IJBPAS, May, 2022, 11(5): 2489-2498

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

QUANTIFICATION OF FLUVOXAMINE IN HUMAN PLASMA BY USING UPLC-MS/MS TECHNIQUE

ARUN KUMAR.V, R. GANDHIMATHI, M. VIJEY AANANDHI, M. SUMITHRA*

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India

***Corresponding Author: Dr. M. Sumithra: E Mail: sumithra.sps@velsuniv.ac.in; Ph. No.: 9444788949**

Received 20th May 2021; Revised 20th June 2021; Accepted 19th July 2021; Available online 1st May 2022

https://doi.org/10.31032/IJBPAS/2022/11.5.6101

ABSTRACT

Bio-analytical is the pathway for studying bioavailability and bioequvalence. The evaluation carried out by using some biological matrix loot. Here proper method development and validation is done with respective to guidelines. A sensitive and selective LC-MS/MS method to quantify Fluvoxamine in K_2EDTA human plasma over the concentration range 2.6260 to 294.0960 ng/mL was developed and validated. Fluvoxamine and its internal standard (Fluvoxamine D4) were selectively extracted from 250 µL plasma by liquid-liquid extraction technique. Separation was achieved by liquid-liquid extraction on Hypurity C18 (50 mm x 4.6 mm, 5µm) column. The plasma samples (250 µL) were processed using liquid-liquid extraction technique. The developed work is specific LC-MS/MS procedure for the determination of Fluvoxamine in human plasma to support bioequivalence/bioavailability studies.

Keywords: Bio-Analytical Method, UPLC-MS/MS, Coefficient Variation, Standard Deviation, Lower Limit of Quantification, Upper Limit of Quantification

INTRODUCTION

Bioanalysis is an extraordinary method **[1]** for quantitative determination of analytes in biological matrices **[2]**. Liquid chromatography Mass Spectroscopy (LCMS/MS) has wide range **[3]** of development in science and research field

for new molecule development and validation under guidelines **[4]**. The current trends in research field lies with ICH guidelines **[5]** LC innovation creation area in past 10 years is marvellous growth **[6]**. The proper range for column is 50-200 µm the particle size measures about ~30 -10 µm, and also well modified implemented injection with conventional detector with reducing band for broadening effects **[7]**.

The process discovering new method for evaluation is known for bioanalytical field **[8]**. The method development **[9]** process followed by liquid-liquid extraction .The application of LLE method is to extract drug absorbed in plasma by polarity separation **[10]**. This process of method is helpful to reduce the cost of sample process. The major advantage of this method less time consumption compared to other method development **[11]**. The structure and **[12]** profile detail refer **Table 1** and **Figure 1**. The development of bioequivalence and bioavailability **[13]** studies play a important role in the clinical trials. We are employed the cost effective LLE extraction **[14]** procedure for sample preparation without the interferences of biological matrices **[15]**.

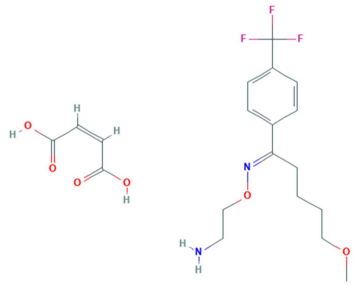

Figure 1: Chemical Structure of Fluvoxamine maleate

Table 1: Profile of Fluvoxamine maleate and IS

Profiles	Fluvoxamine	Fluvoxamine –D4
Drug Name	Fluvoxamine Maleate	Fluvoxamine-D4 Maleate
IUPAC Name	[(E)-[5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone O-(2-Aminoethyl)oxime Maleate	(E)-5-Methoxy-1-[4-(triflurormethyl)phenyl]-1-pentanone O-(2-Aminoethyl-d4)oxime Maleate
Chemical Formula	$C_{15}H_{21}F_3N_2O_2$	$C_{19}H_{21}D_4F_3N_2O$
Molecular Weight	318.33 g/mol	327.23 g/mol
Solubility	Crystalline powder freely soluble in ethanol and chloroform	Crystalline powder freely soluble in ethanol and chloroform
Physical Properties	Appearance : White to off white Colour : Crystalline powder Odour : Odourless	Appearance : White to off white Colour : Crystalline powder Odour : Odourless
Half life	12 to 13 hour	12 to 13 hour

