Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (13):226-232
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

UV, UV derivative and Visible Spectrophotometric methods for the analysis of Trifluoperazine a phenothiazine antipsychotic drug in bulk and pharmaceutical formulations

L Kalyani[1] and Chava Venkata N Rao[2*]

[1]Department of Chemistry, NRI Institute of Technology, Perecharla, Guntur Dt., A.P. India.
[2]Department of Chemistry, NRI Institute of Technology, Pothavarappadu, Agieripalli Mandal, Krishan dt., A.P., India.

ABSTRACT

A simple, sensitive and accurate UV, first derivative, second derivative and two visible spectrophotometric methods were developed for the analysis of Trifluoperazine in pharmaceutical formulations. Method M1, M2 and M3 were based on the UV absorption of drug and first and second derivative calculation in UV region that shows absorption maxima at 220nm. Method M4 and M5 were based on the oxidative coupling reaction of drug with 1, 10 phenanthroline and Potassium ferricyanide that shows maximum absorbance at 520nm and 720nm respectively. Linearity range was found to be 2-12µg/ml for UV method [M1, M2 and M3], 8- 20µg/ml for 1,10 - phenanthroline method [M4] and 2-20µg/ml for Potassium ferricyanide method [M5]. The methods were validated as per ICH guidelines. The proposed methods have been applied for the estimation of Trifluoperazine in tablets. The developed method was simple, accurate, reliable and economical. The proposed method is specific and interference of excipients. Hence it can be used for the routine analysis of Trifluoperazine in bulk and in pharmaceutical formulation.

Key words: Trifluoperazine, Validation, UV and Vis - Spectrophotometric methods

---

INTRODUCTION

Trifluoperazine is a typical antipsychotic belongs to the class of organic compounds known as phenothiazines [1-5]. It is used for the treatment of anxiety disorders, depressive symptoms secondary to anxiety and agitation. Trifluoperazine blocks postsynaptic mesolimbic dopaminergic D1 and D2 receptors in the brain; depresses the release of hypothalamic and hypophyseal hormones and is believed to depress the reticular activating system thus affecting basal metabolism, body temperature, wakefulness, vascomotor tone, and emesis [6-12]. The drug is sold as tablet, liquid and 'Trifluoperazine-injectable USP' for deep intramuscular short-term use [10, 13].

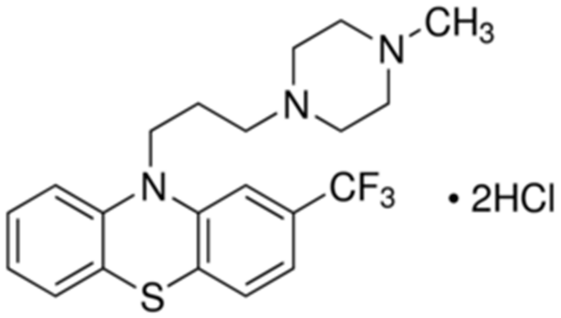

Figure 1: chemical structure of Trifluoperazine

Very few analytical methods have been reported with Trifluoperazine individually and along with other drugs as a combined dosage forms. Among that there are ultraviolet methods [14, 15] individually and UV method for simultaneous estimation [16], visible method [17], derivative method [18] and HPLC methods [19-28] are reported. As very few spectro-photometric methods are reported with Trifluoperazine in single form for formulation, it is therefore taken up the present study to develop all the Ultraviolet, visible and derivative methods for formulation analysis.

MATERIALS AND METHODS

2.1 Instrumentation:
Tec comp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10 mm path length are used for analysis. Standard drug was weighed by using Denver electronic analytical balance (SI-234).

2.2 Chemicals and reagents:
The working standard Trifluoperazine pure was kindly provided as a gifted sample by from Sun Pharmaceutical Industries Ltd. The chemicals used like methanol, 1,10- phenanthroline and ferric chloride were of laboratory analytical grade and were purchased from Merck chemicals private limited, Mumbai, India. The marketed formulation brand Trazine was purchased in local pharmacy.

