AM-V-0206-0300

ANALYTICAL METHOD PROCEDURES

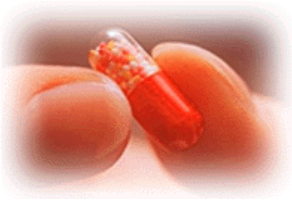
Total Number of Pages: 24

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

FLUOXETINE HCl

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

STABILITY INDICATING ASSAY VALIDATION



Method is suitable for:

- ☒ In-process control
- ☑ Product Release
- ☑ Stability indicating analysis (Suitability - US/EU Product)

CAUTION

FLUOXETINE HYDROCHLORIDE IS A HAZARDOUS CHEMICAL AND SHOULD BE HANDLED ONLY UNDER CONDITIONS SUITABLE FOR HAZARDOUS WORK.

IT IS HIGHLY PRESSURE SENSITIVE AND ADEQUATE PRECAUTIONS SHOULD BE TAKEN TO AVOID ANY MECHANICAL FORCE (SUCH AS GRINDING, CRUSHING, ETC.) ON THE POWDER.

ED. N0: 04	Effective Date:	APPROVED:			
Ed. Status : Supcds 03	DD/MM/2000	*Anne* ANALYST	*Bella* SUPERVISOR	*Edanna* QC	*Carol* HEAD

ISSN 0793 694X US/Canada ISSN 0793 7784 Euro ISSN 0793 7822 Pacific Rim

AM-V-0206-0300

ANALYTICAL METHOD PROCEDURES

Total Number of Pages: 24

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

TABLE OF CONTENTS

ED. N0: 04	Effective Date:	APPROVED:			
Ed. Status : Supcds 03	DD/MM/2000	*Anne* ANALYST	*Bella* SUPERVISOR	*Edanna* QC	*Carol* HEAD

ISSN 0793 694X US/Canada ISSN 0793 7784 Euro ISSN 0793 7822 Pacific Rim

AM-V-0206-0300

ANALYTICAL METHOD PROCEDURES

Total Number of Pages: 24

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

BACKGROUND

Therapeutically, Fluoxetine hydrochloride is a classified as a selective serotonin-reuptake inhibitor. Effectively used for the treatment of various depressions. Fluoxetine hydrochloride has been shown to have comparable efficacy to tricyclic antidepressants but with fewer anticholinergic side effects. The patent expiry becomes effective in 2001 (US).

INTRODUCTION

Fluoxetine capsules were prepared in two dosage strengths: 10mg and 20mg dosage strengths with the same capsule weight. The formulas are essentially similar and geometrically equivalent with the same ingredients and proportions. Minor changes in non-active proportions account for the change in active ingredient amounts from the 10 and 20 mg strength.

The following validation, for the method SI-IAG-206-02 , includes assay and determination of Meta-Fluoxetine by HPLC, is based on the analytical method validation SI-IAG-209-06.

Currently the method is the in-house method performed for Stability Studies. The Validation was performed on the 20mg dosage samples, IAG-21-001 and IAG-21-002.

In the forced degradation studies, the two placebo samples were also used.

PRECISION

SYSTEM REPEATABILITY

Five replicate injections of the standard solution at the concentration of 0.4242mg/mL as described in method SI-IAG-206-02 were made and the relative standard deviation (RSD) of the peak areas was calculated.

SAMPLE	PEAK AREA
#1	5390
#2	5406
#3	5405
#4	5405
#5	5406
Average	**5402.7**
SD	**6.1**
% RSD	**0.1**

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

PRECISION - Method Repeatability

The full HPLC method as described in SI-IAG-206-02 was carried-out on the finished product IAG-21-001 for the 20mg dosage form. The method repeated six times and the relative standard deviation (RSD) was calculated.

SAMPLE Number	%ASSAY of labeled amount
I	96.9
II	97.8
III	98.2
IV	97.4
V	97.7
VI	98.5
(%) Average	97.7
SD	0.6
(%) RSD	0.6

PRECISION - Intermediate Precision

The full method as described in SI-IAG-206-02 was carried-out on the finished product IAG-21-001 for the 20mg dosage form. The method was repeated six times by a second analyst on a different day using a different HPLC instrument. The average assay and the relative standard deviation (RSD) were calculated.

