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ICH GUIDELINES IN PRACTICE: DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR MORNIFLUMATE IN BULK AND IN TABLET DOSAGE FORM

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Abstract

A simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for the determination of morniflumate from tablet dosage form. The stability indicating ability of the RP-HPLC method was demonstrated by performing forced degradation studies on morniflumate under wet heat, hydrolytic (acidic and basic) dry heat, oxidative and photolytic conditions. Separation of drug from its major degradation products was achieved by isocratic elution using a mobile phase consisting of methanol**:** phosphate buffer {pH 7; 0.02M} {95:5 v/v} using Shodex C_{18} column (250 X 4.6mm, 5µm) at a flow rate of 1 ml/minwith UV detection at a wavelength of 287 nm at ambient temperature. The method was validated according to ICH guidelines. The retention time of morniflumate was found to be 6.4± 0.005 min. The drug undergoes excessive degradation under alkaline hydrolysis and wet heat conditions. Methanolic solution of the drug was found to degrade appreciably under photolytic conditions. Around 10-15% degradation was observed when the drug was stressed under acidic and oxidative conditions. The developed method was found to be linear over a range of 2.5-15 µg/ml.The method was demonstrated to be precise, accurate, specific and robust. The results obtained verify that the method can be used for its intended purpose.

Keywords: Forced degradation, morniflumate, RP-HPLC, ICH guidelines.

Introduction

A stability-indicating test method is one that accurately and selectively quantifies intact drug in the presence of degradation products and other solution components. The ICH guidelines Q1A on Stability Testing of New

Drug Substances and Products emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and/or efficacy must be done by validated stability-indicating assay methods. Hence, these guidelines require conduct of forced degradation studies under a variety of conditions like pH, light, oxidation, dry heat etc. and separation of drug from its degradation product.[1-3]

Morniflumate is chemically 3-pyridinecarboxylic acid 2-[[3-(trifluoromethyl) phenyl]-amino]-2-(4-morpholinyl)-ethyl ester (Figure 1). The pharmacological activity of Morniflumate is likely to be attributed to the niflumic acid levels to which it gives rise in the plasma, and consequently at the anatomical sites of inflammation, following the very rapid hydrolysis of the ester to the free acidic form in the plasma. Morniflumate acts as an anti-inflammatory, analgesic and an anti-pyretic by inhibiting the 5-lipoxygenase pathway of the Arachidonic acid cascade as well as the synthesis of cyclooxygenase products. This dual inhibitory property has represented an improvement in the anti-inflammatory therapy.

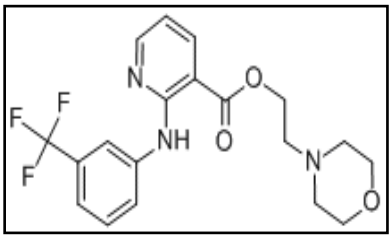

Figure 1: Chemical structure of morniflumate.

The literature survey reveals that high-performance liquid chromatography method has been developed for the simultaneous determination of morniflumate and its major active metabolite, Niflumic acid, in human plasma.[4-5]However there are no reports for the development of a stability indicating RP-HPLC method for morniflumate. Hence the objective of the present research work was to develop and validate a simple, accurate and precise stability indicating method for estimation of morniflumate in bulk and in its tablet dosage form.

Materials and Methods

Reagents and chemicals:

The gift sample of morniflumate was provided by Enaltec labs, Ambernath. It was used without further purification. Methanol, monobasic potassium dihydrogen-o-phosphate and dibasic potassium hydrogen phosphate were purchased from Molychem Manufacturers & Importers of Laboratory Reagents & Fine Chemicals. All other chemicals & reagents used were of analytical grade purchased from Molychem India. Double distilled water used in the study was prepared in house using the borosil glass distillation unit.

