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Development and Validation of Spectroscopic Analytical Method for Simultaneous Estimation of Mupirocin and Satranidazole in Bulk and Topical Formulation

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ABSTRACT

The aim of the present investigation was to develop and validate a simple, precise and cost effective UV spectroscopic method for simultaneous estimation of mupirocin and satranidazole in bulk and its formulation using methanol and water (50:50) as solvent. The method was proposed in the present work, the maximum absorbance was shown at 220 nm for mupirocin and 213 nm for satranidazole in zero order derivative spectroscopy. The concentration range was 1-5 and 0.5-2.5 µg/mL with correlation coefficient 0.999 for both mupirocin and satranidazole. The drugs followed Beer-Lambert's law in the selected concentration range and exhibited good correlation coefficient (r2 =0.999). The proposed methods are effectively applied to its ointment formulation across all validation studies as per ICH guidelines. Accuracy of the method was verified by performing recovery studies using simultaneous equation method and found to be 100.69-101.51% for mupirocin and 99.86-102.96 % for satranidazole. Excellent mean recovery studies for precision, repeatability, ruggedness and sensitivity results showed that the method has been validated successfully, the results are also in accordance with the % RSD values obtained within specified limits. The proposed method was applied to the determination of MUP and SATRA, the mean % amount was found to be 99.11 and 100.35 (SATRA) with % RSD values was NMT 2.0% indicates the developed method was successfully applied for analysis of formulation. The developed spectrophotometric method can be employed for routine analysis of mupirocin and satranidazole in bulk as well as in the commercial ointment formulation.

INTRODUCTION

Analysis is important in every product but it is vital in medicines as it involves life. The assurance of quality is achieved through analysis of drug product. Marketed survey revealed day by day new drugs and their combination with another drugs are being introduced in market as they have more patient compliance than a single drug. Analytical methodology should be used for quality control and stability studies. Analytical methods are necessary to assure the identity, strength, quality, purity and bioavailability of drug product and stability.

Analytical methods are measure of quality of the drugs which play a very comprehensive role in drug development and follow-up activities to assure that a drug product meets the established standard and will continue to meet reported quality throughout its shelf life (Ahuja *et al*., 2001). Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable (Scypinski *et al*., 2001). Analytical methods should be used with in good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols setup in the international conference on harmonization (ICH) guidelines (Q2A and Q2B) (ICH Steering Committee, 1996).The development of method, an important issue is for the drug in the form of a formulation due to the interference caused by the formulation excipients in a short time period and minimum trials. The UV-visible spectrophotometry is one of the most frequently employed methods in pharmaceutical analysis. It involves the measurement of amount of ultraviolet (190-380 nm) or visible (380-800 nm) radiation absorbed by a substance in solution. Absorption of light in both the ultraviolet and visible regions of the electromagnetic spectrum occurs when the energy of the light matches that required inducing in the molecule an electronic transition, associated vibrational and rotational transitions (Patel, 2008). The spectrophotometric assay of drug rarely involves the measurements of absorbance of samples containing only one absorbing component. The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substances is required in sample known to contain other absorbing substances, which potentially interfere in the assay. If the formula of the sample is known, identify and concentration of the interfering substance are known and the extent of interference in the assay may be determined. MUP is natural crotonic acid derivative extracted from pseudomonas fluorescens (Bageshwar *et al*., 2010).

Mupirocin inhibits bacterial protein synthesis by specific reversible binding to bacterial isoleucyl *t*RNA synthase with excellent activity against gram positive staphylococci and streptococci. It is primarily used for treatment of primary and secondary skin disorder, nasal infection and wound healing. Satranidazole, a novel nitroimidazole possess in a C-N linkage at C2 of the imidazole ring has been examined for its ability to damage DNA. It is stated that the drug produces extensive DNA damage characterized by helix destabilization and strand breakage. Its comparison with other 2 and 5- imidazole indicates it may be more active towards anaerobes than many 5-nitroimidazole. It is due to its relatively high redox potential which make it more resistant to inactivation by oxygen (Fig. 1). It is a highly potent, well tolerated and clinically useful agent for common protozoa (Arulappa *et al*., 2011). Method developed can be conveniently used for quality control and routine determination of drug in pharmaceutical preparation in pharmaceutical industry. A number of modifications to the simple spectrophotometric procedure are available to the analyst, which may eliminate certain sources of interference and permit the accurate determination of all of the absorbing components. Each modification of the basic procedure may be applied if certain criteria are satisfied. The literature survey reveals that various methods are present for the determination of mupirocin and Satranidazole individually or in combination with other drugs. The methods which are developed for mupirocin and satranidazole individually are HPLC, HPTLC. But there is no single method has been reported for combination of these two drugs. Therefore the reported research work aims to develop a simple, accurate, sensitive and reproducible method for mupirocin and satranidazole in ointment dosage form by simultaneous method.