SAMPLE Number	% ASSAY of labeled amount
I	98.3
II	96.3
III	94.6
IV	96.3
V	97.8
VI	93.3
Average (%)	96.1
SD	2.0
RSD (%)	2.1

The difference between the average results of method repeatability and the intermediate precision is 1.7%.

AM-V-0206-0300

ANALYTICAL METHOD PROCEDURES

Total Number of Pages: 24

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

LINEARITY

Standard solutions were prepared at 50% to 200% of the nominal concentration required by the assay procedure. 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
50	0.2244	2853
75	0.3365	4269
100	0.4244	5409
150	0.6617	8466
200	0.8822	11265
Correlation coefficient (RSQ)		**0.99999**
Slope		**12810.3**
$\frac{\text{Y - Intercept}}{\text{response at 100\%}} * 100\ (\%)$		**0.5%**

RANGE

Different concentrations of the sample (IAG-21-001) for the 20mg dosage form were prepared, covering between 50% - 200% of the nominal weight of the sample.

Conc. (%)	Conc. (mg/mL)	Peak Area	% Assay of labeled amount
50	0.20116	2350	96.7
70	0.27935	3340	99.2
100	0.39734	4632	96.6
150	0.64480	7577	97.5
200	0.79448	9394	97.9
(%) Average			**97.6**
SD			**1.0**
(%) RSD			**1.0**

ED. N0: 04	Effective Date:	APPROVED:			
Ed. Status : Supcds 03	DD/MM/2000	*Anne* ANALYST	*Bella* SUPERVISOR	*Edanna* QC	*Carol* HEAD

ISSN 0793 694X US/Canada ISSN 0793 7784 Euro ISSN 0793 7822 Pacific Rim

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

RANGE (cont.)

The results demonstrate linearity as well over the specified range.

Correlation coefficient (RSQ)0.99981 Slope 11808.3

$$\frac{\text{Y - Intercept}}{\text{response at 100\%}} * 100\ (\%) \quad 0.3\%$$

ACCURACY

ACCURACY OF STANDARD INJECTIONS

Five (5) replicate injections of the working standard solution at concentration of 0.4242mg/mL, as described in method SI-IAG-206-02 were made.

INJECTION NO.	PEAK AREA	% ACCURACY
I	5392	99.7
II	5405	99.9
III	5404	99.9
IV	5406	100.0
V	5407	100.0
Average	**5402.8**	**99.9%**
SD	**6.1**	**0.1**
RSD, (%)	**0.1**	**0.1**

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

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

ACCURACY OF THE DRUG PRODUCT

Admixtures of non-actives (placebo, batch IAG-21-001) with Fluoxetine HCl were prepared at the same proportion as in a capsule (70%-180% of the nominal concentration).

Three preparations were made for each concentration and the recovery was calculated.

Conc. (%)	Placebo Wt. (mg)	Fluoxetine HCl Wt. (mg)	Peak Area	% Accuracy	Average (%)
70%					
70	79.47	7.84	3465	102.2	
70	79.68	7.87	3427	100.7	
70	79.61	8.01	3465	100.0	101.0
100%					
100	79.62	11.25	4763	97.9	
100	80.80	11.42	4917	99.6	
100	79.60	11.42	4854	98.3	98.6
130%					
130	79.72	14.90	6405	99.4	
130	80.31	14.75	6328	99.2	
130	81.33	14.76	6402	100.3	99.6
180	79.99	20.10	8636	99.3	
180	79.38	20.45	8794	99.4	
180	80.08	20.32	8748	99.5	99.4
Placebo, Batch Lot IAG-21-001					

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

VALIDATION OF FLUOXETINE HCl AT LOW CONCENTRATION

LINEARITY AT LOW CONCENTRATIONS

Standard solution of Fluoxetine were prepared at approximately 0.02%-1.0% of the working concentration required by the method SI-IAG-206-02. Linear regression analysis demonstrated acceptability of the method for quantitative analysis over this range.

ACCURACY OF FLUOXETINE HCl AT LOW CONCENTRATION

The peak areas of the standard solution at the working concentration were measured and the percent deviation from the true value, as determined from the linear regression was calculated.