Instrumentation:

The HPLC system employed was Agilent {1200 series} comprising of quaternary pump, variable wavelength programmable UV detector and Rheodyne sample injector fitted with 20µL capacity loop. Data acquisition was obtained using the software chemstation (B.02.01). 50µL Hamilton injection syringe was used for sample injection. UV detection was performed using a double beam Jasco V-630 spectrophotometer with Spectramanager software. All chemicals were weighed using Shimadzu electronic balance, Model BL-220H. Measurement of pH of buffer solutions was done using Equip-Tronics digital pH meter with magnetic stirrer. Oscar Ultrasonics bath sonicator was used for degassing the mobile phase.

Chromatographic Conditions: The chromatographic separation was achieved on a reverse phase Shodex C 18-4E column (5 µm; 250 × 4.6 mm, Showa Denko America Inc., USA) using a mobile phase comprising of a mixture of methanol and phosphate buffer, pH 7.0 (0.02M, 95:5 *v/v*) at a flow rate of 1.0 ml/min. The mobile phase was filtered through Millipore nylon membrane filter (0.45 µm) and was degassed by sonicating for 20 min. The column was maintained at room temperature. The injection volume was 20 µl. The wavelength for UV detection was 287 nm.

Preparation of Standard Stock Solution: Accurately about 10 mg of morniflumate was weighed and transferred to a 10 ml volumetric flask. The volume was then made up to the mark with methanol to get a standard solution of morniflumate having a concentration of 1000 µg/ml.

Preparation of working standard solutions:

Working standard solutions for HPLC injections were prepared on a daily basis. The standard solution was prepared by dilution of the stock solution with mobile phase to give a solution of concentration 10 µg/ml.

Forced degradation studies of morniflumate:

Forced degradation studies were carried out as per ICH guidelines.[6-7]All samples of degradation were prepared at a drug concentration of 1 mg/ml. Morniflumate was subjected to a variety of stress conditions to effect degradation up to about 10 %. The drug was degraded under a variety of conditions like hydrolysis (acid and base), wet heat, oxidation, photolysis and dry heat.

The degraded samples were diluted with the mobile phase to a final concentration of 10µg/ml in terms of the drug and subjected to chromatographic separation using the developed mobile phase to resolve the drug from any potential degradation products.

Calibration experiments:

The stock solution of morniflumate was diluted suitably with the mobile phase to get concentrations of 2.5, 5, 10, 15, and 20 µg/ml.

These were analyzed in three replicates to get the drug peak areas that were then plotted against the concentration and subjected to least square linear regression analysis.

Method Validation:

The developed method was validated as per ICH guidelines Q2 to demonstrate that it is suitable for the intended purpose. The method was validated for system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.[8-10]

a. System Suitability:

System suitability parameters were studied to ensure that the instrument is suitable for the intended purpose. Retention time, tailing factor and theoretical plates were evaluated.

The drug solution was injected five times into chromatographic system under the optimized conditions and the parameters were evaluated.

b. Accuracy and Precision:

Accuracy and precision were evaluated at three concentration levels (80,100,120) by determining % relative error and % relative standard deviation (%R.S.D.) respectively, for the results of recovery of morniflumate from pre-analyzed tablet powder spiked with morniflumate at three levels. The analysis was performed in triplicate at each level over three days and intra-day and inter-day variations were evaluated.

c. Specificity:

The specificity of the method was inferred from the resolution of the drug peak from that of the possible degradation products produced in the forced degradation experiments. Specificity was also studied by analyzing powder of blank tablets before and after subjecting to forced degradation. Lack of interfering peaks at the retention time of the drug was taken as an indication of specificity.

d. Limit of Detection and Limit of Quantification:

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve. The sensitivity of the method was established by the LOD and the LOQ values.

e. Robustness:

Robustness was established by introducing small deliberate changes in the HPLC optimized conditions which include the change in wavelength, flow rate and percentage of acetonitrile in the mobile phase. This was studied using three replicates at a concentration level of 10µg/ml.