MATERIALS AND METHODS

Materials

Mupirocin and satranidazole were received as gift sample from Kopran Pharma limited, Mumbai, India. Methanol, Acetonitrile, Ethanol, Soft paraffin, Lanolin, Propylene glycol was procured from Merck limited, India. All the chemicals and reagents were used of analytical grade.

Methods

Instrumentation

Spectrophotometric analysis was performed on UV Analytical Tech (UV 1800), Software UV analyst double beam Spectrometer, Mobile phase methanol: water (50:50v/v), detection wavelength 213 nm and 220 nm were selected to develop an accurate method.

Optimization of mobile phase and selection of wavelength

The standard solution of MUP and SATRA was scanned over the range of 200-400 nm wavelengths. The wavelength of absorption was found to be 220.0 nm and 213.0 nm for mupirocin and satranidazole respectively. For the development of spectrophotometric method initially distilled water (100%) was tried solvent, but in that solvent mupirocin and satranidazole were not properly soluble and the spectra were not obtained. Then in second trial methanol: water (20:80%) was used for better solubility but in that solvent, satranidazole got some turbidity hence the spectra was not properly obtained. After that conc. methanol: water (40:60%) was used wherein less turbid solution and spectra on 215nm and 221nm for mupirocin and satranidazole respectively were obtained but those wavelengths were not matched with standard spectra of drug. Then methanol: water (50:50) was tried wherein the drug was dissolved in methanol and volume was makeup with dist. water to get clear solution as well as proper spectra with standard wavelength which is 220 and 213 for mupirocin and satranidazole respectively.

Preparation of standard solutions

Accurately weighed each about 10 mg of mupirocin (MUP) and satranidazole (SATRA) standard were transferred to separate 100.0 mL volumetric flask. About 50.0 mL of methanol was added to each of the volumetric flasks and sonicated to dissolve the drug. The solution was cooled to the room temperature and made up to the mark with distilled water to get concentration 100 µg/mL of both solutions. From above solution 0.1, 0.2, 0.3, 0.4, 0.5mL of the stock solution for MUP was further diluted with solvent (methanol: water, 50:50) to a five 10 mL volumetric flasks individually with solvent to get concentration of 1,2,3,4 and 5µg/mL. Similarly for SATRA, 0.05, 0.1, 0.15, 0.2, 0.25 mL of the stock solution for MUP was further diluted to a five 10 mL volumetric flasks individually with solvent to get concentration of 0.5, 1, 1.5, 2 and 2.5µg/mL.

Formulation of ointment containing MUP and SATRA

Firstly high melting point base was melted in porcelain dish which was placed on water bath and other bases were added according to the high melting point to completely melt. The porcelain dish was removed from water bath and spread on tile. Drug was added to the base in a geometric manner till it persists ointment consistency (Cooper *et al*., 2008). The final ointment was placed inappropriate aluminum tube and stored in a cool and dry place. The compositions of ointment are illustrated in Table 1.

Estimation of MUP and SATRA in formulated ointment preparation

Extraction process

1g of ointment formulation was taken in centrifuge tube and diluted to 10mL with solvent. The centrifuge tube was heated at 70°C for 10min and mixed occasionally during heatingprocess. After heating, tube was centrifuged at 1000 rpm for 10min. Aliquot of the liquid layer was filtered using 0.4µm filter paper. From filtrate, 2 mL further diluted to 10mL with solvent and noted the absorbance at 213 nm and 220 nm.

Validation of UV-Spectrophotometric method

When method development and optimization are complete, it is necessary to accomplish method validation. For validation of analytical method, the guidelines of the international conference on the harmonization of technical requirements for the registration of pharmaceuticals for human use has recommended validation characteristics including system suitability, accuracy (%recovery), linearity, precision (%RSD) were investigated.

