DOI: 10.21276/sajp.2017.6.4

Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2017; 6(1): 27-33 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Original Research Article

RP-HPLC method development and validation for the simultaneous estimation of Miconazole and Clindamycin in pharmaceutical dosage forms

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Abstract: A simple, Accurate, precise method was developed for the simultaneous estimation of the Miconazole and Clindamycin in Tablet dosage form by RP-HPLC. Chromatogram was run through kromasil 150mm x 4.6 mm, 5µ. Mobile phase containing Buffer and Acetonitrile taken in the ratio 20:80 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.01N KH_2PO_4solution. Temperature was maintained at 30°C. Optimized wavelength for Miconazole and Clindamycin was 210nm. Retention time of Miconazole and Clindamycin were found to be 2.076min and 2.795min. % RSD of the Miconazole and Clindamycin were and found to be 0.99 and 1.05 respectively. Percentage assay was obtained as 100.23% and 99.25% for Miconazole and Clindamycin respectively. LOD, LOQ values are obtained from regression equations of Miconazole and Clindamycin were 1.42ppm, 0.60ppm and 4.31pm, 1.82ppm respectively. Regression equation of Miconazole is y = 19688x + 25044, and y = 18253x + 2735.5 of Clindamycin.
Keywords: Miconazole, Clindamycin, RP-HPLC, Retention time, Assay.

INTRODUCTION

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance [7-10], separation of the components of a solution or mixture, or determination of structure of chemical compounds. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, microorganisms, minerals and various synthetic products.

Miconazole [1-6] is an imidazole antifungal agent, developed by Janssen Pharmaceutical, commonly applied topically to the skin or to mucous membranes to cure fungal infections. It works by inhibiting the synthesis of ergosterol, a critical component of fungal cell membranes. It can also be used against certain species of Leishmania protozoa which are a type of unicellular parasite that also contain ergosterol in their cell membranes. In addition to its antifungal and antiparasitic actions, it also has some antibacterial properties. It is marketed in various formulations under various brand names. Miconazole is also used in Ektachrome film developing in the final rinse of the Kodak E-6 process and similar Fuji CR-56 process, replacing formaldehyde. Fuji Hunt also includes Miconazole as a final rinse additive in their formulation of the C-41RA rapid access color negative developing process.

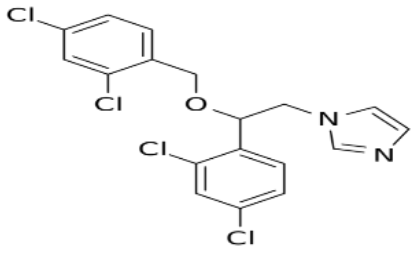

Fig-1: Chemical structure of Miconazole

Clindamycin [7-10] is an antibiotic of the lincosamide class, which blocks the ribosomes of microorganisms. It is not only used to treat infections caused by anaerobic bacteria, but also used to treat protozoal diseases, such as malaria. It is a common topical treatment for acne and can be useful against some methicillin-resistant Staphylococcus aureus (MRSA) infections. The most severe common adverse effect of Clindamycin is Clostridium difficile-associated diarrhea (the most frequent cause of Pseudomembranous colitis). Although this side effect occurs with almost all antibiotics, including beta-lactam antibiotics, it is classically linked to Clindamycin use.

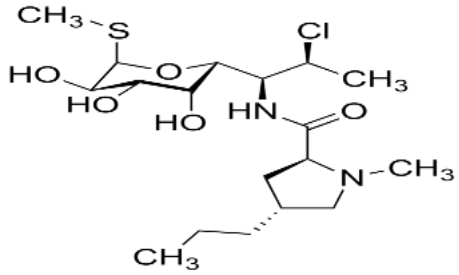

Fig-2: Chemical structure of Clindamycin

MATERIALS AND METHODS

MATERIALS

All the reagents used in the experiment were HPLC grade solvents, Miconazole and Clindamycin, Combination of Miconazole and Clindamycin capsules, milli-Q water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial aceticacid, methanol, potassium dihydrogen phosphate buffer, tetrahydrofuran, triethyl amine, ortho-phosphoric acid etc.

Instrument

HPLC instrument was used of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Miconazole and Clindamycin solutions.

