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Validated Stability -Indicating UPLC Method for Determination of Dapoxetine and Fluoxetine: Characterization of Their Hydrolytic Degradation Products, Kinetic Study and Application in Pharmaceutical Dosage Forms

Suzan Mahmoud Soliman[1*], Heba MY El-Agizy[1] and Abd El Aziz El Bayoumi[2]

[1]*National Organization for Drug Control and Research (NODCAR), Giza, Egypt*
[2]*Analytical Chemistry Departments, Faculty of Pharmacy, Cairo University, Cairo, Egypt*

Abstract

New isocratic stability-indicating reversed phase UPLC method was developed for determination of two antidepressant drugs dapoxetine hydrochloride (DAP) and fluoxetine hydrochloride (FLX) in the presence of their hydrolytic degradation products namely; (+)-N, N-dimethyl-1-phenyl-3-propanolamine (DAP Deg I), N-methyl-3-hydroxy-3-phenyl propyl amine (FLX DegI), α, α, α-Trifluorotoluene (FLX Deg II) and application in their pharmaceutical dosage forms.

UPLC method using small sub-1.8 µm particle was developed for separation and determination of the selected drugs using Agilent Eclipse XDB C18 (50 mm x 2.1 mm i.d., 1.8 µm) column. Upon using UPLC, the run time could be reduced 5-fold and the solvents consumption decreased 10 tims. Quantification is achieved by detection wavelength at 210 nm, based on peak area. The linear ranges were 0.05-100 µg/mL and 0.30-100 µg/mL with LOD of 0.01 and 0.09 µg mL^{-1} and mean recoveries of 99.41 ± 1.02 and 100.05 ± 0.89 for DAPand FLX, respectively, the developed method was successfully applied to analysis of DAP and FLX in bulk powder, laboratory-prepared mixtures containing different percentages of degradation products and pharmaceutical dosage forms.

UPLC method was also directed to investigate the degradation kinetic processes of both drugs. It was followed pseudo-first order reactions with a degradation reaction rate constant (k) of 0.0575 (h^{-1}) and 0.965 (h^{-1}) and half-life ($t_{1/2}$) of 12.04 and 0.75 (h) for DAP and FLX, respectively. The degradation rate (k) obeyed Arrhenius equation and the activation energies were calculated. The degradation products (I-III) were separated by UPLC and subjected to MS spectrometry to confirm their structures and elucidate degradation pathway. The developed methods were validated as per ICH guidelines.

Keywords: Dapoxetine; Fluoxetine; Stability-indicating UPLC-MS; Kinetic investigation; Structural elucidation

Introduction

Major depressive disorder (MDD), depression, is one of the most common and widespread mental disorders in our society. It affects approximately 216 million people (3% of the world's population) [1,2].

Dapoxetine HCl (DAP) and fluoxetine HCl (FLX) are antidepressant drugs of the selective serotonin reuptake inhibitors (SSRIs) class. They are used for the treatment of major depressive and obsessive–compulsive disorders. Clinical studies have shown that after treatment with SSRI drugs, intravaginal ejaculation latency time gradually increased as a side effect in men [3]. Dapoxetine is a short acting oral SSRI, it cannot permanently cure premature ejaculation PE but has increased in importance due to fewer side effects. It has a much lower ejaculation-delaying effect compared with FLX. SSRIs appear to be the most important therapeutic drugs for PE with regard to their multiple sites of action in the regulation of the complex mechanisms involved in ejaculation. Therefore, SSRIs have opened a new potential avenue for PE treatment. According to the European Association of Urology (EAU) guidelines, daily treatment with SSRI has become the first-choice treatment in PE. It is postulated that on-demand (acute) treatment with SSRI, such as dapoxetine, will not produce an ejaculation delay equivalent to daily long-term (chronic) treatment of SSRI, such as fluoxetine [3,4].

Chemically DAP is recognized as (S)-N, N-dimethyl-3-(naphthalen-1-yloxy)-1-phenyl propan-1- amine hydrochloride [5]. Whereas FLX is (±) - [N-methyl-3-phenyl-3-[4-(trifluoromethyl)-phenoxy] propan-1-amine] hydrochloride [5].

Dapoxetine is not official in any Pharmacopeia. In the literature survey, several reports have been established for analysis of DAP either alone or in combined pharmaceutical dosage forms such as synchronous fluorescence spectroscopy [6] TLC densitometry [7], CZE [8], and liquid chromatography [9,10] using different columns and various mobile phases.

Few methods have been published for the stability indicating determination of DAP in the presence of unknown degradation products formed through forced degradation studies. Most of the methods were related with chromatographic techniques such as HPLC [11-15] using different mobile phases and various detection modes, but no attempt has been carried out for characterization of degradation products. To best of our knowledge, there is only one stability-indicating UPLC with diode array detection was carried out for quantification of DAP along with sildenafil [16]. However, these

***Corresponding author:** Suzan Mahmoud Soliman, National Organization for Drug Control and Research (NODCAR) El Agoza, Giza, 12311, Egypt, Tel: +20226496077; Fax: +20223379445; E-mail: suzansoliman1961@hotmail.com

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methods were associated with some major drawbacks such as the use of gradient-system program that had increased solvents consumption and quantified the drug in high cost of analysis [13,15,16], also there was a lack of selectivity and relatively high retention time [15].

On the other hand, the quantification of FLX in pharmaceutical formulations were addressed in several reports including; Spectrophotometry [17], Spectro fluorimetry [17], GC [18], CE [19], HPTLC [20,21] and, liquid chromatography methods [20-23] using various detection modes.

In the literature survey, few methods have been developed for the stability indicating assay of FLX in combination with other drugs including; HPTLC densitometry [24] and HPLC via diode array detection [25] and UV detection [26].

Unfortunately, these methods were associated some limitations such as increasing the cost of analysis, solvent consumption and analysis time, particularly due to the use of gradient elution mode [25] and the relatively high retention time (20 min) [26]. In addition, no data published for identification and characterization of FLX degradation products [24-26].

Recently UPLC methods for the simultaneous determination of FLX and its active metabolite in biological samples have been reported with Risperidone [27] or Clomipramine [28]. To best of our knowledge, there is no stability-indicating UPLC for the determination of FLX in presence of its degradarion products (I, II) with its application in pharmaceutical formulation.

UPLC is a recent technique giving new possibilities in liquid chromatographic separation, which enables significant decrease of time of analysis and solvent consumption. UPLC system is designed in a special way to withstand high system back-pressures. Special columns packed with 1.7 µm particles size are used about this UPLC system. It allows reduction in time of analysis up to 9 folds comparing to the conventional HPLC using 5 µm particle packed columns [29].

