Analytical Methods

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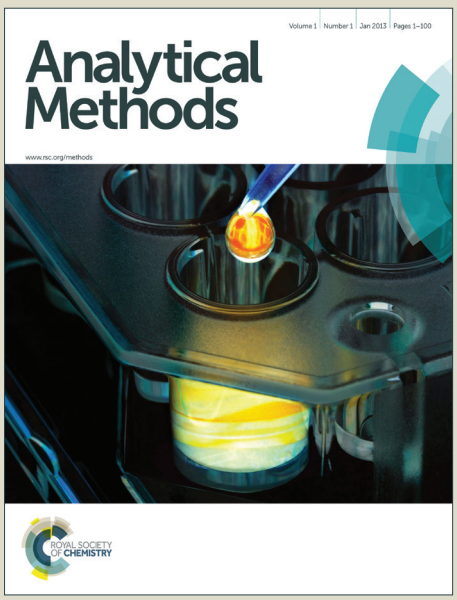

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A fast and reliable LC-MS/MS method for simultaneous quantitation of Fluoxetine and Mirtazapine in human plasma

Pallavi Alegete,[a, b] Prasad Kancherla,[c] Satyanarayana Boodida,[* a] and Saeed S. Albaseer, [d]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

A rapid and sensitive ultra-performance liquid chromatographic / tandem mass spectrometric assay (LC-MS/MS) was developed to simultaneously quantify fluoxetine and mirtazapine in human plasma using fluoxetine-D5 and olanzapine as internal standards (IS), respectively. The analytes and the internal standards (IS) were extracted from 400µL aliquots of human plasma through liquid-liquid extraction. Chromatographic separation was achieved in a run time of 2.0 min on X-terra RP8 (50×4.6 mm, 5µm particle size) column. Isocratic mobile phase consisting of a mixture of acetonitrile and 10 mM ammonium acetate (90:10, v/v), at a flow-rate of 0.50 mL/min was found to be optimum. Quantitation of analytes was performed by electrospray ionization tandem mass spectrometry, operating in positive-ion and multiple reactions monitoring (MRM) acquisition mode. The protonated precursors to product ion transitions monitored for fluoxetine, mirtazapine, fluoxetine-D5 and olanzapine were at m/z 310.20 → 148.17, 266.35 → 195.31, 315.20 → 153.17 and 313.19 → 256.12, respectively. The method was validated over the concentration range of 0.050 – 50.037 ng/mL for fluoxetine and 0.100–100.000 ng/mL for mirtazapine in human plasma. The method has shown high reproducibility with intra-batch and inter-batch precision (CV %) less than 10.16% across four quality control levels for both the analytes. The assay was linear over the concentration range of 0.050–50.037 ng/mL for fluoxetine (r^2 =0.9988) and 0.100–100.000 ng/mL for mirtazapine (r^2 =0.9975). The method is suitable for measuring accurate concentration of the two analytes in bioequivalence study and therapeutic drug monitoring following combined administration.

1 Introduction

Fluoxetine (FLU) [*N*-methyl-3-(4-trifluoromethylphenoxy) propylamine], is an antidepressant drug that enhances serotoninergic neurotransmission through the selective inhibition of neuronal reuptake of serotonin [1] .It is widely prescribed for a variety of psychopathological conditions, including mood and eating disorders, obsessive-compulsive disorders, depression in the elderly, and dysthymia [2-4]. Mirtazapine (MIR); [1,2,3,4,10,14b-Hexahydro-2-methylpyrazino-[2,1a] pyrido [2,3-c] benzazepine [5] is a new second generation antidepressant. Chemical structures of fluoxetine, mirtazapine, fluoxetine-D5 (ISTD for fluoxetine) and olanzapine are shown in Figure 1. Chemically, MIR is a piperazinoazepine compound which belongs to the class of noradrenergic and specific serotonergic antidepressants (NaSSA) and its mechanism of action probably involves the increased release of serotonin and nor

epinephrine due to the antagonism on auto-receptors and α_2-adrenergic hetero-receptors. This mechanism, which differs from that of most second-generation antidepressants, grants it good efficacy in the treatment of patients who are non-responder to the other members of second-generation antidepressants [6]. The drug is currently used against generalized anxiety disorder [5],

