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RESEARCH ARTICLE

HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ALVIMOPAN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of alvimopan in bulk and capsule dosage form. A column of Altima 150mm x 4.6 mm, 5µ was used. The mobile phase comprises of potassium dihydrogen orthophosphate buffer and acetonitrile in the ratio of 60:40 (v/v). The flow rate was 1.0 ml/min and the effluents were monitored at 260 nm. The retention time was found to be 4.001. The detection concentration was linear over 30-180ppm.Regression equation of alvimopan was found to be y = 6065.6x + 795.43with regression co-efficient 0.999. The developed method was successfully validated in accordance to ICH guidelines. Hence, this method can be conveniently adopted for the routine analysis in quality control laboratories.

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INTRODUCTION

Alvimopan (trade name Entereg) is an oral, gastro-prokinetic, peripherally acting, mu-opioid receptor (PAMOR) antagonist drug. It is indicated in patients to avoid postoperative ileus following partial large or small bowel resection with primary anastomosis. Alvimopan accelerates the gastro-intestinal recovery period as defined by time to first bowel movement or flatus (Neary, 2005; Schmidt, 2001; FDA, 2008; Alvimopan, 2008; Goldstein, 2007; Delaney, 2008; Wolff, 2006 and Foss, 2008). Chemically Alvimopan[[2(S)-[[4(R)-(3-hydroxyphenyl) -3(R),4-dimethyl-l-piperidinyl] methyl]-l-oxo-3-phenylpropyl] amino]acetic acid di-hydrate with chemical formula $C_{25}H_{32}N_2O_4$ and molecular weight 424.53 g/mol (https://en. wikipedia.org/wiki/Alvimopan). Rambabu Kuchi *et al.* (2012) had reported a simple, rapid and precise reverse phase high performance liquid chromatography method for the analysis of alvimopan. Chromatographic separation of alvimopan was performed by using a waters C18 column (250 x 4.6mm, 5 µm) as stationary phase with a mobile phase comprising of Methanol: Acetonitrile in the ratio 80:20 (v/v) at
a flow rate of 1.0ml/min and UV detection at 261 nm and 10µl sample was injected. The linearity of alvimopan is in the range of 40 ppm to 100 ppm. The proposed method was found to be accurate, precise and rapid for the analysis of alvimopan. The retention time for alvimopan was 5.3 min.

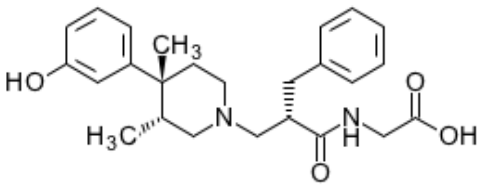

Figure 1. Structure of alvimopan

The percentage RSD for precision and accuracy of the method was found to be less than 2%. The present method was successfully validated in accordance to ICH guidelines (International Conference on Harmonization, 2005). The results of the study showed that the proposed RP-HPLC method is useful for the routine determination of the drug in bulk drug and in its pharmaceutical dosage form.

MATERIALS AND METHODS

Alvimopan was obtained as a gift sample from Hetero Drugs Ltd. Hyderabad. Acetonitrile, methanol, potassium dihydrogen phosphate and ortho-phosphoric acid used were of analytical

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grade. Commercially available alvimopan capsules (Entereg ®-12mg) was procured from local market.

Instruments

Quantitative HPLC was performed on Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing powered with Empower-2 Software. An Altima column of 150mm 4.6mm: i.d and 5µ particle size was used. PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was used for UV measurements.

Standard Solution Preparation

Accurately weighed 12 mg of alvimopan working Standard was transferred into a 10 ml clean dry volumetric flask, 7ml of diluent was added and sonicated for 30 minutes and made up to the final volume with diluents to obtain Stock solution of 200µg/ml.

Working standard preparation

From the above stock solution, 10 ml was pipetted out in to a 100ml volumetric flask and then made up to the final volume with diluents to obtain 120µg/ml of alvimopan.

Sample Preparation

Accurately weighed capsule powder equivalent to 12mg of alvimopan and transfer into a 10ml volumetric flask. The volume was made up to the mark with diluents and sonicated for 30mins and filtered (Stock- 1200 µg/ml of alvimopan). From the above sample stock solution, 1ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluents (120µg/ml of alvimopan).

Preparation of Buffer

1.36gm of Potassium dihydrogen ortho phosphate was transfer into a 1000ml volumetric flask, added about 900ml of milli-Q water, degased to sonicate and finally madeupto the volume with water. The pH was adjusted to 3.3 with orthophosporic acid.

