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Research Article

Stability-Indicating Spectrophotometric Methods for Determination of Milnacipran HCl and Duloxetine HCl in Bulk Drug and Pharmaceutical Formulations

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ABSTRACT

The present study describes accurate and sensitive stability-indicating spectrophotometric methods for the determination of Milnacipran HCl in presence of its acid, base degradates, and Duloxetine HCl in presence of its base degradates; including Dual-wavelength, Ratio difference, and Ratio derivative techniques. The developed methods were validated according to the International Conference on Harmonisation (ICH) guidelines. The recovery percentage of Milnacipran HCl and Duloxetine HCl were found to be in the ranges 99.42-100.42% and 100.17-100.3%, respectively. The low relative standard deviation of precision results confirms the suitability of the proposed methods for the estimation of the studied drugs in pure form, laboratory-prepared mixtures, and pharmaceutical formulations. Statistical comparison of the proposed methods with the reported methods revealed that there were no significant differences concerning the accuracy and precision of the adopted techniques. Validation studies demonstrated that the proposed methods are easy, specific, and rapid for the determination of Milnacipran HCl and Duloxetine HCl.

Keywords: *Fibromyalgia; Duloxetine HCl; Milnacipran HCl; Spectrophotometric methods; Dual-wavelength*

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1. INTRODUCTION

Fibromyalgia means "muscle and connective tissue pain" derived from new Latin, fibro-, meaning "fibrous tissues", Greek myo, "muscle", and Greek algos, "pain" **[1]**.

Fibromyalgia is an idiopathic, chronic, nonarticular pain syndrome, which commonly begins after a physical trauma, surgery, infection or significant psychological stress **[2]**.

The widespread occurrence of fibromyalgia is common in the female gender and increasing age. The pathophysiology of fibromyalgia is not fully understood, however, exercise, relaxation, and stress-reduction were found useful in decreasing fibromyalgia symptoms. In addition, drugs increasing serotonin and norepinephrine activity have an important role in treating fibromyalgia

[3]. Antidepressants as Milnacipran HCl (**Fig. 1**) and Duloxetine HCl (**Fig. 2**) can be used for the treatment of fibromyalgia.

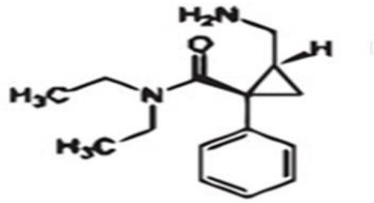

Fig. 1. Milnacipran HCl [(1*R**,2*S**)-2-(aminomethyl)-*N*,*N*-diethyl-1-phenylcyclopropanecarboxamide]

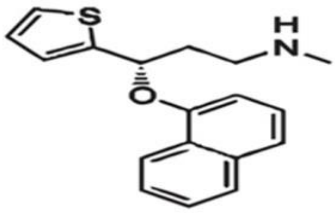

Fig. 2. Duloxetine HCl [(+)-(*S*)-*N*-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine]

Duloxetine is a mixed serotonin/norepinephrine-reuptake inhibitor. Both the antidepressant activities and pain inhibitory properties of duloxetine are believed to be related to its potentiation of serotonergic and noradrenergic activity in the CNS, although the exact mechanisms in humans are unknown **[4]**.

Milnacipran is a serotonin-norepinephrine reuptake inhibitor in a ratio of 1:3. Treatment of both depression and fibromyalgia is achieved by increasing both neurotransmitters concentration simultaneously **[5]**.

Major Pharmacopeias like USP, EP, and BP have not reported spectrophotometric methods for the determination of Milnacipran HCl and Duloxetine HCl. Some sophisticated analytical methods for estimation of Milnacipran HCl **[6-13]** and Duloxetine HCl **[14-18]** were stated in the literature. However, there hasn't been a stability-indicating spectrophotometric method yet developed for their determination in bulk and pharmaceutical formulations.

The proposed methods are considered the first spectrophotometric methods for the determination of Milnacipran and Duloxetine in presence of their degradation products by utilizing dual-wavelength **[19]**, ratio difference **[20]**, and ratio derivative **[21, 22]** techniques. The scientific novelty of the developed methods is that they were found to be easier, more rapid, less expensive and less time-consuming than the sophisticated HPLC methods for the determination of the studied drugs.

2. MATERIALS AND METHODS

2.1. Reagents and Pharmaceutical Formulations

(a) Methanol A.R, hydrochloric acid, sodium hydroxide, potassium permanganate, sulphuric acid, sodium sulfite - Scharlau (Scharlab S.L, Sentmenat, Spain)

(b) Bidistilled water - produced by Millipore Milli-Q plus water purification system (Millipore Corp., Billerica, MA).

(c) 25 and 50 mg Tablets of Milnacipran HCl (Myodonia®) - Amoun Pharmaceuticals Inc. (Cairo, Egypt).

(d) 30 and 60 mg capsules of Duloxetine HCl (Cymbatex®) - EVA Pharmaceuticals (Cairo, Egypt).

(e) Samples of Milnacipran HCl and Duloxetine HCl - Hetero Drugs Ltd (Medak, India).

2.2. Equipments

(a) Double-beam UV-Vis spectrophotometer (Shimadzu 1650 PC) with UVPC personal spectroscopy software version 2.42 (Shimadzu) to process absorption and derivative spectra- (Shimadzu Corp., Kyoto, Japan).

(b) Ultrasonic bath - Elma (Danbury, CT).

(c) pH-meter (Orion) - Equipped with a combined glass electrode (Thermo Scientific).

(d) Hotplate (WiseStir) with temperature controller - (Daihan Scientific Co. Ltd, Korea).

2.3. Solutions preparation

ICH guidelines Q2(R1)**[23]** and Q1A(R2) **[24]** didn't mention specified conditions or reagents for stress testing of the drug substances. Studying the acid and base hydrolysis of a drug can be carried out by refluxing the drug in 0.1N HCl or NaOH for 8 h. If acceptable degradation was achieved, the trial can be stopped. Nevertheless, if inacceptable degradation occurs under these conditions, the drug should be refluxed in acid or base of higher concentrations and for longer periods. So, mild conditions (0.1N HCl or 0.1N NaOH) were tried at first but didn't give complete degradation. Therefore, drastic conditions were used to achieve complete degradation.

2.3.1. Milnacipran HCl

(1) Stock Standard Solution (1 mg/mL)

In a 100-mL volumetric flask, 100 mg of the intact drug was dissolved in methanol A.R., sonicated and completed to volume with the same solvent.

(2) Working Solution (100 µg/mL)

In a 100-mL volumetric flask, 10 mL of the stock standard solution was diluted to 100 mL with methanol A. R.

(3) Acid-induced Forced Degradation of Milnacipran HCl (20 µg/mL)

In a conical flask, 2 mL stock standard solution (1 mg/mL) were added on 12 mL concentrated HCl, heated for 13 h on a hot plate (adjusted at 180 ^{o}C), cooled, then excess acid was neutralized with 10M NaOH using a pH meter, and completed to 100 mL with methanol A.R.

(4) Base-induced Forced Degradation of Milnacipran HCl (40 µg/mL)

In a conical flask, 4 mL stock standard solution (1 mg/mL) was added on 4 mL 10 M NaOH, heated for 120 min. on a hot plate (adjusted at 150 ^{o}C), cooled, then the excess base was neutralized with 6 M HCl using a pH meter, and completed to 100 mL with methanol A. R.

