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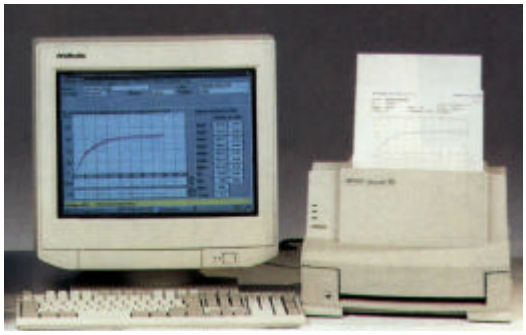
FLUOXETINE VALIDATION - DISSOLUTION TEST.



ANALYTICAL METHOD VALIDATION

DISSOLUTION TEST

SOLIDS DOSAGE FORMS

FLUOXETINE CAPSULES
10 + 20mg

Method Number SI-230-03
Edition Number 03
Effective Date DD/MM/200Y

Prepared by: _____ Date: _____

Reviewed by: _____ Date: _____

Approved by: _____ Date: _____

Edition No: 03	Effective Date:	APPROVED			
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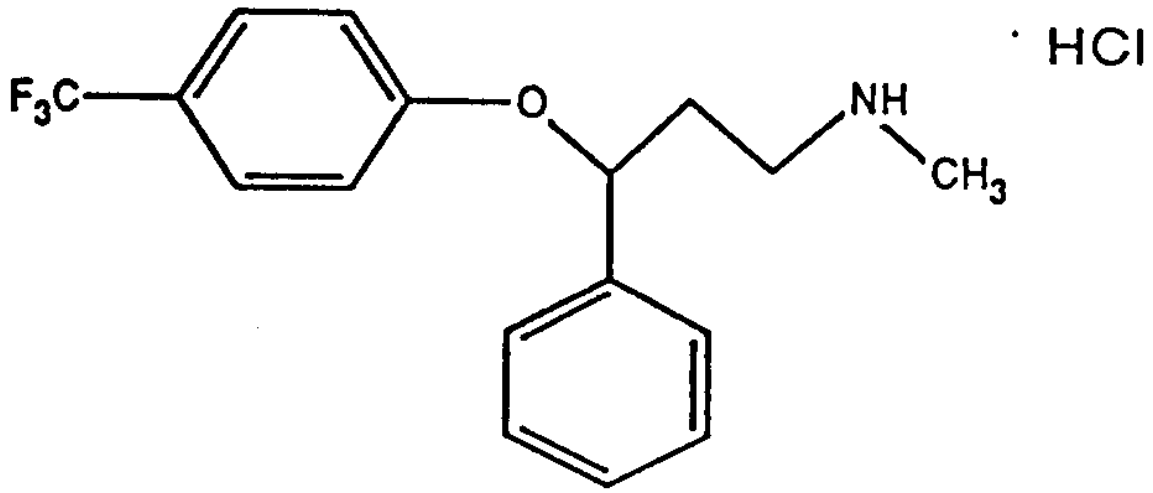
FLUOXETINE VALIDATION - DISSOLUTION TEST.

INTRODUCTION

Fluoxetine Hydrochloride acts as a selective Serotonin-reuptake inhibitor and is clinically effective for the treatment of certain depression categories. Fluoxetine Hydrochloride has been demonstrated to exhibit comparable efficacy to the tricyclic antidepressant group, but with relatively fewer anti-cholinergic side effects to the patients.



$C_{17}H_{18}F_3NO \bullet HCl$ M.W. = 345.79 CAS — 59333-67-4

Fluoxetine Capsules are available in two dosage forms Fluoxetine HCl equivalent to 10mg or 20mg Fluoxetine per capsule. The 10mg and 20mg are manufactured to the same capsule weight.

This ANALYTICAL METHOD VALIDATION DISSOLUTION TEST was carried out for the method **SI-230-03** which includes the Dissolution determination by HPLC. It is in-house method and currently used method for stability studies. The validation was performed on the 20mg dosage samples, from the PIVOTAL batch, Lot #001.

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FLUOXETINE VALIDATION - DISSOLUTION TEST.

PRECISION

System Repeatability

Ten replicate injections of the standard solution at the concentration of 0.5432mg/100mL as described in method SI-230-03 were made and the relative standard deviation of the peak areas was calculated.