Mobile phase

Buffer and Acetonitrile are taken in the ratio 60:40.

Experimentation

System Suitability

A Standard solution of alvimopan working standard was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections are within range and results were shown in Table 1.

All the system suitability parameters are within range and satisfactory as per ICH guidelines (International Conference on Harmonization, 2005), (Figure 2)

Table 1. System suitability data ofAlvimopan

S.No	Name of the drug & conc (120µg/ml)	RT (min)	Peak Area	USP Plate Count	USP Tailing
1	Alvimopan (std)	4.001	730064	7325	1.69
2	Alvimopan (std)	4.002	723381	7386	1.68
3	Alvimopan (std)	4.003	721505	7291	1.69
4	Alvimopan (std)	4.004	720970	7243	1.68
5	Alvimopan (std)	4.008	726077	7254	1.69
6	Alvimopan (std)	4.013	719446	7602	1.67
Mean			723574		
Std.Dev.			3912.1		
%RSD			0.5		

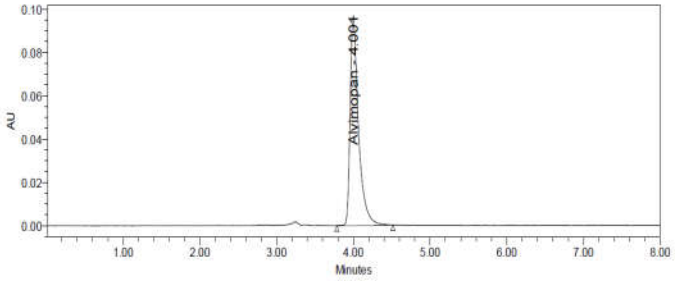

Figure 2. System suitability chromatogram

Linearity

To demonstrate the linearity by assay method, five standard solution samples were injected with concentrations ranging from 30 to 180 µg/ml of. A graph of concentration versus peak area was plotted. Slope obtained was 6065, Y-intercept was 795.4 and correlation co-efficient was found to be 0.999. Regression equation of alvimopan was found to be y = 6065.6X+795.4 (Figure 3).

Table 2. Calibration data ofAlvimopan

Linearity Level (%)	Concentration (ppm)	Area
25	30	182769
50	60	367179
75	90	548561
100	120	728204
125	150	900947
150	180	1099245

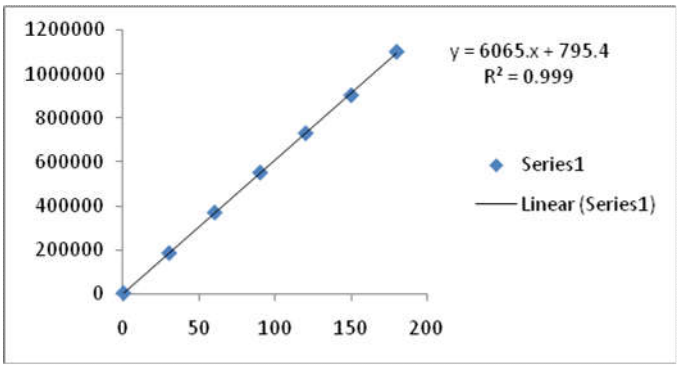

Figure 3. Calibration Curve of Alvimopan

Assay studies

Standard preparations were prepared from the standards and sample solutions of formulation (International Conference on Harmonization, 2007). Six homogeneous samples of both

sample and standard were injected. Percentage assay of the drug in the formulation was estimated. From the data it was found that the sample and standard retention times are similar i.e. 4.001. From linearity Table, it was found that the drug obeys linearity within the concentration range of 30-180ppm for alvimopan. By using this method, assay of marketed formulation was carried out and it was found to be 99.76%. (Table 3)

Table 3. Assay data Alvimopan

Sample No	Name of the drug	Retention time	%Assay
1	Alvimopan	4.001	99.89
2	Alvimopan	4.003	99.40
3.	Alvimopan	4.008	99.44
4.	Alvimopan	4.013	100.04
5.	Alvimopan	4.004	100.16
6.	Alvimopan	4.002	99.61
AVG		4.005	99.76
STD DEV			0.3166
%RSD			0.32

mean ($\bar{x}$) - $\frac{\text{Sum of six observations (X)}}{6\ (N)}$

Standard deviation (S) - $\sqrt{(\Sigma(x-\bar{x})^2 /N-1)}$ where x is absorbance