The proposed UPLC method for analysis of DAP and FLX in their pharmaceutical preparations has been developed (for the first time) in presence of their major degradation products confirmed as (+)-N, N-dimethyl-1-phenyl-3-propanolamine, N-methyl-3-hydroxy-3-phenyl propyl amine and, α, α, α-Trifluorotoluene. Up till now neither isocratic stability-indicating UPLC nor degradation kinetic study of DAP and FLX has been reported, therefore our target is to develop a simple, rapid, economical, reproducible, fully validated and alternative isocratic stability-indicating UPLC method for quantifying DAP and FLX in presence of their degradates (I-III) with good detection limits in drug substances and products that will overcome the above-mentioned drawbacks (Figure 1).

Experimental Instruments

UPLC system Agilent, USA, 1200 series, consisted of a binary pump SL(G131213) equipped with an autosampler injector ALS SL(G132913) and a photo diode array detector (PDAD) (G1315C). The chromatographic separations were performed at 25°C using Agilent Eclipse XDB C18 (50 mm x 2.1 mm i.d., 1.8 µm particle size). pH measurements were carried out using a digital pH meter (Hanna pH211, Romania). TLC aluminum sheets (20 x 20 cm, 0.2 mm) Silica gel F254 (Merck KGaA Darmstadt, Germany) and a UV lamp with a short wavelength of 254 nm (Desaga Germany) were used for identification by TLC. The mobile phases were filtered through 0.45 µm Millipore membrane filters (Sartorius, Germany). A thermostatic water bath (Memmert, Germany), a Rotavapor (Buchi, Switzerland), a sonicator (crest, New York) were used. Hamilton syring with a 10 µl capacity was used.

LC/MS

The HPLC apparatus consisted of Accela 1200 LC-10AD pump, auto sampler Accela and a Hypersill gold Phenomenex (50 mm × 2.0 mm i.d., 2.1 µm particle size) column preceded by a C18 security guard cartridge Gemini C18 Phenomenex (4 mm x 3 mm i.d., 5 µm particle size).

Mass spectrometric analysis was carried out using a TSQ Quantum Access MAX triple quadrupole detection system. Data acquisition was performed in Full scan mode. Processing of data is performed using Thermo Scientific Xcalibur 2.1 software.

Materials

Authentic samples

Dapoxetine HCl standard material (99.22 ± 1.35%) was kindly supplied by Inspire Pharma. Its purity was assessed using the reported HPLC method. The determination of the drug was done using a mobile phase consisting of Methanol- H_2O (80:20 v/v) with quantification at a wavelength at 239 nm [12].

Fluoxetine HCl standard material was kindly supplied by Eli Lilly Company. Its purity was certified to be (99.29 ± 1.54%) using cetonitrile-methanol-0.032 M ammonium acetate buffer (45:05:50, v/v/v) as the mobile phase as the mobile phase with quantitation at 235 nm [21].

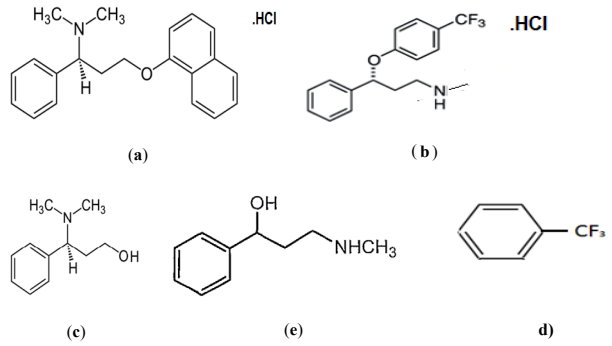


Figure 1: Chemical structures of the studied drugs and their degradation products: (a) Dapoxetine HCl DAP, (b) Fluoxetine HCl FLX, (c) (+)-N,N-dimethyl-1-phenyl-3-propanolamine (DAP Deg I), (d) N-methyl-3-hydroxy-3-phenylpropylamine (FLX Deg I) and (e) α, α, α-Trifluorotoluene (FLX Deg II).

Market samples

Joypox® tablets (batch No # 0912109) were kindly supplied by Inspire Pharma (Cairo,Egypt). Each tablet was labeled to contain 60 mg dapoxetine base.

Prozac® capsules (batch No # 1014B) were kindly supplied by Lilly S.A (Spain) (Cairo, Egypt), Each capsule was labeled to contain 20 mg fluoxetine base.

Chemicals and solvents

Chromatographic grade, scetonitrile and methanol were obtained from (Lab-Scan, Poland). Ammonium acetate (Fischer Chemical, UK), triethylamine (LOBA Chemie, India) and acetic acid glacial (Biosolve Chimie SARL, France) were of analytical grade. Double distilled water was used to prepare all necessary solutions.

Standard solutions

Stock standard solutions of 1 mg mL^{-1} of each of DAP and FLX were prepared in the mobile phase, the working standard solutions were separately prepared by convenient dilution of their stock standard solutions with the corresponding mobile phase to obtain standard solutions of 0.1 mg/ml and 0.2 mg mL^{-1} of DAP and FLX, respectively.

Chromatographic conditions

The chromatographic separation was achieved on a reversed phase Agilent Eclipse XDB C18 (50 mm x 2.1 mm i.d., 1.8 µm particle size) column. An isocratic mobile phase consisting of a mixture of aqueous 0.03 M ammonium acetate, pH 4.2-acetonitrile (35: 65, v/v) was used for separation and quantification of DAP. A mixture of aqueous 0.03 M triethylamine, pH 4.3 (adjusted with glacial acetic acid) -acetonitrile (40:60, v/v) was used for the chromatographic analysis of FLX. The prepared mobile phase was degassed in an ultrasonic bath for 10 min and then filtered using a vacuum pump. Determination was performed at 0.2 mL min^{-1} flow rate with injection volumes 0.8 µL and detection wavelength 210 nm. All measurements were carried out at ambient temperature (25°C ± 2) for both drugs. To achieve good equilibrium, the column was pre-conditioned with the mobile phase before analysis at ambient temperature about 20 min.

