Development And Validation Of Hplc Method For The Isobutylglutarmonoamide

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Abstract

To obtain the necessary chromatographic separation for Isobutylglutarmonoamide, a high-pressure liquid chromatography (HPLC) technology was applied. Aiming for accuracy, precision, linearity, and stability, the analytical approach was created. A quantitative HPLC analysis of Isobutylglutarmonoamide was performed utilizing a technique that was created and improved for accuracy, precision, linearity, robustness, and stability. With a stationary phase of Hypersil BDS C18 Chromatography was used to separate Isobutylglutarmonoamide by using a column with the following parameters: (diameter: 250mm, internal diameter: 4.6mm), particle size: 5, mobile phase consisting of buffer and acetonitrile in an 80:20 ratio, flow rate of 1.0 ml/min, UV detector at 210nm. The squared correlation coefficient, often known as R2, was found to have a value that was more than 0.999 for a concentration range that ranged from 12.5 to 75 g/ml. The retention time of Isobutylglutarmonoamide was observed 8.0 1.0 minutes. Studies on forced degradation of Isobutylglutarmonoamide were conducted utilizing acid, base, heat, photolysis, and hydrogen peroxide oxidation. The main peak was unaffected by the degradation products that are produced under stress condition. To get findings that were exact, accurate, and selective in the presence of degrading impurities, this analytical technique was developed and validated for all the parameters listed in the ICH guidelines. This developed analytical method is precise, efficient, quick, accurate, economical and rapid. The recommended technique is highly useful for regular analysis and quality control in bulk drug manufacturing for Isobutylglutarmonoamide.

Keywords: Isobutylglutarmonoamide, validation, development, stability indicating methodology.
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Pregabalin, an anticonvulsant medication used to treat neuropathic pain, is synthesized chemically From Isobutylglutarmonoamide (R)-3-(Carbamoylmethyl)-5-Methylhexanoic Acid.

Isobutylglutarmonoamide is an intermediate of Pregabalin, which has a below structure.

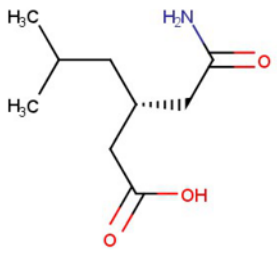


Figure-1: Structure of Isobutylglutarmonoamide (Pregabalin Intermediate) IUPAC name for Isobutylglutarmonoamide is (3R)-3-(2-amino-2-oxoethyl)-5-methyl acid hexanoic. After through the processes of chemical synthesis and purification, it is converted into pregabalin, which is then administered to patients suffering from epilepsy or anxiety. Additionally, it can be used to relieve discomfort caused by nerves. Diabetes and shingles are just two of the many conditions that can bring on nerve discomfort, but there are many more as well. The release of neurotransmitters like glutamate, norepinephrine, substance P, and calcitonin gene related peptide can be inhibited by pregabalin because it binds to the 2 (alpha2delta) subunits of the voltage-dependent calcium channel in the central nervous system. This keeps neuro-transmitters from being released. As a result of its ability to suppress nerve transmission, pregabalin can both reduce pain and the frequency of seizures. The active drug was separated from any potential impurities using the HPLC instrumental method. In order to determine Isobutylglutarmonoamide in the presence of possible contaminants, a user-

friendly, specific, accurate, cost effective and exact high pressure liquid chromatography-based technology was developed. Since there was no reference in the literature to such an approach for identifying the chemical and its degradants. The chromatographic technique is often used for the analysis of active drug and intermediates because it is specific, precise, exact, and user-friendly. Chromatographic methods produce high-quality findings while eliminating time-consuming extraction and isolation procedures. During the chromatographic multistage separation procedure, the components of the sample are distributed in the phase transition between the fixed and the mobile states. The liquid that is sustained by a solid, gel, or solid constitutes the stationary phase of the system. A correctly applied stationary phase that is arranged in columns, rolled out into layers, and spread out as thin films. It is possible for the mobile phase to be either a gas, a liquid, or a supercritical fluid. Adsorption, ion exchange, mass distribution (partitioning), and changes in the physicochemical characteristics of the molecules, such as the molecules' mass, size, and volume, are some of the ways that substances can be separated from one another. This particular kind of chromatography may be applied for quantitative as well as qualitative examination by employing a wide range of analytical methods.

Method and Requirements

Instrument

Shimadzu HPLC components include the injector, column oven, UV detector, and pump.

Chromatographic column

Hypersil BDS C18 having dimensions: 250 mm x 4.6 mm, 5 micron.

