

**INTERNATIONAL JOURNAL OF UNIVERSAL
PHARMACY AND BIO SCIENCES**

Pharmaceutical Sciences

Research Article.....!!!

**DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR
SIMULTANEOUS ESTIMATION OF KETOCONAZOLE AND
SALICYLIC ACID IN BULK AND DOSAGE FORM**

Sonali B. Gire*, P. A. Datar, R. V. Shete, K. J. Kore, V.R. Harnaskar

Department of Quality Assurance Technique, Rajgad Dnyanpeeth's College of Pharmacy,
Bhor, Savitribai Phule Pune University, Maharashtra.**KEYWORDS:**

Ketoconazole, Salicylic acid, Ultraviolet spectrophotometer, Simultaneous equation method, Q- Analysis.

For Correspondence:**Sonali B. Gire*****Address:**Department of Quality Assurance Technique,
Rajgad Dnyanpeeth's College of Pharmacy,
Bhor, Savitribai Phule Pune University,
Maharashtra.**ABSTRACT**

Two new simple, accurate and economic spectrophotometric methods in UV/VIS region have been developed for the determination of Ketoconazole and Salicylic Acid in bulk and lotion formulation. Due to mutual interference, quantitation was carried out by the proposed methods such as simultaneous equation Method and absorbance ratio Method. The wavelengths selected for simultaneous equation method were 261.00 nm and 255.00 nm i.e. the respective λ max of both the drugs. In absorbance ratio method, two wavelengths 261.00 nm, λ max of Ketoconazole and 295.00 nm, the iso-absorptive point were selected. Two methods follow Beer's linearity in the range of 10-35 μ g/ml for Ketoconazole and 10-35 μ g/ml for salicylic acid with correlation coefficient r^2 of 0.999 for Ketoconazole and Salicylic acid respectively. According to ICH guidelines the parameters linearity, precision, accuracy, limit of detection, and limit of quantification, robustness, ruggedness were studied, the results of analysis were validated statistically and by recovery studies. Recovery studies for Ketoconazole and Salicylic acid were performed and the percentage recovery for both the drugs was obtained in the range of 98.36-102.84% (Method A) and 97.48-100.7% (Method B) confirming the accuracy of the proposed method. The proposed methods were simple, cost effective and were successfully applied to the determination of these drugs in quality control of combined pharmaceutical dosage.

1. INTRODUCTION:

Ketoconazole and Salicylic Acid are available in tablet, cream and lotion formulation. Chemically Ketoconazole(1-[4-(4-{[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazine-1-yl]ethan-1-one). The drug is a highly effective broad spectrum antifungal agent. It is the traditional pharmaceutical substance. It has antiseptic and antifungal properties. It shows keratoplastic and keratolytic effect. Literature survey reveals many analytical methods for determination of Ketoconazole such as UV Spectrophotometry, HPLC, and Capillary electrophoresis methods from pharmaceutical preparations.¹

Salicylic acid is a (2-hydroxyl benzoic acid), and naturally occurring in the bark of willow tree (Salix alba). It is an important active metabolite of aspirin, which acts as a prodrug to salicylic acid. The salts and esters of salicylic acid are known as salicylates that are widely used as rubefacient and analgesic in several topical formulations. Salicylic acid alleviates peeling of intercellular cement and binds with scales in the stratum corneum, thereby loosening the keratin. This keratolytic effect also renders an antifungal effect as removal of the stratum corneum suppresses the fungal growth. It exerts antiinflammatory activity by suppressing the cyclooxygenase (COX) activity. Therefore, it is widely used for the treatment of several skin diseases like acne, psoriasis, seborrhoeic dermatitis, calluses, keratosis pilaris, and warts due to its keratolytic, fungicidal, bacteriostatic, and photoprotective properties.^{2,3}

Few analytical methods for determination of Salicylic Acid using UV Spectroscopy, HPLC and other chromatographic methods in plasma and pharmaceutical formulation have been reported. However, there are no reported methods for simultaneous estimation of both drugs in combination or in lotion formulation. This paper presents two simple, rapid, reproducible and economical methods for the simultaneous analysis estimation of both the drugs in bulk and pharmaceutical dosage form.

2. MATERIALS AND METHODS

Instruments

UV-Vis Spectrophotometer (Jasco V- 530 Spectrophotometer)

Digital balance(Shimadzu)

Sonicator (Cintex)

Materials

Standard gift samples of Ketoconazole were procured from (Ciron Drugs and Pharmaceutical Pvt. Ltd. Boisar), and Salicylic Acid were procured from (Research-Lab Fine Chem Industries

Mumbai). Lotion formulation containing both drugs are Kenz-sal Lotion (KLM Laboratories Pvt. Ltd.) purchased from local market.

3. STOCK SOLUTIONS

Standard stock solutions of ketoconazole and Salicylic acid were prepared by separately dissolving accurately weighed quantities (100 mg each) of ketoconazole and salicylic acid in 40 ml methanol and transferred it into 100 ml volumetric flask. Volume was made up to mark with methanol to obtain stock solution of 1000 µg/ml.

4. DETERMINATION OF λ MAX:

The standard solutions of ketoconazole (20µg/ml) and salicylic acid (20µg/ml) were scanned separately in the wavelength range of 200 – 400 nm and the λ max was found to be 261 nm and 255 nm.

