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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE DETERMINATION OF ALVIMOPAN IN BULK AND PHARMACEUTICAL FORMULATION

Ramesh Devi

Government Polytechnic for Women, Warangal - 506002, Telangana, India.

Keywords:

Alvimopan, Method development, Recovery, Stability, Validation

Correspondence to Author:
Ramesh Devi

Lecturer,
Government Polytechnic for Women, Warangal - 506002, Telangana, India.

E-mail: rameshdevi07@rediffmail.com

ABSTRACT: Objective: The present investigation demonstrates a simple, sensitive and accurate high-pressure liquid chromatographic (HPLC) method for the determination of Alvimopan (**AMP**) in bulk and dosage form. The chromatographic separation was achieved within 10 minutes by using acetonitrile: potassium dihydrogen phosphate buffer pH 3.0 adjusted with orthophosphoric acid (50:50) as mobile phase on Altima Grace Smart C-18 column (5µ; 250 × 4.6 mm) at flow rate of 1.0 ml/min with injection volume 20µL at wavelength of 261nm. The retention time of the drug was found to be 5.02 min. This method was validated as per United States Food & Drug Administration (US-FDA) guidelines. The results of the validation parameters were found to be within the acceptance limits. The method was linear in the concentration range from 500-20000 ng/mL (r^2 = 0.9998) and the accuracy of drug was found to be 95.83-104.63% for **AMP**. The lower limit of quantification was found to be ng/ml and the stability of recovered samples at different conditions was found to be more than 95%. The developed method possesses good selectivity, specificity, there was no interference found in the blank sample at retention times of **AMP**. We found a good correlation between the peak area and concentration of the drug under prescribed conditions. Furthermore, the method can also be used to estimation of drug in the dosage form for future studies of AMP.

INTRODUCTION: Narcotic medicines that are often used to reduce the pain caused by gastrointestinal surgery. However, these medicines can cause a variety of side effects, such as nausea, vomiting, constipation and stomach pain, which are further leads to delay recovery in patients undergoing gastrointestinal surgery. Therefore, medicines those are required to prevent the above-mentioned side effects without diminishing the pain-relieving effect of narcotic medicines.

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Alvimopan (AMP) (Entereg), the only drug approved by the Food & Drug Administration (FDA) for the treatment of postoperative ileus [1, 2]. This drug behaves as a peripherally acting µ-opioid antagonist. Since, the AMP has limited ability to cross the blood–brain barrier, many of the undesirable side-effects of the narcotic medicines are minimized without affecting analgesia or precipitating withdrawal [3, 4].

In order to comprehend the antagonist effect of AMP further, it is imperative to determine the pharmacokinetic parameters through estimation of the AMP in plasma using simple estimation methods. Several liquid chromatography-tandem mass spectrometric (LC–MS/MS) assay methods have been employed for the determination of various drugs in the form of single and combined

dosage forms [5-9]. In addition, estimation of AMP in plasma and pharmaceutical formulation has also been carried out using LC-MS/MS method. In general, this method is highly sensitive to separate and identify a multitude of compounds in low concentration in a complex mixture with little assay optimization [10, 13].

The present contribution provides simple and regular estimation method for determination of AMP in using high pressure liquid chromatography (HPLC). **Fig. 1** gives the molecular structure of AMP. As the above-mentioned, AMP is the only narcotic antagonist approved by the FDA for the treatment of postoperative surgery of gastro intestine.

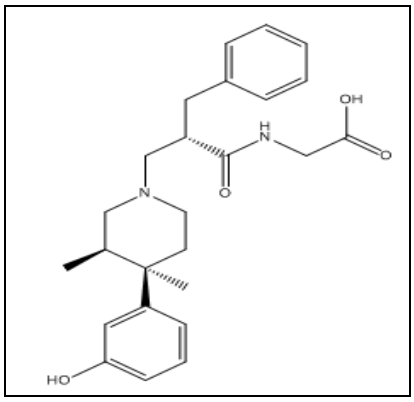

FIG. 1: CHEMICAL STRUCTURE OF 2-([(2S)-2-([(3R, 4R) - 4 - (3 - HYDROXYPHENYL) - 3, 4-DIMETHYL – PIPERIDIN - 1 - YL] METHYL) - 3 - PHENYL-PROPANOYL] AMINO) ACETIC ACID

MATERIALS AND METHODS:

Instrumentation: The instruments employed in this study are; HPLC- Shimadzu HPLC SPD 10A VP UV-Visible Detector with manual injector. Sonicator- Sharp Analytical, Hyderabad, India. Analytical balance- Sartorius, German. Millipore Direct-Q 3 U.V. USA. pH meter- Systronics, Ahmadabad, India.