(5) Sample Preparation (1 mg/mL)

The contents of five (Myodonia®) 25 mg and 50 mg tablets were crushed and mixed separately. In a 100-mL volumetric flask, an accurately weighed amount (equivalent to 100 mg of Milnacipran HCl) was diluted to the mark with methanol A. R, and then the flask was sonicated for 15 min and filtered.

2.3.2. Duloxetine HCl

(1) Stock Standard Solution (1 mg/mL)

In a 100-mL volumetric flask, 100 mg of the intact drug was dissolved in methanol A.R, sonicated and completed to volume with the same solvent.

(2) Working Solution (100 µg/mL)

In a 100-mL volumetric flask, 10 mL of the stock standard solution was diluted to 100 mL with methanol A. R.

(3) Acid-induced Forced Degradation of Duloxetine HCl (200 µg/mL)

In a conical flask, 10 mL stock standard solution (1 mg/mL) were added on 10 mL 1 M HCl, heated for 40 min on a hot plate (adjusted at 90 ^{o}C), cooled, then excess acid was neutralized with 10 mL 1 M NaOH using a pH meter, and completed to 50 mL with methanol A. R.

(4) Sample Preparation (100 µg/mL)

The contents of five Cymbatex® 30 mg and 60 mg capsules were emptied, crushed and mixed

separately. In a 100-mL volumetric flask, an accurately weighed amount (equivalent to 100 mg of Duloxetine HCl) was diluted to the mark with methanol A. R., and then the flask was sonicated for 15 min and filtered. Then, 10 mL was further diluted to 100 mL with methanol A. R.

2.4. Procedures

2.4.1. Spectral Characteristics

(1) Milnacipran HCl and its acid-induced and base-induced degradates

The zero-order absorption spectra of Milnacipran HCl (10 µg/mL) and (20 µg/mL) were measured in the range 240 nm to 300 nm against methanol A. R. The zero-order absorption spectra of acid-induced degradates (10 µg/mL) and base-induced degradates (20 µg/mL) were measured at the same wavelength range, where the absorbance difference at 252.8 nm and 259.7 nm, at 255 nm and 261.4 nm was found to be zero for acid-induced degradates and base-induced degradates; respectively.

(2) Duloxetine HCl and its acid-induced degradates

The zero-order absorption spectrum of Duloxetine HCl (4 µg/mL) was recorded over the spectral wavelength range 220 nm to 340 nm against methanol A. R. The zero-order absorption spectrum of acid-induced degradates (4 µg/mL) was measured at the same wavelength range where the absorbance difference at 323 nm and 274.5 nm was found to be zero.

2.4.2. Method Validation

2.4.2.1. Milnacipran HCl

2.4.2.1.1. Linearity

(1) Dual Wavelength

Into a series of 100-mL volumetric flasks, accurately measured aliquots from the working solution (100 µg/mL) were separately transferred to produce 6-14 µg/mL and then completed to the mark with methanol A. R. Each of these solutions was measured in triplicate in the wavelength range from 240 nm to 300 nm; then the absorbance was determined at 252.8 nm and 259.7 nm, where the difference in absorbance of acid-induced degradation was found to be zero. A calibration curve was constructed by plotting the difference in absorbance at the selected wavelengths against Milnacipran concentrations.

Similarly, another set of 100-mL volumetric flasks were prepared, accurately measured aliquots of working solution (100 µg/mL) were transferred to produce 4-30 µg/mL and then completed to the mark with methanol A. R. Each of these solutions was measured in the same wavelength range. The absorbance was determined at 255 nm and 261.4 nm, where the difference in absorbance of base-induced degradation was found to be zero. A calibration curve was constructed by plotting the difference in absorbance at the selected wavelengths against Milnacipran concentrations.

(2) Ratio Difference Spectrophotometric Method

Into a series of 100-mL volumetric flasks, accurately measured aliquots from the working solution (100 µg/mL) were separately transferred to produce 2-18 µg/mL and then completed to the mark with methanol A. R. Each of these solutions was measured in triplicate in the wavelength range from 240 nm to 300 nm. Each of these spectra was divided by acid-induced degradation of Milnacipran HCl (10 µg/mL) as a divisor to get the corresponding ratio spectra. Similarly, another set of 100-mL volumetric flasks were prepared, accurately measured aliquots of working solution (100 µg/mL) were transferred to produce 4-30 µg/mL and then completed to the mark with methanol A. R. Each of these solutions was measured in the same wavelength range.

Each of these spectra was divided by base-induced degradates of Milnacipran HCl (20 µg/mL) as a divisor to get the corresponding ratio spectra. In the case of acid-induced degradates, the absorbance was determined at 249.6 nm and 270.6 nm. In the case of base-induced degradates, the absorbance was determined at 255 nm and 270.9 nm. Two calibration curves were constructed relating the absorbance difference at the selected wavelengths versus the corresponding concentrations of Milnacipran in each case.

(3) Ratio Derivative Spectrophotometric Method

Into a series of 100-mL volumetric flasks, accurately measured aliquots from the working solution (100 µg/mL) were separately transferred to produce 2-15 µg/mL and then completed to the mark with methanol A. R. Each of these solutions was measured in triplicate in the wavelength range from 240 nm to 300 nm. Each of these spectra was divided by acid-induced degradation of Milnacipran HCl (10 µg/mL) as a divisor to get the corresponding ratio spectra. The first derivative of each of the obtained ratio spectra was computed using a scaling factor 10 and $\Delta\lambda$= 4 nm. Peak amplitudes were measured at 272.7 nm and plotted against Milnacipran HCl concentrations in the prepared solutions to construct a calibration curve.

The same procedure was applied to the ratio spectra of the solutions 4-30 µg/mL prepared into a series of 100-mL volumetric flasks, from measured aliquots of working solution (100 µg/mL), measured in triplicate in the same wavelength range mentioned above and divided by base-induced degradates of Milnacipran HCl (20 µg/mL) as a divisor. Peak amplitudes were measured at 273.6 nm and plotted against Milnacipran HCl concentrations in the prepared solutions to construct a calibration curve.

2.4.2.1.2. Accuracy

Accuracy of the mentioned spectrophotometric methods under Linearity (**2.4.2.1.1**) was calculated by determining the %recoveries of 8 - 12 µg/mL standard solutions, prepared from working solution (100 µg/mL) (in case of intact drug with acid-induced degradates) and 14-30 µg/mL standard solutions, prepared from working solution (100 µg/mL) (in case of intact drug with base-induced degradates), measured in triplicate using the corresponding regression equations.

2.4.2.1.3. Precision

(a) The intra-day precision

Three replicates of Milnacipran HCl at three different concentration levels of 8, 10, and 12 µg/mL (in case of intact drug with acid-induced degradates) and 16, 20, 24 µg/mL (in case of intact drug with base-induced degradates) were analyzed on the same day by applying the same procedures under Linearity (**2.4.2.1.1**), and the relative standard deviation was calculated.

(b) The inter-day precision

The above-mentioned concentrations of Milnacipran HCl were analyzed on three successive days by applying the same procedures under Linearity (**2.4.2.1.1**) and the relative standard deviation was calculated.

2.4.2.1.4. Specificity

For acid degradation, into three separate 50-mL volumetric flasks, accurately measured aliquots of working solution (100 µg/mL) were transferred to produce 8, 10, 12 µg/mL each, accurately measured aliquots of acid-induced degradates were added to produce 10, 8, 6 µg/mL, each on a flask, volume was completed to the mark with methanol A. R. Each of these solutions was measured in triplicate using the procedures mentioned under Linearity (**2.4.2.1.1**).