SAMPLE	CONC. µg/100mL	AREA FOUND	% ACCURACY
I	470.5	62584	99.7
II	470.5	63590	98.1
III	470.5	61585	101.3
IV	470.5	61940	100.7
V	470.5	62525	99.8
VI	470.5	62715	99.5
(%) Average	Slope = 132.73952		99.9
SD	Y-Intercept = -65.87237		1.1
(%) RSD			1.1

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

System Repeatability

Six replicate injections of standard solution at 0.02% and 0.05% of working concentration as described in method SI-IAG-206-02 were made and the relative standard deviation was calculated.

SAMPLE	FLUOXETINE HCl AREA	
	0.02%	0.05%
I	1017	3623
II	1150	3731
III	1010	3475
IV	1062	3390
V	1039	3315
VI	1095	3235
Average	**1062**	**3462**
RSD, (%)	**5.0**	**5.4**

Quantitation Limit - QL

The quantitation limit (QL) was established by determining the minimum level at which the analyte was quantified. The quantitation limit for Fluoxetine HCl is 0.02% of the working standard concentration with resulting RSD (for six injections) of 5.0%.

Detection Limit - DL

The detection limit (DL) was established by determining the minimum level at which the analyte was reliably detected. The detection limit of Fluoxetine HCl is about 0.01% of the working standard concentration.

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

VALIDATION FOR META-FLUOXETINE HCl

(EVALUATING POSSIBLE IMPURITIES)

Meta-Fluoxetine HCl linearity at 0.05% - 1.0%

Relative Response Factor (F)

Relative response factor for Meta-Fluoxetine HCl was determined as slope of Fluoxetine HCl divided by the slope of Meta-Fluoxetine HCl from the linearity graphs (analysed at the same time).

$$F = \frac{132.73952}{74.859534} = 1.8$$

Detection Limit (DL) for Fluoxetine HCl

The detection limit (DL) was established by determining the minimum level at which the analyte was reliably detected.

Detection limit for Meta Fluoxetine HCl is about 0.02%.

Quantitation Limit (QL) for Meta-Fluoxetine HCl

The QL is determined by the analysis of samples with known concentration of Meta-Fluoxetine HCl and by establishing the minimum level at which the Meta-Fluoxetine HCl can be quantified with acceptable accuracy and precision.

Six individual preparations of standard and placebo spiked with Meta-Fluoxetine HCl solution to give solution with 0.05% of Meta Fluoxetine HCl, were injected into the HPLC and the recovery was calculated.

AM-V-0206-0300

ANALYTICAL METHOD PROCEDURES

Total Number of Pages: 24

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

META-FLUOXETINE HCl

[RECOVERY IN SPIKED SAMPLES].

Approx. Conc. (%)	Known Conc. (µg/100ml)	Area in Spiked Sample	Found Conc. (µg/100mL)	Recovery (%)
0.05	21.783	3261	25.735	118.1
0.05	21.783	3268	25.821	118.5
0.05	21.783	2920	21.557	99.0
0.05	21.783	3241	25.490	117.0
0.05	21.783	2872	20.969	96.3
0.05	21.783	3285	26.030	119.5
(%) AVERAGE	The recovery result of 6 samples is between 80%-120%.			111.4
SD				10.7
(%) RSD	QL for Meta Fluoxetine HCl is 0.05%.			9.6

Accuracy for Meta Fluoxetine HCl

Determination of Accuracy for Meta-Fluoxetine HCl impurity was assessed using triplicate samples (of the drug product) spiked with known quantities of Meta Fluoxetine HCl impurity at three concentrations levels (namely 80%, 100% and 120% of the specified limit - 0.05%).

