

Micellar High Performance Liquid Chromatographic Method for Simultaneous Determination of Clonazepam and Paroxetine HCl in Pharmaceutical Preparations Using Monolithic Column

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Abstract

A new and accurate micellar high performance liquid chromatographic method coupled with ultraviolet detection was developed for simultaneous determination of Clonazepam (CLZ) and Paroxetine (PRX) using a monolithic C-18 column, and mobile phase consisting of 0.175M Sodium dodecyl sulphate, 12% n-propanol prepared in 0.02M phosphoric acid at pH 6.0. The analysis was performed at a flow rate of 1 mL/min with ultraviolet- detection at 300 nm. The method was linear over the concentration range (1.0-20 µg) and (4.0-250 µg) with limits of detection of 0.277, 2.675 µg/mL and limits of quantification of 0.838, 8.106 µg/mL for CLZ and PRX respectively. The average % recovery was found to be 100.08 ± 1.31 and 100.22 ± 1.15 for CLZ and PRX respectively. Method validation according to ICH Guidelines recommendation was evaluated. Statistical analysis of the results obtained by the proposed method was compared successfully with those obtained using the reference one. There was no significance difference between the two methods regarding accuracy and precision respectively.

Keywords: Micellar liquid chromatography; Ultraviolet detection; Clonazepam; Paroxetine; Co-formulated tablets; Method validation

Introduction

Clonazepam (5-(2-Chlorophenyl)-7-nitro-2,3-dihydro-1,4-benzodiazepin-2-one) (Figure 1) is a benzodiazepine drug having anxiolytic, anticonvulsant, muscle relaxant, sedative, and hypnotic properties [1]. Clonazepam is used to eliminate seizure activity, anxiety, mania, panic disorders and schizophrenia as it calms brain and nerves [2]. For these reasons, Clonazepam has been identified as a promising drug. Some of the commonly side effects are restless, changing mood, hyperactive and aggressive [3]. The British Pharmacopoeia [4] recommends non-aqueous titration with perchloric acid for the determination of clonazepam. For dosage forms, high-pressure liquid chromatography (HPLC) is recommended by US Pharmacopoeia [5]. Several methods have been reported for determination of this compound including spectrophotometric methods [6-8], potentiometric methods [9,10], voltammetry [11], polarography [12], HPLC [12-14] and gas chromatography coupled with mass spectrometry (GC-MS) [13,15].

Paroxetine ((3S,4R)-3-[(2H-1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl) piperidine) (Figure 1) is an antidepressant drug of the selective serotonin reuptake inhibitor (SSRI) type. Paroxetine is used to treat major depression, obsessive-compulsive disorder, panic disorder, anxiety, posttraumatic, generalized anxiety disorder and vasomotor symptoms (e.g., hot flashes and night sweats) associated with menopause [16,17] in adult outpatients. Paroxetine is primarily used to treat major depression, obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD), panic disorder, generalized anxiety disorder (GAD) [18] social phobia/social anxiety disorder [19] premenstrual dysphoric disorder (PMDD) [20] and menopausal hot flashes.

Paroxetine was the first antidepressant formally approved in the United States for the treatment of panic attacks [21].

The previously published methods that concerned with quantitative determination of PRX in tablets include voltammetry [22,23], densitometry [24,25], high-performance liquid chromatography [26-31], gas chromatography [32-34], capillary electrophoresis [35] and spectrofluorimetry [36].

Paroxetine co administered with clonazepam demonstrated significant improvement by endpoint. Combined treatment with paroxetine and clonazepam resulted in more rapid response than with the SSRI alone [37].

Up till now there was not any micellar HPLC method for simultaneous determination of Paroxetine and Clonazepam in the tablet dosage forms. There is one method for the simultaneous determination of clonazepam and paroxetine by RP-HPLC [38]. Our method is more sensitive and we use a smaller amount of organic solvent comparing to the other method (using 60% acetonitrile). For these reasons we are encouraged to perform the present work which determines CLZ and PAX simultaneously using monolithic column.

