CHAPTER-5

SERTRALINE

5.1 A ***survey of literature*** for Sertraline indicated the estimation reported by the following methods, Viz., High-performance liquid chromatography-electrospray ionization mass spectrometry, High-performance liquid chromatography, Capillary isotachophoresis.Gas chromatography-mass spectrometry, UV Spectrophotometric method , Micellar electro kinetic chromatography, Gas chromatography-mass spectrometry. The brief information on above analytical methods are follows.

Rittner,et al [166] have reported screening method for seventy psychoactive drugs or drug metabolites in serum based on high-performance liquid chromatography-electro spray ionization mass spectrometry . Kobayashi K et al [166] have reported High-performance liquid chromatography determination of N- and O-demethylase activities of chemicals in human liver microsomes: application of postcolumn fluorescence derivatization using Nash reagent. BuzinkaiovaT et al [167] have reported Determination of four selective serotonin reuptake inhibitors by capillary isotachophoresis. Maurer HH, et al [168] have reported screening procedure for detection of antidepressants of the selective serotonin reuptake inhibitor type and their metabolites in urine as part of a modified systematic toxicological analysis procedure using gas chromatography-mass spectrometry. Lucca A. et al [169] have reported simultaneous determination of human plasma levels of four selective serotonin reuptake inhibitors by HPLC. TI: Goeringer KE et al [170] have reported Post-mortem forensic toxicology of selective serotonin re-uptake inhibitors: a review of pharmacology and report of 168 cases. Dhake et al [171] have reported spctrophotometric determination of sertraline hydrochloride in pharmaceutical preparations

Lacassie E et al [172] have reported methods for the determination of seven selective serotonin reuptake inhibitors and three active metabolites in human serum using high-performance liquid chromatography and gas chromatography. Lucangioli,-SE et al [173] have reported analysis cis-trans isomers and enantiomers of sertraline by cyclodextrin-modified micellar electrokinetic chromatography. Eap CB. et al [174] have

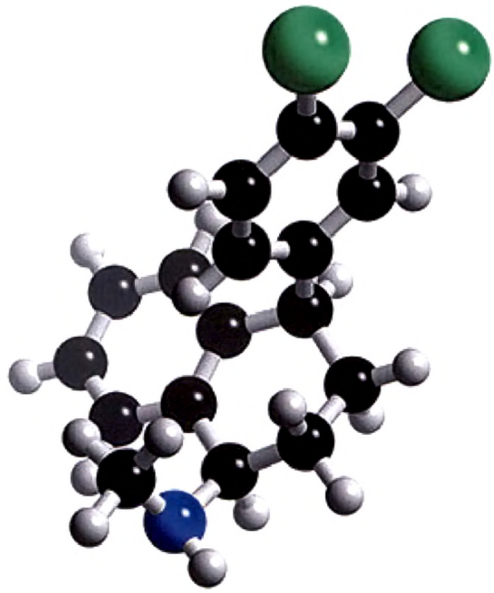
reported simultaneous determination of human plasma levels of citalopram, paroxetine, sertraline, and their metabolites by gas chromatography-mass spectrometry. Bebawy,-LI; et al [175] have reported spectrophotometric determination of fluoxetine and sertraline using chloranil, 2,3-dichloro-5,6-dicyanobenzoquinone and iodine.

Vatassery GT et al [176] have reported analysis of sertraline and desmethyl sertraline in human plasma and red blood cells. Eap CB et al [177] have reported analytical methods for the quantitative determination of selective serotonin re-uptake inhibitors for therapeutic drug monitoring purposes in patients. Patel J. et al [178] have reported HPLC of sertraline and norsertraline in plasma or serum.Rogowsky D et al [179] have reported determination of sertraline and desmethyl sertraline in human serum using copolymeric bonded-phase extraction, liquid chromatography and gas chromatography-mass spectrometry.Logan BK. et al [180] have reported analysis of sertraline (Zoloft) and its major metabolite in post-mortem specimens by gas and liquid chromatography.Wiener HL. et al [181] have reported separation and determination of sertraline and its metabolite, demethylsertraline, in mouse cerebral cortex by reversed-phase high-performance liquid chromatography.Tremaine LM et al [182] have reported automated gas-chromatographic - electron-capture assay for the selective serotonin uptake-blocker, sertraline.Fouda HG et al [183] have reported Gas-chromatographic - mass-spectrometric analysis and preliminary human pharmacokinetics of sertraline, a new antidepressant drug.

Even though there are several methods reported to estimate sertraline from plasma samples as well as matrices but no where sertraline separations and estimations from its related compound and process impurities are reported Hence we have under taken this problem for current work. Subsequently we have proposed to develop a simple and cost effective stability indicating assay method on HPLC, which will suit even small scale manufacturers keeping ICH guide lines and various regulatory requiremnets mind. Developed HPLC methods are validated thoroughly to check the suitability and corrective ness as per ICH guidelines.

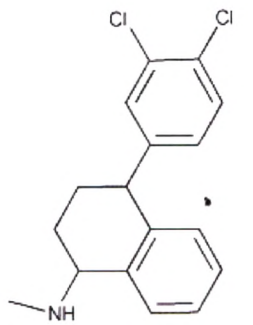
5.2 ***Method development for the analysis of related substances in Sertraline by HPLC***

Setraline 3 dimensional structure is given below.



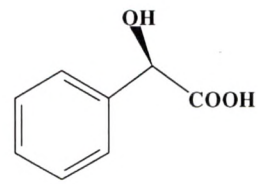
Sertraline 3 D structure STR-1

Sertraline and sertraline impurity structures are given below.