% RSD - S*100/ $\bar{x}$

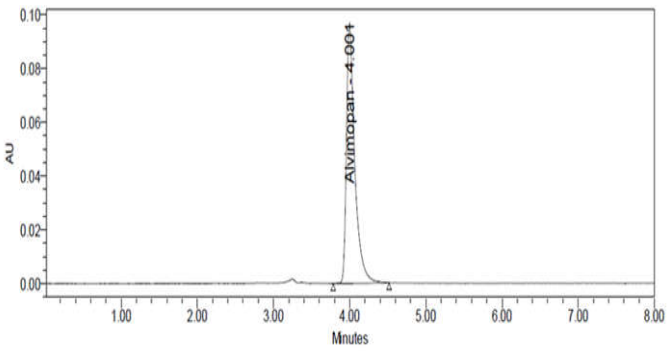

Figure 4. Assay chromatogram of Alvimopan

Precision

Six working sample solutions are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.5 (Figure 3)

Table 4. Precision data of alvimopan

S.No	Name of the drug	Retention time	Peak Area
1	Alvimopan	4.001	682973
2	Alvimopan	4.008	677407
3	Alvimopan	4.013	674154
4	Alvimopan	4.004	676799
5	Alvimopan	4.003	681115
6	Alvimopan	4.001	675250
AVG		4.005	677950
STD. DEV			3422.5
%RSD			0.5

mean ($\bar{x}$) - $\frac{\text{Sum of six observations (X)}}{6\ (N)}$

Standard deviation (S) - $\sqrt{(\Sigma(x-\bar{x})^2 /N-1)}$ where x is absorbance

% RSD - S*100/ $\bar{x}$

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD* and LOQ** of the drug were calculated using the following equations designated by International Conference on Harmonization (ICH) guideline (International Conference on Harmonization (ICH) 2005).

* LOD = 3.3 × /S
**LOQ = 10 × κS

Where p= the standard deviation of the response *
S = Slope of calibration curve

LOD and LOQ values for alvimopan were found to be 0.43ppm and 1.31ppm respectively. (Figure 5 and Figure 6)

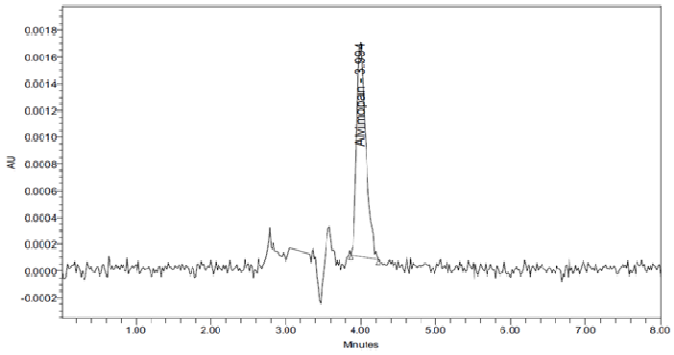

Figure 5. LOD Chromatogram ofAlvimopan

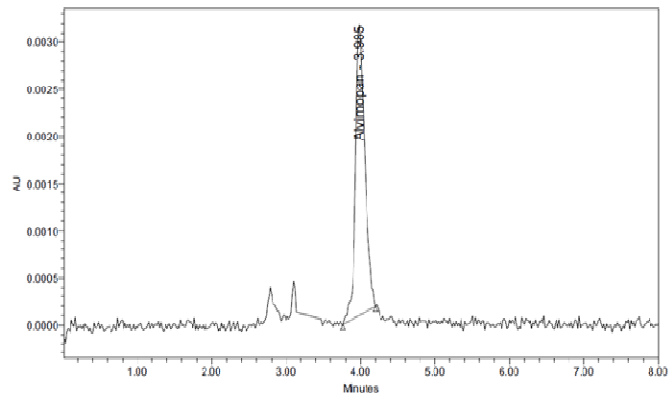

Figure 6. LOQ Chromatogram ofAlvimopan

Robustness

The samples were anylised by making minor changes in the optimised parameters like flow rate,mobile phase and temperatue. %RSD in each of the above condition calculated and tabulated (Table 5).