METHODS

Preparation of buffer

1ml of OPA was taken in 1000 ml volumetric flask and make up to the mark with milli-Q water.

Standard Preparation (200µg/ml Miconazole and 100µg/ml Clindamycin)

Accurately Weighed and transferred 20mg&10mg of Miconazole and Clindamycin working Standards into 50ml and 50ml clean dry volumetric flasks separately, add 3/4th volume of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above each stock solution, 1 ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Sample Preparation

20 capsules were weighed and calculate the average weight of each tablet then the tablet powder weight equivalent to 200mg of Miconazole and 100mg of Clindamycin was transferred into a 500ml volumetric flask, 200ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pippete out into a 10 ml volumetric flask and made upto 10ml with diluent.

Linearity

Linearity solutions are prepared such that 0.20ml, 0.5ml, 0.70ml, 1ml, 1.20ml, 1.5ml from the Stock solutions Miconazole and Clindamycin are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 10ppm, 20ppm, 30ppm, 40ppm, 50ppm, 60ppm of Miconazole and 5ppm, 10ppm, 15ppm, 20ppm, 25ppm, 30ppm of Clindamycin.

Accuracy

Standard Preparation: (200µg/ml Miconazole and 100µg/ml Clindamycin)

Accurately Weighed and transferred 20mg&10mg of Miconazole and Clindamycin working Standards into 50ml and 50ml clean dry volumetric flasks separately, add 3/4th volume of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above each stock solution, 1 ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Preparation of 50% Spiked Solution

Accurately Weighed and transferred 100mg & 50mg of Miconazole & Clindamycin working Standards int 500 ml clean dry volumetric flasks, add 3/4th volume of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution of Miconazole, 1 ml was pipette out in to a 10ml Volumetric flask and then make up to the final volume with diluent, and 1 ml of Clindamycin solution was pipette out in to a 10ml Volumetric flask and then make up to the final volume with diluents.

Preparation of 100% Spiked Solution

Accurately Weighed and transferred 200mg & 100mg of Miconazole & Clindamycin working Standards int 500 ml clean dry volumetric flasks, add 3/4th volume of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution of Miconazole, 1 ml was pipette out in to a 10ml Volumetric flask and then make up to the final volume with diluent, and 1 ml of Clindamycin solution was pipette out in to a 10ml

Volumetric flask and then make up to the final volume with diluents.

Preparation of 150% Spiked Solution

Accurately Weighed and transferred 300mg & 150mg of Miconazole & Clindamycin working Standards int 500 ml clean dry volumetric flasks, add 3/4th volume of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution of Miconazole, 1 ml was pipette out in to a 10ml Volumetric flask and then make up to the final volume with diluent, and 1 ml of Clindamycin solution was pipette out in to a 10ml Volumetric flask and then make up to the final volume with diluent.

Method Development

Many trials were done by changing columns and Mobile phases and were reported the optimized method below. Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column Used : Kromasil 150 x 4.6 mm, 5µ.
Buffer : 0.01N KH_2PO_4 solution
Mobile phase : buffer: Acetonitrile (45:55A)
Flow rate : 1.0 ml/min
Diluent : water: Acetonitrile:: 30:70
Wavelength : 210nm
Temperature : 30°C
Injection Volume: 10µl

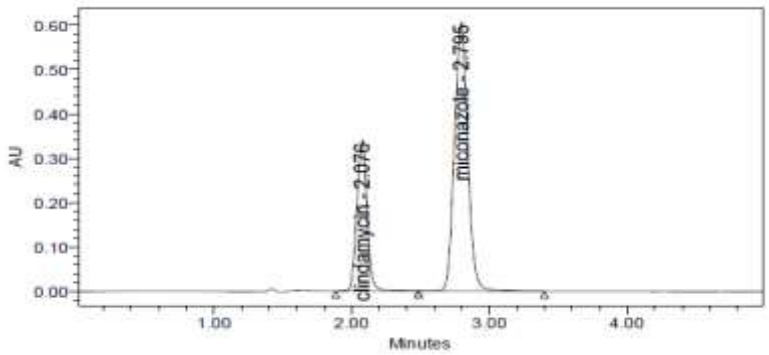

Fig-3: Optimized chromatogram of Miconazole and Clindamycin

Method Validation

All the solutions were prepared according to the procedures given under preparation of standard and sample solutions. The developed method was validated as per ICH guidelines.

