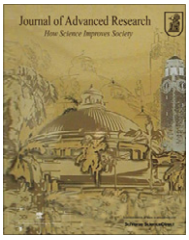
Journal of Advanced Research (2013) 4, 173–180

Cairo University

Journal of Advanced Research



ORIGINAL ARTICLE

Simultaneous determination of olanzapine and fluoxetine hydrochloride in capsules by spectrophotometry, TLC-spectrodensitometry and HPLC

Mahmoud A. Tantawy *, Nagiba Y. Hassan, Nariman A. Elragehy, Mohamed Abdelkawy

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr el Aini Street, 11562 Cairo, Egypt

Received 10 April 2012; revised 18 May 2012; accepted 20 May 2012
Available online 23 June 2012

KEYWORDS

Spectrophotometry;
TLC-spectrodensitometry;
HPLC;
Olanzapine;
Fluoxetine HCl

Abstract This paper describes sensitive, accurate and precise spectrophotometric, TLC-spectrodensitometric and high performance liquid chromatographic (HPLC) methods for simultaneous determination of olanzapine and fluoxetine HCl. Two spectrophotometric methods were developed, namely; first derivative (D^1) and derivative ratio (DD^1) methods. The TLC method employed aluminum TLC plates precoated with silica gel GF_{254} as the stationary phase and methanol: toluene:ammonia (7:3:0.1, by volume) as the mobile phase, where the chromatogram was scanned at 235 nm. The developed HPLC method used a reversed phase C18 column with isocratic elution. The mobile phase composed of phosphate buffer pH 4.0:acetonitrile:triethylamine (53:47:0.03, by volume) at flow rate of 1.0 mL min^{-1}. Quantitation was achieved with UV detection at 235 nm. The methods were validated according to the International Conference on Harmonization (ICH) guidelines. The selectivity of the proposed methods was tested using laboratory-prepared mixtures. The developed methods were successfully applied for the determination of olanzapine and fluoxetine HCl in bulk powder and combined capsule dosage form.

Introduction

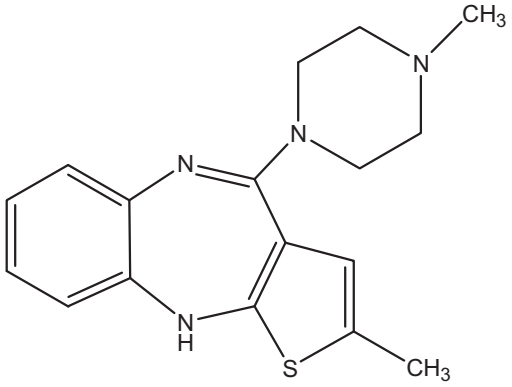
Olanzapine (OLZ) is an atypical antipsychotic drug, approved by the FDA for the treatment of schizophrenia and bipolar disorder. It is chemically designated as 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno(2,3-*b*)(1,5)benzodiazepine, Fig. 1A. It has a higher affinity for 5-HT_2 serotonin receptors than D_2 dopamine receptors. The mode of action of Olanzapine's antipsychotic activity is unknown [1]. Fluoxetine HCl (FLX) is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class. It is chemically designated as *N*-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine, Fig. 1B. It is used for the treatment of depression. Being one of SSRI drugs, it acts by increasing the extracellular level of the neurotransmitter serotonin by inhibiting its reuptake into the cell [1].

* Corresponding author. Tel.: +20 223639307; fax: +20 223628426.
E-mail address: matantawy@hotmail.com (M.A. Tantawy).

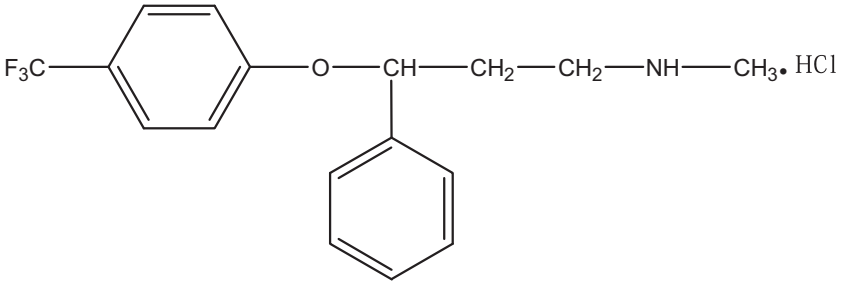
Peer review under responsibility of Cairo University.