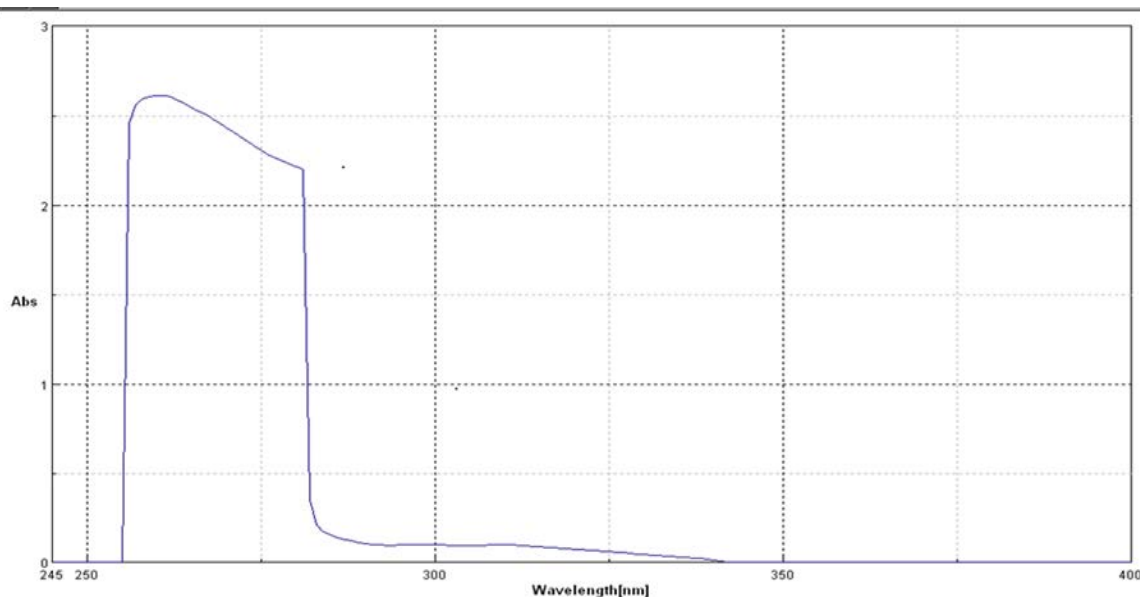


Fig. - UV Spectra of Ketoconazole at conc. 20µg/ml.

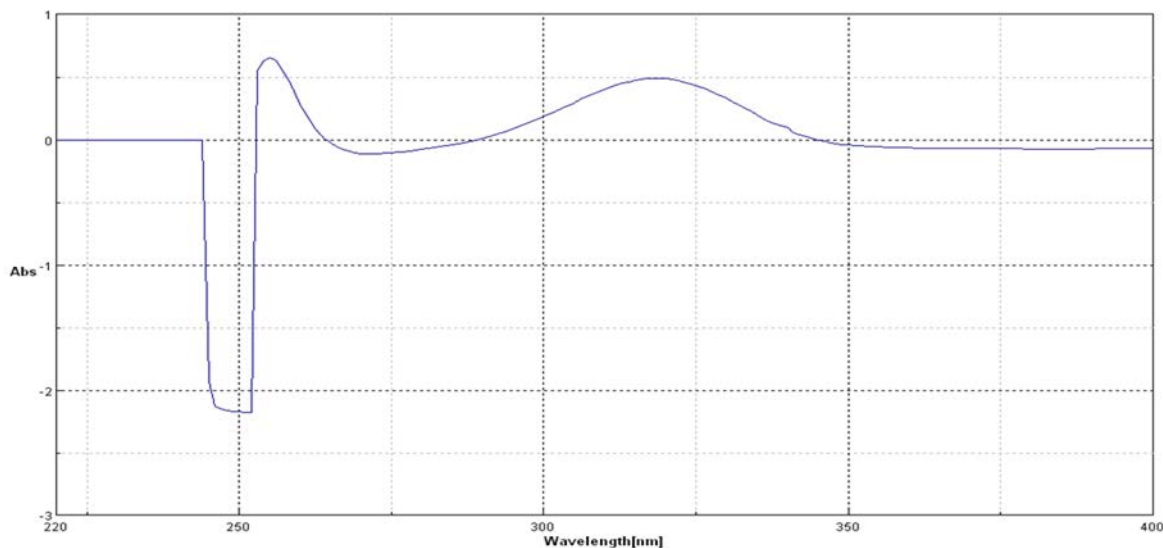


Fig. - UV Spectra of Salicylic acid at conc. 20µg/ml

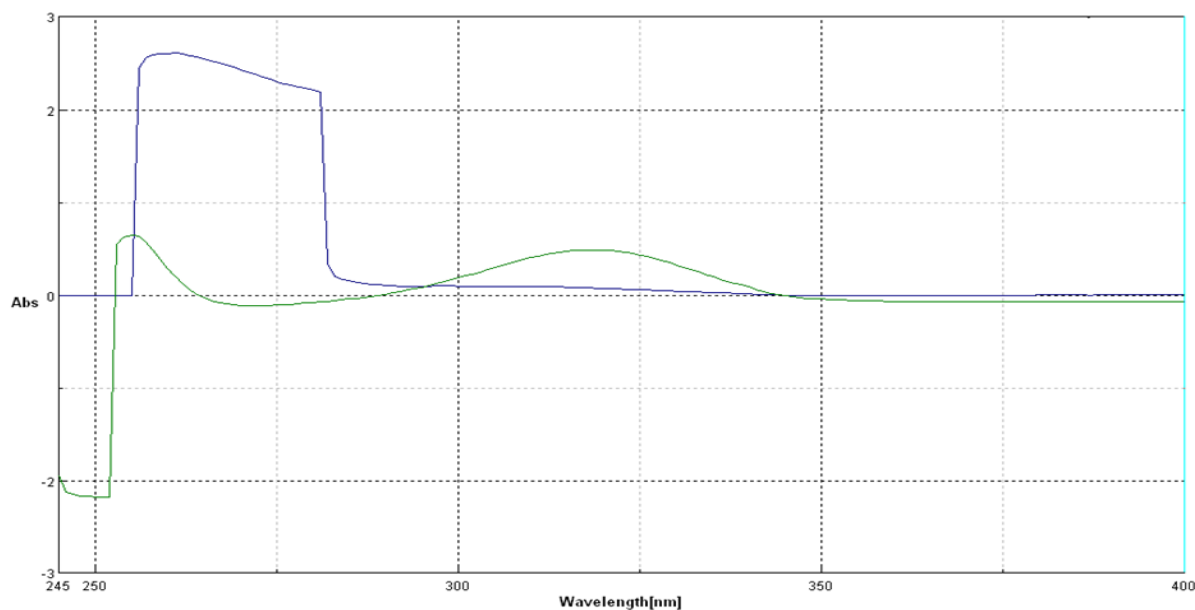


Fig. - Overlain UV-Spectra of Ketoconazole and Salicylic acid

5. METHODS

Both drugs overlay at the wavelength 295.00 nm and according to overlain spectra of Ketoconazole and salicylic acid two methods have been carried out for estimation of both the drugs i.e., Simultaneous equation method and Absorbance ratio method (Q-Absorbance method).

METHOD A : SIMULTANEOUS EQUATION METHOD

20µg/mL solutions of Ketoconazole and Salicylic acid were prepared separately in Methanol and the solutions were scanned against blank in the entire UV range to determine the λ_{max} values.

Clear peaks were observed at 261nm for Ketoconazole and 255nm for Salicylic acid. Hence these wavelengths were chosen as the λ_{\max} values for each drug respectively. Standard solutions of Ketoconazole and Salicylic acid in the concentration range of 10-35 μ g/ml for Ketoconazole and 10-35 μ g/ml for Salicylic acid respectively were prepared in methanol and the absorbance of these solutions was measured at 261nm and 255 nm. Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs.

