A COMPARATIVE STUDY ON VALIDATION OF RANOLAZINE AND LACOSAMIDE IN PHARMACEUTICAL FORMULATIONS BY RP-HPLC METHOD

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ABSTRACT

A reversed phase high performance liquid chromatographic (RP-HPLC) method was developed for the determination of ranolazine and lacosamide in pharmaceutical formulations. A comparative study was made on the results obtained to evaluate the strong and weak aspects of the reported method. Hypersil BDS-C_8 column with sodium acetate-acetonitrile (50:50) mobile phase was employed for the determination of ranolazine in the range 0 to 150 µg mL$^{-1.}$ Completely resolved peaks were observed at 4.74 min and at 5.00 min retention times for standard and samples of ranolazine respectively. The precision (RSD = 0.2%) and accuracy (recovery = 100.4 – 100.5%) of the proposed method were found to be good. Lacosamide in pharmaceutical formulations was determined using Inert sustain HP C_{18} as stationary phase, a mixture of potassium dihydrogen phosphate (pH 2.0) and acetonitrile (90 : 10 v/v) as mobile phase A, ,mixture of acetonitrile and water (60 : 40 v/v) as mobile phase B. The method with high precision (RSD 0.04 %) and good accuracy (recovery 98.8 – 101.4 %) is useful for the determination of lacosamide in the range 0 to 150 µg mL$^{-1.}$ Both the methods were found to be highly robust.

INTRODUCTION

Ranolazine is an anti-anginal medication. It works by improving blood flow to help the heart work more efficiently. Ranlazine inhibits the late inward sodium current in heart muscle. This leads to reduction in elevated intracellular calcium levels leading to reduced tension in the heart wall. Lacosamide is a functionalized amino acid. It inhibits the repetitive neuronal firing, reduces long-term channel availability without affecting physiological function. It also modulates collapsin response mediator protein-2 (CRMP-2) preventing the formation of abnormal connections in the brain. The structures of ranolazine and lacosamide are shown in figure 1 and 2.
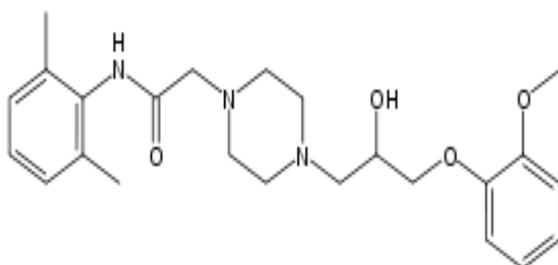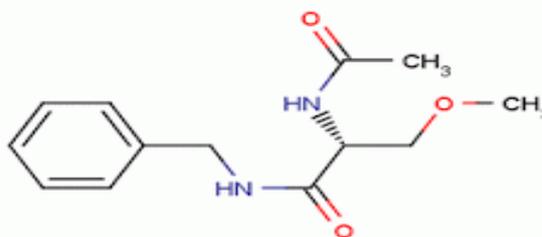

Fig 1: structure of ranolazine

Fig 2: structure of lacosamide

Good number of HPLC methods[1- 9] were reported for the determination of ranolazine. V.D. Patel et. Al[10] reviewed the analytical methods for its determination. The analytical data of some of the reported chromatographic methods[11-19] for the determination of lacosamide were reviewed. We are now reporting a simple, precise and sensitive reverse phase HPLC method for the determination of ranolazine and lacosamide employing new chromatographic columns and novel chromatographic conditions. A comprehensive comparison has been made for the analytical results obtained.

EXPERIMENTAL

Agilent HPLC system with variable wavelength detector, quaternary solvent manager, sample manager and column heating compartment was employed for recording the chromatograms. Ranolazine standard solution was prepared by dissolving 50 mg of pure ranolazine in 35mL of diluent, sonicated and diluted to the volume in 50mL volumetric flask. 5mL of this

solution was diluted to 50 mL with diluent. The diluent was prepared by dissolving 200mg of NaOH in 1000 mL of methanol and mixing this NaOH solution with water in 80:20 v/v ratio. The sample solution of ranolazine was prepared by dissolving 250 mg of powdered ranolazine tablet in 200 mL of methanolic NaOH and diluting to 250mL with distilled water. 5 mL of this solution was further diluted to 50 mL with diluent. 1.93 grams of CH_3COONH_4 were dissolved in 1000 mL of distilled water and the pH of resultant solution was adjusted to pH 5.5 with dilute acetic acid. A mixture of this buffer and acetonitrile in 50:50 (v/v) ratio was used as mobile phase.

