

SIMULTANEOUS ESTIMATION OF OFLOXACIN AND ORNIDAZOLE IN SOLID DOSAGE FORM BY U V SPECTROPHOTOMETRY USING MIXED SOLVENCY CONCEPT

Sanjay Jain^{1*}, R. K. Maheshwari², Rajesh Kumar Nema³ and Indrajeet Singhvi⁴

¹Research Scholar, Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur.

²Department of Pharmacy, Shri G.S. Institute of Technology and Science, Indore 452003, Madhya Pradesh, India.

³Lakshmi Narain College of Pharmacy (RCP), Indore 453331, Madhya Pradesh, India.

⁴Department of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.

Article Received on
25 Dec. 2017,

Revised on 15 Jan. 2018,
Accepted on 05 Feb. 2018

DOI: 10.20959/wjpr20184-10944

*Corresponding Author

Sanjay Jain

Research Scholar, Faculty
of Pharmacy, Pacific
Academy of Higher
Education and Research
University, Udaipur.

ABSTRACT

In present research work a new simple, specific, precise, accurate, robust and economical UV-Spectrophotometric method for simultaneous estimation of ofloxacin and ornidazole in tablet dosage form using mixed solvency concept was developed and validated. In the present work 10% sodium caprylate, 10% niacinamide and 10% sodium benzoate blend was used as a hydrotropic solvent to increase the solubility of poorly water soluble ofloxacin and ornidazole. The analytical wavelength for ofloxacin and ornidazole are 330nm and 318 nm respectively. The developed method was validated as per ICH guidelines in terms of linearity and range, specificity, accuracy, precision and sensitivity. The percent drug estimated in tablet

formulation was 95.10 ± 0.800 for ornidazole and 97.05 ± 0.530 for ofloxacin by method B. and similarly it was 95.16 ± 0.519 for ornidazole and 100.15 ± 0.300 for ofloxacin by method A respectively. The range of percent recoveries varied from 95.13 ± 0.355 to 95.66 ± 1.07 for ornidazole and 96.82 ± 0.480 to 100.15 ± 0.515 for ofloxacin. Based on the results obtained the proposed method can be regarded a simple, precise, accurate, reliable, cost effective and eco-friendly for simultaneous estimation of ofloxacin and ornidazole.

KEYWORDS: Ofloxacin, Ornidazole, UV-Spectrophotometry, solid dosage formulation, mixed solvency concept.

INTRODUCTION

Increasing the aqueous solubility of Insoluble and slightly soluble drugs has been done by various methods to avoid the usage of organic solvents. Because of toxicity, volatility, and also high cost of organic solvents, an alternative method has been developed. Mixed solvency concept is one of the methods to enhance the aqueous solubility of less water soluble drugs. Mixed solvency concept may be a proper choice to preclude the use of organic solvents. So there is a broad scope for mixed solvency concept in quantitative estimation of other less water soluble drugs. By application of this concept, innumerable solvent system can be developed. Maheshwari^[1-6] is one of the opinions that each substance possesses solubilizing power. He has given several ecofriendly methods in the area of drug estimations and formulations precluding the use of toxic organic solvents. The solubility of large number of poorly soluble drugs has been enhanced by mixed solvency concept.^[1-31]

The present research work also provides an ecofriendly method for simultaneous estimation of ofloxacin and ornidazole in solid dosage form by UV Spectrophotometry by using mixed solvency concept. Ofloxacin is (\pm)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1, 2, 3-de]-1,4-benzoxazine-6-carboxylic acid. Ornidazole is 1(3-chloro-2-hydroxypropyl)-2-methyl-5-nitro Imidazole; α -(chloromethyl)-2-methyl-5-nitro-1H-Imidazole-1-ethanol. Ofloxacin is Antibacterial, ocular anti-infective. Ornidazole is antiprotozoal agent and anti-infective agent.