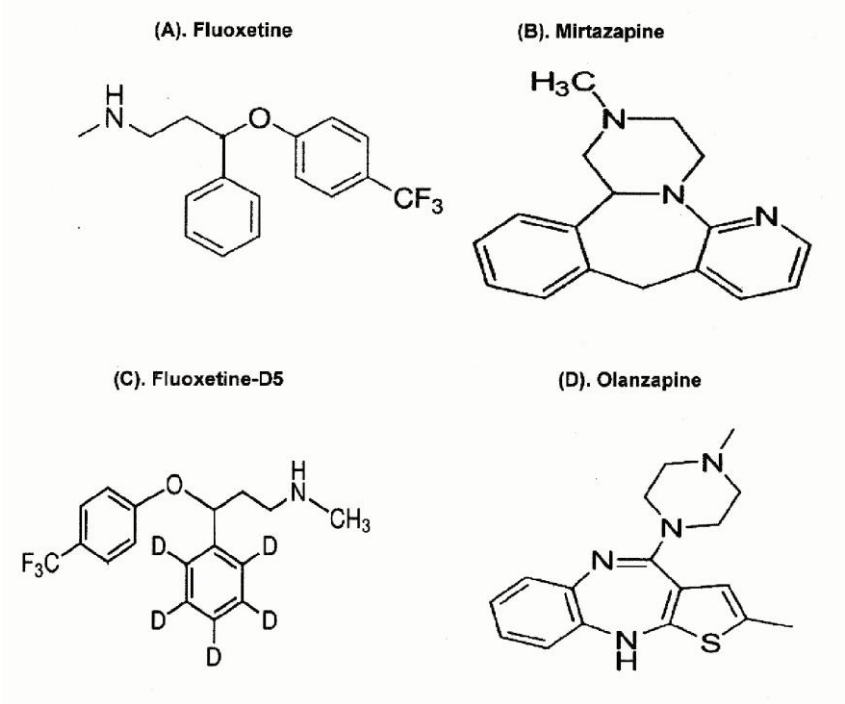

Figure 1: Chemical structures of Fluoxetine, Mirtazapine, Fluoxetine-D5 and Olanzapine

obsessive–compulsive disorder [7], and post-traumatic stress disorder [8]. MIR also shows promise in preventing post-chemotherapy nausea and vomiting due to its anti-emetic effects [9]. For the determination of fluoxetine in human plasma, several methods based on high-performance liquid chromatography (HPLC) with UV [11,12], detection and LC-MS [13,14] have been published. Similarly, a number of methods for the determination of mirtazapine in human plasma have been developed based on HPLC [15]), spectrofluorimetry [16], and LC-MS/MS [17,18]. However, as per the available literature to the best of our knowledge, there was no method reported in the literature for the simultaneous determination of these two analytes using LC-MS/MS technique. The present method makes it possible to simultaneous determination of the two analytes with the advantage of being more sensitive, with lesser run time and plasma volume.

2 Experimental

2.1 Reagents and chemicals

Reference standards of Fluoxetine (Potency (w/w 99.90%), Mirtazapine (Potency (w/w 98.60 %) and Olanzapine (Potency w/w 99.70 %) were obtained as gifted samples from Dr.Reddy's Laboratory, (Hyderabad, India). Fluoxetine-D5 (Potency w/w 99.92 %) was obtained from Clearsynth Labs (P) LTD, (Mumbai, India). HPLC Grade methanol and acetonitrile were purchased from J.T. Baker (Philipsburg, USA). Analytical-grade ammonium acetate was purchased from SD fine chemicals (Mumbai, India.), HPLC Grade tert-Butyl methyl

ether and n-Hexane were purchased from Merck specialties (Mumbai, India). Polypropylene vials were obtained from Torsens products Pvt Ltd, (Kolkata, India). Water used in the entire analysis was prepared by a Milli-Q water purification system (Millipore, Bangalore, India). The human K2EDTA control plasma was obtained from Vuppala venkaiah memorial blood bank, (Hyderabad, India).

2.2 Instrumentation

Chromatographic separation was carried out using a waters Acquity UPLC system consisting of binary solvent delivery capability (Milford, MA, USA). The analytical column used was X-terra RP8 (50×4.6 mm, 5-µm particle size) from waters, (Milford, MA, USA). The flow-rate of the mobile phase under isocratic condition was kept at 0.500 mL/min. The mobile phase consisted of acetonitrile: 10 mM ammonium formate (90:10, v/v). The column oven temperature was maintained at 35°C and the total LC run time was about 2.0 min.

Detection of analytes and IS was carried out on a triple quadrapole mass spectrometer, (WATERS, Quattro Micro LC-MS/MS, Milford, MA, USA), equipped with electrospray ionization, and operating in positive ion mode using three multiple reaction monitoring (MRM) functions (Table 1). For the optimization of MS/MS parameters, standard solutions of fluoxetine, mirtazapine, and the IS solutions prepared in methanol was infused at a flow-rate of 10 mL/min using a syringe pump (Harvard Apparatus, Holliston, MA, USA). The dwell time assay set at 500ms. Mass Lynx software version 4.1 was used to control all parameters of LC-MS/MS.