In the determination of lacosamide, a mixture of water and acetonitrile in the ratio 90:10 (v/v) was employed as the diluent. The standard solution of lacosamide was prepared by dissolving 50 mg of the drug and diluting to 50 mL with the diluent. 5 mL of the resultant solution was diluted to 50 mL with the diluent. 5mL of lacosamide injection were dissolved and diluted to 100 mL with diluent. 5mL of this solution were diluted to 25mL with diluent to get the working solutions of sample solutions. The buffer solution (pH 2.0) was prepared by dissolving 1.36 grams of KH_2PO_4 in 1000 mL of distilled water. Mobile phase-A was obtained by mixing the buffer solution and acetonitrile in 90:10(v/v) ratio. A mixture of acetonitrile and water in 60:40 (v/v) was used as mobile phase-B

RESULTS AND DISCUSSIONS
Typical chromatograms for the standards and the sample solutions of ranolazine and lacosamide were recorded under optimal conditions and are shown in figures 3-6.
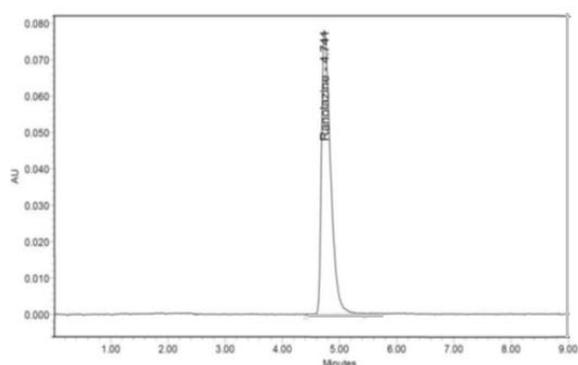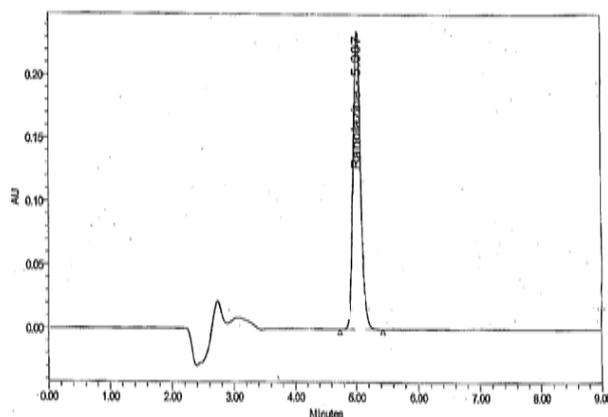

Fig-4: Chromatogram of ranolazine sample.

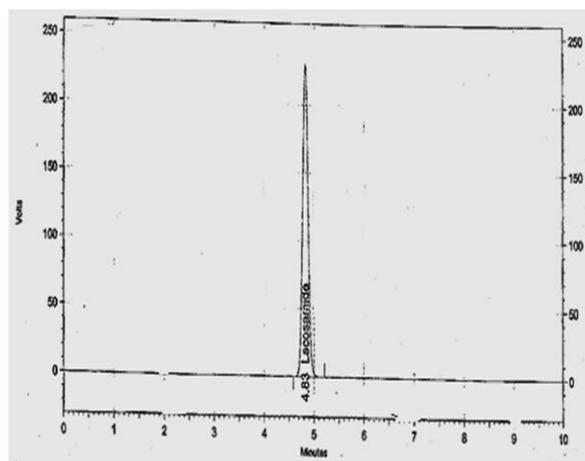**Fig-5: Chromatogram of lacosamide standard.**

