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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SYNCHRONIZED APPRAISAL OF PIROXICAM, VENLAFAXINE IN BULK AND MARKETED FORMULATION BY RP-HPLC

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ABSTRACT

The aim of this study is to develop, evaluate, and validate Piroxicam and Venlafaxine in bulk and market formulations. The separation was carried out by using Hypersil BDS column (250x4.6,5µm) at ambient temperature to develop this method. Acetonitrile, Methanol, and ammonium acetate buffer($C_2H_7NO_2$) (40:30:30 v/v/v) were fed into the column at a flow rate of 1.0 mL/min at a wavelength of 234nm. Piroxicam and venlafaxine reported linearity concentrations of 10-50µg/ml and 100-200µg/ml, respectively, with correlation coefficients (R2) of 0.9990 and 0.9991. With % RSD values of NMT 2.0, the approach was verified to be precise. The results revealed that the procedure was accurate, with a % recovery of 99.74% for Piroxicam and 100.04 % for Venlafaxine. The ICH guidelines Q2 (R1) were utilized to validate this method. All parameters, including theoretical plates, resolution, tailing factor, and

percent RSD, were within acceptable ranges. The selected two drugs piroxicam, venlafaxine was not estimated in combination earlier. As a result, the focus of this analysis is a modest, innovative rapid analytical procedure. As a result, the proposed method can be used on a routine basis.

Keywords: Piroxicam, Venlafaxine, HPLC, ICH

INTRODUCTION:

Piroxicam (PIR) [4-hydroxy-2-methyl-N-(2-pyridyl)-H-1,2-benzothiazine-3-carboxamide-1,1-di-oxide] is an anti-inflammatory and analgesic non-steroidal medication. Piroxicam is a nonsteroidal anti-inflammatory drug that binds to and chelates cyclooxygenase isoforms, reducing phospholipase A2 activity and the conversion of arachidonic acid to prostaglandin precursors at the rate-limiting cyclooxygenase enzyme step. PIR suppresses neutrophil activation, which helps to explain why this has anti-inflammatory properties **[1] (Figure 1)**.

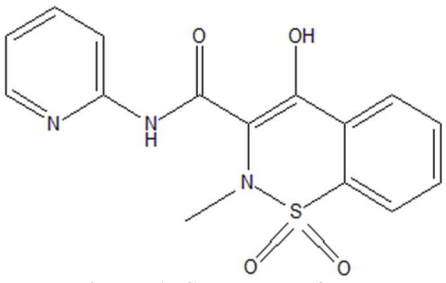

Figure 1: Structure of PIR

Venlafaxine (VEN) is a serotonin-norepinephrine reuptake inhibitor (SNRI) that belongs to the 1-[2-(dimethylamine)-1-(4-methoxyphenyl) ethyl] cyclohexan-1-ol class of antidepressants. It is a bicyc1ic phenylethylamine derivative that inhibits noradrenaline and serotonin presynaptic reuptake (5-hydroxytryptamine; 5-HT). This helps neurotransmission by extending the time these neurotransmitters are engaged with postsynaptic receptor sites. Dysregulation of these neurotransmitters has been connected to the genesis of depressive disorder **[2] (Figure 2)**.

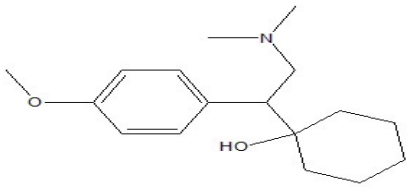

Figure 2: Structure of VEN

MATERIALS AND METHODS:

Materials:

Methanol, acetonitrile, $C_2H_7NO_2$ buffer was obtained from national scientific products. The formulation containing 20mg of PIR and 75mg of VEN were procured from local market.

Chromatographic Conditions:

A PDA detector was included by means of HPLC. Chromatographic analysis was performed on a Hypersil BDS (250x4.6,5µm) column. Detected at 234 nm and used with mobile phase of Acetonitrile: Methanol: $C_2H_7NO_2$ buffer (40:30:30 v/v/v). The

injection volume was 20µL, with a 1mL/min flow rate.

Preparation of Standard Stock Solution:

Approximately 10mg of PIR and VEN were weighed and transferred individually into a 10mL volumetric flask, in which they were dissolved in acetonitrile: methanol (50:50v/v) and made up to 1000µg/mL.