For base-induced degradation, into three separate 50-mL volumetric flasks, accurately measured aliquots of working solution (100 µg/mL) were transferred to produce 16, 20, 24 µg/mL each, accurately measured aliquots of base-induced degradates were added to produce 20, 16, 12 µg/mL, each on a flask, volume was completed to the mark with methanol A. R. Each of these solutions was measured in triplicate using the procedures mentioned under Linearity (**2.4.2.1.1**).

2.4.2.1.5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Approaches based on the standard deviation of the intercept and the slope were used for determining the LOD and LOQ, where

LOD = 3.3 * S.D. / slope

LOQ = 10 * S.D. / slope

2.4.2.2. Duloxetine HCl

2.4.2.2.1. Linearity

(1) Dual Wavelength

Into a series of 100-mL volumetric flasks, accurately measured aliquots from the working solution (100 µg/mL)**,** were separately transferred, to produce 1-8 µg/mL and then completed to the mark with methanol A. R. Each of these solutions was measured in triplicate in the wavelength range from 220 nm to 340 nm. The absorbance was determined at 323 nm and 274.5 nm, where the difference in absorbance of acid-induced degradation was found to be zero. A calibration curve was constructed between the difference in absorbance at the selected wavelengths and Duloxetine concentrations.

(2) Ratio Difference Spectrophotometric Method

Each of the spectra of the solutions (1-8 µg/mL) obtained in the previously mentioned method (**IV.B.1.6.2.1.1.)** was divided by acid-induced degradation of Duloxetine HCl (4 µg/mL) as a divisor to get the corresponding ratio spectra. The absorbance was measured at 320 nm and 257 nm. A calibration curve was constructed by plotting the absorbance difference at the selected wavelengths versus the concentrations of Duloxetine HCl.

(3) Ratio Derivative Spectrophotometric Method

The first derivative of each of the ratio spectra of the solutions (1-8 µg/mL) obtained in the previously mentioned method (**IV.B.1.6.2.1.2.**) was computed using a scaling factor 10 and $\Delta\lambda$= 4 nm. Peak amplitudes were measured at 322 nm and plotted against Duloxetine HCl concentrations in the prepared solutions to construct a calibration curve.

2.4.2.2.2. Accuracy

Accuracy of the mentioned spectrophotometric methods under Linearity (**2.4.2.2.1**) was calculated by determining the %recovery of 2-6 µg/mL standard solutions, prepared from working solution (100 µg/mL), measured in triplicate using the corresponding regression equations.

2.4.2.2.3. Precision

(a) The intra-day precision:

Three replicates of Duloxetine HCl at three different concentration levels of 3, 4, and 5 µg/mL were analyzed on the same day by applying the procedures under Linearity (**2.4.2.2.1**)**,** and the relative standard deviation was calculated.

(b) The inter-day precision

The above-mentioned concentrations of Duloxetine HCl were analyzed on three successive days by applying the procedures under Linearity (**2.4.2.2.1**) and the relative standard deviation was calculated.

2.4.2.2.4. Specificity

Into three separate 100-mL volumetric flasks, accurately measured aliquots of working solution (100 µg/mL) were transferred to produce 3, 4, 5 µg/mL each, accurately measured aliquots of acid-induced degradates were added to produce 5, 4, 3 µg/mL, each on a flask, volume was completed to the mark with methanol A. R. Each of these solutions was measured in triplicate using the procedures mentioned under Linearity (**2.4.2.2.1**).

2.4.2.2.5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Approaches based on the standard deviation of the intercept and the slope were used for determining the LOD and LOQ, where

LOD = 3.3 * S.D. / slope

LOQ = 10 * S.D. / slope

2.4.3. Assay in Pharmaceutical Dosage Forms

2.4.3.1. Milnacipran HCl

For determination of Milnacipran HCl in Myodonia® 25 mg or 50 mg tablets, in 100-mL volumetric flasks, aliquots from sample solutions prepared under (**2.3.1.5**), were transferred to produce 10 µg/mL (in case of acid-induced degradates) and 20 µg/mL (in case of base-induced degradates), volume was completed to the mark with methanol A. R, then measured in triplicate using the procedures mentioned under Linearity (**2.4.2.1.1**), regression equations were used to calculate the concentrations of Milnacipran HCl.

Further, the standard addition technique was followed:

Into three separate 50-mL volumetric flasks, accurately measured aliquots of sample solution prepared using 25 mg tablets as under (**2.3.1.5**) were transferred separately to produce 6 µg/mL and 12 µg/mL each, accurately measured aliquots of working solution of Milnacipran HCl as prepared under (**2.3.1.2**) were added to produce 2, 4, 6 µg/mL and 4, 8, 12 µg/mL each on a flask; respectively, volume was completed to the mark with methanol A. R. Each of these solutions was measured in triplicate using the procedures mentioned under Linearity (**2.4.2.1.1**).

The same steps were repeated with solutions prepared using 50 mg tablets as under (**2.3.1.5**).

2.4.3.2. Duloxetine HCl

For determination of Duloxetine HCl in Cymbatex® 30 mg or 60 mg capsules, in 100-mL volumetric flasks, aliquots from the sample solutions prepared under (**2.3.2.4**), were transferred to produce 4 µg/mL, completed to volume with methanol A.R and then measured in triplicate using the procedures mentioned under Linearity (**2.4.2.2.1**). The corresponding regression equation was used to calculate Duloxetine concentration.

Further, the standard addition technique was followed:

Into three separate 100-mL volumetric flasks, accurately measured aliquots of sample solution Cymbatex® 30 mg capsules (**2.3.2.4**) were transferred to produce 2 µg/mL each, accurately measured aliquots of working solution (**2.3.2.2**), were added to produce 1, 2, 3 µg/mL each on a flask, volume was completed to the mark with methanol A.R. Each of these solutions was measured in triplicate using the procedures mentioned under Linearity (**2.4.2.2.1**).

The same steps were repeated with sample solution Cymbatex® 60 mg capsules (2**.3.2.4**).

3. RESULTS AND DISCUSSION

Srinivas et al **[12]** and Hussain et al **[13]** developed colorimetric methods for the determination of Milnacipran HCl in bulk and pharmaceutical formulations. Samal and Prusty **[16]** developed a zero-order UV

spectrophotometric method for the determination of Duloxetine HCl in bulk and pharmaceutical formulations. The advantage of the methods discussed in this paper over the methods in literature is that they are specific stability-indicating methods used to determine Milnacipran in presence of its acid and base degradation products; and Duloxetine in presence of its acid degradation products.

The absorption spectra of Milnacipran HCl and its degradates are strongly overlapped, so the application of the traditional spectral techniques failed to resolve this problem. However, the resolving power of the proposed methods could be demonstrated by this spectral overlapping. **Fig. 3** shows both the overlap between acid, base-induced degradation spectra and Milnacipran HCl spectrum; and the overlap between acid-induced degradation spectrum and Duloxetine HCl spectrum.