Linearity

Linearity curve was plotted for the quantitative estimation of mupirocin and satranidazole. Linearity of the method was confirmed by preparing standard curves for the analytical range of 1-5 µg/mL and 0.5-2.5 µg/mL for MUP and SATRA respectively. The calibration curves were prepared in between absorbance and concentrations were subjected to least square linear regression analysis to generate the calibration equations and calculate correlation coefficients.

Limit of detection and limit of quantitation

Limit of detection and limit of quantitation was measured by calculating standard deviation and slope of the calibration curves. ICH guidelines describe several approaches to determine the detection and quantitation limits. The LOD and LOQ are the lowest level and lowest concentration of the analyte respectively in a sample that would yield signal -to- noise ratio of 3.3 for LOD and 10 for LOQ. These are determined from the standard deviation of the peak response and the slope of the calibration curve.

Accuracy

Weighed 1g of placebo (without API), diluted to 10 mL with solvent in centrifuge tube. Centrifuge tube was heated at 70°C in water bath for 10 min followed by centrifugation at 1000 rpm for 10 min. The mixture was filtered and from filtrate 2 mL of the solution was taken and diluted to 10 mL.

Standard preparation

20 mg of mupirocin and 10 mg of satranidazole were weighed and dissolved in 10 mL solvent in two different 10mL volumetric flasks. From the above stock solution 1mL of mupirocin and satranidazole pipette respectively and diluted to 10 mL. The concentration of this solution was 200 µg/mL mupirocin and 100 µg/mL satranidazole respectively. From 200 stock of mupirocin 0.8, 1.0, 1.2 mL of mupirocin was added to three different 10 mLvolumetric flasks which were labeled as 80%, 100%, and 120%. 1mL of placebo was added to each flask. To another three 10mL volumetric flasks 0.8, 1.0, 1.2mL of standard satranidazole (100µg/mL) was added. 1mL of placebo was added to each volumetric flask and dilute to 10mL with solvent. The absorbance of the above solution was noted against blank. The concentration of the above solution was determined by substituting the absorbance value in simultaneous equation method. The equations are constructed as per simultaneous equation as given follow (Beckett et al., 1988);

$A_1 = a_{x1}bc_x + a_{y1}bc_y$…….. (1)

$A_2 = a_{x2}bc_x + a_{y2}bc_y$…….. (2)

Determination of E (1%1cm) of drugs at selected wavelengths

Aliquot portion from MUP stock solution was transferred to 10 mL of volumetric flask and volume was adjusted to mark to obtain the concentration of 2µg/mL. Similarly, aliquot portion from SATRA stock solution was transferred to 10 ml volumetric flask; volume was adjusted to mark to obtain concentration of 1 µg/mL. The absorbance of these solutions was recorded at two wavelengths 220 nm and 213nm.

E (1%1cm) = absorbance/ concentration (g/100ml)

Concentration of C_x and C_y of MUP and SATRA respectively in g/100 mL in the sample solution can be obtained as;

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

A_1 and A_2 are the absorbances of the sample solution measured at 220 and 213 nm.

Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Six replicates of 10µg/ml of mupirocin and 10 µg/ml of satranidazole were prepared together and the absorbance was noted at two wavelengths (213nm and 220nm). The precision is reported in terms of % RSD.

Intermediate precision

Intra-day and inter-day variations are determined by analyzing three different solutions of MUP and SATRA within the same day and three different days over a period of week. Intra-day precision was estimated by analyzing 2, 3 and 4µg/mL of MUP and 1, 1.5 and 2µg/mL of SATRA for three times within the same day. Inter-day precision was estimated by analyzing above mentioned concentrations of both the drugs for three different days over a period of week.

Repeatability

The tests were performed by collecting data from five replicate of standard solutions. An accurately weighed quantity of MUP about 10 mg and SATRA about 5 mg were transferred separately into 100.0 mL volumetric flask. About 50.0 mL of methanol was added to the volumetric flask and sonicated to dissolve the drug. The solution was cooled to the room temperature and made up to the mark with distilled water to get the final concentrations of 100.0 µg/mL MUP and 50.0 µg/mL SATRA respectively. Further the solution was diluted to get 4 µg/Ml MUP and 2 µg/mL SATRA.