Production and hosting by Elsevier

2090-1232 © 2012 Cairo University. Production and hosting by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jare.2012.05.004



(a) The structure of olanzapine.
$C_{17}H_{2O}N_4S$=312.4



(b) The structure of fluoxetine HCl.
$C_{17}H_{18}F_3NO$=309.3

Fig. 1 The structures of olanzapine and fluoxetine HCl.

Determination of OLZ was carried out by HPLC [2–6], UV spectrophotometry [2,7], CZE [2] and linear voltammetry [2]. For FLX, it was determined by UV spectrophotometry [8–10] and HPLC [11,12].

There is no official method for the determination of OLZ and FLX in dosage form. There are few reported methods for their simultaneous analysis including three HPLC methods [13–15] and two HPTLC methods [14,15].

So, the aim of this work was to develop recent, simple, sensitive and validated spectrophotometric methods, TLC-spectrodensitometric method and HPLC chromatographic method for the simultaneous determination of OLZ and FLX in their pure powdered form, laboratory prepared mixtures and in their pharmaceutical capsule dosage form. The spectrophotometric methods applied are first derivative (D^1) and derivative ratio (DD^1) method. The developed methods can be successfully applied in routine analysis and quality control laboratories.

Experimental

Apparatus

Spectrophotometric measurements were carried out on a dual beam Shimadzu (Kyoto, Japan) UV–Vis. spectrophotometer, model UV-1601 PC connected to IBM compatible with an Hp 600inkjet printer. The bundle software, UV PC personal spectroscopy software version 3.7 (Shimadzu, Kyoto, Japan) was used to process absorption and derivative spectra, the spectral band width was 2 nm and scanning speed was 2800 nm min^{-1}.

The TLC system comprised a Camag Linomat autosampler (Switzerland), Camag microsyringe (100-μL), and Camag TLC scanner 35/N/30319 with winCATS software, a short wavelength UV lamp emitting at 254 nm (Desaga,Germany) and TLC plates precoated with silica gel GF_{254} 20×20 cm, 0.25 mm thickness (E. Merck, Darmstadt, Germany).

The HPLC system comprised an Agilent pump with different flow rates (model 1100 series, Agilent, USA), equipped with a variable wavelength detector and a 20-μL volume injection loop. A Zorbax ODS (5 μm, 25×4.6 mm i.d.) column was used as stationary the phase. The samples were injected with a 50-μL Hamilton analytical syringe.

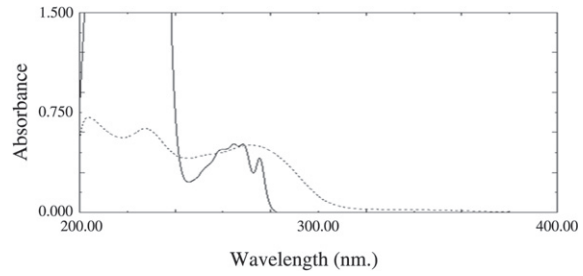
Materials

Pure samples

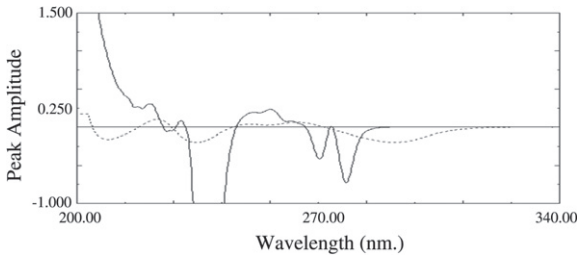
Olanzapine and fluoxetine HCl were kindly supplied by Eli Lilly Company – Egypt. Their purity was found to be 100.00% and 99.92% for OLZ and FLX, respectively according to a reported HPLC method [14].

Pharmaceutical dosage form

Symbyax® (3 mg/25 mg) (Eli Lilly and Company – USA) Batch No. A588272A, labeled to contain 3 mg olanzapine and 25 mg fluoxetine HCl per capsule.



(a) Zero order absorption spectra of 7.5 μg mL^{-1} olanzapine (- - -) and 200 μg mL^{-1} fluoxetine HCl (——) using methanol as a blank.



(b) First derivative absorption spectra of 7.5 μg mL^{-1} olanzapine (- - -) and 200 μg mL^{-1} fluoxetine HCl (——) using methanol as a blank.

Fig. 2 D^0 and D^1 Spectra of olanzapine and fluoxetine HCl.