The concentration of two drugs in mixture was calculated by using following equations.

$$C_X = (A_2 \times a_{y1} - A_1 \times a_{y2}) / (a_{x2} \times a_{y1} - a_{x1} \times a_{y2})$$

$$C_Y = (A_1 \times a_{x2} - A_2 \times a_{x1}) / (a_{x2} \times a_{y1} - a_{x1} \times a_{y2})$$

Where, C_X and C_Y are the concentrations of Ketoconazole and Salicylic acid respectively in mixture and in sample solutions. A_1 and A_2 are the absorbencies of sample at 261nm and 255nm, respectively, a_{x1} and a_{x2} are the absorptivities of Ketoconazole and a_{y1} and a_{y2} are the absorptivities of the Salicylic acid at 261nm and 255nm, respectively. All standard and sample solutions absorbance was measured at 261nm and 255nm with their respective blanks.^{4,5}

METHOD B : ABSORBANCE RATIO METHOD/ Q-ANALYSIS

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length. E.g. two dilutions of the same substance give the same absorbance ratio A_1 / A_2 . In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbance's are measured at two wavelengths, one being the λ_{\max} of one of the components (λ_2) and the other being a wavelength of equal absorptivity of the two components (λ_1), i.e., an iso-absorptive point. A series of standard solutions of Ketoconazole and Salicylic acid in the concentration range of 10-35 μ g/ml for Ketoconazole and 10-35 μ g/ml for Salicylic acid respectively were prepared in methanol and the absorbance of these solutions was measured at 295nm (iso-absorptive point) and 261 nm (λ_{\max} of Ketoconazole) . The concentration of the individual components, C_X and C_Y can be calculated by using the following equations.

$$C_X = (Q_M - Q_Y / Q_X - Q_Y) \times (A / Q_A)$$

$$C_Y = (Q_M - Q_X / Q_Y - Q_X) \times (A / Q_B)$$

Where A are absorbance of Formulation at iso-absorptive point (295nm), Q_M = absorbance of formulation at selected wavelength divided by absorbance of formulation at iso- absorptive point ,

Q_X and Q_Y are the division of respective drugs absorbances at selected wavelength and iso-absorptive point. Q_A = absorbance of first drug at iso-absorptive point divided by concentration of drug. Q_B = absorbance of second drug at iso-absorptive point divided by concentration of drug.^{4,5}

VALIDATION OF UV- VISIBLE SPECTROPHOTOMETRIC METHODS

LINEARITY AND RANGE

Five aliquots of each drug solutions were taken from standard stock solution and transferred to 10 ml volumetric flask to get a final concentration of 10, 15, 20, 25, 30, 35 μ g/ml of Ketoconazole and 10, 15, 20, 25, 30 and 35 μ g/ml of Salicylic acid and the volume was completed with the distilled water and each flask content was measured to determine the absorbance at all the selected wavelength. For simultaneous equation method the absorbance of all standard solutions were measured at 261nm and 255nm, the calibration curves of absorbance vs. concentration was plotted and correlation coefficient and regression line equations for both Ketoconazole and Salicylic acid were determined. For Q-Absorption ratio method the wave lengths selected were 295nm (iso-absorptive point) and 261nm (λ max of ketoconazole). The absorbance at these two wavelengths for all standard solutions of both Ketoconazole and Salicylic acid were measured and the calibration curves and linear regression equation of Ketoconazole and Salicylic acid at 295nm and 261nm were determined. the calibration curves of absorbance vs. concentration was plotted and correlation coefficient and regression line equations for both Ketoconazole and Salicylic acid were determined.

ACCURACY AND RECOVERY STUDIES

To check the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels according to ICH guidelines. A series of solutions of Ketoconazole and Salicylic acid at 80%, 100%, and 120% of the standard preparation in the ratio of the formulation were prepared and checked for accuracy by determining the absorbance values at λ max of 261nm and 255 nm (Simultaneous equation method) 295nm and 261nm (Absorbance ratio method) respectively. To a fixed concentration of the formulation, varying concentrations of pure drug solutions were added and percentage recoveries calculated.

PRECISION

In intra-day study concentration of two drugs were calculated on the same day at an interval of one hour. In inter-day study the concentration of drug contents were calculated on three different days study expresses with in laboratory variation in different days. In both intra and inter-day precision

study for the methods %RSD were calculated. The %RSD values found to be less than 2 for intra-day and inter-day precision, which indicate that the proposed method is precise for analysis.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION:

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations, y intercept was calculated and the standard deviation of the y intercept was computed. From these values, the parameters Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined by using equation as $3.3\sigma/S$ and $10\sigma/S$, respectively.

RUGGEDNESS

The ruggedness of the proposed method was determine for 20 $\mu\text{g/ml}$ concentration of ketoconazole and 20 $\mu\text{g/ml}$ concentration of salicylic acid by analysis of aliquots from a homogenous slot by two analyst using same operational and environmental conditions .The result was indicated as % RSD.

ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Therefore, the proposed method was considered as robust.

7. RESULT AND DISCUSSION

1. LINEARITY AND RANGE

The linearity of Ketoconazole and Salicylic acid was found to be in the range of 10-35 $\mu\text{g/ml}$ for Ketoconazole and 10-35 $\mu\text{g/ml}$ for Salicylic acid with correlation coefficient of 0.9998 and 0.9996.and Linear regression equation was found to be $Y = 0.0246x - 0.1338$ and $Y = 0.0494x - 0.275$. The calibration data is expressed in the Table No. 1.1. Calibration curve is shown in Figure No. 1 and 2. For Absorption ratio method the concentrations range i.e. 10-35 $\mu\text{g/ml}$ for Ketoconazole and 10-35 $\mu\text{g/ml}$ for Salicylic acid with correlation coefficient of 0.9998 and 0.9996 for ketoconazole and 0.9997 and 0.9999 for Salicylic acid. Linear regression equation for ketoconazole was found to be $Y = 0.0224x - 0.0111$, $Y = 0.0176x - 0.1072$ and Linear regression equation for Salicylic acid was found to be $Y = 0.0196 X - 0.132$, $Y = 0.0199 X - 0.0142$.The calibration data is expressed in the Table No.:1.2. Calibration curve is shown in Figure No.: 3, 4, 5 and 6.