Table 1. MRM (4 channels) parameters of the analytes and IS

Analyte	Precursor /Ion product (m/z)	Dwell time (Secs)	Cone voltage (eV)	Collision energy (eV)
Mirtazapine	266.35 > 195.31	0.500	35.0	27.0
Fluoxetine	310.20 > 148.17	0.500	10.0	13.0
Olanzapine	313.19 > 256.12	0.500	35.0	27.0
Fluoxetine-D5	315.20 > 153.17	0.500	10.0	13.0

2.3 Preparation of stock and working solutions

The standard stock solutions of 1 mg/mL fluoxetine, 1 mg/mL mirtazapine and ISTD's were prepared by dissolving their accurately weighted amounts in methanol. The standard stock solutions of analytes were then serially diluted with a mixture of water: methanol (40:60, v/v) to obtain working solutions of the required concentration range. All the solutions were stored at 2–8°C and brought to room temperature before use.

2.4 Preparation of Calibration standards and Qc samples in plasma

The calibration standards and quality control (QC) samples were prepared by spiking 2% of the total plasma volume with working

solutions. Calibration standards were prepared at the concentrations of 0.050, 0.100, 0.500, 5.003, 10.007, 25.017, 40.027 and 50.037ng/mL for fluoxetine and 0.100, 0.200, 1.000, 10.000, 20.000, 50.000, 80.000 and 100.000 ng/mL for mirtazapine. Quality control samples were prepared at 0.050 ng/mL (lower limit of quantitation, LLOQ), 0.149 ng/mL (low quality control, LQC), 22.551 ng/mL (medium quality control, MQC) and 38.060 ng/mL (high quality control, HQC) for fluoxetine and 0.100 ng/mL (LLOQ), 0.297 ng/mL (LQC), 45.030 ng/mL (MQC) and 76.000 ng/mL (HQC) for mirtazapine. Aliquots of spiked plasma samples were taken in micro-centrifuge tubes and stored at -70°C.

2.5 Sample preparation

Prior to analysis, all frozen subjects samples, calibration standards and quality control samples were thawed and allowed to equilibrate at room temperature. To select the best extraction method for Fluoxetine and Mirtazapine, we tried SPE and liquid-liquid extraction. Finally, LLE was chosen as it gave better extraction efficiency. To optimize LLE, several solvents (Ethyl acetate, MTBE, n-Hexane, diethyl ether and pentane) were checked alone and in combination. Although ethyl acetate gave the best results in terms of extraction recovery and matrix effect, precision and accuracy were poor with high % CV when ethyl acetate was used. So, MTBE was picked as extraction solvent, which gave good extraction efficiency with little matrix effect. To eliminate matrix effect, n-Hexane was added to MTBE (to decrease the polarity of solvent) in a ratio of 1:4, respectively. This solvent combination proved optimum for as it resulted in clean chromatogram for both blank and spiked plasma samples with good reproducibility and negligible matrix effect. The final extraction procedure was as follows: to an aliquot of 400 µL of spiked plasma sample, 50µL internal standard was added and vortexed for 20 s. To these samples , 2.5 mL of extraction solvent (MTBE : n Hexane 80:20, v/v) was added and samples were extracted on extractor at 2500 rpm for 10 min. Centrifugation of the samples was done at 10°C, for 10 min at a centrifugal speed of 4000 rpm. An amount of 2.0 mL supernatant was separated and evaporated to dryness under nitrogen at 50° C ± 0.50 °C at 15 psi for 15 min. The dried samples were reconstituted with 400 µL of the mobile phase and 10 µL was injected into the chromatographic system.

3 Method validation

The method was validated for selectivity, sensitivity, linearity, precision and accuracy, recovery, matrix effect, dilution integrity and stability according to US Food and Drug Administration (FDA) guidelines (FDA, 2001) [19] .

3.1 Selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. The selectivity of the method was evaluated by processing six different lots of blank plasma sample. These samples were spiked with LLOQ concentration along with IS to confirm the lack of interference at their retention time and absence of lot-to-lot variation.

3.2 Linearity and LLOQ

The calibration curves of fluoxetine and mirtazapine were constructed using standard plasma samples at eight nonzero concentrations. Curves were best fitted using a least square linear regression model y = mx + b weighted by 1/x2, in which y is the peak area ratio of

analyte to IS, m is slope of the calibration curve, b is the y-axis intercept of the calibration curve and x is the analyte concentration. The lowest concentration on the standard curve with detector response ten times greater than the control human plasma was considered as the LLOQ.

3.3 Accuracy and precision.

Inter-batch precision and accuracy of the assay were evaluated by running three validation batches on three separate days, whereas intra-batch precision and accuracy were evaluated within a day batch. Each batch consisted of one set of calibration standards and six replicates of quality control samples at four levels (LLOQ, LQC, MQC and HQC). A comparison was done between the obtained values and the theoretical values. Precision was expressed as percentage of coefficient of variation (CV%).