Preparation of Working Standard Solution:

0.3mL PIR and 1.5mL VEN were pipette out from the stock solution, and the volume was made up with acetonitrile: methanol (50:50v/v).

Preparation of Sample Solution:

With a mortar and pestle, 20 tablets were weighed and crushed into fine powder. One tablet powder equivalent weight was transferred into a 25mL volumetric flask and made up with 3/4th of the diluent, then sonicated for 1 hour with intermediate shaking, the capacity diluted with diluent, and filtered over a 0.45 µ Millipore Nylon filter. 0.5mL was pipette out of the above stock solution and transferred to a 10mL volumetric flask, in which the volume was made up with Acetonitrile: Methanol (50:50v/v).

Method Validation:

The method was validated using the International Conference on Harmonization (ICH) regulations for evaluating analytical processes in order to evaluate linearity, specificity, and the analyte required precision, accuracy.

Optimized chromatographic conditions:

Column: Hypersil BDS (250x4.6,5µm)

Mobile phase: Acetonitrile: Methanol: $C_2H_7NO_2$ buffer (40:30: 30v/v/v)

Wavelength **:** 234nm

Flow rate **:** 1.0mL/min

Injection volume **:** 20µL

Run time **:** 7 mins

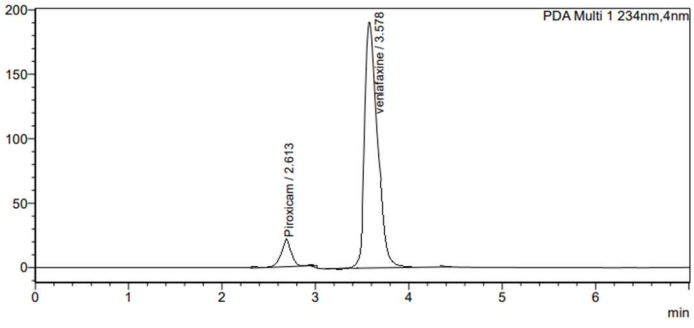

Figure 3: Standard Chromatogram

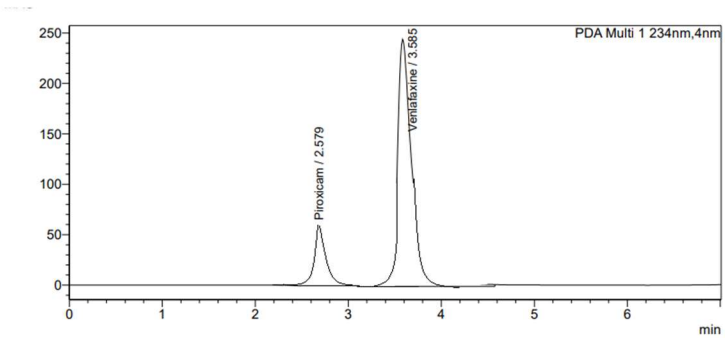

Figure 4: Sample Chromatogram

A standard solution containing PIR and VEN was evaluated for changes in characteristics such as retention duration, theoretical plate number, percent RSD, and the tailing factor in order to assess the system's compatibility. Data was gathered in the acceptance requirements as displayed in **Table 1**.

Specificity:

PIR, VEN specificity is assessed by comparing the blank, standard, and sample chromatogram, as well as any type of analyte interference. Standard, sample, and diluent solutions were prepared according to the test procedure and injected into the chromatographic system.

Linearity:

The method linearity was investigated by creating calibration curves with various concentrations of standard solutions. The PIR, VEN linearity was demonstrated over the concentration range 10-50µg/mL and 100-200µg/mL respectively. Parameters such as slope, intercept, and regression equation were calculated from the resulting data. R^2 (Correlation coefficient) of 0.999 and the linearity data contains in the **Table 2**.

Precision:

Six injections of Piroxicam and Venlafaxine were used to test system and method precision. Data was represented in **Table 3**.

Accuracy:

By creating known amounts of samples at 50%, 100 %, and 150% levels, the exactness was proved. They were injected in a triplicate at each level and data was represented in **Table 4**.

Limit of detection (LOD) and Limit of quantification (LOQ):

The LOD of PIR and VEN were determined to be 1µg/mL and 10µg/mL respectively. The LOQ of PIR and VEN was found to be 3µg/ml and 10µg/ml respectively.