Reagents and chemicals

Water (HPLC garde), Acetonitrile (HPLC garde), Ammonium dihydrogen phosphate (Analytical grade), and Orthophosphoric acid (Analytical grade).

Mobile phase preparation

Ammonium dihydrogen phosphate weighs 4.75 grams; it dissolves in 1000 ml of HPLC-grade water. 10% orthophosphoric acid is used to get the solution's pH to 3.00 ± 0.05 (Buffer). Use this buffer preparation with Acetonitrile using ration of 80:20. Use a 0.45 membrane filter to filter this mobile phase, then sonicate to degas.

Diluent preparation

Mobile phase

Standard stock (1000ppm) solution

Weigh accurately 50 mg of standard of Isobutylglutarmonoamide and transfer in to 50 ml volumetric flask. Dissolve in about 30 ml of diluent, and then gradually increase the amount of diluent being used until the desired concentration is achieved (1000ppm solution).

Working standard solution

Working standard solutions with concentrations of 12.5, 25, 37.5, 50, 62.5, and 75 ppm were prepared with the use of the standard stock solution. For the purposes of these research on stress degradation, the "working standard" was used containing the concentration of 50 ppm. At a wavelength of 210 nm, both the standard solution and the sample solution, which each had a concentration of 50 ppm, were analysed.

Sample solution

After accurately weighing the test sample of Isobutylglutarmonoamide at 50 mg, transfer it into a 50 ml volumetric flask. After dissolving in approximately 30 ml of diluent, add more diluent until the volume reaches the desired level (1000ppm solution). Further dilutions were performed in order to achieve a concentration in the final stock that was equivalent to 50 ppm.

Before settling on the HPLC parameters and validate the analytical method, such as the mobile phase, its ratio, and the flow rate, a large number of experiments were performed with the goal of finding the optimal settings for the chromatographic apparatus. After that, the one that worked the best was improved. The chromatographic estimation of Isobutylglutar-monoamide and its separation from degradation products were achieved by using a Hypersil BDS C18 analytical column (Dimension: Length 250 mm, 4.6 mm internal diameter, and particle size 5), a mobile phase consisting of ammonium dihydrogen phosphate buffer with a pH of 3.00±0.05, and acetonitrile in the ratio of 80:20, with a flow rate of 1.0 ml UV detection was carried out at a wavelength of 210 nm.

Validation of proposed method

The suggested methodology's compliance with the requirements for specificity, linearity, precision,

accuracy, robustness, etc. was verified using ICH guidelines [9].

System suitability test

To test the system suitability parameters, six replicates of Isobutylglutarmonoamide were injected at 50 ppm, and the chromatograms were recorded. [3].

Forced degradation studies

In line with ICH Q1A, Isobutylglutarmonoamide was subjected to forced degradation testing for acid and base, oxidation and thermal stress conditions, as well as photo stability testing. The oxidative hydrolysis was performed with 10% hydrogen peroxide, the acid hydrolysis was performed with 1M hydrochloric acid, and the base hydrolysis was performed with 0.1M sodium hydroxide. Samples of Isobutylglutar-monoamide were tested for thermal and photolytic degradation [10].

Acid hydrolysis

In order to conduct out acid degradation, a stock solution of Isobutylglutarmonoamide with a concentration of 1000 ppm was added to 1 M hydrochloric acid. This solution was put at a temperature of 70 degrees Celsius for a period of 4.0 hours. After that, the resultant solutions were diluted to a concentration of 50 ppm and neutralized with sodium hydroxide at a concentration of 1 M.

Base hydrolysis

In order to carry out base-induced forced degradation, a stock stock solution containing 1000 ppm of Isobutylglutarmonoamide was added to 0.1M sodium hydroxide. After that, the solution that was produced was diluted to a concentration of 50 ppm and then neutralized with 0.1 M of hydrochloric acid.

Oxidation stress

A 10% hydrogen peroxide solution was added in to Isobutylglutarmonoamide stock solution (1000 ppm) and heated up to 70°C for four hours. To assess the impact of oxidative conditions, this solution was then diluted to a concentration of 50 ppm.

Thermal degradation

A sample (solid) was subjected to temperature testing by being heated to 105 degrees Celsius for a period of one day. The final concentration of the Isobutylglutarmonoamide solution was 50 ppm.

Photolytic degradation

Isobutylglutarmonoamide standard stock solution, which had an initial concentration of 1000 ppm, was further diluted until it reached a final concentration of 50 ppm after being subjected to ultraviolet radiation in a UV chamber for about 24 hours.

Results

Mobile phase was used as the diluent because Isobutylglutarmonoamide dissolved in a water and acetonitrile solution. Since they do not completely complete solubility and result in hazy solutions, For the purpose of dilution, single solvents such as water or acetonitrile were not employed.