The results are within specifications:

For 0.4% and 0.5% recovery of 85% -115%

For 0.6% recovery of 90%-110%

AM-V-0206-0300

ANALYTICAL METHOD PROCEDURES

Total Number of Pages: 24

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

META-FLUOXETINE HCl

[RECOVERY IN SPIKED SAMPLES]

Approx. Conc. (%)	Known Conc. (µg/100mL)	Area in spiked Sample	Found Conc. (µg/100mL)	Recovery (%)
[0.4%]				
0.4	174.26	14283	182.66	104.82
0.4	174.26	14606	187.11	107.37
0.4	174.26	14351	183.59	105.36
[0.5%]				
0.5	217.83	17344	224.85	103.22
0.5	217.83	16713	216.15	99.23
0.5	217.83	17341	224.81	103.20
[0.6%]				
0.6	261.39	18367	238.95	91.42
0.6	261.39	20606	269.81	103.22
0.6	261.39	20237	264.73	101.28
RECOVERY DATA DETERMINED IN SPIKED SAMPLES				

ED. N0: 04	Effective Date:	APPROVED:			
Ed. Status : Supcds 03	DD/MM/2000	*Anne* ANALYST	*Bella* SUPERVISOR	*Edanna* QC	*Carol* HEAD

ISSN 0793 694X US/Canada ISSN 0793 7784 Euro ISSN 0793 7822 Pacific Rim

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

REPEATABILITY

Method Repeatability - Meta Fluoxetine HCl

The full method (as described in SI-IAG-206-02) was carried out on the finished drug product representing lot number IAG-21-001-(1). The HPLC method repeated serially, six times and the relative standard deviation (RSD) was calculated.

IAG-21-001 20mg CAPSULES - FLUOXETINE

Sample	% Meta Fluoxetine	% Meta-Fluoxetine[1] in Spiked Solution
1	0.026	0.095
2	0.027	0.086
3	0.032	0.077
4	0.030	0.074
5	0.024	0.090
6	0.028	0.063
AVERAGE (%)	**0.028**	**0.081**
SD	**0.003**	**0.012**
RSD, (%)	**10.3**	**14.5**

[1]**NOTE**:

All results are less than QL (0.05%) therefore spiked samples with 0.05% Meta Fluoxetine HCl were injected.

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

Intermediate Precision - Meta-Fluoxetine HCl

The full method as described in SI-IAG-206-02 was applied on the finished product IAG-21-001-(1) .

It was repeated six times, with a different analyst on a different day using a different HPLC instrument.

The difference between the average results obtained by the method repeatability and the intermediate precision was less than 30.0%, (11.4% for Meta-Fluoxetine HCl as is and 28.5% for spiked solution).

IAG-21-001 20mg - CAPSULES FLUOXETINE

Sample N°:	Percentage Meta-fluoxetine	% Meta-fluoxetine[1] in spiked solution
1	0.026	0.069
2	0.027	0.057
3	0.012	0.061
4	0.021	0.058
5	0.036	0.055
6	0.027	0.079
(%) AVERAGE	**0.025**	**0.063**
SD	**0.008**	**0.009**
(%) RSD	**31.5**	**14.5**

[1]NOTE:

All results obtained were well below the QL (0.05%) thus spiked samples slightly greater than 0.05% Meta-Fluoxetine HCl were injected. The RSD at the QL of the spiked solution was 14.5%

AM-V-0206-0300

ANALYTICAL METHOD PROCEDURES

Total Number of Pages: 24

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

SPECIFICITY - STABILITY INDICATING EVALUATION

Demonstration of the Stability Indicating parameters of the HPLC assay method [SI-IAG-206-02] for Fluoxetine 10 & 20mg capsules, a suitable photo-diode array detector was incorporated utilizing a commercial chromatography software managing system[2], and applied to analyze a range of stressed samples of the finished drug product.

GLOSSARY of PEAK PURITY RESULT NOTATION (as reported[2]):

Purity Angle - is a measure of spectral non-homogeneity across a peak, i.e. the weighed average of all spectral contrast angles calculated by comparing all spectra in the integrated peak against the peak apex spectrum.

Purity Threshold - is the sum of noise angle[3] and solvent angle[4]. It is the limit of detection of shape differences between two spectra.

Match Angle - is a comparison of the spectrum at the peak apex against a library spectrum.

Match Threshold - is the sum of the match noise angle[3] and match solvent angle[4].

[3]**Noise Angle** - is a measure of spectral non-homogeneity caused by system noise.

[4]**Solvent Angle** - is a measure of spectral non-homogeneity caused by solvent composition.