Chemicals and reagents

All chemicals used throughout the work were of analytical grade and solvents were of spectroscopic and HPLC grade:

Methanol (Merck, Germany), acetonitrile (Merck, Germany), triethylamine (Sigma–Aldrich, Belgium), phosphate buffer solution pH 4.0 [16], toluene (Adwic, Egypt), ammonia solution 33% (Adwic, Egypt) and double distilled deionized water (Otsuka, Cairo, Egypt).

Solutions

Stock standard solutions

Stock standard solutions of OLZ (4 mg mL^{-1}) and FLX (20 mg mL^{-1}) were prepared in methanol.

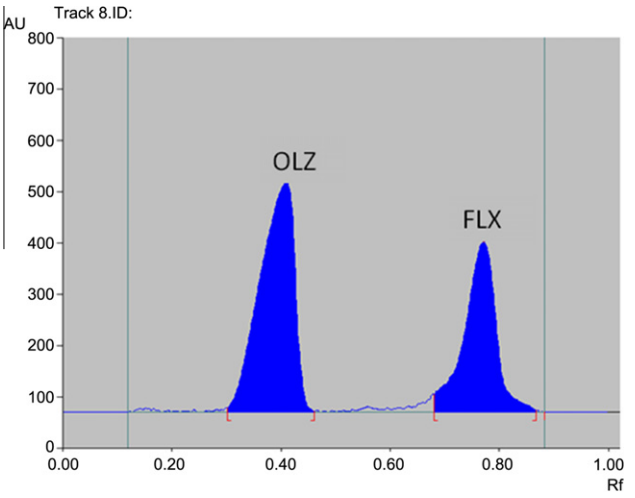


Fig. 4 TLC chromatogram of a resolved mixture of olanzapine (6 μg band^{-1}) and fluoxetine HCl (25 μg band^{-1}).

Working standard solutions

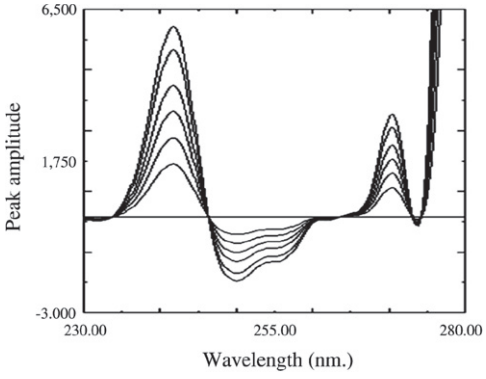
For spectrophotometric methods. Working solutions of OLZ (50 μg mL^{-1}) and FLX (1 mg mL^{-1}) were prepared from their respective stock solutions using methanol as a solvent.

For TLC-spectrodensitometric method. Working solutions of OLZ (1 mg mL^{-1}) and FLX (10 mg mL^{-1}) were prepared from their respective stock solutions using methanol as a solvent.

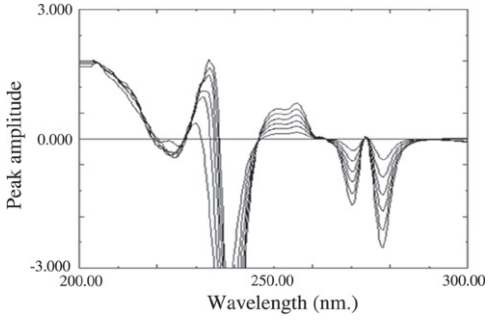
For HPLC method. Working solutions of OLZ (100 μg mL^{-1}) and FLX (1 mg mL^{-1}) were prepared from their respective stock solutions using methanol as a solvent.

Laboratory-prepared mixtures

Solutions containing different ratios of OLZ and FLX were prepared by transferring aliquots from their working solutions into a series of 10-ml volumetric flasks and the volume of each was completed to the mark with methanol in case of spectrophotometry and TLC spectro-densitometry. For HPLC, the volume was completed to the mark with the mobile phase.



(a) First derivative of ratio spectra of olanzapine 5 – 17.5 μg mL^{-1} using the spectrum of 200 μg mL^{-1} of fluoxetine HCl as a divisor, methanol was used as a blank.



(b) First derivative of ratio spectra of fluoxetine HCl 100 – 600 μg mL^{-1} using the spectrum of 12.5 μg mL^{-1} of olanzapine as a divisor, methanol was used as a blank.