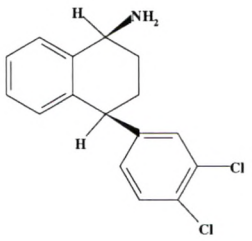


Chemical Formula: $C_{17}H_{17}Cl_2N$

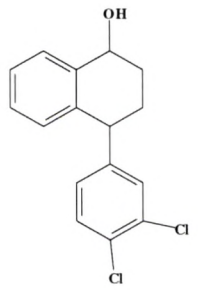
Sertraline structure STR-2



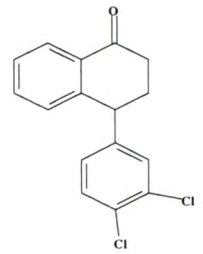
Mandelic acid STR-2A



Desmethyl Impurity STR-3

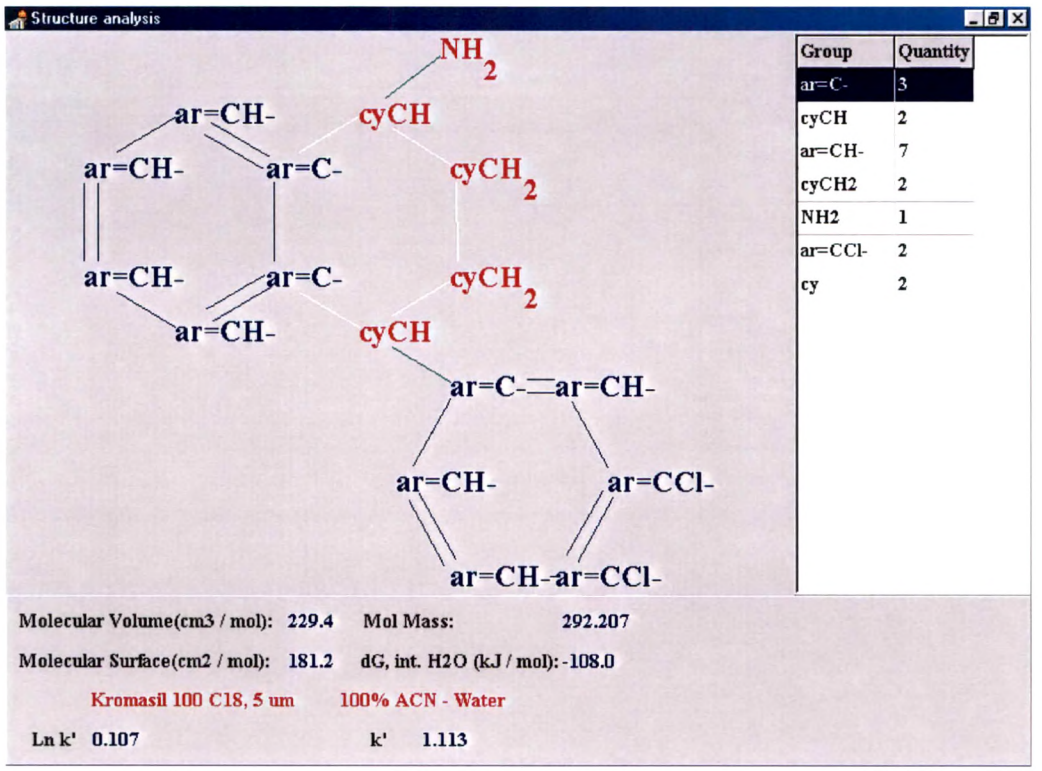


Impurity A STR-4

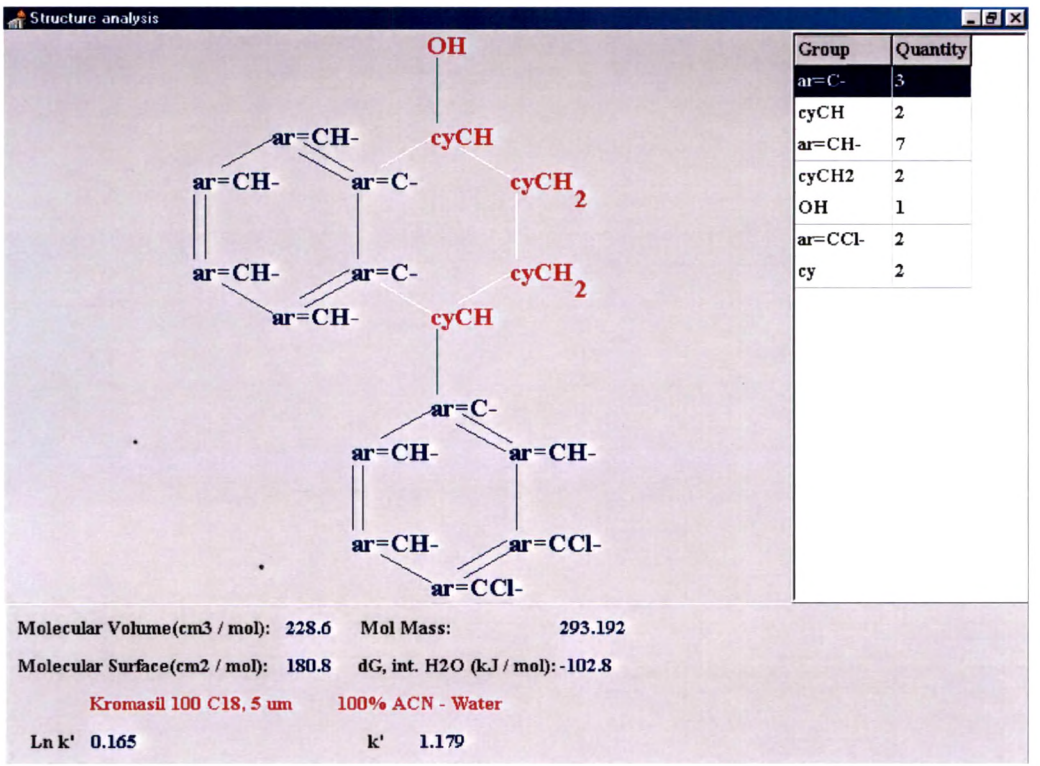


Sertraline stage-1 STR-5

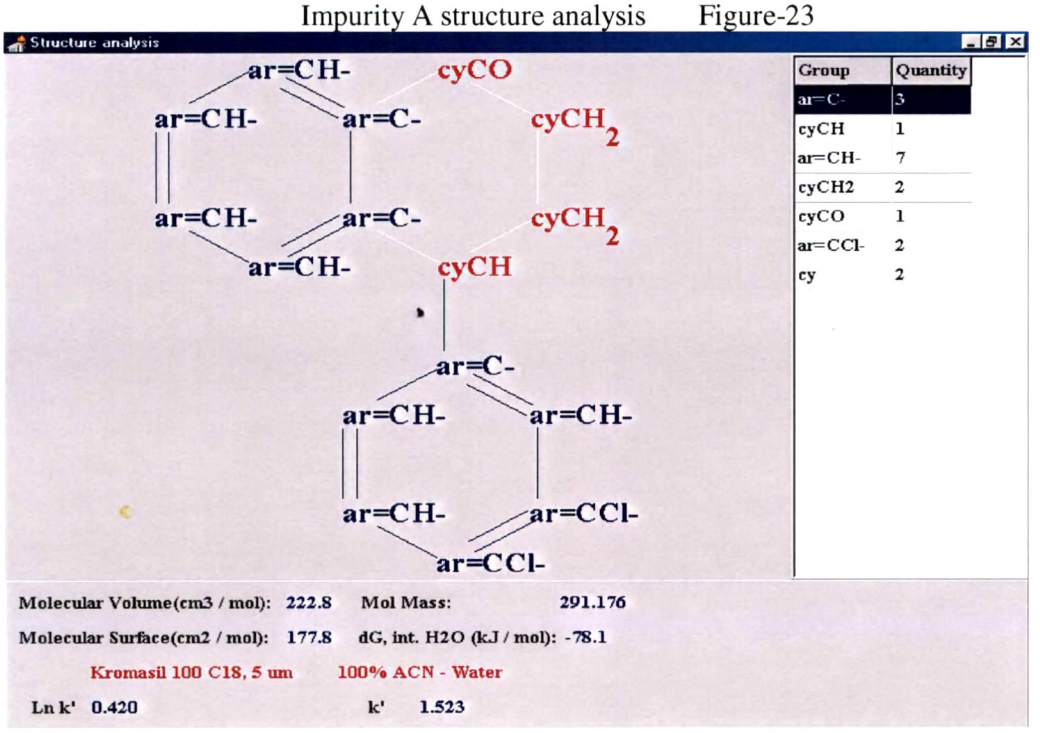
All structures are loaded into chromsword HPLC method development software to deduce structure analysis for method development. Figure-22 to 26 shows Sertraline and all impurities structure analysis charts .



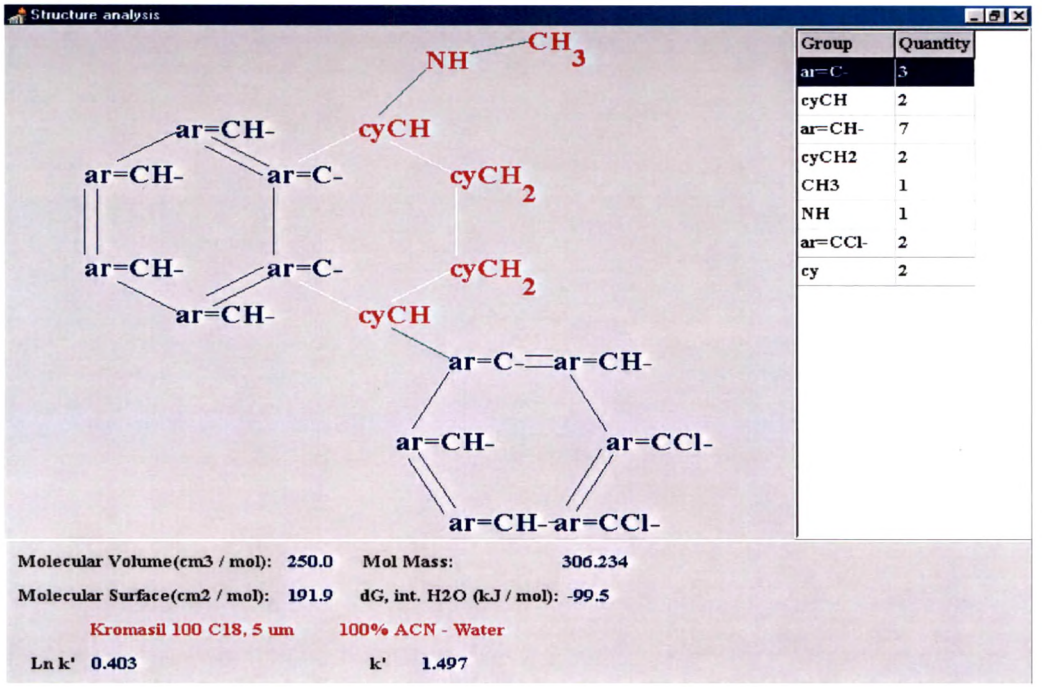
Desmethyl Impurity structure analysis Figure-22