Ruggedness

Robustness was tested using so called factor 'one factor at a time' method. The factors evaluated were mobile phase composition, flow rate, wavelength and change analyst.

RESULTS AND DISCUSSION

An attempt was made to develop simple UV spectrophotometric method for the simultaneous estimation of mupirocin and satranidazole in formulation with solvent. Optimization of spectrophotometric conditions was done by initially taking distilled water (100%), but in that solvent mupirocin and satranidazole were not properly soluble. Thensecond trial, we used methanol: water (20:80%) for better solubility, but some turbidity obtained hence the spectra was not properly obtained. After that conc. methanol: water (40:60%) was used wherein less turbid solution and spectra on 215nm and 221nm for mupirocin and satranidazole respectively were obtained but those wavelengths were not matched with standard spectra of drug. Then methanol: water (50:50) was tried wherein the drug was dissolved in methanol and volume was makeup with dist. water to get clear solution as well as proper spectra with standard wavelength which is 220 and 213 for mupirocin and satranidazole respectively.

The λ_{max} is the point at which both the drugs in a particular combination will have same absorbance at a single wavelength. From the overlay spectra, two wavelengths 220.0 nm (λ_{max} of MUPI) and 213.0 nm (λ_{max} of SATRA) were selected for estimation of drugs using Simultaneous Equation Method (SEM). The isosbestic point of mupirocin and satranidazole was found to be 249.0 nm. The maximum absorbance was found to be at 220 nm for mupirocin and 213 nm for satranidazole. The linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of the analyte with a given range.

Beer's law states that absorbance is proportional to the concentration of the absorbing species (ICH IC, 2005). Both the drug was linear in the concentration range of 1-5µg/mL and 0.5-2.5µg/mL. The correlation coefficients calculated from calibration curve were 0.999 and 0.999 for mupirocin and satranidazole respectively (Table 2). The result shows an excellent correlation between the absorbance and the concentrations of drugs in the selected range. UV- Visible spectra and overlay spectra of MUPI and SATRA are shown in Fig.2. It is evident from the standard calibration curve that there exists an excellent linearity characteristic with r^2 value of 0.999 %. Under the experimental condition described, the spectra showed linear relationship. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were y = 0.141x + 0.049 (r^2 = 0.998) at 220 nm for mupirocin and y = 0.172x + 0.038 (r^2 = 0.997) for satranidazole at 213 nm. From the data obtained standard deviation (SD) and % RSD were calculated. The % RSD should be less than 2.0%. The relative standard deviation of six replicates measurements of standard solution was found to be 0.26% (limit NMT 2.0%), which indicates that the system is precise to analyze the sample. Placebo solution was prepared in the same manner as standard and sample preparation. No interference of placebo was found. The placebo showed the highest absorbance at same wavelength spectrophotometrically. No interference of placebo was found. Accuracy is the closeness of the best result obtained by the method to the true value. The concentration recovered should be within ±2% to the true value. To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. Amount of the drug recovered was calculated using simultaneous equation method for accuracy. The percentage of the standard added to the pre analyzed sample was calculated and it was found to be 100.69-101.51% for mupirocin and 99.86-102.96% for satranidazole indicates good accuracy of the methodfor the determination of MUP and SATRA in bulk drug (Table 3 and 4).The recovery study results with statistical validation have shown in Table 5 shows accuracy of the method and level of interference of excipients for the proposed method.% RSD of the intermediate precision studies were found in between1.13-1.94 % for mupirocin and 0.82-1.47% for satranidazole which indicate that method was precise. The % RSD of repeatability precision was found to be 0.48% for mupirocin and 0.72% for satranidazole respectively (Table 6). The amount was found with %RSD (NMT than 2%) which was in agreement with system suitability. Therefore, the proposed method for the determination of MUP and SATRA in a tablet was found to be sufficiently precise. Repeatability was determined by the analyzing MUP (4µg/ml) and