Analysis of tablet dosage form of morniflumate:

To determine the content of morniflumate in tablets (label claim 250mg per tablet}, 20 tablets were weighed, and average weight was calculated. Tablets were triturated and powder equivalent to average weight was weighed. The drug was extracted from the tablet powder with methanol. The resulting solution was injected in HPLC and drug peak area was noted. Regression equation was generated using peak areas of standard solutions. Using the regression equation and peak area of the sample the amount of morniflumate in the sample was calculated. The amount of morniflumate per tablet was thus found.

Results and Discussion

Optimization of chromatographic conditions:

When the working standard solution of morniflumate was subjected to RP-HPLC analysis using the mobile phase comprising of methanol: phosphate buffer, pH 7.0 (0.02M, 95:5 *v/v*), the drug was eluted at an adequate retention time of 6.4± 0.005 min. The chromatographic peak showed good peak shape and resolution with a symmetry of 0.82.[11]This optimized mobile phase was then used for chromatographic analysis of the forced degradation samples of the drug to achieve separation between the drug and any potential degradation products. Figure 2 depicts the representative chromatogram of standard solution of morniflumate.

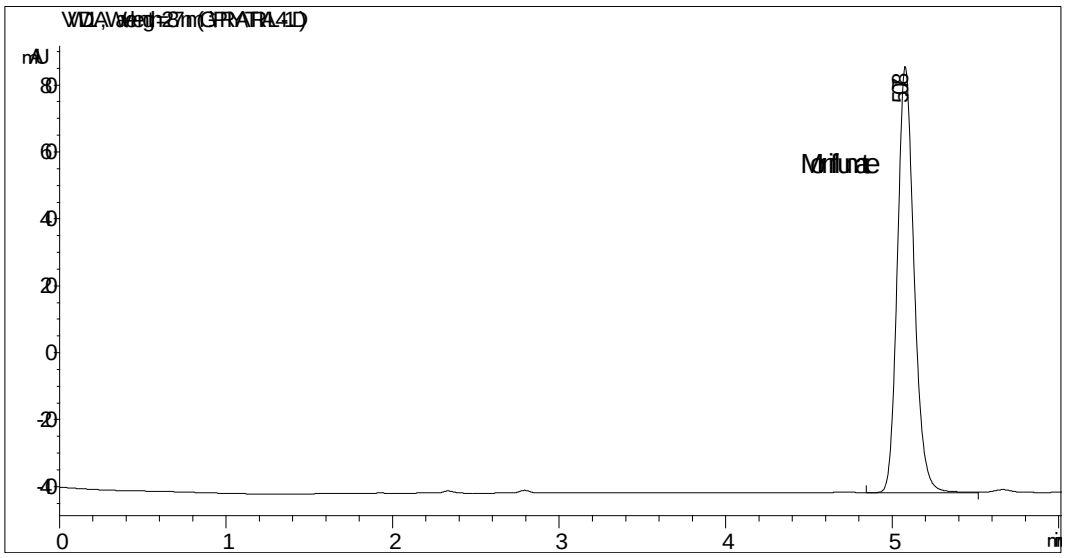

Figure 2: Chromatogram of standard morniflumate (10 µg/ml).

Forced degradation studies:

When morniflumate was subjected to a variety to of stress conditions and subsequently to chromatographic analysis; it was found to degrade under all conditions except dry heat and photolytic conditions in the solid state. In case of acid induced degradation, when morniflumatewas heated under reflux for nearly 2hr in 0.05N HCL, around 10% degradation resulted with one degradation product being formed as shown in Figure 3. Likewise, morniflumate was found to be extremely unstable in alkaline conditions showing more than 90% degradation within a minute even at room temperature (Figure 4).When morniflumate mixed with water was boiled for 30min under reflux nearly 50% degradation resulted with the formation of one degradation product as observed in Figure 5. On exposure of the drug to 3% H_2O_2 for 48h, 15% degradation resulted with degradation products formed as shown in Figure 6. Although no degradation was apparent when the drug powder was exposed to direct sunlight for 24 hours, when methanolic solution was given the same treatment, 26% degradation occurred with the formation of one degradation product as shown in Figure 7. The drug was stable under dry heat conditions with no degradation resulting on heating in oven at 80^0C for 48h. Table 1 summarizes the results obtained after forced degradation studies of morniflumate under a variety of conditions.

