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Research Article

Development and Validation of Analytical Method for Simultaneous Estimation of Ketoconazole and Salicylic Acid in Bulk and Dosage Form.

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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 µg/ml for Ketoconazole and 10-35 µg/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.

KEYWORDS

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

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

2.1. Instruments

UV-Vis Spectrophotometer (Jasco V- 530 Spectrophotometer)

Digital balance (Shimadzu)

Sonicator (Cintex)

2.2. 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.

2.3. 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.

2.4. Determination of λmax:

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.

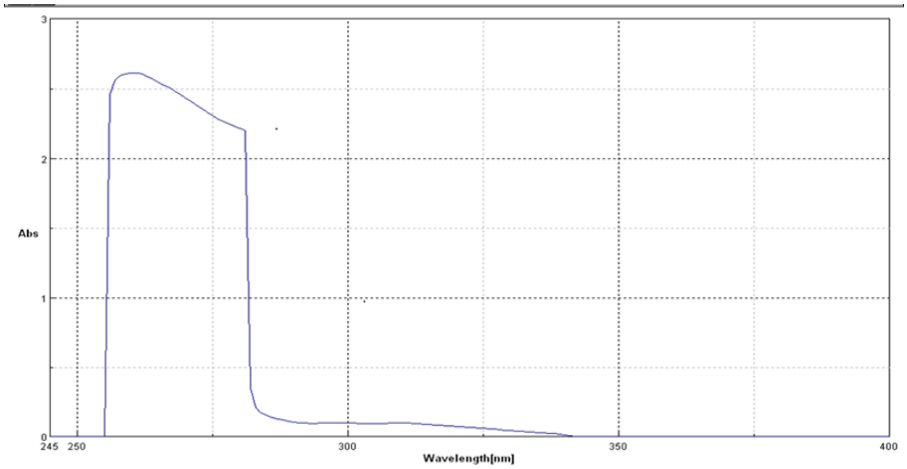

Fig. 1. -UV Spectra of Ketoconazole at conc. 20µg/ml.