Robustness:

By altering the chromatographic conditions, PIR and VEN standard solutions were administered by changing the flow rate and mobile phase. The flow rate was modified to 0.8mL/min and 1.2mL/min, and the mobile phase was changed from Acetonitrile: Methanol: $C_2H_7NO_2$ buffer (40:30:30 v/v/v) to (50:20:30 v/v/v) and (35:35:30 v/v/v). Data was represented in **Table 5**.

Assay:

Percentage purity of PIR and VEN was obtained at 99.85% and 99.21% respectively.

Table 1: Data of System Suitability

Injection No	PIR		VEN	
	R_t(min)	Peak area	R_t(min)	Peak area
1	2.592	1020680	3.573	4302394
2	2.589	1016897	3.573	4370088
3	2.588	1043055	3.574	4367116
4	2.587	1025446	3.573	4354751
5	2.585	1014493	3.574	4235746
6	2.583	1007705	3.573	4358977
Mean		1021379	4331512	
Standard Deviation		12176.7	53045.5	
% RSD		1.19	1.22	

Acceptance Criteria: The % Relative Standard Deviation should be less than 2.0%.

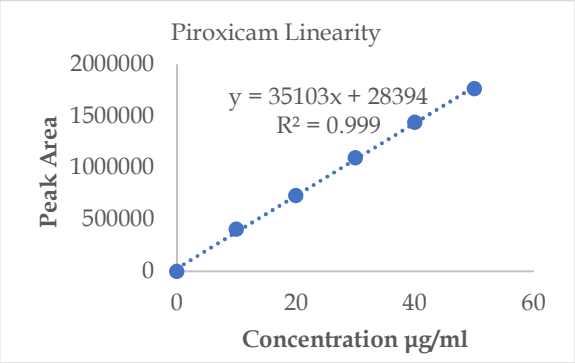

Figure 5: calibration curve of PIR

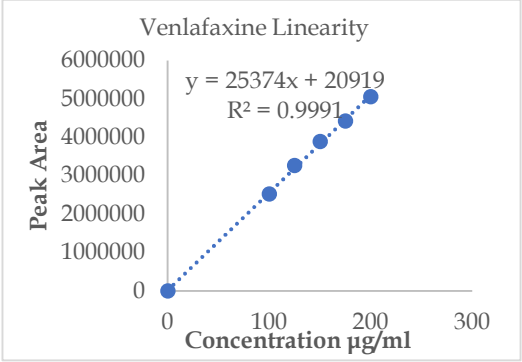

Figure 6: calibration curve of VEN

Table 2: Data of Linearity

PIR		VEN	
Concentration(µg/ml)	Peak Area	Concentration(µg/ml)	Peak Area
10	405637	100	2524224
20	730892	125	3263069
30	1095554	150	3891399
40	1440178	175	4424884
50	1763553	200	5052283
R^2= 0.999		R^2= 0.9991	

Acceptance Criteria: The R^2 ought to be NLT 0.999.

Table 3: Data of system precision & method precision

s. no	System Precision		Method Precision	
	PIR Peak area	VEN Peak area	PIR Peak area	VEN Peak area
1	1022570	4202365	1020680	4122175
2	1016897	4170256	1017623	4070159
3	1033062	4261128	1023112	4056152
4	1032463	4344352	1032356	4142258
5	1014563	4234346	1014753	4125327
6	1008509	4208977	1005705	4027877
Mean	1021349	4236904	1019038	4090658
SD	9925.08	60915.1	8885.89	45629.3
%RSD	0.97	1.44	0.87	1.12

Acceptance Criteria: NMT 2.0 should be the percent relative standard deviation for the Peak area.

Table 4: Data of Accuracy

% Level	PIR				VEN			
	Standard Peak area	Sample Peak area	% Recovery	Mean % Recovery	Standard Peak area	Sample Peak area	% Recovery	Mean % recovery
50%	1021379	508547	99.25	99.74%	4331512	2185461	100.04	100.04%
	1021379	509458	99.33		4331512	2198542	100.54	
	1021379	508956	99.27		4331512	2194562	100.40	
100%	1021379	1021548	99.79		4331512	4332512	99.27	
	1021379	1022584	99.88		4331512	4328956	99.17	
	1021379	1022459	99.89		4331512	4345611	99.58	
150%	1021379	1525891	99.38		4331512	6552141	100.09	
	1021379	1545894	100.67		4331512	6589521	100.65	
	1021379	1539564	100.24		4331512	6591562	100.66	

Acceptance criteria: The Mean % recovery at each level should be NLT 98% and NMT 102%.