Mobile phase Optimization

Due to the fact that testing with methanol: water showed peak merging with other contaminants, buffer and acetonitrile was selected to be the final mobile phase. Because moving the ratio to gradient mode caused peaks to merge together and the peak form to become less distinct, the isocratic ratio of mobile phase was selected. Since peak merging occurred at flow rates of 0.8 or 1.2 ml/min, the ideal flow rate was determined to be 1.0 ml/min in this study.

Table 1: Mobile phase Preparation

Mobile phase	
Buffer	4.75gm Ammonium dihydrogen phosphate dissolve in 1000 ml of water (HPLC grade) and adjusted the pH 3.00 ± 0.05 with the solution of 10% orthophosphoric acid.
Acetonitrile	Liquid Chromatographic grade
Buffer: Acetonitrile	80 : 20

Chromatographic conditions optimization

Specifically locating the analyte peak in the presence of any possible contaminants is the aim of analytical method optimization. To separate Isobutylglutarmonoamide from its degradants using high pressure liquid chromatography, Hypersil BDS C18 analytical column was used in conjunction with mobile phase at a flow rate of 1.0 ml/min and a detection wavelength of UV 210 nm. The dimension of the column was as follows: length: 25 cm; internal diameter: 4.6 mm; and particle size: 5 microne.

Table 2: Optimized chromatographic conditions and parameters

Parameters	Chromatographic conditions
Stationary phase	Hypersil BDS C18 (250mm x 4.6mm) 5µ
Mobile phase Flow	1.0ml/min
Injection volume	10µl
UV wavelength	210 nm
Run Time	30.0 min.
Diluent	Mobile Phase

System suitability test"

The outcome for system suitability test is displayed in Table 3 below. The outcome was within the permitted range. Consequently, the HPLC system was appropriate for the proposed analytical method.

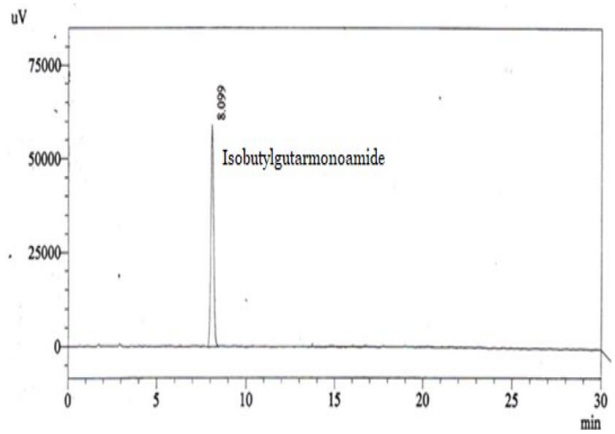


Figure-2: HPLC chromatogram of Isobutylglutarmonoamide during system suitability test.

Chromatographic conditions

Mobile phase Ammonium dihydrogen phosphate buffer and acetonitrile (80:20 ration) with the flow rate of 1.0 mL/min; UV detection was at 210 nm; stationary phase Hypersil BDS C18 (Length 250 mm, 4.6 mm internal diameter), and particle size 5 micron.

Table 3: System suitability Results"

System Suitability Results			
Parameters	**%RSD for results (n=6)**	**Acceptance Criteria**	**Result**
%RSD, (Repeatability)	0.22	%RSD <2.0	Method Passed with System Suitability Criteria.
Tailing Factor	1.13	<2.0	
Theoretical Plates	81807	>5000	

Validation Studies

Linearity

For Isobutylglutarmonoamide, the linearity was shown between 12.5 ppm and 75 ppm in concentration. After doing so, the equation of the regression line was drawn between the concentration and the peak area. The correlation coefficient was observed as R2=0.99983.

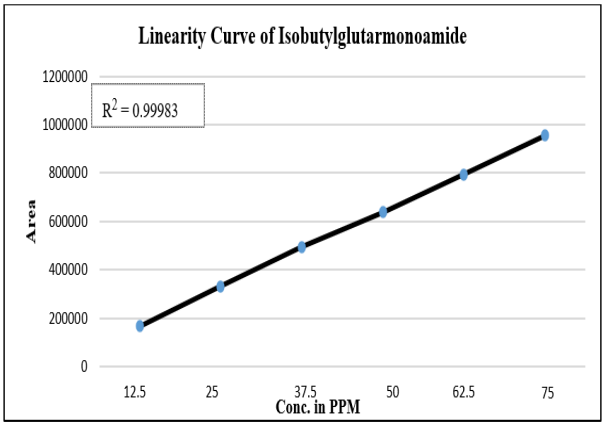


Figure-3: Linearity curve of Isobutylglutarmonoamide

Table 4: Linearity data of Isobutylglutarmonoamide

Linearity of Isobutylglutarmonoamide	
Concentration in ppm	**Area**
12.51	166444
25.04	331154
37.54	494919
50.04	639100
62.48	795042
75.02	955195
Correlation coefficient	**0.99983**

Specificity

Figure 2 displays the specificity findings. Due to blank and degradants in Isobutylglutarmonoamide, no interference was seen.