Fig 3: Chromatogram of ranolazine standard.
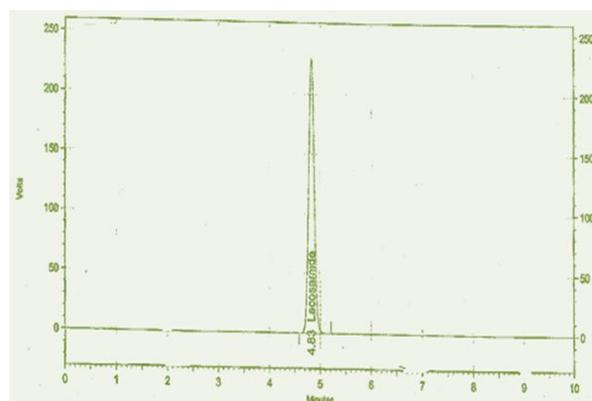
Fig-6: Chromatogram of lacosamide sample.

Table 1: Chromatographic conditions.

Parameter	Ranolazine	Lacosamide
Column	Hypersil BDS-C₈of 250 mm x 4.6 mm with 5μm particle size	Inert sustain HPC-18 of 100 mm x 4.6 mm dimensions 3 μm particle size
Mobile phase	50:50 (v/v) mixture of ammonium acetate buffer (pH 5.5) and acetonitrile	A : mixture of KH_2PO_4 buffer (pH 2.0) and acetonitrile in the ratio 90:10 (v/v) B : mixture of acetonitrile and water 60:40 (v/v)
Flow rate	1.0 mL min⁻¹	1.5mL min⁻¹
Injection volume	10 μL	10 μL
Run time	9 minutes	10 minutes
Detection wave length	220 nm	210 nm

The flow rate, injection volume, run time and detection wave lengths are very similar for both ranolazine and lacosamide.

Table 2: Systems suitability results.

Parameter	Ranolazine	Lacosamide
Relative standard deviation (RSD,%)	0.2	0.05
Number of theoretical plates	8176	9960
Asymmetry	1.2	1.1

The results in the above table indicate that the proposed method is more sensitive for lacosamide than for ranolazine.

Table 3: Specificity results.

Sample	Retention time (min)	
	Ranolazine	Lacosamide
Blank	-	-
Placebo	-	-
Standard	4.741	4.83
Sample	5.007	4.83

The results in Table 3 reveal that neither the blank nor the placebo exhibit any peak at the retention time where the drugs are showing significant peaks. Further, both standard and sample solutions of lacosamide eluted at the same retention time whereas the standards and sample solutions of ranolazine were eluted with slightly different retention times.

Table 4: Precision results.

Injection no.	Peak Areas	
	Ranolazine	Lacosamide
1	2002174	28732316
2	2001654	28713167
3	2009977	28714203
4	2001737	28717820
5	2001432	28744780
6	2006846	28722577
Average	2003970	28724094
Standard deviation (SD)	3588.1	12283.3
RSD (%)	0.2	0.04

The data in the above table shows that the proposed method is highly precise for the determination of both ranolazine and lacosamide. However, it is more precise for lacosamide than for ranolazine.

Table 5: Accuracy Results.

Amount added (mg)		Amount found (mg)		Recovery (%)		Average Recovery (%)		R.S.D (%)	
Ranolazine	Lacosamide	Ranolazine	Lacosamide	Ranolazine	Lacosamide	Ranolazine	Lacosamide	Ranolazine	Lacosamide
62.26	12.58	62.59	12.68	100.5	100.8				
62.41	12.67	62.41	12.69	100.5	100.2	100.4	100.3	0.1	0.5
62.34	72.69	62.34	12.68	100.3	99.9				
125.22	24.88	126.14	25.00	101.5	100.5				
125.18	24.98	126.90	25.00	101.7	100.1	101.4	100.4	0.4	0.3
125.33	24.92	125.60	25.07	101.0	100.6				
250.11	49.50	252.82	49.26	101.9	99.5				
248.48	49.72	249.70	48.86	100.1	98.3	100.5	101.4	0.6	0.1
250.01	49.76	250.96	49.05	100.4	98.6				
374.87	75.22	349.48	76.33	101.2	101.5				
375.37	75.13	374.63	76.22	100.1	101.4	100.5	101.4	0.6	0.1
374.03	75.28	375.23	76.28	100.3	101.3				

The results in Table 5 reveal that the proposed method is highly and equally accurate for the determination of both ranolazine and lacosamide.