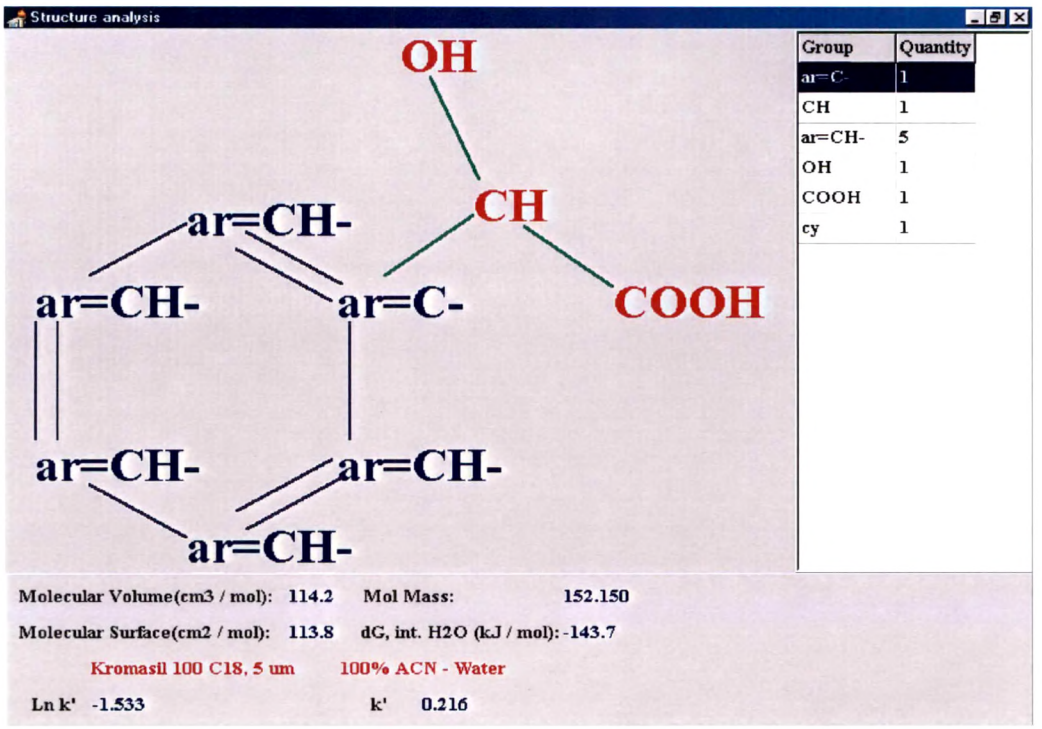
Impurity A structure analysis Figure-23



Sertraline stage-1 structure analysis Figure-24

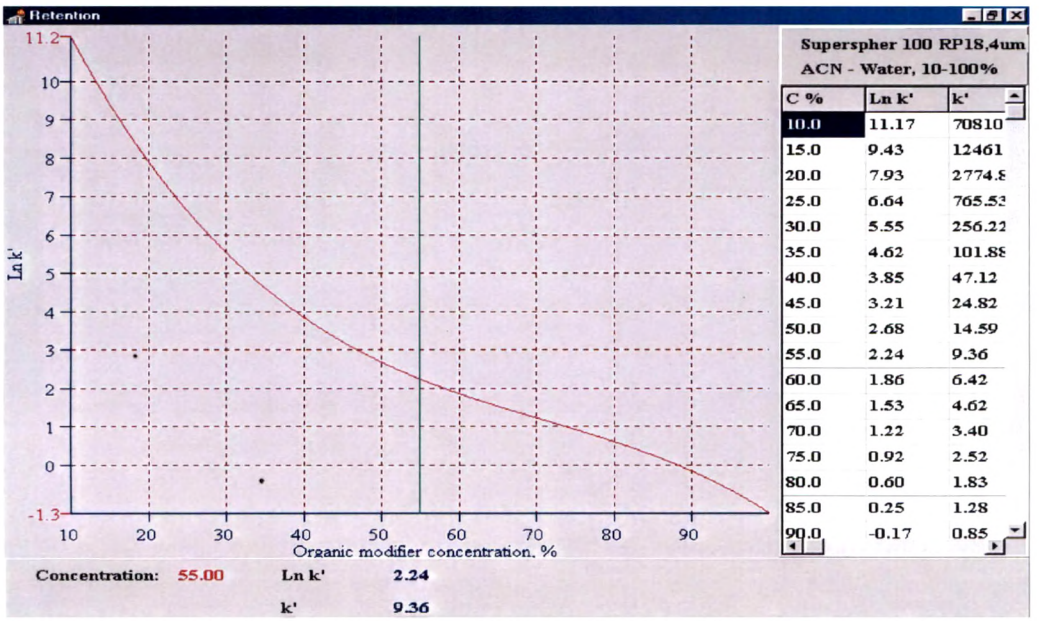


Sertraline structure analysis Figure-25

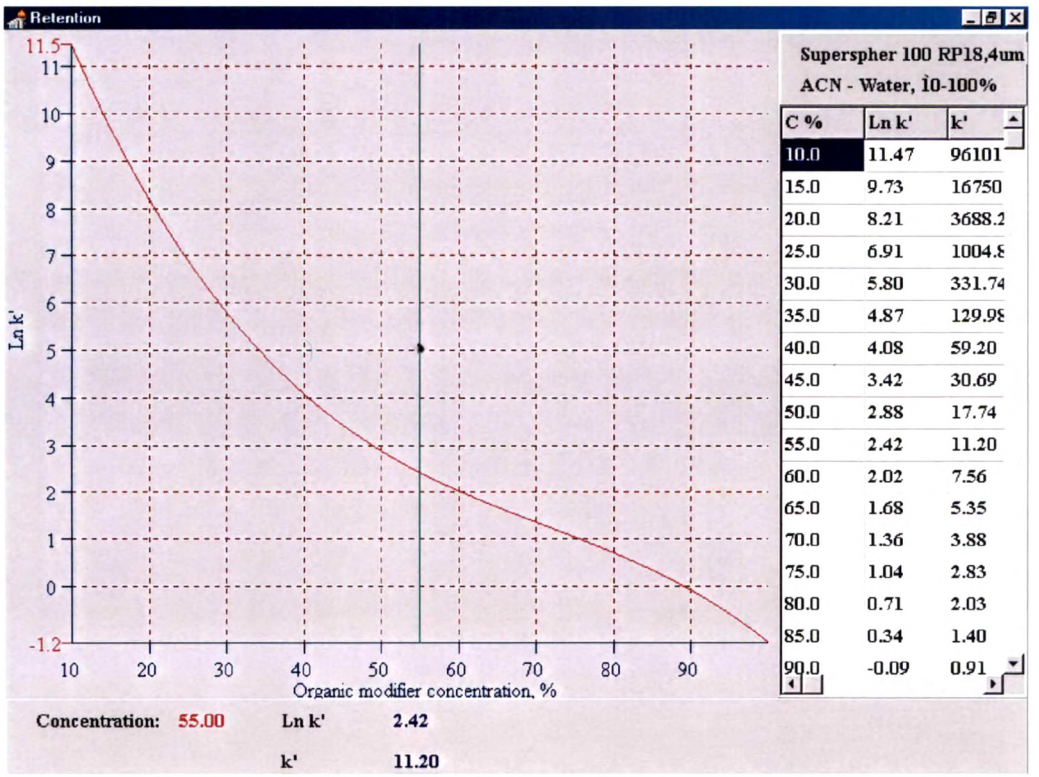


Mandelic acid structure analysis Figure-26

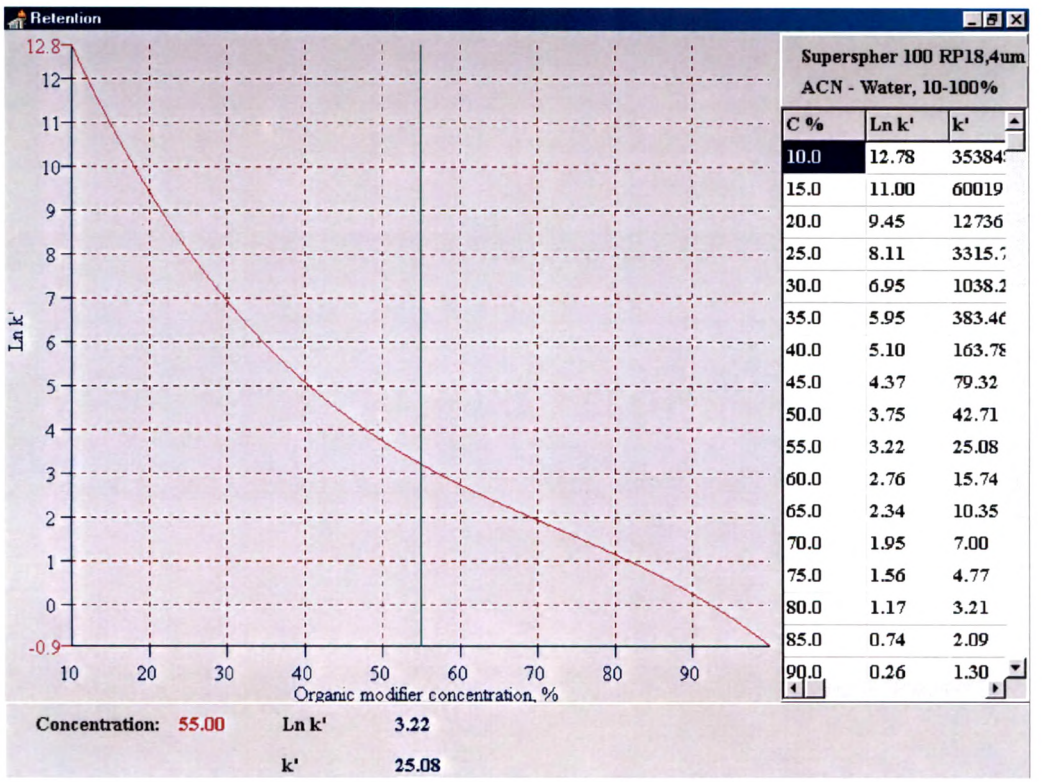
Figure-27 to 31 shows Sertraline and all impurities retention analysis charts .



