A New L.C method development and validation for analysis of Atvimopin in Formulations.

Addanki Padmini. Sudharani Utala, Gumparthi Prasad, Adigarla Bennayyanaidu, Koyyuri Raju, Rambabu Kuchi*

Dept of P.G Chemistry, D. N. R College, Bhimavaram, West Godavari (D.T) Andra Pradesh, India

ABSTRACT

A simple, rapid and precise reverse phase high performance liquid chromatography method was developed for the analysis of Atvimopin Chromatographic separation of Atvimopin was performed by using a waters C_{18} column (250 x 4.6mm, 5 µm) as stationary phase with a mobile phase comprising of Methanol:Acetonitrile80:20 (v/v/v) at a flow rate of 1.0ml/min and UV detection at 261 nm and 20µl sample was injected. The linearity of Atvimopin 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 Atvimopin. The retention time for Atvimopinwas 5.3 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Atvimopintablet dosage form and bulk drug.

Keywords: Atvimopin, HPLC method, Method development, Validation, L.O.D, L.O.Q, Entereg

INTRODUCTION

Alvimopan is a drug which behaves as a peripherally acting µ-opioid antagonist. With limited ability to cross the blood–brain barrier, many of the undesirable side-effects of the opioid agonists such as constipation are minimized without affecting analgesia or precipitating withdrawals. The Food and Drug Administration reviewed the safety and efficacy data for Alvimopan and approved its use in May 2008. Alvimopan competitively binds to mu-opioid receptor in the gastrointestinal tract. Unlike methylnaltrexone (another peripherally acting mu-receptor antagonist) that bears a quaternary amine, alvimopan owes its selectivity for peripheral receptors to its kinetics. Alvimopan binds to peripheral mu-receptors with a Ki of 0.2 ng/mL and dissociates slower than most other ligands.

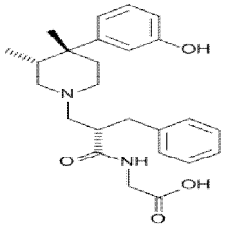


Figure.1 Structure of Atvimopin

1. H.P.L.C Method development and validation

Materials and Methods:

Methanol, Acetonitrile, water Acetic acid, Methanol used was analytical grade. Chromatographic separation was performed with PEAK high performance liquid chromatography having LC-P7000 isocratic pump, equipped with PEAK LC-UV7000 variable wavelength detector. Chromatograms and data were recorded by means of PEAK Chromatographic Software version 1.06.

Preparation of standard solution:

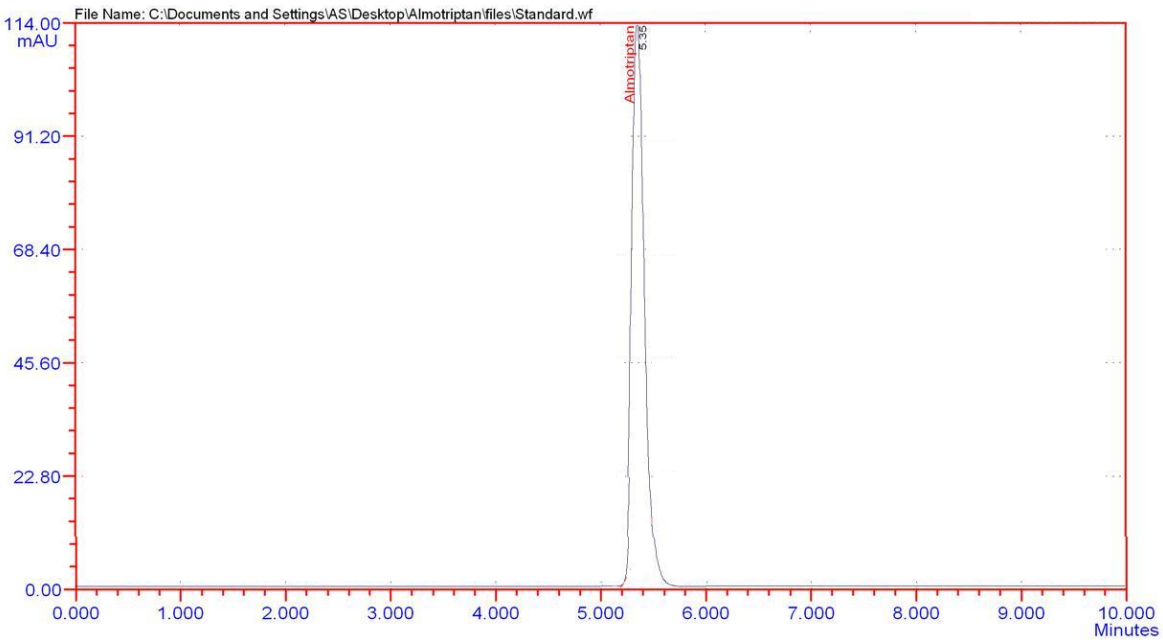
10mg of Atvimopin was taken in a 100ml volumetric flask and 100ml of mobile phase was added to obtain 100 ppm of Atvimopin standard solution.