Table 1: Summary of Forced Degradation of Morniflumate.

Condition	Retention time(s) of degradation products (mins)	% Degradation	Figure
Refluxed in 0.05N HCL for 115 mins.	2.3	10.86	6.3.8
In 0.01N NaOH at RT (Zero min)	2.3, 5.3	93.12	6.3.9
Refluxed in H_2O for 30 mins	2.3	48.03	6.3.10
Kept in 3%H_2O_2 for 48h	2.3, 5.2	15.00	6.3.11
Kept in oven at 80º for 48h	No Degradation	-	6.3.12
Exposed to direct sunlight for 24h (Powder)	No Degradation	-	6.3.13
Exposed in direct sunlight for 24h (Methanolic solution)	5.4	26.66	6.3.14

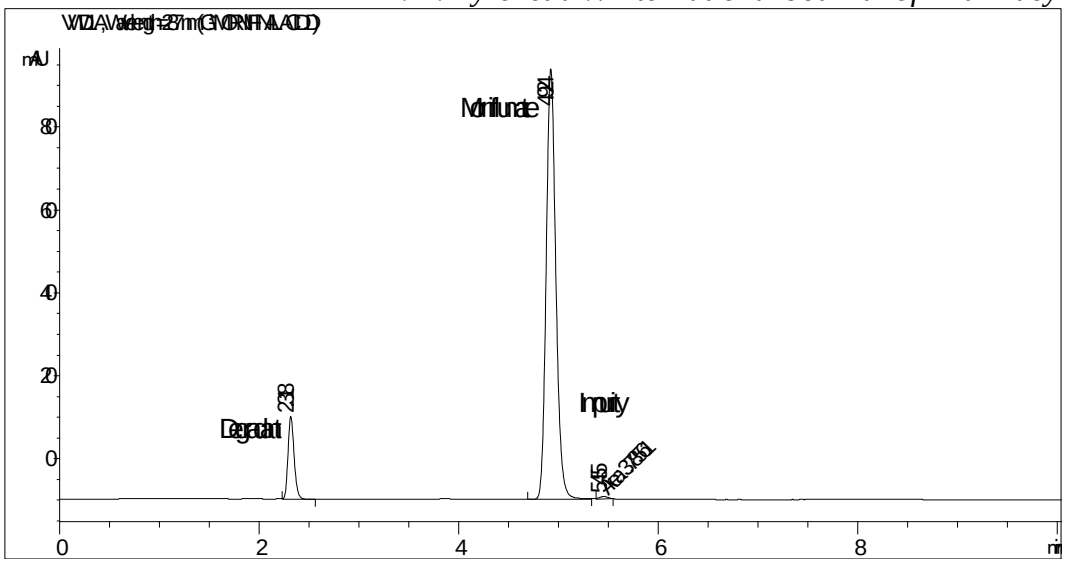

Figure 3: Chromatogram of morniflumate (10 µg/ml) after acid induced hydrolysis.