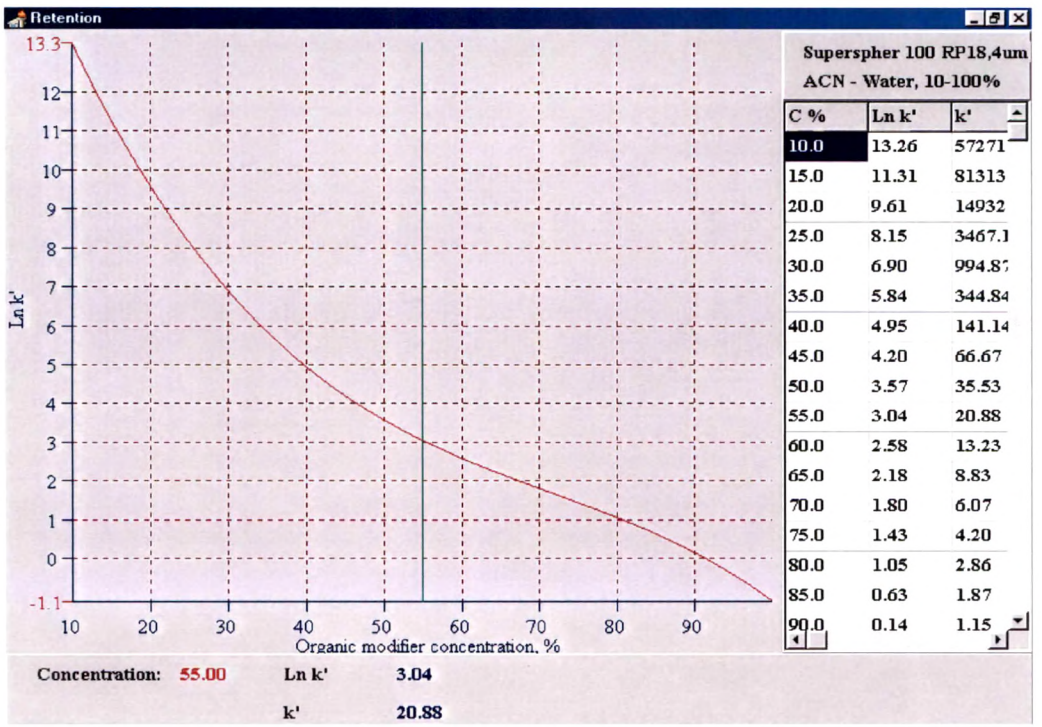
Desmethyl retention analysis Figure-27



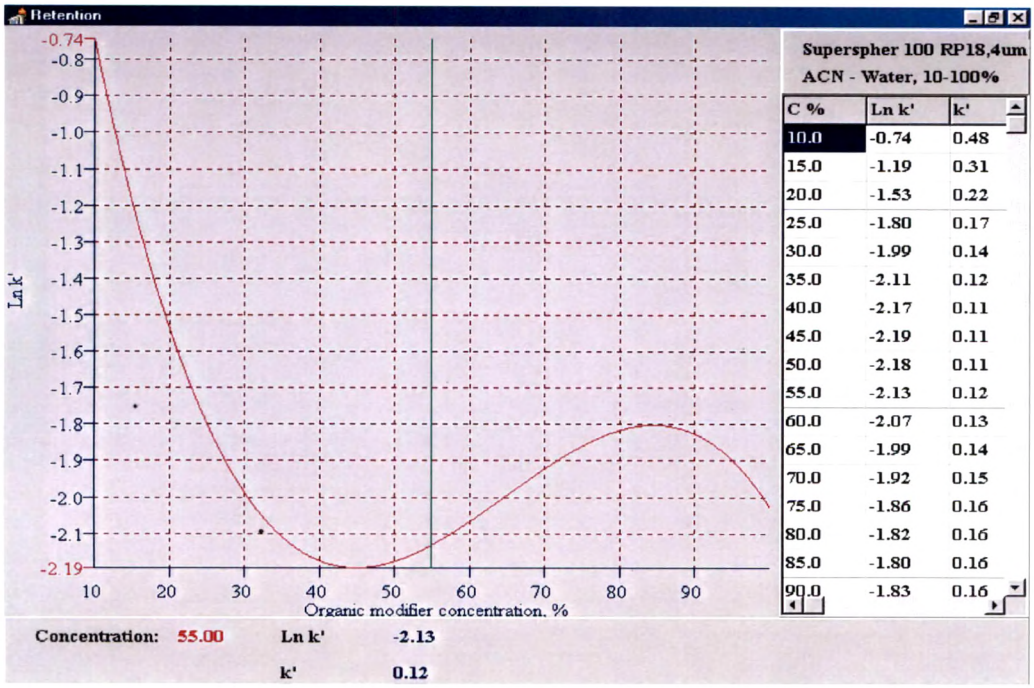
Impurity A retention analysis Figure-28



Sertraline stage-1 retention analysis Figure-29

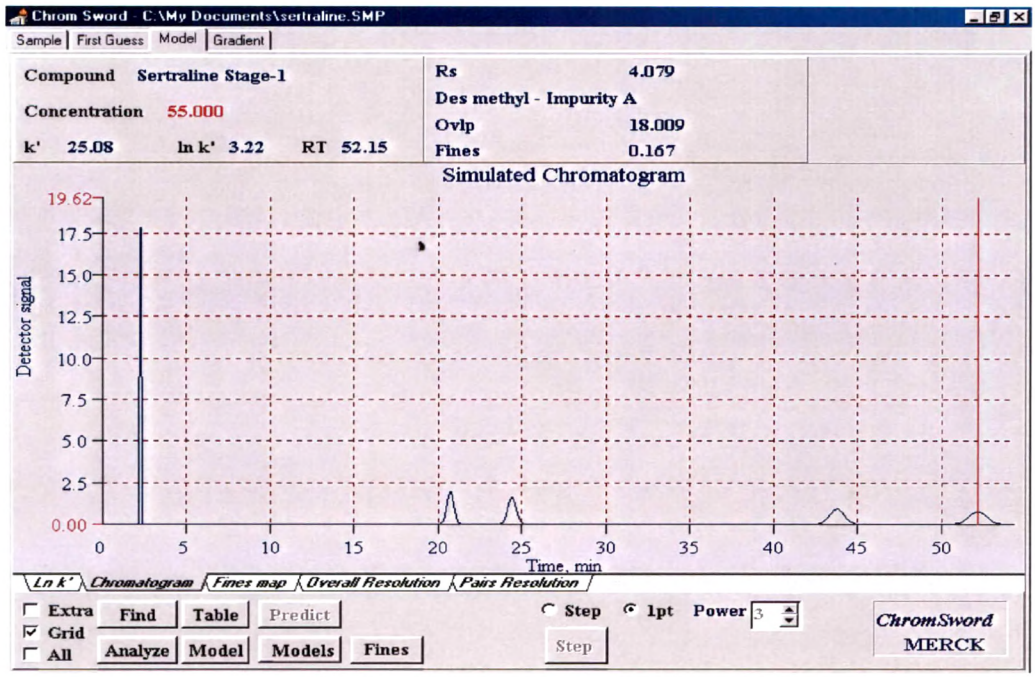


Sertraline retention analysis Figure-30

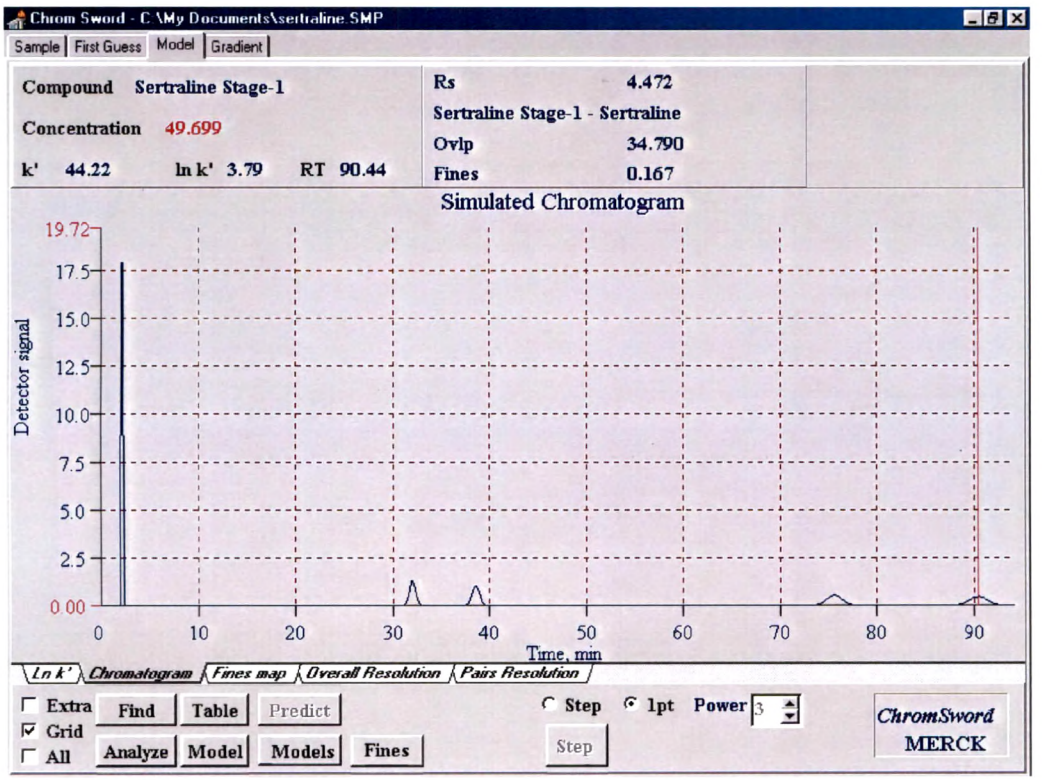


Mandelic acid retention analysis Figure-31

Figure-32 to 33 shows Sertraline and all impurities simulated chromatogram at two different concentrations of organic solvent.



Simulated chromatogram 1 Figure-32



Simulated chromatogram 2 Figure-33

Analysis: Software had predicted all components separation looking at molecular volumes and molecular surfaces as given in structure analysis charts. When organic concentration is about 50 %, two impurities are eluting about 76, 91 minutes suggesting to optimize a gradient analysis to resolve all impurities within a reasonable run time.. Ammonium dihydrogen orthophosphate buffer with pH to 3.0 ± 0.1 is selected as mobile phase A and acetonitrile as mobile phase B. 40 volumes of buffer was added to mobile phase B to reduce gradient noise and optimized method is given below.

5.2.1 *Analytical Method :*

Reagents and chemicals :

1) Ammonium dihydrogen orthophosphate (anhydrous) : AR grade
2) Acetonitrile : HPLC grade
3) Water : Milli Q Water
4) Orthophosphoric acid : AR grade

Buffer solution :Transfer 14 g of ammonium dihydrogen orthophosphate (anhydrous) into a 1000ml volumetric flask. Dissolve in and dilute up to the mark with water. Adjust the pH to 3.0 ± 0.1 with 10% v/v orthophosphoric acid.

Mobile phase :

Mobile phase A : Prepare sufficient quantity by mixing 40 volume of buffer, 25 volume of water and 35 volume of acetonitrile. Filter and degas prior to use.

Mobile phase B . Prepare sufficient quantity by mixing 40 volume of buffer and 60 volume of acetonitrile Filter and degas prior to use.

System suitability :Transfer about 3 mg, accurately weighed, -sertraline WRS and Impurity - B into a 100 ml volumetric flask Dissolve in and dilute up to mark with mobile phase A (30 µg/ml).

Sample preparation :Transfer about 50mg accurately weighed -sertraline sample in to a 50 ml volumetric flask. Dissolve in and dilute up to mark with mobile phase (1000 µg / ml).

Standard solution :Transfer about 10mg accurately weighed each of impurity A, Impurity B, Mandelic acid; sertraline/1 and -sertraline WRS into a 100ml volumetric flask. Dissolve in and dilute up to mark with mobile phase A. Pipette out 3 0 ml of this solution into a 100 ml volumetric flask and make up the volume with mobile phase A (3µg / ml of each)

Chromatographic system :Use a suitable high pressure liquid chromatography system equipped with a UV detector set to 235 nm and a column of 250 mm x 4 6mm containing 5µ C8 packing material (suggested column - Hypersil C8 BDS).The system is also equipped to deliver the two phases in a programmed manner as shown in the table below.

Total flow rate : 1.5ml / minute

Time (in Mins.)	Mobile Phase A (per cent v/v)	Mobile Phase B (per cent v/v)
0	100	0
7	100	0
15	80	20
25	10	90
35	10	90
36	100	0
42	100	0

Procedure :Inject 20 µl system suitability solution in duplicate and calculate the resolution between -sertraline and Impurity-B should not be less than 3.0. Inject 20 µl of standard and test preparation. Calculate the impurity present in a sample.

Calculations :

1) Calculate the amount of impurity-A, Impurity-B, Mandelic acid and stage-I using formula :

$$\frac{C_R}{C_S} \times \frac{r_S}{r_R} \times 100$$

Where :

C_R : Concentration of impurity A, impurity B, Mandelic acid & stage-I individually in standard solution.

r_S : Detector response of impurity A, impurity B, Mandelic acid & stage-I individually in sample chromatogram.

C_S : Concentration of sample preparation

r_R : Detector response of impurity A, impurity B, Mandelic acid & stage-I individually in standard solution chromatogram.

2) Calculate the amount of any other impurity using the formula :

$$\frac{C_c}{C_S} \times \frac{r_i}{r_c} \times 100$$

Where

C_c : Concentration of Sertraline in standard solution.

r_i : Detector response of any other impurity individually in sample chromatogram.

C_S : Concentration of sample preparation

r_c : Detector response of -sertraline in standard solution chromatogram.

Limits

1 Impurity A	(NMT 0 3%)
2 Impurity B	: (NMT 0.3%)
3.Mandelic acid	(NMT 0 3%)
4.Sertraline/I	: (NMT 0 3%)
5 Any other impurity, individually.	(NMT 0.1%)
6.Total impurities	: (NMT 1 5%)

5.3 Experimental Protocol

Purpose :

The purpose of this experiment is to establish the precision, accuracy, linearity of detector response and ruggedness of the analytical method through number of scientific studies and discussions of the data

Scope:

This method, upon validation, can be used for the analysis of related substances in Sertraline.

Analytical Method : As per method described in method development section 5.2.1

5.3.1 The experiments are designed to study

a.System suitability

b.Identification of individual component

c.Instrument precision

d.Method precision

e.Linearity and range

f.Accuracy

g.Minimum detection limit

h.Minimum quantitation limit

i.Ruggedness of the method

5.3.2 Stock Solutions :

Solution 1 :Transfer about 37.5mg, accurately weighed, impurity-A into a 25ml volumetric flask. Dissolve in & dilute up to mark with mobile phase A (1500 µg/ml).

Solution 2 :Transfer about 37.5mg, accurately weighed, impurity-B into a 25ml volumetric flask. Dissolve in and dilute up to mark with mobile phase A (1500µg/ml).

Solution 3 Transfer about 37.5mg, accurately weighed, sertraline/1 into a 25ml volumetric flask. Dissolve in and dilute up to mark with mobile phase A (1500µg/ml).

Solution 4 :Transfer about 37.5mg, accurately weighed, Mandelic acid into a 25ml volumetric flask. Dissolve in and dilute up to mark with mobile phase A (1500µg/ml).

Solution 5 :Transfer about 37 5mg, accurately weighed, -sertraline into a 25ml volumetric flask. Dissolve in and dilute up to mark with mobile phase A (1500µg/ml).

Solution 6 :Pipette out 5.0 ml each of solution 1, 2, 3, 4 in to 100ml volumetric flask. Dilute up to the mark with mobile phase A (75 µg/ml each of impurity A, impurity B, sertraline/1 and mandelic acid).

Solution 7:Pipette out 5.0 ml of *Solution 5* into a 100 ml volumetric flask and dilute to mark with mobile phase A (75 µg / ml).

Standard Solution : Pipette out 2.0 ml of *Solution 6* and *Solution 7* into a 50 ml volumetric flask and dilute to mark with mobile phase A (3 µg / ml of each).

5.3.3 System suitability :

System Suitability solution :

Pipette out 1ml each of solution 2 and solution 5 into a 50ml volumetric flask. Mix and dilute to volume with mobile phase A (30µg/ml each of impurity of B and -sertraline)

i) Set the chromatographic system as mentioned in the analytical method.

ii) Inject 20 µL of the system suitability solution in duplicate and record the chromatograms upto 42 mın.

iii) Calculate the resolution between the Impurity (B) and -sertraline peaks.

Acceptance limits :

Resolution factor *R* is not less than 3.0

5.3.4 Identification

i) Pipette out 1 0 ml of *Solutıon 1* ınto a 10 ml volumetric flask, dılute to volume wıth mobile phase A (about 150 µg/ml of Impurity-A)

ii) Pipette out 1.0 ml of *Solutıon 2* into a 10 ml volumetric flask, dilute to volume wıth mobile phase A (about 150 µg/ml of Impurity - B).

iii) Pipette out 1.0ml of *Solutıon 3* into a 10 ml volumetric flask, dılute to volume wıth mobıle phase A(about 150 µg/ml of sertraline/1).

iv) Pıpette out 1.0 ml of *Solutıon 4* into a 10 ml volumetric flask, dilute to volume with mobile phase A (about 150 µg/ml of mandelic acid).

v) Pıpette out 1 0 ml of *Solutıon 5* into a 10 ml volumetric flask, dılute to volume with mobıle phase A (about 150 µg/ml of -sertraline).