Experimental

Chemicals and Reagents

Pharmaceutical grade Ofloxacin and Ornidazole was a gift from Modern Laboratories Pvt. Ltd. Indore and its dosage formulation Zenotas-OZ was purchased from local market. All other chemicals were of analytical grade.

Instrumentation

UV Visible spectrophotometer (Model 1800, Shimadzu, Japan) with 10 –mm path length connected to a computer was used for spectrophotometric analysis.

Selection of solvent

10% sodium caprylate, 10% niacinamide and 10% sodium benzoate blend was selected as the solvent after considering the solubility and stability factor of both the drugs as well as the interference due to excipients present in the formulation.

Preparation of stock solution

The 50 mg each ofloxacin and ornidazole were accurately weighed and dissolved in 6 ml of blend respectively and then volume make up to 10ml in 10ml volumetric flask to get a concentration of 5000 $\mu\text{g/ml}$, appropriate volumes of this solutions were further diluted with distilled water to obtain final concentrations in the range of 10-50 $\mu\text{g/ml}$. The absorbances of these standard solutions were noted at 330 nm, 319 nm and 303 nm against respective reagent blanks.

Selection of appropriate wavelength

Standard solution of appropriate concentration of ofloxacin and ornidazole were prepared separately and scanned in the range of 400 to 200 nm at a slow scan speed. The absorption maximas of ofloxacin was found at 330 nm while for ornidazole at 319 nm. The overlain spectra of ofloxacin and ornidazole were recorded as shown in fig.1.

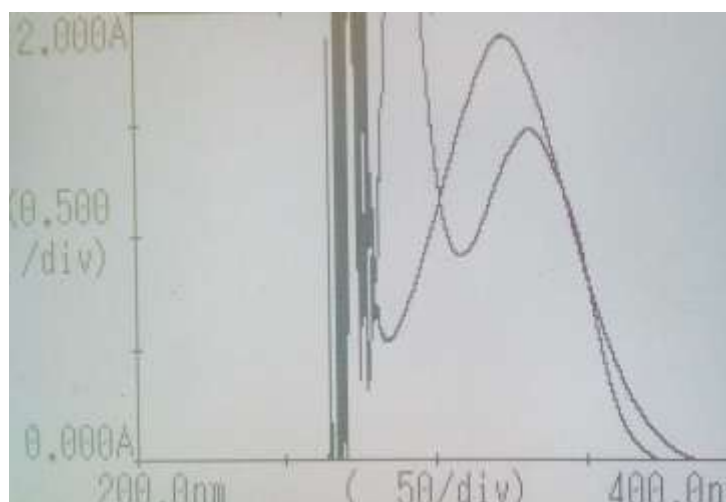
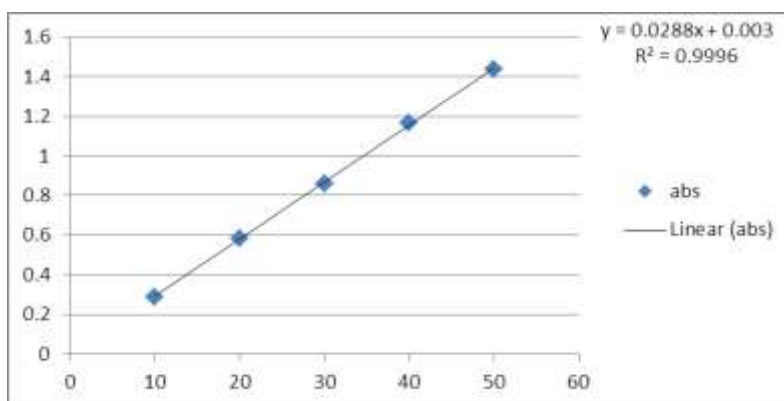


Fig. 1: Overlain spectra of ofloxacin and ornidazole with 10% sodium caprylate, 10% niacinamide and 10% sodium benzoate blend.