SATRA (2µg/ml) of drug solution for five replicates and results are shown in Table 7 and 8. The repeatability again shows the closeness of the observed results that enhance the reliability of the above method. LOD for mupirocin and satranidazole were found to be 0.13 and 0.19 $\mu g.mL^{-1}$ respectively. LOQ for mupirocin and satranidazole were found to be 0.46 and 0.58 $\mu g.mL^{-1}$ respectively. The mean standard deviation is 0.004 and 0.0029 and slope is 0.049 and 0.038 for mupirocin and satranidazole respectively. The ruggedness of the method was checked by changing the analyst worked. Ruggedness of proposed method is determined by analysis of aliquots from homogenous slot by two analysts using same operation and environmental condition; the results are given in Table 9. The % RSD was found to be 0.11-0.38 % for mupirocin and 1.38-1.64% for satranidazole respectively. Lastly, the specificity, as well as selectivity, ensures that the observed data are totally free of any interference as the placebo interference is believed to be negligible. So we can assure that the proposed method for the analytical evaluation of mupirocin and satranidazole is validated.

Application of developed method for ointment drug content

Ointment preparation was prepared by suitable standard method containing MUPI (200 mg) and SATRA (100 mg) for the application of the proposed method. An absorptivity value of MUP and SATRA are calculated and represented shown in Table 10.From the absorptivity value, the concentration of drugs in the sample solution was determined by Vierodt's method (United State Pharmacopoeia, 1998). The results of estimation of MUP and SATRA are shown in Table 11 and 12.The proposed method was applied to the determination of MUP and SATRA in ointment formulation. The mean % amount found was 99.19 (MUP) and 100.35 (SATRA) with % RSD values was NMT 2.0% indicates the developed method was successfully applied for analysis of formulation. All the results found were in good agreement

In the present investigation, a simple, sensitive, reproducible and economical analytical method was developed and validated for the assay of the mupirocin and satranidazole by UV spectrophotometry. Method developed can be conveniently used for quality control and routine determination of drug in pharmaceutical dosage forms in pharmaceutical industry. The result of analysis of ointment formulation and recovery studies obtained by spectrophotometric method was statistically validated and high percentage of recovery studies suggest that the developed method was free from interferences of excipients generally used in ointment formulation. The developed method was statically validated in terms of accuracy, precision, linearity and reproducibility. Hence, above method can be employed in

quality control to estimate the amount of mupirocin and satranidazole in bulk and commercial semisolid formulation.

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Table 1: Ointment formulation containing MUP and SATRA

Sr. No.	Compositions	Quantity (100 g)	Quantity (10 g)
1	Mupirocin	2%	0.2%
2	Satranidazole	1%	0.1%
3	Soft paraffin (base)	25%	2.5%
4	Lanoline (base)	45%	4.5%
5	Liquid paraffin (base)	18%	1.8%
6	Propylene glycol	9%	0.9%

Table 2: Calibration of mupirocin and satranidazole in methanol: water (50:50)

Sr. No.	Conc. (µg/ml)		Absorbance (± S.D)	
	Mupirocin	Satranidazole	MUP (220)	SATRA(213)
1	1	0.5	0.20±0.003	0.12±0.002
2	2	1	0.32±0.004	0.22±0.003
3	3	1.5	0.48±0.003	0.29±0.001
4	4	2	0.61±0.005	0.39±0.007
5	5	2.5	0.76±0.007	0.47±0.001
	Slope		0.141	0.172
	Intercept		0.049	0.038
	Correlation coefficient (R^2)		$R^2 = 0.9986$	$R^2 = 0.9978$

Table 3: Recovery studies for mupirocin

Sr. No.	Level of % Recovery	Initial amount present (µg/ml)	Amount of standard Added (µg/ml)	Total Amount present (µg/ml)	Total amount Recovered (µg/ml)	% Recovery
		2	1.6	3.6	3.63	101.86
1	80	2	1.6	3.6	1.61	101.15
		2	1.6	3.6	1.62	100.17
		2	2	4.0	4.03	101.80
2	100	2	2	4.0	4.02	101.50
		2	2	4.0	4.03	101.80
		2	2.4	4.4	2.41	100.41
3	120	2	2.4	4.4	2.42	100.97
		2	2.4	4.4	2.40	100.0

Table 4: Recovery studies for satranidazole

Sr. No.	Level of % Recovery	Initial amount present (µg/ml)	Amount of standard Added (µg/ml)	Total Amount present (µg/ml)	Total amount Recovered (µg/ml)	% Recovery
		1	0.8	1.79	0.79	98.75
1	80	1	0.8	1.80	0.80	100.16
		1	0.8	1.80	0.80	100.16
		1	1	2.03	1.03	102.96
2	100	1	1	2.02	1.02	102.38
		1	1	2.01	1.01	101.12
		1	1.2	2.23	1.23	102.71
3	120	1	1.2	2.22	1.22	101.66
		1	1.2	2.22	1.22	101.66