2.3 Preparation of reagents:
o-Phenonthroline: Weighed accurately 200 mg of o-phenonthroline and was dissolved in 100 ml of distilled water with warming.

Fe (III) solution: Accurately 250 mg of anhydrous ferric chloride was weighed and taken in a 100 ml graduated volumetric flask. It was dissolved in little amount of distilled water and the final volume was made up to the mark with distill water.

Potassium ferricyanide solution: Accurately 100 mg of Potassium ferricyanide was weighed and taken in a 100ml graduated volumetric flask. It was dissolved in double distilled water and made up to the mark.

2.4 Preparation of working standard drug solution:
The standard Trifluoperazine (100 mg) was weighed accurately and transferred to volumetric flask (100 ml). It was dissolved properly and diluted up to the mark with diluents prepared by mixing methanol and water in the ratio of 1:1 (v/v) to obtain final concentration of 1000 µg /ml (stock solution I). 10ml, 20 ml of stock solution I was diluted to 100ml with same diluent (stock solution II- 100µg/ml and stock solution III-200µg/ml). The resulting solutions were used as working standard solution.

2.5 Preparation of Sample solution:
An amount of finely ground tablet powder equivalent to 10mg of Trifluoperazine (Trazine ® -10mg) was accurately weighed into a 10 ml calibrated flask along with 6 ml of methanol and shaken for 20 min. Then, the volume was made up to the mark with water, mixed well and filtered by using a Whatmann No 42 filter paper. The filtrate on

subsequent portion (1000µg/ml Trifluoperazine) was diluted appropriately to get suitable concentrations for analysis by proposed methods.

3 Method Developments:

3.1 *Method 1: UV Spectrophotometric method* [M1]:

Different solvents were used for the preparation of standard drug Trifluoperazine and the wavelength maximum was determined for each of the prepared solution. The solvent which gives the best wavelength maximum and high absorbance was selected as suitable solvent for the analysis of Trifluoperazine. A solvent composition of methanol and water in the ratio of 1:1 (v/v) was found to show maximum absorbance in UV region for Trifluoperazine. From the standard stock solution, aliquots of drug (0.5 to 3.5ml; 100µg/ml) are pipette in to 25ml volumetric flasks. The final volume was made up to the mark with the diluents selected for the analysis. The absorbance maximum was determined with one solution. The absorbance of all other solutions was measured at the same wavelength maxima against blank.

3.2 *First order derivative spectrophotometer method* [M2]***:***

The UV absorption spectra for the dilutions prepared for the construction of calibration curve was used for the development of first order derivative spectrophotometer method. The UV absorption spectra of Trifluoperazine were measured against reagent blank. The first derivative spectra are smoothened using spectrophotometer software.

3.3 *Second order derivative spectrophotometer method* [M3]***:***

The UV absorption spectra for the dilutions prepared for the construction of calibration curve was used for the development of first order derivative spectrophotometer method. The UV absorption spectra of Trifluoperazine were measured against reagent blank. The first derivative spectra are smoothened using spectrophotometer software.

3.4 *1,10- Phenonthroline method* [M4]:

From the standard stock solution III, aliquots of drug (0.4 to 1ml; 200µg/ml) are pipetted out in to 10 ml volumetric flasks and to which 0.5 ml $FeCl_3$ solution and 2 ml of 1, 10- Phenonthroline were added. The tube was heated in water bath for 30 min. and after cooling the tube 2 ml of 1N HCl was added and made up to the mark with water. The absorbance maximum was determined with one solution and the absorbance of all the solution was measured in the same wavelength maxima against reagent blank.