Procedures

Linearity and construction of the calibration curves

Aliquots of DAP (0.1 mg mL^{-1}) and FLX (0.2 mg mL^{-1}) working standard solutions were separately transferred into series of 10 ml volumetric flasks to get final concentration range of (0.05-100 µg mL^{-1}) and (3-100 µgmL^{-1}) for DAP and FLX, respectively, in the corresponding mobile phase. Triplicate 0.8 µL injections were performed for each concentration and chromatographed under the specified chromatographic conditions described previously. The peak area values were plotted against corresponding concentrations, and the regression equations were computed.

Stability study of DAP and FLX

100 mg of each of DP and FLX powder was transferred into 250 ml round bottom flask, dissolved in 100 ml of 5M HCl solution. The two flasks were tightly closed away from light and left for 30 and 2 h at thermostatically controlled water bath set at 90°C and 80°C for DAP and FLX, respectively. The complete degradation processes of the two drugs were followed by TLC using toluene-methanol (8.5:1.5 v/v) and toluene -glacial acetic acid (5:5 v/v) as developing systems to achieve complete degradation of DAP and FLX, respectively. The developed plates were visualized at 254 nm under a UV lamp.

The acidic degradation products solutions were neutralized with 5M Na OH to pH 7 and then evaporated until dryness in Rotavapor water bath at 70°C and cooled. The residues of the hydrolytic degradation products were separately dissolved in 50 ml methanol and filtered to exclude sodium chloride formed during the neutralization process. The solutions were further re-evaporated until dryness. The residues of degradation products were separately dissolved in 100 mL methanol to get stock solutions of (1 mg mL^{-1}) for each degradate. Further dilution was prepared in mobile phase to obtain working standard solutions of (0.1 mg/ml of DAP degradate) and (0.2 mg mL^{-1} of FLX degradate).

The obtained degradation products were identified by mass spectrometry.

a) Laboratory prepared mixtures containing different ratios of DP and its hydrolytic degradation product (DegI)

Several laboratory prepared mixtures of DAP and its degradation product (I) were prepared in the range of (10-90%w/w).

b) Laboratory prepared mixtures containing different ratios of FLX and its hydrolytic degradation products (DegI&II)

Several laboratory prepared mixtures of FLX and its degradation products (I&II) were prepared in the same range.

The prepared mixtures were analyzed by the proposed method as described under chromatographic conditions where the concentrations of the intact drug in each mixture was calculated from the corresponding regression equation.

Application to pharmaceutical formulation

The content of ten Joypox® tablets and content of ten Prozac® capsules was separately weighed and ground. A portion of the ground powder equivalent to 100 mg of each drug was accurately weighed, transferred separately to 100 ml volumetric flask, dissolved in 80 mL of the mobile phase using ultrasonic bath (15 min), filtered and completed to the volume with the corresponding mobile phase. Further dilution was carried out with the mobile phase to reach the specified calibration range. Triplicate 0.8 µL of the prepared solutions of both drugs were injected into the chromatographic column under the previously described chromatographic conditions. The concentration of each drug was calculated using the corresponding regression equation and standard addition technique was applied.

Kinetic investigation of the hydrolytic degradation products by UPLC

One milliliter of each of DAP and FLX stock standard solution (1 mg mL^{-1}) was separately transferred into a series of test tubes followed by the addition of 1 mL of 5N HCl solution. The test tubes were stoppered and placed in a thermostatic water bath at different temperatures (60, 70, 80, 90°C and (60, 70, 80°C) for DAP and FLX, respectively. Every 40-min starting from zero time until 4 hours for DAP and every time interval of 20 min starting from zero time until 1.7 hour for FLX, the content of each tube was neutralized to pH 7 by using 1 mL of 5N NaOH. Each tube immediately transferred to 10 mL volumetric flasks, filtered and then the volume was completed with the corresponding mobile phase. The prepared solutions were chromatographed as the previously described under linearity and construction of the calibration curve.

The concentrations of each remaining drug were calculated at each time interval and temperature. Logarithm of the percentage of the remaining drug concentration was plotted against the corresponding time interval in (h) for each temperature, and the regression equations were computed. Arrhenius plots were used to determine the activation energy (Ea), half-life ($t_{1/2}$) and frequency factor (A) of each drug.

Results and Discussion

Optimization of chromatographic conditions

A RP-isocratic stability-indicating UPLC method was developed as an alternative method for determination of DAP and FLX in their pharmaceutical formulations in presence of their hydrolytic degradation products(I-III). The achievement of complete separation of the target drugs from all other interferences and excipients within a short analysis time, low solvent consumption and acceptable efficiency is one of the favorable advantage of the proposed UPLC method.

Agilent Eclipse XDB C18 (50 x 2.1 mm i.d., 1.8 µm), zorbax SB C18 (100 x 2.1 mm i.d., 1.8 µm) and Agilent Eclipse XDB C18 (100 x 2.1 mm i.d., 1.8 µm) were tested as stationary phases. Agilent Eclipse XDB C18 (50 x 2.1 mm i.d., 1.8 µm) column was chosen for best separation with shorter retention times.

Mobile phase systems matching trials were performed to achieve the best system that provides well resolved peaks of the drugs from their degradations. Our preliminary trials using water and acetonitrile in different ratios were tried; it was observed that retention time was found to be increase with the increase in proportion of water. This may be due to the presence of non-polar naphthalene and benzene rings in the structure of the cited drugs which increase the affinity for the non-polar stationary phase. After addition of 0.1% Phosphoric acid to acetonitrile as a mobile phase, it resulted in broad peak of DAP and FLX. As the combination of aqueous 0.03 M ammonium acetate at pH 4.2 or aqueous 0.03 M triethylamine at pH 4.3 with acetonitrile, peak shape of the target drugs was significantly improved.

To optimize the peak separation, the flow rate was varied between at 0.1 and 0.5 mL min^{-1}. The best resolution flow rate was at 0.2 mL min-1 for both drugs. The column temperatures between 25 and 45 °C were tested. The column temperature at 25°C was chosen. The quantitation's of the cited drugs were performed at 210 nm using photodiode array detector DAD which has the advantage of selecting the best wavelength for analysis.

DAD has a major advantage for keeping both the sensitivity and stability indicating power of the proposed method by quantifying the studied drugs and detecting the degradants at their optimum wavelength. The effect of pH on some system suitability parameters was also studied including resolution and retention time. The retention time of the basic drugs DAP (pk_a 8.6) and FLX (pk_a 9.8) was found to be increased with the increasing of pH value as indicated in Table 1.