3.4 The relative recovery, absolute matrix effect and process efficiency

The relative recovery, matrix effect and process efficiency were assessed as recommended by Matuszewski [20] at Std-1, Std-3, Std-5, Std-6 and Std-8 levels in six replicates. Relative recovery (RR) was calculated by comparing the mean area response of extracted samples (spiked before extraction) to that of unextracted samples (spiked after extraction) at each QC level. The recovery of IS was similarly estimated. Absolute matrix effect (ME) was assessed by comparing the mean area response of unextracted samples (spiked after extraction) with mean area of neat standard solutions. The overall 'process efficiency' (PE%) was calculated by comparing the mean area response of extracted samples (spiked before extraction) to that with mean area of neat standard solutions at each QC level. The assessment of relative matrix effect was based on direct comparison of the MS/MS responses (peak areas) of the analytes spiked into extracts originating from different lots of plasma. The variability in these responses, expressed as CV%, was considered as the measure of relative matrix effect.

3.5 Stability studies and dilution integrity

Stability experiments were performed to evaluate the analyte stability in stocks solutions and in plasma samples under different conditions, which may occur during sample analysis. . Stock solution stability was performed by comparing area response of stability samples of analytes and the IS with the area response of sample prepared from fresh stock solutions. Bench-top stability, extracted sample stability (wet extract stability), freeze–thaw stability, dry extract stability and long-term stability were performed at LQC and HQC level using six replicates at each level. The dilution integrity experiment was intended to validate the dilution test to be carried out on higher analyte concentrations (above HQC), which may be encountered during real subject samples analysis. The test was carried out at a concentration which was twice the HQC. Six replicate samples of half and one-fifth concentration were prepared and their concentrations were calculated by applying the dilution factor of 2 and 5 respectively against the freshly prepared calibration curve.

4 Results and discussion

4.1 Method development

The goal of this work was to develop and validate a simple, rapid and sensitive assay method for the simultaneous estimation of

fluoxetine and mirtazapine, suitable for bioequivalence and therapeutic drug monitoring studies. To achieve this goal, different method parameters were evaluated to optimize mass spectrometry, chromatography and sample extraction conditions. Mass spectrometry conditions were optimized by infusing standard solutions of the tested compounds of appropriate concentrations having an electrospray ionization source (ES) and operating in the multiple reaction monitoring (MRM) mode. Tuning was done in both positive and negative-ion modes. The intensity obtained in the positive ion mode was higher for both the analytes and their respective internal standards (IS), as they are basic in nature and have the ability to accept protons and give protonated species $[M + H]^+$ ions in Q1 mode. Precursor → Product ion (m/z) transitions for fluoxetine, mirtazapine,

fluoxetine-D5 and olanzapine were observed at m/z 310.20→148.17, 266.35→195.31, 315.2→153.17 and 313.19→256.12 respectively. Figure 2 shows the production mass spectra of fluoxetine and mirtazapine, respectively. The dwell time for each transition was 500 ms. The chromatographic conditions were aimed at obtaining adequate response, sharp peaks and a short run time per analysis for the analytes and IS. The typical chromatograms for human plasma samples spiked with fluoxetine, mirtazapine and IS at LLOQ and HQC are shown in Figure 3 and Figure 4, respectively. The parameters optimized included mobile phase, column type, and flow-rate, among others. Initially, separation was tried using buffers like formic acid, ammonium acetate and ammonium formate in varying combinations with methanol or acetonitrile on different columns like Symmetry C8, Symmetry C18, Hypersil Gold C18, X-terra RP8, and Inertsil-ODS. Using trial and error procedure mobile phase was optimized and a mixture consisting of acetonitrile-10 mM ammonium formate (v/v) (90:10) was found to be optimum. As for the column, the use of an X-terra RP8 column (50×4.6 mm, 5-µ m) helped in achieving good peak shape and response even at LLOQ level for both the analytes and internal standards (IS), as well. After selecting the optimum mobile phase and column, fine tuning of source dependent parameter was done based on the flow-rate of mobile phase and the optimum flow rate was found to be 0.500 mL/min. In addition, the use of shorter column helped in the elution of all the analytes in a short run time.