Table 5: Statistical validation for recovery studies of mupirocin and satranidazole

	Mupirocin			Satranidazole		
Level of % Recovery	80	100	120	80	100	120
% Mean Recovery	101.51	101.65	100.69	99.86	102.6	102.1
Standard Deviation	0.5	0.21	0.4	1.7	0.41	0.74
% RSD	0.49	0.21	0.39	1.7	0.4	0.73

Table 6: Result of precision

Drug	Amount Taken [µg/ml]	Intra-day (n=3)		Inter-day (n=3)	
		Amount Found (µg/ml)	% RSD	Amount Found (µg/ml)	% RSD
MUP	2	1.99	1.13	2.03	1.54
	3	2.98	1.93	3.05	1.37
	4	4	1.94	4.05	1.35
SATRA	1	1	1.35	1	1.03
	1.5	1.52	0.82	1.46	0.29
	2	1.96	1.47	2.05	0.65

Table 7: Statistical validation for repeatability and precision for MUP

Sample Conc. (µg/ml)	Number of measurement	Absorbance	Precision for drug	
			Amount found (µg/ml)	% of Label claim
4	1	0.6212	4.05	101.24
4	2	0.6134	4.00	100.00
4	3	0.6145	4.01	100.26
4	4	0.6157	4.02	100.50
4	5	0.6178	4.03	100.85
Mean			100.57	
Standard Deviation			0.02	
% RSD			0.48	
Coefficient of variance			0.003	
Standard mean error			0.122	
Lower 95% Confidence limit			99.517	
Upper 95% confidence limit			100.149	

Table 8: Statistical validation for repeatability and precision for SATRA

Sample Conc. (µg/ml)	Number of measurement	Absorbance	Precision for drug Amount found (µg/ml)	% of Label claim
2	1	0.3917	2.06	102.81
2	2	0.3911	2.02	102.64
2	3	0.3901	2.05	102.64
2	4	0.3905	2.05	102.50
2	5	0.3913	2.05	102.70
Mean		102.66		
Standard Deviation		0.11		
% RSD		0.72		
Coefficient of variance		0.003		
Standard mean error		0.122		
Lower 95% Confidence limit		99.517		
Upper 95% confidence limit		100.149		

Table 9: Result of ruggedness

Sr. No.	Analyst	Amount Found (%)		% RSD	
		MUP	SATRA	MUP	SATRA
1	Analyst -1	100.46	100.63	0.12	1.41
2	Analyst -2	100.31	102.64	0.11	1.38
3	Analyst -3	101.04	99.27	0.38	1.64

Table 10: Absorptivity values of drugs at selected wavelengths

Absorptivity values	Wavelength at λ_{max}	
	220 nm	213nm
ax_1	165000	-
ax_2	-	21000
ay_1	16000	-
ay_2	-	22000

$ax_1$and ax_2= Absorptivity of MUP

$ay_1$and ay_2=Absorptivity of SATRA

Table 11: Statistical data for estimation of MUP in ointment formulation

Sr. No.	Conc. (µg/ml)	Absorbance	Amount found Assay(mg)	Drug Assay (%)
1	2.00	0.3283	1.66	99.01
2	2.00	0.3298	1.67	99.57
3	2.00	0.3282	1.66	99.00
	Mean	0.328767	1.663333	99.19333
	SD	0.000896	0.005774	0.326241
	% RSD	0.272622	0.347104	0.328894

Table 12: Statistical data for estimation of SATRA in ointment formulation

Sr. No.	Conc. (µg/ml)	Absorbance	Amount found Assay(mg)	Estimation % Drug Assay (%)
1	1.00	0.2098	0.99	99.88
2	1.00	0.2110	1.00	100.58
3	1.00	0.212	1.01	100.59
	Mean	0.210933	1	100.35
	SD	0.001102	0.01	0.407063
	% RSD	0.52221	1	0.405643