RESULTS AND DISCUSSIONS

System suitability

All the system suitability parameters are within range and satisfactory as per ICH guidelines

Table-1: System suitability studies of Miconazole and Clindamycin

Property	Miconazole	Clindamycin
Retention time (R)	2.161min	2.906min
Theoretical plates (N)	3248 ± 63.48	5729 ± 63.48
Tailing factor (T)	1.59 ± 0.117	1.34± 0.117

Linearity

Six Linear concentrations of Miconazole (50-300ppm) and Clindamycin (25-150ppm) are prepared and injected. Regression equation of the Miconazole and Clindamycin are found to be, y = 19688x +25044, and y = 18253x + 2735.5. And regression co-efficient was 0.999.

Table-2: Calibration data of Miconazole and Clindamycin

S.no	Miconazole (µg/ml)	Response	Clindamycin (µg/ml)	Response
1	50	995088	25	479043
2	100	2018499	50	930547
3	150	2966078	75	1353885
4	200	4045421	100	1789034
5	250	4940768	125	2272070
6	300	5881466	150	2777619

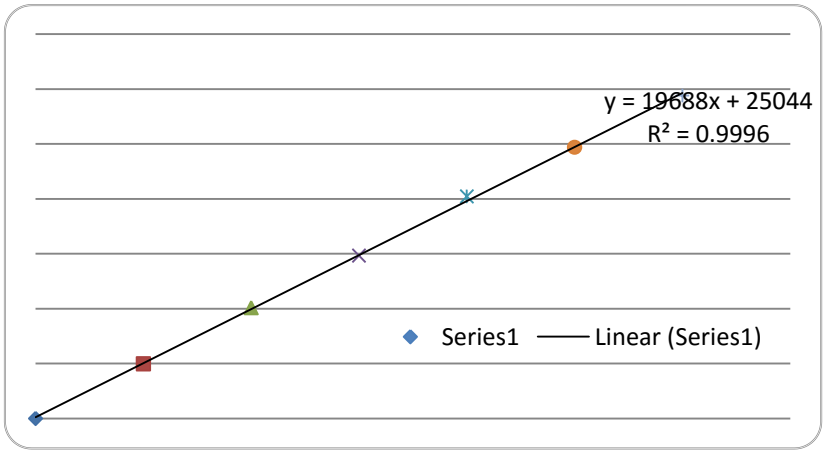

Fig-4: Calibration curve of Miconazole

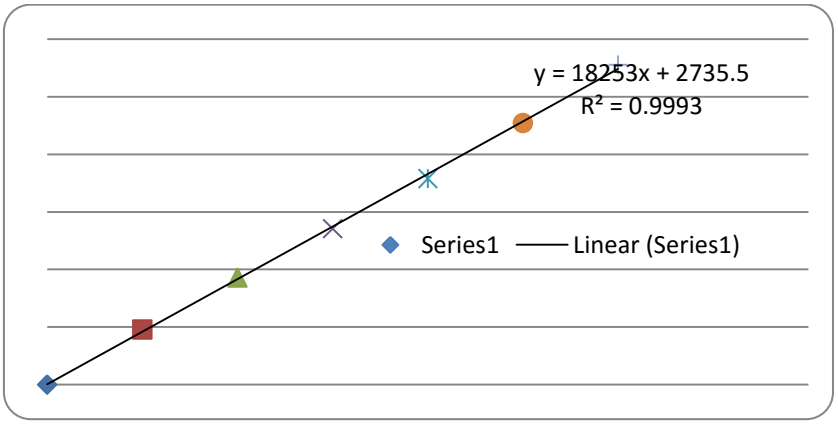

Fig-5: Calibration curve of Clindamycin

Precision

Intraday precision (Repeatability)

Intraday Precision was performed and % RSD for Miconazole and Clindamycin were found to be 0.99% and 1.05% respectively. Results were showed in Table-3.

Inter day precision

Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Miconazole and Clindamycin were 0.33% and 0.44%. Results were showed in Table-3.

