ORIGINAL ARTICLE   **OPEN ACCESS**

A Simple HPLC Method for *in-Vitro* Dissolution Study of Sertraline Hydrochloride in Tablets

Sushil P. Narkhede*[1], Santosh Kumar Singh[1], G. Vidyasagar[1]
[1]Suresh Gyan Vihar University, Mahal, Jagatpura, Jaipur-302 025, India
E-mail: sushil.narkhede@gmail.com
Contact: +91-8446231031

ABSTRACT
The main aim of present work was to develop and validate a dissolution test for Sertraline Hydrochloride in tablets using HPLC method. The dissolution established conditions were: 900 ml of 0.05M Acetate buffer pH 4.5 as dissolution medium, using a paddle apparatus at a stirring rate of 75 rpm. The chromatographic condition employed a C-18 column (Eurospehere, 250 x 4.6mm, 5μm), isocratic elution with Acetonitrile:Methanol:Phosphate buffer pH 3 (20:50:30 V/V/V) and UV detection at 274 nm. The method was validated to meet requirements for a global regulatory filing. The dissolution test developed was adequate for its purpose and could be employed for routine quality control of Sertraline Hydrochloride tablets.
Keywords: Sertraline Hydrochloride, Dissolution method, HPLC, Validation

INTRODUCTION

The Dissolution test has emerged in the pharmaceutical field as a very important tool to characterize drug product performance. It provides measurements of the bioavailability of a drug as well as can demonstrate bioequivalence from batch-to-batch. Besides, dissolution is a requirement for regulatory approval for product marketing and is a vital component of the overall quality control program [1].

Sertraline Hydrochloride (SER), (1S,4S)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronaphthalen-1-amine hydrochloride (Fig. 1) is white or almost white powder, slightly soluble in water, freely soluble in anhydrous ethanol, slightly soluble in acetone and in isopropanol. SER is primarily selective serotonin reuptake inhibitor (SSRI), belongs to the category of antidepressant drug [2].

Literature survey reveals that no instrumental method is reported for estimation of percent release of SER from tablet. Hence an attempt has been made to develop an ideal dissolution method for determination of percent release of drug from their dosage form [3-19].

MATERIAL AND METHODS

The drug Sertraline HCl (SER) was obtained as a gift sample from Matrix Laboratories Ltd., Nashik (MS, India). Acetonitrile, and Methanol of analytical grade were purchased from Priya Scientific Pvt. Ltd., Vapi. The HPLC was of Waters Alliance System equipped with DAD. The Dissolution Apparatus (Type II) was of ELECTROLAB, Model: TDT 06P. In addition an electronic balance (CONTECH), Digital pH meter (AE-MAX), Ultrasonicator (CITIZEN), Hot air oven (LABLINE) was used in this study. All the glass apparatus used in this study was of BOROSIL.

Preparation of Stock Solution and Dissolution Medium:
Dissolve 136 gm of sodium acetate and 77 gm of ammonium acetate in water and dilute with water to 1000 ml. Add 250 ml of glacial acetic acid and mix. Stock solution was prepared by weighing 50 mg of SER. Transfer the weighed drug to 50 ml volumetric flask and make up the volume up to the mark with methanol to obtain solution containing 1000 μg/ml of SER. The solution was further diluted with dissolution medium to obtain final concentration of 10-60 μg/ml of SER.

Selection of Sampling Wavelength:

Solutions containing of SER (30 μg/ml) was scanned from 200 nm to 400 nm and spectra were recorded. The wavelength of maximum absorption (λ $_{max}$) was observed at 274 nm and selected for estimation. It is shown in Fig. 2

Linearity Study:
Suitable aliquots of SER stock solution were taken in different 10 ml volumetric flask and diluted up to the mark with dissolution medium to obtain final concentration of 10-60 μg/ml of SER. Chromatographic peak area was measured at 274 nm. Calibration curve were constructed by plotting Peak area *versus* Concentration as shown in Fig 3.