SAMPLE	PEAK AREA
I	3638
II	3611
III	3618
IV	3657
V	3623
VI	3618
VII	3611
VIII	3632
IX	3659
X	3617
Average	**3629.4**
SD	**16.6**
RSD. (%)	**0.5**

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PRECISION (cont.)

Method Repeatability

The full method as described in SI-230-03 was carried-out on the finished product Lot #001 for the 20mg dosage form, repeated six times and the relative standard deviation was calculated.

SAMPLE	ASSAY %
I	89.5
II	86.8
III	90.3
IV	89.3
V	90.6
VI	93.2
Average(%)	**89.9**
SD	**2.1**
RSD (%)	**2.3**

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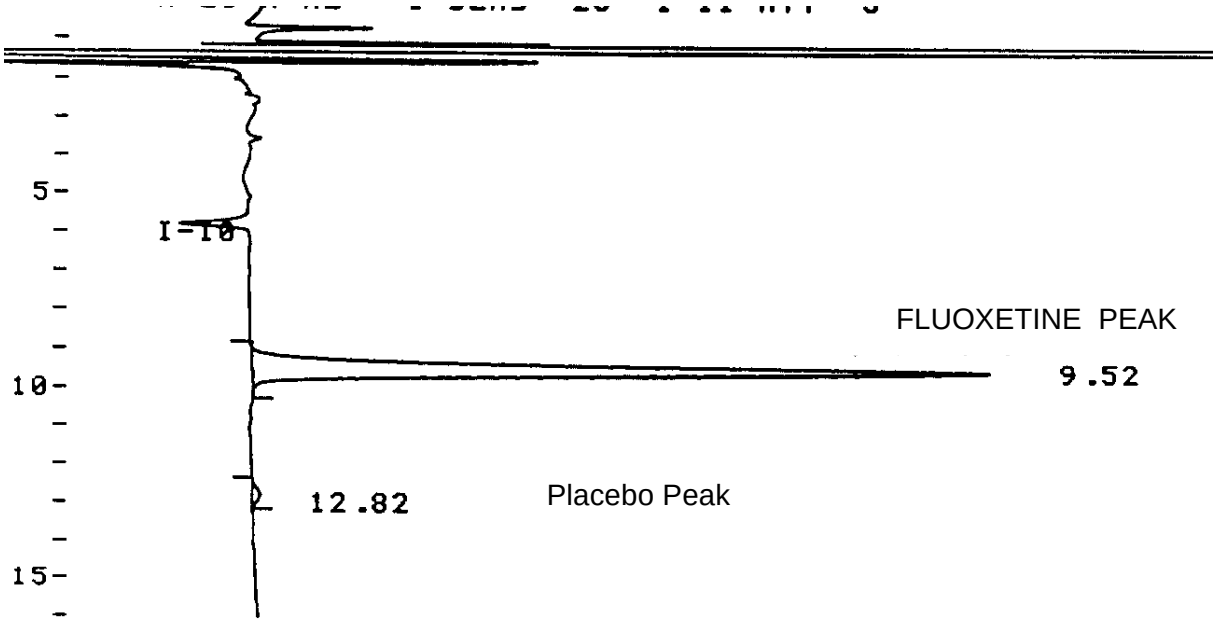
FLUOXETINE VALIDATION - DISSOLUTION TEST.

TYPICAL CHROMATOGRAM



The peak at RRT = 1.3 refers to the placebo PREPARATION

FLUOXETINE VALIDATION - DISSOLUTION TEST.

PRECISION (cont.)

Intermediate Precision

The full method as described in SI-230-03 was carried-out on the finished product #-001 for the 20mg dosage form. It was repeated six times by another analyst in a different day with a different HPLC.

The average and the relative standard deviation were calculated.

SAMPLE	ASSAY %
I	95.1
II	98.7
III	99.3
IV	98.9
V	98.8
VI	98.8
Average(%)	**98.3**
SD	**1.6**
RSD (%)	**1.6**

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FLUOXETINE VALIDATION - DISSOLUTION TEST.

LINEARITY

Standard solutions were prepared from 20% to 120% of the nominal concentration required by the method SI-230-03. Linear regression analysis demonstrated acceptability of the method for quantitative analysis over the concentration range required. Y-Intercept was found to be insignificant.