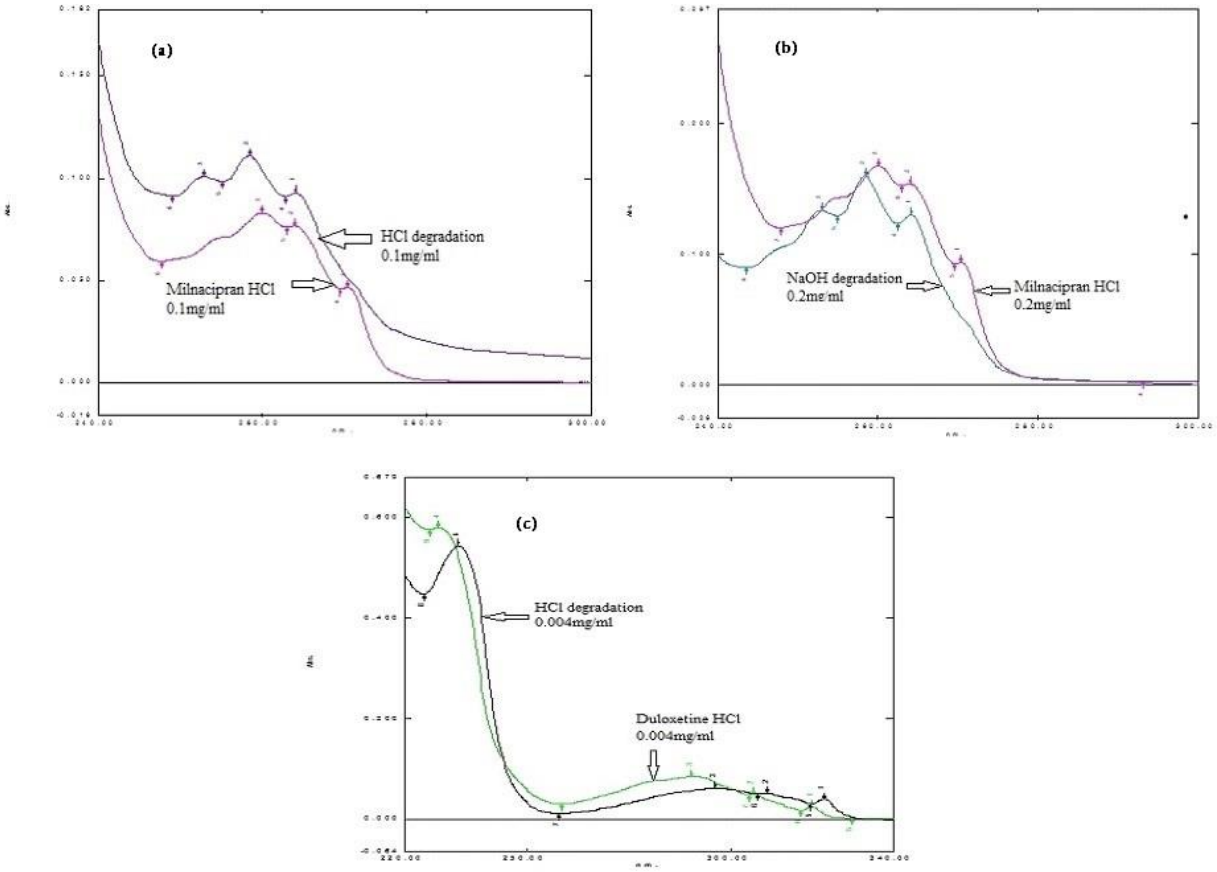

Fig. 3. Zero order absorption spectra of intact Milnacipran HCl (10 µg/mL) and its
(a) acid degradation product (10 µg/mL) in methanol
(b) base degradation product (20 µg/mL) in methanol
(c) intact Duloxetine HCl (4 µg/mL) and its acid degradation product (4 µg/mL) in methanol

Dry heat degradation and photodegradation were tried but no significant change in the peak area appeared, indicating the stability of Milnacipran HCl and Duloxetine HCl to thermal and photodegradation.

Optimization of the influence of different variables as the choice of the divisor and the working wavelengths on the developed spectrophotometric methods was studied, consequently, in case of ratio difference method, different concentrations of acid-induced

degradates (6, 8, 10, 12 µg/mL) of Milnacipran HCl were tried as divisors and the concentration of 10 µg/mL was selected as the one giving better selectivity and minimum noise. Similarly, different concentrations of base-induced degradates (12, 16, 20, 24 µg/mL) of Milnacipran HCl were tried as divisors and the concentration of 20 µg/mL was selected. Finally, different concentrations of acid-induced degradates (2, 3, 4, 5 µg/mL) of Duloxetine HCl were tried as divisors and the concentration of 4 µg/mL was selected as the one giving minimum noise and better selectivity.

Concerning wavelength selection, in case of dual-wavelength, 252.8 nm and 259.7 nm were selected, where the difference in Milnacipran HCl acid-induced degradation absorbance was found to be zero; 255 nm and 261.4 nm were selected, where the difference in Milnacipran HCl base-induced degradate absorbance was found to be zero and 323 nm and 274.5 nm were selected, where the difference in Duloxetine HCl acid-induced degradation absorbance was found to be zero allowing determination of Milnacipran HCl and Duloxetine HCl without interference from their acid and base degradates. While ratio difference, the absorbance was measured at 249.6 nm and 270.6 nm in case of Milnacipran HCl acid-induced degradates; at 255 nm and 270.9 nm in case of Milnacipran HCl base-induced degradates and 320 nm and 257 nm in case of Duloxetine HCl acid-induced degradates. For the ratio derivative method, different trials were performed to optimize the scaling factor and $\Delta\lambda$ where 10 and 4 were found to give the best spectrum shapes respectively, and absorbances were determined at peak amplitude in each case.

3.1. Method Validation

Method validation was performed according to the ICH guidelines **[23]** for the suggested spectrophotometric methods.

Linearity was assessed by analyzing different concentrations of Milnacipran HCl in the ranges of 6-14 µg/mL, 2-18 µg/mL and 2-15 µg/mL for dual-wavelength, ratio difference and ratio derivative; respectively (in case of acid-induced degradates) and 4-30 µg/mL for the three methods (in case of base-induced degradates). Similarly, the proposed methods were found to be linear in the range of 1-8 µg/mL for Duloxetine HCl. Calibration curves were constructed between either the average absorbance difference - in dual-wavelength and ratio difference methods - or average peak amplitude in ratio derivative method, and the corresponding concentrations of Milnacipran or Duloxetine.

Spectra of all studied calibration concentrations by the proposed methods are shown in **Fig. 4-6** for Milnacipran and **Fig. 7** for Duloxetine; respectively. Results are summarized in **Tables 1, 2** and **Fig. 8-11**; respectively.

The accuracy of the results was assured by utilizing the proposed methods for calculating % recoveries of different concentrations 8, 9, 10, 11, 12 µg/mL (in case of Milnacipran acid-induced degradates); 14, 16, 20, 24, 30 µg/mL (in case of Milnacipran base-induced degradates) and 2, 3, 4, 5, 6 µg/mL (in case of Duloxetine acid-induced degradates) of the intact drug in bulk powder. The corresponding regression equations were applied for calculating the concentrations in each case. The mean recoveries ± SD are shown in **Tables 3 and 4**, respectively.