MATERIALS AND METHOD

Materials

The Fluvoxamine (Working standard), Fluvoxamine D4 (internal standard) are collected from MTS Lab, Chennai. The sample are authenticated by melting and solubility studies.The UPLC –MS/MS (Waters Acquity) containing Liquid chromatography with triple quadrupole mass spectrometry and Mass Lynx 4.2 as software. The LLE extraction carried out and sample are dried by Low Volume Evaporator **[13]**. The following solvent are used in the process **[14]**. The Acetonitrile

(HPLC Grade), Acetone-M and water are used in HPLC grade. The Ammonium formate, ethyl acetate, formic acid are analytical grade **[15]**.

Preparation of 5.0 mM Ammonium Formate Solution with 0.1% Formic Acid buffer 1 solution:

Weighed 315.30 mg of Ammonium formate and transfer into 1000 mL volumetric flask containing water. Mix and Filter through 0.2 µm nylon membrane filter.

Preparation of Mobile Phase (Acetone-M: Buffer-1 (75:25, v/v))

Measure 750 mL of Acetone-M and 250 mL of buffer-1, transfer into 1000 mL reagent bottle. Mix well and filter through 0.2 µm nylon membrane filter. Degas the solution.

Prepartion of Buffer-2: (0.2 mM Sodium dihydrogen ortho phosphate dehydrate)

Weigh about 0.312 g of Sodium dihydrogen ortho phosphate dihydrate in 1000 mL of HPLC grade water.

Preparation of Fluvoxamine Stock Solution for CC (1000 µg/mL)

Weigh accurately about 2 mg of Fluvoxamine WS and transfer into a 2 mL volumetric flask. Add 2 mL of Acetone-M, dissolve and make up to the volume with Acetone-M. Calculate the final concentration of Fluvoxamine in µg/mL by following formula:

$$\frac{\text{Weight of Fluvoxamine taken in mg}}{\text{2 mL}} \times \frac{\text{Potency (as is basis)}}{100} \times \frac{M_1}{M_2} \times 1000$$

Where, M_1 is the molecular weight of Fluvoxamine (salt free) and M_2 is the molecular weight of Fluvoxamine (salt)

Preparation of Internal Standard Stock Solution (1000 µg/mL)

Weigh accurately about 2 mg of Fluvoxamine D4 and transfer into a 2 mL volumetric flask. Dissolve and make up to the volume with Acetone-M. Calculate the final concentration of Fluvoxamine D4 in µg/mL by following formula:

$$\frac{\text{Weight of Fluvoxamine D4 taken in mg}}{\text{2 mL}} \times \frac{\text{Potency (as is basis)}}{100} \times \frac{M_1}{M_2} \times 1000$$

Where, M_1 is the molecular weight of Fluvoxamine D4 (salt free) and M_2 is the molecular weight of Fluvoxamine D4 (salt). Store the prepared solution in refrigerator at 2°C to 8°C.

Internal standard solution

Prepare a solution of internal standard in the concentration of 1000 ng/mL for Fluvoxamine D4 in diluent as described in **Table 2**.

Spiked Calibration Curve Standards

Transfer 0.200mL aliquot of corresponding concentrations of standard Fluvoxamine stock solution into each 10 mL glass volumetric flask for separation and make up to the volume with screened, pooled, K_2EDTA plasma to attain the spiked amount of analyte to form calibration curve standards described in the **Table 3**.