Standards and Chemicals: Alvimopan (AMP) was gift sample obtained from Aurobindo Pharma (Hyderabad, India). Purified water was obtained from a Millipore Direct-Q 3 U.V. Acetonitrile of HPLC grade, o-phosphoric acid and potassium dihydrogen phosphate were of A.R. Grade were purchased from Merck, Pvt. Ltd (Mumbai, India).

Stock and Working Solution Preparation:

Preparation of Standard Stock Solution: Accurately weighed and transferred 10mg of AMP into a 10mL volumetric flask dissolved with small amount of methanol and made up of the volume with methanol. Daily working standard solutions of mixture were prepared by suitable dilution of the stock solution with the mobile phase.

Preparation of a Buffer: Accurately weighed 2.72g of potassium dihydrogen orthophosphate dissolved in 1000ml of HPLC grade water and pH was adjusted to 3.0 by using orthophosphoric acid.

Chromatographic Conditions: The chromatographic separation was done by using Grace Smart Altima C18 column (250X4.6 mm, 5µ) with mobile phase acetonitrile: phosphate buffer pH 3.0 (50:50 % v/v) at a flow rate of 1mL/min and detection wavelength was 261nm with 20µL of injection volume.

Method Development:

Method Optimization: The method development, top priority was given for the sharp and separation of drug from solvent peaks. The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic solvent and buffer used in the mobile phase and composition of the mobile phase on trial error basis by varying one parameter and keeping all other conditions constant.

Method Validation: The validation parameters like linearity, sensitivity, accuracy, precision, recovery and stability of drugs were studied according to the ICH guidelines [14].

Selectivity: Selectivity was studied by comparing the chromatograms obtained from the blank sample with the chromatogram obtained from a standard drug mixture.

Linearity: The linearity of this method was evaluated by linear regression analysis, using least square method and found linear in the concentration range of 0.5-20µg/mL for AMP. Calibration standards are prepared by spiking required volume of working standard (100µg/mL) solution into different 10 mL volumetric flasks and volume made up with mobile phase to yield concentrations of 0.5, 1, 2, 5, 10, 15, 20 µg/mL of AMP. The resultant peak area of drug was measured. Calibration curve is plotted between

peak areas of drug against concentration of the drug.

Sensitivity: The LOD and LOQ of this method were verified based on the standard deviation of response, slope.

Intra-day and Inter-day Precision and Accuracy, Recovery: Intra- and inter-day accuracy and precision of this method was determined at three different concentration levels in 3 different days. On each day, three replicates were analyzed with independently prepared calibration curves. The accuracy and precision were expressed as percentage accuracy and relative standard deviation (R.S.D., %) respectively.

Robustness: Robustness of the method was done by changing slight variation in the parameters like mobile phase composition, flow rate and wavelength. Present method didn't show any significant change when the critical parameters were modified (i.e. Mobile phase composition, flow rate and pH of buffer).

Solution Stability: The stability of the drug solution was determined for the short-term stability, auto-sampler stability. Short-term stability was carried out by keeping at room temperature (25°C) for 24 h to determine any degradation during the analysis. Each sample injected three times into HPLC, and concentrations obtained were compared with the nominal values of the quality control (QC) samples.

Analysis of Marketed Formulation: 20 Capsules (Entereg) were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 12mg of AMP [equivalent to one capsule] was extracted with different extraction solvents like acetonitrile, methanol, water and mobile phase.

The powder equivalent one tablet was transferred and extracted with 50:50 methanol and acetonitrile in a 100ml volumetric flask and sonicated for 15 min. This solution was filtered through Whatmann No.1 filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined, filtered through 0.22µ syringe filter and injected into HPLC. The amount of drug recovered was calculated from the respective linear graph.

RESULTS AND DISCUSSION: During the method development, top priority was given for the complete separation of drugs. The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic solvent and buffer used in the mobile phase and composition of the mobile phase on trial error basis. Phosphate buffer in various strengths are tried along with methanol and acetonitrile as organic solvent.

A mixture of acetonitrile and phosphate buffer with different pH was tried. At pH 3.0 the separation was good enough, and then the proportions of acetonitrile and phosphate buffer pH 3.0 were tested as a mobile phase with Grace Smart C-18 column. The mobile phase composition of 50:50 v/v phosphate buffer: acetonitrile was shown good resolution, retention time with minimal tailing factor in acceptable range.

The method was optimized with the mobile phase composition of acetonitrile and phosphate buffer 50:50 (v/v). Buffer molarity of 10, 20 and 50 mM was tested. There were no significant changes in the chromatographic response and peak shape with change in buffer molarity. A buffer molarity of 20 mM was selected for further analysis.

After several trials, the method was optimized as a mixture of 20mM potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile (50:50 v/v), at a flow rate of 1mL/min, at 261nm by using Grace Smart, Altima C-18 column.