CONC. (%)	STD. CONC. (mg/mL)	PEAK AREA
20	0.001086	70468
30	0.001630	104845
50	0.002716	178940
100	0.005432	360638
120	0.006584	439690
Linear Regression		**0.99997**
Slope		**67221605**
$\frac{\text{Y - Intercept}}{\text{responce at 100\%}} * 100\ (\%)$		**1.0%**

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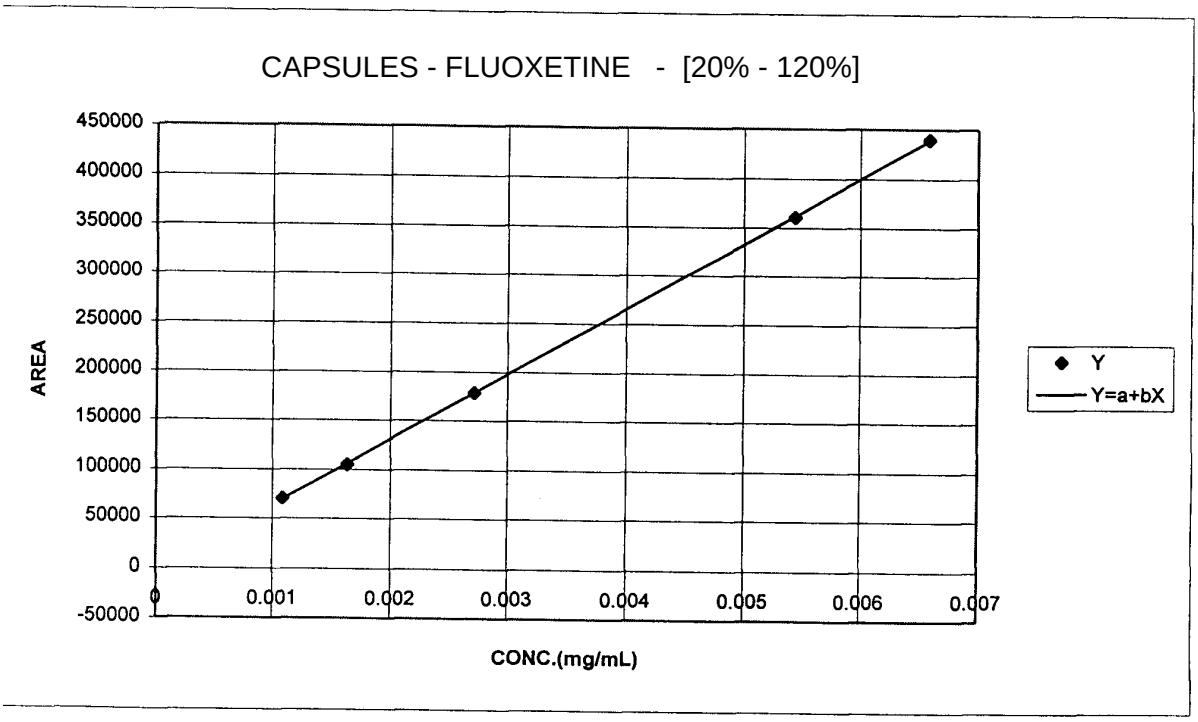
LINEARITY GRAPH



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FLUOXETINE VALIDATION - DISSOLUTION TEST.

ACCURACY

Accuracy of Standard Injections

Five replicate injections of the working standard solution were prepared at a concentration of 0.5432mg/100mL, as described in the assay method.

The percent deviation from the true value was determined from the linear regression line.

INJECTION NO.	PEAK AREA	% ACCURACY
I	363802	100.6
II	361774	100.1
III	361061	99.9
IV	365287	101.1
V	362692	100.3
Average	**362923.2**	**100.4%**
SD	**1673.4**	**0.5**
RSD (%)	**0.5**	**0.5**

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FLUOXETINE VALIDATION - DISSOLUTION TEST.

ACCURACY (Cont.)

Accuracy of the Drug Product

Known amount of Fluoxetine HCl at THREE concentrations: 60% (Q-20), 80%(Q) and 100% was added into the dissolution vessels which contained capsules with Placebo.

Duplicate samples were prepared for each concentration.

The dissolution vessels were operated under the conditions described in the dissolution method.

The Accuracy Test was done for the two dosage forms: 10mg and 20mg, using Placebo batches: #003 for 10mg caps and #004 for 20mg caps.