Table 2: Preparation of Internal Standard solution for Fluvoxamine D4

Stock Concentration (ng/mL)	Stock Aliquot (µL)	Diluent Added (mL)	Final Volume (mL)	Final Concentration (ng/mL)
1000000.0000	100.000	99.900	100.000	1000.0000

Table 3: Preparation of Fluvoxamine Spiked Calibration Curve Standards

Stock CCID	Calibration Stock Concentration (µg/mL)	Calibration Stock Aliquot (mL)	Plasma Added (mL)	Final Volume (mL)	Final Concentration (ng/mL)	Spiked CC-ID
AQ-H	14.7342	0.200	9.800	10.000	294.6840	STD H
AQ-G	12.5241	0.200	9.800	10.000	250.4820	STD G
AQ-F	6.2621	0.200	9.800	10.000	125.2420	STD F
AQ-E	3.1311	0.200	9.800	10.000	62.6220	STD E
AQ-D	1.5656	0.200	9.800	10.000	31.3120	STD D
AQ-C	0.6262	0.200	9.800	10.000	12.5240	STD C
AQ-B	0.3757	0.200	9.800	10.000	7.5140	STD B
AQ-A	0.1315	0.200	9.800	10.000	2.6300	STD A

Pipette 350 µL aliquot of each spiked calibration standard into an appropriate polypropylene capped tubes and store in ultra low temperature freezer at -70°C ± 15°C.

Preparation of Fluvoxamine Stock Solution for QC (1000 µg/mL)

Weigh accurately 2 mg of Fluvoxamine WS and transfer into a 2 mL volumetric flask. Add 1 mL of Acetone-M, dissolve and make up to the volume with Acetone-M. Calculate the final concentration of Fluvoxamine in µg/mL as follows in **Table 4**.

$$\frac{\text{Weight of Fluvoxamine taken in mg}}{\text{2 mL}} \times \frac{\text{Potency (as is basis)}}{100} \times \frac{M_1}{M_2} \times 1000$$

Table 4: Preparation of Stock solutions of Fluvoxamine for QC

Stock Concentration (µg/mL)	Stock Aliquot (mL)	Diluent Added (mL)	Final Volume (mL)	Final Concentration (µg/mL)	Stock QC-ID
736.7076	0.100	1.900	2.000	36.8354	QCSTD
36.8354	0.680	1.320	2.000	12.524	AQ-HQC
12.524	1.000	1.000	2.000	6.262	AQ-MQC
6.262	0.500	1.500	2.000	1.5655	AQ-INTQC
1.5655	0.500	1.500	2.000	0.3914	AQ-LQC
0.3914	0.690	1.310	2.000	0.1350	AQ-LOQQC

Method Validation: Sample Preparation

To vortex the thawed samples to ensure complete mixing of contents. Add 50 µL of internal standard solution (Fluvoxamine D4 10 ng/mL) to all the samples except blank. Pipette 250 µL of

samples into respectively labeled RIA vials containing internal standard solution and vortex. Add 200 µL of Buffer-2 toall the samples and vortex. Add 2.5 mL of Ethyl Acetate to all the samples and cap them. Centrifuge the samples at 3500 rpm for 5 minutes in a refrigerated centrifuge between 2°C to 8°C. Transfer 1.0 mL of supernatant in respectively labeled RIA vials. Dry all the samples under nitrogen evaporator at 40°C and 15 psi. Reconstitute the dried residue with 500 µL of mobile phase and vortex. Transfer the sample into respectively labeled auto-injector vials. Load the processed samples into LC-MS/MS.

LCMS/MS conditions:

The UPLC-MS/MS condition described in the **Table 5** the mode of ionization positive.