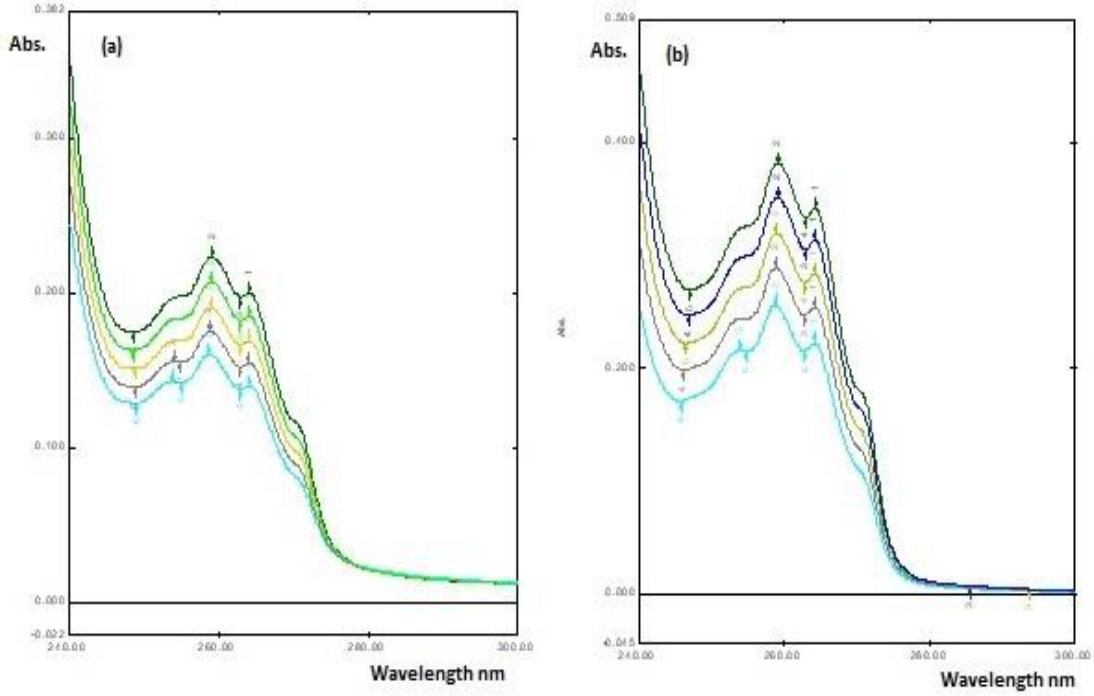

Fig. 4. Zero order absorption spectra of (a) intact Milnacipran HCl (6-14 µg/mL) with its acid degradation product (10 µg/mL) (b) intact Milnacipran HCl (4-30 µg/mL) with its base degradation product (20 µg/mL) using dual wavelength method

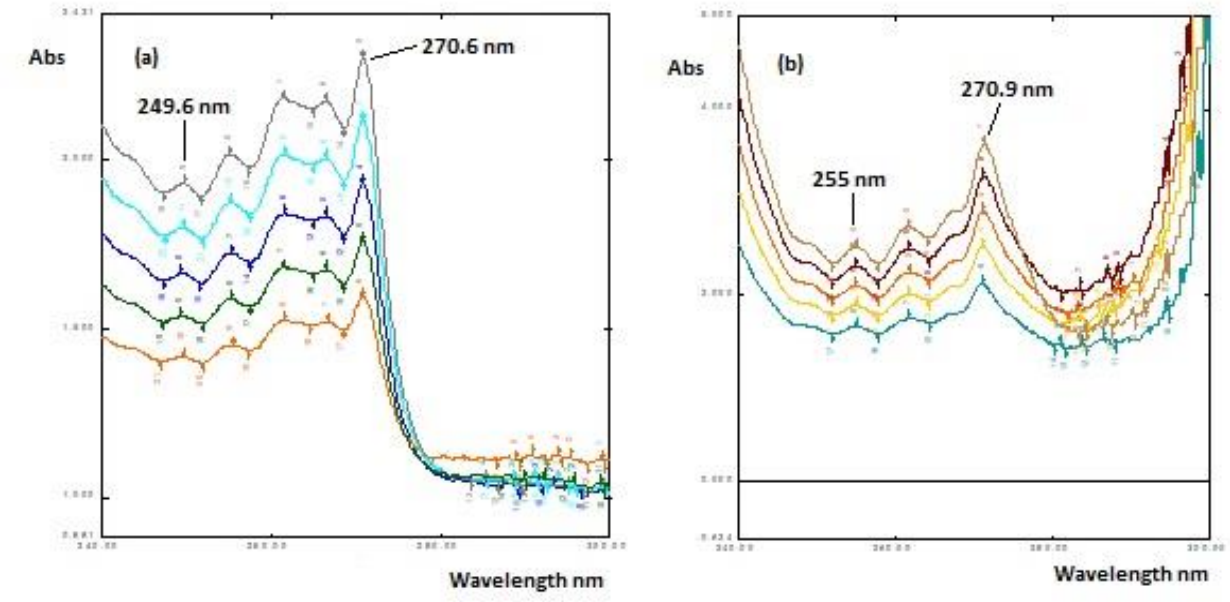

Fig. 5. Ratio absorption spectra of (a) intact Milnacipran HCl (2-18 µg/mL) after resolution from its acid degradation product (10 µg/mL) (b) intact Milnacipran HCl (4-30 µg/mL) after resolution from its base degradation product (20 µg/mL) using ratio difference method at the specified wavelengths

Table 1. Results of assay validation parameters of Dual-wavelength, ratio difference and ratio derivative for determination of Milnacipran HCl in presence of its acid or base-induced degradates

Item	Dual-wavelength		Ratio difference		Ratio derivative	
	Acid degradate	Base degradate	Acid degradate	Base degradate	Acid degradate	Base degradate
Range of linearity (µg/mL)	6 - 14 µg/mL	4 - 30 µg/mL	2 - 18 µg/mL	4 - 30 µg/mL	2 - 15 µg/mL	4 - 30 µg/mL
Regression equation ($Y = bC + a$)[1]	y = 0.002x - 0.0039	y = 0.0006 x + 0.0088	y = 0.0107x + 0.0622	y = 0.0221 x + 0.7294	y = 0.05 x + 0.3054	y = 0.0404 x + 0.5974
Correlation coefficient	0.9998	0.9998	0.9998	0.9996	0.9996	0.9995
LOD (µg/mL)	0.19	0.65	0.38	0.96	0.51	1.10
LOQ (µg/mL)	0.58	1.96	1.16	2.91	1.53	3.34
Precision (intraday)[2] RSD%	1.185	0.327	1.413	0.885	0.832	0.921
Precision (interday)[2] RSD%	0.593	0.316	1.173	1.298	0.254	0.371

1) a = Intercept, b = slope, and C = concentration of drug in µg/mL, n=5 (in triplicates)
2) relative standard deviation n=3 (in triplicates) of concentrations 8, 10, 12 µg/mL of intact drug with acid degradates and 16, 20, 24 µg/mL of intact drug with base degradates

Table 2. Results of assay validation parameters of Dual wavelength, ratio difference and ratio derivative for determination of Duloxetine HCl in presence of its acid-induced degradates

Item	Dual wavelength	Ratio difference	Ratio derivative
Range of linearity (µg/mL)	1 - 8 µg/mL	1 - 8 µg/mL	1 - 8 µg/mL
Regression equation ($Y = bC + a$)[1]	y = 0.0145x + 0.0002	y = 0.1423x + 0.3225	y = 0.1604x + 0.0052
Correlation coefficient	0.9999	0.9998	0.9999
LOD (µg/mL)	0.06	0.19	0.05
LOQ (µg/mL)	0.19	0.59	0.15
Precision (intraday)[2] RSD%	0.395	0.751	0.151
Precision (interday)[2] RSD%	1.132	0.685	0.182

1) a = Intercept, b = slope, and C = concentration of drug in µg/mL, n=5 (in triplicates)
2) relative standard deviation n=3 (in triplicates) of concentrations 3, 4, 5 µg/mL of intact drug with acid degradates

The %RSD were calculated for three concentrations of Milnacipran HCl measured in triplicates (8, 10, and 12 µg/mL) in case of acid-induced degradates, (16, 20, 24 µg/mL) in case of base-induced degradates and (3, 4, and 5 µg/mL) of Duloxetine HCl within the same day to evaluate intra-day precision and on three successive days to evaluate inter-day precision. The low values of %RSD obtained in both cases by the proposed methods suggest excellent precision. The results of precision are shown in **Tables 1 and 2**.

