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BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SERTRALINE HCL IN RAT PLASMA BY HPLC

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ABSTRACT

Objective: An accurate, simple, reproducible and sensitive bioanalytical method for the determination of sertraline HCL in pharmaceutical dosage form was developed and validated using a reversed-phase C18 column (250 mm X 4.6 mm i. d, 5 µm particle size) with isocratic elution. **Methods:** A mixture of Methanol: Water (90:10 v/v), was used as a mobile phase at the flow rate of 1.5 ml/min and detector wavelength at 273 nm. The retention time of Sertraline was found to be 6.3 minutes (min). The method was statistically validated for the linearity, accuracy, precision and robustness. **Results:** The linearity of Sertraline Hydrochloride was in the range of 20 to 400µg/ml. This method showed an excellent linear response with the correlation coefficient (R^2) value of 0.994 for the Sertraline HCl. The recovery of the drug was ranged from 99.75 to 99.81 %. An intra-day and inter- day precision study of the new method was less than the maximum allowable limit (% RSD<2.0). **Conclusion:** The proposed method was cost effective, which can be used for the estimation of Setraline in bulk and in solid dosage forms.

KEYWORDS: Sertraline, HPLC, Isocratic, Retention time, Validation.

INTRODUCTION

Sertraline (SER) is one of the most accepted Antidepressant and is used in the treatment in panic attack, depression, anxiety. SER exists in varied dosage forms like tablets, capsules, with molecular formula of $C_{17}H_{18}Cl_3N$ and molecular weight of 342.688 g/mol. Its chemical structure is given in fig. 1.

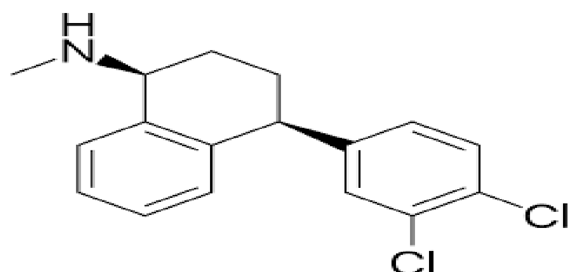


Fig. 1: Chemical structure of Sertraline.

The proper mechanism of action sertraline is not fully known, but the drug appears to selectively inhibit the reuptake of serotonin at the presynaptic membrane. This results in an increased synaptic concentration of serotonin in the CNS, which leads to numerous functional changes associated with enhanced serotonergic neurotransmission. It is suggested that these modifications are responsible for the antidepressant action observed during long term administration of antidepressants.

The purpose of the current effort is to develop and validate a new analytical method for the determination of SER in tablet dosage form. In this proposed method, we have put an effort to develop a simple, accurate, precise, rapid, and robust High Performance Liquid Chromatography (HPLC) method with enough data of validation parameters.

MATERIALS AND METHODS

All the chemicals and reagents were used in this method were analytical reagent grade and Like Methanol and Water were obtained from Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune. SER API was obtained from Wokhart Aurangabad. The HPLC Agilent1120 Compact LC was equipped with UV detector, gradient pump and Rheodyne injector with 20µl and the analyte was monitored at 273 nm. Chromatographic analysis was performed on a C18 column having 250 cm X4.6 mm i. d and 5 µm particle size. The mobile phase was filtered through 0.45µm membrane filter and degassed. The determination of SER was performed at 25°c temperature.

1. Reagents and chemicals used

a. Acetronitrile (HPLC Grade)
b. Methanol (HPLCGrade)
c. HPLC Grade water

2. Preparation of mobile phase

The mobile phase Methanol: Water (90:10 v/v) was prepared and filtered through 0.45 µm membrane filter and sonicated on ultrasonic bath for 15 min.

3. Preparation of standard stock solution

Sertraline hydrochloride standard stock solution was prepared by transferring 100mg of Sertraline hydrochloride into, approximately 10ml HPLC grade Methanol was added and sonicated for 20 min. The volume was made up to 20 ml with HPLC grade methanol to get the concentration of 5000µg/ml. This solution was filtered through a 0.45µm pore size Nylon 66 membrane filter. These subsequent dilutions were prepared by diluting stock solution with the methanol.