OVERVIEW

The assay of the main peak in each stressed solution is calculated according to the assay method SI-IAG-206-02, against the Standard Solution, injected on the same day.

If the Purity Angle is smaller than the Purity Threshold and the Match Angle is smaller than the Match Threshold, no significant differences between spectra can be detected. As a result no spectroscopic evidence for co-elution is evident and the peak is considered to be pure.

The stressed condition study indicated that the Fluoxetine peak is free from any appreciable degradation interference under the stressed conditions tested. Observed degradation products peaks were well separated from the main peak.

[1]® PDA-996 Waters™ ; [2][Millennium 2010]

ED. N0: 04	Effective Date:	APPROVED:			
Ed. Status : Supcds 03	DD/MM/2000	Anne	Bella	Edanna	Carol
		ANALYST	SUPERVISOR	QC	HEAD

ISSN 0793 694X US/Canada ISSN 0793 7784 Euro ISSN 0793 7822 Pacific Rim

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

FORCED DEGRADATION OF FINISHED PRODUCT & STANDARD

1. UNSTRESSED SAMPLE

1.1. Sample IAG-21-001 (2) (20mg/capsule) was prepared as stated in SI-IAG-206-02 and injected into the HPLC system. The calculated assay is 98.5%.

SAMPLE - UNSTRESSED

Fluoxetine:	Purity Angle	:0.075	Match Angle	:0.407
	Purity Threshold	:0.142	Match Threshold	:0.425

1.2. Standard solution was prepared as stated in method SI-IAG-206-02 and injected into the HPLC system. The calculated assay is 100.0%.

Fluoxetine:	Purity Angle	:0.078	Match Angle	:0.379
	Purity Threshold	:0.146	Match Threshold	:0.427

2. ACID HYDROLYSIS

2.1. Sample solution of IAG-21-001 (2) (20mg/capsule) was prepared as in method SI-IAG-206-02 : An amount equivalent to 20mg Fluoxetine was weighed into a 50mL volumetric flask. 20mL Diluent was added and the solution sonicated for 10 minutes. 1mL of conc. HCl was added to this solution The solution was allowed to stand for 18 hours, then adjusted to about pH = 5.5 with NaOH 10N, made up to volume with Diluent and injected into the HPLC system after filtration.

Fluoxetine peak intensity did NOT decrease. Assay result obtained - 98.8%.

SAMPLE- ACID HYDROLYSIS

Fluoxetine peak:	Purity Angle	:0.055	Match Angle	:0.143
	Purity Threshold	:0.096	Match Threshold	:0.371

2.2. Standard solution was prepared as in method SI-IAG-206-02 : about 22mg Fluoxetine HCl were weighed into a 50mL volumetric flask. 20mL Diluent were added. 2mL of conc. HCl were added to this solution. The solution was allowed to stand for 18 hours, then adjusted to about pH = 5.5 with NaOH 10N, made up to volume with Diluent and injected into the HPLC system.

Fluoxetine peak intensity did NOT decrease. Assay result obtained - 97.2%.

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

STANDARD - ACID HYDROLYSIS

Fluoxetine peak:	Purity Angle	:0.060	Match Angle	:0.060
	Purity Threshold	:0.099	Match Threshold	:0.371

3. BASE HYDROLYSIS

3.1. Sample solution of IAG-21-001 (2) (20mg/capsule) was prepared as per method SI-IAG-206-02 : An amount equivalent to 20mg Fluoxetine was weight into a 50mL volumetric flask. 20mL Diluent was added and the solution sonicated for 10 minutes. 1mL of 5N NaOH was added to this solution. The solution was allowed to stand for 18 hours, then adjusted to about pH = 5.5 with 5N HCl, made up to volume with Diluent and injected into the HPLC system.

Fluoxetine peak intensity did NOT decrease. Assay result obtained - 99.3%.

SAMPLE - BASE HYDROLYSIS

Fluoxetine peak:	Purity Angle	:0.063	Match Angle	:0.065
	Purity Threshold	:0.099	Match Threshold	:0.362

3.2. Standard stock solution was prepared as per method SI-IAG-206-02 : About 22mg Fluoxetine HCl was weighed into a 50mL volumetric flask. 20mL Diluent was added. 2mL of 5N NaOH was added to this solution. The solution was allowed to stand for 18 hours, then adjusted to about pH=5.5 with 5N HCl, made up to volume with Diluent and injected into the HPLC system.