The LOD and LOQ were calculated using the calibration curves of the proposed methods and the obtained values are shown in **Tables 1 and 2**.

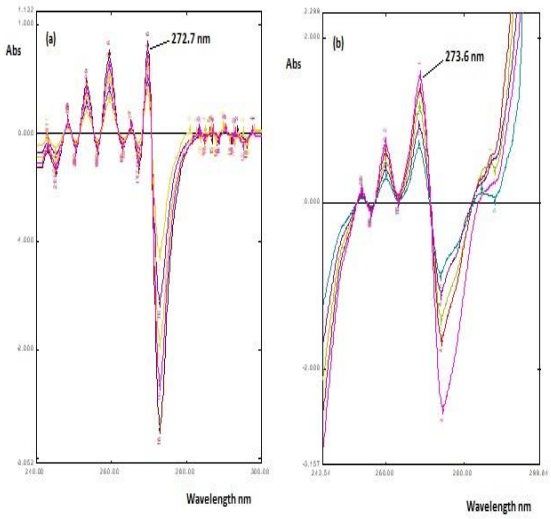

Fig. 6. First derivative of ratio spectra for (a) Milnacipran HCl (2-15 µg/mL) with its acid degradation product using (10 µg/mL) acid degradation product as the divisor (b) Milnacipran HCl (4-30 µg/mL) with its base degradation product using (20 µg/mL) base degradation product as the divisor

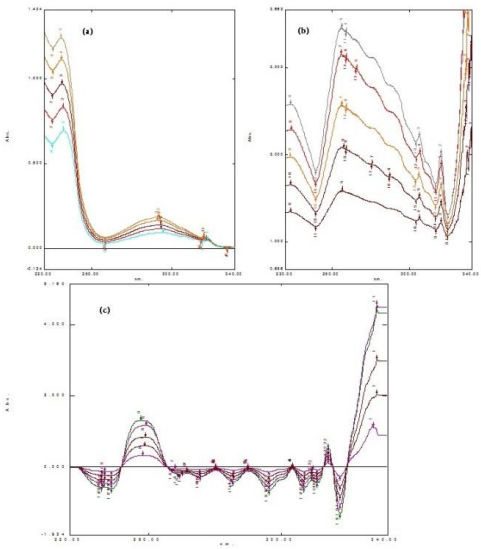

Fig. 7. (a) Zero-order absorption spectra of intact Duloxetine HCl (1-8 µg/mL) with its acid degradation product (4 µg/mL) using the dual-wavelength method

(b) Ratio absorption spectra of intact Duloxetine HCl (1-8 µg/mL) after a resolution from its acid degradation product (4 µg/mL) using the ratio difference method at the specified wavelengths

(c) The first derivative of ratio spectra for solutions of Duloxetine HCl (1-8 µg/mL) with its acid degradation product using (4 µg/mL) acid degradation product as the divisor

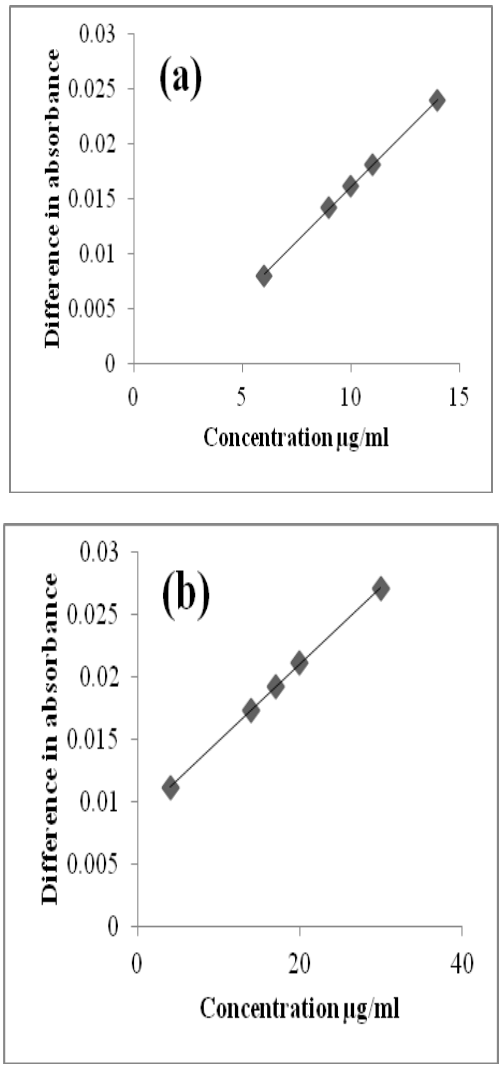

Fig. 8. Linearity of the absorbance difference corresponding to the concentration of

(a) Milnacipran HCl (6-14 µg/mL) with its acid degradation and
(b) Milnacipran HCl (4-30 µg/mL) with its base degradation; respectively using dual-wavelength method

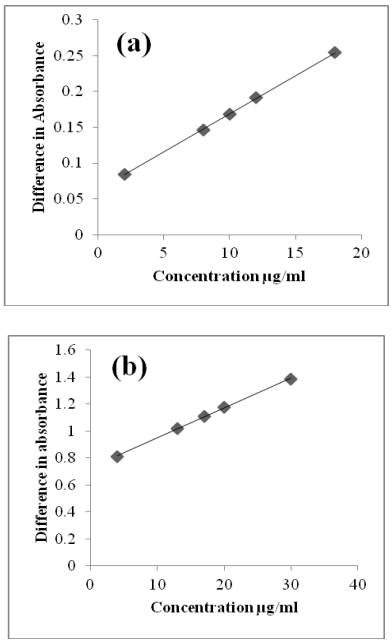

Fig. 9. Linearity of the absorbance difference corresponding to the concentration of
(a) Milnacipran HCl (2-18 µg/mL) with its acid degradation and
(b) Milnacipran HCl (4-30 µg/mL) with its base degradation; respectively using the ratio difference method

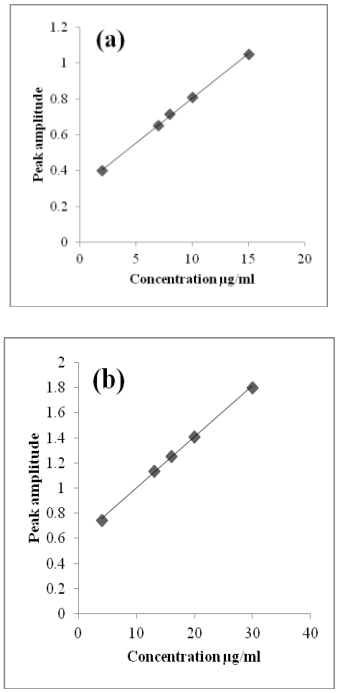

Fig. 10. Linearity of the peak amplitude corresponding to the concentration of
(a) Milnacipran HCl (2-15 µg/mL) with its acid degradation and
(b) Milnacipran HCl (4-30 µg/mL) with its base degradation; respectively using the ratio derivative method