Fig. 3 DD^1 spectra of olanzapine and fluoxetine HCl.

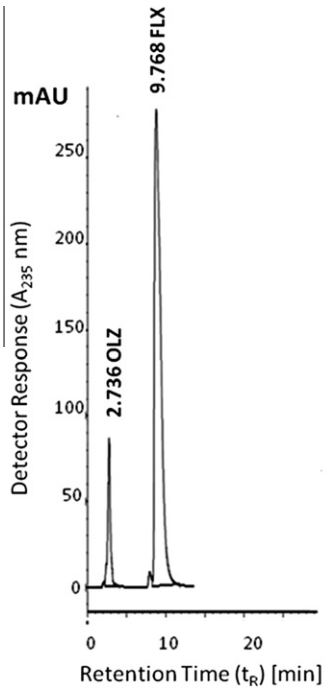


Fig. 5 HPLC chromatogram of 30 μg mL^{-1} olanzapine and 500 μg mL^{-1} fluoxetine HCl.

Procedures

Construction of the calibration curves

For spectrophotometric method. For D^1 spectrophotometric method. Aliquots equivalent to 50–175 μg of OLZ and 1000–6000 μg of FLX were accurately measured and transferred from their working solutions into a set of 10-ml volumetric flasks and the volumes were completed to the mark with methanol. The zero order and the first derivative spectra were recorded. The peak amplitudes of the obtained first derivative spectra were measured at 292 nm for OLZ and at 270 nm for FLX.

For DD1 spectrophotometric method. The zero order absorption spectra of OLZ (5–17.5 μg mL^{-1}) and FLX (100–600 μg mL^{-1}) were measured and divided by the absorption spectra of 200 μg mL^{-1} FLX and 12.5 μg mL^{-1} OLZ, respectively. The first derivative of the obtained spectra was recorded. The peak amplitudes of the obtained DD^1 spectra were measured at 270 nm for OLZ and at 278 nm for FLX.

For TLC-spectrodensitometric method. Aliquots equivalent to 1–8 mg of OLZ and 10–60 mg of FLX were accurately measured and transferred from their working standard solutions into a set of 10-ml volumetric flasks and the volumes were completed to the mark with methanol. A 10-μL aliquot of each solution was applied to the TLC plates, and the plates were developed to a distance of about 9.5 cm by the ascending technique using methanol: toluene: ammonia (7: 3: 0.1, by volume) as the mobile phase. The plates were then removed, air-dried, and the spots were visualized under a UV lamp at 254 nm. The chromatogram was scanned at 235 nm. Two calibration curves representing the relationship between the recorded area under the peak and the corresponding concentrations of the drugs in micrograms per band were plotted.

For HPLC method. Aliquots equivalent to 200–1000 μg of OLZ and 1000–6000 μg of FLX were accurately measured and transferred from their working solutions into a set of 10-ml volumetric flasks and the volumes were completed to the mark with the mobile phase [Phosphate buffer pH 4.0: acetonitrile: triethylamine (53:47:0.03, by volume)]. A 20-μL aliquot of each solution was injected onto a Zorbax ODS column (5 μm, 250 × 4.6 mm i.d.), using the mobile phase, at flow rate 1.0 mL min^{-1} and detection at 235 nm. Two calibration curves were constructed by plotting the peak area ratios, using 50 μg mL^{-1} of OLZ and 200 μg mL^{-1} of FLX as the external standards (the divisors), against the corresponding concentration of each drug in micrograms per milliliter.

Assay of laboratory-prepared mixtures

For spectrophotometric methods. The absorption spectra of the laboratory-prepared mixtures were scanned, processed as under calibration for each of the proposed methods and the concentration of OLZ and FLX in each mixture was calculated using the specified regression equation.

For TLC-spectrodensitometric and HPLC methods. The peak areas or peak area ratios of the laboratory-prepared mixtures

Table 1 Assay parameters and validation sheet for determination of olanzapine and fluoxetine HCl by the proposed methods.