Inject 20 µL each of the above diluted solutions (about 150µg/ml) individually and record the chromatograms upto 42 min. Note the retention time of each for ıdentıfication.

5.3.5 Instrument precision

System precision solutıon .

Transfer about 50 mg, accurately weighed, -sertraline standard ınto a 50ml volumetric flask Pıpette out 2.0ml *Solutıon 6* into it. Dissolve by addıng 20ml mobıle phase A and dılute to volume wıth mobile phase A. (3 µg/ml each of Impurity-A, Impurity-B, Mandalic acıd and sertralıne/I corresponding to 0.3% of the -sertraline concentration of 1000 µg/ml)

i) Set up the system as mentioned under the chromatographic conditions.

ii) Inject 20 µL of the system precision solution six times and record the chromatograms up to 42 min.

iii) Calculate the relative standard deviation of the detector response for each component.

Acceptance limit :

RSD of detector response for each component is ≤ 5.0 %

5.3.6 Method precision

i) Prepare a sample solution as directed under the procedure (about 1000µg/ml).

ii) Set the chromatographic conditions as mentioned under the method, inject 20 µL of the system suability solution in duplicate and record the chromatograms upto 42 min.

iii) Inject 20 µL of *Standard Solution* (prepared as mentioned in section 2.2) in duplicate and record the chromatograms upto 42 mın. Use this for calculatıons.

iv) Inject 20 µL of the sample solution in duplicate and record the chromatograms upto 42 min.

v) Calculate the amounts of the Impurity present in the sample.

vi) Prepare six sets of thıs sample as directed under the method. Spike impurities upto the target levels in each of the sample preparation.

vii) Inject 20 µL of each sample preparation in duplicate in to the chromatograph set to the condition mentioned under the method and record the chromatograms upto 42 min

viii) Calculate the % of impurity and its RSD for each.

Acceptance limit :

RSD of the calculated impurities in the six sets ≤ 5.0 %.

5.3.7 Linearity and range :

i) Use *solutıon-6* & *solutıon-7* (mentioned under section 4.2.2) for preparing following solution.

ii) Linearity solutıons.

Table 5.3.7.1

Sr. No.	Level % of target	Vol. of. *Solution 6*	Vol. Of *Solution 7*	Final Dilution	Final Concentrations				
		ml	*ml*	*ml*	*Impurity-A µg/ml*	*sertraline/I µg/ml*	*mandelic acid µg/ml*	*Impurity-B µg/ml*	*sertraline µg/ml*
L1	50	1 0	1.0	50	1 500	1.500	1 500	1.500	1.500
L2	75	1 5	1.5	50	2.250	2.250	2.250	2.250	2.250
L3	100	2.0	2.0	50	3.000	3.000	3 000	3.000	3.000
L4	125	2 5	2.5	50	3 750	3.750	3.750	3.750	3.750
L5	150	3.0	3.0	50	4.500	4.500	4.500	4 500	4.500

iii) Inject 20 µL each of the linearity solution in triplicate into the chromatographic system set to the conditions mentioned under the method and record the chromatograms upto 42 min.

iv) Calculate the mean and RSD of the detector responses for each linearity level individually for each component.

v) Plot a graph of the concentration versus mean area count and perform mathematical regression for each component individually

Acceptance limits :

RSD of area counts for individual component at each level is ≤ 5.0%

Plot of concentration versus detector response for each component is linear The regression correlation coefficient (r^2) ≥ 0.99

5.3.8 ***Accuracy :***

Use *Solution 6* [75µg/ml each of Impurity] as mentioned in section 4.2.2 for preparing the following solutions.

i) Prepare five sets for five level (70, 85, 100, 115 and 130% of target concentration) recovery study by transferring about 50 mg, accurately weighed, -sertraline standard into five 50 ml volumetric flasks. Pipette out appropriate volumes of *Solution 6* as shown in the table below and dilute to volume with mobile phase A

Table 5.3.8.1

Sr. No.	Level % of target	Vol. of *Solution 6*	Final Dilution	Final Concentrations		Final Concentrations	
		ml	*ml*	*Impurity-A µg/ml*	*mandelic acid µg/ml*	*Stage-1 µg/ml*	*Impurity B µg/ml*
R1	70	1.4	50	2.10	2.10	2.10	2.10
R2	85	1.7	50	2 55	2 55	2.55	2.55
R3	100	2 0	50	3.00	3.00	3.00	3.00
R4	115	2.3	50	3 45	3 45	3.45	3.45
R5	130	2 6	50	3.90	3 90	3.90	3 90

ii) Inject 20µL of standard solution, prepared as mentioned above, in triplicate and record the chromatograms upto 42 min Calculate the mean area counts of the standard

iii) Inject 20 µL each of the recovery solution R1, R2, R3, R4 and R5 into the chromatograph in triplicate and record the chromatograms upto 42 min.

iv) Calculate the mean and RSD of the detector responses for individual impurity in each recovery set and standard solution.

v) Calculate the amount of each impurity for each set of the recovery sample and calculate the percentage of recovery.

Acceptance limit .

Percentage recovery is 95.0 to 105.0 %

RSD of detector response for each component is ≤ 5.0%

5.3.9 Minimum Quantitation level (MQL)and Minimum Detection level (MDL)

i) Prepare a MDL stock solution for this study by pipetting out 1 0 ml of *Solution 6* into a 50 ml volumetric flask. Dilute to volume with mobile phase A [1.5 µg/ml each of Impurity] - MDL Stock solution

ii) Prepare subsequent diluted solutions as shown in the table below and inject them in triplicate and record the chromatograms upto 42 min.

iii) Calculate the RSD of the triplicate injections for each level.

Table 5.3.9.1

Level	Vol. of MDL stock soln (ml)	Final Dilution (ml)	Concentrations of components in µg/mL		Concentrations of components in µg/mL	
			impurity A	*mandelic acid*	*Stage-1*	*Impurity-B*
D1	---		1.500	1 500	1.500	1 500
D2	5	10	0 750	0.750	0 750	0.750
D3	2 5	10	0.375	0.375	0 375	0.375
D4	1	10	0 150	0 150	0 150	0.150
D5	0.5	10	0.075	0.075	0.075	0.075
D6	0.2	10	0.030	0 030	0.030	0.030
D7	0 1	10	0 015	0 015	0.015	0 015
D8	0.1	25	0 006	0.006	0 006	0.006
D9	0.1	50	0.003	0.003	0.003	0.003

Acceptance limit :

The MQL of each component is the lowest concentration at which the RSD of the triplicate injections ≤ 5.0 %

The MDL of each component is that concentration at which the detector shows a positive response.

5.3.10 ***Ruggedness of the method :*** A sample previously analysed for related substances is reanlaysed by another analyst independently by this method and the results are compared.

5.4 Validation - Experimental

5.4.1 Reagents and chemicals :

i. Ammonium dihydrogen orthophosphate (anhydrous) : AR grade (S d Fine Chemical)

ii. Acetonitrile : HPLC grade (Ranbaxy)

iii. Water Milli Q Water

iv. Orthophosphoric acid . AR grade (S d. Fine Chemical)

5.4.2 Working standards and sample :

sertraline WRS . # 170/119/sertraline

Impurity A	:	# 170/199C/sertraline/Imp-A
Impurity B	.	# 155/87/sertraline/Imp-B
Mandelic acid	:	Yamakawa Chem. Ind. Ltd., Japan
sertraline/I	:	# 35/30R/sertraline/I

5.4.3 Chromatographic system :

Column	:	4.6 mm x 25 cm, 5 µm, Hypersil - C8
Detector	:	UV-235 nm
Flow rate	:	1 5 ml
Injection volume	:	20 µl

- Shimadzu LC-10AT solvent delivery pump with SIL-10AXL Autoinjector
- Shimadzu SPD-10A UV detector with CLASS LC 10 computer software.

5.4.4 Buffer solution :

42 09g of Ammonium dihydrogen orthophosphate (anhydrous) was transferred in to a 5 lit beaker. 3000 ml of water, measured with a measuring cylinder, was added to dissolve. Finally, the pH of this solution was adjusted to 3.0 with 10%v/v orthophosphoric acid.

5.4.5 Mobile phase :

Mobile phase A

5 Lit of mobile phase was made by mixing 2000 ml of buffer solution and 1250 ml of water and 1750ml of acetonitrile. The entire mixture is filtered and degassed

Mobile phase B

2.5 Lit of mobile phase was made by mixing 1000 ml of buffer solution and 1500ml of acetonitrile. The entire mixture is filtered and degassed.

5.4.6 Stock Solutions :

Solution 1 : 39.0 mg of impurity A was dissolved and diluted to 25 ml in mobile phase A (1560 µg/ml of impurity A)

Solution 2 : 37.7 mg of impurity B was dissolved and diluted to 25 ml in mobile phase A (1508 µg/ml of impurity B)

Solution 3 : mg of sertraline/I was dissolved and diluted to 25 ml in mobile phase A (1500 µg/ml of sertraline/I).

Solution 4 : 37.9 mg of mandelic acid was dissolved and diluted to 25 ml in mobile phase A (1516 µg/ml of mandelic acid).

Solution 5 : 37.5 mg of -sertraline was dissolved and diluted to 25 ml in mobile phase A (1500.0 µg/ml of -sertraline).

Solution 6 : 5.0ml each of stock solution 1, solution 2, solution 3 and solution 4 was pipetted out into a 100ml volumetric flask and diluted to mark with mobile phase A (78.0µg/ml of impurity A, 75.4 µg/ml of impurity B, 75.0 µg/ml of sertraline/I and 75.8 µg/ml of mandelic acid).

Solution 7 : 5.0ml of stock solution 5 was pipetted out into a 100ml volumetric flask, dissolve in and diluted to mark with mobile phase A (75 µg/ml of -sertraline).

5.4.7 System suitability solution :

1.0ml each of solution 2 and solution 5 pipetted out in to a 50ml volumetric flask, dissolve in and diluted to mark with mobile phase A (30.16 µg/ml of impurity-B and 30.0 µg/ml of -sertraline)

5.4.8 Standard solution :

2.0ml each of solution 6 and solution 7 pipetted out in to a 50ml volumetric flask, dissolve in and diluted to mark with mobile phase A.

Final concentration of Standard Solution :

sertraline WRS	:	3.00 µg/ml
Impurity A	:	3.02 µg/ml
Impurity B	:	3.12 µg/ml

Mandelic acid	:	3.03 µg/ml
sertraline/I	:	3 00 µg/ml

5.5 Results and Discussions :

5.5.1 Identification :

1.0 ml of solution 1, solution 2, solution 3 and solution 4 and solution 5 are individually pipetted out in 5 separate 10 ml volumetric flasks and diluted to volume with mobile phase A. 20 µl each of this solution is injected individually and the chromatograms recorded upto 42 min.

Results & discussions :

Figure- 85 to Figure-88 (Appendix) shows typical chromatograms of individual components.

Retention time :

Mandelic acid	.	RT about 2.5 min.
Impurity B	:	RT about 11.5 min.
Sertraline	.	RT about 12.9 min.
Impurity A	:	RT about 28.7 min.
sertraline/I	:	RT about 31 7 min.

The above results show that all the components are clearly separated and identifiable.