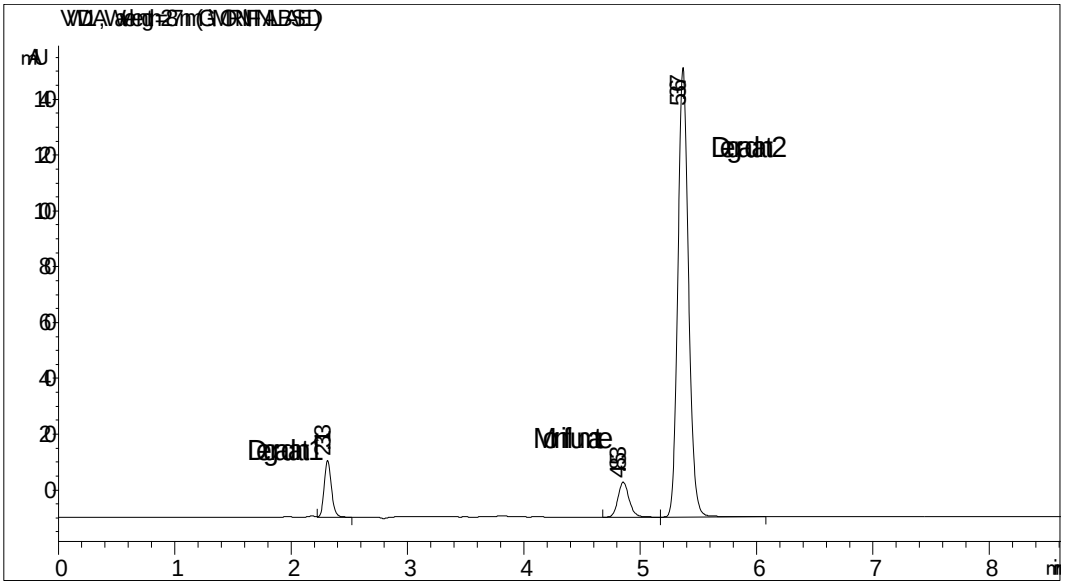

Figure 4: Chromatogram of morniflumate (10 µg/ml) after base induced hydrolysis.

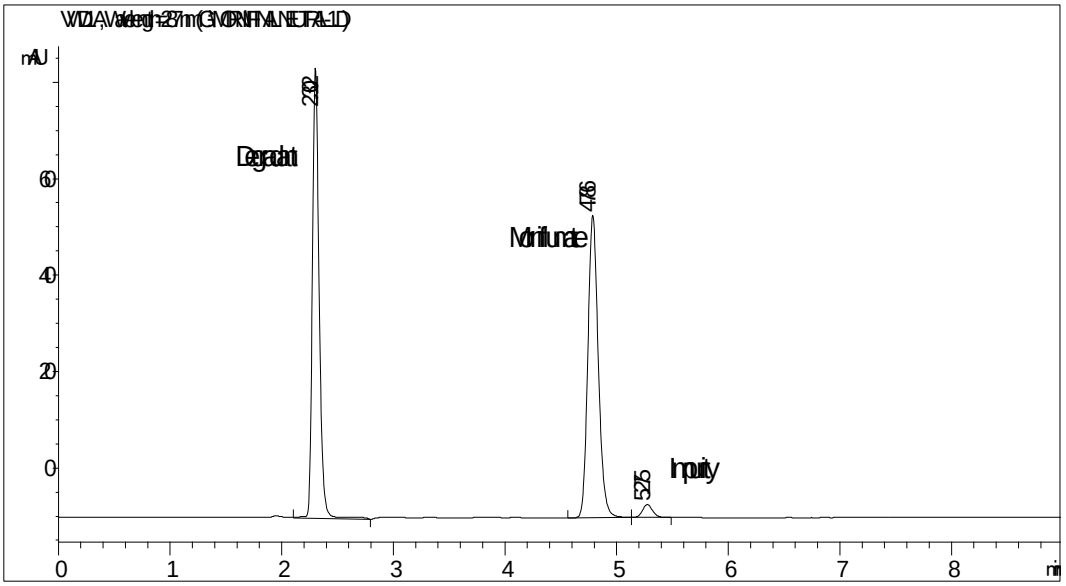

Figure 5: Chromatogram of morniflumate (10 µg/ml) after wet heat induced hydrolysis.