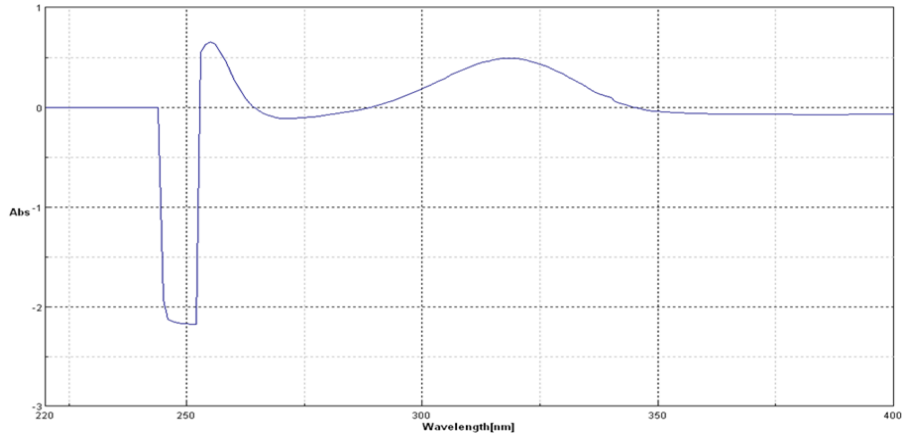

Fig. 2 - UV Spectra of Salicylic acid at conc. 20µg/ml

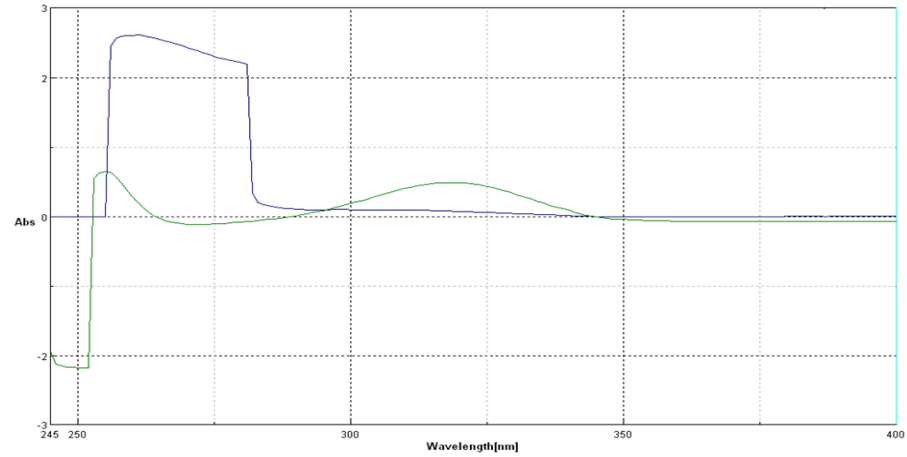

Fig. 3.-Overlain UV-Spectra of Ketoconazole and Salicylic acid

2.5. 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 preparedseparately in Methanol and thesolutions were scanned against blank in the entireUV range to determine the λmax values. Clear peakswere observed at 261nm for Ketoconazole and 255nm for Salicylic acid. Hence these wavelengths were chosen as theλmax values for each drug respectively.Standard solutions of Ketoconazole and Salicylic acid in theconcentration range of 10-35µg/Ml for Ketoconazole and 10-35µg/mL for Salicylic acid respectively were prepared in methanol andthe absorbance of these solutions was measured at 261nm and 255 nm. Calibration curves were plottedto verify the Beer's law and the absorptivity valuescalculated at the respective wavelengths for both thedrugs. The concentration of two drugs in mixture was calculated by using following equations.

$C_X = (A2 \times ay1 - A1 \times ay2)/(ax2 \times ay1 - ax1 \times ay2)$

$C_Y = (A1 \times ax2 - A2 \times ax1)/(ax2 \times ay1 - ax1 \times ay2)$

Where, C_X and C_Y are the concentrations of Ketoconazole and Salicylic acid respectively in mixture and in sample solutions. A1 and A2 are the absorbencies of sample at 261nm and 255nm, respectively, ax1 and ax2 are the absorptivities of Ketoconazole and ay1 and ay2 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 thesimultaneous equation procedure. It depends on theproperty that for a substance, which obeys Beer's lawat all wavelength, the ratio of absorbance at any twowavelengths is constant value independent ofconcentration or path length. E.g. two dilutions of thesame substance give the same absorbance ratio A1 /A2. In the USP, this ratio is referred to as Q value. Inthe quantitative assay of two components in admixtureby the absorbance ratio method, absorbance's aremeasured at two wavelengths, one being the λ max ofone of the components (λ2) and the other being awavelength of equal absorptivity of the two components(λ1), i.e., an iso-absorptive point. A series of standard solutions of Ketoconazole and Salicylic acid in the concentration range of 10-35µg/mL for Ketoconazoleand 10-35µg/mL for Salicylic acid respectively were prepared inmethanol and the absorbance of thesesolutions was measured at 295nm (iso-absorptivepoint) and 261 nm(λmax of Ketoconazole) . The concentration of the individual components, Cx and C_Y can be calculated by using the following equations.

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

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

Where A are absorbance of Formulationat 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 dividation of respective drugs absorbances at selected wavelength and iso-absorptive point. Q_A = absorbance of first drugs 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]

2.6. Validation of UV- Visible Spectrophotometric Methods

Linearity and Range

Five aliquots of each drug solutions were taken from standard stock solution and transferred to 10ml volumetric flask to get a final concentration of 10, 15, 20, 25,30,35µg/ml of Ketoconazole and 10,15,20,25,30 and 35µg/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 (λmax 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 λmax 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 3.3σ/S and 10σ/S, respectively.

Ruggedness

The ruggedness of the proposed method was determine for 20µg/ml concentration of ketoconazole and 20 µg/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 robustnessof 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.