Accuracy for 10mg Caps

Conc. (%)	Placebo Capsules Wt. (mg)	Fluoxetine HCl Wt. (mg)	Peak Area	% Recovery	Average (%)
60	219.0	6.66	1708	85.6	
60	217.2	6.84	1992	97.2	91.4
80	217.7	8.98	2780	103.4	
80	216.4	9.05	2708	99.9	101.7
100	217.2	11.39	3503	102.7	
100	209.6	11.94	3659	102.3	102.5

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FLUOXETINE VALIDATION - DISSOLUTION TEST.

ACCURACY (Cont.)

Accuracy for 20mg Caps

Conc. (%)	Placebo Capsules Wt. (mg)	Fluoxetine HCl Wt. (mg)	Peak Area	% Recovery	Average (%)
60	209.1	13.39	1952	97.3	
60	207.9	13.65	2148	105.1	101.2
80	209.0	17.65	2748	104.0	
80	208.7	17.34	2796	107.7	105.9
100	206.6	22.76	3418	100.3	
100	205.4	22.87	3611	105.4	102.9

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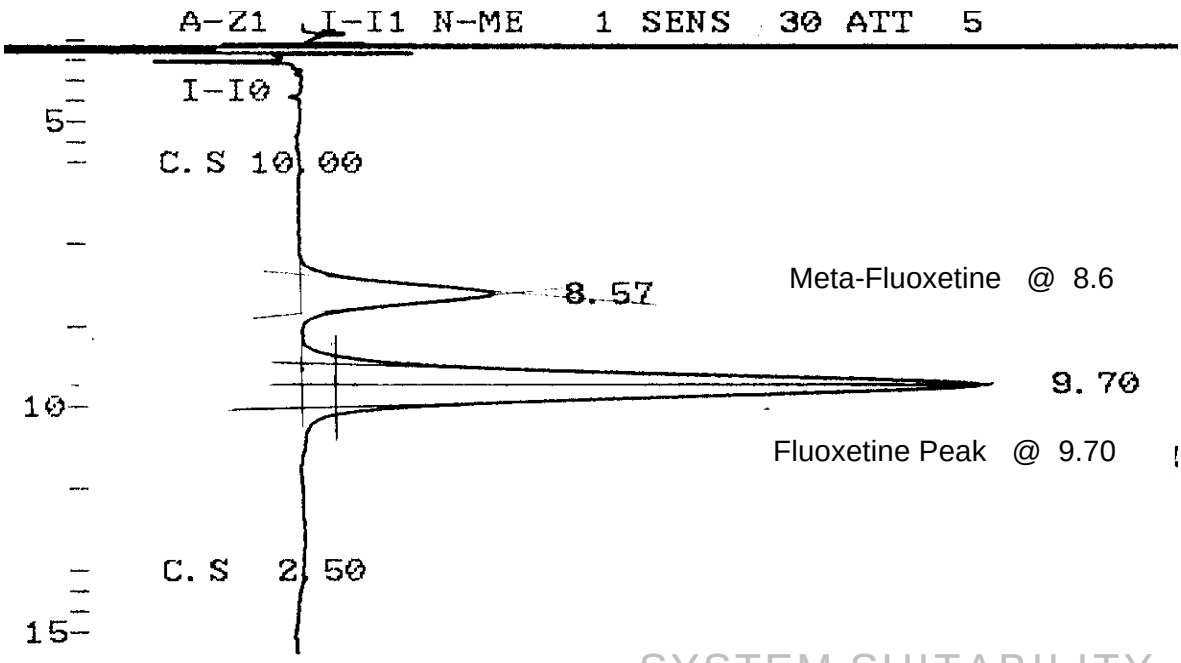
FLUOXETINE VALIDATION - DISSOLUTION TEST.

SPECIFICITY

System Suitability

A test for System Suitability was performed as described in the method. The resolution calculated (according to USP) between Meta-Fluoxetine peak and Fluoxetine peak should be not less than 2.0. The tailing factor for Fluoxetine peak should be not more than 2.0. Typical retention time of Fluoxetine peak is approcximately 9.5 min.

TYPICAL CHROMATOGRAM



SYSTEM SUITABILITY SOLUTION

FLUOXETINE CAPS.
Hypersil BDS C-8, 5u, 4. 6*150mm
DISSOLUTION-
55 A:45 B
Flow=1. 5ml/min, Press=147

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FLUOXETINE VALIDATION - DISSOLUTION TEST.