Table 5. Robustness data

Parameter	%RSD
Flow Minus	0.6
Flow Plus	0.5
Mobile phase Minus	0.4
Mobile phase Plus	0.6
Temperature minus	0.4
Temperature plus	0.5

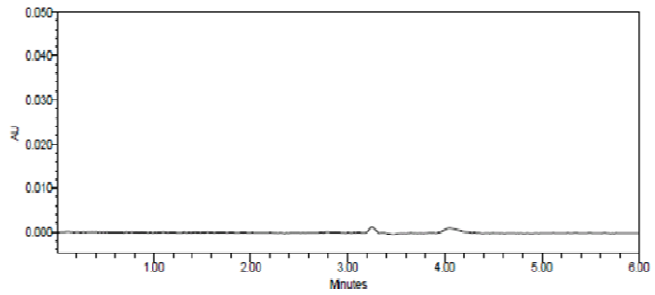

Figure 7. Blank Chromatogram of Alvimopan

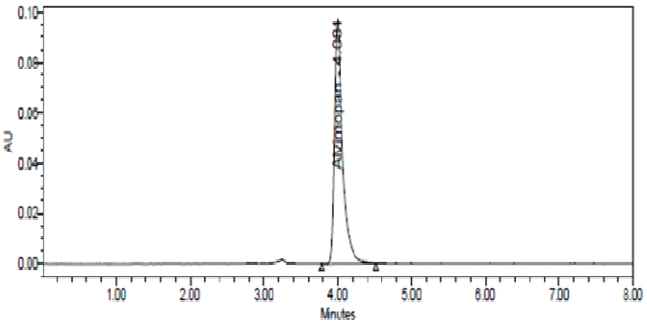

Figure 8. Chromatogram of standard solution of Amlodipine

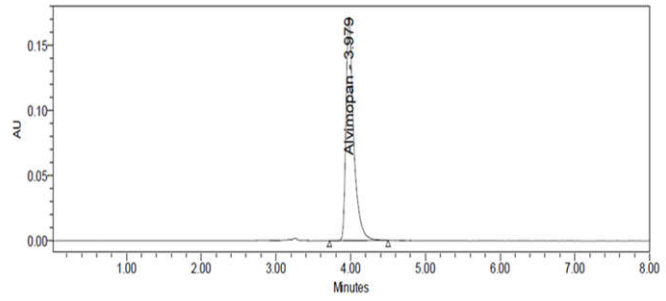

Figure 9. Typical chromatogram of sample solution of Amlodipine

RESULTS AND DISCUSSION

From the typical chromatogram of drug as shown in Figure 8, the retention time were found to be alvimopan was found to be 4.001. The mobile phase comprises of potassium dihydrogen orthophosphate buffer and acetonitrile in the ratio of 60:40 (v/v). In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved.

A good linear relationship (r=0.9998) was observed between the concentration range of 30-180µg/ml.Low values of standard deviation are indicative of the high precision of the method. The assay of alvimopan was 99.76% (Table 3). The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the formulation. The limit of detection (LOD) and limit of quantification (LOQ) for alvimopan were 0.43ppm and 1.31ppm. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and Tablets dosage form of the drugs within a short analysis time.

REFERENCES

Addanki Padmini. Sudharani Utala, Gumparthi Prasad, Adigarla Bennayyanaidu, Koyyuri Raju and Rambabu Kuchi, 2012. A New L.C method development and validation for analysis of Atvimopin in Formulations. *Research Desk*, 1(2).60-65

Alvimopan Product Label as approved by the FDA on May 20, 2008.

Delaney, C.P. 2004. Clinical perspective on postoperative ileus and the effect of opiates. *Neurogastroenterol Motil*., 6(Suppl 2):61–66.

FDA press release - FDA Approves Entereg to Help Restore Bowel Function Following Surgery.

Foss, J.F., Fisher, D.M. and Schmith, V.D. 2008. Pharmacokinetics of alvimopan and its metabolite in healthy volunteers and patients in postoperative ileus trials. *Clinical Pharmacology and Therapeutics*, 83(5):770-6.

Goldstein, J.L., Matuszewski, K.A. and Delaney, C.P. 2007. Inpatient economic burden of postoperative ileus associated with abdominal surgery in the United States. P&T. 32(2):82–90.

https://en.wikipedia.org/wiki/Alvimopan

International Conference on Harmonization (ICH), Q2R1, Validation of Analytical Methods: Methodology, Yokohama, Japan, (2005)

Neary, P., Delaney, P. 2005. "Alvimopan". Expert OpinInvestig Drugs14 (4): 479–88.

Schmidt, W.K. 2001. "Alvimopan* (ADL 8-2698) is a novel peripheral opioid antagonist". *Am J Surg*., 182 (5A Supp): 27–38.

Wolff BG, Leslie JB, Saclarides TJ. *et al.* 2006. Integrating new agents for postoperative ileus into multimodal protocols. *Contemp Surg*., (Suppl):1–24.

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