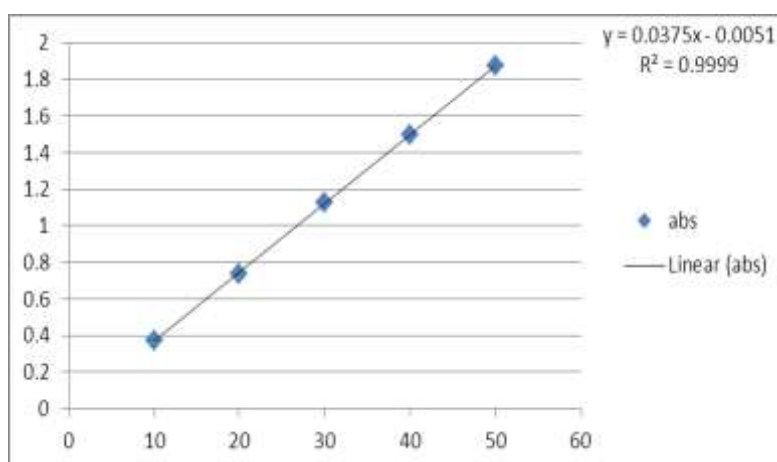
Calibration curve

Appropriate volumes of stock solution were further diluted with distilled water to obtain final concentrations in the range of 10-50 $\mu\text{g/ml}$ for ofloxacin and ornidazole separately. The absorptions of these standard solutions were noted at 319 nm, 330 nm, and 303 nm (Isobestic

point) against respective reagent blanks. A calibration curve of absorbance against concentration was plotted and the regression coefficient (R^2) was determined for both the drugs separately as shown in Fig.2. The absorptivity of for both the drugs is presented in Table-I.



[A]OFLOXACIN



[B]ORNIDAZOLE

Fig. 2: Calibration curves of absorbance against concentration.

Table 1: Absorbitivity* values for ofloxacin and ornidazole.

Concentration (µg/ml)		Absorbitivity at 303 nm		Absorbitivity at 319 nm		Absorbitivity at 330 nm	
ofloxacin	Ornidazole	ofloxacin	Ornidazole	ofloxacin	Ornidazole	ofloxacin	Ornidazole
10	10	235	230	250	370	282	330
20	20	225	248	258	378	292	342
30	30	238	235	244	364	284	331
40	40	222	243	264	384	290	341
50	50	230	239	254	374	287	336
mean	mean	230	239	254	374	287	336

Absorbitivity* = Absorbance/Con. In gm per 100ml.

The assay of above multicomponent system is done by

A- Simultaneous equation Method

This spectrophotometric technique is employed when the two absorbing drugs in the sample X and Y absorbs at the λ max of each other provided that the following criteria are fulfilled;

The criteria are that the ratios between

$$\frac{A_2}{A_1} / \frac{ax_2}{ax_1} \text{ And } \frac{ay_2}{ay_1} / \frac{A_2}{A_1}$$

Should lie outside the range 0.1-0.2 for precise determination of Y and X respectively.

Where ax_1 and ax_2 are the absorptivities of drug X at λ_1 and λ_2 , ay_1 and ay_2 are the absorptivities of drug Y at λ_1 and λ_2 . A_1 and A_2 are the absorbances of the diluted sample at λ_1 and λ_2 .

Based on the fact that the absorbance of the mixture is the sum of the individual absorbance's of X and Y, two simultaneous equation can be built for the system as;-

$$A_1 = a_{x1}.b.Cx + a_{y1}.b.Cy \dots\dots\dots 6.$$

$$A_2 = a_{x2}.b.Cx + a_{y2}.b.Cy \dots\dots\dots 7.$$

When the path length is 1cm, the equation 2 can be rearranged in terms of

$$C_y = \frac{A_2 - ax_2 C_x}{ay_2}$$

Substituting the value of C_y in equation (6), we get

$$C_x = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \dots\dots\dots 8.$$

Similar,

$$C_y = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2} \dots\dots\dots 9.$$