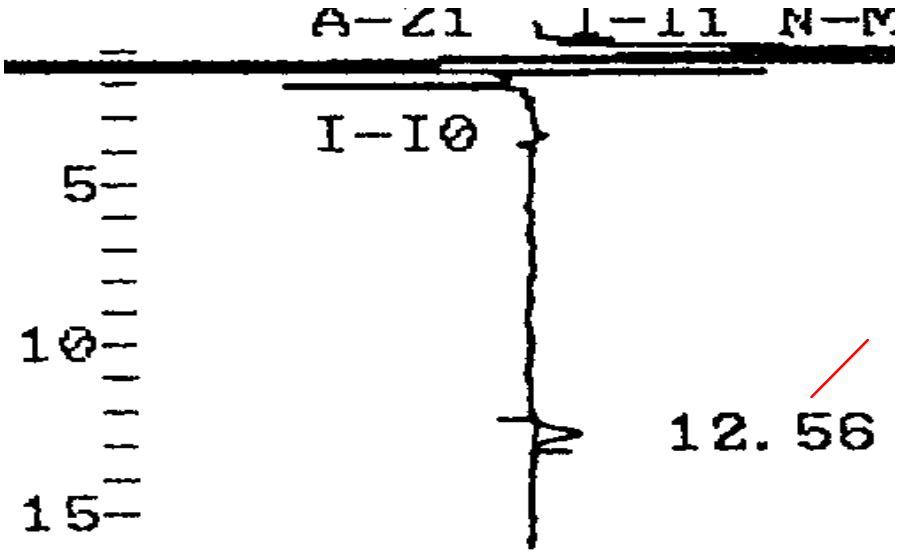
SPECIFICITY (cont.)

Placebo

Capsules containing only non actives excipients (Placebo capsules, Lot # 003 for 10mg dosage and Lot # 004 for 20mg dosage) were prepared as in method.

- No interfering peaks were observed.
- The peak at RRT = 1.3 belong to Placebo.

PLACEBO CHROMATOGRAM



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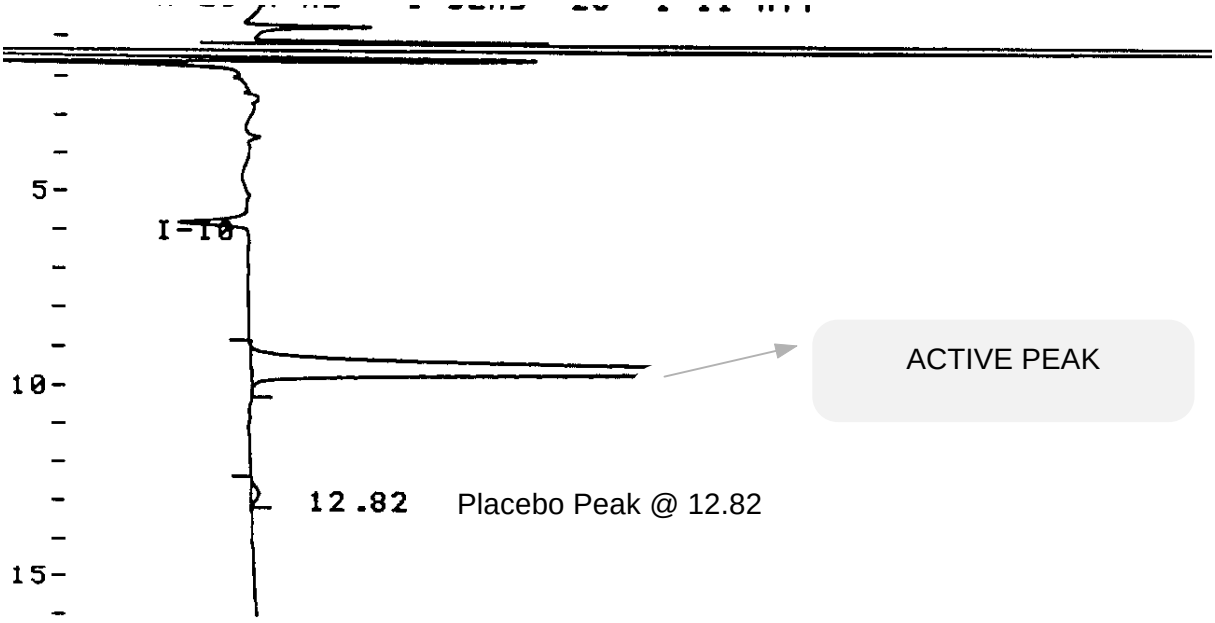
FLUOXETINE VALIDATION - DISSOLUTION TEST.