3. RESULTS AND DISCUSSION

3.1. Linearity and Range

The linearity of Ketoconazole and Salicylic acid was found to be in the range of 10-35µg/ml for Ketoconazole and 10-35µg/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 theconcentrations range i.e. 10-35µg/ml for Ketoconazole and10-35µg/ml forSalicylic acid with correlation coefficient of 0.9998 and 0.9996 for ketoconazole and 0.9997 and 0.9999 for Salicylic acid. Linear regression equationfor ketoconazole was found to be Y = 0.0224x-0.0111, Y= 0.0176x-0.1072 and Linear regression equationfor Salicylic acidwas found to beY= 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, 5and 6.

For Simultaneous Equation Method

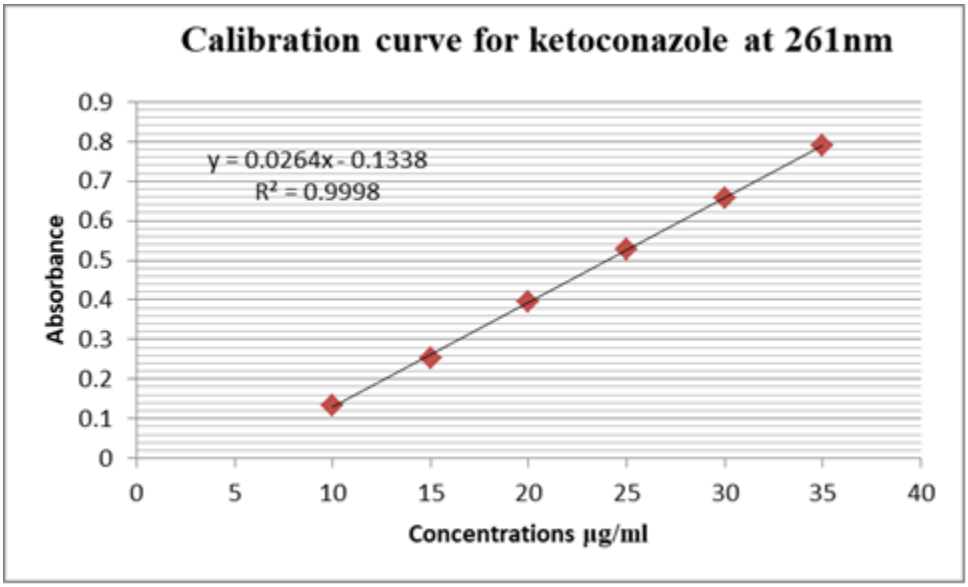

Fig-1: Calibration curve of Ketoconazole at 261nm

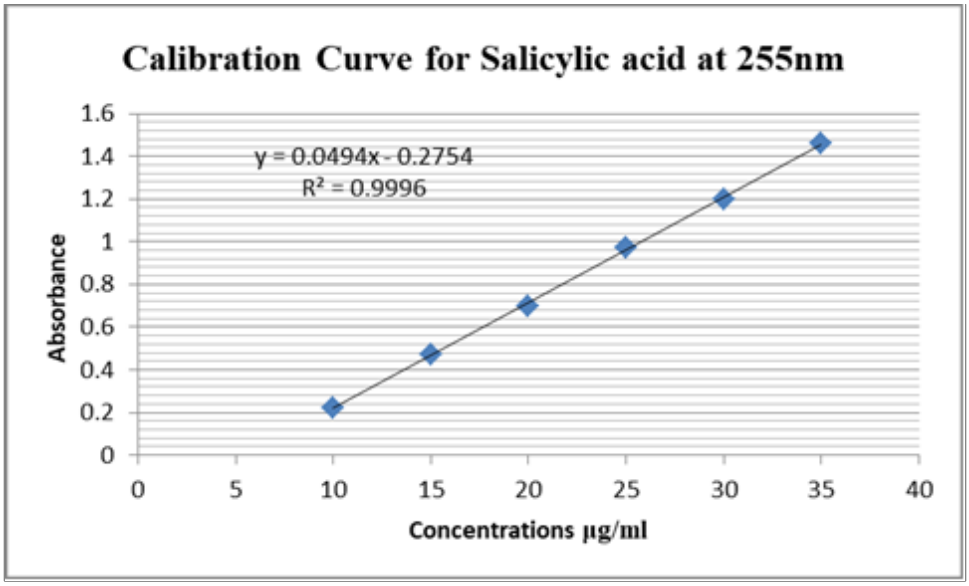

Fig-2: Calibration curve of Salicylic acid at 255nm

FOR ABSORBANCE RATIO METHOD-

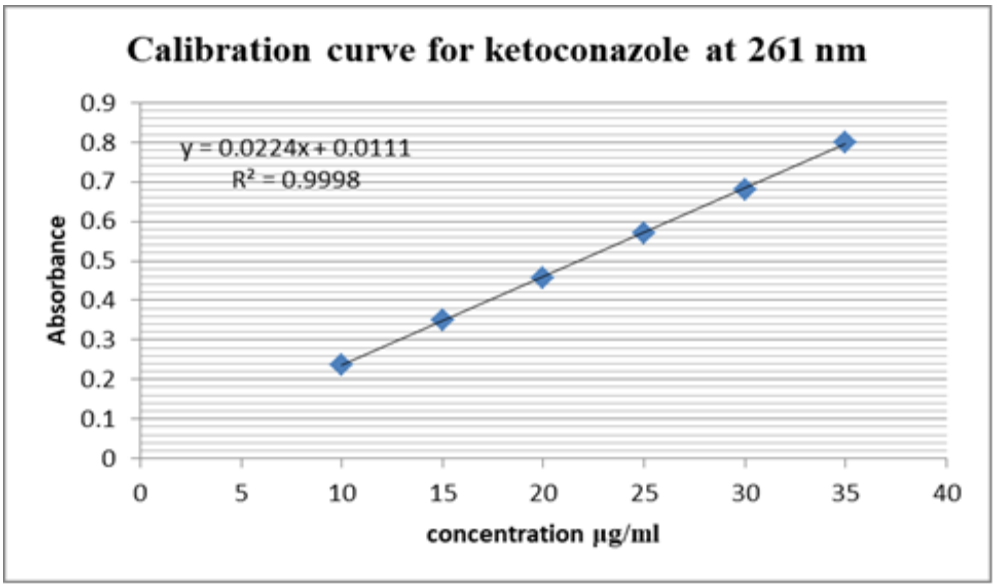

Fig-3: Calibration curve of ketoconazole at 261 nm

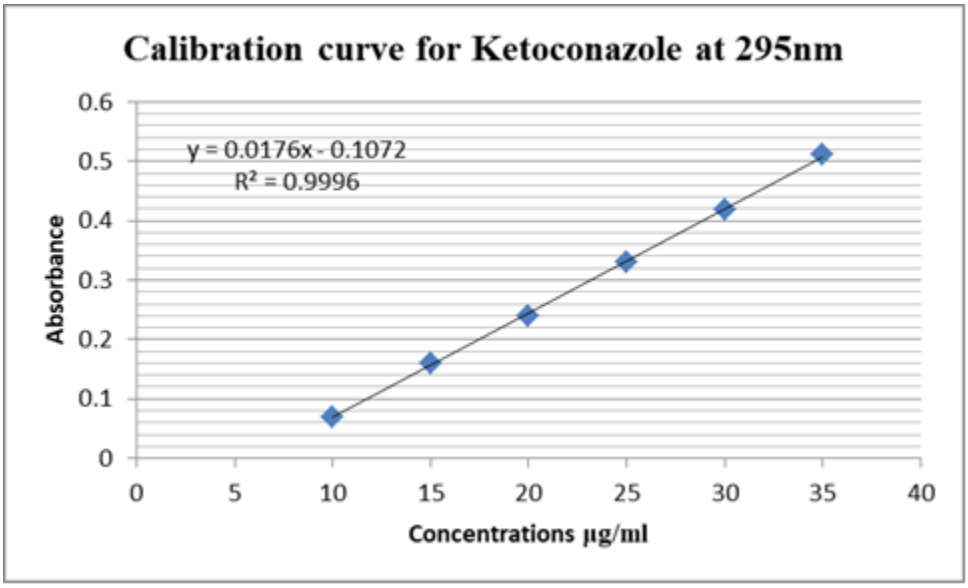

Fig-4: Calibration curve of ketoconazole at 295 nm