Experimental

Apparatus

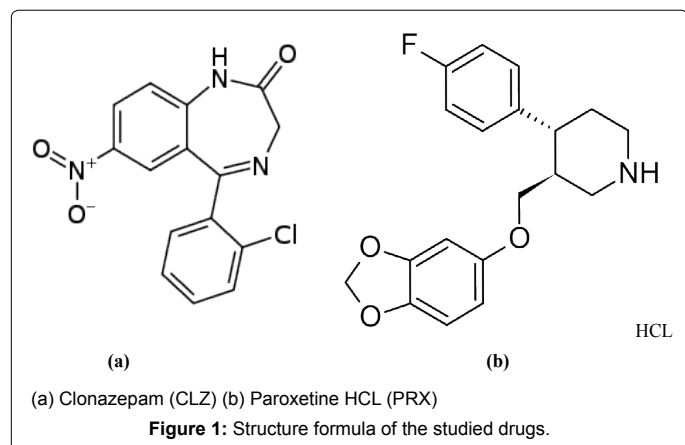
HPLC using a Chromatograph separation was carried out using a (Merck Hitachi model L-7100) equipped with a Rheodyne injector valve with a 20 µL loop, and an ultraviolet detector (Merck Hitachi L-7400), operated at 300 nm. The chromatograms were recorded on a Shimadzu C-R6A integrator. Mobile phase was filtered using membrane filters (Millipore, Ireland) and degassed using Merck solvent L-7612 degasser. pH-meter used is Consort P-901. Ultrasonic bath, model SS 101 H 230, USA.

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Materials and reagents

- Clonazepam CLZ: was kindly provided from Egyptian International Pharmaceutical Industry Company (EIPICO), with a purity of 99.3% as determined by the comparison method.
- Paroxetine HCl: was kindly provided by Pharaonia pharmaceuticals company (Alexandria, Egypt), with a purity of 99.8% as determined by the comparison method.
- Sodium dodecyl sulphate (SDS) 90% and orthophosphoric acid 85% were obtained from Riedel-deHäen (Germany).
- Methanol and n-propanol (HPLC grade) were obtained from Sigma-Aldrich (Germany).
- Amotril[®] tablets product of (Amoun Pharmaceutical Co. El Obour city, Cairo, Egypt), labeled to contain 0.5 mg clonazepam. (Batch no. ≠131777).
- Seroxat[®] CR tablets product of (GlaxoSmithKline Inc, 7333 Mississauga Road North, Canada) labeled to contain 12.5 mg Paroxetine HCl. (batch no. ≠A103035).
- Laboratory prepared co-formulated tablets (0.25 mg clonazepam, 12.5 mg Paroxetine HCl, 20 mg talc powder, 15 mg starch, 15 mg lactose and 10 mg magnesium stearate per tablet).
- All the pharmaceuticals used were obtained from Egyptian market.

Chromatographic conditions: Chromolith[®] speed ROD C-18 (50 mm × 4.6 mm i.d., 2 μm particle sizes), Merck, Germany. Mobile phase: a solution consists of a mixture of 12% n-propanol and 0.175 MSDS and the pH was adjusted to 6.0 using orthophosphoric acid. The mobile phase was filtered through Millipore membrane filter. Flow rate: 1 mL/min. Ultraviolet detection: 300 nm.

Standard solutions: Stock solutions were prepared by dissolving either 10.0 mg of CLZ or 10.0 mg PRX in 100.0 mL of methanol to give solution containing 100 μg/ml using ultrasonic bath for good solubility. Working standard solutions were prepared by appropriate dilution of the stock solutions with methanol. All solutions were stored in the refrigerator at 2°C and found to be stable for at least 7 days.

Procedures

Different volumes of the drug working standard solutions were accurately conveyed into a series of 10.0 mL volumetric flasks to obtain the final concentrations in the range of 1.0-20 μg/mL for CLZ and 4.0-

250 μg/mL for PRX. Then the volumes of solutions were completed to the mark with the mobile phase and pH was adjusted at 6.0 and mixed well.

Volumes of 20.0 μL were injected (triplicate) and eluted with the mobile phase under the optimum chromatographic conditions. The peak areas against the final concentration of the drugs in μg/mL were drawn. And the corresponding regression equations were obtained.