3.5 *Ferricyanide method* [M5]:

From the standard stock solution II, aliquots of drug (0.2-2.0ml; 100µg/ml) solution were transferred and 1.0 ml of Fe (III) solution is added. The tubes were stoppard immediately and shaken well for 5 min. Then 0.5ml of potassium ferricyanide solution was added into each tube and closed with lids immediately. After 5 min, 1ml of 1N HCl was added and the final volume was made up to 10 ml with double distilled water. The absorbance maximum was determined with one solution and the absorbance of all the solution was measured at the same wave lenght.

4 Method Validations:

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result, or a product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like linearity, accuracy, precision, specificity, robustness, Limit of Detection (LOD), and Limit of Quantification (LOQ).

RESULTS AND DISCUSSION

UV-Visible spectrophotometry is one of the most frequently employed techniques in the pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Five simple UV, visible and UV derivative spectrophotometric methods were developed for the analysis of Trifluoperazine in pharmaceutical formulations.

UV method [M1] was based on the UV light absorption of the standard drug Trifluoperazine in dilute solution. It was confirmed that at a wavelength of 220nm (Figure 2) was found to be suitable for the analysis of Trifluoperazine, where the drug absorb maximum UV light. M2 and M3 methods are based on the derivative spectra observed for standard drug. The maximum absorbance was observed same as UV absorbance i.e. 220nm (Figure 2) for both first and second derivative methods.

M4 is based on the mechanism of oxidation followed by complex formation, wherein the initial reaction, the anti-oxidant undergoes oxidation in presence of ferric chloride and then the oxidized ferric chloride reacts with 1,10-phenanthroline and the drug to form a orange red colored complex which exhibits maximum absorption at wavelength of 520nm (Figure 2).

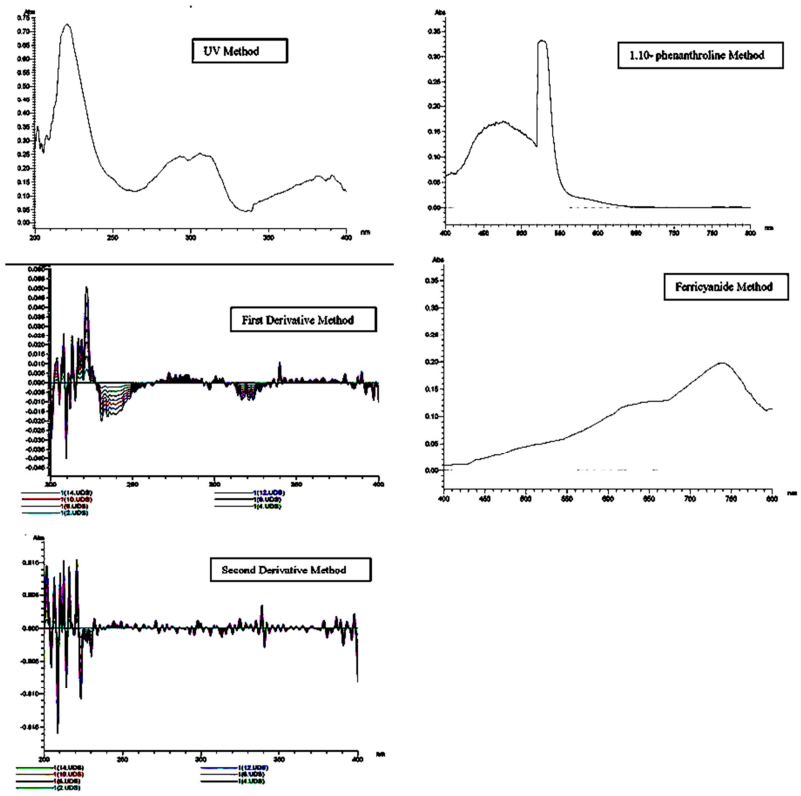

Figure 2: Wavelength scanning spectra for Trifluoperazine in the proposed methods