Fluoxetine peak intensity did NOT decrease - 99.5%.

STANDARD - BASE HYDROLYSIS

Fluoxetine peak:	Purity Angle	:0.081	Match Angle	:0.096
	Purity Threshold	:0.103	Match Threshold	:0.363

4. OXIDATION

4.1. Sample solution of IAG-21-001 (2) (20mg/capsule) was prepared as per method SI-IAG-206-02. An equivalent to 20mg Fluoxetine was weighed into a 50mL volumetric flask. 20mL Diluent added and the solution sonicated for 10 minutes.

1.0mL of 30% H_2O_2 was added to the solution and allowed to stand for 5 hours, then made up to volume with Diluent, filtered and injected into HPLC system.

Fluoxetine peak intensity decreased to 95.2%.

SAMPLE - OXIDATION

Fluoxetine peak:	Purity Angle	:0.090	Match Angle	:0.400
	Purity Threshold	:0.154	Match Threshold	:0.429

4.2. Standard solution was prepared as in method SI-IAG-206-02 : about 22mg Fluoxetine HCl were weighed into a 50mL volumetric flask and 25mL Diluent were added. 2mL of 30% H_2O_2 were added to this solution which was standing for 5 hours, made up to volume with Diluent and injected into the HPLC system.

Fluoxetine peak intensity decreased to 95.8%.

STANDARD - OXIDATION

Fluoxetine peak:	Purity Angle	:0.083	Match Angle	:0.416
	Purity Threshold	:0.153	Match Threshold	:0.429

5. SUNLIGHT

5.1. Sample solution of IAG-21-001 (2) (20mg/capsule) was prepared as in method SI-IAG-206-02 . The solution was exposed to 500w/hr. cell sunlight for 1hour. The BST was set to 35°C and the ACT was 45°C. The vials were placed in a horizontal position (4mm vials, National + Septum were used). A Dark control solution was tested. A 2%w/v quinine solution was used as the reference absorbance solution.

Fluoxetine peak decreased to 91.2% and the dark control solution showed assay of 97.0%.

The difference in the absorbance in the quinine solution is 0.4227AU.

Additional peak was observed at RRT of 1.5 (2.7%).

The total percent of Fluoxetine peak with the degradation peak is about 93.9%.

SAMPLE - SUNLIGHT

Fluoxetine peak:	Purity Angle	:0.093	Match Angle	:0.583
	Purity Threshold	:0.148	Match Threshold	:0.825

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

SUNLIGHT (Cont.)

5.2. Working standard solution was prepared as in method SI-IAG-206-02 . The solution was exposed to 500w/hr. cell sunlight for 1.5 hour. The BST was set to 35°C and the ACT was 42°C. The vials were placed in a horizontal position (4mm vials, National + Septum were used). A Dark control solution was tested. A 2%w/v quinine solution was used as the reference absorbance solution.

Fluoxetine peak was decreased to 95.2% and the dark control solution showed assay of 99.5%.

The difference in the absorbance in the quinine solution is 0.4227AU.

Additional peak were observed at RRT of 1.5 (2.3).

The total percent of Fluoxetine peak with the degradation peak is about 97.5%.

STANDARD - SUNLIGHT

Fluoxetine peak:	Purity Angle	:0.067	Match Angle	:0.389
	Purity Threshold	:0.134	Match Threshold	:0.819

6. HEAT OF SOLUTION

6.1. Sample solution of IAG-21-001-(2) (20 mg/capsule) was prepared as in method SI-IAG-206-02 . Equivalent to 20mg Fluoxetine was weighed into a 50mL volumetric flask. 20mL Diluent was added and the solution was sonicated for 10 minutes and made up to volume with Diluent. 4mL solution was transferred into a suitable crucible, heated at 105°C in an oven for 2 hours. The sample was cooled to ambient temperature, filtered and injected into the HPLC system.

Fluoxetine peak was decreased to 93.3%.