Parameter	OLZ				FLX			
	D^1	DD^1	TLC	HPLC	D^1	DD^1	TLC	HPLC
Range	5–17.5 μg mL^{-1}		1–8 μg band^{-1}	20–100 μg mL^{-1}	100–600 μg mL^{-1}		10–60 μg band^{-1}	100–600 μg mL^{-1}
Slope	−0.0261	0.1819	Slope 1[a] = −307.257	0.018	−0.0021	−0.0041	Slope 1[a] = −3.369	0.005
			Slope 2[a] = 7226.1				Slope 2[a] = 511.8	
Intercept	−0.0006	−0.0096	1319.8	0.086	0.0066	−0.0593	7362.3	−0.004
Mean of R (%)	99.98	100.04	99.93	100.00	100.33	100.26	99.99	99.83
SD of R (%)	0.828	0.608	0.725	0.890	0.521	0.421	0.297	0.729
Variance	0.686	0.370	0.525	0.792	0.271	0.177	0.088	0.531
Correlation coefficient (r)	0.9998	0.9999	0.9999	0.9998	1.0000	0.9999	1.0000	0.9999
Repeatability[b] (%)	0.500	0.721	0.789	0.982	0.252	0.431	0.151	0.511
Intermediate precision[b] (%)	0.729	0.743	0.812	0.959	0.270	0.404	0.162	0.533

[a] Slope 1 and 2 are the coefficients of X^2 and X, respectively. Following a polynomial regression $A = ax^2 + bx + c$ Where, A is the integrated peak area, x is the concentration of Olanzapine or Fluoxetine (μg band^{-1}), a and b are coefficients 1 and 2, respectively and c is the intercept.
[b] Average of three determinations.

were scanned and processed as described for the calibration for each of the proposed TLC and HPLC methods, respectively. The concentrations of OLZ and FLX in each mixture were calculated using the specified regression equations.

Application to pharmaceutical preparations

For spectrophotometric methods. Twenty capsules of Symbyax® (3 mg/25 mg) were evacuated, accurately weighed and finely powdered. Accurately weighed portions equivalent to 12 mg OLZ and 100 mg FLX, respectively were transferred into 100-mL beakers, sonicated in 30 mL methanol for 10 min and filtered into 100-mL volumetric flasks. The residues were washed three times each using 10 mL methanol and the solution was completed to the mark with the same solvent. Aliquots of 1.0 mL were transferred from the prepared solutions to 10-mL volumetric flasks and diluted with methanol for spectrophotometric determination of both drugs. The general procedure previously described under each method was followed to determine the concentration of each drug in the prepared dosage form solutions.

For TLC-spectrodensitometric and HPLC methods. Forty capsules of Symbyax® (3 mg/25 mg) were evacuated, accurately weighed and finely powdered. Accurately weighed portions equivalent to 60 mg OLZ and 500 mg FLX respectively, were transferred into 100-mL beakers, sonicated in 30 mL methanol for 10 min, and filtered into 100-mL volumetric flasks. The residues were washed three times each using 10 mL methanol and the solution was completed to the mark with the same solvent. Aliquots of 5.0 mL were transferred from the prepared solutions to 10-mL volumetric flasks and diluted with methanol for TLC-spectrodensitometric determination of both drugs,

Table 2 Determination of olanzapine and fluoxetine HCl in laboratory prepared mixtures by spectrophotometric methods.

Concentration (μg mL^{-1})		OLZ		FLX	
OLZ	FLX	D^1	DD^1	D^1	DD^1
17.5	100	99.95	100.00	101.05	99.20
12.0	100	100.60	100.45	100.88	99.99
05.0	100	99.96	100.60	100.60	100.42
17.5	200	100.67	100.30	100.70	99.70
10.0	100	100.20	100.00	100.65	99.65
Mean		100.28	100.27	100.78	99.79
RSD		0.344	0.268	0.186	0.451

Table 3 Determination of olanzapine and fluoxetine HCl in laboratory prepared mixtures by TLC spectro-densitometric and HPLC methods.

OLZ: FLX	OLZ		FLX	
	TLC	HPLC	TLC	HPLC
3:25	98.83	100.63	99.76	99.76
1:2	99.00	100.78	99.90	98.65
1:3	99.00	100.66	100.23	99.43
1:4	99.20	100.64	100.05	100.36
Mean	99.01	100.68	99.99	99.55
RSD	0.153	0.069	0.202	0.716

Table 4 Determination of olanzapine and fluoxetine HCl in Symbyax® capsules and application of standard addition technique using the proposed methods.

