluated by injecting six replicates of the mix standard preparation in the chromatographic system. The relative standard deviation (RSD) of the area response and the retention time were evaluated. The RSD values for area response was found to be 0.16 and 0.48 for OZ and MZ respectively. The RSD values for retention time was found to be 0.28 and 0.18 forOZ and MZrespectively.

Method precision

Method precision was evaluated by injecting six preparations each of the two marketed formulations. The RSD for the back calculated % label claim of the active components was evaluated. The RSD values for the assay of OZ and MZ was found to be 0.98% and 0.64% respectively.

Linearity and range

The response against concentration relationship was evaluated using a seven point calibration curve. Mixed linearity levels were prepared having concentration of 50, 60, 80 100, 120, 140 and 150 µg/ml for both OZ and MZ. The detector response of a 1 µl injection volume was measured and was plotted against the nominal concentration of each concentration level for both the analytes. The analytical method was found to be linear between 50 to 150µg/ml for both Ornidazole and Miconazole. The regression coefficient value were 0.9949and 0.9979 for the analytes OZ and MZ

respectively. The linearity plot for OZ and MZ are shown in **Fig. 8** and **9** respectively.

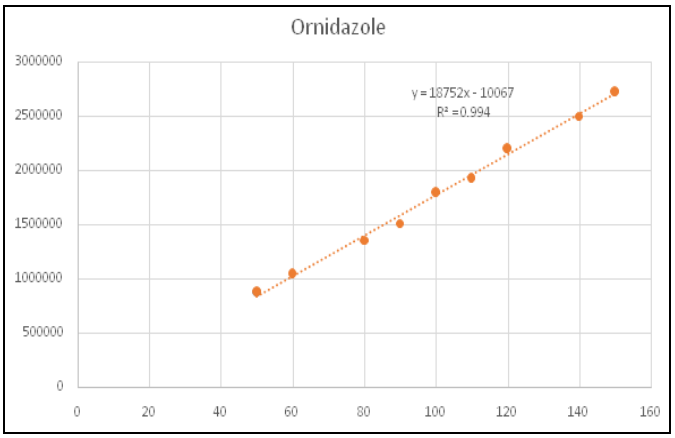

FIG. 8: LINEARITY PLOT FOR ORNIDAZOLE

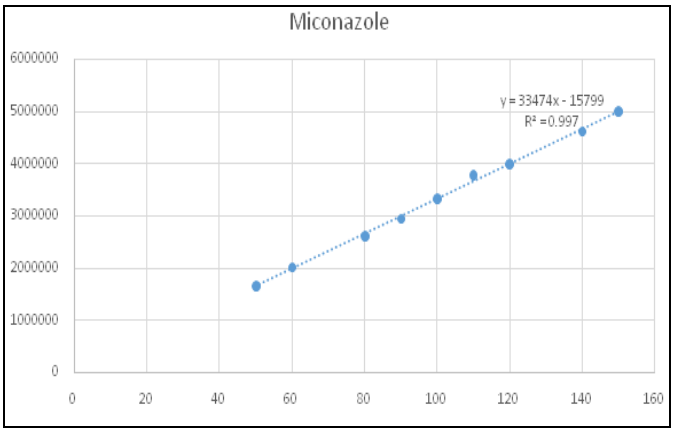

FIG. 9: LINEARITY PLOT FOR MICONAZOLE

Accuracy:

Accuracy of the method was checked using recovery method i.e. by spiking the WS solution in formulation sample and checking the recovery. Since the formulations under investigation were procured from the market, the placebo was not available; hence the recovery was conducted by adding the standard of each component in the cream formulation and then checking the recovery for added standard. The Accuracy of the method was evaluated at three levels i.e. 50, 100 and 150 % of the working concentration.

Three replicate weighing at each of the three levels were prepared. These samples were then processed as per the sample processing method. The resultant solution was injected into the chromatographic system. The back calculated content of each of the three replicates at an individual level were evaluated. The accuracy was found to be consistent for both OZ and MZ across the three concentration levels. The accuracy for OZ was found to be between 99.91% and 100.55% and for MZ was found to be between 99.47% and 100.13%.

Ruggedness (Intermediate precision):

For ruggedness experiment the sample preparation and analysis was performed by another analyst using the same method of analysis. Six replicates of each of the marketed formulation were prepared and chromatographed on the next day of the method precision experiment. Intermediate assay method precision was evaluated by injecting six preparations each of the two marketed formulations.

The RSD for the back calculated % label claim of the active components was evaluated. The RSD values for the assay of OZ and MZ was found to be 1.18% and 0.85% respectively. The cumulative RSD values for the 12 samples for assay of OZ and MZ was found to be 1.23% and 0.77% respectively.

Solution stability:

The sample solutions prepared for the assay method precision experiment were re-injected after intervals of 12, 24, 36 and 48 hrs after initial injections. The stability of the analytes in the sample solution was evaluated by comparing the back calculated assay values for both OZ and MZ. The analytes were found to be stable in the sample solution for at least 48 hrs. The stability was found to be 99.67% and 99.12% for OZ and MZ respectively.

Ruggedness:

As a part of the method validation, minor changes were done to the chromatographic parameters to determine their impact on the analysis results. The flow rate was changed from 1.0 ml/min to 0.9 ml/min and 1.1 ml/min. No merging of any peaks with the analytes peaks was observed. The results of the analysis in both the cases were found to be consistent with the precision experiment results. The initial column oven temperature was changed from 180 ºC to 170 ºC and 190 ºC. No merging of any peaks with the analyte peaks of interest was observed. The results of the analysis in both the cases were found to be consistent with the precision experiment results.

Application of method to marketed formulations:

The assay of OZ and MZ was performed on commercial marketed samples of the cream formulation. Purchased samples of Candimale and Candifem were analysed using the analytical method. The assay results were 100.55% and 99.94% for Candimale and 99.23% and 100.44% for Candifem. Assay testing performed on different days showed similar results.

CONCLUSION: Ornidazole and Miconazole have distinct boiling points and polarity index thus developing a simultaneous method of analysis was a difficult task. The preservatives present in the formulations methyl paraben and propyl paraben are the other components to be resolved from the peaks of OZ and MZ and which can also be quantified using this method.

The GC-MS assay method has been developed and validated for quantification of OZ and MZ from cream formulations. The validation data demonstrate good precision and accuracy and high specificity for the analytes. The method was robust and did not encounter any variation with minor changes in the method parameters. This method was applied for the analysis of marketed formulations and were found to provide consistent and accurate results. This method significantly improves upon the drawbacks of the previously reported methods. It has also been observed that placebo interference is drastically reduced in the present method compared to the reported methods.

This assay method for simultaneous quantification of OZ and MZ will be beneficial for the routine and Quality control analysis of cream formulations containing these active ingredients, by saving the time and efforts of the analyst. This method can also be applied for the quantification of methyl and propyl parabens as well with suitable validation.

Compliance with Ethical Standards:

Conflict of interest: The work presented herein has been conducted as part of doctoral research work and the researcher has not received any grant for the said work. None of the authors have any conflict of interest in the presented work.

This work does not contain any research involving humans and/ or animals.

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