Table-3: Repeatability results for Miconazole and Clindamycin.

S.No.	Intraday precision		Inter day precision	
1	4056806	1753689	4061879	1748881
2	4080217	1767216	4083520	1748881
3	4108521	1741336	4062401	1738937
4	4152240	1721718	4062401	1754379
5	4054132	1751050	4072790	1759290
6	4134282	1721981	4043238	1759372
Mean	4097700	1742832	4064372	1751623
Std. Dev.	40758.1	18236.2	13402.5	7776.9
%RSD	0.99	1.05	0.33	0.44

Accuracy

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 4.

LOD

Limit of detection was calculated by standard deviation method of Miconazole and Clindamycin and LOD for Miconazole and Clindamycin were found to be 1.42 and 0.60 respectively.

Table-4: Table of Accuracy

Sample	Recovery Level	Recovery (%)	% RSD
Miconazole	100	100.19	1.61
	200	99.91	1.51
	300	99.35	1.18
Clindamycin	50	100.18	1.17
	100	101.14	0.65
	150	99.58	1.02

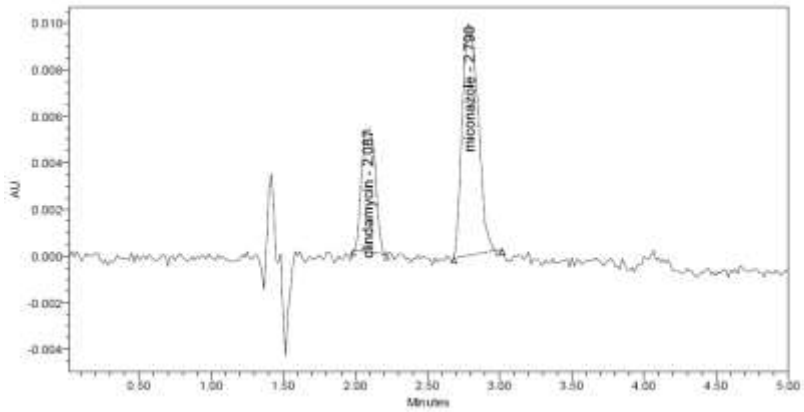

Fig-6: LOD Chromatogram of Miconazole and Clindamycin

LOQ

Limit of Quantification was calculated by standard deviation method of Miconazole and Clindamycin and LOQ for Miconazole and Clindamycin were found to be 4.31 and 1.82 respectively.

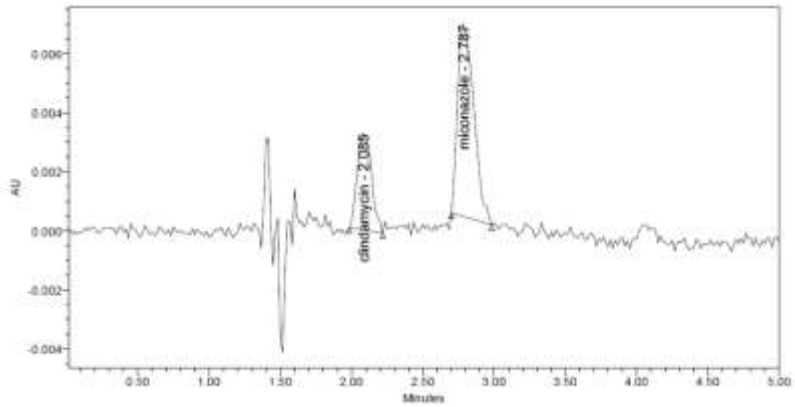

Fig-7: LOQ Chromatogram of of Miconazole and Clindamycin.

Robustness

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Table-5: Robustness data of Miconazole and Clindamycin

S.NO	Robustness condition	Miconazole %RSD	Clindamycin %RSD
1	Flow minus	0.2	1.7
2	Flow Plus	0.6	0.2
3	Mobile phase minus	0.3	0.3
4	Mobile phase Plus	0.3	0.3
5	Temperature minus	0.3	0.3
6	Temperature Plus	0.5	4.6

Assay

Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.23% and 99.25% for Miconazole and Clindamycin respectively.