5.5.2 System suitability :

Before starting a set of analysis, the system suitability solution is injected in duplicate. The resolution between the impurity B and sertraline is calculated.

Results & discussion :

Figure-82 shows (Appendix) the typical chromatogram of blank and Figure -24 (Appendix) shows the system suitability chromatogram

The Resolution factor R between Impurity-B and sertraline = 3.59[Limit NLT 3.0]

As the resolution meets the system suitability requirements the chromatographic system was used for further studies

5.5.3 Instrument precision

System Precision Solution ·

50 2 mg /sertraline standard was transferred into a 50ml volumetric flask. 2 0ml of solution 6 is pipetted out into it. Dissolve in and diluted to mark with mobile phase A (3.03 µg/ml mandelic acid ; 3.02 µg/ml impurity-B 3 12 µg/ml impurity A, 3.00 µg/ml sertraline/I and 1004 µg/ml -sertraline)

Individual area counts and % RSD values are shown in Table 5 5.3.1:

Results & discussion :

Table 5.5.3.1 : Instrument precision

Injection	Detector Response (Area counts)				
	Mandelic acid	**Impurity B**	**Sertraline**	**Impurity A**	**sertraline/I**
1	11415	43011	13187406	61681	89108
2	11424	43487	13329299	63594	88344
3	11454	43201	13259357	63095	89389
4	11414	43062	13194462	62240	88201
5	11536	43352	13253607	62891	89219
6	11534	43584	13440051	62457	88275
Mean	11463	43283	13277364	62660	88756
SD	58	231	94887	677	538
RSD (%)	0.5	0 5	0 7	1 1	0.6

[Limit : RSD NMT 5.0 %]

The above results are well within the acceptance limits and indicates instrument precision

5.5.4 Method Precision :

The Table 5.5 4 1 shows the weights of sertraline sample (Batch No. #170/119/sertraline) taken into 50 ml volumetric flask separately. 2.0 ml of *Solution 6 is* pipetted into each flask, dissolved by adding about 20 ml of mobile phase A and made to volume with mobile phase A. Each flask contains impurities at about target levels 0 3% each of Madalic acid, Impurity A, Impurity B, and sertraline/I)

Results & discussion :

Table 5.5.4.1 : Method Precision

Set	Wt. of Sample (mg)	Final Dilution (ml)	Concentration of Sample (µg/ml)	Observed Results in percentage			
				Mandelic acid	*impurity B*	*Impurity A*	*Sertraline /I*
T1	50.3	50	1006	0.2983	0 3125	0.2976	0.2956
T2	50.1	50	1002	0.2948	0.3098	0.2951	0.2863
T3	50.2	50	1004	0.2978	0.3145	0.2976	0.2917
T4	50.1	50	1002	0 2954	0 3163	0.2957	0.2936
T5	50.3	50	1006	0 3004	0.3271	0.2931	0.2975
T6	50.4	50	1008	0 3069	0 3164	0.2943	0.2901
Mean				0.2989	0.3161	0.2956	0.2925
RSD %				1.47	1.88	0.61	1.37

[Limit : RSD NMT 5.0 %]

The above results are well within the acceptance limits and indicates method precision. Figure-23 shows typical chromatogram for system precision , method precision .

5.5.5 Linearity and range :

The linearity of detector (UV) response for impurities was determined by preparing and injecting solutions in the concentration range of 50-150 % of limit conc. [0.3 % (3µg/ml) each of mandelic acid, impurity B, impurity A and sertraline/I].

Solution 6 and *Solution 7*, prepared as under Section 5.4.6, are used for making the linearity solutions as shown in Table 5.5 5 1

Table 5.5.5.1 : Dilutions and Concentration for Linearity Study

Sr. No	Level % of target	Vol. of *Solution 6*	Vol. of *Solution 7*	Final Dilution	Final Concentrations				
					Mandelic acid	*Impurity B*	*Sertraline*	*Impurity A*	*sertraline/I*
		ml	*ml*	*ml*	*µg/ml*	*µg/ml*	*µg/ml*	*µg/ml*	*µg/ml*
L1	50	1 0	1 0	50.0	1.516	1.508	1.500	1.560	1.500
L2	75	1 5	1.5	50.0	2.274	2.262	2.250	2.340	2.250
L3	100	2.0	2.0	50.0	3.032	3.016	3.000	3.120	3.000
L4	125	2.5	2.5	50.0	3 790	3.770	3 750	3.900	3.750
L5	150	3.0	3.0	50.0	4.548	4.524	4.500	4.680	4.500

Results and Discussion :

The results of individual impurities is shown in Table 5.5.5.2 to 5.5 5.4

Table 5.5.5.2 : Linearity of Mandelic acid

Level	Detector response (area counts)				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD %*
L1	5688	5615	5647	5650	0.64
L2	8763	8788	8816	8789	0.30
L3	11267	11338	11393	11333	0.56
L4	14290	14253	14313	14285	0.21
L5	17021	16963	17231	17072	0.83
Slope				3738.79	
Intercept				89.8	
Correlation coefficient (r^2)				0.9993	

Figure-30 shows the typical linearity plot for mandelic acid

Table 5.5.5.3 : Linearity of Impurity B

Level	Detector response (area counts)				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD %*
L1	18067	18255	18297	18206	0.67
L2	30039	30204	29962	30068	0.41
L3	40446	41260	41140	40949	1.07
L4	52677	52290	52525	52497	0.37
L5	62990	62179	62383	62247	0.55
Slope				14657	
Intercept				-3411	
Correlation coefficient (r^2)				0.999	

Figure-91 (Appendix) shows the typical linearity plot for Impurity B

Table 5.5.5.4 : Linearity of sertraline

Level	Detector response (area counts)				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD %*
L1	18011	18647	18440	18366	1.76
L2	29046	28827	28926	28933	0.38
L3	39907	39853	40091	39950	0.31
L4	51902	52422	52829	52384	0.89
L5	61648	61464	64635	62582	2.84
Slope				14918	
Intercept				-4310.22	
Correlation coefficient (r^2)				0.9992	

Figure-92 shows the typical linearity plot for sertraline.

Table 5.5.5.5 : Linearity of Impurity A

Level	Detector response (area counts)				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD %*
L1	29746	29924	30633	30101	1.55
L2	45762	45155	46180	45699	1.13
L3	61741	61598	62247	61862	0.55
L4	77973	75196	76828	76666	1.82
L5	89821	92379	89515	90572	1.74
Slope				19476	
Intercept				216.40	
Correlation coefficient (r^2)				0.9992	

Figure-93 shows the typical linearity plot for Impurity A

Table 5.5.5.6 : Linearity of sertraline/ stage I

Level	Detector response (area counts)				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD %*
L1	40782	40190	42656	41209	3.12
L2	64661	65127	65443	65077	0.60
L3	85769	87035	88438	87081	1.53
L4	108428	109115	109884	109142	0.67
L5	128879	12480	125581	126423	1.71
Slope				28599.07	
Intercept				-10.80	
Correlation coefficient (r^2)				0.9985	

Limits : RSD $\leq$ 5.0 %
Correlation coefficient (r^2) $\geq$ 0.99

Figure-94 shows the typical linearity plot for stage-1

5.5.6 Accuracy :

The five level (70, 85, 100, 115 and 130 % of 0.3 % mandelic acid, Impurity B, Impurity A and sertraline/I each) recovery study is performed.

The *Solution 6* prepared as under section 5 4 6 are used.

The Sertraline (# 170/119/sertraline) was used for recovery study

Table 5.5.6.1 : Dilutions and Concentration for recovery study

Sr. No.	% of target Level	Wt. of sertraline	Vol. of *Solution 6*	Final Dilution	Final Concentrations			
					Mandelic acid	*Impurity B*	*Impurity A*	*sertraline/I*
		mg	*ml*	*ml*	*µg/ml*	*µg/ml*	*µg/ml*	*µg/ml*
R1	70	50.3	1.4	50.0	2.122	2.111	2.184	2.100
R2	85	50.4	1.7	50 0	2 577	2.564	2.652	2.550
R3	100	50 3	2.0	50.0	3 032	3 016	3.120	3 000
R4	115	50.2	2.3	50.0	3.487	3 468	3.588	3.450
R5	130	50 1	2.6	50 0	3 942	3 921	4.056	3.900

Results & discussion .

Table 5.5.6.2 : Recovery of Mandelic acid from -sertraline

Sr No.	Level (%)	Actual amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery = Amt found ---------- x 100 Amt. added
R1	70	2 122	2 154	101.51
R2	85	2.577	2 592	100.58
R3	100	3.032	3 025	99.77
R4	115	3.487	3.449	98.91
R5	130	3.942	3 993	101 29
Mean				100.41
% RSD				1.08

Table 5.5.6.3 : Recovery of Impurity B from sertraline

Sr. No.	Level (%)	Actual amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery = Amt found ---------- x 100 Amt. added
R1	70	2.111	2.109	99.91
R2	85	2.564	2.547	99.34
R3	100	3.016	3.015	99.97
R4	115	3.468	3.476	100.23
R5	130	3.921	3.992	101.81
Mean				100.25
% RSD				0.93

Table 5.5.6.4 : Recovery of Impurity A from sertraline

Sr. No.	Level	Actual amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery = Amt found ---------- x 100 Amt. added
R1	70	2.184	2.182	99.91
R2	85	2.652	2.626	99.02
R3	100	3.120	3.112	99.74
R4	115	3.588	3.593	100.14
R5	130	4.056	4.178	103.01
Mean				100.36
% RSD				1.53

Table 5.5.6.5 : Recovery of sertraline/I from sertraline

Sr. No.	Level	Actual amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery = Amt found ---------- x 100 Amt. added
R1	70	2.100	2.136	101.71
R2	85	2.550	2.597	101.84
R3	100	3.000	3.029	100.97
R4	115	3.450	3.510	101.74
R5	130	3.900	4.073	104.44
Mean				102.14
% RSD				1.30

[Limit : Recovery - 95.0 % - 105.0 %]

5.5.7 Limit of Detection and Quantitation :

The limit of detection is established by injecting a solution containing about 1.5µg/ml of mandelic acid, impurity B, -sertraline, impurity A and sertraline/I impurity, further diluting the solution and injecting consecutively and recording the detector response. Table 5.5.7.1 shows the dilutions used. Table 5.5.7.2 to 5 5.7.6 shows the detector response for each impurity. Summary of LOD & LOQ is given in 5.5.7.7

The *Solution 6,* and *Solution 7* prepared as mentioned under section 5.4.6 were used

Solution A (MDL Stock Solution) :

2.0 ml each of *Solution 6* and *Solution 7* were pipetted out into a 100 ml volumetric flask and diluted to volume with mobile phase A.