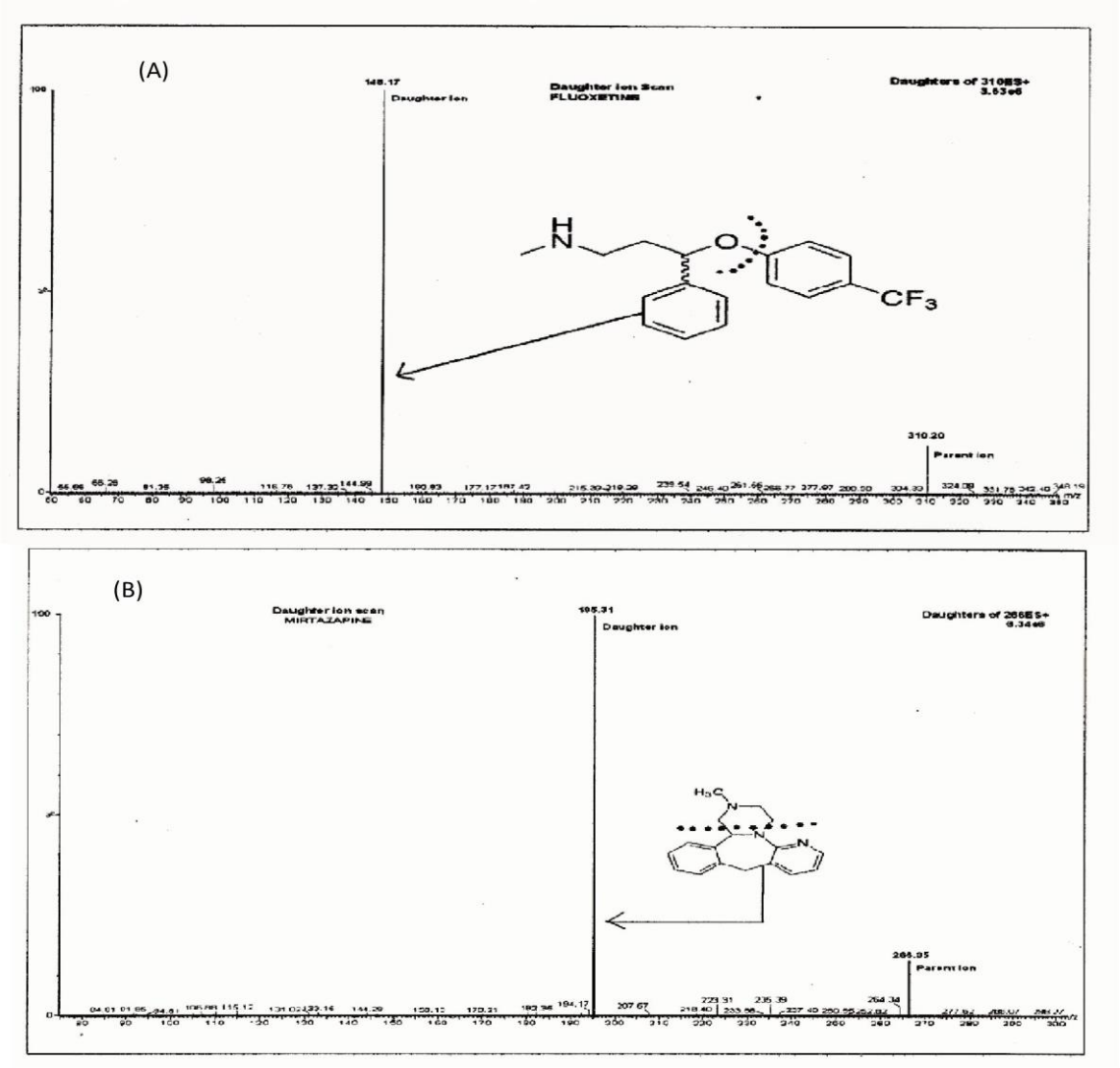

Figure 2: Mass spectra of (A) fluoxetine, and (B) mirtazapine

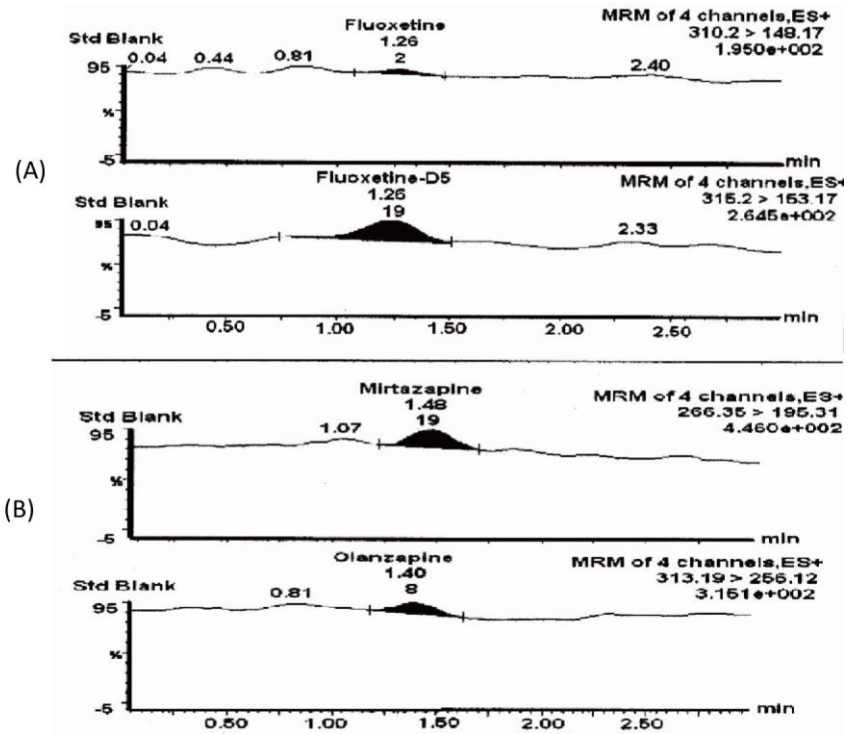

Figure 3: The typical chromatograms for human plasma samples spiked with (A): fluoxetine and (B): mirtazapine and respective IS at LLOQ levels

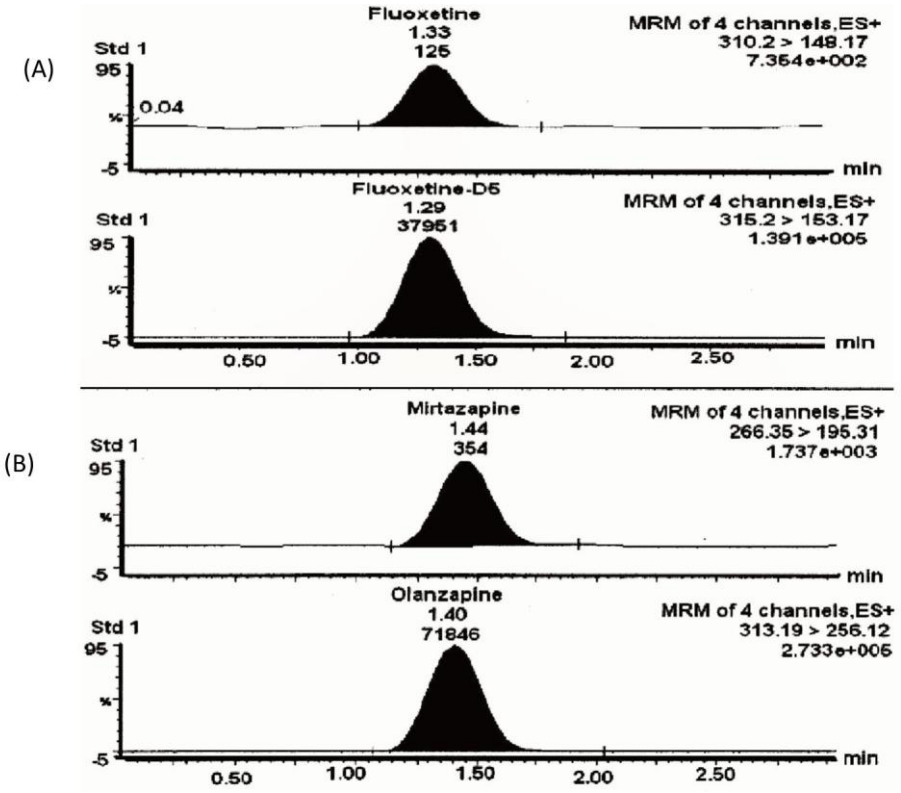

Figure 4: The typical chromatograms for human plasma samples spiked with (A): fluoxetine and (B): mirtazapine and respective IS at HOQ levels

4.2 Selectivity.

No interfering peaks from endogenous compounds were observed at the retention times of analytes and IS. The retention times of fluoxetine, mirtazapine, fluoxetine-D5 and olanzapine were 1.33, 1.29, 1.44 and 1.40 min, respectively. The total chromatographic run time was 2.0 min.

4.3 Linearity and LLOQ.

The assay was linear over the concentration range of 0.050–50.037 ng/mL for fluoxetine and 0.100–100.000 ng/mL for mirtazapine. Typical equations for calibration curves (n =4) are: r^2 =0.9988, slope 0.0258, intercept = 0.0004 for fluoxetine and r^2 =0.9975, slope 0.0180, intercept = 0.0004 for mirtazapine, and these limits were adequate for quantifying analytes in human pharmacokinetic samples following oral administration of therapeutic doses for both the analytes.