Instrument Analysis Condition:
For HPLC Instrument:
Chromatographic Condition for Analysis:

Instrument	**:** Waters Alliance System (Software- Empower 3)
Column	**:** Eurosphere-100 C$_{18}$, 250×4.6 mm, particle size 5 μm.
Mobile Phase	Acetonitrile:Methanol:Phosphate buffer pH 3 (20:50:30 V/V/V)
Flow Rate	**:** 1.0 ml/min.
Wavelength	**:** 274 nm.
Injection Volume	**:** 20 μl.
Column temperature	**:** Ambient

For Dissolution Apparatus:

Apparatus	:	Electrolab USP, Type II (Paddle)
Dissolution Medium	:	Acetate buffer pH 4.5 (900ml)
Speed	:	75 rpm
Temperature	:	37°C ±0.5°C
Time	:	45 min (Profile 5, 10,15,30,45 min)

Analysis of Marketed Formulations:
Prepare standard solution of Sertraline Hydrochloride (SER) by dissolving 50 mg SER in 50mL mobile phase in volumetric flask. Dilute 3 ml of this solution to 100 ml with dissolution medium in volumetric flask. Filter this solution through MDI syringe filter and fill up the sample cuvette. The 20 μl of standard solution is injected into the HPLC system and quantification was done from regression line equation of calibration curve.

The tablets having 50mg of Sertraline Hydrochloride (SER) were studied in developed dissolution medium at 37 ± 0.5°C. Place one tablet in 900 ml dissolution medium and immediately run dissolution apparatus up to 45 min. The dissolution was carried out in USP apparatus II at 75 rpm. The samples withdrawn after time interval of 5, 10, 15, 30, 45 mins, diluted with mobile phase solutions as above, filtered through 0.45μm membrane filter. Measure the peak area of solution and quantification was done from regression line equation of calibration curve. The % of drug release is listed in Table 2 and the results were represented. The in vitro release profile of marketed formulation (Product-A, Product-B and Product-C) of Sertraline Hydrochloride (SER) is represented in Figures 4. The sample withdrawn after time interval of 45 mins chromatogram is shown in Figure 5.

Method Validation [20-25]:
The dissolution test was validated as per the recommendations of ICH and USP for the parameters like accuracy, linearity, precision, Specificity, detection limit, quantitation limit and ruggedness. The accuracy of the method was determined by calculating percentage recovery of SER. Recovery studies were carried by addition of standard solution containing 20 μg/ml of SER to the preanalyzed sample solution after 10, 15, 30 min. time interval. Chromatographic peak area of the solution was measured and percent recovery was calculated. Results are reported in Table 2.

Variation of results within the same day (intra-day), variation of results between days (inter day) were analyzed. Intra-day precision was determined by analyzing SER for three times in the same day. Inter-day precision was determined by analyzing SER daily for three days. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formulae: LOD= 3.3(SD)/S and LOQ= 10 (SD)/S, where SD=standard deviation of response and S= average of the slope of the calibration curve. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Robustness is the measure of the capacity of a method to remain unaffected by small variations in the method parameters. Robustness of the method was determined in triplicate at a concentration level of 30 μg/ml of Sertraline Hydrochloride. After small changes in this parameter effect peak areas were determined and mean and RSD of peak areas calculated.
a) Change in % organic phase of mobile phase by ± 5.0 %
b) Change in pH of buffer of mobile phase by ± 0.5 of set pH

c) Change in the flow rate of the mobile phase by ± 10 % of the original flow rate.
d) Change in detection wavelength by ± 5.0 nm
e) Change in column temperature by ± 5.0 ºC
The method was found to be robust, as small but deliberate changes in method parameters have no detrimental effect on the method performance as shown in Table 6. The low value of percentage relative standard deviation indicates that the method is robust.
Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by different analyst.
To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 30µg/ml for Sertraline Hydrochloride (SER), respectively to check the reproducibility of the system and results obtained are summarized in Table 8.