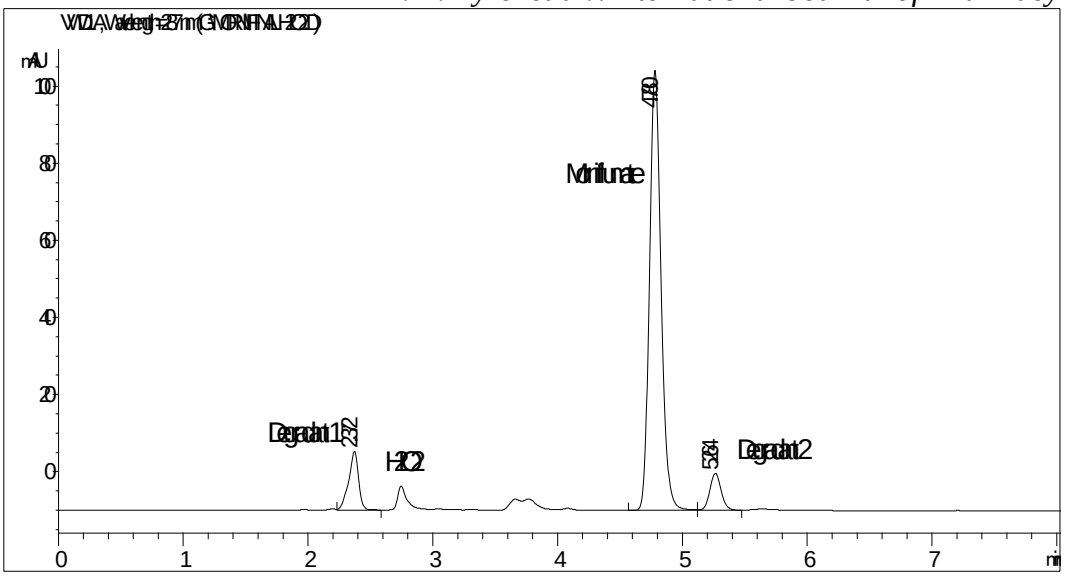

Figure 6: Chromatogram of morniflumate (10 µg/ml) after oxidation.

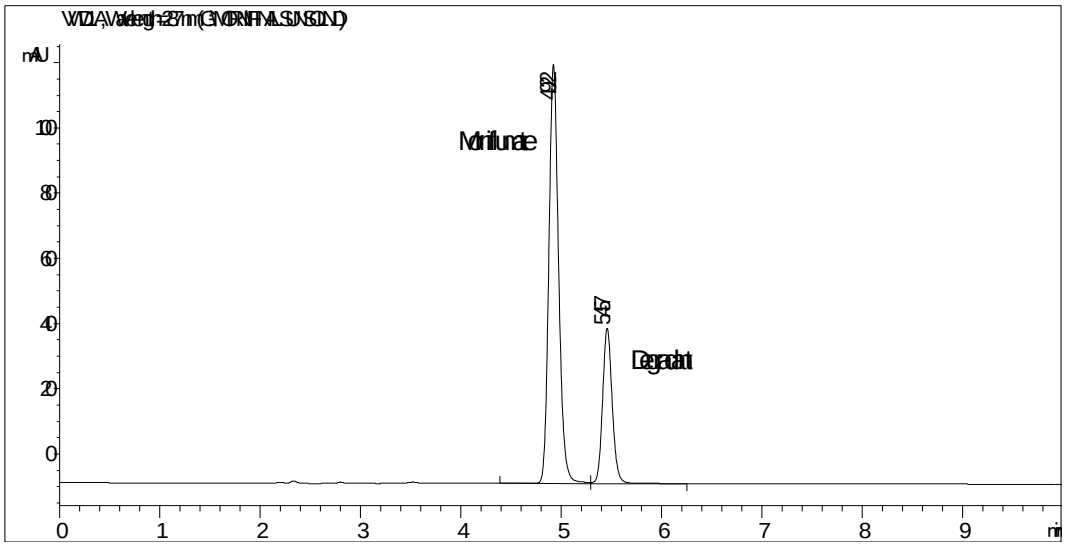

Figure 7: Chromatogram of morniflumate (10 µg/ml) after photolysis (methanolic solution).