4.4 Accuracy and precision.

Six replicates at LLOQ, LQC, MQC and HQC levels were analysed for method accuracy calculation. Method accuracy expressed in terms of recovery percentage was ≥99.8% for fluoxetine and ≥99% for mirtazapine. At HQC level, intra-batch and inter-batch precision (CV%) was ≤3.55, ≤2.83 and ≤0.84, ≤1.58 for Fluoxetine and Mirtazpine, respectively. At LLOQ level, intra-batch and inter-batch precision (CV%) was ≤8.95, ≤8.31 and ≤6.78, ≤10.16 for Mirtazapine and Fluoxetine, respectively (Table 2).

Table.2 Intra- and inter-day precision and accuracy data for Fluoxetine and Mirtazapine (six replicates at each level)

		Intra-batch					Inter-batch				
Level	**Quality control samples (ng/mL)**	***N***	**Mean concentration observed (ng/mL)**	**Percentage bias**	**CV%**	***RE%***	***N***	**Mean concentration observed (ng/mL)**	**Percentage bias**	**CV%**	**RE%**
Fluoxetine											
LLOQ	0.050	6	0.050	0.00	6.77	100	24	0.051	-2.00	10.16	102
LQC	0.149		0.159	-6.71	4.31	106.7		0.157	-5.37	5.52	105
MQC	22.551		24.202	-7.32	0.70	107.3		23.998	-6.42	4.28	106.4
HQC	38.060		37.991	0.18	0.83	99.8		37.956	0.27	1.57	99.7
Mirtazapine											
LLOQ	0.100	6	0.099	1.00	8.94	99	24	0.102	-2.00	8.30	102
LQC	0.297		0.324	-9.09	5.22	109		0.315	-6.06	5.52	106
MQC	45.030		49.944	-10.91	2.95	110.9		48.664	-8.07	6.76	108
HQC	76.000		76.787	-1.04	3.54	101		76.567	-0.75	2.82	100.7

4.5 Extraction recovery, process efficiency and matrix effect results

The relative recovery, absolute matrix effect and process efficiency were calculated at five concentration levels (Std-1, Std-3, Std-5, Std-6 and Std-8 levels); six replicates for each concentration level. The mean recoveries for fluoxetine, mirtazapine, fluoxetine-D5 and

Olanzapine in human plasma were 86.10 %, 83.82, 81.30 and 77.25 %, respectively. Further, the extent of matrix effect in different lots of plasma (spiked after extraction) was within the acceptable limits. Table 3 shows the results for comparative data of absolute matrix effect, relative recovery and process efficiency for fluoxetine (FLU) and mirtazapine (MTZ) and their respective internal standards.

Table 3. Comparison data of absolute matrix effect, relative recovery and process efficiency for Fluoxetine (FLU) and Mirtazapine (MTZ)

Analyte	A[a] (%CV)	B[b] (%CV)	C[c] (%CV)	Absolute matrix effect (%ME) [d]	Relative recovery (%RE) [e]	Process efficiency (%PE) [f]
STD 1						
Fluoxetine	87 (7.3)	92 (6.09)	81 (4.24)	105.78	88.34	93.45
Mirtazapine	353 (2.15)	348 (5.23)	295 (4.00)	98.63	84.86	83.70
Fluoxetine-D5	60482 (0.76)	60191 (0.46)	50564 (1.12)	99.52	84.01	83.60
Olanzapine	164936 (3.99)	152220 (1.95)	121990 (3.80)	92.29	80.14	73.96
STD 3						
Fluoxetine	807 (1.12)	849 (4.26	724 (2.72)	105.21	85.25	89.69
Mirtazapine	3278 (2.59)	3392 (2.83)	2861 (3.70)	103.46	84.36	87.28
Fluoxetine-D5	59830 (1.42)	58914 (0.43)	50696 (1.14)	98.47	86.05	84.73
Olanzapine	161575 (6.63)	166338 (3.59)	127380 (4.50)	102.95	76.58	78.84
STD 5						
Fluoxetine	15223 (2.23)	15806 (4.12)	13825 (0.68)	103.83	87.47	90.82
Mirtazapine	63100 (2.72)	63278 (2.02)	48994 (1.07)	100.28	77.43	77.65
Fluoxetine-D5	53741 (0.96)	53303 (1.28)	44902 (0.63)	99.18	84.24	83.55
Olanzapine	156942 (4.21)	154633 (3.25)	115153 (2.14)	98.53	74.47	73.37
STD 6						
Fluoxetine	31009 (0.62)	32144 (1.51)	28172 (3.52)	103.66	87.64	90.85
Mirtazapine	146653 (0.50)	145391 (0.59)	117151 (2.79)	99.14	80.58	79.88
Fluoxetine-D5	47192 (0.50)	47001 (0.41)	39433 (0.82)	99.60	83.90	83.56
Olanzapine	146989 (0.77)	146568 (0.99)	115111 (0.470	99.71	78.54	78.31
STD 8						
Fluoxetine	53907 (0.67)	53282 (0.92)	43593 (0.50)	98.84	81.82	80.87
Mirtazapine	40194 (0.61)	39710 (1.00)	32131 (0.44)	98.79	80.91	79.94
Fluoxetine-D5	285763 (0.15)	286514 (1.03)	227181 (0.76)	98.79	80.91	79.94
Olanzapine	143546 (0.25)	146401 (2.78)	117380 (3.00)	101.99	80.18	81.77