Procedure for analysis of Tablet formulation

Twenty tablets weighed accurately. The average weight was determined and then ground to a fine powder. A quantity equivalent to 50 mg of ornidazole and 20 mg of ofloxacin was transferred to a 10ml volumetric flask. The contents were ultra sonicated for 10 min with blend made to volume and filtered through Whatmann filter paper no.41. One ml of the above

solution diluted to 100 ml with distilled water to give concentration of 20 µg/ml of ofloxacin and 50µg/ml of ornidazole (expected) respectively. Absorbances of these solutions were measured at 319 nm (ornidazole) and 330 nm (ofloxacin) as A₁ and A₂ respectively and concentration of these two drugs in the sample were calculated using equation 6 and 7. Results of the analysis of tablet formulations are reported in table 2.

Table 2: Determination of ofloxacin and ornidazole in combined tablet dosage form.

Component	Methods	Labelled Drug(mg/tablet)	Amount obtained(mg)	%Amount Found	S.D.*	%R.S.D.*
OF	A	200	200.30	100.15	0.300	0.299
	B	200	194.10	97.05	0.530	0.546
OR	A	500	475.80	95.16	0.519	0.545
	B	500	475.50	95.10	0.800	0.841

S.D.*=Standard deviation, n=3, R.S.D. *=Relative standard deviation.

Table Formulation Zenotas-OZ, manufactured by-INTAS Pharmaceuticals LTD

Recovery studies

To perform the recovery studies standard ofloxacin and ornidazole drugs were mixed to form a uniform mixture of both the drugs in the ratio of 2:5 respectively and added in the quantity of 68.00mg, 85.00mg and 102.00mg separately to the pre-analyzed tablet powder equivalent to 50 mg of ornidazole and 20.00mg of ofloxacin and the drug contents were determined by the proposed method. Results of analysis were reported in Table 3.

Table 3: Statistical validation of Recovery studies.

Level of % recovery	Methods	% Recovery*		% R.S.D.*	
		OF	OR	OF	OR
80	A	100.05	95.21	0.699	0.945
	B	96.82	95.22	0.480	0.259
100	A	100.15	95.13	0.515	0.355
	B	97.15	95.16	0.927	0.345
120	A	100.37	95.66	0.311	1.07
	B	97.25	95.60	0.127	0.439

*Denotes average of three estimations at each level of recovery.

METHOD VALIDATION

The developed UV-spectrophotometric method was validated as per ICH guidelines in terms of linearity, and range, specificity, precision, sensitivity and accuracy.

In order to determine linearity range of developed method a series of solutions were prepared using ofloxacin and ornidazole stock solution at concentration range of 10-50µg/ml. The

absorbances of the resultant solutions were measured at 319 and 330 nm against reagent blank. The calibration curves were constructed by plotting concentration on X axis and absorbance on Y axis. R^2 value not less than 0.999 was regarded as acceptance criteria (Figure 1).

Table 4: Developed UV method specification.

Instrument and specification	UV-Spectrophotometer Shimadzu 1800
Scanning Range	200 nm to 400 nm
Solvent Used	Hydrotropic Solvent
Strength of Solvent	10% sodium caprylate, 10% niacinamide and 10% sodium benzoate
Composition of Solvent	10% sodium caprylate, 10% niacinamide and 10% sodium benzoate
Wavelength Maxima of ofloxacin and ornidazole	330 nm and 319 nm

Specificity was performed to exclude the possibilities of interference of solvent in the region of maximum absorbance peaks of ofloxacin and ornidazole. The specificity of the method was tested under the normal conditions and results of the tests proved that the components other than ofloxacin and ornidazole did not produce the deductible peaks at the maximum absorbance peaks of the drug.

Accuracy of the developed method was determined by recovery studies at three different levels. The pre analyzed samples were spiked with 80, 100 and 120% of mixed standard solution. The mixtures were analyzed and the recoveries were determined. The recovery study was carried out in triplicate. The mean % recovery of the ofloxacin and ornidazole at each level should not be less than 98% and not more than 102% was considered as the acceptance criteria.