4. Collection of blood and Separation of Plasma from Rat blood

- Wistor rats (n=9) of either sex (220-250 gm) were obtained from Aagharkar research institute, Pune. The rats were housed at temperature of 25± 10 C and relative humidity at 45-55 % under 12:12 light-dark cycle. The animals had free access to feed pellets and water ad libitum. The experimental protocol was approved (Approval no. DYPCOP/IACE/2018/04) and performed in accordance the guidelines of CPCSEA, Government of India an animal experimentation.
- The blood (1.5ml) from each rat, under mild ether anesthesia was removed by retro orbital method of blood collection. Blood was collected into purple top EDTA tubes and centrifuged (3000 rpm) at 4°C for 20 minutes. After centrifugation using clean pipette technique place 1.0 ml of plasma was placed into 1.5ml Eppendorf tube labeled with tracking number and "plasma".

5. Preparation of sample solution

Sample solution was prepared by taking 0.9ml of rat plasma and the aliquots of standard stock solution like 0.4ml, 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml were transfer to a series of 10 ml of volumetric flask to get concentration 200,500,1000,1500,2000,2500,3000,3500 and 4000µg/ml respectively and 1 ml of precipitating agent (Acetonitrile) to precipitate plasma protein, were added and mixed. The resulting solution was centrifuged at 3000 rpm for 10 min. at $2\text{-}4^{0}$C. The supernatant layer was separated and analyzed. The sample solution was injected with the above chromatographic condition and the chromatograms were recorded. The retention time 6.30 min, was recorded respectively. The response factor (peak area ratio of standard peak area and sample peak area were calculated and the concentration of the Sertraline Hydrochloride present in the plasma samples was calculated.

Table 1: Spiking Sertraline Hydrochloride in plasma.

Concentration (µg/ml)	Vol. of Spiking (ml)	Vol. of plasma (ml)	Final vol. (ml)	Final conc. (µg/ml)
200	0.1	0.9	1	20
500	0.1	0.9	1	50
1000	0.1	0.9	1	100
1500	0.1	0.9	1	150
2000	0.1	0.9	1	200
2500	0.1	0.9	1	250
3000	0.1	0.9	1	300
3500	0.1	0.9	1	350
4000	0.1	0.9	1	400

METHOD VALIDATION

All the method validation parameters such as accuracy, linearity, precision, detection limit, quantification limit and robustness were validated as per the International Conference on Harmonization (ICH) guidelines.

System suitability parameters

To evaluate system suitability parameters such as theoretical plates, tailing factor and retention time of six replicate injections of standard SER of concentration 20µg/ml was used and the % RSD values were calculated.

Linearity and range

The linearity was analyzed through the standard curves ranging from 10µg/ml to 400µg/ml. The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis.

Accuracy

The truthfulness of an analytical method expresses the closeness between the expected value and the value found. In the present study, consecutive analysis (n=3) for three different concentration of the standard mixture (80, 100 and 120% of the nominal concentration) was carried out to verify the accuracy of the proposed method.

Precision

Precision of the method was determined by repeatability

(intra-day precision) and intermediate precision (inter-day precision) of both standard and sample solutions. Precision was determined in six replicates of both standard and sample solutions. The results were expressed as % RSD of the measurements.

1. Recovery

The recovery of an analyte assay is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the pure authentic standard. Recovery pertains to be extraction efficiency of an analytical method within the limit or variability. Recovery of the analyte need not be 100%, but the extent of recovery of an analyte and of the internal standard should be consistent, precise and reproducible. Recovery experiment should be performed by comparing the analytical results for extracted samples at three concentrations (low, medium, high) with unextracted standards that represent 100% recovery.[19,2]

2. Stability

- **Freeze and Thaw stability**

The analyte stability was determined after three freeze and thaw cycles. Three aliquots at each of the low and high concentrations was stored at the intended storage temperature for 24 hours and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 12-24 hours under the same conditions. The freeze–thaw cycle was repeated two more times, and then analyzed on the third cycle.

- **Short term temperature stability**

The short-term stability was performed by three aliquots of each of the low and high concentrations were tested at room temperature and kept at this temperature from 8 hours and analyzed.