CHROMATOGRAPHIC CONDITIONS

Column	**: Hypurity C18 (50 mm x 4.6 mm, 5µm)**
Mobile phase	**: Acetone-M: Buffer-1 (75:25, v/v)**
Injection volume	**: 10 µL**
Flow rate	**: 0.600 mL/min**
Column oven / Auto sampler temperature: 40°C / 10°C	
Expected Retention time	**: For Analyte: 1.25 ± 0.30 minutes**
	For Internal standard: 1.25 ± 0.30 minutes
Total run time	**: 3.0 minutes**

Table 5: LCMS/MS conditions in XEVO TQ MS instrument Parameters

Molecule	Parent (m/Z)	Daughter (m/Z)	Dwell (sec)	Cone (Volts)	Collision energy (eV)
Fluvoxamine	319.30	70.99	0.2	50	20
Fluvoxamine D4	323.23	71.04	0.2	50	20

RESULT AND DISCUSSION

A sensitive and selective LC-MS/MS method to quantify Fluvoxamine in K_2EDTA human plasma over the concentration range 2.6260 to 294.0960 ng/mL was developed and validated. Fluvoxamin**e** and its IS (Fluvoxamine D4) described in the Table 09 were selectively extracted from 250 µL plasma by liquid-liquid extraction technique on Hypurity C18 (50 mm x 4.6 mm, 5µm) column.

Biological Matrix

The Blank K_2EDTA human plasma lots were used for screening loot detail and the characteristics are given in **Table 6**. The Plasma sample are procured from Micro Therapeutic Research Labs Pvt. Limited, Chennai.

Calibration Curve Standards and Quality Control Samples

Calibration curve standards and quality control all samples were prepared as per

ICH guidelines and stored in –70 ± 15ºC and –20º ± 5ºC.

VALIDATION AND CHARACTERISTICS OF METHOD

K_2EDTA Plasma Screening

Selectivity was evaluated by analysing a total of nine lots (six lots of blank K_2EDTA human normal plasma, one lot of hemolysed plasma, one lot of lipemic plasma and one lot of heparin plasma), the demonstrating acceptance criteria were met and the sample are complies the test are described in the **Table 7**. Signal-to-Noise ratios ranged from 9.037 to 3155.376 across the matrix lots evaluated, demonstrating acceptable S/N intensity.

Carry Over Test

Carry over is calculated as the percentage peak area observed in a processed blank plasma injected in duplicate immediately after a processed ULOQ calibration standard which were used from PA batch sample. No significant carry over observed for Fluvoxamine and its internal standard **(Table 8)**.

Linearity

Linearity concentration is carried out by preparing an eight-point standard calibration curve in K_2EDTA human plasma. The Fluvoxamine concentration ranged from 2.6260 to 294.0960 ng/mL using Fluvoxamine D4 as internal standard and for CC curve refer in **Figure 4**.

Precision and Accuracy

Assay precision and accuracy values were by analyzing six replicates each of LLOQC, LQC, MQC, and HQC samples are described in the **Table 9**. For chromatogram of refer in **Figure 2**, Hrefer in **Figure 3**.

Recovery studies of Fluvoxamine

The recovery of Fluvoxamine was determined by comparing the detector response of Analyte at three distinct levels of extracted low, medium and high quality control samples from extracted Calibration curve and quality control samples from PA batch with detector response obtained from un-extracted aqueous samples. The average recovery of Fluvoxamine was 62.74%. The percentage CV for Fluvoxamine was 3.10% at three different QC level. The mean recovery of IS was 84.55 % for Fluvoxamine D4 **(Table 10)**.

Analyte	:	Fluvoxamine
Matrix	:	K_2EDTA Human Plasma Validated
CC range (ng/mL)	:	2.6260 to 294.0960

Table 6: Loot details

Supplier	Micro Therapeutic Research Labs Pvt. Limited, Chennai
# Plasma Batch ID /Lot No.	MAT-0511-II, MAT-0512-II, MAT-0513-II, MAT-0514-II, MAT-0516-II, MAT-0517-II, MAT-0617-III (Heparin), MAT-0515-II (Hemolysed) and MAT-0510-II (Lipemic)