COMPARISON OF PLACEBO PEAK IN A STANDARD CHROMATOGRAM



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FLUOXETINE VALIDATION - DISSOLUTION TEST.

SPECIFICITY (cont.)

Separation of Impurities / Degradation Products

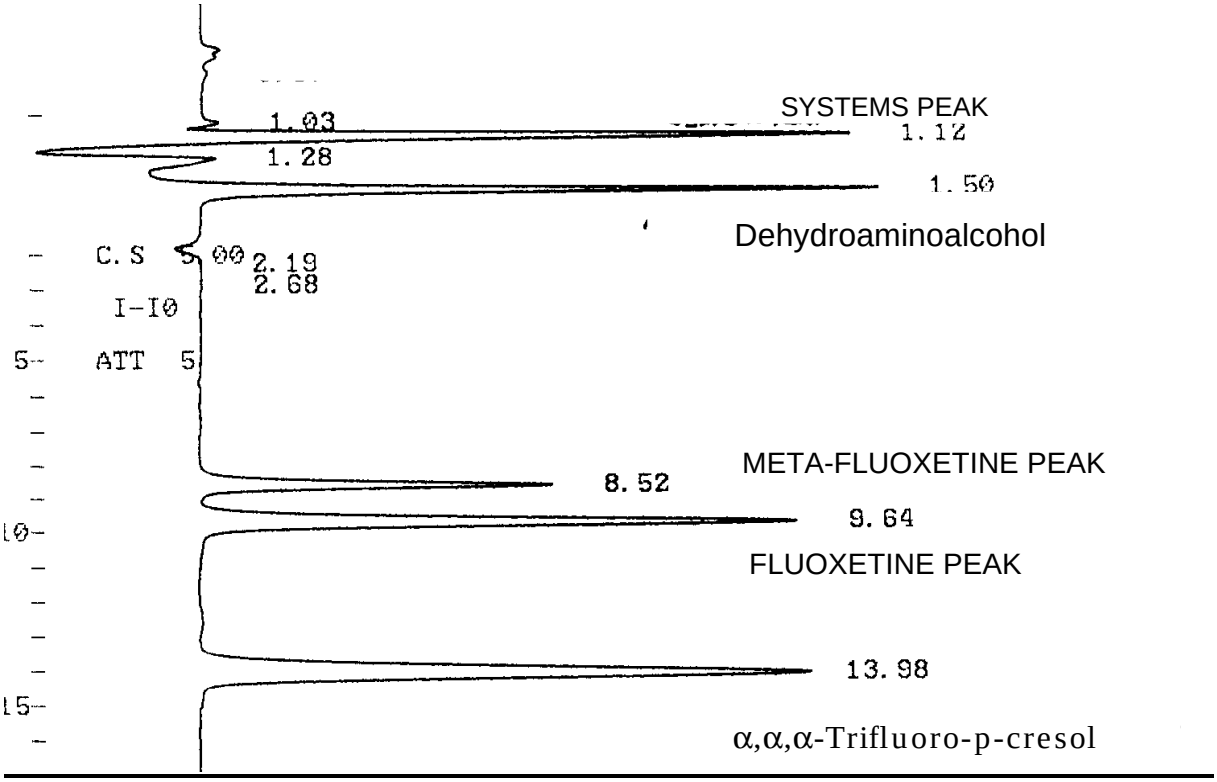
A mixed solution of Impurities./Degradation products (Dehydroaminoalcohol HCl, Meta-Fluoxetine HCl, Aminoalcohol Base and α,α,α-Trifluoro-p-cresol), including Fluoxetine HCl was prepared in concentration of the nominal concentration and chromatographed under the conditions of the method.

It was observed, that all the Impurities/Degradation products are separated each from the other and none of them interfere with Fluoxetine peak.

Dehydroaminoalcohol peak was observed in the solvent front.

Aminoalcohol peak was not observed in the method conditions.

TYPICAL SEPARATION CHROMATOGRAM



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FLUOXETINE VALIDATION - DISSOLUTION TEST.

STABILITY OF STANDARD & SAMPLE SOLUTIONS

Standard Solutions

Two standard solutions were prepared, kept in the refrigerator for 1 week and compared with two new preparation of standard solutions.