Analysis of CLZ/PRX laboratory prepared mixtures by the proposed method: Aliquots of CLZ and PRX standard solutions at a pharmaceutical ratio of 1: 50 [39]. Were transferred into a series of 10.0 mL volumetric flasks. The solutions were diluted to the volume with the mobile phase and mixed well. Procedure described under “Construction of the Calibration Graphs” was then applied. The mean percentage recoveries were calculated by referring to the calibration graphs, or using the corresponding regression equations.

Analysis of the two drugs in their single tablets by the proposed method: The content of ten tablets (Amotril[®], Seroxat[®] CR) were accurately weighed, finely powdered, and thoroughly mixed. Accurately weighed amounts of the powdered tablets equivalent to 0.5 mg of CLZ or 12.5 mg of PRX were transferred into a 100 ml volumetric flask and extracted with 80 mL of methanol. The contents of the flask were sonicated for 30 min, completed to the volume with the same solvent and filtered. Filtration utilizing syringe filter was performed to get clear solutions. Aliquots containing suitable concentrations of the studied drugs were analyzed as described under “construction of the calibration graphs”. The nominal content of each drug was calculated either from a previously plotted calibration graph or using the corresponding regression equation.

Analysis of the two drugs in their laboratory prepared co-formulated tablets by the proposed method: Co-formulated tablets were prepared, according to their pharmaceutical ratio (1:50). Weighed quantity of mixed laboratory prepared tablets equivalent to 0.25 mg CLZ and 12.5 mg PRX were transferred into 100 mL volumetric flasks and about 80 mL of methanol were added. The contents of the flask were sonicated for 30 min, completed to the volume with the same solvent and filtered twice using syringe filters to get highly clear filtrate. Volumes containing different concentrations of CLZ and PRX were taken and analyzed as described under construction of the calibration graphs. The content of each drug was calculated either from the already plotted calibration graphs or by using the corresponding regression equations.

Results and Discussion

A micellar HPLC method coupled with ultraviolet detection was developed and fully validated for the simultaneous determination of CLZ and PRX.

The proposed method can separate CLZ and PRX with good resolution, as the retention time between CLZ and PRX less than 5 min. The experimental parameters influencing the chromatograms of the studied drugs were accurately considered and optimized. The optimal parameter gives the highest number of theoretical plates and the best resolution within a reasonable time. Figure 2 shows a typical chromatogram for laboratory prepared mixture of CLZ and PRX under the described chromatographic conditions and the detection was performed at 300 nm. The separation was achieved within short retention time ($t_r=1.36$ and 4.9 min) for CLZ and PRX, respectively.

Optimization of the chromatographic performance and system suitability

Study of experimental parameters: Different experimental conditions affecting chromatographic behavior and determination of