RESULT AND DISCUSSION
Drug solubility and solution stability are important properties to be considered when selecting the dissolution medium. SER stability in dissolution medium was evaluated at 37.0 ± 0.5ºC for 3 hours. No change in peak area after 3 hours indicates the stability of SER. In this study, 0.05M acetate buffer pH 4.5 is used as dissolution medium. An important aspect it simulates the environment in GI track which monitor biopharmaceutical characteristic of the drug product, 900 ml 0.05M acetate buffer pH4.5 provides *sink* condition for tablet containing 50 mg Sertraline Hydrochloride. USP Type II with Paddle rotating at 75 rpm was selected as a dissolution apparatus was selected for the dissolution study.
UV-spectra of SER showed that the drug absorbed appreciably at 274 nm, so this wavelength was selected as the detection wavelength. The calibration curve for SER was found to be linear over the range of 10-60 µg/ml. The data of regression analysis of the calibration curves is shown in Table 1. The proposed method was successfully applied for the estimation of SER in their tablet dosage form (Table 1, Fig 3).
The accuracy expresses the agreement between the accepted value and the value found. The % Recovery was found between 99.17% – 101.7%, it proved that method is accurate (Table 2).
The precision of the dissolution tests was evaluated through variation in the result in same day (intraday) and variation of the result between days (inter day). These results can demonstrate the good precision of the method for dissolution test (Table 3, 4 and 5).
Limit of detection (LOD) for SER was found to be 2.1 µg/ml and Limit of quantification (LOQ) for SER was found to be and 6.93 µg/ml