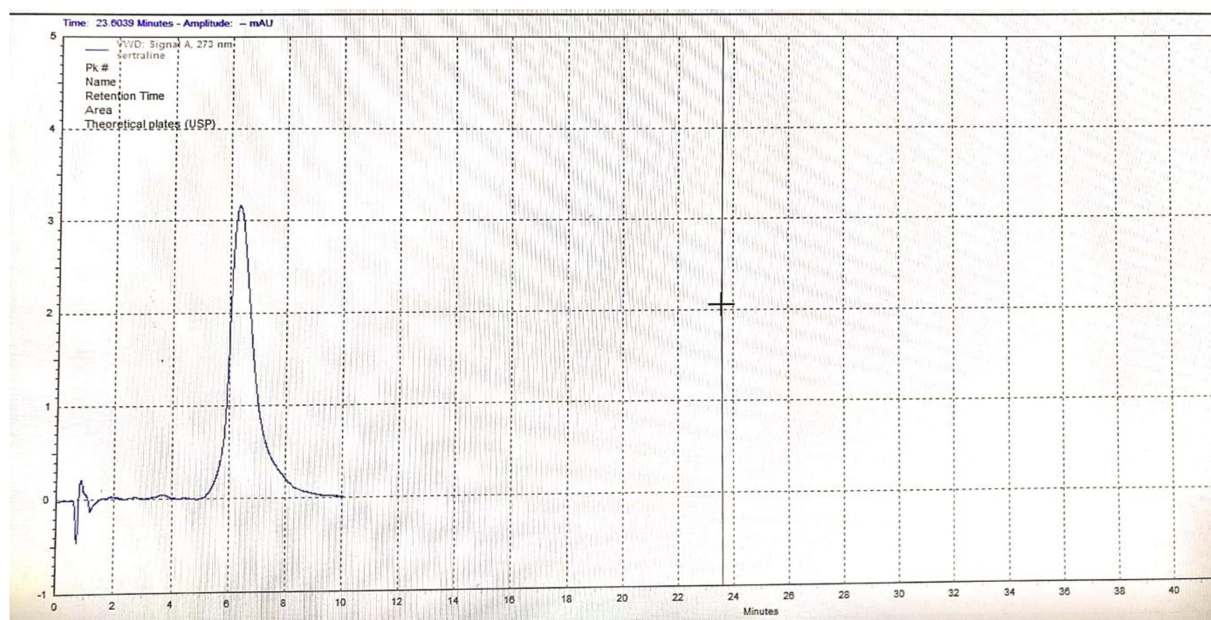
- **Long term stability**

Long-term stability should be determined by storing at least three aliquots of each of the low and high concentrations under the same conditions for 15days.

RESULTS AND DISCUSSION

The main objective of the chromatographic method was to develop a precise, specific bioanalytical HPLC method for the estimation of SER. In order to develop a suitable isocratic HPLC method, organic solvent concentration and column chemistry were applied to achieve the isocratic elution of Sertraline. The mobile phase Methanol: Water (90:10 v/v) with the flow rate of 1.5 mL/min and detector wavelength at 273nm was found to be satisfactory.

The retention time of SER was 6.3 min. Our proposed method has good symmetrical peak shape, theoretical plates and tailing factor as compared to reported studies. The mobile phase used in the present method is simple, precise, accurate, as compared to other studies. This may decrease cost of analysis, which may be economical to quality control labs. The typical chromatogram of the SER is shown in fig. 2.



METHOD VALIDATION

1. LINEARITY

The linearity of the method was demonstrated over the concentration range of 20-400 (µg /ml).

Table 2: Spiking of Sertraline Hydrochloride in plasma.

Concentration (µg/ml)	Vol. of Spiking (µl)	Vol. of plasma (ml)	Final vol. (ml)	Final conc. (µg/ml)
200	100	0.9	1	20
500	100	0.9	1	50
1000	100	0.9	1	100
1500	100	0.9	1	150
2000	100	0.9	1	200
2500	100	0.9	1	250
3000	100	0.9	1	300
3500	100	0.9	1	350
4000	100	0.9	1	400

Table 3: Standard calibration data of Sertraline Hydrochloride Spiked in Plasma.