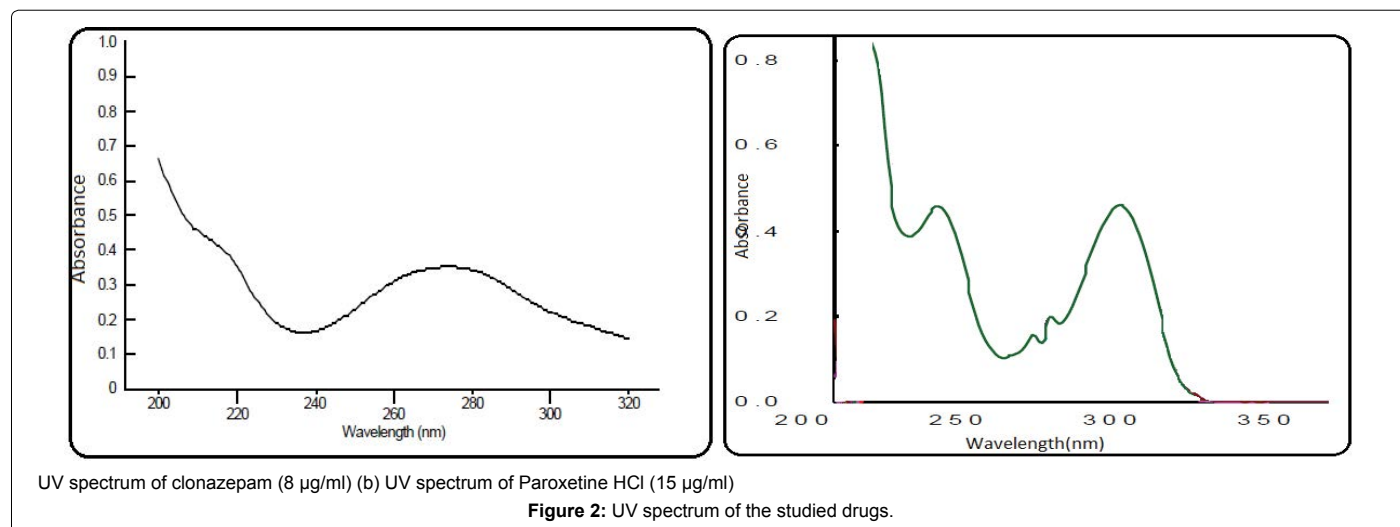


Figure 2: UV spectrum of the studied drugs.

the studied drugs including; type of column, concentration of SDS, pH of the mobile phase, concentration of the organic modifier and detection wavelength were carefully studied and optimized.

Choice of column: Two different columns were tried for performance investigations, including: Promosil ODS C18 column (250 × 4.6 mm i.d., 5 µm particle size), Agela Technologies, USA and Chromolith® speed ROD C-18 (50 mm × 4.6 mm i.d., 2 µm particle sizes), Merck, Germany. Experimental studies revealed that, the second column was the appropriate one, giving symmetrical, well defined peaks with good resolution within reasonable time. The first column was not suitable as it showed disturbed overlapped peaks.

Concentration of SDS: The effect of the concentration of SDS on the selectivity and retention time of the drugs was studied using mobile phase containing concentration of 0.1 to 0.2M SDS. It was found that 0.175M of SDS was the optimum conc.

pH of the mobile phase: The influence of pH changing on the retention time of CLZ and PRX was studied over the range of (4.0-6.5), pH 6.0 was the most appropriate pH as it gives symmetrical peaks within reasonable time and high number of theoretical plates as shown in Table 1.

Type of organic modifier: Different organic modifiers were tried including methanol, n-propanol and n-butanol. It was found that n-propanol was the organic modifier of choice as it gives the most symmetrical separated peaks. Methanol was found to give a precipitate in the mobile phase and n-butanol gives non symmetrical peaks.

Concentration of organic modifier: The effect of increasing the % concentration of n-propanol on the chromatographic behavior was studied over the range of (8% -14%). It was found that 12% (v/v) was the most appropriate concentration as it gives the highest number of theoretical plate and good resolution. As shown in Table 1.

Choice of detection wavelengths: The effect of changing wavelength on the chromatographic behavior of both drugs was investigated over the range (290-320 nm). We use the wavelength that gives the maximum peak for paroxetine to enable us to determine both drugs simultaneously. It was found that 300 nm was the most suitable wavelength found for the determination and separation since it gives the symmetrical peaks for both drugs with high number of theoretical plates and good resolution.

Development and validation of the analytical method

The validity of the proposed method was tested regarding linearity, specificity, accuracy, repeatability and precision according to ICH Q2R1 recommendations [40].

Linearity and range: Using the proposed procedure, a linear regression equation was obtained. The regression plot showed that there was a linear relationship established by plotting the peak area against the drug concentration µg/mL. Linear regression analysis of the data gave the following equation:

$$P = -20657.1 + 53108C \quad (r = 0.9998) \text{ for CLZ}$$

$$P = -19856.2 + 12613.3C \quad (r = 0.9999) \text{ for PRX}$$

Where the P is the peak area, C is the concentration of the drug in µg/mL and r is the correlation coefficient.

Statistical analysis [41] of the data gave a reasonable value of the correlation coefficient (r) of the regression equation, accepted values of the standard deviation of residuals ($S_{y/x}$), standard deviation of intercept (S_a), and standard deviation of slope (S_b), and accepted value of the percentage relative standard deviation and the percentage relative error (Table 2). These data proved the linearity of the calibration curve and low scattering of the points around the calibration curve.