M5 is based on the oxidation of drug by excess of ferric salt (Fe^{3+}) and the reduced state of Fe^{3+} was utilized besides the un reacted Fe^{3+}. The Fe^{2+} has tendency to give colored complex on treatment with Potassium ferricyanide and the developed color can be estimated by using spectrophotometer at a wavelength 720nm (Figure 2). The developed color complex was stable for 90min. The wavelength scanning spectra of placebo, standard drug and formulation reveals that no spectral detection was observed at the wavelength maxima of Trifluoperazine and similar wavelength maxima was observed for standard and sample reveals that 221nm was found to be suitable for method M1. In method M4 and M5, no color change was observed in the blank. Hence the developed methods were found to be specific for Trifluoperazine. The optical parameters were given in Table 1. The developed color complex was stable for 150min.

Table 1: optical parameters for Trifluoperazine in the developed methods

S. No	Parameter	M1	M2	M3	M4	M5
1	Wavelength Max	221nm	221nm	221nm	520nm	720nm
2	Beer-Lambert's Law Limits (µg/ml)	2-14	2-14	2-14	8-20	2-20
3	r^2	0.998	0.998	0.999	0.999	0.998
4	Slope	0.047	0.007	0.0008	0.03267	0.075027
5	Intercept	- 0.046	7E-05	-0.0006	-0.0015	0.045273

Different aliquots of standard drug were measured accurately and the developed method was applied for all the solutions separately. The absorbance of the each of the aliquot was measured in triplicates at the corresponding wavelength against reagent blank. Beers law plot was constructed by taking the concentration on x-axis and average absorbance on y-axis. A linear correlation was found between absorbance at λ max and concentration of

Trifluoperazine reveals the Beers law limit for the developed methods. Beers law range was found to be 2-14µg/ml [y = 0.047x - 0.046; R^2 = 0.998], 2-14µg/ml [y = 0.003x + 7E-05; R^2 = 0.998], 2- 14µg/ml [y = 0.0008x - 0.0006; R^2 = 0.999], 8-20µg/ml [y = 0.032x - 0.004; R^2 = 0.998] and 2-20µg/ml [y = 0.073x + 0.066; R^2 = 0.998] for method M1 to M5 respectively. Very accurate fit linearity graph was obtained for all the methods. The results of the linearity were given in table 2 and calibration curves were given in figure 3.