Table 7: K2EDTA Plasma screening for Fluvoxamine and Internal Standard

Plasma Lot ID	Specificity (Blank)		Selectivity (Spiked LLOQ)		% Interference in Blank		Area Ratio	S/N Ratio (≥5)
	Analyte	IS peak	Analyte	IS peak	Analyte (<20%)	IS (<5%)	Analyte/IS	Analyte
Blank-MAT-0511	1408	3878	67467	986508	2.0869		0.068	2261.256
Blank-MAT-0512	940	3259	66580	981214	1.4118	0.3321	0.068	3155.376
Blank-MAT-0513	790	2675	68098	996960	1.1601	0.2683	0.068	1420.591
Blank-MAT-0514	1016	2588	67538	997731	1.5043	0.2594	0.068	2048.873
Blank-MAT-0516	877	2424	67184	1001293	1.3054	0.2421	0.067	1400.245
Blank-MAT-0517	769	1993	69227	1027938	1.1108	0.1939	0.067	1365.561
Blank-MAT-0617 (Heparin)	996	3373	68292	993727	1.4584	0.3394	0.069	1641.093
Blank-MAT-0515 (Hemolysed)	607	1659	67921	985625	0.8937	0.1683	0.069	1909.837
Blank-MAT-0510 (Lipimic)	985	3201	68527	988887	1.4374	0.3237	0.069	1630.802
			Mean	995542.55556	1.37433	0.26591	0.06811	
						SD	0.000782	
% of Lots passing =	100.00	%				%CV	1.15	
					Result	Pass		

Table 8: Carry Over Test for Fluvoxamine and Fluvoxamine D4

Sample ID	Analyte peak area	IS peak area
Extracted blank	784	10
Extracted LLOQ+IS	58327	572095
Extracted ULOQ+IS	5777505	599557
Extracted blank-I	821	46
Extracted blank-II	1126	43
% Carry Over from Blank I	0.06	0.01
% Carry Over from Blank II	0.59	0.01
Result for blank - I	Pass	Pass
Result for blank - II	Pass	Pass

Table 9: Precision and accuracy study for Fluvoxamine

QC ID	LLOQC	LQC	MQC	HQC
Actual Concentration (ng/mL)	2.6960	7.8120	124.9900	249.9800
Calculated Concentrations (ng/mL)	2.9000	8.8000	124.3000	243.0000
	2.9000	7.4000	122.3000	227.5000
	2.7000	7.5000	116.8000	237.2000
	2.6000	8.3000	120.8000	262.0000
	3.0000	6.9000	122.0000	263.1000
	2.7000	7.9000	120.7000	255.3000
Mean	2.80000	7.80000	121.15000	248.01667
SD	0.154919	0.681175	2.498600	14.416576
%CV	5.53	8.73	2.06	5.81
%Nominal	103.86	99.85	96.93	99.21