Table 5: Data of Robustness

Parameter	PIR			VEN		
	R_t(min)	Peak area	%RSD	R_t(min)	Peak area	%RSD
Change in flow rate 0.8mL/min	3.221	1462339	0.68	4.462	5865105	0.70
	3.221	1476426		4.464	5923715	
Change in flow rate 1.2ml/min	2.203	972514	0.24	2.991	3787648	0.75
	2.200	969164		2.991	3828017	
Change in mobile phase ratio 50:20:30 v/v/v	2.188	6135262	0.20	2.895	3628746	0.71
	2.188	6117783		2.895	3665551	
Change in mobile phase ratio 35:35:30 v/v/v	2.200	2985874	0.97	3.042	3827529	0.55
	2.199	3027125		3.040	3857356	

Acceptance criteria: The % relative standard deviation for the Peak area should be NMT 2.0.

DISCUSSION:

PIR and VEN were eluted at 2.588 and 3.569 minutes, respectively, in an optimized chromatogram. This method was validated acc. to ICH specifications. The linearity concentration ranges for PIR and VEN were 10-50µg/mL and 100-200µg/mL, respectively, with R^2 of 0.9990 and 0.9991. With % relative standard deviation values not more than 2.0, the technique was determined to be precise. The results revealed that the procedure was accurate, with a percent recovery of 99.74 percent for PIR and 100.04 percent for VEN. Separation was unaffected by the modifications in robustness parameters, and % RSD was within not more than 2.0 permissible limits.

CONCLUSION:

For the estimation of PIR and VEN in formulation, a simple, exact Rp-HPLC method was devised. At room temperature, a Hypersil BDS column (250x4.6, particle size 5m) was used to separate the samples. Acetonitrile was utilized as the mobile phase: At a flow rate of 1.0ml/min, Acetonitrile: methanol: $C_2H_7NO_2$ buffer (40:30:30v/v/v) was supplied into the column at a detection wavelength of 234nm. The enhanced method was validated in accordance to the ICH standards. There was no mechanism for calculating PIR and VEN in

tablet dosage form at the same time, acc to the literature study. The method created was novel, precise, and applicable to routine analysis.

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Conflict of Interest:

There are no competing interests among the authors of this paper.

REFERENCES:

[1] Hasan Basan, Nilgun Gulden Goger. Quantitative determination of piroxicam in a new formulation (piroxicam–β-cyclodextrin) by derivative UV spectrophotometric method and HPLC. Journal of pharmaceutical and biomedical analysis. 2001; 26:171-178.

[2] Stephen M. Holliday and Paul Benfield. Venlafaxine a review of its pharmacology and therapeutic potential in depression. Adis international limited.1985;49(2):280-294.

[3] M Yritia, P. Parra. Piroxicam quantitation in human plasma by high-performance liquid chromatography with on- and off-line solid-phase extraction. Journal of Chromatography A. 1999; 846:199-205.

[4] Alina Diana Panainte, Madalina Vieriu. Fast HPLC method for the determination of piroxicam and its application to stability study. Revista de chimie.2017; 68: no.4.

[5] Jeffrey A. Richards, Dale A. Cole. High Performance Liquid Chromatography Assay for Piroxicam in Pharmaceutical Products. Journal of chromatographic sciences. 1987; vol 25:292-295.

[6] Ebenezer B. Asafu-Adjaye a, Patrick J. Faustino. Validation and application of a stability-indicating HPLC method for the in vitro determination of gastric and intestinal stability of venlafaxine. Journal of pharmaceutical and biomedical analysis. 2007; 43:1854-1859.

[7] Subhashini Edla. A novel RP-HPLC method for the determination of venlafaxine in pharmaceutical drug products. International journal of Science and technology.2012; vol.1:11-21.

[8] APPP Rama Rao Nadendla, Siva Prasad M. A Novel synchronic estimation of Metronidazole, Ciprofloxacin and Doxycycline by RP-HPLC in bulk and pharmaceutical formulation. Journal of Pharmaceutical Research international. 2021;33(53)A:354-362.