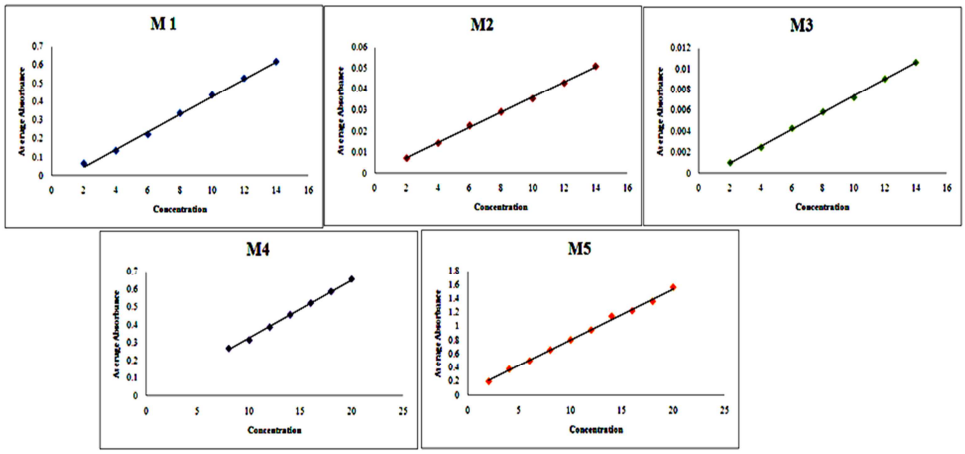

Figure 3: Beers law graphs for Trifluoperazine in the proposed methods

Table 2: Linearity results for Trifluoperazine in the proposed methods

S No	M1		M2		M3		M4		M5	
	Conc*	Abs#	Conc*	Abs#	Conc*	Abs#	Conc*	Abs#	Conc*	Abs#
1	2	0.064	2	0.00713	2	0.001	8	0.268	2	0.199
2	4	0.133	4	0.01429	4	0.00246	10	0.315	4	0.378
3	6	0.221	6	0.02261	6	0.00426	12	0.387	6	0.499
4	8	0.335	8	0.02917	8	0.00584	14	0.456	8	0.656
5	10	0.434	10	0.03543	10	0.00719	16	0.521	10	0.802
6	12	0.525	12	0.04303	12	0.00901	18	0.586	12	0.944
7	14	0.616	14	0.05114	14	0.0106	20	0.656	14	1.142
8	-	-	-	-	-	-	-	-	16	1.219
9	-	-	-	-	-	-	-	-	18	1.355
10	-	-	-	-	-	-	-	-	20	1.557

Precision:

Table 3: Linearity results for Trifluoperazine in the proposed methods

S No	M1 at 8µg/ml@		M2 at 8µg/ml@		M3 at 8µg/ml@		M4 at 16µg/ml@		M5 at 12µg/ml@	
	Intra@	Inter@	Intra@	Inter@	Intra@	Inter@	Intra@	Inter@	Intra@	Inter@
1	0.321	0.321	0.02917	0.02947	0.00571	0.00584	0.521	0.529	0.944	0.956
2	0.32	0.304	0.02958	0.02965	0.00576	0.00586	0.526	0.532	0.943	0.96
3	0.316	0.309	0.02944	0.02946	0.00565	0.00571	0.522	0.536	0.945	0.951
4	0.307	0.307	0.02937	0.02993	0.00581	0.00573	0.529	0.528	0.936	0.963
5	0.317	0.313	0.02981	0.02947	0.00575	0.00562	0.521	0.532	0.951	0.948
6	0.316	0.314	0.02954	0.02965	0.00565	0.00581	0.525	0.521	0.955	0.926
RSD	**1.568**	**1.934**	**0.731**	**0.617**	**1.119**	**1.588**	**0.615**	**0.960**	**0.70**	**1.40**

** Concentration of the solution prepared in µg/ml*
 # Average absorbance obtained in three replicate measurements
@ Intra – Intra-day Precision and Inter – Inter-day Precision

To ensure the accuracy and reproducibility of the results obtained, known amounts of pure drug was added to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The % recovery and % RSD of recovery in each spiked level was studied in each of the method. The % recovery was found to be less than 2 (Table 4) for all the spiked levels for the five developed methods. Hence the developed methods were found to be accurate.

Table 4: Recovery results for Trifluoperazine in the proposed methods

S No	Method	Spiked Level	Target (µg/ml)	Spiked (µg/ml)	Final (µg/ml)	Amount found Mean ± SD*	% Recovered Mean ± SD*	% RSD
1	M1	50%	4	2	6	6.008 ± 0.128	100.7±1.212	1.204
2		100%	4	4	8	7.959±0.059	99.49±0.749	0.753
3		150%	4	6	10	10.01±0.055	100.15±0.584	0.583
1	M2	50%	4	2	6	5.92±0.013	98.67±0.231	0.234
2		100%	4	4	8	7.901±0.009	98.77±0.113	0.114
3		150%	4	6	10	9.950±0.052	99.50±0.521	0.524
1	M3	50%	4	2	6	5.944±0.026	99.06±0.443	0.447
2		100%	4	4	8	7.906±0.004	98.83±0.056	0.057
3		150%	4	6	10	0.004±0.033	99.21±0.335	0.338
1	M4	50%	8	4	12	11.833±0.055	98.61±0.455	0.462
2		100%	8	8	16	15.833±0.097	98.96±0.606	0.612
3		150%	8	12	20	20.33±0.050	101.68±0.251	0.247
1	M5	50%	8	4	12	11.81±0.026	98.416±0.217	0.220
2		100%	8	8	16	15.82±0.065	98.87±0.408	0.413
3		150%	8	12	20	19.713±0.080	98.57±0.401	0.406

** Values indicated are the mean ± standard deviation of three replicate measurements*

All the developed methods were found to be very sensitive. Limit of detection [LOD] and limit of quantification [LOQ] results confirms the sensitivity of the methods. The results of the sensitivity studies were given in Table 5.