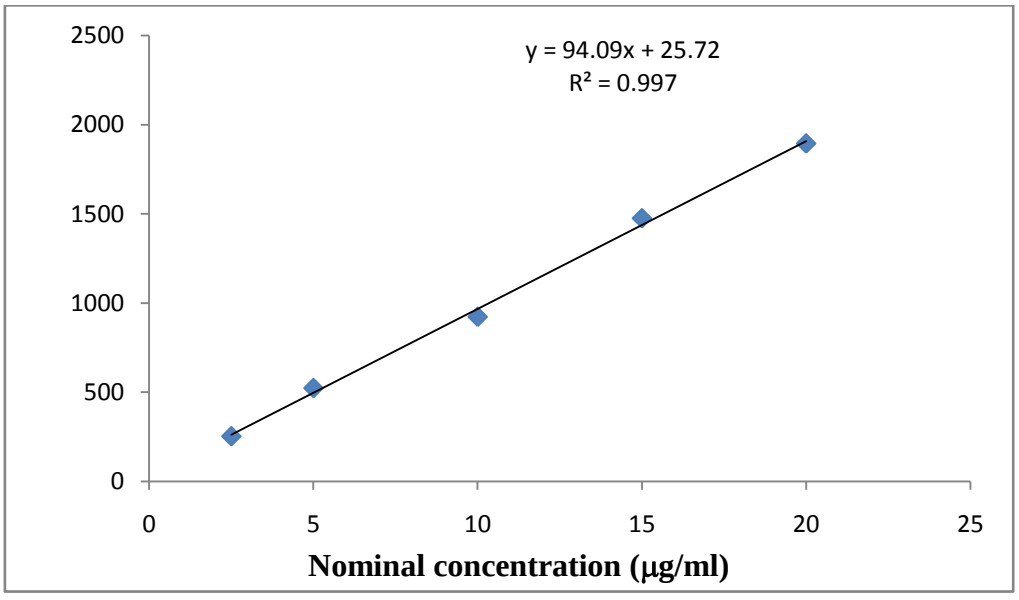

Figure 8: Calibration line of morniflumate.

Calibration experiments:

The data for calibration runs of morniflumate is presented in Table 2. Calibration curve of peak area against concentration was found to be linear in the concentration range of 2.5-15 μg/ml as shown in Figure 8 with the regression equation y= 94.09x + 25.72 and correlation coefficient of 0.997.

Table 2: Linearity Data for Morniflumate.

Calibration standard	Nominal concentration (μg/ml)	Peak area of replicate {mAU.sec}			Mean peak area {mAU.sec}
		1	2	3	
1	**2.5**	237.56	276.28	243.54	252.46
2	5	489.8	508.7	569.8	522.76
3	10	974.7	981.1	811.8	922.53
4	15	1482.5	1451.3	1493.2	1475.7
5	20	1975.5	1858.8	1851.7	1895.33

Method validation:

System suitability parameters indicate high column efficiency with large number of theoretical plates (>2000). The symmetry of the chromatographic peak of morniflumate was found to be 0.82 which does not exceed the critical value of 2. The average retention time was found to be 6.4 min.

The results of intra and inter- day variation of morniflumate at three different concentration levels (80 %, 100 %, and 120 %) are depicted in Table 3. The data indicate that maximum %relative error at 80%, 100% and 120% was –0.7, 1.08 and 0.72 respectively while the maximum %relative standard deviation was 1.45, 1.51 and 1.57 respectively indicating that the method has acceptable accuracy and precision. Also the calculated t-values were lesser than the tabulated t-value of 4.3 for $\alpha = 0.05$ at two degrees of freedom. This indicated that the experimental values were not significantly different from the nominal values, which reflected the accuracy of the method.