Limit of Quantitation (LOQ) and limit of detection (LOD): The limit of quantitation (LOQ) is the minimal concentration which can be determined based on ICH Q2R1 recommendations (40) under which the calibration plot is non linear.

The limit of detection (LOD) is the minimum analyte concentration which can be detected (41).

$$LOQ = 10 S_a / b$$

$$LOD = 3.3 S_a / b$$

Where S_a = standard deviation of the intercept of the calibration curve and b = slope of the calibration curve.

LOQ and LOD values for CLZ and PRX by the suggested method were showed in Table 2. LOQ values are 0.838 and 8.106 µg/mL while LOD values are 0.277 and 2.675 µg/mL for CLZ and PRX, respectively.

Accuracy: The accuracy of the proposed method was proved by comparing the results of the proposed method with those obtained

Parameter		No. of theoretical plates (N)		Mass distribution ratio (Dm)		Resolution (Rs)	Relative retention (α)
		CLZ	PRX	CLZ	PRX		
pH of the mobile phase	4	494	767	0.77	5.62	6.46	7.298
	4.5	1098	807	0.76	5.41	7.08	7.12
	5	1117	745	0.775	5.53	6.9	7.14
	5.5	663	877	0.71	5.29	6.98	7.45
	6	1036	1129	0.71	5.24	8.06	7.39
	6.5	667	862	0.715	5.23	6.89	7.32
Conc. of SDS (M)	0.1	667	862	0.71	5.2	6.89	7.3
	0.125	1044	834	0.71	5.13	7.07	7.22
	0.15	653	950	0.69	5.14	7.11	7.45
	0.175	1058	860	0.727	5.23	7.2	7.19
	0.2	1051	856	0.722	5.21	7.19	7.22
Conc. of n-propanol	8%	1288	659	0.9	6.5	6.8	7.22
	10%	740	844	0.8	5.9	7.15	7.375
	12%	613	1015	0.6	4.9	7.2	8.16
	14%	455	850	0.7	5.17	6.5	7.38
Effect of flow rate(ml/min)	0.8	464	868	0.716	5.26	6.6	7.35
	1.0	1021	904	0.697	5.3	7.5	7.73
	1.2	1029	871	0.7	5.27	7.33	7.52

Where: Number of theoretical plates (N)= $5.54(t_R/W_{n/2})^2$

Mass distribution ratio (Dm)= $t_m - t_m/t_m$

Relative retention (α)= Dm_2/Dm_1

Resolution (R)= $2\Delta t_R/W_1 + W_2$

Table 1: Optimization of the chromatographic conditions for clonazepam and Paroxetine mixture by the proposed method.

Parameter	CLZ	PRX
Linearity range ($\mu\text{g/mL}$)	1.0-20	4.0-250
Intercept (a)	-20657.1	-19856.2
Slope (b)	53108	12613.3
Correlation coefficient (r)	0.9998	0.9999
S.D. of residuals ($S_{y/x}$)	6962.97	17960
S.D. of intercept (S_a)	4448.77	10224.1
S.D. of slope (S_b)	411.73	76.408
Percentage relative standard deviation, % RSD	1.310	1.143
Limit of detection, LOD ($\mu\text{g/mL}$)	0.277	2.675
Limit of quantitation, LOQ ($\mu\text{g/mL}$)	0.838	8.106

Table 2: Analytical performance data for the determination of the studied drugs by the proposed method.

using the comparison chromatographic methods [42,43] for CLZ and PAX respectively. The comparison method for CLZ involved the use of methanol and ammonium phosphate (50:50 v/v) adjusted to pH 8.0 and detected ultravioletly at 254 nm, and for PAX involved the use of dipotassium hydrogen phosphate and Acetonitrile (90: 10 v/v), adjusted to pH 6.5 and detected spectrophotometrically at 295 nm. Statistical analysis of the results obtained by the proposed method and comparison methods using Student's t-test and variance ratio F-test [41] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 3).

Precision

I. **Intra-day precision:** Intra-day precision was assessed through replicate analysis of three concentrations of the studied drugs on three successive times within the same day. The results are shown in Table 4.

II. **Inter-day precision:** Inter-day precision was carried out through replicate analysis of three concentrations of the studied drugs on three successive days. The results are summarized in Table 4.