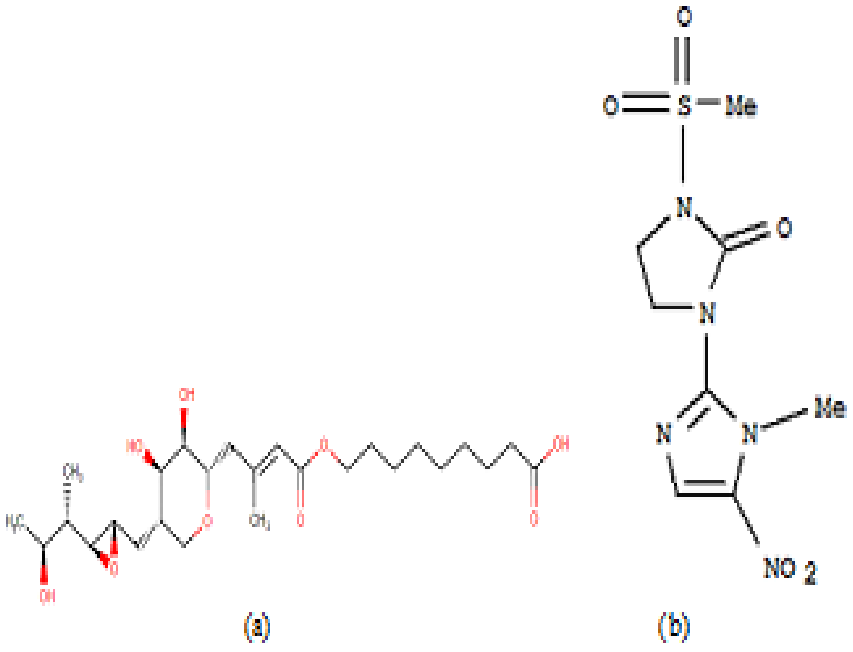

Fig. 1: Chemical structures of (a) Mupirocin (MUP) and (b) Satranidazole (SATRA)

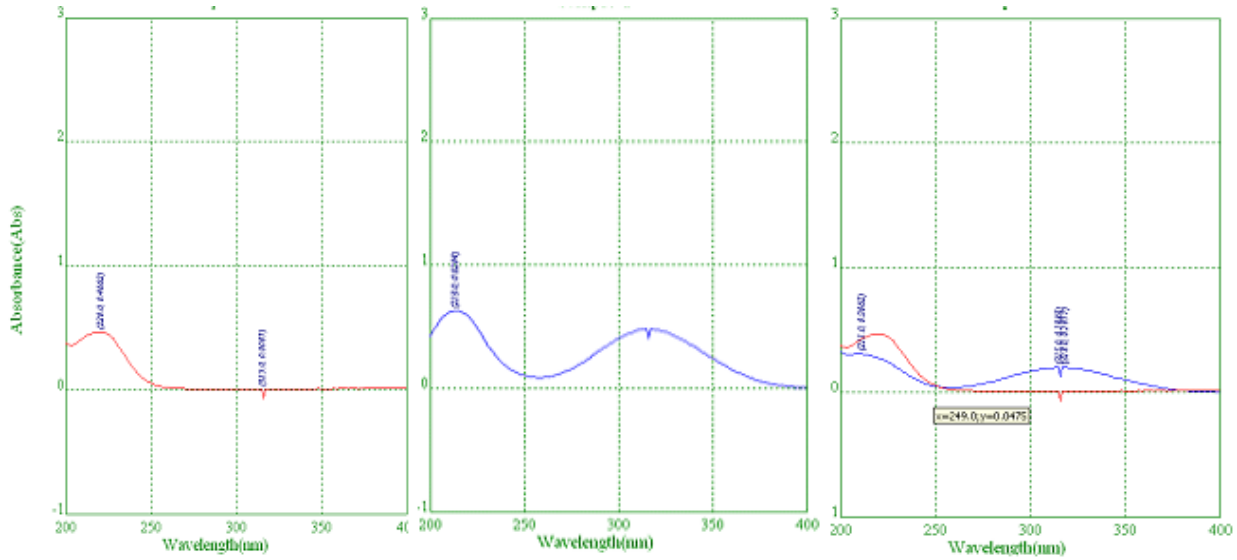

Fig.2: UV- Visible spectrum and overlay spectra of MUP and SATRA

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