Finally, mobile phase consisting of a mixture of aqueous 0.03M ammonium acetate at pH 4.2 with acetonitrile or aqueous 0.03M triethyl amine (TEA) at pH 4.3 with acetonitrile in a ratio of (35: 65, v/v) or (40: 60, v/v) were used for separation of DAP, FLX, and their degradations. The two mobile phase systems were found to be optimal as to achieve minimal back ground noise and obtained well resolved peak shape.

The retention time are 1.58 and 1.85 min for DAP and its degradation product (Deg I) and 2.28, 2.88 and 4.32 min for FLX and its degradants (Deg I&II), respectively (Figures 2-4).

The Linearity between the integrated peak areas and the corresponding concentrations of the drugs was studied. Linear relationship was obtained in the range of 0.05-100 µgml^{-1} for DAP and 0.3-100 µgml^{-1} for FLX. The regression equations were:

Y= 5.447 X + 1.9937 (r = 0.9995) for DAP

Y= 0.3615 X – 0.3633 (r = 0.9997) for FLX

Where Y is the peak area, C is the concentration of the compound in µg mL^{-1} and r is the correlation coefficient (Figure 5).

Forced degradation study

Forced degradation studies of the intact drug can support identification of the major degradates, establishing the degradation pathway, validate stability indicating power of the developed method and asses' stability of the studied drug.

DAP and FLX are ether containing pharmaceutical compounds, they were subjected to acid hydrolysis in aqueous solution to develop stability indicating UPLC chromatographic method.

DP was subjected to different mild and drastic degradation conditions studied according to ICH guidelines for acid stress using 0.1M, 1M HCl for 10 h, 2M, 3M HCl for 10 h, 4M HCl for 20 h and 5 M HCl for 20 and 30 h. Also, stability of FLX was studied using 0.1M, 1M HCl for 1 h, 2M HC, 3M HCl for 1 h and 4 M, 5 M HCl for 2 h as per ICH guideline [30].

Upon refluxing DAP and FLX with 5M HCl, respectively, hydrolysis of the ether group occurs, and a regular decrease in the concentration of the cited drugs with increase time intervals was observed. However, in drastic acid hydrolysis conditions, the drugs are totally degraded. The degradation product of DAP (Deg I) is (+)-N, N-dimethyl-1-phenyl-3-propanolamine, where the other degradation product is Naphthalene (Deg II) which is known to be volatile (Scheme I).

The complete degradation of FLX giving two degradation products namely; N-methyl-3-hydroxy-3-phenyl propyl amine (Deg I) and α, α, α-Trifluorotoluene Deg II) (Scheme II). The degradation study indicates that both the drugs undergo hydrolytic degradation at 900°C and FLX was found to be more susceptible to hydrolysis as compared to DAP in 5M HCl.

The mass spectrum

The mass spectrum of degraded DAP showed mass ion peak at (180.34 m/z) (Figure 6) corresponding to acid degradation product which gave the following suggestion for the molecular formula: $C_{11}H_{17}NO$ (m/z 180.34, Deg I). The proposal of the reaction pathway of DAP with 5M HCl was presented in (Scheme I). The other fragment was suggested to be naphthalene which is known to be volatile.

Selected drug	pH	Resolution	Retention time
	3	1.9	1.7
DAP	4	2.2	1.8
	4.2	2.68	1.83
	4.4	2.55	2.2
	5.2	2.5	2.3
	3	1.5	2
FLX	4	2.1	2.15
	4.3	2.3	2.28
	4.5	2.2	5.2
	5.3	2.1	7.1

Table 1: Effect of changing pH on resolution and retention time of DAP and FLX using two different mobile phase systems.

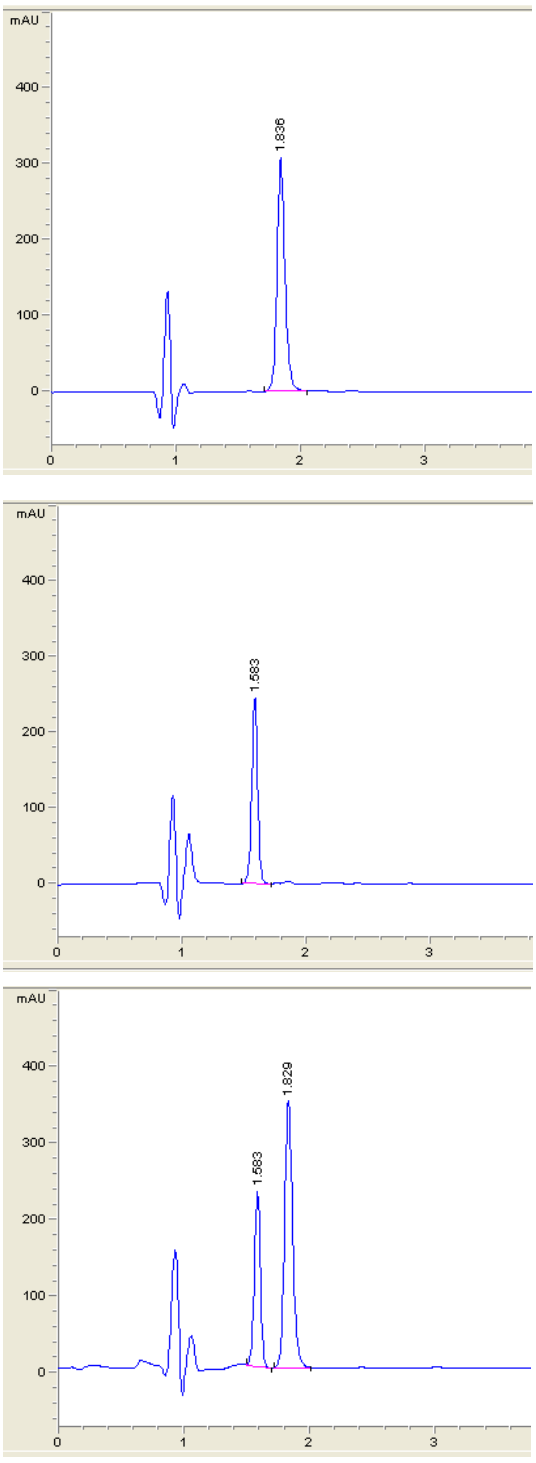


Figure 2: UPLC Chromatograms of (a) DAP, (b) degradation product of DAP, and (c) mixture of DAP and its degradation product, (10 µg/mL each).