Table 5: Results of the sensitivity studies for Trifluoperazine in the proposed methods

S NO	Test	M1	M2	M3	M4	M5
1	LOD	0.03µg/ml	0.03µg/ml	0.03µg/ml	0.075µg/ml	0.08µg/ml
2	LOQ	0.10µg/ml	0.10µg/ml	0.10µg/ml	0.25µg/ml	0.25µg/ml

The proposed methods have been applied for the estimation of Trifluoperazine in tablets without any interference from the additives. A % assay of 99.587, 99.587, 99.587, 98.687 and 99.080 for Trifluoperazine in method M1 to M5 respectively (Table 6) were obtained in the formulation analysis study. This confirms that more than 98% assay was observed for the drug in the proposed methods and the formulation excipients doesn't interference and the results were unaffected by excipients. This confirms that the method was found to be suitable for the routine analysis of Trifluoperazine in fixed dosage forms.

Table 6: Formulation analysis results for Trifluoperazine in the proposed methods

S. No.	Method	Brand name	Available form	Label claim mg	Concentration µg/ml	Amount found µg/ml	% Assay
1	M1	Trazine	Tablet	10	8	7.967	99.587
2	M2	Trazine	Tablet	10	8	7.967	99.587
3	M3	Trazine	Tablet	10	8	7.967	99.587
4	M4	Trazine	Tablet	10	16	15.79	98.687
5	M5	Trazine	Tablet	10	12	11.89	99.080

CONCLUSION

Five simple and economical UV-Visible spectrophotometric methods were developed for the analysis of Trifluoperazine in pharmaceutical formulations. The method discussed in the present work provides a simple, accurate, economical and convenient method for the analysis of Trifluoperazine. The wavelength maxima was found to be 221nm in UV and derivative spectrophotometer methods, 520nm and 720nm for 1,10 phenanthroline and Potassium ferricyanide methods respectively. Linearity range was found to be 2-12µg/ml for UV method first and second derivative methods, 8-20µg/ml for 1,10 phenanthroline method and 2-20µg/ml for Potassium ferricyanide method. These methods were validated as per ICH guidelines. Based on the results obtained in the three developed

methods it can be concluded that the proposed methods were simple, accurate, reliable, economical and specific. Hence the methods can be used for the routine analysis of Trifluoperazine in bulk and in pharmaceutical formulation.