Sr. No	Concentration (µg/ml)	Peak Area* (mAU)	S.D.*
1	20	1912395	0.0215
2	50	3219689	0.0325
3	100	4530881	0.0227
4	150	5349052	0.0743
5	200	6354603	0.0518
6	250	7263412	0.0725
7	300	8175030	0.0636
8	350	8981306	0.0827
9	400	9790126	0.0934

***n=3**

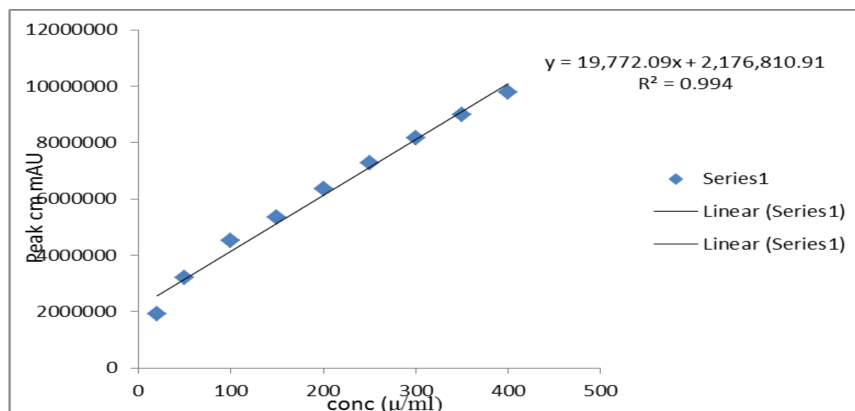


Fig 3: Calibration curve of Sertraline Hydrochloride Spiked in Plasma.

According to graph 7.15 the coefficient correlation was found to be 0.994

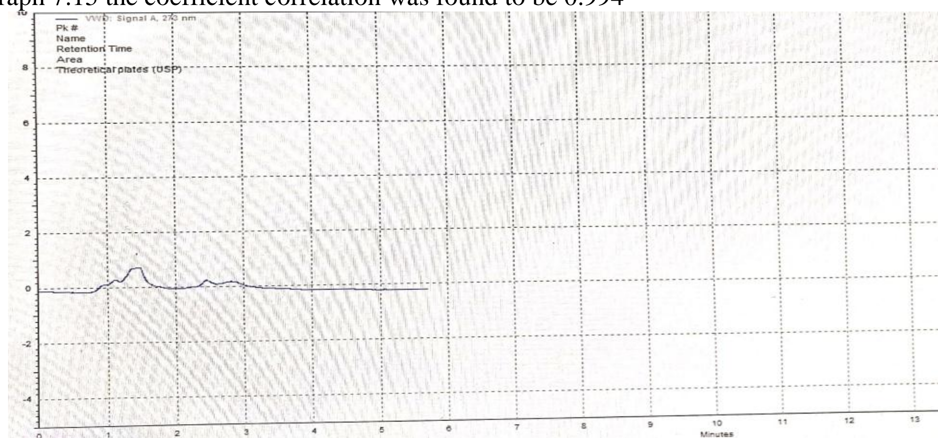


Fig No. 4: Chromatogram of Plasma.

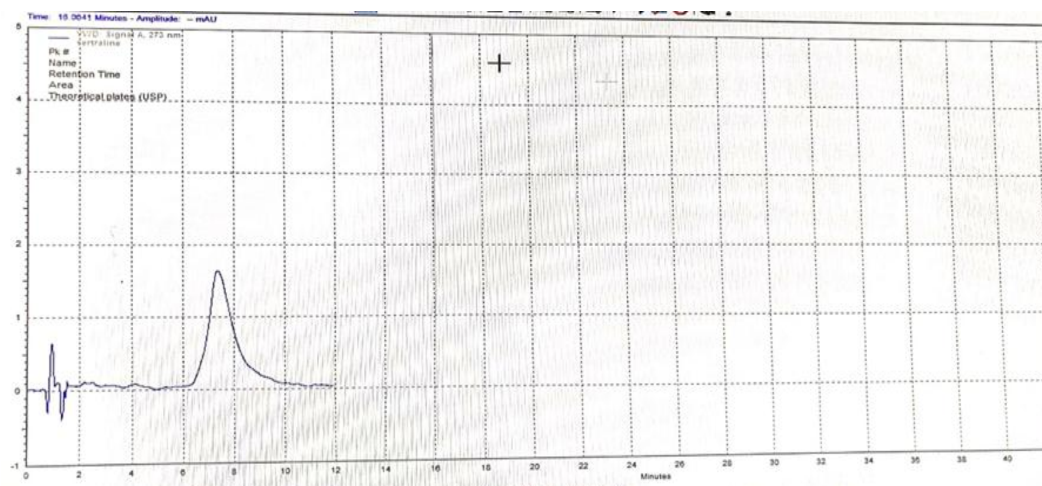


Fig No. 5: Chromatogram of LQC at 273nm.