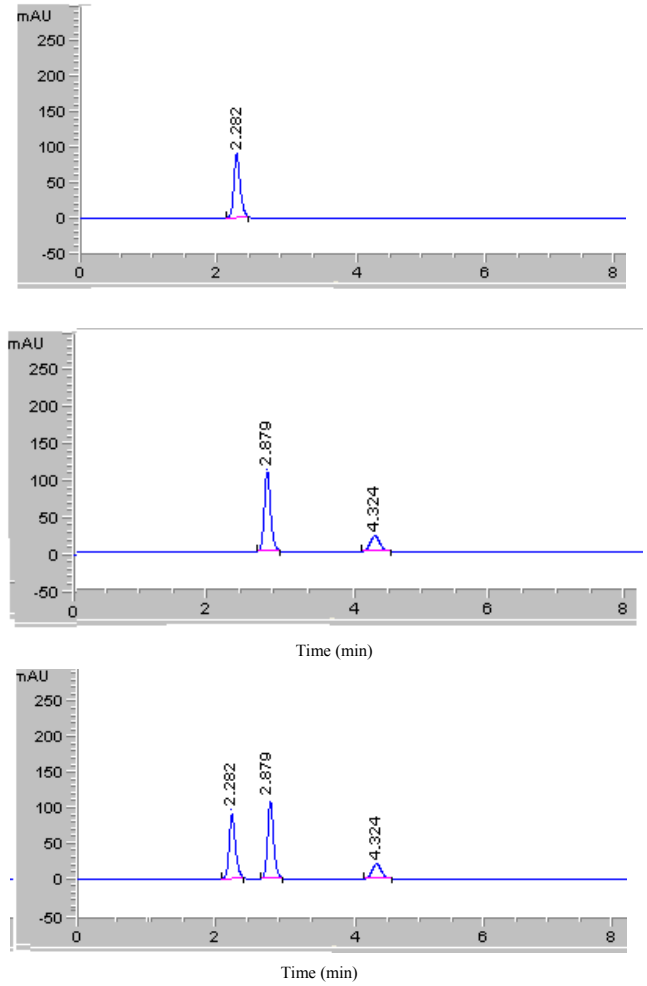


Figure 3: UPLC Chromatograms of (a) FLX, (b) degradation product of FLX (Degl and II), and (c) mixture of FLX and its degradation product, (30 µg/mL each).

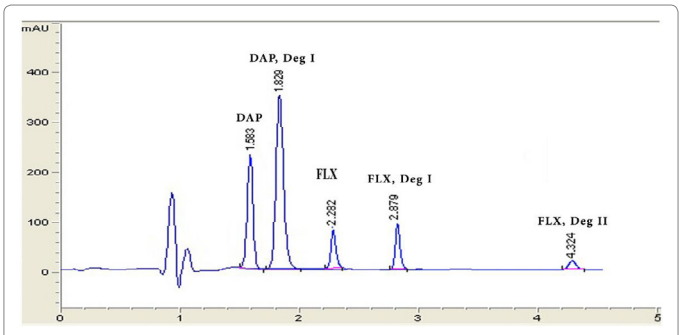


Figure 4: UPLC Chromatogram of DAP, FLX, and their degradation products using 0.03M ammonium acetate, pH 4.2-acetonitrile (35: 65, v/v) or a mixture of aqueous 0.03M triethylamine, pH 4.3 -acetonitrile (40: 60, v/v) as two different mobile phase systems.

On the other hand, the mass spectra of degraded FLX showed ion peak at (164.84 m/z and 145.84 m/z) (Figure 7a and 7b) corresponding to hydrolitic degradation products which gave the following suggestion for the molecular formula: $C_{10}H_{15}NO$ (m/z 164.84, Deg I) and $C_7H_5F_3$ (m/z 145.84, Deg II). The proposal of the reaction pathway of FLX with 5 M HCl was presented in (Scheme II). The Mass spectra support the suggested pathway of acid hydrolysis of DAP and FLX (Schemes I and II).

Kinetic study

In the literature, there is no kinetic study has been reported for the analysis of DAP and FLX applying UPLC technique.

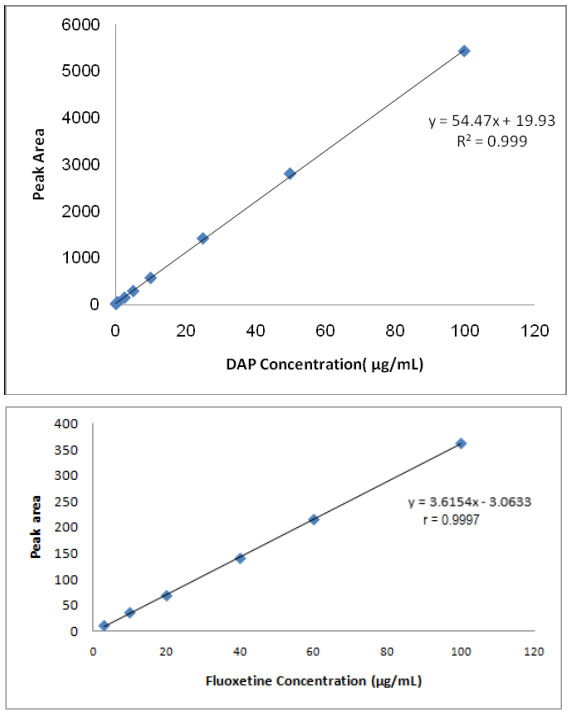
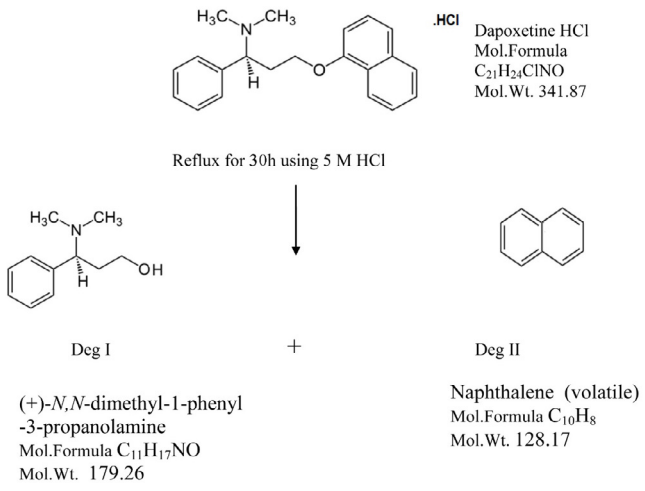


Figure 5: Calibration curves of: (a) DAP (0.05-100 µg/mL) and (b) FLX (3-100 µg/mL by the proposed UPLC method at 210 nm.



Scheme I: Degradation pathway of Dapoxetine HCl.