Standard No.	1	2	3	4
Weight (mg)	11.31	10.67	11.11	10.59
Preparing Date	13.08.97	13.08.97	20.08.97	20.08.97
Response	3477	3274	3486	3295
	3531		3518	
	3537		3452	
	3514		3490	
	3497		3469	
Average	3511.2	3274	3483.0	3296
RSD (%)	0.7	-	0.7	-

Comparison between standards (%)

Standard	2	3	4
1	1.18	0.98	0.25
2	-	2.17	1.43
3	2.17	-	0.73

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FLUOXETINE VALIDATION - DISSOLUTION TEST.

Sample Solutions

The full method as described in the method was carried-out on the finished product #002 for the 20mg dosage form. The dissolution sample solutions taken from the dissolution cells were allowed to stand for 3 days at refrigerator temperatures and thereafter analyzed.

Preparing Date	Analysis Date	% Dissolution	Average
26.08.97	26.08.97	95.2 98.6 99.5 98.7 98.8 98.8	98.3
26.08.97	29.08.97	94.7 98.5 98.1 98.8 98.4 98.3	97.8

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FLUOXETINE VALIDATION - DISSOLUTION TEST.

ROBUSTNESS

Factorial design

The HPLC condition as stated in method are as follows:

Column : Shandon Hypersil BDS C-8, 5µ, 150×4.6mm

Mobile phase : Solution A: solution B (55:45)
Solution A = Triethylamine buffer*: Acetonitrile: Tetrahydrofuran (75:15:10)
Solution B = Triethylamine buffer*: Acetonitrile: Tetrahydrofuran (65:20:15)

Column Temperature : 30°C

Flow rate : 1.5mL/min

Injection volume : 50µL

Detector : UV detector at 227nm, 10mm flow cell.

Diluent A : Water: Acetonitrile (70:30)

Diluent B : Water: Acetonitrile (45:55)

Diluent C : Water: Acetonitrile (65:35)

Factorial design variation were made to the above method with changes of eluent composition, flow rate, Column Temperature and column, using the System Suitability parameters.

Incident	Flow Rate (mL/min.)	% Solution A	Column Temp. (°C)	Column
1	1.5	55	30	Hypersil, BDS C-8, 5u, 150 × 4.6mm
-1	1.7	60	27	A new Hypersil, BDS C-8, 5u, 150 × 4.6mm

[Ruggedness = Small but deliberate changes to the current analytical method.]

FLUOXETINE VALIDATION - DISSOLUTION TEST.

Factorial design (Cont.)

Run #	Flow Rate (mL/min)	% solution A	Temp (°C)	Column	Tailing Factor	Resolution*	Retention Time
1	1	1	1	1	1.0	2.7	9.5
2	1	1	-1	-1	1.2	2.6	10.2
3	1	-1	1	-1	1.2	2.6	10.4
4	1	-1	-1	1	1.1	2.9	11.0
5	-1	1	1	-1	1.2	2.4	8.5
6	-1	1	-1	1	1.1	2.7	9.0
7	-1	-1	1	1	1.0	2.9	9.1
8	-1	-1	-1	-1	1.1	2.6	9.8

CONCLUSION FOR FACTORIAL DESIGN

Considering these results, the method is determined to be robust.

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7. Int. J of Drug R&D Vol. 01 No 06 Analytical Overview of FLUOXETINE
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FLUOXETINE VALIDATION - DISSOLUTION TEST.

VALIDATION CONCLUSIONS.

- Repeatability of assay was proven on the Pivotal batch No #-001.
- The results from the intermediate precision test do not differ significantly from those obtained in the method repeatability test .
- Linear regression analysis demonstrated acceptability of the method for quantitative determinations over the concentration range of **20%** to **120%** of the nominal working concentration.
- The accuracy and recovery of the analytical method was demonstrated in the range of **60%** to **100%** of the label amount.
- Analysis of the non-active formulation components (placebo capsules), showed no interference with the quantitative determination of the active substance.
- The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters.
- The standard and sample solutions were shown to be stable for **72** hours at room temperature. Standard solutions were also shown to be stable for **ONE** week at 4°C in a refrigerator.
- Method SI-230-03 for " **Dissolution**" by HPLC test is demonstrated to be accurate and precise for carrying out **Dissolution** determination analysis as part of the stability studies of Fluoxetine Capsules 10 and 20 mg.

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