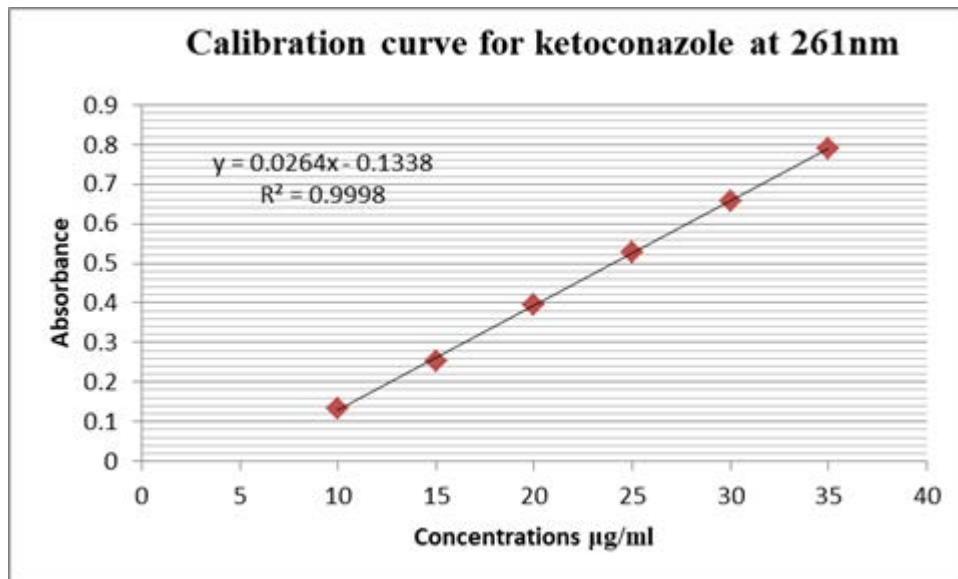


Fig-1: Calibration curve of Ketoconazole at 261 nm

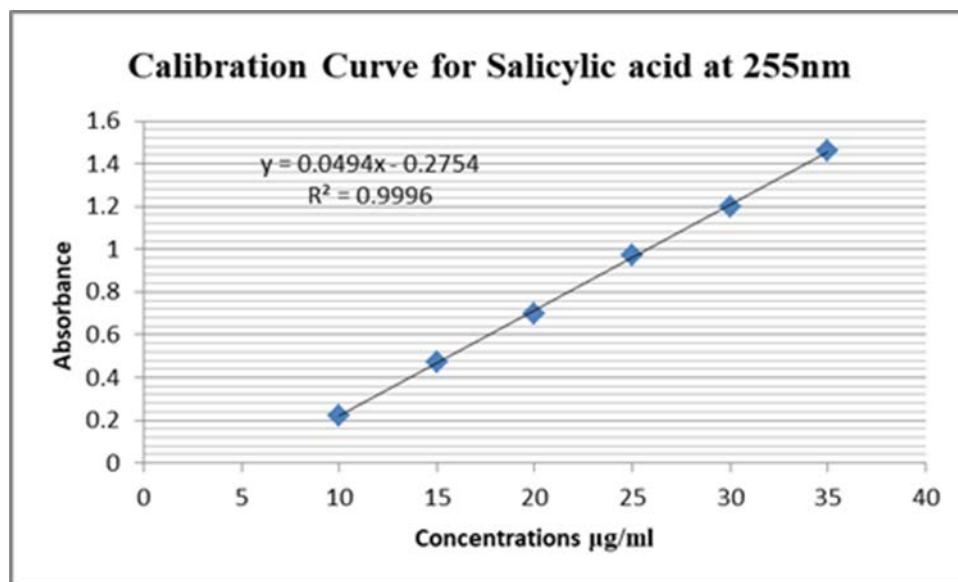


Fig-2: Calibration curve of Salicylic acid at 255nm

FOR ABSORBANCE RATIO METHOD-

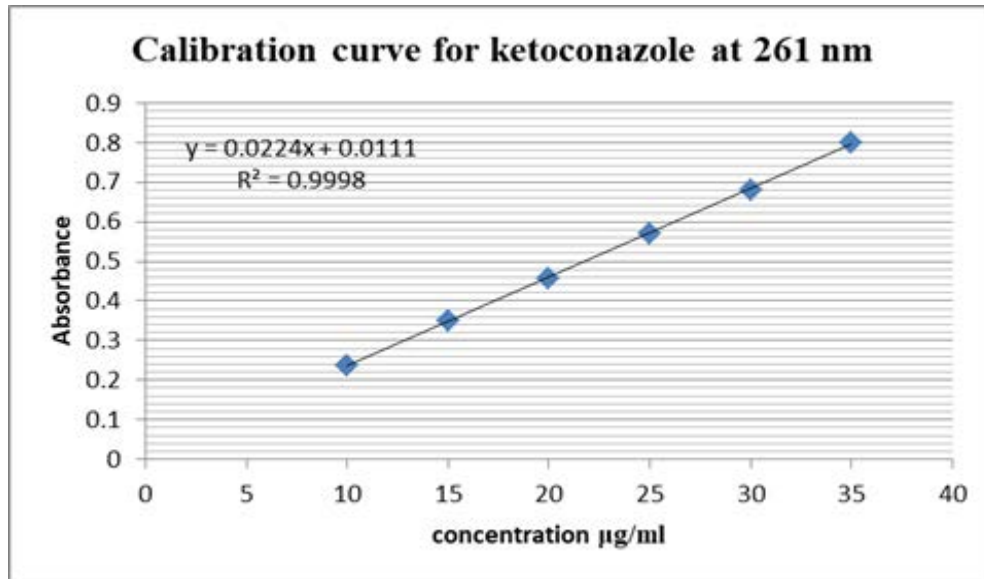


Fig-3: Calibration curve of ketoconazole at 261 nm

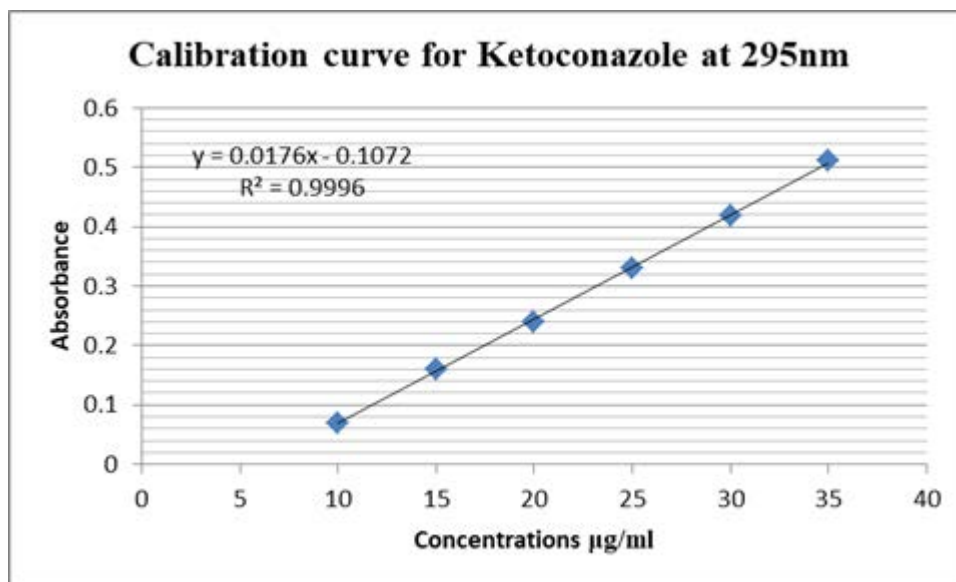


Fig-4: Calibration curve of ketoconazole at 295 nm

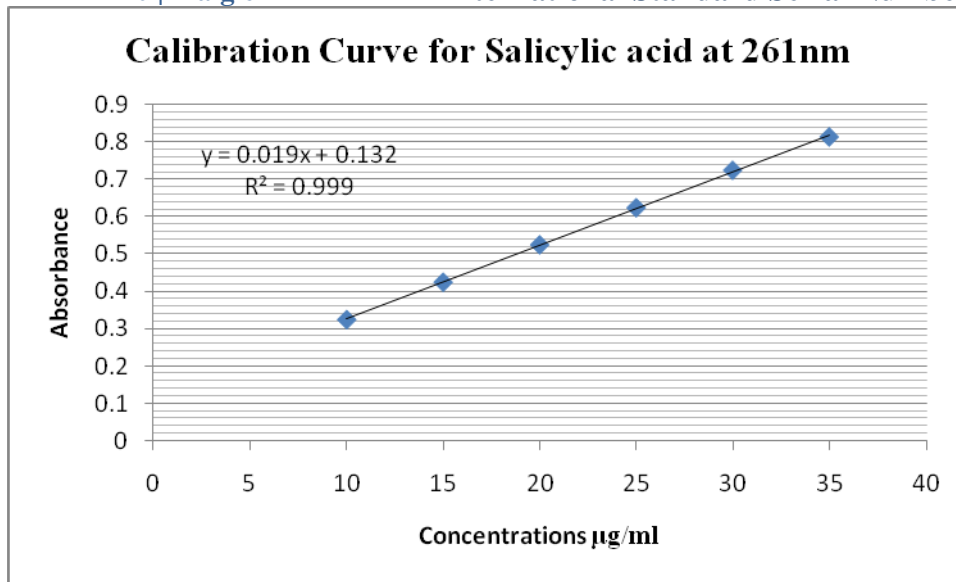


Fig-5: Calibration curve of Salicylic acid at 261 nm

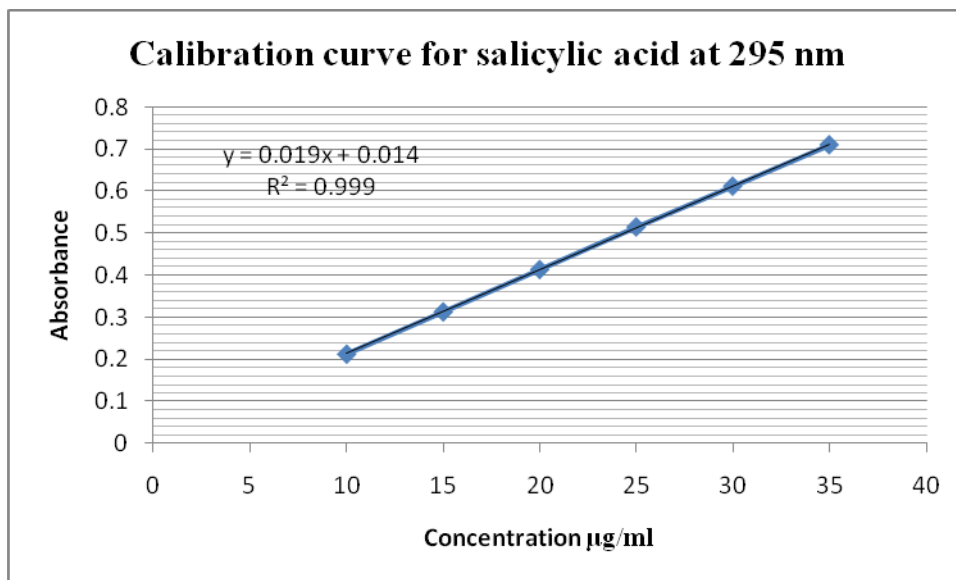


Fig-6: Calibration curve of Salicylic acid at 295 nm

Table No: 1.1: Calibration data of Ketoconazole and Salicylic acid for simultaneous equation method

Sr. No.	Ketoconazole		Salicylic acid	
	Conc.(µg/ml)	Absorbance*at 261 nm	Conc.(µg/ml)	Absorbance*at 255 nm
1	0.0	0.0	0.0	0.0
2	10.0	0.1337	10.0	0.2211
3	15.0	0.2543	15.0	0.4698
4	20.0	0.3954	20.0	0.6987
5	25.0	0.5264	25.0	0.9699
6	30.0	0.6571	30.0	1.1998
7	35.0	0.7882	35.0	1.4581

Table No: 1.2: Calibration data of Ketoconazole and Salicylic acid for Absorbance ratio method

Sr. No.	Ketoconazole			Salicylic acid		
	Conc.(µg/ml)	Absorbance		Conc.(µg/ml)	Absorbance	
		261nm	295nm		261nm	295nm
1	0.0	0.0	0.0	0.0	0.0	0.0
2	10.0	0.2365	0.0697	10.0	0.3256	0.2123
3	15.0	0.3488	0.1598	15.0	0.4253	0.3128
4	20.0	0.4569	0.2398	20.0	0.5247	0.4135
5	25.0	0.5698	0.3296	25.0	0.6235	0.5148
6	30.0	0.6789	0.4196	30.0	0.7233	0.6118
7	35.0	0.7999	0.5111	35.0	0.8122	0.7101

Table No: 1.3: Optical and regression parameters of the calibration curve obtained by UV Spectroscopy method.