The kinetics of the acidic degradation of DAP and FLX were investigated using 5N HCl, since the decomposition rate of DAP and FLX at lower strengths of hydrochloric acid was too slow to obtain reliable kinetic data. The proposed UPLC method was used to determine the order of the hydrolytic degradation rate of the reaction by following the regular decrease in the concentration of the intact drugs with increasing time intervals.

The influence of temperatures on the degradation processes of each drug was studied as shown in Figure 8a and 8b. Logarithm the percentages of the remaining concentration of the drugs were plotted against the corresponding time interval in hours for each temperature, and the regression equations were computed. At

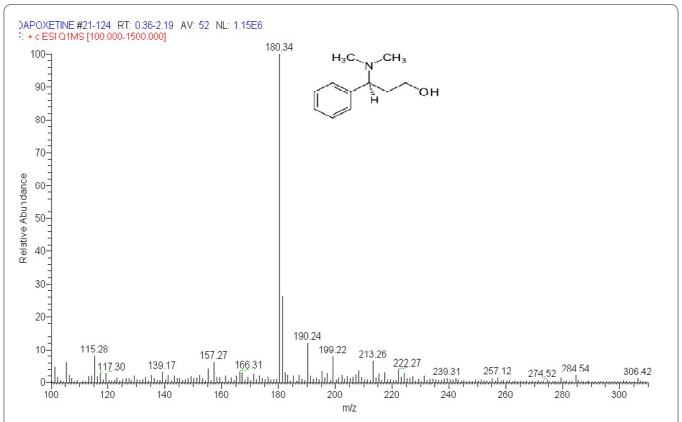


Figure 6: The mass spectrum of the acid degradation product of DAP.

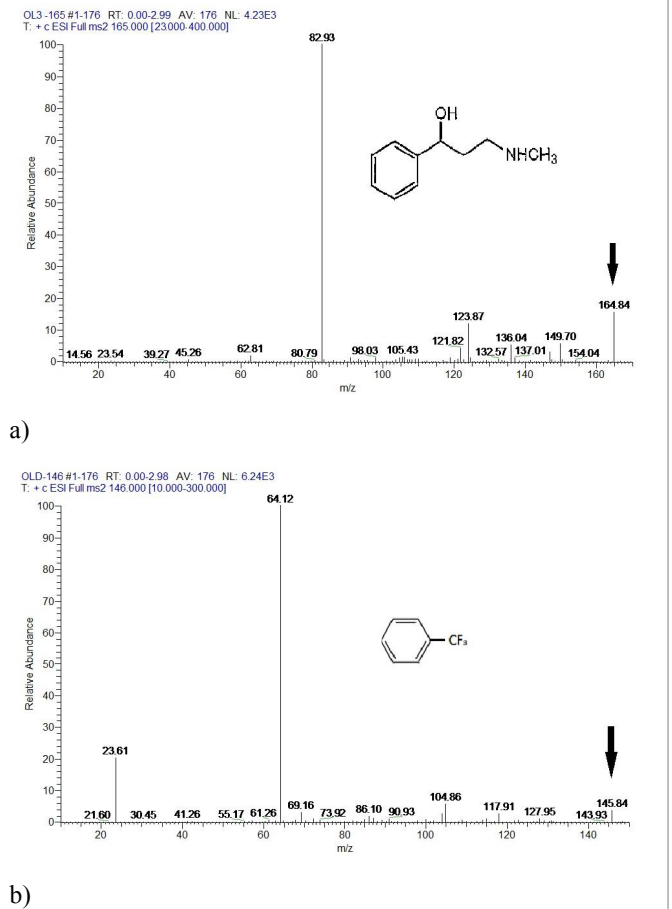
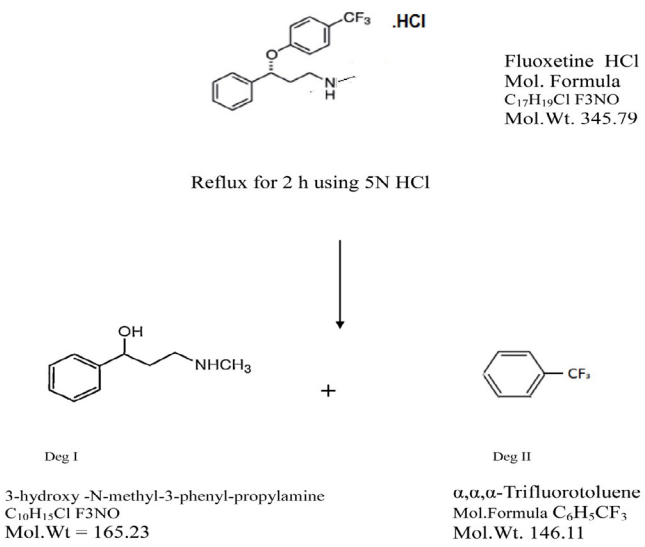
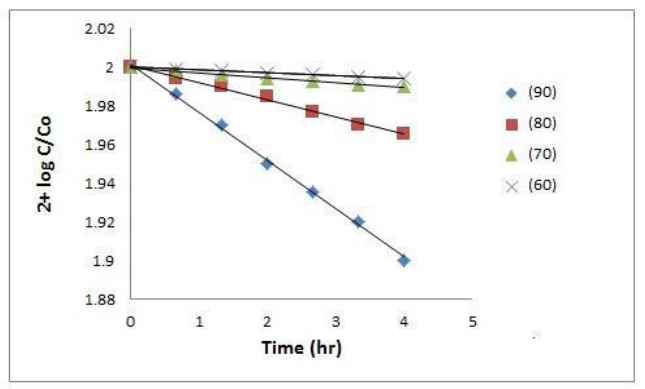


Figure 7: The mass spectra of the acid degradation products of FLX (a) Deg I and (b) Deg II.

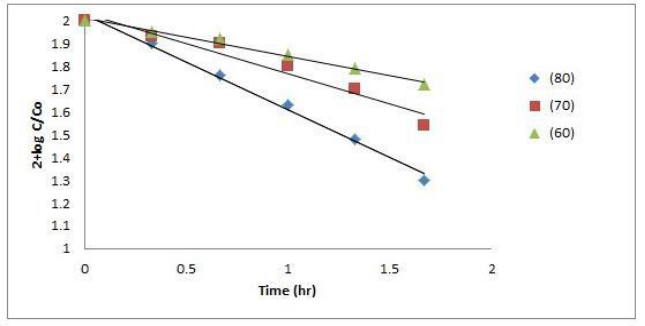
the selected temperatures (90, 80, 70, 60°C) for DAP hydrolytic degradate (80, 70, 60°C) for FLX degradates, since Na OH (5M) was found in excess, so the previous degradation reactions of DAP and FLX followed first order kinetics where the degradation rates depended on the concentration remaining of the studied drugs and the temperature at which the degradations were conducted. Figure 8a and 8b show that increasing the temperature leading to increasing of the reaction rate constant.