Specificity was examined by analyzing a solution of a placebo, which consisted of all the excipients the might be present in tablets except the drug. The excipients were lactose, dibasic calcium phosphate, maize starch, sodium starch glycolate, croscarmellose sodium, talc and magnesium stearate. Their concentrations were determined based in Handbook of Pharmaceutical Excipients and calculated for a medium weight of content. The peak purity index and HPLC chromatogram showed peaks of the drugs (Sertraline Hydrochloride) without any interfering peak and the estimation of the drug were found to be satisfactory (Fig. 6).
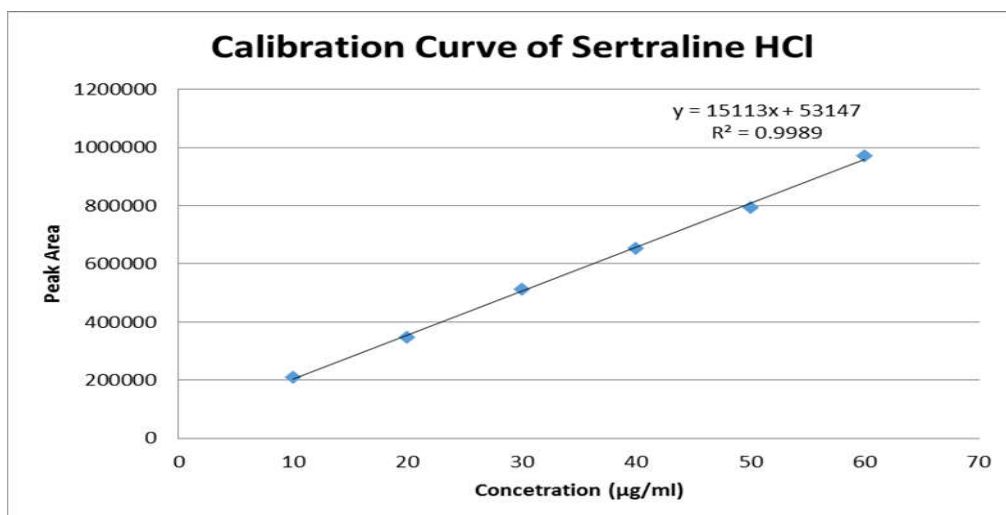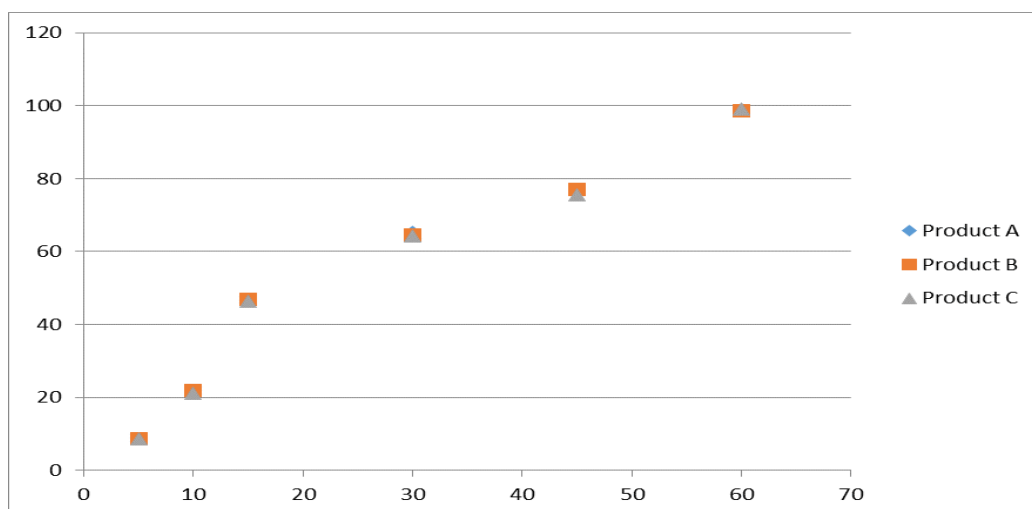The result of Ruggedness evaluation was shown in Table 7.
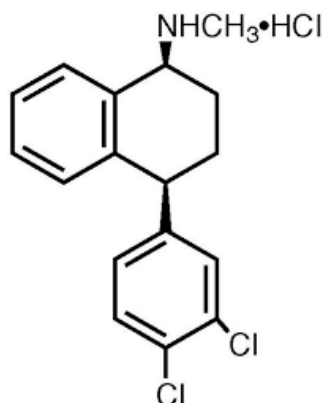
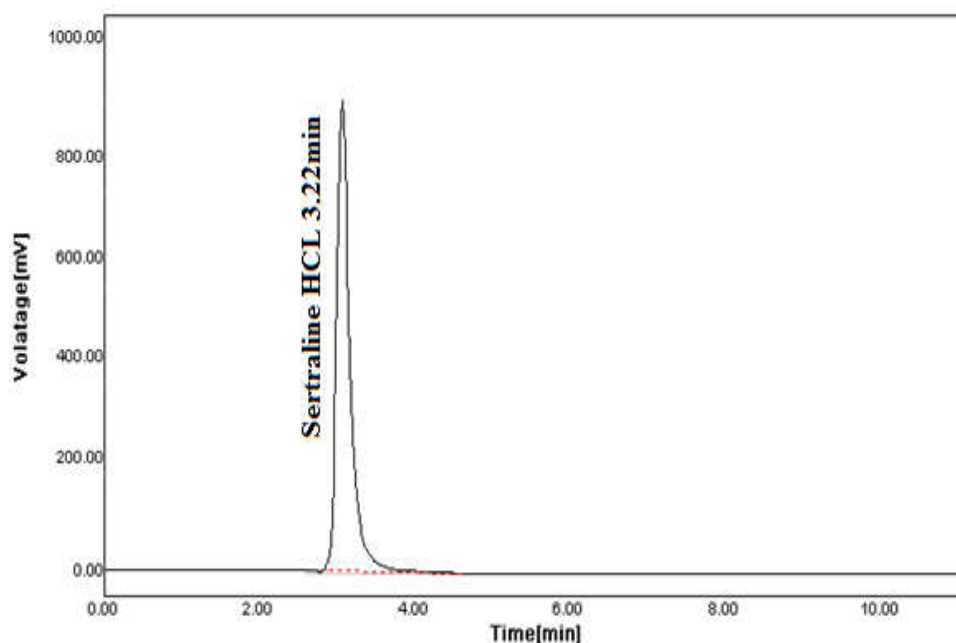**Figure 1: Structure of Sertraline Hydrochloride (SER)**
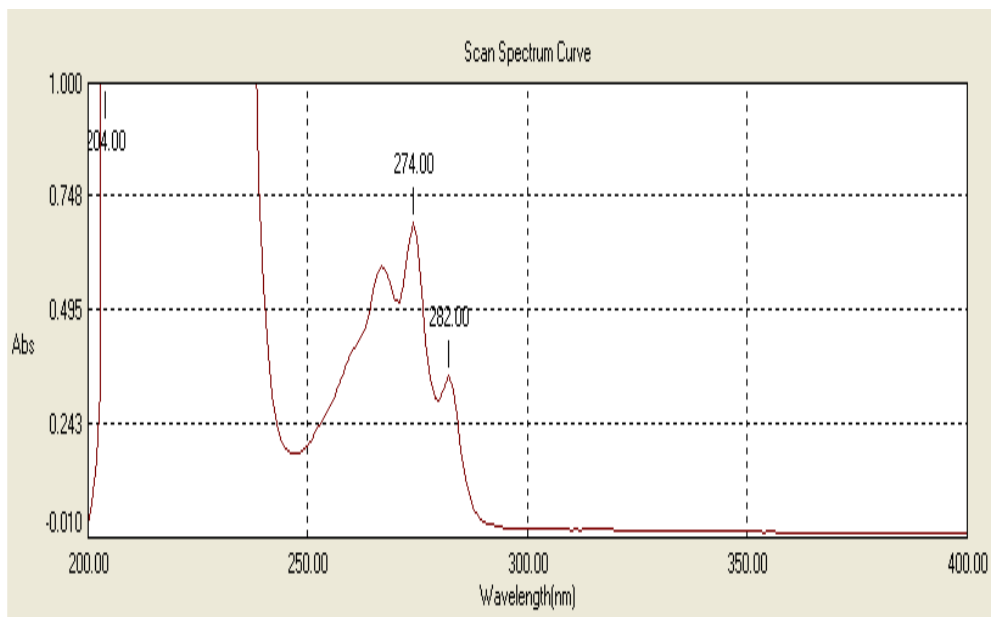
Figure 2: UV-Spectra of Sertraline Hydrochloride in 0.05M Acetate buffer pH 4.5
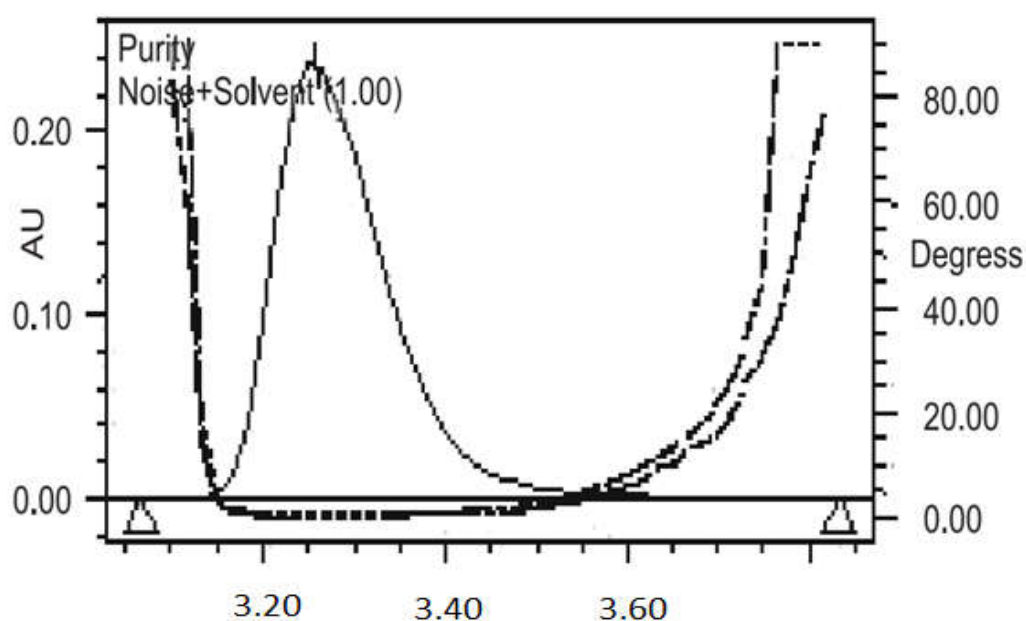