SAMPLE - HEAT OF SOLUTION [105°C]

Fluoxetine peak:	Purity Angle	:0.062	Match Angle	:0.460
	Purity Threshold	:0.131	Match Threshold	:0.818

6.2. Standard Working Solution (WS) was prepared under method SI-IAG-206-02 . 4mL of the working solution was transferred into a suitable crucible, placed in an oven at 105°C for 2 hours, cooled to ambient temperature and injected into the HPLC system.

Fluoxetine peak intensity did not decrease - 100.5%.

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

STANDARD - HEAT OF SOLUTION

Fluoxetine peak:	Purity Angle	:0.105	Match Angle	:0.235
	Purity Threshold	:0.146	Match Threshold	:0.823

7. HEAT OF POWDER

Equivalent to 20mg Fluoxetine were weighed from powder of IAG-21-001-(2) (20mg/capsule) into a suitable test tube with cover and heated to 100°C for 4 hours. The powder after cooled to room temperature was transferred into a 50mL volumetric flask with 20mL Diluent, sonicated for 10 minutes, made up to volume with Diluent and injected into the HPLC system after filtration. - Fluoxetine peak intensity decreased to 86.6%.

SAMPLE - HEAT OF POWDER

Fluoxetine peak:	Purity Angle	:0.070	Match Angle	:0.039
	Purity Threshold	:0.107	Match Threshold	:0.119

About 45mg Fluoxetine HCl were weighed into a suitable test tube with cover and heated to 100°C for 4 hours. The powder after cooled to room temperature was transferred into a 100mL volumetric flask with Diluent, made up to volume with Diluent and injected into the HPLC system.

Fluoxetine peak intensity did not decrease significantly - 95.9%.

STANDARD - HEAT OF POWDER

Fluoxetine peak:	**Purity Angle**	**:0.062**	**Match Angle**	**:0.010**
	Purity Threshold	:0.099	Match Threshold	:0.116

SYSTEM SUITABILITY

A System Suitability Test was performed as described in the method SI-IAG-206-02 .

The resolution calculated (according to USP) between Meta Fluoxetine peak and Fluoxetine peak should be not less than 2.0.

The Tailing Factor [TF] for Fluoxetine peak should be not more than 2.0.

Typical retention time of Fluoxetine peak is about 9.5 - 10.0 minutes.

AM-V-0206-0300

ANALYTICAL METHOD PROCEDURES

Total Number of Pages: 24

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

PLACEBO

A mixture of non-actives (placebo, batch IAG-21-001-1 for 10mg dosage and IAG-21-001-3 for 20mg dosage) was prepared and subjected to analysis. No interfering peaks were observed.

STABILITY OF STANDARD & SAMPLE SOLUTIONS

STANDARD SOLUTION TEST

A Standard Solution (1) was initially prepared, held under refrigerated conditions [4 ^{o} - 8^{o} C] for up to 30 days and tested against two freshly prepared Standard Solutions (2) and (3).

Standard No.	1	2	3
Weight (mg)	21.91	21.83	21.35
Preparation Date	23.03.98	23.03.98	23.02.98
Response	5870 5900 5869 5885 5896	5898 5894	5776 5778
Average RSD (%)	5884 0.3	5895	5776

Comparison between Standards (%)

Standard	2	3
1	0.56	0.75
2	-	0.18
3	0.18	-

CONCLUSION

Under refrigerated conditions 4 ^{o} - 8^{o} C the Standard Solution remains stable for up to 30 days storage

ED. N0: 04	Effective Date:	APPROVED:			
Ed. Status : Supcds 03	DD/MM/2000	*Anne* ANALYST	*Bella* SUPERVISOR	*Edanna* QC	*Carol* HEAD

ISSN 0793 694X US/Canada ISSN 0793 7784 Euro ISSN 0793 7822 Pacific Rim

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

SAMPLE SOLUTIONS

Two sample solutions from batch IAG-21-001 (20mg/capsule) were prepared and analyzed as in method. The sample solution was refrigerated for 48 hours and re-analyzed.