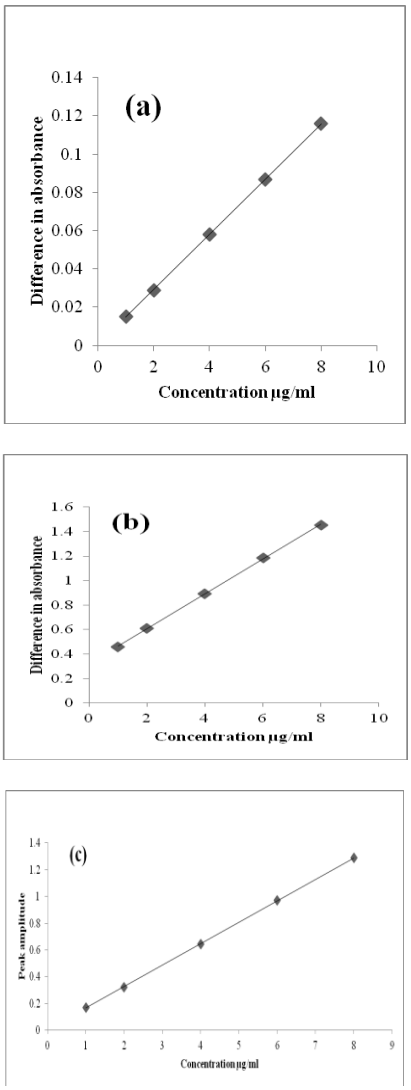

Fig. 11. (a) Linearity of the absorbance difference corresponding to the concentration of Duloxetine HCl (1-8 µg/mL) with its acid degradation using the dual-wavelength method
(b) The linearity of the absorbance difference corresponding to the concentration of Duloxetine HCl (1-8 µg/mL) with its acid degradation using the ratio difference method
(c) The linearity of the peak amplitude corresponding to the concentration of Duloxetine HCl (1-8 µg/mL) with its acid degradation using ratio derivative method

Table 3. Accuracy results for the determination of Milnacipran HCl in bulk powder by the proposed methods

		Taken concentration (µg/mL)	Found concentration (µg/mL)	*%Found
Dual-wavelength	acid degradation	8.00	7.96	99.50%
		9.00	8.85	98.33%
		10.00	9.98	99.80%
		11.00	11.08	100.73%
		12.00	11.85	98.75%
		Mean ± SD		99.42% ± 0.936
	base degradation	14.00	13.80	98.57%
		16.00	15.87	99.19%
		20.00	19.94	99.70%
		24.00	24.18	100.75%
		30.00	29.78	99.27%
		Mean ± SD		99.5% ± 0.809
Ratio difference	acid degradation	8.00	8.15	101.88%
		9.00	9.02	100.22%
		10.00	9.94	99.40%
		11.00	11.20	101.82%
		12.00	11.82	98.50%
		Mean ± SD		100.36% ± 1.487
	base degradation	14.00	13.89	99.21%
		16.00	16.21	101.31%
		20.00	20.01	100.05%
		24.00	23.95	99.79%
		30.00	29.89	99.63%
		Mean ± SD		100.00% ± 0.794
Ratio derivative	acid degradation	8.00	8.02	100.25%
		9.00	8.93	99.22%
		10.00	10.00	100.00%
		11.00	11.04	100.36%
		12.00	12.27	102.25%
		Mean ± SD		100.42% ± 1.118
	base degradation	14.00	14.09	100.64%
		16.00	15.94	99.63%
		20.00	20.00	100.00%
		24.00	23.82	99.25%
		30.00	30.27	100.90%
		Mean ± SD		100.08% ± 0.686

*Mean of three determinations

Table 4. Accuracy results for the determination of Duloxetine HCl in bulk powder by the proposed methods

	Taken concentration (µg/mL)	Found concentration (µg/mL)	*%Found
Dual-wavelength	2.00	1.99	99.50%
	3.00	3.02	100.67%
	4.00	4.03	100.75%
	5.00	5.03	100.60%
	6.00	6.00	100.00%
	Mean±SD	100.30% ± 0.538	
Ratio difference	2.00	2.02	101.00%
	3.00	3.02	100.67%
	4.00	3.98	99.50%
	5.00	5.04	100.80%
	6.00	5.96	99.33%
	Mean±SD	100.26% ± 0.783	
Ratio derivative	2.00	2.00	100.00%
	3.00	3.01	100.33%
	4.00	4.04	101.00%
	5.00	5.01	100.20%
	6.00	5.96	99.33%
	Mean±SD	100.17% ± 0.602	

* Mean of three determinations

Table 5. Specificity results for the determination of Milnacipran HCl in laboratory prepared mixtures with acid and base degradates using the proposed methods

Method	Binary mixture ratio		Taken concentration (µg/mL)	Found concentration (µg/mL)	*Recovery%
Dual-wavelength	Intact drug: Acid	4:5	8.00	8.00	100%
		5:4	10.00	9.83	98.30%
		6:3	12.00	12.12	101.00%
	Mean±SD			99.77% ± 1.365	
	Intact drug: Base	4:5	16.00	15.76	98.50%
		5:4	20.00	19.83	99.15%
		6:3	24.00	24.02	100.08%
	Mean±SD			99.24% ± 0.794	
Ratio difference	Intact drug: Acid	4:5	8.00	8.00	100%
		5:4	10.00	10.00	100%
		6:3	12.00	11.81	98.42%
	Mean±SD			99.47% ± 0.912	
	Intact drug: Base	4:5	16.00	16.21	101.31%
		5:4	20.00	19.93	99.65%
		6:3	24.00	24.16	100.67%
	Mean±SD			100.54% ± 0.837	
Ratio derivative	Intact drug: Acid	4:5	8.00	8.06	100.75%
		5:4	10.00	10.00	100%
		6:3	12.00	11.85	98.75%
	Mean±SD			99.83% ± 1.010	
	Intact drug: Base	4:5	16.00	16.31	101.94%
		5:4	20.00	20.00	100%
		6:3	24.00	24.26	101.08%
	Mean±SD			101.01% ± 0.972	

*Mean of three determinations

Table 6. Specificity results for the determination of Duloxetine HCl in laboratory prepared mixtures with acid-induced degradates using the proposed methods

Method	Binary mixture ratio		Taken concentration (µg/mL)	Found concentration (µg/mL)	*Recovery%
Dual-wavelength	Intact drug: Acid degradate	3:5	3.00	3.02	100.67%
		1:1	4.00	4.02	100.50%
		5:3	5.00	4.94	98.80%
	Mean ± SD			99.99% ± 1.034	
Ratio difference	Intact drug: Acid degradate	3:5	3.00	3.06	102.00%
		1:1	4.00	3.99	99.75%
		5:3	5.00	4.99	99.80%
	Mean ± SD			100.52% ± 1.285	
Ratio derivative	Intact drug : Acid degradate	3:5	3.00	3.04	101.33%
		1:1	4.00	3.97	99.25%
		5:3	5.00	5.00	100.00%
	Mean ± SD			100.19% ± 1.053	