Symbyax® B.N. A588272A (3 mg OLZ & 25 mg FLX) per capsule	OLZ				FLX			
	D^1	DD^1	TLC	HPLC	D^1	DD^1	TLC	HPLC
Mean[a] ± RSD	99.65 ± 0.317	100.92 ± 0.019	98.99 ± 1.350	100.98 ± 0.210	100.33 ± 0.067	100.49 ± 0.112	101.07 ± 0.160	99.26 ± 0.370
Recovery of standard added[a] ± RSD	100.55 ± 0.224	99.95 ± 0.743	99.51 ± 0.523	100.97 ± 0.510	99.81 ± 0.481	99.68 ± 0.246	100.44 ± 1.206	99.15 ± 0.229

[a] Average of five determinations.

where 10 μL was applied onto TLC plates. For HPLC analysis, the last solution was further diluted by transferring 1.0 mL aliquots of it to 10-mL volumetric flasks and the volumes were completed with the HPLC mobile phase. The general procedures described above for each method were followed to determine the concentration of OLZ and FLX in the prepared dosage form solutions.

Results and discussion

Spectrophotometric methods

First derivative method (D^1)

The zero order absorption spectra of OLZ and FLX show severe overlapping that prevents the use of direct spectrophotometry for their analysis without preliminary separation, Fig. 2A. In the first derivative spectrophotometry, the zero order absorption spectra of OLZ and FLX are obtained and then the first derivative of the obtained spectra was recorded using $\Delta\lambda = 4$ nm and a scaling factor of 10, Fig. 2B. The peak amplitudes of the obtained first derivative spectra were measured at 292 nm for OLZ and 270 for FLX. The first derivative spectroscopy was applied to solve the problem of the overlapped absorption spectra of the cited drugs.

The regression equations were computed for OLZ and FLX and found to be:

$$D^1 = -0.0261C - 0.0006 \quad \text{(for OLZ)}$$

$$D^1 = -0.0021C + 0.0066 \quad \text{(for FLX)}$$

where D^1 is the peak amplitude and C is the corresponding concentration in μg mL^{-1}.

Derivative ratio method (DD^1)

In the derivative ratio spectrophotometry, the absorption spectrum of the mixture is obtained and divided by the absorption spectrum of the standard solution of one of the components, and the first derivative of the ratio spectrum is obtained. First derivative ratio spectrophotometric method DD^1 was applied to solve the problem of the overlapped absorption spectra of the cited drugs.

Different concentrations of OLZ and FLX were investigated as divisors. The divisor concentrations 12.5 μg mL^{-1} and 200 μg mL^{-1} of OLZ and FLX, respectively, were found the best regarding average recovery percent when they were used for the prediction of OLZ and FLX concentrations in bulk powder as well as in laboratory-prepared mixtures. The obtained ratio spectra were differentiated with respect to wavelength using scaling factor 10 and $\Delta\lambda = 4$, Fig. 3A and B. The peak amplitudes showed good linearity and accuracy at 270 nm and 278 nm for OLZ and FLX, respectively. The regression equations were computed for OLZ and FLX and found to be:

$$DD^1 = 0.1819C - 0.0096 \quad \text{(for OLZ)}$$

$$DD^1 = -0.0041C - 0.0593 \quad \text{(for FLX)}$$

where DD^1 is the peak amplitude and C is the corresponding concentration in μg mL^{-1}.

TLC-spectrodensitometric method

Several trials were done to choose a developing system which can separate OLZ from FLX. Satisfactory separation was obtained using the system methanol: Toluene: ammonia (7:3:0.1, by volume) as the mobile phase. R_f values were 0.3 ± 0.02 and 0.7 ± 0.02 for OLZ and FLX, respectively as shown in Fig. 4. This separation allows the determination of OLZ and FLX at 235 nm without any interference from each other. A polynomial relationship was found to exist between the integrated area under the peak of the separated spots at the selected wavelength (235 nm) and the corresponding concentration of OLZ in the range of 1–8 μg band^{-1} and in the range of 10–60 μg band^{-1} in case of FLX. The regression equations were computed for OLZ and FLX and found to be:

$$A = -307.257C^2 + 7226.1C + 1319.8 \quad \text{(for OLZ)}$$

$$A = -3.369C^2 + 511.8C + 7362.3 \quad \text{(for FLX)}$$

where A is the integrated peak area under the peak and C is the corresponding concentration in μg band^{-1}.