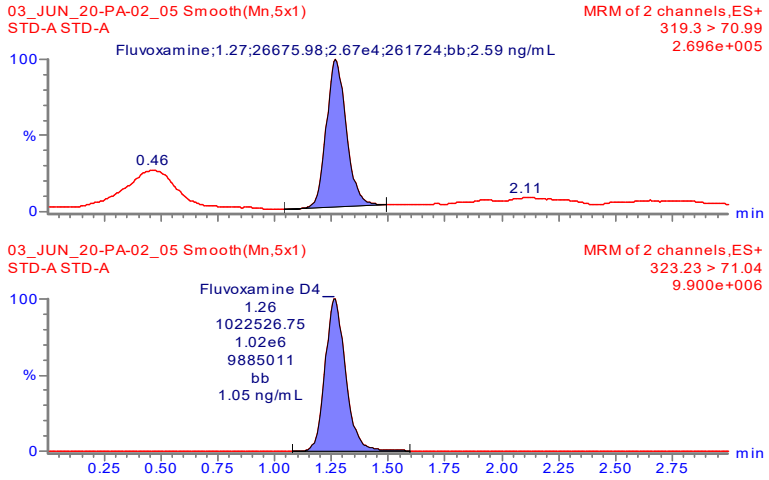

Figure 2: Representative Chromatogram of Standard A for Fluvoxamine

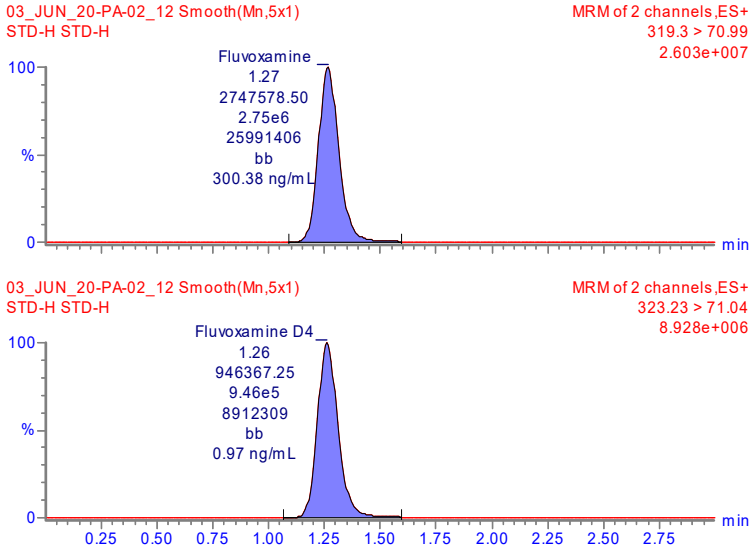

Figure 3: Representative Chromatogram of Standard H Sample for Fluvoxamine

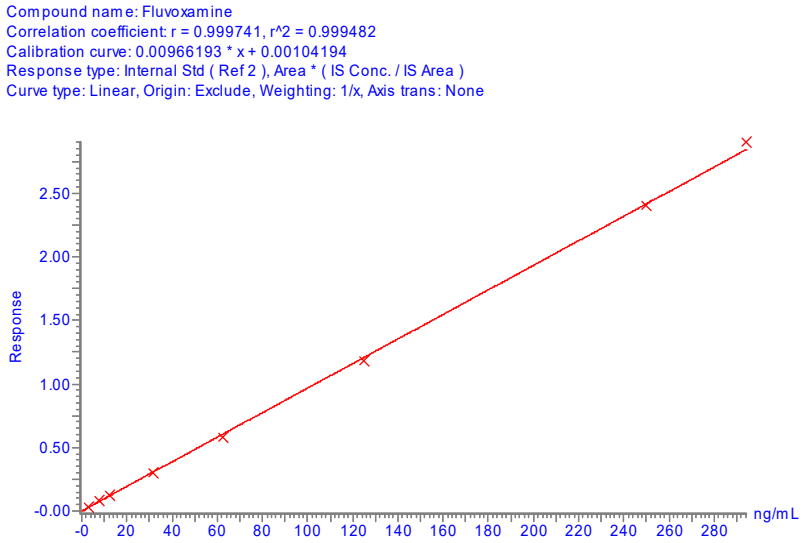

Figure 4: Calibration Curve for Fluvoxamine

Table 10: Summary of Experimental Parameters & Results of Validated LC- MS/MS Method for the Quantification of Fluvoxamine in K_2EDTA Human Plasma

S. No.	Experimental Parameters	Acceptable Range/Criteria (in %)	Result Obtained (in %)
1	Specificity and Selectivity % of passing lots (%CV of Area Ratio)	>80 ≤ 20	100 4.28%
2	Carry Over Test	Analyte < 20 IS < 5	8.36 -0.12
3	Recovery	≤110%	Analyte- 66.72% IS- 83.04%

CONCLUSION

The bio-analytical method development is developed as per ICH guidelines, a successive and sensitive method bought from UPLC-MS/MS and this method is to quantify Fluvoxamine in matrix loot K_2EDTA plasma from human blood and spiked for a range of concentration 2.6260 to 294.0960 ng/mL .It has been successfully validated and the total summary of experimental parameters and resulted value are described in the **Table 10**. This cost efficient and time saving LLE method has more positive advantages and this new updated method is very suitable for LLE method of sample analysis to bring BA/BE studies involving in the API formulations of Fluvoxamine.