Parameter	Simultaneous equation method		Q-Absorption ratio method			
	Keto	SA	Keto	Keto	SA	SA
	261nm	255nm	261nm	295nm	261nm	295nm
Linearity range (µg/ml)	10- 35	10- 35	10- 35	10-35	10- 35	10-35
Slope	0.0263	0.0494	0.0224	0.0175	0.0195	0.0199
Intercept	0.1338	0.2754	0.0111	0.1072	0.1320	0.0142
Regression coefficient (r ²)	0.9998	0.9996	0.9998	0.9996	0.9997	0.9999

2. ACCURACY (RECOVERY):

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The % recoveries found for the simultaneous equation method was 98.36-99.30 and 99.54-102.84 for ketoconazole and salicylic acid simultaneously. For Q-Absorption ratio method the %recoveries found to be 97.92-100.1(261nm) and 98.06-99.49(295nm) for ketoconazole and 97.48-99.70(261nm) and 98.28-100.7 (295nm) for salicylic acid, The recovery studies are reported in Table No.: 1.4, 1.5.

Table No.:1.4: Result of % Recovery and % RSD of Ketoconazole and Salicylic acid for simultaneous equation method

Drugs	Recovery level	Initial conc. (µg/ml)	Conc. of std. drug added. (µg/ml)	% Recovery	% RSD
Keto	80%	20	16	99.00	0.0092
	100%	20	20	99.30	0.18
	120%	20	24	98.36	0.57
SA	80%	20	16	102.84	0.24
	100%	20	20	99.54	0.29
	120%	20	24	99.95	0.11

*Average of 3 determinations

Table No: 1.5: Result of % Recovery and % RSD of Ketoconazole and Salicylic acid for Absorbance ratio method

Drugs	Recovery level	Initial conc. (µg/ml)	Conc. of std. drug added (µg/ml)	% Recovery		% RSD	
				261nm	295nm	261nm	295nm
Keto	80%	20	16	97.92	98.96	0.22	0.23
	100%	20	20	100.12	99.49	0.17	0.17
	120%	20	24	98.78	98.06	0.04	0.14
SA	80%	20	16	99.48	100.7	0.56	0.08
	100%	20	20	99.70	98.28	0.40	0.03
	120%	20	24	97.48	100.4	0.40	0.25

*Average of 3 determinations

3. PRECISION:

The precision of the method was expressed in terms of % relative standard deviation (%RSD). For Intra-day precision, %RSD found for the simultaneous equation method in the range of 0.11-0.61 for ketoconazole and 0.37-1.85 for salicylic acid. The %RSD found for Q Absorption ratio method in the range of 0.19-0.68 (261nm) and 0.16-0.43 (295nm) for ketoconazole, 0.13-0.89(261nm) and 0.03-0.28(295nm) for salicylic acid, respectively. For Inter-day precision, %RSD found for the simultaneous equation method in the range of 0.14-0.64 for ketoconazole and 0.31-1.27 for salicylic acid. The %RSD found for Q Absorption ratio method in the range of 0.25-0.72 (261nm) and 0.12-0.30 (295nm) for ketoconazole, 0.18-0.48(261nm) and 0.09-0.67 (295nm) for salicylic acid, respectively. The result is expressed in Table No: 1.6, 1.7, 1.8 and 1.9

Table No: 1.6: Result of Intra-day precision of Ketoconazole and Salicylic acid for Simultaneous equation method

Sr. No	Concentration ($\mu\text{g/ml}$)		Absorbance* Mean \pm S.D. (n = 3)		%RSD	
	Keto	SA	Keto(261nm)	SA (255nm)	Keto	SA
1	15	15	0.3427 \pm 0.0021	0.5341 \pm 0.0075	0.61	1.41
2	20	20	0.8225 \pm 0.0031	0.7880 \pm 0.0029	0.38	0.37
3	25	25	0.7167 \pm 0.00083	1.2309 \pm 0.0228	0.11	1.85

*Average of 3 determinations

Table No.: 1.7: Result of Intra-day precision of Ketoconazole and Salicylic acid for Absorbance ratio method

Sr. No	Concentration ($\mu\text{g/ml}$)		Absorbance* Mean \pm S.D.		% RSD	
	Keto	SA	Keto	SA	Keto	SA
1	15	15	0.7105 \pm 0.0048 (261 nm)	0.6397 \pm 0.0028 (261 nm)	0.68 (261 nm)	0.89 (261 nm)
			0.1413 \pm 0.0003 (295 nm)	0.3112 \pm 0.0009 (295 nm)	0.21 (295 nm)	0.28 (295 nm)
2	20	20	1.4293 \pm 0.0039 (261 nm)	0.4853 \pm 0.0033 (261 nm)	0.27 (261 nm)	0.67 (261 nm)
			0.6098 \pm 0.0010 (295nm)	0.1768 \pm 0.0005 (295 nm)	0.16 (295 nm)	0.28 (295 nm)
3	25	25	1.2630 \pm 0.0024 (261 nm)	0.8945 \pm 0.0012 (261 nm)	0.19 (261 nm)	0.13 (261 nm)
			0.2448 \pm 0.0010 (295 nm)	0.3347 \pm 0.0001 (295 nm)	0.43 (295 nm)	0.03 (295 nm)

*Average of 3 determinations

Table No: 1.8: Result of Inter-day precision of Ketoconazole and Salicylic acid for Simultaneous equation method

Sr. No	Concentration ($\mu\text{g/ml}$)		Absorbance* Mean \pm S.D. (n = 3)		%RSD	
	Keto	SA	Keto(261nm)	SA (255nm)	Keto	SA
1	15	15	0.7173 \pm 0.0010	0.9513 \pm 0.0077	0.14	0.81
2	20	20	1.4235 \pm 0.0091	0.9007 \pm 0.0028	0.64	0.31
3	25	25	1.2641 \pm 0.0062	1.1978 \pm 0.0152	0.49	1.27

*Average of 3 determinations

Table No: 1.9: Result of Inter-day precision of Ketoconazole and Salicylic acid for Absorbance ratio method

Sr. No	Concentration ($\mu\text{g/ml}$)		Absorbance* Mean \pm S.D.		%RSD	
	Keto	SA	Keto	SA	Keto	SA
1	15	15	0.710 \pm 0.0020 (261nm)	0.6442 \pm 0.0012 (261nm)	0.28 (261nm)	0.18 (261nm)
			0.1435 \pm 0.0001 (295nm)	0.3131 \pm 0.0002 (295nm)	0.12 (295nm)	0.09 (295nm)
2	20	20	1.4259 \pm 0.0036 (261nm)	0.4878 \pm 0.0017 (261nm)	0.25 (261nm)	0.35 (261nm)
			0.6097 \pm 0.0013 (295nm)	0.1761 \pm 0.0011 (295nm)	0.22 (295nm)	0.67 (295nm)
3	25	25	1.2638 \pm 0.0091 (261nm)	0.8869 \pm 0.0043 (261nm)	0.72 (261nm)	0.48 (261nm)
			0.2456 \pm 0.0007 (295nm)	0.3342 \pm 0.0008 (295nm)	0.30 (295nm)	0.24 (295nm)