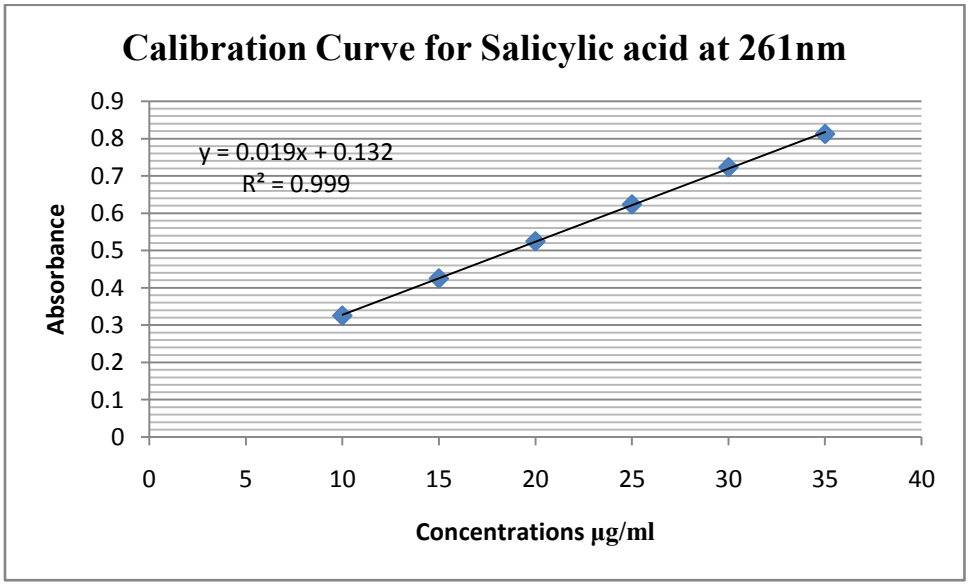

Fig-5: Calibration curve of Salicylic acid at 261 nm

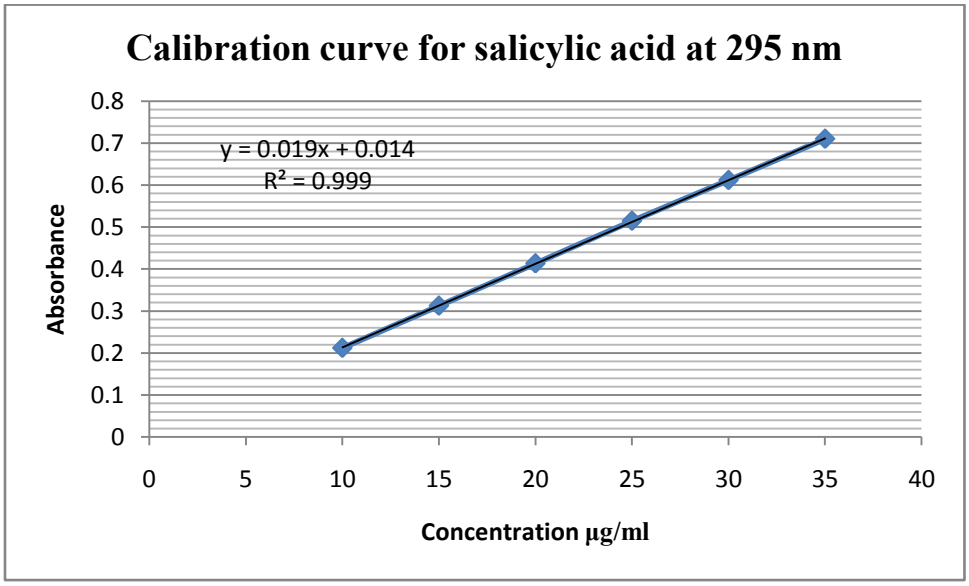

Fig-6: Calibration curve of Salicylic acid at 295 nm

Table 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	25.0	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 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	25.0	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 1.3: Optical and regression parameters of the calibration curve obtained by UV Spectroscopy method.