REFERENCES

[1] Rex William Cowdry, David L. Gardner, *Arch. Gen. Psychiatry*, **1988**, 45 (2), 111–119.
[2] David S. Baldwin, Polkinghorn, *International Journal of Neuropsychopharmacology*, **2005**, 8, 293–302.
[3] L.Tang, P.K. Shukla, Z. J. Wang, *Neuroscience Letters*, **2006**, 397 (1–2), 1–4. Science Daily (2006-02-13).
[4] Clive Ballard, Marisa Margallo Lana, Megan Theodoulou, Simon Douglas, Rupert McShane, Robin Jacoby, Katja Kossakowski, Ly-Mee Yu, Edmund Juszczak, *PLOS Medicine*, **2008**, 5 (4), e76.
[5] J. Huerta-Bahena, R. Villalobos-Molina, J. A. García-Sáinz, *Molecular Pharmacology*, **1983**, 23 (1), 67–70.
[6] P. Seeman, T. Lee, M. Chau-Wong, K. Wong K, *Nature*, **2005**, 261 (5562), 717–719.
[7] I. Creese, D. R. Burt, S.H. Snyder, *The Journal of Neuropsychiatry and Clinical Neurosciences*, **1996**, 8 (2), 223–226.
[8] Ebadi, Manuchair S, "Trifluoperazine Hydrochloride". CRC desk reference of clinical pharmacology (illustrated ed.). CRC Press, 1998.
[9] L.O. Marques, M.S. Lima, S.B.G. Marques, Luciana de Oliveira, ed. "Trifluoperazine for schizophrenia". *Cochrane Database of Systematic Reviews(1)*, 2004.
[10] K. Koch, K. Mansi, E. Haynes, C. E. Adams, S. Sampson, V. A. Furtado, "Trifluoperazine versus placebo for schizophrenia". *Cochrane Database of Systematic*, Reviews (1), 2014, 1-117.
[11] R.A. Smego, D.T. Durack, *Archives of Internal Medicine*, **1982**, 142 (6), 1183–1185.
[12] D. Hedges, K. Jeppson, P. Whitehead, *Drugs of Today* (Barcelona, Spain:1998), **2003**, 39 (7), 551–557.
[13] D. J. Boet, *Documenta Ophthalmologica. Advances in Ophthalmology*, **1970**, 28 (1), 1–69.
[14] C.M.Bhaskar Reddy, G.V.Subba Reddy, N. Ananda Kumar Reddy, *International Journal of Scientific and Research Publicati*ons, ISSN 2250-3153, **2012**, 2(8), 1-5
[15] Nief Rahman Ahmed et al, *Journal of Pharmaceutical Analysis*, 2014, 3(2).
[16] R. B. Saudagar, Swarnlata Saraf, S Saraf, *Indian Journal of Pharmaceutical Sciences*, **2007**, 69 (1), 149-152.
[17] Zuhair A-A. Khammas, Rana Abbas Rashid, *Science Journal of Analytical Chemistry*, **2015,** 3(5): 61-70.
[18] M.C. Sharma, S. Sharma, *World Journal of Chemistry*, 2011, 6 (2), 80-84.
[19] Jameel M. Dhabab, Salam A.H. Al-Ameri, Assaf H. Taufeeq, *Journal of the Association of Arab Universities for Basic and Applied Science*s, **2013,** 13, 14-18.
[20] Dontinineni Kalyan, Punna Venkateswarlu, *Asian J Pharm. Research*, **2015**, 1(5), 7-12.
[21] Suman Pattanayak, Y. Asha Rani, *International J of Pharmaceutical Sciences and Drug Research* **2015**, 7(1), 105-109.
[22] Shashi Daksh, Aju Hoyal Chakshu K Pandiya, *International J of Pharmaceutical Research and Analysis*, **2015**, 5(1), 38-45.
[23] P. Shetti, A. Venkatachalam, *E-Journal of Chemistry*, **2010**, 7(S1), S299-S313.
[24] Jameel M Dhabab, Assaf H Taufeeq, Tarik Ak Nasser, *J Int. Environmental Application and Science*, **2012**, 7(3), 503-510.
[25] Komal V Patel, Mandev B Patel, Nishith K Patel, *J Pharm. Sci. Bioscientific Res.*, **2015**, 5(6), 556-564.
[26] Komal Patel, Ankit Chaudhary, Bhadani Sjhweta, Ekta Patel Rajiv, *International J of Current Research in Pharmacy*, **2015**, 1(1), 50-59.
[27] P. Shetti, A. Venkatachalam, *J Pharmaceutical and Biomedical Sciences*, **2011**, 9(07), 1-10.
[28] R. Sukanya, P. Bharath Rathna Kumar, R. Venu Priya, K. B. Chandrasekhar, *J of Global Trends in Pharmaceutical Sciences*, **2015**, 6(2), 2555-2561.