Table 6: Linearity Results.

Ranolazine		Lacosamide	
Amount (μgmL^{-1})	Average peak area	Amount (μgmL^{-1})	Average peak area
25.00	1057498	24.99	7201341
50.00	2124590	49.98	14530291
75.00	3177753	74.97	21473272
100.00	4201027	99.96	28911508
124.99	5250072	124.95	35986449
149.99	6277596	149.94	42897586
Correlation coefficient	0.9989		0.9999
Slope	241852		286749
y-intercept	16717		73900

The peak areas were plotted against the amount of the drug and shown in figure 7 and 8 for ranolazine and lacosamide respectively. The plots indicate that the proposed method is suitable for the determination of ranolazine and lacosamide in the concentration range 0 – 150 μg mL^{-1}. The slope of the linear plots and correlation coefficient values justify the validity of the method
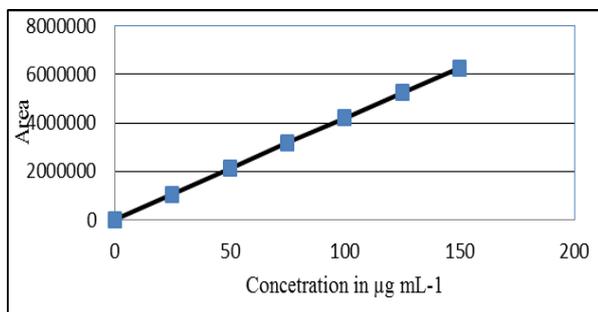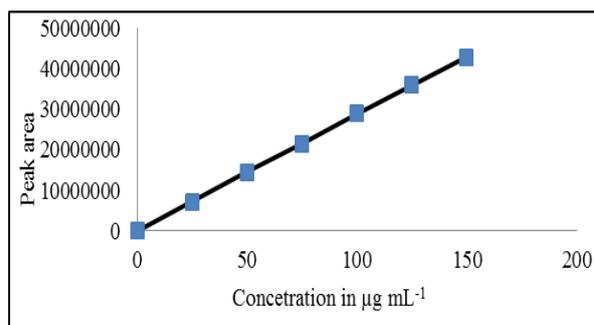

Fig 8: Linearity Curve of Lacosamide.

Fig 7: Linearity curve of ranolazine.

Table 6: Robustness Results.

Ranolazine				Lacosamide			
Condition	**RSD(%)**	**Number of theoretical plates**	**Asymmetry**	**Condition**	**RSD(%)**	**Number of theoretical plates**	**Asymmetry**
Normal condition	0.04	14934	1.2	Normal condition	0.05	9960	1.1
Buffer solution of pH 5.3	0.60	14619	1.2	Buffer solution of pH 1.8	0.10	8981	1.2
Buffer solution of pH 5.7	0.10	15274	1.1	Buffer solution of pH 2.2	0.02	9016	1.2
Column temperature 25°C	0.50	14074	1.3	Column temperature 40°C	0.03	9603	1.1
Column temperature 35°C	0.10	15247	1.2	Column temperature 50°C	0.03	9598	1.2
Flow rate of 0.9 mL min^{-1}	0.10	15737	1.2	Flow rate of 1.35 mL min^{-1}	0.10	10230	1.2
Flow rate of 1.1 mL min^{-1}	0.10	13888	1.1	Flow rate of 1.65 mL min^{-1}	0.03	9272	1.2
Organic compartment (-5%)	0.10	15046	1.3	Organic compartment (-5%)	0.10	9332	1.1
Organic compartment (+5%)	0.10	14095	1.2	Organic compartment (-5%)	0.10	9246	1.2

The results in Table 7 prove that the number of theoretical plates and asymmetry of the chromatograms are very similar with changing chromatographic conditions. This shows that the proposed method is highly robust.

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