HPLC method

Good chromatographic separation of the two drugs in their binary mixtures could be achieved by using a Zorbax ODS column (5 μm, 250 × 4.6 mm i.d.) with a mobile phase consisting of Phosphate buffer pH 4: acetonitrile: triethylamine (53:47:0.03, by volume) followed by UV detection at 235 nm, Fig. 5. Several trials have been undertaken to reach the optimum stationary/mobile phases matching. The suggested chromatographic system allows complete base line separation at reasonable time. The linearity of the detector's response of the studied drugs was determined by plotting peak area ratios (calculated following the external standard technique using 50 μg mL^{-1} of OLZ and 200 μg mL^{-1} of FLX as the external standards) versus concentrations and linear correlation was obtained.

Table 5 Parametrs required for system suitability test of TLC-spectrodensitometric and HPLC methods.

Parameters	TLC		HPLC		Reference values [18,19]
	OLZ	FLX	OLZ	FLX	
Retention time (t_R) [min.]			2.74	9.77	
Retardation factor (R_f)	0.30	0.70			
Resolution (R_s)		3.56		12.88	$R_s > 2$
Tailing factor (T)	0.833	0.714	0.9	1.1	$T = 1$ for a typical symmetric peak
Capacity factor (K')			2.053	8.743	$1 < K' < 10$
Selectivity factor (α)		2.333		4.259	$\alpha > 1$
Column efficiency (N)			2774.01	2334.89	$N > 2000$
Height equivalent to theoretical plate (HETP) (mm)			0.090	0.107	

Table 6 Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of olanzapine and fluoxetine HCl.

Parameter	OLZ				FLX				Reported[a] HPLC method [14]	
	D^1	DD^1	TLC	HPLC	D^1	DD^1	TLC	HPLC	OLZ	FLX
Mean	99.98	100.04	99.93	100.00	100.33	100.26	99.99	99.83	100.00	99.92
SD	0.828	0.608	0.720	0.890	0.521	0.421	0.300	0.730	0.450	0.410
Variance	0.686	0.370	0.525	0.792	0.271	0.177	0.088	0.531	0.200	0.170
n	6	6	6	9	6	6	7	9	6	6
F-test	3.417 (5.05)[b]	1.847 (5.05)[b]	2.62 (5.05)[b]	3.96 (4.82)[b]	1.601 (5.05)[b]	1.045 (5.05)[b]	1.92 (4.39)[b]	3.13 (4.82)[b]		
Student's t-test	0.065 (2.23)[b]	0.126 (2.23)[b]	0.197 (2.23)[b]	0.007 (2.16)[b]	1.506 (2.23)[b]	1.422 (2.23)[b]	0.376 (2.20)[b]	0.260 (2.16)[b]		

[a] HPLC method using C-18 analytical column, acetonitrile: methanol: 0.032 M ammonium acetate buffer (45:05:50 by volume) as the mobile phase at flow rate 1.5 ml min^{-1} and detection at 235 nm.

[b] These values represent the corresponding tabulated values of t and F at $p = 0.05$.

The regression equations were computed for OLZ and FLX and found to be:

$$A = 0.018C + 0.086 \quad \text{(for OLZ)}$$

$$A = 0.005C - 0.004 \quad \text{(for FLX)}$$

where A is the peak area ratio and C is the corresponding concentration in μg mL^{-1}.

Validation of the proposed methods was done according to the ICH guidelines. For all the proposed methods, the intermediate precision and repeatability, the assay parameters of the regression equations and the concentration ranges are shown in Table 1.

The proposed methods were successfully applied to the analysis of OLZ and FLX in their laboratory prepared mixtures, Tables 2 and 3 and in capsule dosage form, Table 4. The validity of the proposed methods was assessed by applying the standard addition technique, Table 4.

After the proposed TLC-spectrodensitometric and HPLC methods have been validated, an overall system suitability testing was done to determine if the operating system is performing properly. All peak parameters of resolution efficiency were calculated and satisfactory results were obtained, Table 5.

Statistical comparison between the results obtained by the proposed methods and those obtained by the reported HPLC method was done [14]. The calculated t- and F-values [17] were found to be less than the corresponding theoretical ones, confirming good accuracy and excellent precision, Table 6.

Conclusion

The proposed methods are simple, sensitive, and precise and could be easily applied in quality control laboratories for the simultaneous determination of OLZ and FLX.

The advantages of the proposed HPLC method over the reported ones [13–15] are better resolution (12.88), wider range (we can determine up to 100 μg mL^{-1} olanzapine and 600 μg mL^{-1} fluoxetine HCl) and less tailed (more symmetric) peaks. The proposed TLC-spectrodensitometric method has also the advantages of better resolution and wider range (we can determine up to 8 μg $band^{-1}$ olanzapine and 60 μg $band^{-1}$ fluoxetine HCl) over the reported ones [14,15].