Scheme II: Degradation pathway of fluoxentine HC1.



a)



b)

Figure 8: Kinetic plot for the effect of temperature on the rate of (a) Dapoxetine HCl degradation reaction and (b) Fluoxetine HCl degradation reaction with 5 M HCl using the proposed UPLC method.

From the slopes of the regression lines, it was possible to calculate the apparent first order degradation rate constants (K obs) and the half-life at each temperature was calculated in according to the following equations [31]:

$\text{Log } (C_t / C_o) + 2 = -K_{obs}\, t$, $t½ = 0.693/ K_{obs}$, $K_{obs} = -2.303$ slope

Where C_t = concentration remaining at time t, C_o = initial concentration, K_{obs} = apparent rate constant, t ½ is the half-life.

From the above equation, the half-lives of the drug are constant at each temperature and independent on the drug concentration.

By plotting Log K_{obs} values versus 1/T (inverse absolute temperature), the Arrhenius plot was obtained and found to be linear at the selected temperature ranges (Figure 9a and 9b). The activation energy was calculated by applying the following equations [31]:

$\text{Log } K_{obs} = \log A - Ea\ 2.303\ RT$

Slope = -Ea/ 2.303 R

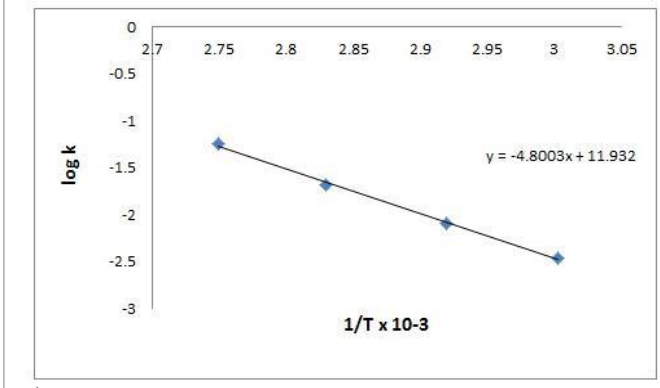
Where: K is the reaction rate constant, A is the frequency factor, Ea is the activation energy, R is the universal gas constant, T is the absolute temperature in Kelvin.

The activation energy of DAP degradation reaction was calculated and found to be 23.3 Kcal.mol-1 with frequency factor (A) = 7.9×10^{10} s^{-1}. Also, Ea of FLX degradation reaction was found to be 14.5 Kcal. mol-1 with frequency factor (A) = 9.1×10^{8} s^{-1}.

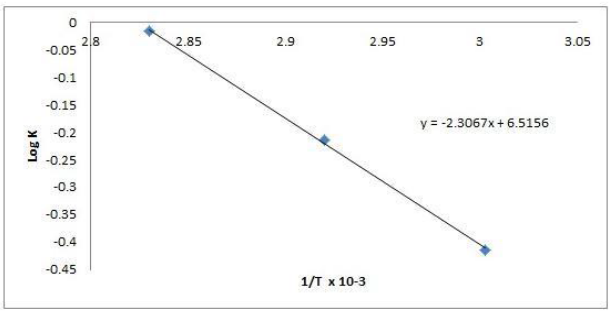
Kinetic parameters for the degradation of the cited drugs in 5M HCL were calculated by the proposed UPLC method and the results were represented in Table 2, which indicate instability of the studied drugs towards acidic degradation and that the activation energy of the reaction is the same at different temperature.

System suitability

System suitability parameters were calculated as per USP [32] guidelines to verify that the chromatographic system was adequate for



a)



b)

Figure 9: Arrhenius plot of: (a) acidic degradation process of Dapoxetine HCl and (b) degradation process of Fluoxetine HCl, in 5M HCl using the proposed UPLC method.

the analysis. The parameters to be checked during the analysis were capacity factor (k'), selectivity (α), resolution (Rs), tailing factor (T), column efficiency, HETP and RSD% of peak areas of six replicate injections, as illustrated in Table 3.

Validation of the Method

Validation of the proposed method was performed as per ICH guidelines [33].

Linearity

Different concentrations of the standard drug solutions were injected and analyzed in triplicates under the optimized chromatographic conditions. The calibration curves were constructed between the integrated peak areas and the corresponding concentrations in the range of 0.05-100 $\mu g ml^{-1}$ for DAP and 0.3-100 $\mu g ml^{-1}$ for FLX (Figure 5). Parameters of the regression data were represented in Table 4.

Accuracy Precision and accuracy

Repeatability as intra-day precision and intermediate precision were studied using three different concentrations assayed three times repeatedly in the same lab, on the same day and in triplicate on three successive days determine variation arising from the UPLC method, which was expressed as percent relative standard deviation (%RSD). The results of precision analysis are summarized in Table 3. Moreover, the standard addition technique was applied to evaluate the matrix effect on the recovery, the data indicate satisfactory accurate results as listed in Table 4.

The optimized methods were successfully applied for quantification of DAP and FLX in their pharmaceutical products (Joypox® tablets and Prozac® capsules), respectively. Accuracy and possibility of interference of excipients with the analysis of the studied drugs in their dosage forms were evaluated by recovery tests after addition of known concentrations of authentic drug to various pre-analyzed samples dosage forms.

Recovery experiments from Joypox® tablets and Prozac® capsules showed the reliability of the method. The recovery results are listed in Table 4.

Parameters	DAP	Deg 1	FLX	Deg1	Deg 2	Recommended value*
Retention time (min)	1.583	1.829	2.282	2.879	4.324	The small value indicates rapid resolution
k' Capacity factor	0.96	1.3	3.5	4.8	7.6	1-10 acceptable
Selectivity(α)	1.35		1.37 1.6			>1
Resolution(R_s)	2.68		2.3 3.1			>2
Tailing factor(T)	1.122	1.003	1.116	1.166	1.196	≤ 2 T=1 for a typical symmetric peak
(N) Theoretical plates	4228	5056	11706	14184	15551	>2000 Increases with efficiency of the separation
HETP	1.2×10^{-3}	5056	2.1×10^{-3}	1.8×10^{-3}	1.6×10^{-3}	The smaller the value, the higher the column efficiency
RSD% of peak areas	0.23	0.42	0.41	0.09	0.11	RSD <1, n =6

Table 2: Kinetic parameters for the degradation of Dapoxetine and Fluxetine hydrochlorides in 5M HCL; Degradation rate constants, half-life, activation energy and frequency factor by the proposed UPLC method.