* Mean of three determinations

Table 7. Application of standard addition technique on pharmaceutical formulations for determination of Milnacipran HCl using the proposed spectrophotometric methods

	Pharmaceutical dosage forms	*Found% ± S.D.			Claimed taken (µg/mL)	Pure added (µg/mL)	Pure found (µg/ml)			*Recovery % of pure added		
		DWL	RDiff	RDer			DWL	RDiff	RDer	DWL	RDiff	RDer
Acid degradation	25 mg	100.62%±0.000	100.48%±0.000	100.02%±0.000	6.00	2.00	1.99	1.97	1.98	99.50%	98.50%	99.00%
						4.00	3.99	3.99	4.04	99.75%	99.75%	101.00%
						6.00	5.98	6.11	6.00	99.67%	101.83%	100%
							Mean ±SD			99.64% ±0.128	100.03% ±1.682	100.% ±1.000
	50 mg	101.85%±0.000	99.98%±0.000	99.82%±0.000	6.00	2.00	2.02	2.02	1.99	101.00%	101.00%	99.50%
						4.00	4.07	3.95	4.03	101.75%	98.75%	100.75%
						6.00	5.96	6.11	5.98	99.33%	101.83%	99.67%
							Mean ±SD			100.69% ±1.239	100.53% ±1.594	99.97% ±0.678
Base degradation	25 mg	101.73%±0.000	100.15%±0.000	100%±0.000	12.00	4.00	3.97	4.06	4.06	99.25%	101.50%	101.50%
						8.00	7.93	7.85	8.15	99.13%	98.13%	101.88%
						12.00	12.06	11.78	12.19	100.50%	98.17%	101.58%
							Mean ±SD			99.63% ±0.759	99.27% ±1.934	101.65% ±0.200
	50 mg	100.94%±0.001	99.67%±0.000	100%±0.000	12.00	4.00	4.02	4.06	4.06	100.50%	101.50%	101.50%
						8.00	8.10	7.87	8.14	101.25%	98.38%	101.75%
						12.00	11.79	11.81	12.17	98.25%	98.42%	101.42%
							Mean ±SD			100% ±1.561	99.43% ±1.790	101.56% ±0.172

*Mean of three determinations

DWL: Dual-wavelength, RDiff: Ratio difference, RDer: Ratio derivative

Table 8. Application of standard addition technique on pharmaceutical formulations for determination of Duloxetine HCl using the proposed methods

Pharmaceutical dosage forms	*Found% ± S.D.			Claimed taken (µg/mL)	Pure added (µg/mL)	Pure found (µg/mL)			*Recovery % of pure added		
	DWL	RDiff	RDer			DWL	RDiff	RDer	DWL	RDiff	RDer
30 mg	99.83%±0.000	100.22%±0.001	99.92%±0.001	2.00	1.00	1.01	1.01	0.99	101.00%	101.00%	99.00%
					2.00	2.02	2.03	1.98	101.00%	101.5%	99.00%
					3.00	3.02	3.06	2.99	100.67%	102.00%	99.67%
						Mean ± SD			100.89% ± 0.191	101.5% ± 0.500	99.22% ± 0.387
60 mg	101.38%±0.000	98.89%±0.002	100.13%±0.001	2.00	1.00	1.01	1.00	1.01	101.00%	100%	101.00%
					2.00	2.00	2.03	1.99	100%	101.50%	99.50%
					3.00	3.05	3.06	2.99	101.67%	102.00%	99.67%
						Mean ± SD			100.89% ± 0.840	101.17% ± 1.041	100.06% ± 0.821

*Mean of three determinations

DWL: Dual-wavelength, RDiff: Ratio difference, RDer: Ratio derivative

Table 9. Statistical comparison between the results of the proposed Spectrophotometric methods and the reported method for determination of Milnacipran HCl

Statistical term	Reported methods	Proposed methods					
		Dual wavelength		Ratio difference		Ratio derivative	
		Acid degradate	Base degradate	Acid degradate	Base degradate	Acid degradate	Base degradate
Mean recovery	99.81	99.42	99.50	100.36	100.00	100.42	100.08
RSD%	0.757	0.941	0.813	1.482	0.794	1.113	0.685
SD	0.756	0.936	0.809	1.487	0.794	1.118	0.686
Variance	0.572	0.876	0.654	2.211	0.630	1.250	0.471
t (2.306)*		0.725	0.626	0.737	0.388	1.011	0.591
F (6.388)*		1.531	1.143	3.865	1.101	2.185	1.214

*Figures in parentheses are the theoretical t and F values at (p=0.05), n=5
reported method: aliquots of standard solutions in methanol containing 5 - 50 µg/mL Milnacipran HCl was determined by RP-HPLC method using C_{18} column as stationary phase and [0.0125 M potassium dihydrogen phosphate buffer + 0.3% triethylamine, pH adjusted to 3.65: acetonitrile (72:28 v/v)] as mobile phase, at flow rate 1 mL/min and wavelength 220 nm

Table 10. Statistical comparison between the results of the proposed Spectrophotometric methods and the reported method for determination of Duloxetine HCl

		Proposed methods		
Statistical term	Reported methods	Dual-wavelength	Ratio difference	Ratio derivative
Mean recovery	100.14	100.30	100.26	100.17
RSD%	1.289	0.536	0.781	0.601
SD	1.291	0.538	0.783	0.602
Variance	1.667	0.289	0.613	0.362
t (2.306)*		0.256	0.178	0.047
F (6.388)*		5.768	2.719	4.605

*Figures in parentheses are the theoretical t and F values at (p=0.05), n=5
reported method: aliquots of standard solutions in methanol containing 10-50 µg/mL Duloxetine HCl were determined by RP-HPLC method using C_{18} column as stationary phase and [methanol: acetonitrile (50:50 v/v)] as mobile phase, at flow rate 1 mL/min and wavelength 278 nm

Specificity was checked in laboratory prepared mixtures to ensure the ability of each proposed method to measure the intact drug response without interference from its degradation products. Recovery and standard deviations were calculated. Satisfactory results were obtained as shown in **Tables 5 and 6**.

3.2. Application to Pharmaceutical Formulations

The developed methods were successfully used to determine Milnacipran HCl and Duloxetine HCl in their pharmaceutical formulations. Moreover, the standard addition technique was applied to assess the validity of the proposed methods as shown in **Tables 7 and 8**.

A statistical comparison of the results obtained by the proposed methods and the reported methods **[6, 14]** for the determination of Milnacipran HCl and Duloxetine HCl was done. Statistical comparison between the results was performed using a student's t-test and F-test at a 95% confidence level where there was no significant difference concerning accuracy and precision between the proposed methods and the reported methods **[6, 14]** as shown in **Tables 9 and 10**; respectively.

4. CONCLUSION

This paper describes simple, rapid, and cost-effective methods for the determination of Milnacipran and Duloxetine. The proposed methods are sensitive and depend on the use of simple and cost-effective chemicals and techniques; however, they provide a sensitivity equivalent to that obtained by sophisticated and expensive techniques like HPLC. In addition, they are considered stability-indicating assay methods as they can separate each intact drug from its degradation products.

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing interests

The authors declare that no competing interests exist

Funding Statement

No funding source was received.

Authors' contributions

All authors have read, revised and approved the final manuscript.

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