These chromatographic conditions achieved satisfactory resolution, retention time and tailing for AMP. The **Fig. 2** shows that chromatogram of standard drug.

The standard mixture solution was used as a system suitability solution, and it was injected into HPLC five times. The retention time, tailing factor, resolution and theoretical plates of drug were observed. Then, calculated the percentage relative standard deviation (% RSD) of five consecutive injections for each parameter. The system's suitability parameters of the present method were found to be within acceptable limits.

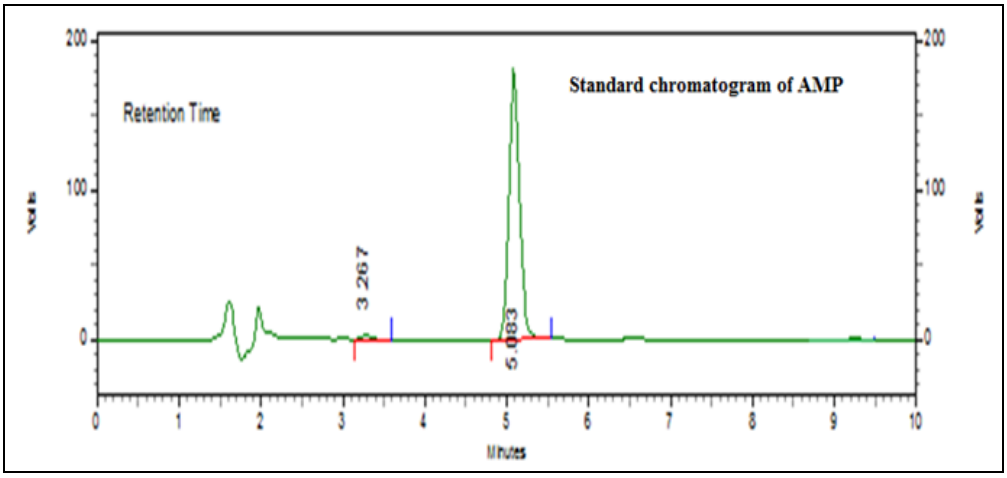

FIG. 2: STANDARD CHROMATOGRAM OF AMP

TABLE 1: SYSTEM SUITABILITY PARAMETERS OF AMP

Parameters (n=5)	Results	Required limits
Retention time in minutes (R_t)	5.02±0.01, 0.30	RSD≤2
Theoretical plates (N)	6433±99, 1.54	N>2000
Tailing Factor (T)	1.17±0.01, 1.48	T≤2
Peak Area	1125898±13464, 1.19	-

The data of system suitability parameters were represented in **Table 1**. The acceptable limits of the resolution between two adjacent peaks should be ≥2 and tailing factor should be ≤2 [15] and the %RSD of these values should be ≤2. System suitability tests confirmed that the chromatographic system was adequate for the analysis planned to be done.

The method was found to be selective for AMP as there is no interference that was found at the retention time of drug (AMP) when comparing the chromatograms of blank (mobile phase) and drug solution (AMP). The linearity was performed, and calibration curve is plotted between peak areas of drug against concentration of the drug. The curve was linear over the range of 0.5-20µg/mL for AMP and the graph shown in **Fig. 3.**

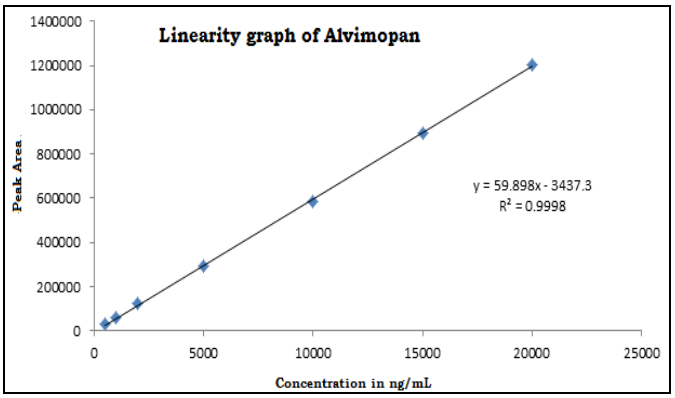

FIG. 3: LINEARITY GRAPH OF AMP

The regression equations of drug was y=59.898x – 3437.3(r^2=0.9998) for AMP. The results of intra and inter day precision was shown in **Table 2**. The % RSD was found to be less than 2 for all the drugs which indicating that the method is precise.

TABLE 2: INTRA AND INTER-DAY ACCURACY AND PRECISION OF AMP

Concentration (ng/mL)	Intra-day (n=6)		Inter-day (n=9)	
	mean±SD	RSD (%)	mean±SD	RSD (%)
1500	104.63±0.55	0.53	104.58±1.20	1.15
8000	98.15±1.34	1.36	95.83±1.77	1.85
15000	100.19±0.40	0.40	99.35±0.18	0.19

Values expressed Mean±SD

Present method didn't show any significant change when the critical parameters were modified. The tailing factor for the drug was always less than 2.0 and the components were well separated under all the changes carried out (i.e. Mobile phase composition, flow rate and pH of buffer). Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may indicate the method found to be robust. The robustness data of the method was presented in **Table 3**.