Precision was studied to find out intra- day and inter-day variations in the test method of ofloxacin and ornidazole, Intra- day assay precision was found by analysis of standard drug thrice on the same day in different intervals of time. Inter-day assay precision was carried out on three different days and percentage relative standard deviation (%RSD) was calculated. The %RSD should not be more than 2.0%.

Sensitivity of proposed method was estimated in terms of limit of Detection (LOD) and Limit of quantification (LOQ). The LOD and LOQ of ofloxacin and ornidazole by proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3s/S$ and

10s/S respectively .where S is the slope of calibration curve and s is standard deviation of response.

Table 5: Optical character and Validation Data of Ofloxacin and Ornidazole.

Parameters	Ofloxacin		Ornidazole	
	Method A	Method B	Method A	Method B
Maximum Absorbance	330nm	----	319nm	----
Linearity	10-50µg/ml	10-50µg/ml	10-50µg/ml	10-50µg/ml
Correlation coefficient	0.999	-----	0.999	-----
Precision	0.299	0.546	0.545	0.841
% Recovery	100.37	97.25	95.66	95.60
LOD	0.683	-----	0.619	-----
LOQ	2.050	-----	1.858	-----
Tablet Assay	100.15	97.05	95.16	95.10

B-Absorbance ratio or Q analysis method

From the overlain spectrum of ofloxacin and ornidazole, two wavelengths were selected, one at 303 nm Isoabsorptive point for both the drugs and the other at 319 nm max of ornidazole. The absorbances of the standard and the sample solutions were measured in same manner as in previous method. The absorptivity values for both the drugs at the selected wavelengths are presented in table. The methods employ Q values; the concentrations of drugs in sample solution were determined by using the following formula.

For ofloxacin

$$C_x = \frac{Q_m - Q_y / Q_x - Q_y * A_1 / ax_1}{Q_x - Q_y * A_1 / ax_1}$$

For Ornidazole

$$C_y = \frac{Q_m - Q_x / Q_y - Q_x * A_1 / ay_1}{Q_y - Q_x * A_1 / ay_1}$$

- $Q_m =$ Absorbance of sample at 319 nm/ Absorbance of sample at 303 nm
- $Q_x =$ Absorptivity of ofloxacin at 319 nm/ Absorptivity of ofloxacin at 303 nm
- $Q_y =$ Absorptivity of ornidazole at 319 nm/ Absorptivity of ornidazole at 303 nm

$A_1 =$ Absorbance of sample at Isoabsorptive point

$ax_1 =$ Absorptivity of ofloxacin at Isoabsorptive point

$ay_1 =$ Absorptivity of ornidazole at Isoabsorptive point

RESULTS AND DISCUSSION

The solubility of ofloxacin and ornidazole in distilled water was found to be 0.02 % at room temperature. Approximate solubility of ofloxacin and ornidazole in aqueous solution of 10%

phenol and 20% sodium benzoate was 1.0% w/v. It is evident from table-2 that the percent drug estimated in tablet formulation was 95.10 ± 0.800 for ornidazole and 97.05 ± 0.530 for ofloxacin by method B. and similarly it was 95.16 ± 0.519 for ornidazole and 100.15 ± 0.300 for ofloxacin by method A respectively. These values are very close to 100, indicating the precision of the proposed analytical method. Further table-3 shows that the range of percent recoveries varied from 95.13 ± 0.355 to 95.66 ± 1.07 for ornidazole and 96.82 ± 0.480 to 100.15 ± 0.515 for ofloxacin which are again very close to 100, indicating the accuracy of the proposed method. Proposed analytical method is further supported significantly by small values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error (table 3). The limit of detection and the limit of quantification were found to be $0.683 \mu\text{g/ml}$ and $2.050 \mu\text{g/ml}$ for ofloxacin and 0.619 and 1.858 for ornidazole.