*Average of 3 determinations

4. LOD AND LOQ

The limit of detection found to be 0.539 and 0.659 for simultaneous equation method for both ketoconazole and salicylic acid, respectively, the limit of quantification found to be 1.634 and 1.997 for both ketoconazole and salicylic acid, respectively. For Q-Absorption ratio method the

limit of detection found to be 0.531 at (261nm), 0.662 at (295nm) and 0.603 at (261nm), 0.266 at (295nm) for ketoconazole and salicylic acid, respectively, the limit of quantification found to be 1.610 at (261nm) and 2.000 at (295nm), 1.830 at (261nm) and 0.807 at (295nm) for both ketoconazole and salicylic acid. The result is expressed in Table No.: 1.10 and 1.11

Table No.: 1.10: Result of LOD & LOQ of Ketoconazole and Salicylic acid for Simultaneous equation method

Sr.No.	Drugs	LOD($\mu\text{g/ml}$)	LOQ ($\mu\text{g/ml}$)
1.	Keto	0.539	1.634
2.	SA	0.659	1.997

Table No: 1.11: Result of LOD & LOQ of Ketoconazole and Salicylic acid for Absorbance ratio method

Sr.No.	Drugs	LOD($\mu\text{g/ml}$)	LOQ ($\mu\text{g/ml}$)
1.	Keto	0.531 (261nm)	1.610(261nm)
		0.662(295nm)	2.000(295nm)
2.	SA	0.603(261nm)	1.830(261nm)
		0.266(295nm)	0.807(295nm)

5. ROBUSTNESS

The respective absorbance's were noted and the result was indicated as % RSD. The results were obtained at different wavelengths. For simultaneous equation method % RSD found to be 0.105, 0.079, 0.078 and 0.094, 0.093, 0.087 for both ketoconazole and salicylic acid. For Q-Absorption ratio method the % RSD found to be 0.105, 0.079, 0.078 and 0.050, 0.086, 0.053 for ketoconazole and 0.448, 0.710, 0.396 and 0.330, 0.221, 0.218 for salicylic acid. The result of Robustness was expressed in Table No.1.12, 1.13 and 1.14

Table No: 1.12: Result of Robustness of Ketoconazole and Salicylic acid for Simultaneous equation method

Sr.no	Drugs	Keto			SA		
		259	261	263	253	255	257
	Conc.µg/ml						
1	20	0.650	0.653	0.657	0.531	0.535	0.536
2	20	0.650	0.653	0.658	0.531	0.534	0.536
3	20	0.651	0.653	0.658	0.531	0.534	0.537
4	20	0.651	0.653	0.657	0.532	0.534	0.536
5	20	0.651	0.654	0.657	0.532	0.535	0.537
6	20	0.652	0.654	0.657	0.532	0.535	0.536
7	Mean	0.65083	0.65333	0.65666	0.5315	0.5345	0.53633
8	SD	0.00068	0.00051	0.00051	0.0005	0.0005	0.00047
9	%RSD	0.10555	0.07904	0.07863	0.0940	0.09354	0.08763

*Average of 6 determinations

Table No: 1.13: Result of Robustness of Ketoconazole for Absorbance ratio method

Sr.no	Drugs	Keto (at selected wavelength)			Keto (at Isobestic wavelength)		
		259	261	263	293	295	297
	Conc.µg/ml						
1	20	0.650	0.653	0.657	0.931	0.934	0.937
2	20	0.650	0.653	0.658	0.931	0.934	0.937
3	20	0.651	0.653	0.658	0.931	0.935	0.936
4	20	0.651	0.653	0.657	0.932	0.936	0.936
5	20	0.651	0.654	0.657	0.932	0.935	0.936
6	20	0.652	0.654	0.657	0.931	0.936	0.937
7	Mean	0.65083	0.65333	0.65666	0.93133	0.93500	0.9365
8	SD	0.00068	0.00051	0.00051	0.00047	0.00081	0.0005
9	%RSD	0.10555	0.07904	0.07863	0.05046	0.08663	0.05339

*Average of 6 determinations

Table No: 1.14: Result of Robustness of Salicylic acid for Absorbance ratio method

Sr.no	Drugs Conc.µg/ml	SA (at selected wavelength)			SA (at Isobestic wavelength)		
		259	261	263	293	295	297
1	20	0.111	0.113	0.118	0.223	0.226	0.229
2	20	0.111	0.113	0.118	0.223	0.225	0.229
3	20	0.112	0.114	0.119	0.224	0.225	0.229
4	20	0.112	0.115	0.119	0.224	0.226	0.228
5	20	0.112	0.114	0.119	0.225	0.226	0.228
6	20	0.111	0.115	0.119	0.223	0.225	0.228
7	Mean	0.1115	0.1140	0.1186	0.2236	0.2255	0.2285
8	SD	0.0005	0.00081	0.00047	0.00074	0.0005	0.0005
9	%RSD	0.44843	0.71052	0.39629	0.33094	0.22172	0.21881

*Average of 6 determinations

6.RUGGEDNESS

The ruggedness of the proposed method was determined for 20µg/ml concentration of ketoconazole and salicylic acid .The result was indicated as % RSD. For simultaneous equation method % RSD found to be 0.09, 1.18 for both ketoconazole and salicylic acid for analyst 1 and 1.35, 1.44 for both ketoconazole and salicylic acid for analyst 2. For Q-Absorption ratio method the % RSDfound to be 0.39 (261nm), 0.24(295nm) and 0.41(261nm), 0.61(295nm) for both ketoconazole and salicylic acid for analyst 1 and 0.37(261nm), 0.17(295nm) and 0.87(261nm), 0.229295nm) for both ketoconazole and salicylic acid for analyst 2.The result expressed in Table No.1.15 and 1.16

Table No.: 1.15: Result of Ruggedness of Ketoconazole and Salicylic acid for Simultaneous equation method

Sr. No.	Drugs	Conc. (µg/ml)	Analyst I		Analyst II	
			SD	%RSD	SD	%RSD
1	Keto	20	0.0017	0.09	0.0265	1.35
2	SA	20	0.0122	1.18	0.0149	1.44

*Average of 6 determinations

Table No.: 1.16: Result of Ruggedness of Ketoconazole and Salicylic acid for Absorbance ratio method