Precision

The results of the analysis are summarized in Table 9. The% RSD was discovered to be within the permitted limit.

Table 5: System precision

System precision				
Injections	**Peak Area**	**Average**	**Standard Deviation**	**%RSD**
Injection-1	645888	649470	2288	0.35
Injection-2	650114			
Injection-3	649886			
Injection-4	651153			
Injection-5	647699			
Injection-6	652077			

Table 6: Method precision (Analyst-1)

Method Precision (Analyst-1)						
Samples	**Peak Area**	**Ave. Area**	**% Assay**	**%Ave. Assay**	**Standard Deviation**	**% RSD**
Sample -1	640750	640921	99.4	99.6	0.28	0.277
	641091					
Sample -2	650109	650792	99.5			
	651475					
Sample -3	644171	643144	99.7			
	642117					
Sample -4	644012	644084	100.1			
	644156					
Sample -5	648621	648322	99.3			
	640022					
Sample -6	645688	645154	99.5			
	644619					

Table 7: Method precision (Analyst-2)

Method Precision (Analyst-2)						
Samples	**Peak Area**	**Average Area**	**% Assay**	**%Average Assay**	**Standard Deviation**	**% RSD**
Sample 1	642526	641785	99.8	99.7	0.259	0.259
	641043					
Sample 2	643915	644345	99.4			
	644775					
Sample 3	643239	644123	100.1			
	645007					
Sample 4	644123	644722	99.8			
	645321					
Sample 5	642146	642831	99.7			
	643516					
Sample 6	645985	645398	99.5			
	644810					

Table 8: Intermediate precision (Cumulative results)

Intermediate precision					
Analysts	**Test Sample**	**% Average Assay**	**%Assay Overall**	**Standard Deviation**	**% RSD**
1st Analyst	Sample 1	99.4	99.6	0.264	0.27
	Sample 2	99.5			
	Sample 3	99.7			
	Sample 4	100.1			
	Sample 5	99.3			
	Sample 6	99.5			
2nd Analyst	Sample 1	99.8			
	Sample 2	99.4			
	Sample 3	100.1			
	Sample 4	99.8			
	Sample 5	99.7			
	Sample 6	99.5			

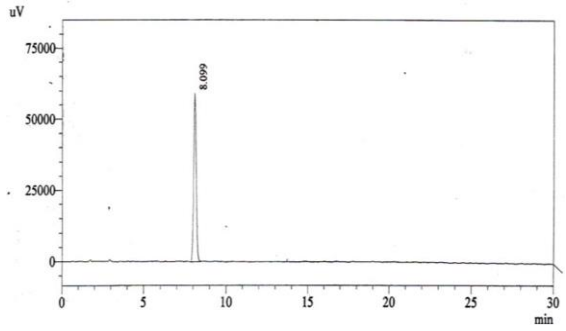
Robustness

In order to evaluate the resilience of the technique, chromatographic settings and parameters such column oven temperature, flow rate, and mobile phase pH were subjected to a series of controlled and purposeful changes. The findings demonstrated that the flow rate, column oven temperature, and mobile phase pH were not found to have any discernible effects on the results.

Forced degradation

Isobutylglutarmonoamide was put to a number of different types of stress, such as hydrolysis by acids and bases, photolysis, oxidation, and thermal stress. The findings are summarized in Table 10.

Figure 4: Isobutylglutarmonoamide test before treatment



Acid and base hydrolysis

Degradation was successfully completed under acid and base degradation stress conditions. The breakdown rates for Isobutylglutarmonoamide are 8.5% and 5.8%, respectively, under the acid and base hydrolysis-imposed stress conditions. The chromatograms demonstrated the presence of degraded products. (Figure 5 and Figure 6)