Table 3: Results of Accuracy and Precision for Morniflumate.

Day	Amount added	Amount found				% error	Relative	% RSD	Calculated t-values
		1	2	3	Mean				
1	200	197.2	202.7	198.4	199.43	-0.28		1.45	0.113
	250	256.7	249.1	252.3	252.7	1.08		1.51	0.408
	300	295.5	297.9	304.6	299.33	-0.22		1.57	0.081
2	200	201.2	203.1	199.6	201.3	0.65		0.87	0.428
	250	248.3	249.1	253.2	250.2	0.08		1.05	0.044
	300	303.4	301.7	298.1	301.06	0.35		0.89	0.227
3	200	197.7	199.2	198.9	198.6	-0.7		0.39	1.018
	250	252.6	251.3	250.9	251.6	0.64		0.35	1.039
	300	301.1	301.8	303.6	302.16	0.72		0.43	0.969

The HPLC chromatograms recorded for the blank tablets and blank tablets exposed to the degradation conditions showed no peaks at the retention time of morniflumate and also the representative chromatograms of stressed samples under various stress conditions **{**Figures 3 to 7**}** showed that morniflumate was well resolved from its degradation products, indicating the specificity of the method. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 0.125 µg/ml and 0.5 µg/ml respectively.

The results of robustness study are given in Table 4. It was found that there was no drastic change in the resolution of morniflumate when deliberate changes were introduced in the optimized chromatographic conditions thus confirming robustness of the developed method.

Table 4: Robustness Study

Parameter	Optimized	Variation	Mean peak area (mAU)	Mean Retention time ±S.D. (min)	Mean No. of theoretical plates ±S.D.	Mean Tailing factor± S.D.
Detection wavelength	287nm	286nm	934.12	6.1±0.002	6026±10.3	0.83±0.004
		--	940.04	6.1±0.001	6210±14.5	0.82±0.007
		288nm	931.76	5.9±0.004	6104±12.7	0.84±0.002
Flow rate	1mL/min	0.8mL/min	942.67	6.3±0.001	6020±26.9	0.79±0.001
		--	939.38	6.1±0.003	6201±15.3	0.83±0.003
		1.2mL/min	935.81	5.8±0.002	6350±34.1	0.81±0.002
% of acetonitrile in mobile phase	40%	39%	930.03	6.0±0.005	6240±28.3	0.80±0.001
		--	943.49	6.1±0.002	6321±16.7	0.81±0.005
		41%	936.01	5.8±0.003	6120±34.9	0.83±0.002

Analysis of tablet dosage form of morniflumate:

The chromatograms of the drug samples extracted from tablets did not show a change in the retention time. There was no interference from the excipients, which are commonly present in the tablets. The drug content was found to be 101.39 % with a % RSD of 1.35 as shown in Table 5**.** Therefore, it may be concluded that, degradation of morniflumate had not occurred in the tablet formulations. The % low RSD value indicated the suitability of the method for the routine analysis of morniflumate in pharmaceutical formulation.

Table 5: Analysis of Tablet Formulation.

Amount per tablet {mg}	Mean amount recovered {mg}	% recovered ±SD	Mean ±SD
250	253.1	101.2±1.03	100.7±1.34
250	255.2	102.1±0.76	
250	247.2	98.9±0.51	

Conclusion

The isocratic R+P-HPLC method developed for quantitative determination of morniflumate is precise, selective and robust. The validation of the assay method shows satisfactory data for the parameters tested. The method well separated the drug from its degradation products, thus indicating its stability indicating ability.

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Conflict of Interest: The authors do not have any conflict of interest.

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