Table 5.5.7.1 : Dilutions and Concentration for Limit of Detection and Limit of Quantitation Study

Level	Vol. of MDL stock soln (ml)	Final Dilution (ml)	Concentrations of components in µg/mL				
			Mandelic acid	*Impurity B*	*- sertraline*	*Impurity A*	*sertraline/I*
D1	-	-	1.516	1.508	1.500	1.560	1.500
D2	5.0	10	0.758	0.754	0.500	0.780	0.750
D3	2.5	10	0.379	0.377	0.250	0.390	0.375
D4	1.0	10	0.152	0.151	0.150	0.156	0.150
D5	0.50	10	0.075	0.075	0.075	0.078	0.075
D6	0.25	10	0.030	0.030	0.030	0.031	0.030
D7	0.10	10	0.015	0.015	0.015	0.016	0.015
D8	0.10	25	0.006	0.006	0.006	0.006	0.006
D9	0.10	50	0.003	0.003	0.003	0.003	0.003

Results & discussion :

Table 5.5.7.2 : Limit of Detection and Limit of Quantitation Study (Mandelic acid)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD %*
D1	1.516	5624	5670	5389	5561	2.71

D2	0.758	2542	2501	2494	2512	1.03
D3	0.379	1247	1742	1769	1586	18.53
D4	0.152	599	604	594	599	0.8
D5	0.075	309	462	227	333	35.85
D6	0.030	-	-	-	-	-

Table 5.5.7.3 : Limit of Detection and Limit of Quantitation Study (Impurity B)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD %*
D1	1.508	19687	19381	19671	19580	0.88
D2	0.754	7377	7320	7339	7352	0.39
D3	0.377	5134	5767	5395	5230	2.74
D4	0.151	2169	2444	2037	2217	9.37
D5	0.075	1000	1888	1576	1488	30.28
D6	0.030	-	-	-	-	-

Table 5.5.7.4 : Limit of Detection and Limit of Quantitation Study (-sertraline)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD %*
D1	1.500	18141	18683	18360	18395	1.48
D2	0.500	10049	9659	9892	9867	1.98
D3	0.250	5020	5328	4492	4947	8.53
D4	0.150	2140	2576	2211	2309	10.13
D5	0.075	912	183	2345	1145	95.83
D6	0.030	-	-	-	-	-

Table 5.5.7.5 : Limit of Detection and Limit of Quantitation Study (Impurity A)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD %*
D1	1.560	31099	29833	29639	30190	2.63
D2	0.780	14543	14465	14545	14518	0.31
D3	0.390	7326	7501	6827	7218	4.84
D4	0.156	3191	3307	3148	3215	2.56
D5	0.078	2138	1936	1906	1993	6.33
D6	0.031	-	-	-	-	-

Table 5.5.7.6 : Limit of Detection and Limit of Quantitation Study (sertraline/I)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD %*
D1	1.500	43930	44308	41280	43173	3.82
D2	0.750	18409	18484	18512	18468	0.29
D3	0.375	7831	7591	6897	7440	6.52
D4	0.150	3096	3079	3221	3132	2.47
D5	0.075	1400	932	1071	1134	21.21
D6	0.030	-	-	-	-	-

The limit of quantitation and detection for each impurity is as follows summary table :

Table 5.5.7.7 : Limit of Detection and Limit of Quantitation Summary

	Limit of quantitation		**Limit of detection**	
	(µg/ml)	**(%)**	**(µg/ml)**	**(%)**
Mandelic acid	0.758	0 076	0.075	0.0075
Impurity B	0.377	0.038	0.075	0.0075
-sertraline	0.750	0.075	0.075	0.0075
Impurity A	0.390	0.039	0.078	0.0078
sertraline/I	0.750	0.075	0.075	0.0075

5.5.8 Ruggedness :

Method ruggedness is established by a sample previously analysed for related substances is reanalysed by another analyst independently by this method and the results are compared.

Analyst : A.L.Prasad

Table 5.5.8.1 : Ruggedness experiment-1

Batch No.	**Mandelic acid**	**Impurity B**	**Impurity A**	**sertraline/I**	**Any other unknown impurity**
# 170/119/sertraline	Not detected	Not detected	Not detected	Not detected	i) 0.023% ii) 0.051%
# 170/132/sertraline	Not detected	Not detected	Not detected	Not detected	i) 0.011% ii) 0.023% iii) 0.2048%

Ruggedness
Analyst : YC

Table 5.5.8.2 : Ruggedness experiment-2

Batch No.	Mandelic acid	Impurity B	Impurity A	sertraline/I	Any other unknown impurity
# 170/119/sertraline	Not detected	Not detected	Not detected	Not detected	i) 0.023% ii) 0.055%
# 170/132/sertraline	Not detected	Not detected	Not detected	Not detected	i) 0.024% ii) 0.23%

Figure-29 shows the typical chromatographs for ruggedness experiments.

Analytical results compared between two experiments concluded that the method is passing in ruggedness.

5.6 Summary And Conclusions

	Acceptance limit	Actual results			
System suitability Resolution between Impurity B & sertraline	NLT 3.0 %	3.59, 3.4, 3.6			
		Mandelic acid	**Impurity B**	**Impurity A**	**sertraline/I**
Precision					
Instrument - RSD of Detector Response for each impurity	≤ 5.0 %	0.5%	0.5%	1.11%	0.6%
Method - RSD of each Impurity %	≤ 5.0 %	1.47%	1.88%	0.61%	1.37%
Linearity and Range					
Correlation coefficient (r^2)	≥ 0.99	0.9993	0.999	0.9992	0.997
RSD of detector responses	≤ 5.0 %	max. 0.83%	max. 2.84%	max. 1.82%	max. 3.12%
Accuracy Percentage recovery	95.0 % - 105.0 %	100.41%	100 25%	100.36%	102.14 %
Minimum quantitation level		0.076%	0.038%	0.039%	0.075%
Minimum detection level		0 008%	0 008%	0.008%	0.008%

The results of the study indicates that this method for related substances in -sertraline is precise, accurate, linear in detector response and rugged.

5.7 Method Development For The Analysis Of Sertraline assay By HPLC

Sertraline assay method was developed on a simple isocratic system to suite many small manufacturers how ever specificity study performed during validation indicated developed method is stability indicating and robust method.Method details are given below.

Analytical Method

Reagents .

1)	Potassium dihydrogenorthophosphate	:	AR grade (S.D. Fine Chem)
2)	Methanol	:	HPLC grade (Ranbaxy)
3)	Triethylamine	.	AR grade (S.D Fine Chem)
4)	Milli Q water		

Working standard and sample :

1) Sertraline WRS : # 155/54/7015 (Assay 98 84 % on as is basis)
2) Sertraline sample · # 35/42/7015

Mobile phase .

Buffer solution :

Transfer 1.36 g of potassium dihydrogen orthophosphate into a 1000 ml volumetric flask. To this add 500 ml of water and swirl to dissolve. Make up the volume to 1000 ml with water

Prepare filtered and degassed mixture of buffer, methanol and triethylamine in the proportion of (70 . 30 . 0.1).

Standard preparation :

Transfer about 50 mg of accurately weighed, Sertraline WRS into a 50 ml volumetric flask. Dissolve in and dilute upto mark with mobile phase.

Test preparation :

Transfer about 50 mg, accurately weighed, sample into a 50 ml volumetric flask. Dissolve in and dilute upto mark with mobile phase.

Instrumental conditions :

Use a suitable High Performance Liquid Chromatograph (HPLC) with the following conditions.

Column	.	HYPERSIL C8 (25cm x 4.6mm) 5 µ (Shandon, U.K)
Flow rate		1.0 ml/min
Detector	.	UV set at 235 nm
Attenuation	:	Set appropriately
Run time	:	About 20 min
Injection volume	:	10 µl

System suitability and precision .

Determine the instrument precision with five replicate injections of Sertraline standard preparation (1000 µg/ml). RSD is not more than 2 0 % and retention time of Sertraline is about 6 5 min

Procedure :

(1) Set up chromatographic system as described under instrumental conditions.

(2) Inject equal volume of the standard and test preparation in duplicate into the chromatograph and record the chromatograms.

Calculation :

$$\% \text{ Assay of Sertraline (on dried basis)} = \frac{AT}{AS} \times \frac{WS}{WT} \times \frac{DT}{DS} \times \frac{P \times 100}{100 - Q}$$

Where .

AT	=	Average area count of Sertraline peak in test preparation
AS	=	Average area count of Sertraline peak in standard preparation
WT	=	Weight of sample in mg
WS	=	Weight of Sertraline standard in mg
DS	=	Dilution factor of standard preparation
DT	=	Dilution factor of test preparation
P	=	% purity of Sertraline standard (as is basis)
Q	=	Loss on drying at 105°C for 3 hrs

5.8 Validation Of Stability Indicating HPLC Method For Assay Of Sertraline

Purpose

Purpose of this document is to generate supporting validation data for assay of sertraline by the HPLC method The validation data to demonstrate its specificity, stability indicating nature, accuracy, precision and linearity is described in the following sections

Analytical Method : As per method described in method development section 5.7

General :

(a) For these validation studies, following equipments were used for all experiments unless specified otherwise.

- Shimadzu LC-10AS solvent delivery pump with SIL-10A Autoinjector
- Shimadzu SPD-10A UV detector
- CLASS LC-10 computer software
- HYPERSIL C8 (25cm x 4 6mm) 5 μ (Shandon, UK)

(b) System suitability parameters, i.e. RSD of six replicate injections.

(c) The following limits are considered for acceptance .

5 8 1 **System suitability and reproducibility** ·

The relative standard deviation of response for Sertraline standard solution with six replicate injection is not more than 2 0 % and retention time of Sertraline is about 6.5 min.

5.8.2 **Method Precision** .

The relative standard deviation of the 6 sets of assay from the same Sertraline sample is not be more than 2.0 %.

5.8.3. **Specificity (degradation study) :**

The peak purity index of Sertraline peak as measured with a photodiode array detector from each degradation study is ≥ 0 999

5 8.4 **Linearity and range** :

The plot of detector responses for a concentration range of 50 to 150 % of the assay level (1000 μg/ml) is linear and the regression correlation coefficient is ≥ 0.999

5.8.5. **Accuracy (Recovery study) :**

In the 5 level (70, 85, 100, 115 and 130 % of assay conc.) recovery study of drug from the sample, the recovery is between 98.0 and 102.0 %. The relative standard deviation of recovery is not more than 2.0 %.

5.8.6. **Ruggedness** :

The assay of a sample carried out by deliberately changing some of the parameters should not differ by more than ± 0.5 %.

5.9 System suitability and reproducibility :

The reproducibility of injection was checked by injecting the Sertraline standard preparation 964 µg/ml (48.2 mg was dissolved in 50 ml mobile phase) six times. Individual area counts and % RSD values are shown in Table – 5.9 1 below ·

Figure-96 shows the typical chromatogram for system precision

[Limit : RSD NMT 2.0 %]

Table – 5.9.1 : Results of reproducibility study

Injection	Sertraline (964 µg/ml)
1	7468572
2	7442377
3	7463267
4	7436865
5	7432024
6	7438522
Mean	7446938
Standard deviation	± 15167
Relative standard deviation	0.204 %

5.10 Method precision :

Five solutions of Sertraline sample were prepared on same day for analysis by the HPLC method. Results of these analysis are shown in Table - 5.10.1.