Figure 5: Degradation by Acid

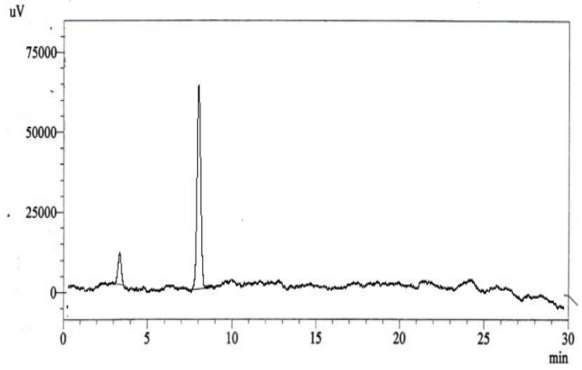
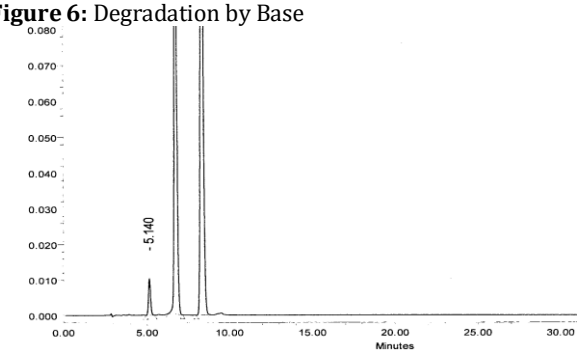


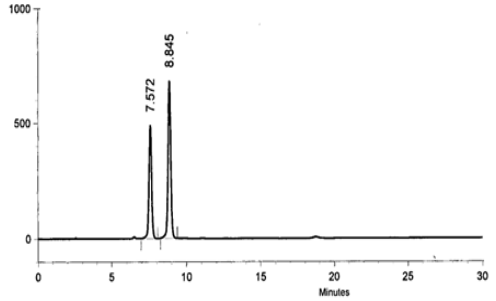
Figure 6: Degradation by Base



Oxidation degradation

It has been demonstrated that Isobutylglutarmonoamide is quite stable in the presence of oxidative stress. It said that the amount of Isobutylglutarmonoamide was 2.6%.

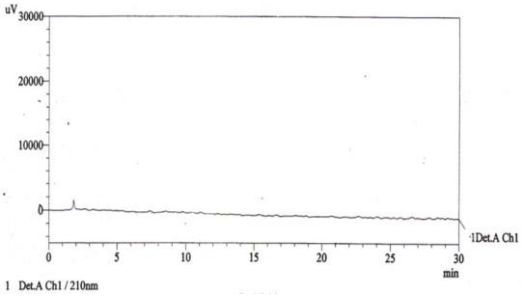
Figure 7: Oxidation degradation



Thermal degradation

It was discovered that Isobutylglutarmonoamide was quite stable under thermal degradation conditions, i.e., 0.4% for Isobutylglutarmonoamide.

Figure 8: Thermal degradation



Photolytic degradation

In photolytic stress conditions, Isobutylglutarmonoamide was found to be quite stable, and it deteriorated up to 1.8% when stress conditions were applied.

Table 9: Ssummary of Validation

Summary of Validation		
Parameters	**Results**	
Range 50% to 150%	12.5ppm to 75ppm	
Correlation coefficient	0.99983	
Accuracy (%)	Recovery (%)	RSD (%)
50	99.9	0.31
100	99.6	0.02
150	99.9	0.30
Precision	Concentration	RSD (%)
Repeatability n=6	50ppm	0.22
Precision (Intraday) n=6	50ppm	0.28
Precision (Interday) n=6	50ppm	0.26
Cumulative Results n=12	50ppm	0.27
Method Robustness: With intentional adjustments to the technique parameters, no significant difference was seen.		

Table 10: Result of Stress condition and degradation studies

Stress Condition	**% Degradation**
Initial	No Degradation
5ml, 0.1 M Sodium Hydroxide Solution 0.0 hours	5.8
5ml, 1 M Hydrochloride Acid, Heated at 70°C for 4.0 hours	8.4
5ml, 10% Hydrogen peroxide, heat 70°C for 4.0 hours	2.6
Solid Test heat at 105°C for 24.0 hours	0.4
Test solution in UV light for 24 hours	1.8

Conclusions

According to ICH criteria, the current studies are highly helpful for predicting the stability behaviour of Isobutylglutarmonoamide. Under stressful circumstances, Isobutylglutarmonoamide was found to be more stable. Using this analytical method, it was discovered as precise, accurate, with consistent and good recoveries at studied at all range. This shows there aren't any degradants or other contaminants interfering impurities for this methodology's assessment of the drug content. RSD also observed less than 2% demonstrating the proposed method's high level of precision. This approach to Analysis is precise, quick, accurate, and economical. The suggested approach can be used for Isobutylglutarmonoamide routine analysis and can also be very beneficial for quality control and assurance in manufacturing in bulk

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