Sr. No.	Drugs	Conc. (µg/ml)	Analyst I		Analyst II	
			SD	%RSD	SD	%RSD
1	Keto	20	0.0075 (261 nm)	0.39 (261 nm)	0.0074 (261 nm)	0.37 (261 nm)
			0.0017 (295 nm)	0.24 (295 nm)	0.0012 (295 nm)	0.17 (295 nm)
2	SA	20	0.0018 (261 nm)	0.41 (261 nm)	0.0039 (261 nm)	0.87 (261 nm)
			0.0010 (295 nm)	0.61 (295 nm)	0.0003 (295 nm)	0.22 (295 nm)

*Average of 6 determinations

7. ANALYSIS OF MARKETED FORMULATION (KENZ-SAL LOTION, KLM Pvt.ltd.) BY UV SPECTROPHOTOMETRIC METHOD

The percentage of Ketoconazole and Salicylic acid in the estimated formulation was found to be 99.70% and 98.35% for Ketoconazole and Salicylic acid respectively for simultaneous equation method. For Q-Absorption ratio method the percentage of Ketoconazole and Salicylic acid in the estimated formulation was found to be 97.80 and 98.50% as shown in Table 1.17

Table-1.17: Results of analysis of Lotion dosage forms containing Ketoconazole and Salicylic acid

Methods	Simultaneous equation method		Absorbance ratio method	
	Keto	SA	Keto	SA
Active content estimated	19.94	19.67	19.50	19.70
% Assay	99.70	98.35	97.80	98.50

*Average of 6 determinations

8. CONCLUSION

Two new, simple, sensitive and economical UV spectrophotometric methods were developed for the simultaneous analysis of Ketoconazole and Salicylic acid in bulk and in pharmaceutical

formulations. The developed methods were validated as per ICH guidelines and from the statistical data, it was found that the methods were linear, accurate and precise and can be successfully applied for the analysis of pharmaceutical formulations without interference of excipients.

9. ACKNOWLEDGEMENT

The authors are grateful to Rajgad Dnyanpeeth's College of Pharmacy, Bhor Savitribai Phule Pune University, for providing necessary facilities. All teaching and non-teaching staff of college for their help.

10. REFERENCES

1. Fraihat S., Bahgat K. Spectrophotometric Methods for the Determination of Ketoconazole in Pharmaceutical Dosage Forms. *Tropical Journal of Pharmaceutical Research*.13,9 (2014)1511-1514.
2. Trivedi M., Branton A., Trivedi D., Shettigar H., Bairwa K. and Jana S. Fourier Transform Infrared and Ultraviolet-Visible Spectroscopic Characterization of Biofield Treated Salicylic Acid and Sparfloxacin. *Natural Products Chemistry & Research*. 3, 5 (2015) 720-723.
3. Chocholous P., Holik P., Satinsky D., Solich P. A novel application of Onyx™ monolithic column for simultaneous determination of salicylic acid and triamcinolone acetonide by sequential injection chromatography. *Journal of Science direct Talanta*. 72 (2007) 854–858.
4. Sivasubramanian L., Lakshmi K., Tintu.T. Simultaneous Spectrophotometric Estimation of Paracetamol And Lornoxicam In Tablet Dosage Form. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2, 4 (2010) 166-168.
5. Gummadi S., Thota D., Varri V., Vaddi P., Lakshmi V., Seshagiri N., Jillella R. Development and validation of UV spectroscopic methods for simultaneous estimation of ciprofloxacin and tinidazole in tablet formulation. *International Current Pharmaceutical Journal*. 1,10(2012), 317-321.
6. Pinki S., Patel D., Meshram D., Desai S. First Order Derivative Spectrophotometric Method For Simultaneous Estimation Of Escitalopram Oxalate And Flupentixol Dihydrochloride In Pharmaceutical Dosage Form. *Indo American Journal of Pharmaceutical Research*, 6, 2(2016) 4544-4553.
7. Kaur S., Kaur T., Kaur G. Verma S. Development And Validation Of Uv-Spectrophotometric Method For Estimation Of Hydroquinone In Bulk, Marketed Cream

And Prepared Nlc Formulation. International Journal of Applied Pharmaceutics. 9, 5(2017) 102-108.

8. Gunji R., Nadendla R., Ponnuru V. Simultaneous UV-Spectrophotometric determination and validation of Diclofenac Sodium and Rabeprazole Sodium using Hydrotropic agents in its tablet Dosage Form. International Journal of Drug Development & Research. 4,1(2012) 316-324.
9. Joshi R., Pawar N., Katiyar S., Zope D and Amol T. Development and validation of UV spectrophotometric methods for simultaneous estimation of Paracetamol and Ibuprofen in pure and tablet dosage form. Pelagia Research Library Der Pharmacia Sinica, 2,3 (2011)164-171.
10. Ahmad I. and Vaid F. Determination of Benzoic Acid and Salicylic Acid in Commercial Benzoic and Salicylic Acids Ointments by Spectrophotometric Method. Pakistan Journal of Pharmaceutical Science. 22,1(2009)18-22.
11. ICH–Guidelines Q2 (R1), Validation of Analytical procedures: Text and Methodology. 2005.
12. Patel H., Patel M. Development and Validation of UV Spectrophotometric Method for Simultaneous estimation of Terbinafine hydrochloride and Mometasone furoate in Combined Dosage Form. Asian Journal of Research Chem. 6, 1(2013) 29-34.
13. Naveed S., Jaweed L. UV spectrophotometric assay of Ketoconazole oral formulations. American Journal of Biology and Life Sciences 2,5(2014) 108-111.
14. Shou M., Galinada W., Wei Y., Tang Q., Markovich R., Rustum A. Development and validation of a stability-indicating HPLC method for simultaneous determination of salicylic acid, betamethasone dipropionate and their related compounds in Diprosalic Lotion. Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 356–361.
15. Vertzoni M., Reppas C., Archontaki H. Optimization and validation of a high-performance liquid chromatographic method with UV detection for the determination of ketoconazole in canine plasma. Science Direct Journal of Chromatography B. 839 (2006) 62–67.
16. Hamdy D., Brocks D. High performance liquid chromatographic assay for the simultaneous determination of midazolam and ketoconazole in plasma. Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 617–622.

17. Pirola R., Bareggi S., Benedittis G. Determination of acetylsalicylic acid and salicylic acid in skin and plasma by high-performance liquid chromatography. *Journal of Chromatography B*, 705 (1998) 309–315.
18. Mikami E., Goto T., Ohno T., Matsumoto H., Nishida M. Simultaneous analysis of dehydroacetic acid, benzoic acid, sorbic acid and salicylic acid in cosmetic products by solid-phase extraction and high-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis* 28 (2002) 261–267.