Preparation Date	Analysis Date	% Assay	Average (%)
26.12.96	26.02.98	96.8 97.9	97.4
	30.02.98	96.9 98.9	97.9

ROBUSTNESS

EXTRACTION EFFICIENCY PROFILE

Demonstration of the method's extraction efficiency was achieved using triplicate sample solutions as described in the HPLC method via the selection of the three extraction times - 5 minutes apart - as shown in the table.

EXTRACTION EFFICIENCY		
Sonication Time (min.)	**Assay Percentage**	**Average Percentage**
5	95.3 97.7 98.0	97.0
10	98.4 98.3 96.8	97.8
15	98.5 97.9 98.1	98.2
20mg dose	Lot : IAG-21-001	

The results demonstrated that an extraction time of 10 minutes by sonication is suitable to ensure a complete extraction of the active material.

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

ROBUSTNESS - (Cont.)

SPECIFICATION - FACTORIAL DESIGN

The HPLC condition as performed in the analytical method SI-IAG-206-02 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)

Flow rate : 1.4 - 1.6mL/min

Temperature : 30°C (±2°C)

Injection volume : 10µL

Detector : UV detector λ = 227nm, Flow Cell = 10mm.

Diluent : SOLUTION B

The factorial design variations were performed on the above set of specifications. Changes were made to the following parameters, using the system suitability parameters.

percentage eluent composition
flow rate parameters
column temperature
column [tpe/make]

Incident	Column Temp. (°C)	Flow Rate (mL/min.)	% of Solution A	HPLC COLUMN TYPE
1	30	1.5	55	Recommended HPLC column [Shandon] Hypersil, BDS C-8, 5u, 150 x 4.6mm
-1	27	1.0	60	Alternative HPLC column Hypersil, BDS C-8, 5u, 150 x 4.6mm

HPLC ASSAY with DETERMINATION OF META-FLUOXETINE HCl.
ANALYTICAL METHOD VALIDATION
10 and 20mg Fluoxetine Capsules HPLC Determination

FACTORIAL DESIGN (CONT.)

Run Number	% Solution A	Column	Flow Rate (mL/min)	Temp (°C)	Tailing Factor [TF]	Resolution[1]	Retention Time [min]
1	1	1	1	1	1.5	2.6	9.4
2	1	-1	1	-1	1.3	2.6	9.6
3	-1	-1	1	1	1.3	2.7	9.9
4	-1	1	1	-1	1.4	2.7	10.6
5	1	-1	-1	1	1.5	2.8	13.4
6	1	1	-1	-1	1.3	2.8	14.8
7	-1	1	-1	1	1.5	2.8	15.2
8	-1	-1	-1	-1	1.4	2.8	15.7

NOTES

[1]The resolution is calculated between the Fluoxetine and Meta-Fluoxetine peaks

Conclusion of results from Factorial Design - based on the obtained results, the analytical procedures are determined to be robust.

CONCLUSION

HPLC Method SI-IAG-206-02 for assay evaluation was demonstrated to be both accurate and precise for performing the stability indicating assay and the determination of Meta-Fluoxetine HCl analysis for product release and stability studies and profiling of Fluoxetine Capsules 10 and 20mg.

REFERENCES/HISTORY

1. Millennium 2010 chromatography manager, PDA software user's guide, manual No. 038993 revision 2, chap. 9, January 1993.
2. FLUOXETINE CAPSULES (Fluoxetine HCl equivalent to 10mg or 20mg Fluoxetine per capsule ASSAY AND DETERMINATION OF META-FLUOXETINE HCl-ANALYTICAL METHOD VALIDATION REPORT IAG-SI-V-01- EU IAG-206-02 /10/20-013.
3. USP/NF XXIV Rockville Maryland USA
4. Martindale Pharmaceutical UK
5. DAB FRG and Eu. Pharm.
6. IAG-SI -221X8-03 FLUOXETINE CAPSULES 03-97-03 Containing: Fluoxetine HCl equivalent to 10mg or 20mg Fluoxetine per capsule ASSAY FOR STABILITY STUDIES.
7. Int. J of Drug R&D Vol. 01 No 06 Analytical Overview of FLUOXETINE
8. Stark, P. And Hardison, C.D. (1985). J. Clin. Psychiatry, 46, (3, Sec. 2), 53)