Robustness of the method: The robustness of the method was assessed by evaluating the influence of small variation of experimental variables: concentrations of organic modifier (12% \pm 1), pH (6.0 \pm 0.1),

and conc. of SDS (0.175M \pm 0.01) on the analytical performance of the method. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time. The minor changes in these experiment parameters did not significantly affect the peak areas; recovery percentage in case of CLZ is 100.08 \pm 1.31 and in case of PRX is 100.22 \pm 1.15 respectively.

Selectivity: The proposed MLC method was considered selective by detecting any change resulted from common tablet additives such as lactose, starch, magnesium stearate, and talc. The high mean % recovery and high accuracy with low SD indicated that excipients did not affect the results of the proposed method.

Applications

Application of the proposed method to the analysis of CLZ/ PRX laboratory prepared mixtures: CLZ and PRX can be determined in laboratory prepared mixtures simultaneously by the suggested method in ratios of 1:50 (Figure 3). Both drugs can be quantitated in the laboratory prepared mixtures concerning the linear regression equations of the calibration plots.

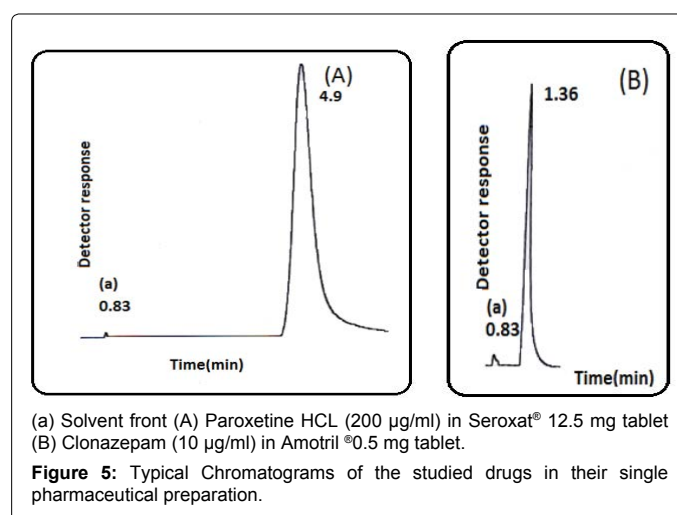
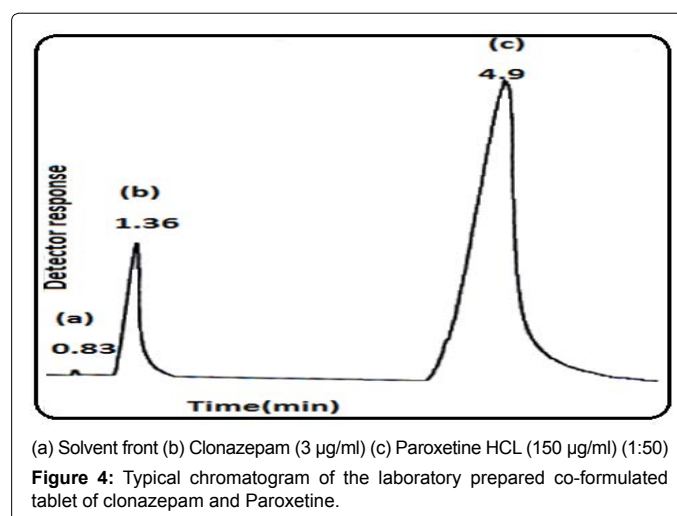
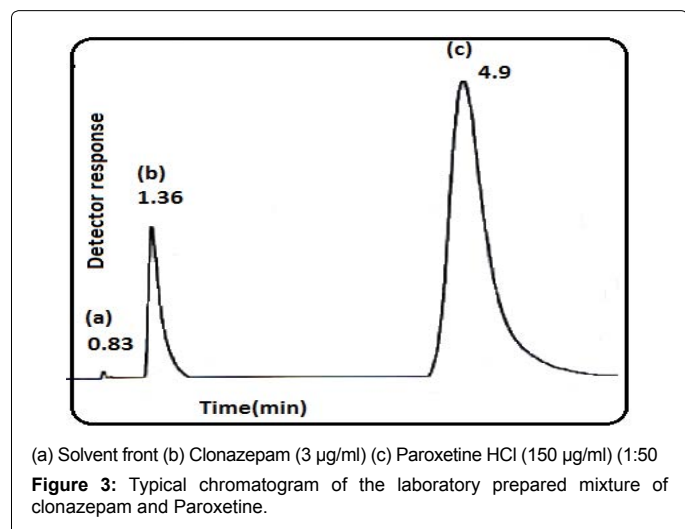
The results shown in Table 5 are in good agreement with those obtained using the official methods [42,43]. Student's t-test and variance ratio F-test statistically analyzed the results [41] and showed no significant difference between the two methods concerning the accuracy and precision, respectively. Good recoveries, 99.86 \pm 1.58 and 100.25 \pm 1.443 were achieved for CLZ and PRX, respectively.