a Mean area response of six replicate samples prepared in Mobile phase neat samples
b Mean area response of six replicate samples prepared by spiking in post extracted blank
c Mean area response of six replicate samples prepared by spiking in plasma before extraction
d %Matrix effect: Post extracted mean response/Aqueous (Neat) mean response x 100
e %Recovery: Extracted mean response / Post extracted mean response x 100
f %Process efficiency: Extracted mean response / Aqueous Mean response x 100

4.6 Stability studies and dilution integrity.

Using the mean of six replicates (n = 6), short-term and long-term stability was assessed for stock solutions of fluoxetine, mirtazapine and IS. It was found that these analytes were stable at room temperature for a minimum period of about 8 h, and for about 8 days when

stored at a temperature of 2–8°C. Fluoxetine and mirtazapine in control human plasma (bench top samples) were stable for at least 6 h at ambient temperature and for minimum of four freeze/thaw cycles. Under autosampler conditions maintained at 10°C, spiked quality control samples were satisfactorily stable up to 24 h. Long-term stability of the spiked quality control samples was achieved when these samples were stored at -70°C. The samples were stable for a period of more than 5 days, Table 4.

The dilution integrity experiments were performed with an aim to validate the dilution test to be carried out at high analyte concentration above the upper limit of quantification (ULOQ), which maybe encountered during real subject sample analysis. The precision and accuracy values for 1/5th and 1/10th dilutions were 0.92%, 3.58 % and 97.74%, 98.29% for fluoxetine, respectively, and 2.95%, 2.44 % and 99.89%, 99.05% for mirtazapine, respectively.

Table 4. Stability summary of fluoxetine and mirtazapine in human plasma

			Fluoxetine			Mirtazapine		
Stability	**Storage Condition**	**Level**	**Quality control samples (ng/mL)**	**RE (%)**	**CV%**	**Quality control samples (ng/mL)**	**RE (%)**	**CV%**
Freeze thaw	After four cycles at -70°C	LQC	0.149	101.3	4.21	0.297	107.5	7.32
		HQC	38.06	99.6	1.31	76.000	100.8	2.53
Dry extract	24 h at 2-8°C	LQC	0.149	97.3	8.62	0.297	101.9	4.85
		HQC	38.06	104.7	7.78	76.000	101.6	5.77
Wet extract	24 h at 2-8°C	LQC	0.149	101.0	5.86	0.297	99.9	3.26
		HQC	38.06	107.1	6.59	76.000	96.5	6.59
Autosampler	Autosampler (10°C, 24 h)	LQC	0.149	100.7	5.75	0.297	97.8	3.36
		HQC	38.06	100.1	0.47	76.000	105.5	5.39
Bench top	After six hours (at RT)	LQC	0.149	99.3	5.41	0.297	103.7	3.76
		HQC	38.06	97.9	3.87	76.000	101.0	2.71
Long term	25 days at -70°C	LQC	0.149	102.6	6.70	0.297	100.9	5.74
		HQC	38.06	99.3	3.05	76.000	102.4	3.27

Conclusion

The present LLE-LC-MS/MS method was successfully developed for the simultaneous determination of fluoxetine and mirtazapine in human plasma. This method is highly sensitive and selective with a short turnaround time. No interferences from endogenous plasma components were observed. The validation data demonstrates good precision and accuracy of the method. In addition, the established LLOQ is sufficient to monitor the concentration of fluoxetine and mirtazapine in human plasma for bioequivalence and therapeutic drug monitoring studies, especially that the two drugs Lack pharmacokinetic interaction [21].

Funding

This study was financially supported by Spectrum pharma research solutions, Hyderabad, AP, India.

Notes and references

[a] *Department of Chemistry, JNTU College of Engineering Jagityal, Nachupally, Karimnagar, Andhra Pradesh, India. Tel: 9010069233; E-mail: bsnarayana77@gmail.com*

[b] *Spectrum Pharma Research Solutions, Kukatpally, Hyderabad, Andhra Pradesh, India. E-mail: pallavi.a09@gmail.com*

[c] *Centre for Chemical Sciences & Technology, Institute of Science & Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Andhra Pradesh, India. Email: prsdkancherla11@gmail.com*

[c] *Department of Chemistry, Faculty of Education, Thamar University, Thamar, Yemen.Tel:00967-773651108 Email: sshalbaseer@yahoo.co.uk*

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