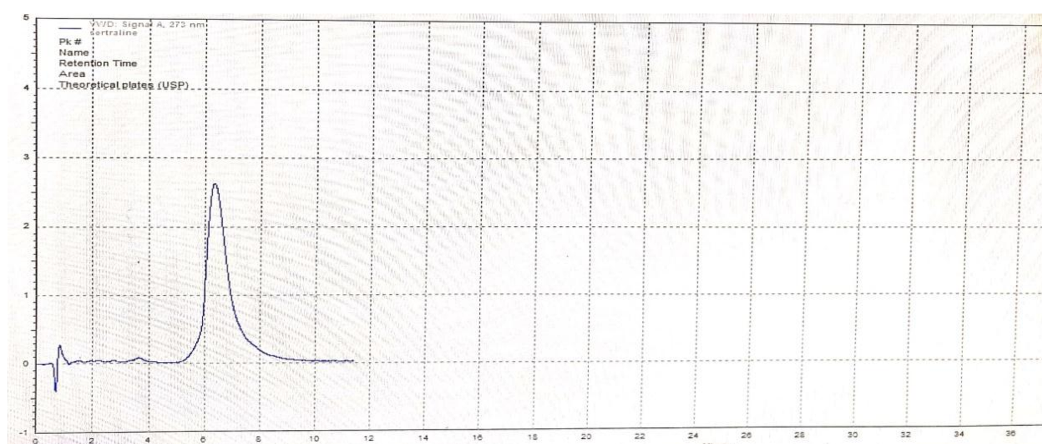


Fig No. 6: Chromatogram of MQC at 273nm.

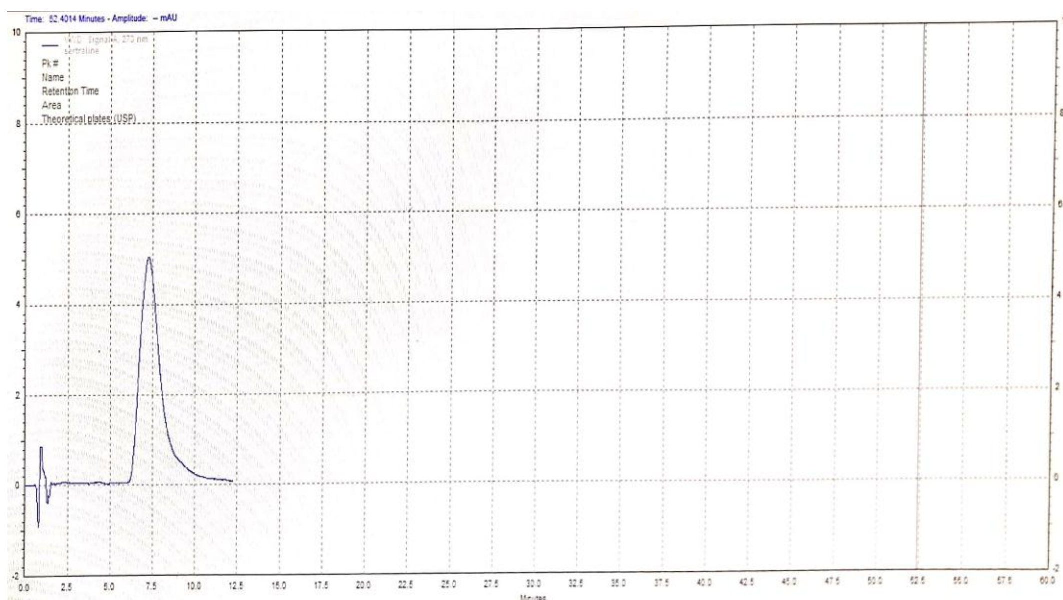


Fig no. 7: Chromatogram of HQC at 273nm.