Parameters	DAP	FLX
Range (mg mL^{-1})	0.05-100	0.3-100
Regression data		
Slope	5.447	3.6154
SD of slope	0.015	0.043
Intercept	19.937	-3.0633
SD of intercept	0.21	0.18
Correlation coefficient (r)	0.9995	0.9997
SD of (r)	0.017	0.05
LOD/LOQ (mg mL^{-1}) a	0.013/0.040	0.089/0.27
Accuracy b		
Drug substance	99.41 ± 1.02	100.05 ± 0.89
Drug product	99.57 ± 0.91	100.10 ± 0.71
Added standard	99.98 ± 0.26	100.24 ± 0.80
Specificityc (± RSD %)	100.26 ± 0.70	99.68 ± 1.14
Precision (± RSD %)		
Intra-day d	± 0.55	± 0.71
Inter-daye	± 0.59	± 0.92

[a] Mean of nine determinations . [b] Mean of six determinations ± SD
[c] Specificity (mean ± SD) for the laboratory prepared mixtures.
[d] The intra-day (n=9), RSD of three concentrations (5, 10, 25 μg mL^{-1}) of DAP and (10, 20, 40 μg mL^{-1}) of FLX repeated three times within the day.
[e] The inter-day (n=9), RSD of three concentrations (5, 10, 25 μg mL^{-1}) of DAP/(10, 20, 40 μg mL^{-1}) of FLX repeated three times in three successive days.

Table 3: Parameters of assay validation results of the UPLC methods for the determination of DAP and FLX in their drug substances.

Selectivity

The Selectivity of UPLC method was proved by analysis of Laboratory-prepared mixtures containing different percentages of the cited drugs and their degradation products. The proposed method was specific for the determination of DAP and FLX in presence of up to 90% in presence of their degradation products without interference as shown in Table 5.

Statistical omparisons were performed on the results of both drugs assay. Students t- and F- values revealed no significant difference between the proposed and the reported methods with respect to accuracy and precision (Table 6).

Robustness of the proposed method

The effect of a small change in the mobile phase composition and variation in pH value (± 0.2) was studied on the peak area. The RSD % of peak areas was calculated for each parameter and was found to be less than 2%. The low values of RSD % indicated that the method is robust.

Degradation% (w/w)	Recovery [a] of intact drug %	
	DAP	FLX
5	99.89	99.68
10	100.30	101.10
30	99.30	100.40
50	101.10	98.20
80	100.70	99.00
90	99.90	100.05
Mean ± RSD	100.26 ± 0.70	99.68 ± 1.14

[a] Average of three determinations.

Table 4: Selectivity of the proposed UPLC methods for the determination of Dapoxetine and Fluxetine hydrochlorides with their degradation products in laboratory prepared mixtures.

Parameter	pure forms		Reported methods [12,21]		pharmaceutical preparation		Reported methods [12,21]	
	DAP	FLX	DAP	FLX	Joypox ® tablets	Prozac ® capsules	DAP	FLX
Mean	99.28	100.33	99.22	99.45	99.57	100.10	99.51	99.39
SD	0.77	0.63	1.35	1.01	0.91	0.71	0.43	0.65
Variance	0.5929	0.3969	1.8225	1.0201	0.8215	0.4985	0.1832	0.6532
n	5	5	5	5	5	5	5	5
t-value (2.306)*	0.09	1.65			0.13	1.65		
F -value (6.400)*	3.07	2.57			4.48	1.55		

*The values between parentheses are the theoretical values of *t* and *F* at (P = 0.05)

Table 5: Statistical comparison between the proposed UPLC method and the reported methods for the determination of DAP and F LX in pure forms and pharmaceutical preparations.

Dapoxetine hydrochloride					Fluoxetine hydrochloride			
Temp °C	K (h^{-1})	$t_{1/2}$ (h)	Ea Kcal.mol^{-1}	A(s^{-1})	K (h^{-1})	$t_{1/2}$ (h)	Ea Kcal. mol^{-1}	A(s^{-1})
60	0.00345	200.8	23.3	7.9×10^{10}	0.385	1.8	14.5	9.1×10^{8}
70	0.00576	120.3	23.3	7.9×10^{10}	0.61	1.13	14.5	9.1×10^{8}
80	0.02049	33.5	23.3	7.9×10^{10}	0.965	0.75	14.5	9.1×10^{8}
90	0.05757	12.04	23.3	7.9×10^{10}				

Table 6: Kinetic parameters for the degradation of Dapoxetine and Fluxetine hydrochlorides in 5M HCL; Degradation rate constants, half-life, activation energy and frequency factor by the proposed UPLC method.

Conclusion

New isocratic UPLC method was developed for separation of DAP and FLX in presence of their major degradation products confirmed as (+)-N, N-dimethyl-1-phenyl-3-propanolamine, N-methyl-3-hydroxy-3-phenyl propyl amine and, α, α, α-Trifluorotoluene. Significant advantages over other methods proposed in literature were found in decrease time of analysis, minimize solvent consumption and significant reduction in run time when compared with other reported methods.

Another advantage of the method over the already reported ones [13,15,16,25,26] was that, the suffered organic solvent for each analysis was 0.4 mL 2 min^{-1} and 0.5 mL 5 min^{-1} for DAP and FLX. Total solvent consumption using the proposed UPLC was significantly reduced over the already published method [16] which used 0.5 mL min^{-1} flow rate with the gradient program and consumed nearly 20 mL acetonitrile and about 80 ml buffer for each assay. The proposed method is rapid and consumed less solvent than the published one [16,25,26]. Solvent consumption, analysis time and cost of analysis are important in quality control laboratories. The proposed UPLC method could be used for analysis of the two SSRI drugs in presence of three known hydrolytic degradation products, irrespective to the diversity in their chemical structures. The UPLC systems are superior in analysis time and solvent consumption. These advantages give the method great value as alternative in routine analysis of the SSRI drugs in their pharmaceutical formulations.

Conflicts of Interest

The authors stated that "There are no conflicts of interest to declare".

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