Pharmaceutical application

Application of the proposed method to the analysis of laboratory prepared co-formulated tablets: The proposed method was successfully applied to the estimation of CLZ and PRX in their laboratory prepared co-formulated tablets. The results listed in Table 6 show a good agreement with those by the official method [42,43]. Student's t-test and variance ratio F-test [41] analyzed the results obtained by the proposed method and showed no significant difference

Compound	Proposed Method			Comparison method (42)
	Amount taken (µg/mL)	Amount found (µg/mL)	% Found	% Found
CLZ	1	1.16	101.59	100.4 99.85 99.36 101.65
	2	2.01	100.40	
	5	4.92	98.39	
	8	7.89	98.59	
	10	9.96	99.62	
	12	12.23	101.88	
	14	14.12	100.83	
	20	19.87	99.33	
Mean			100.08	100.32
± S.D.			1.31	0.99
t-test			0.315 (2.23)*	
F-test			1.77 (8.89)*	
				Comparison method (43)
PRX	4	4.04	100.96	100.2 100.36 99.32 101.27
	10	10.13	101.33	
	50	50.39	100.78	
	75	74.09	98.79	
	100	98.51	98.51	
	150	151.74	101.16	
	200	201.77	100.88	
	250	248.33	99.33	
Mean			100.22	100.61
± S.D.			1.15	0.58
t-test			0.109 (2.23)*	
F-test			2.05 (8.89)*	

Table 3: Determination of the studied drugs in pure form by the proposed and comparison methods.



between it and the official method [42,43] concerning the accuracy and precision, respectively. Good recoveries, 100.02 ± 0.59 and 100.19 ± 1.33 were achieved for CLZ and PRX respectively, from their prepared tablets in 1:50 ratio, respectively. Figure 4 shows chromatograms indicating good resolved peaks of CLZ and PRX in their laboratory prepared co-formulated tablets.

Analysis of CLZ and PRX tablets: The proposed method was successfully applied to the assay of the studied drugs in single dosage forms. The results of the proposed method were favorably compared with those obtained using the comparison method [42,43]. Mean percent recoveries from Amotril® 0.5 mg CLZ tablets and Serostat® CR 12.5 mg PRX tablets were 99.96 ± 0.59 and 99.99 ± 1.27 , respectively.

The results shown in Table 7 are in good agreement with those obtained with the comparison method [42,43]. Statistical analysis of the results obtained using Student's t-test and variance ratio F-test [41] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively. Figure 5 shows chromatograms indicating good resolved peaks of CLZ and PRX in their dosage forms.

Conclusion

A simple, accurate and validated chromatographic method with ultraviolet detection was proposed for the simultaneous determination

Parameters		CLZ Conc. (µg/mL)			PRX Conc. (µg/mL)		
		2	4	6	50	100	150
Intraday	% Found	99.44	100.74	99.59	101.91	99.80	98.69
		99.17	100.03	100	100.62	98.81	98.41
		100.04	98.97	100.76	99.92	100.36	99.79
	(\bar{x})	99.54	99.91	100.12	100.82	99.66	98.96
	± S.D.	± 0.46	± 0.89	± 0.59	± 1.01	± 0.79	± 0.73
	%RSD	0.46	0.89	0.59	1.00	0.79	0.74
Interday	% Found	99.44	100.74	99.59	101.91	99.80	98.41
		99.74	99.32	100.29	101.33	99.39	98.13
		100.66	100.38	101.23	100.98	100.22	99.24
	(\bar{x})	99.95	100.15	100.37	101.41	99.80	98.59
	± S.D.	± 0.64	± 0.74	± 0.82	± 0.47	± 0.42	± 0.58
	%RSD	0.64	0.74	0.82	0.46	0.42	0.59

Table 4: Precision data for the determination of CLZ and PRX in pure form by the proposed method.