2. ACCURACY AND PRECISION

The accuracy, precision, inter-day and intra- day precision was carried out by preparing three individuals samples of LQC, MQC and HQC and the percentage C.V. and % accuracy was calculated.

Table 4: Accuracy and Precision within batch.

Quality control Sample	Amt. Added (µg/ml)	Peak Area*	Amt found (µg/ml)	% Accuracy	% C. V
LQC	50	3219521	49.46	97.3	0.2580
	50	3118964	49.56	97.8	
	50	3219023	49.50	97.5	
MQC	100	4442846	99.90	99.90	0.5701
	100	4543102	99.86	98.84	
	100	4542961	99.95	99.72	
HQC	400	9789906	399.98	99.99	0.1647
	400	9789841	399.90	99.97	
	400	9789574	399.95	99.98	

***Average of three determination**

Table 5: Inter batch Accuracy and Precision.

Quality control sample	Amt. Added (µg/ml)	Peak Area*	Amt. found (µg/ml)	% Accuracy	% C. V
LQC	50	3217521	49.69	97.70	0.3469
	50	3218964	49.72	98.20	
	50	3219023	49.40	98.35	
MQC	100	4442846	89.95	98.53	0.1469
	100	4543102	89.83	98.68	
	100	4444961	89.95	98.82	
HQC	400	9788908	499.99	99.10	0.3799
	400	9789841	498.95	98.74	
	400	9789571	498.99	98.35	

***Average of three determinations** the coefficient of variation (CV) of each concentration of LLQC, LQC, MQC and HQC was found to be less than 15% as shown in table no.7.16 and 7.17. Therefore, the proposed method was found to be precise as per ICH guidelines.

3. RECOVERY

The recovery of an analyte in an assay is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the pure authentic standard. The percentage recovery of the drug was found to be 86.30 %.

Table 6: Recovery study.

Conc. (µg/ml)	Peak Area* (Extracted)	Peak Area* (Un-extracted)	% Recovery
50	3247201	3305132	88.96
100	4485214	4674158	86.92
400	9784990	9889423	82.50

***Average of three determinations**

According to table no. 7.18 the recovery study was found to less than 100% and the recovery of analyte was found to be consistent, precise and reproducible.

4. STABILITY

a. Freeze and thaw stability

Table 7: Freeze and Thaw stability.

Conc.	Peak Area	Conc. Found	% Purity*	S. D	% C. V
50 µg/ml	3253201	49.60	99.06	0.5132	0.5180
400 µg/ml	9747140	399.20	99.83	0.5686	0.5753

***Average of three determinations**

Coefficient of Variation for the above samples was found to be 0.5180 and 0.5753 for 50 µg/ml and 400µg/ml respectively. The CV was within the limit of ICH guidelines.

Short term stability

Table 8: Short term temperature stability.

Conc.(µg/ml)	Peak Area	Conc. Found(µg/ml)	% Purity	S. D	% C. V
50	3154712	49.70	99.43	0.2255	0.2267
400	9752631	399.40	99.76	0.7371	0.7539

***Average of three determinations**

Coefficient of Variation for the above samples was found to be 0.2267 and 0.7539 for 50µg/ml and 400 µg/ml respectively. The C.V was within the limit of ICH guidelines.

b. Long term stability

Table 9: Long term stability.

Conc.	Peak Area	Conc. Found(µg/ml)	% Purity	S. D	%C. V
50 µg/ml	3074168	48.50	98.20	0.6133	0.6245
400 µg/ml	9558746	398.70	98.56	0.7024	0.7199

***Average of three determinations**

Coefficient of Variation for the above samples was found to be 0.6245 and 0.7199 for 50µg/ml & 400µg/ml respectively. The CV was within limit of ICH guideline.

CONCLUSION

The proposed study describes HPLC method for the bioanalytcal identification and quantification of SER. The method was validated and found to be simple, sensitive, rapid, accurate and precise. The developed bioanalytical method was cost effective as compared to the reported methods. The high percentage of recovery shows that the method can be successfully used for routine analysis. Hence the present HPLC method is suitable for the quality control analysis of raw materials, formulation and stability studies

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