Figure 3: Calibration curve for SER

Figure 4: Dissolution profile of Sertraline Hydrochloride (SER) in 0.05M Acetate buffer pH 4.5 at 75 rpm

Figure 5: HPLC Chromatogram of sample solution of Sertraline Hydrochloride (SER) after time interval of 45 minutes

Fig 6: Peak Purity of Sertraline Hydrochloride

Table 1: Linearity data of SER by HPLC Method

Parameters	Sertraline Hydrochloride
Linear Range	10 – 60 µg/ml
Slope	15113
Intercept	53147
Regression Coefficient (r^2)	0.998
Standard deviation of slope	15.452
Standard deviation of intercept	19.854
LOD (µg/ml)	2.1 µg/ml
LOQ (µg/ml)	6.93 µg/ml

Table 2: Accuracy Study

Sr. No.	Level	Amount of drug present in sample (μg/ml)	Amount of Std. drug added (μg/ml)	Total amount of Drug (μg/ml)	Amount of drug found (μg/ml)	% Recovery ± SD
1	0	20	0	20	19.91	99.55 ± 0.661
2	50	20	10	30	30.45	101.5 ± 1.41
3	100	20	20	40	39.67	99.17 ± 0.625
4	150	20	30	50	50.85	101.70 ± 1.112

Table 3: Repeatability study

Time of withdrawal (Minutes)	% of Drug release (Mean ± SD)	% RSD
10	21.393 ± 0.295	1.380
15	64.687 ± 0.378	0.584
30	76.220 ± 0.621	0.814
45	98.850 ± 0.216	0.219

Table 4: Intraday Precision

Time of withdrawal (Minutes)	% of Drug release (Mean ± SD)	% RSD
10	21.454 ± 0.269	1.253
15	64.687 ± 0.173	0.269
30	76.22 ± 0.621	0.814
45	98.968 ± 0.205	0.208

Table 5: Interday Precision

Time of withdrawal (Minutes)	% of Drug release (Mean ± SD)	% RSD
10	21.377 ± 0.153	1.717
15	64.383 ± 0.097	1.13
30	75.790 ± 1.069	0.472
45	98.793 ± 0.763	1.19

Table 6: Robustness Study

Parameters	Change in condition	%RSD
Mobile Proportion Changed acetonitrile: methanol: phosphate buffer pH 3 20:50:30 (v/v/v)	Acetonitrile: Methanol: Phosphate buffer pH 3 (25:55:30 (v/v/v)	1.13
	Acetonitrile: Methanol: Phosphate buffer pH 3 (20:55:25 (v/v/v)	0.73
	Acetonitrile: Methanol: Phosphate buffer pH 3 (25:45:30 (v/v/v)	0.91
pH of mobile phase changed (pH=3.0)	2.7	0.92
	3.3.	1.58
Flow rate Changed (1 ml/min)	0.9	0.71
	1.1	1.62
Column Temperature (30ºC)	25ºC	0.89
	35ºC	1.05
Detection wavelength (274nm)	269 nm	0.68
	279 nm	1.45

Table 7: Ruggedness Study

Sr. No.	Time (Min)	Analyst I* Conc. ± SD	% CV	Analyst II* Conc. ± SD	% CV
1	10	15.84 ± 0.067	0.152	15.76 ± 0.031	0.148
2	15	23.62 ± 0.033	0.144	23.95 ± 0.029	0.098
3	30	42.83 ± 0.051	0.159	42.84 ± 0.075	0.173

Table 8: System Suitability Test Result

Sr. No.	Rt. (min)	Peak area	Tailing factor (T)	No. of Theoretical plates (N)	Capacity factor(K')	Selectivity (α)
1	3.21	526231	1.74	6261.4	2.05	2.11
2	3.25	530461	1.75	6387.35	2.09	2.13
3	3.22	523446	1.69	6378.73	2.12	2.14
4	3.33	526316	1.73	6290.03	2.11	2.07
5	3.27	541211	1.75	6269.98	2.14	2.12
Mean	3.256	529533	1.732	6317.498	2.102	2.114
S.D	0.05	6991.79	0.02	60.80	0.03	0.03
% RSD	1.47	1.32	1.44	0.96	1.63	1.28

CONCLUSION

The dissolution test developed and validated for SER tablets was considered satisfactory. The conditions that allowed the dissolution determination were 900 ml of 0.05M Acetate buffer pH 4.5 at 37.0 ± 0.5 ºC, paddle apparatus, 75 rpm stirring speed and filtration with quantitative filter. In these conditions, the SER stability was guarantee. The % drug delivery was higher than 90% in 45 minutes for all evaluated products. The High Performance Liquid Chromatography (HPLC) method was validated and showed to be specific, linear, precise and accurate. Hence, the dissolution test developed was adequate for its purpose and could be employed for routine quality control of Sertraline Hydrochloride tablets.