REFERENCE

[1] Arun Kumar V, Vijey Aanandhi M, Gandhimathi R, Sumithra M. A review on bioanalytical method development and pre-method validation concepts using SPE and LLE process by LC-MS/MS method. International Journal of Research in Pharmaceutical Sciences. 2021; 12(2): 1353-1359.

[2] Kang L, Weng N, Jian W. LC–MS bioanalysis of intact proteins and peptides. Biomedical Chromatography. 2019; 34(1).

[3] Mejía-Carmona K, Soares da Silva Burato J, Borsatto J, de Toffoli A, Lanças F. Miniaturization of liquid chromatography coupled to mass spectrometry. TrAC Trends in Analytical Chemistry. 2020; 122: 115735.

[4] Kohira T, Kita Y, Tokuoka S, Shiba M, Satake M, Shimizu T. Characterization of supported liquid extraction as a sample pretreatment method for eicosanoids and related metabolites in biological fluids. Journal of Chromatography B. 2019; 1124: 298-307.

[5] ICH, Q2 (R1), Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization, IFPMA, Geneva, 2005.

[6] Soares da Silva Burato J, Vargas Medina D, Toffoli A, Vasconcelos Soares Maciel E, Mauro Lanças F. Recent advances and trends in miniaturized sample preparation

techniques. Journal of Separation Science. 2019; 43(1): 202-225.

[7] Chen Z, Li Q, Yang T, Zhang Y, He M, Zeng H *et al*. Sequential extraction and enrichment of pesticide residues in Longan fruit by ultrasonic-assisted aqueous two-phase extraction linked to vortex-assisted dispersive liquid-liquid microextraction prior to high performance liquid chromatography analysis. Journal of Chromatography A. 2020; 1619: 460929.

[8] Zheng W, Xiang Y, Cai D, Yang X, Zhang L, Zheng W *et al*. Adjunctive Fluvoxamine for Schizophrenia: A Meta-analysis of Randomized Double-Blind, Placebo-Controlled Trials. Journal of Clinical Psychopharmacology. 2020; 40(4): 386-390.

[9] Liu X, Li X, Zhang C, Sun M, Sun Z, Xu Y *et al*. Efficacy and tolerability of fluvoxamine in adults with social anxiety disorder. Medicine. 2018; 97(28): e11547.

[10] Vignali C, Moretti M, Quaiotti J, Freni F, Tajana L, Osculati A *et al*. Distribution of Fluvoxamine and Identification of the Main Metabolite in a Fatal Intoxication. Journal of Analytical Toxicology. 2020; 45(4): e1-e5.

[11] Kim W, Fu Y, Dobson-Stone C, Hsiao J, Shang K, Hallupp M *et al*. Effect of Fluvoxamine on Amyloid-β Peptide Generation and Memory. Journal of Alzheimer's Disease. 2018; 62(4): 1777-1787.

[12] Xu D, Wang C, Zhu X, Zhao W, Jiang B, Cui S *et al*. The antidepressant-like effects of fluvoxamine in mice involve the mTOR signaling in the hippocampus and prefrontal cortex. Psychiatry Research. 2020; 285: 112708.

[13] Tanna N, Lame M, Wrona M. Development of an UPLC/MS–MS method for quantification of intact IGF-I from human serum. Bioanalysis. 2020; 12(1): 53-65.

[14] Buziau A, Scheijen J, Stehouwer C, Simons N, Brouwers M, Schalkwijk C. Development and validation of a UPLC-MS/MS method to quantify fructose in serum and urine. Journal of Chromatography B. 2020; 1155: 122299.

[15] Iqbal M, Ezzeldin E, Herqash R, Anwer M, Azam F. Development and validation of a novel UPLC-MS/MS method for quantification of delafloxacin in plasma and aqueous humour for pharmacokinetic analyses. Journal of Chromatography B. 2020; 1138: 1219.