Parameter	**Simultaneous equation method**		**Q-Absorption ratio method**			
	Keto 261nm	**SA 255nm**	**Keto 261nm**	**Keto 295nm**	**SA 261nm**	**SA 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 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
	80%	20	16	102.84	0.30
SA	100%	20	20	99.54	0.50
	120%	20	24	99.95	0.16

*Average of 3 determinations

Table 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
	80%	20	16	99.48	100.7	0.56	0.08
SA	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 QAbsorption 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 QAbsorption 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 1.6: Result of Intra-day precision of Ketoconazole and Salicylic acid for Simultaneous equation method

Sr. No	**Concentration (µg/ml)**	**Absorbance* Mean ± S.D. (n = 3)**	**%RSD**

	Keto	SA	Keto(261nm)	SA (255nm)	Keto	SA
1	15	15	0.3427±0.0021	0.5341±0.0075	0.61	1.41
2	20	20	0.8225 ±0.0031	0.7880±0.0029	0.38	0.37
3	25	25	0.7167±0.00083	1.2309±0.0228	0.11	1.85

*Average of 3 determinations

Table 1.7: Result of Intra-day precision of Ketoconazole and Salicylic acid for Absorbance ratio method

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

*Average of 3 determinations

Table 1.8: Result of Inter-day precision of Ketoconazole and Salicylic acid for Simultaneous equation method

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

*Average of 3 determinations

Table 1.9: Result of Inter-day precision of Ketoconazole and Salicylic acid for Absorbance ratio method

Sr. No	Concentration (µg/ml)		Absorbance* Mean ± S.D.		%RSD	
	Keto	SA	Keto	SA	Keto	SA
1	15	15	0.710±0.0020 (261nm)	0.6442±0.0012 (261nm)	0.28 (261nm)	0.18 (261nm)
			0.1435±0.0001 (295nm)	0.3131±0.0002 (295nm)	0.12 (295nm)	0.09 (295nm)
2	20	20	1.4259±0.0036 (261nm)	0.4878±0.0017 (261nm)	0.25 (261nm)	0.35 (261nm)
			0.6097±0.0013 (295nm)	0.1761±0.0011 (295nm)	0.22 (295nm)	0.67 (295nm)
3	25	25	1.2638±0.0091 (261nm)	0.8869±0.0043 (261nm)	0.72 (261nm)	0.48 (261nm)
			0.2456±0.0007 (295nm)	0.3342±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 1.10: Result of LOD & LOQ of Ketoconazole and Salicylic acid for Simultaneous equation method

Sr.No.	Drugs	LOD(µg/ml)	LOQ (µg/ml)
1.	Keto	0.539	1.634
2.	SA	0.659	1.997

Table 1.11: Result of LOD & LOQ of Ketoconazole and Salicylic acid for Absorbance ratio method

Sr.No.	Drugs	LOD(µg/ml)	LOQ (µg/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, 0.087for both ketoconazole and salicylic acid.For Q-Absorption ratio method the % RSDfound 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, 1.13and 1.14

Table 1.12: Result of Robustness of Ketoconazole and Salicylic acid for Simultaneous equation method

Sr.no	**Drugs**	**Keto**			**SA**		
	Conc.µg/ml	**259**	**261**	**263**	**253**	**255**	**257**
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 1.13: Result of Robustness of Ketoconazole for Absorbance ratio method

Sr.no	**Drugs**	**Keto (at selected wavelength)**			**Keto (at Isobestic wavelength)**		
	Conc. µg/ml	**259**	**261**	**263**	**293**	**295**	**297**
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 1.14: Result of Robustness of Salicylic acid for Absorbance ratio method

Sr.no	**Drugs**	**SA (at selected wavelength)**			**SA (at Isobestic wavelength)**		
	Conc. µg/ml	**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 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 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	
Parameters	**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

4. CONCLUSION

Two new, simple, sensitive and economical UV spectrophotometric methods were developed for the simultaneous analysis of Ketoconazole and Salicylic acidin 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

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