Table – 5.10.1 : Results of method precision

	Final conc. (µg/ml)	**Area of individual injection**				**% Assay of** Sertraline
		1	*2*	*3*	*Mean Area counts*	
Sertraline (WRS)	966	7428486	7441879	7428733	7433033	
Sertraline (sample)						
1	1004	7629588	7663563	7677036	7656729	99.00
2	1034	7927748	7864401	7879370	7890506	99 07
3	1028	7848610	7838396	7850940	7845982	99.10
4	1014	7750979	7745056	7743002	7746346	99.18
5	1012	7698535	7717752	7756550	7724279	99.10
6	1058	8016742	8128721	8144124	8096529	99.36
Mean						99.14
Standard deviation						± 0.125
Relative standard deviation						0.126 %

5.11 Specificity of the method :

Specificity of the method was established by demonstrating no interference from degradation products. This was demonstrated by carrying out forced degradation of the sample by adding 0.1M HCl, 0.1M NaOH, 3 % H_2O_2, 0.1M $KMNO_4$ separately. The sample was also subjected to exposure under UV light for 24 hrs. and oven (105°C) degradation by heating it in a oven at 105°C for 3 hrs. The samples were prepared as given below and were injected into HPLC with a Shimadzu SPD M-10A photodiode array detector. The chromatograms were recorded upto 20 min. to check for degraded peaks. In each case peak purity index of Sertraline was determined to examine interference from degradation products.

5.11.1 Acid degradation :

50.7 mg Sertraline was transferred into a 50 ml volumetric flask containing 5 ml water. Swirled to disperse. To this was added 5 ml of 0.1M HCl. The sample was kept for 24 hrs. After that, pH of the solution was adjusted to 7.0 with 0.1M NaOH and volume was made up with mobile phase. This solution was injected into the HPLC. Peak purity of Sertraline peak as determined by diode array detector was > 0.9900. No significant degradation was found as determined by comparing area counts with the standard.

5.11.2 Alkali degradation :

51.9 mg Sertraline was transferred into a 50 ml volumetric flask containing 5 ml water. Swirled to disperse. To this was added 5 ml of 0.1M NaOH. The sample was kept for 24 hrs. After that, pH of the solution was adjusted to 7.0 with 0.1M HCl and volume was made up with mobile phase. This solution was injected into the HPLC. Peak purity of Sertraline peak as determined by diode array detector was > 0.9900. However, no significant degradation was found in alkali.

5.11.3 Peroxide degradation :

51.4 mg Sertraline was transferred into a 50 ml volumetric flask containing 5 ml water. Swirled to disperse. To this was added 1 ml of 3 % H_2O_2. The sample was kept for 24 hrs. After that, volume was made with mobile phase. This solution was injected into the HPLC. Peak purity of Sertraline peak as determined by diode array detector was > 0.9900. No significant degradation was found as determined by comparing area counts with the standard.

5.11.4 Degradation with $KMnO_4$:

50.8 mg Sertraline was transferred to 50 ml volumetric flask containing 5 ml water. Swirled to disperse. To this was added 1 ml 0.1M $KMnO_4$. The sample was kept for 24 hrs. After that the volumes were made with mobile phase and filtered. This solutions were injected into the HPLC. Figure-102 shows HPLC chromatograms for degradation with $KMnO_4$. Peak purity of Sertraline as determined by diode array detector was > 0.9900. However significant degradation was observed.

5.11.5 Degradation under UV light :

50.3 mg Sertraline (previously kept under UV light at 254 nm for 24 hrs.) was transferred into a 50 ml volumetric flask containing 5 ml water, dissolved in and diluted to volume with mobile phase. This solution was injected into the HPLC. Figure-100 shows HPLC chromatogram for degradation under UV light. Peak purity of Sertraline peak as determined by diode array detector was > 0.9900 However no significant degradation was observed.

5.11.6 Sunlight degradation :

49.1 mg Sertraline was transferred to 50 ml volumetric flask. Expose to sunlight for 24 hrs. Then add 5 ml water Swirled to disperse After that volume was made with mobile phase. This solution was injected into the HPLC. Figure 101 shows HPLC chromatogram for sunlight degradation. Peak purity of Sertraline as determined by diode array detector was > 0.9900 No significant degradation was observed.

5.11.7 In oven at 105°C for 3 hr. degradation :

52 6 mg Sertraline was transferred into a 50 ml beaker. The sample was heated in a oven at 105°C for 3 hr After cooling sample was transferred into 50 ml volumetric flask and volume was made with mobile phase This solution was injected into the HPLC. Figure-99 shows HPLC chromatogram for oven (105°C) degradation. Peak purity of Sertraline as determined by diode array detector was > 0.9900. No significant degradation was observed

Table – 5.11.1 : Specificity of the method

Degradation	Condition	Retention time (min.)	Peak purity	Acceptance criteria (Peak purity)
Acid degradation	50 7 mg Sertraline & 5 ml 0 1M HCl kept for 24 hrs.	6.4 6.4	Up 0.9995 Dn. 0.9994 Up 0.9994 Dn 0.9993	NLT 0.9900
Alkali degradation	51.9 mg Sertraline & 5 ml 0 1M NaOH kept for 24 hrs	6 4	Up 0.9992 Dn. 0.9991	NLT 0.9900

		6.4	Up 0.9993 Dn. 0.9991	
Peroxide degradation	51.4 mg Sertraline & 1 ml of 3.0 % H_2O_2 water kept for 24 hrs.	6.3 6.3	Up 0.9994 Dn. 0.9992 Up 0.9994 Dn. 0.9992	NLT 0.9900
$KMnO_4$ degradation	50.8 mg Sertraline & 1 ml 0.1M $KMnO_4$ kept for 24 hrs.	6.3 6.3	Up 0.9999 Dn. 0.9999 Up 0.9998 Dn 0.9999	NLT 0.9900
Sunlight degradation	49.1 mg Sertraline kept for 24 hrs. for sunlight exposure	6.3 6.3	Up 0.9995 Dn. 0.9995 Up 0.9995 Dn. 0.9995	NLT 0.9900
UV degradation	50.3 mg Sertraline kept under UV light at 254 nm for 24 hrs.	6.3 6.3	Up 0.9994 Dn. 0.9993 Up 0.9995 Dn. 0.9994	NLT 0.9900
Oven (at 105°C) degradation	52.6 mg Sertraline and heated in oven at 105°C for 3 hrs.	6.3 6.3	Up 0.9994 Dn. 0.9992 Up 0.9994 Dn. 0.9992	NLT 0.9900

5.12 Linearity and range :

(i) The linearity of detector (UV) response for Sertraline was determined by preparing and injecting solutions in the concentration range of 500 - 1500 µg/ml (50-150 % of assay conc.) for Sertraline standard. Figure-97 shows linearity chromatogram and Figure-98 shows graphs of Sertraline and Table - 5.12.1 shows values of slope, intercept and correlation coefficient of linear plot.

Stock solution : 250.3 mg of drug was dissolved in 100 ml of mobile phase (2503 µg/ml)

Table – 5.12.1 : Linearity study

Vol. of stock solution (ml)	Final dilution (ml)	Final conc. (µg/ml)	Area counts			Mean Area counts (n = 3)	RSD (%)
			1	2	3		
2 0	10	500.6	4037492	4017349	4010154	4021665	0.35
3.0	10	750.9	5763043	5771027	5749873	5749873	0 19

4.0	10	1001.2	7536686	7524827	7508310	7523275	0.19
5.0	10	1251.5	9220597	9209557	9232918	9221022	0.13
6.0	10	1501.8	10801923	10746950	10792453	10780442	0.27
Slope						6787.3	
Intercept						663734	
Correlation coefficient (r^2)						0.9995	

[Limit : Correlation coefficient (r^2) - NLT 0.999]

[Limit : RSD of triplicate injections - NMT 2.0 %]

5.13 Accuracy (Recovery study) :

Recovery of drug from Sertraline sample :

The recovery of added drug from Sertraline sample was performed at 70-130 % of assay concentration level (1000 µg/ml). Each level was done in triplicate.

Standard stock solution : 250.3 mg/100 ml with mobile phase (2503 µg/ml)

Sample stock solution : 250.1 mg/100 ml with mobile phase (2501 µg/ml)

Table – 5.13.1 Recovery study (details of dilutions)

Recovery level (%)	Amount of sample stock solution taken (ml)	Amount of standard stock solution added (ml)	Final dilution (ml)	Final conc. (µg)
70	4.0	2.8	10	1701.48
85	4.0	3.4	10	1851.54
100	4.0	4.0	10	2002.40
115	4.0	4.6	10	2151.66
130	4.0	5.2	10	2301.72

Table – 5.13.2 Results of recovery study

Recovery level (1000 µg/ml) (%)	Amount of Sertraline sample (µg)	Amount of Sertraline added (µg)	Amount of Sertraline found (µg)	% Recovery = Amt found ---------- x 100 Amt. added
70	1000.4	700.84	690.02	98.46
85	1000.4	851.02	857.99	100.82
100	1000.4	1001 20	984.98	99.38
115	1000.4	1151.38	1159.41	100.70
130	1000.4	1301.56	1281.12	98.43

Average		99.36
% RSD		1.29 %

[Limit : 98.0 % - 102.0 %]

[Limit : RSD for recovery levels - NMT 2.0 %]

5.14 Ruggedness :

Method ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as instrument and analyst.

Parameter	:	**Normal condition** (I)	**Changed condition** (II)
Column	:	Hypersil C8 25cm x 4.6mm, 5 µ, (Shandon, U.K.)	Lichrosphere (R) 60 RP-select-B (5 µ) (Merck)
Flow rate	:	**1.0 ml/min.**	**0.8 ml/min.**
Mobile phase	:	Methanol : 70 Buffer : 30 Triethylamine : 0.1 pH adjusted to : 3 0	Methanol : 65 Buffer 35 Triethylamine : 0.1 pH adjusted to : 3.1
Pump	:	**LC-10AT**	**LC-10AS**
Detector	:	SPD-10A	SPD-10A
Software	:	**Shimadzu Class LC 10**	**Oracle - 2 Computer software**
Injection volume	:	10 µl	10 µl
Assay	:	**99.62 %**	**99.66 %**

[Limit : Variation ± 0.5 %]

Conclusion : No significant change in assay was found even under the deliberately changed conditions. Thus the ruggedness of the method is established.

5.15 Stability in analytical solution :

Solution of standard (964 µg/ml) was injected at different time intervals and peak areas were recorded..

Table – 5.15.1 : Stability of drug in analytical solution

Time (hrs.)	Sertraline (peak area)
0.0 10 6 36.5	7468572 7428480 7418187
Mean	7438416
Relative standard deviation	0.36 %

No significant change was observed for Sertraline peak areas upto 36 5 hrs.

5.16 Summary and conclusions :

Table – 5.16.1 : Validation results

Sr. No.	Acceptance criteria	Observed value	Limit
1.	System suitability and reproducibility	RSD = 0.204 %	RSD NMT 2 0 %
2.	Accuracy	Recovery = 99 36 % RSD = 1.29 %	Recovery : 98 0 % - 102.0 % RSD : NMT 2.0 %
3	Linearity range	Correlation coefficient (r^2) = 0.9995	Correlation coefficient (r^2) NLT 0 999
4	Precision	RSD 0.127 %	RSD NMT 2 0 %
5	Ruggedness	Variation = - 0.06 %	Variation ± 0.5 %

All these observations indicate that this method for assay of Sertraline is specific, accurate, precise and is also stability indicating