The stability of the drug was studied for short-term stability using the QC samples. The samples were analyzed and compared with freshly analyzed QC samples, no differences were found in accuracy and precision. The stability studies indicate there were no major changes observed in this study.

The method was applied for the determination of AMP in formulation (Entereg) and the amount of drug (AMP) was found to be 12.07mg, the accuracy was found to be 100.05%. The chromatogram of formulation was shown in **Fig. 4** and the data was presented in **Table 4**.

TABLE 3: ROBUSTNESS DATA OF AMP

Parameter	Variation	System suitability		
		Retention time	Theoretical plates	Peak Asymmetry
Flow Rate (mL/min)	0.9	5.04	6560	1.19
	1.0	5.02	6433	1.17
	1.1	5.00	6469	1.20
	Mean±SD	5.02±0.02, 0.39	6487±67, 1.00	1.20±0.04, 1.28
% ACN	52 % v/v	4.99	6498	1.14
	50 % v/v	5.02	6433	1.17
	48 % v/v	5.03	6539	1.15
	Mean±SD	5.01±0.02, 0.41	6490±53, 0.82	1.15±0.01, 1.32
pH	2.92	5.02	6589	1.17
	3.00	5.02	6433	1.17
	3.11	5.04	6487	1.18
	Mean±SD	5.02±0.01, 0.22	6503±79, 1.21	1.17±0.00, 0.49

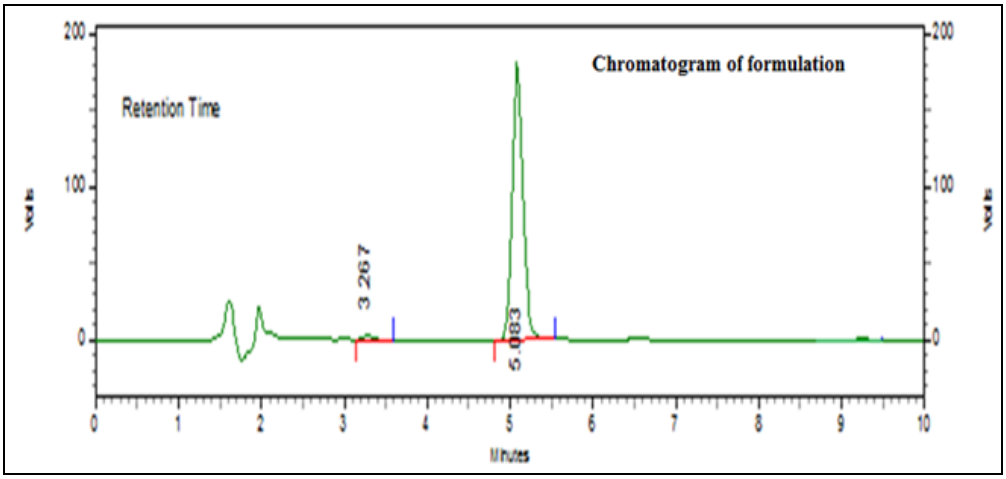

FIG. 4: CHROMATOGRAM OF AMP FROM FORMULATION

TABLE 4: RECOVERY STUDY FROM FORMULATION (N=5) OF AMP

Brand Name	Labeled amount (mg)	Peak Area	Amount of drug (mg)	Percentage of drug recovery
Entereg™	12	69808	12.22	101.89
		68975	12.08	100.73
		68124	11.95	99.55
		68974	12.08	100.73
		68457	12.00	100.01
	Mean±SD		12.07±0.10	100.58±0.88

Values are expressed in Mean ±SD, n=5

CONCLUSION: The developed method possesses good selectivity, specificity, there is no interference found in the blanks at retention time of AMP and good correlation between the peak area and concentration of the drug under prescribed conditions. The recovery studies are found to be 100.58% for the drug. The observation of % RSD less than 2 for both intra- and inter-day measurements indicates a high degree of precision. In the present method, a Grace Smart, Altima C-18 column has been used and at a flow rate of 1mL/min and pH of the buffer were 3.0 which are within the limits specified by the manufacturers. The method was optimized with low injection

volume. The stability of AMP was found to be within the limits indicating that there is no degradation of drug during the daily analysis. This method was applied for the determination of AMP in tablet dosage form. From the results we are concluding that the developed method was accurate, precise, and robust. Hence the method can be applied for further investigation of AMP.

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CONFLICT OF INTERESTS: The authors report no conflict of interest.

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