CONCLUSION

A rapid, simple, and non-toxic UV spectrophotometric method has been developed for simultaneous estimation of ofloxacin and ornidazole in solid dosage form by UV Spectrophotometry.

The present method also validated as per ICH guidelines for linearity, precision, accuracy. The results of all these parameter shows that the present UV spectrophotometric methods found to be precise, linear, rapid, and accurate and can be used for routine quality control analysis of ofloxacin and ornidazole in tablet dosage formulation in any laboratory. A further research can be done to improve the percent of drug estimated and percent recoveries.

REFERENCES

1. Maheshwari RK. "Mixed-solvency approach"- Boon for solubilization of poorly water-soluble drugs. *Asian J Pharm*, 2010; 4(1): 60-3.
2. Maheshwari RK. Solubilization of ibuprofen by mixed solvency approach. *Indian Pharm*, 2009; 8(87): 81-4.
3. Maheshwari RK. "Mixed- solvency" – A novel concept for solubilization of poorly water-soluble drugs. *Delving J. Tech Eng Sci*, 2009; 1(1): 39-43.
4. Maheshwari RK. "Solid as solvent"- Novel spectrophotometric analysis of satranidazole tablets using phenol as solvent". *Indian Pharm*, 2014; 12: 37-40.
5. Maheshwari RK. "Solid as solvent"- Novel spectrophotometric analysis of norfloxacin tablets using phenol as solvent". *Int J Curr. Pharm Res*, 2014; 6: 76-8.

6. Maheshwari RK. Potentiation of solvent character by mixed solvency concept: A novel concept of solubilization. *J Pharm Res*, 2010; 3(2): 411-3.
7. Maheshwari RK, Shilpkar R. Formulation development and evaluation of injection of poorly soluble drug using mixed solvency concept. *Int J Pharm Biosci*, 2012; 3(1): 179-89.
8. Maheshwari RK, Upadhyay N, Jain J, Patani M, Mathuria KC. New spectrophotometric estimation of naproxen tablet formulation employing mixed solvency concept (at 331 nm). *Int J Pharm Technol*, 2011; 3(4): 3618-23.
9. Maheshwari RK, Rajagopalan R. Formulation and evaluation of ornidazole syrup made by mixed-solvency concept. *Der Pharm Lett.*, 2011; 3(6): 266-71.
10. Maheshwari RK, Karawande VU, Application of novel concept of mixed solvency in the design and development of floating microspheres of furosemide. *Int J Pharm Sci* 2013; 15: 167-95.
11. Maheshwari RK, Upadhyay N, Jain J, Patani M, Pandey R. New spectrophotometric analysis of gatifloxacin tablets utilizing mixed solvency concept (at 288 nm). *Der Pharm Lett.*, 2012; 4(1): 1-4.
12. Prashant B, Rawat S, Mahajan YY, Galgatte UC, Maheshwari RK. Formulation development and evaluation of aqueous injection of poorly soluble drug made by novel application of mixed solvency concept. *Int J Drug Delivery*, 2013; 2: 152-66.
13. Maheshwari RK, Rajagopalan R. Formulation and evaluation of paracetamol syrup made by mixed-solvency concept. *Der Pharm Lett.*, 2012; 4(1): 170-4.
14. Chandna C, Maheshwari RK. Mixed solvency concept in reducing surfactant concentration of self-emulsifying drug delivery systems of candesartan cilexetil using D-optimal mixture design. *Asian J Pharm*, 2013; 7(2): 83-91.
15. Maheshwari RK, Upadhyay N, Jain J, Patani M, Mathuria KC. New spectrophotometric estimation of naproxen tablet formulation employing mixed solvency concept (at 331 nm). *Int J Pharm Technol*, 2011; 3(4): 3618-23.
16. Agrawal A, Maheshwari RK. Formulation development and evaluation of in situ nasal gel of poorly water soluble drug using mixed solvency concept. *Asian J Pharm*, 2011; 5(3): 131-40.
17. Bhawsar N, Maheshwari RK, Ansari A, Saktawat Y. New spectrophotometric estimation of gatifloxacin in the tablets using mixed solvency approach. *Int J Pharm Sci*, 2011; 2(2): 270-4.