The proposed methods could be successfully applied for the routine analysis of the studied drugs either in their pure bulk powders or in their dosage forms without any preliminary separation step.

References

[1] Moffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons. London: Pharmaceutical Press; 2004.

[2] Raggi MA, Casamenti G, Mandrioli R, Izzo G, Kenndler E. Quantitation of olanzapine in tablets by HPLZ, CZE, derivative spectrometry and linear voltammetry. J Pharm Biomed Anal 2000;23(6):973–81.

[3] Zhang G, Terry Jr AV, Bartlett MG. Simultaneous determination of five antipsychotic drugs in rat plasma by high performance liquid chromatography with ultraviolet detection. J Chromatogr B Analyt Technol Biomed Life Sci 2007;856(1–2):20–8.

[4] D'Arrigo C, Migliardi G, Santoro V, Spina E. Determination of olanzpine in plasma by reversed-phase high-performance

liquid chromatography with ultraviolet detection. Ther Drug Monit 2006;28(3):388–93.
[5] Dusci LJ, PeterHackett L, Fellows LM, Ilett KF. Determination of Olanzapine in plasma by high-performance liquid chromatography using ultraviolet absorbance detection. J Chromatogr B Analyt Technol Biomed Life Sci 2002;773(2):191–7.
[6] Olesen OV, Linnet K. Determination of olanzapine in serum by high-performance liquid chromatography using ultraviolet detection considering the easy oxidability of the compound and the presence of other psychotropic drugs. J Chromatogr B Biomed Sci Appl 1998;714(2):309–15.
[7] Krebs A, Starczewska B, Puzanowska-Tarasiewicz H, Sledz J. Spectrophotometric determination of olanzapine by its oxidation with N-bromosuccinimide and cerium (IV) sulfate. Anal Sci 2006;22(6):829–33.
[8] Afkhami A, Madrakian T, Khalafi L. Spectrophotometric determination of fluoxetine by batch and flow injection methods. Chem Pharm Bull (Tokyo) 2006;54(12):1642–6.
[9] Darwish IA. Development and validation of spectrophotometric methods for determination of fluoxetine, sertraline, and paroxetine in pharmaceutical dosage forms. J AOAC Int 2005;88(1):38–45.
[10] Prabhakar AH, Patel VB, Giridhar R. Spectrophotometric determination of fluoxetine hydrochloride in bulk and in pharmaceutical formulations. J Pharm Biomed Anal 1999;20(3):427–32.
[11] LLerena A, Dorado P, Berecz R, González A, Jesús Norberto M, de la Rubia A, et al. Determination of fluoxetine and norfluoxetine in human plasma by high performance liquid chromatography with ultraviolet detection in psychiatric patients. J Chromatogr B Analyt Technol Biomed Life Sci 2003;783(1):25–31.
[12] Zarghi A, Kebriaeezadeh A, Ahmadkhaniha R, Akhgari M, Rastkari N. Selective liquid chromatographic method for determination of fluoxetine in plasma. J AOAC Int 2001;84(6):1735–7.
[13] Pathak A, Rajput SJ. Development of stability-indicating HPLC method for simultaneous determination of olanzapine and fluoxetine in combined dosage forms. J Chromatogr Sci 2009;47(7):605–11.
[14] Patel S, Patel NJ. Simultaneous RP-HPLC and HPTLC estimation of fluoxetine hydrochloride and olanzapine in tablet dosage forms. Indian J Pharm Sci 2009;71(4):477–80.
[15] Shah Charmy R, Shah Nehal J, Suhagia Bhanubhai N, Patel Natvarlal M. Simultaneous assay of olanzapine and fluoxetine in tablets by column high- performance liquid chromatography and high-performance thin layer chromatography. J AOAC Int 2007;90(6):1573–8.
[16] Moffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons. London: Pharmaceutical Press; 2004, p. 517.
[17] Spiegel MR, Stephns LJ. Schaum outline of theory and problems of statistics. New York: Schaum Outline Series; 1999.
[18] Andrea W, Phyllis R. HPLC and CE principles and practice. London: Academic Press; 1997, p. 7–15.
[19] Adamovics AJ. Chromatographic analysis of pharmaceuticals. New York: Marcel Dekker; 1997, p. 11–17.