Table-6: Assay Data of Miconazole and Clindamycin Tablet

S. No.	Miconazole %Assay	Clindamycin %Assay
1	99.23	99.87
2	99.80	100.64
3	100.50	99.17
4	101.57	98.05
5	99.17	99.72
6	101.13	98.06
AVG	100.23	99.25
Std.Dev	0.997	1.04
%RSD	0.99	1.05

All the validated parameters were checked by applying statistical formulas such as standard deviation and relative standard deviation. The results were found to fall within the prescribed limits.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Miconazole and Clindamycin in Tablet dosage form. Retention time of Miconazole and Clindamycin were found to be 2.076min and 2.795min. %RSD of the Miconazole and Clindamycin were and found to be 0.99 and 1.05 respectively. %assay was obtained as 100.23% and 99.25% for Miconazole and Clindamycin respectively. LOD, LOQ values are obtained from regression equations of Miconazole and Clindamycin were 1.42ppm, 0.60ppm and 4.31ppm, 1.82ppm respectively. Regression equation of Miconazole is y = 19688x + 25044, and y = 18253x + 2735.5 Of Clindamycin. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in industries.

REFERENCE

1. Satoskar RS, Bhandarkar SD, Ainapure SS. Pharmacology and Pharmacotherapeutics, 17th edition, Popular Prakashan, Mumbai, India, 2001.
2. Burger's Medicinal Chemistry and drug discovery", 6th edition, Wiley Interscience, New Jersey, 2007.
3. "Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry", 11th edition, Lippincott Williams & Wilkins, New york, 2004.
4. Korolkovas A. "Essentials of Medicinal Chemistry", 2nd edition, Wiley Interscience, New Jersey, 1988.
5. "Goodman and Gilman's The Pharmacological Basis of Therapeutics", 9th edition, McGraw-Hill health professions division, New york, 1996.
6. Foye WO. Foye's principles of medicinal chemistry. Lemke TL, Williams DA, editors. Lippincott Williams & Wilkins; 2008.
7. USP 31/NF 26, United States Pharmacopoeia, 31st rev. and the National Formulary, 26 ed. United States Pharamcopoeial Convention, Rockville, 2008.
8. Daum RS: Clinical practice. Skin and soft-tissue infections caused by methicillin-resistant Staphylococcus aureus. N Engl J Med. 2007 Jul 26; 357(4):380-90.
9. Klempner MS, Styrt B: Clindamycin uptake by human neutrophils. J Infect Dis. 1981 Nov; 144(5):472-9.
10. Lamont RF: Can antibiotics prevent preterm birth--the pro and con debate. BJOG. 2005 Mar;112 Suppl 1:67-73.
11. Plaisance KI, Drusano GL, Forrest A, Townsend RJ, Standiford HC: Pharmacokinetic evaluation of two dosage regimens of clindamycin phosphate. Antimicrob Agents Chemother. 1989 May; 33(5):618-20.
12. White TC, Marr KA, Bowden RA: Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev. 1998 Apr; 11(2):382-402
13. Ghannoum MA, Rice LB: Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin Microbiol Rev. 1999 Oct; 12(4):501-17.
14. Edlind T, Smith L, Henry K, Katiyar S, Nickels J: Antifungal activity in Saccharomyces cerevisiae is modulated by calcium signalling. Mol Microbiol. 2002 Oct; 46(1):257-68.

15. Georgopapadakou NH, Walsh TJ: Antifungal agents: chemotherapeutic targets and immunologic strategies. Antimicrob Agents Chemother. 1996 Feb; 40(2):279-91.
16. Heneedak HM, Salama I, Mostafa S, Sadek M.El. J Chromatogr Sci, 2012; 50(10): 855-861.
17. Cemal Akaya, Sibel A Özkanb. II Farmaco, 2003; 57(11):953–957.
18. Prashant K. Pager, Chandrakant S. Satpute, Varsha M. Jadhav. Scholars Research Library Der Pharmacia Lettre, 2012; 4 (6):1793-1804.
19. Ashour S, Kattan N. Simultaneous determination of miconazole nitrate and metronidazole in different pharmaceutical dosage forms by gas chromatography and flame ionization detector (GC-FID). Int J Biomed Sci. 2010 Mar 1; 6(1):13-8.