18. Soni LK, Solanki SS, Maheshwari RK. Solubilization of poorly water soluble drug using mixed solvency approach for aqueous injection. *Br J Pharm Res*, 2014; 4(5): 549-68.
19. Maheshwari RK, Gupta S, Gharia A, Garg SK, Shilpkar R. Simple eco-friendly spectrophotometric estimation of ornidazole tablets by application of mixed-solvency technique. *Bull Pharm Res*, 2011; 1(1): 22-5.
20. Maheshwari RK. "Solid as solvent"-Novel spectrophotometric analysis of ornidazole tablets using melted phenol as solvent. *Asian J Pharm Res*, 2015; 5(1): 21-24.
21. Maheshwari RK, Putliwala M, Padiyar A. Novel approach for spectrophotometric estimation of naproxen in tablet dosage form using solids (eutectic liquid of phenol and niacinamide) as solubilising agents. *Asian J Pharm Res*, 2015; 5(1): 25-28.
22. Jain Sanjay, Maheshwari RK, Nema RK, Singhvi I. Development and validation of simple uv-spectrophotometric method of quantization of Nifedipine in solid dosage formulation using mixed solvency concept. *World J of Pharm res.*, 2017; 6(13): 1014-1021.
23. Jain Sanjay, Maheshwari RK, Nema RK, Singhvi I. Development and validation of simple uv-spectrophotometric method of quantization of Domperidone in solid dosage formulation using mixed solvency concept. *International J of Pharm .sci. and drug research.*, 2017; 9(5): 252-255
24. Jain Sanjay, Maheshwari RK, Nema RK, Singhvi I. Development and validation of simple uv-spectrophotometric method of quantization of Diazepam in solid dosage formulation using mixed solvency concept. *International J of current Pharm res*, 2017; 6(9): 15-17.
25. Jain Sanjay, Maheshwari RK, Nema RK, Singhvi I. Development and validation of simple uv-spectrophotometric method of quantization of Domperidone in solid dosage formulation using mixed solvency concept. *A journal of drug design and discovery*, 2017; 2(4): 19-23.
26. Jain Sanjay, Maheshwari RK, Nema RK, Singhvi I. Development and validation of simple uv-spectrophotometric method of quantization of Domperidone in solid dosage formulation using mixed solvency concept. *European journal of biomedical and pharmaceutical sciences*, 2017; 4(12): 48-51.
27. Jain Sanjay, Maheshwari RK, Nema RK, Singhvi I. simultaneous estimation of ofloxacin and tinidazole in solid dosage form by u v Spectrophotometry using mixed solvency concept. *European Journal of pharmaceutical and medical Research*, 2017; 4(12): 413-418.

28. Jain Sanjay, Maheshwari RK, Nema RK, Singhvi I. simultaneous estimation of norfloxacin and tinidazole in solid dosage form by u v Spectrophotometry using mixed solvency concept. World Journal of pharmaceutical and medical Research, 2018; 4(1): 112-117.
29. Jain Sanjay, Maheshwari RK, Nema RK, Singhvi I. Development and validation of simple uv-spectrophotometric method of quantization of ornidazole in solid dosage formulation using mixed solvency concept. World journal of Pharmacy and pharmaceutical sciences, 2018; 1(7): 824-832.
30. Jain Sanjay, Maheshwari RK, Nema RK, Singhvi I. Development and validation of simple uv-spectrophotometric method of quantization of Indomethacin in solid dosage formulation using mixed solvency concept. The Pharma Innovation Journal, 2017; 6(12): 453-456.
31. Jain Sanjay, Maheshwari RK, Nema RK, Singhvi I. New Spectrophotometric Estimation of Frusemide in the Tablets using mixed solvency concept Approach. International Journal of current Advanced Research, 2017; 6(12): 8510-8513.