ACKNOWLEDGEMENT

REFERENCES

1. Dressman J, Kramer J, (2005). Pharmaceutical dissolution Testing, Taylor & Francis Group, LLC.
2. British Pharmacopoeia, (2009). HMSO Publication: London; Vol.I and II, 5379.
3. Adams AIH; Bergold, AM. (2001). *Journal of Pharmaceutical and Biomedical Analysis*, 26, 505–508.
4. Chen D, Jiang S, Chen Y, Hu Y. (2004). *Journal of Pharmaceutical and Biomedical Analysis*, 34:1, 239-245.
5. Jain DS, Sanyal M, Subbaiah G, Pande UC, Shrivastav P.(2005). *Journal of Chromatography B*, 829:1-2, 69-74.
6. Kim K. M, Jung B. H, Woo J. S, Paeng K. J, Chung B. C.(2002). *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 769:2, 333-339.
7. Lories I, Bebawy LI, El-Kousy N, Suddik JK, Shokry M. (1999). *Journal of Pharmaceutical and Biomedical Analysis*, 21, 133–142.
8. Lucangioli SE, Hermida LG, Tripodi VP, Rodriguez VG, Lopez EE, Rouge PD, Carducci CN. (2000). *Journal of Chromatography A*, 871, 207–215.
9. Mandrioli R, Saracino MA, Ferrari S, Berardi D, Kenndler E, Raggi MA.(2006). *Journal of Chromatography B*, 2006, 836:1-2, 116-119.
10. Wiener HL.(1990). *Journal of Chromatography*, 527, 467-472.
11. Zhou MX, Foley JP. (2004). *Journal of Chromatography A*, 1053, 13–23.
12. Green JM. In; (1996). A Practical Guide to Analytical Method Validation, Anal.Chem. News & Features, May 1, 1996, 305A/309A.
13. Sivaiah Sangaraju, Rao BM, Shyam Kumar BM, Someswararao N.(1999) "Rasayan J. Chem.Vol 2 [1] 42-48
14. Rubel Ratnia, Virendra Yadav, Amit Kumar, (2015)."International Journal of Pharma Research and Health Sciences" Volume 3 (2), Page-616-620
15. B. Victoria Rani, Parthiban C, Sudhakar M. (2015). "Int J Pharm Pharm Sci.", Vol 7, Issue 2, 465-468
16. Mohamed I Walash, Fathallah F Belal, Nahed M El-Enany and Heba Elmansi,(2011). "Chemistry Central Journal", 5:61, 1-11
17. Sameena Kouser, Humera Reshma,(2014). "Der Pharmacia Sinica", 5(6):36-46
18. E. Anka Rao, "Pharmanest: (2010). An International Journal of Advances In Pharmaceutical Sciences", Vol.1 (2), 293-297
19. Zhang M, Gao F, Cui X, Zhang Y, Sun Y, Gu J.(2011) "J Chromatogr Sci.";49(2):89-93
20. ICH / CPMP Guidelines Q2B, (1996). Validation of Analytical Procedures Methodology.
21. ICH/CPMP guidelines Q2A, (1994). Text on Validation of Analytical Procedures.

22. The United State Pharmacopoeia (USP 27). (2004). National Publishing Philadelphia, Asian Ed. 2622-2624.
23. US FDA, (1987). General Principles of Validation, Rockville, MD, Center for Drug Evaluation and Research (CDER).
24. US FDA, (1987). Guidelines for Submitting Samples and Analytical Data for Method Validation,Rockville, MD, Center for Drugs and Biologics Department of Health and Human Services.
25. Rowe RC, Sheskey PJ, Quinn ME, (2009). Handbook of Pharmaceutical Excipients, 6th Ed, PhP pharmaceutical press USA.