Laboratory prepared mixtures	Proposed method						Comparison Method (42)	Comparison Method (43)
	Conc. taken (µg/mL)		Conc. found (µg/mL)		% Found		% Found	% Found
	CLZ	PRX	CLZ	PRX	CLZ	PRX	CLZ	PRX
0.25 mg CLZ + 12.5 mg PRX (1 : 50 ratio)	2	50	1.96	50.96	98.21	101.91	98.51	100.33
	4	100	4.08	99.66	101.93	99.66	100.28	100.65
	6	150	5.95	147.82	99.23	98.55	100.55	98.90
	8	200	8.00	201.57	100.07	100.78	100.61	100.15
\bar{X}				99.86	100.23	99.99	100.01	
± SD				1.58	1.45	1.00	0.77	
% RSD				1.578	1.443			
% Error				0.788	0.723			
T-test				0.136 (2.4)*	0.27 (2.4)*			
F-test				2.5 (9.27)*	3.56 (9.27)*			

N.B. *The value between parenthesis are the tabulated t and F values at P= 0.05

Table 5: results for the analysis of CLZ and PRX in their laboratory prepared mixtures.

Laboratory prepared co-formulated tablets	Proposed method						Comparison Method (42)	Comparison Method (43)
	Conc. taken (µg/mL)		Conc. found (µg/mL)		% Found		% Found	% Found
	CLZ	PRX	CLZ	PRX	CLZ	PRX	CLZ	PRX
0.25 mg CLZ + 12.5 mg PRX /tablet	2	50	2.01	50.97	100.36	101.93	101.4	101.2
	4	100	3.97	98.75	99.28	98.75	98.85	99.36
(1 : 50 ratio)	6	150	6.04	149.6	100.61	99.74	100.36	101.32
	8	200	7.99	200.68	99.81	100.34	100.65	100.36
\bar{X}					100.02	100.19	100.32	100.56
± SD					0.59	1.33	1.07	0.91
% RSD					0.593	1.329		
% Error					0.297	0.666		
T-test					0.49 (2.44)*	0.46 (2.44)*		
F-test					3.26 (9.27)*	2.15 (9.27)*		

N.B.

*The value between parenthesis are the tabulated t and F values at P=0.05

Table 6: Results for the analysis of CLZ and PRX in their prepared tablets by the proposed and comparison methods.

Pharmaceutical preparation	Proposed method			Comparison method
	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Found	% Found
Amotril® tablets (0.5 mg CLZ/tablet)	2	1.99	99.44	100.4
	4	4.03	100.74	101.98
	6	5.98	99.59	99.65
	8	8.01	100.08	100.12
AX; \bar{E}			99.96	100.54
± SD			0.59	1.01
% RSD			0.586	
% Error			0.293	
t-test			0.98 (2.44)*	
F-test			2.97 (9.27)*	
Seroxat® CR tablets (12.5 mg PRX/tablet)	50	50.08	100.16	98.96
	80	79.01	98.77	99.43
	100	101.69	101.69	101.39
	120	119.21	99.34	99.98
AX; \bar{E}			99.99	
± SD			1.27	
% RSD			1.269	
% Error			0.635	
T-test			0.06 (2.44)*	
F-test			1.45 (9.27)*	

N.B.

*The value between parenthesis are the tabulated t and F values at $P=0.05$

Table 7: Determination of CLZ and PRX in their single tablets by the proposed and comparison methods.

of CLZ and PRX in binary mixtures. In addition, it could be applied to the analysis of both drugs in their single and laboratory prepared co-formulated dosage forms without any interference from the common excipients and the results show good agreement with those obtained by the comparison method.

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