System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

S.No	Test	Result
1	Elution	Isocratic
2	API conc	60 ppm
3	Mobile phase	Methanol: Acetonitrile 80:20
4	P^H	5.8
5	Column	C18
6	Wave length	261nm
7	Flow	1ml/min
8	Runtime	6 min
9	Retention time	2.80 min
10	Area	291140
11	Theoretical plates	2374
12	Tailing factor	0.95
13	Pump pressure	12.6 psi

Table 1: Chromatographic Conditions



HPLC Figure.2 Atvimoprin chromatogram

Linearity:

In order to check the linearity for the developed method, solutions of five different concentrations ranging from 40 ppm-100 ppm were prepared. The chromatograms were recorded and the peak areas were given in table 2.linear relationship between areas versus concentrations was observed in about linearity range. This range was selected as linear range for analytical method development of Almotriptan. Linearity graph was shown in figure: 3

S.No	**Concentrations**	**Peak area**
1	40	195421
2	50	245121
3	60	291140
4	70	345210
5	80	405682
6	90	449652
7	100	510232
Linearity range 40-100	Slope Intercept cc	5086.685 -6252.19 0.999393

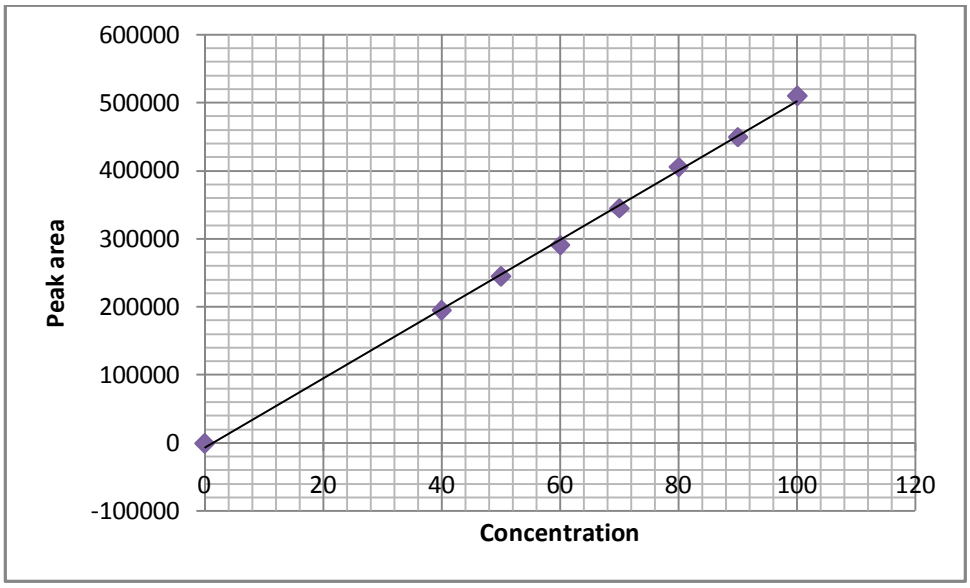


Fig3: Calibration curve for Atvimopin

Precision (Repeatability):

60ppm standard solution was prepared to calculate the precision for the developed method. The prepared solution was injected into injector at same concentrations and same chromatographic conditions. The chromatograms were recorded. %R.S.D for the values calculated for intraday and interday precision was1.56 and 1.0250, the developed method shows precision.

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

The LOQ and LOD were established at a signal to noise ratio. The LOD of Atvimopin is 0.02 ppm. The LOQ of Atvimopin is 0.05 ppm.

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in flow rate of the mobile phase, composition of the mobile phase and wavelength of the detector. Atvimopin at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in table 4.

S.NO	Parameter	Change	Area	% of Change
1	Standard	.....	291140	.....
2	Mobile Phase	Methanol:ACN: 90:10 70:20	291260 291850	0.04 0.15
3	P^H	5.9 5.7	291620 291480	0.16 0.11
4	Wave length	263 nm 259nm	291543 291856	0.13 0.24

Table.4: Robustness results of Atvimopin

Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation. The %RSD was found to be 0.76

Recovery studies:

Recovery

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e. 60ppm, 80ppm, 100ppm. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 99.3to 101.2 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in table.5

Table:5 Recovery results

	Target Conc (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Recovery
50%	40	20	60	60.2	100.3
	40	20	60	59.6	99.3
	40	20	60	59.9	99.8
100%	40	40	80	81.2	101.5
	40	40	80	80.8	101
	40	40	80	79.4	99.2

150%	40	60	100	101.2	101.2
	40	**60**	**100**	**99.8**	**99.8**
	40	60	100	100.6	100.6

Table. 6: Formulation Analysis

Formulation	Dosage	Concentration	Amount found	% Assay
Entereg	12 mg	80 ppm	79.64	99.55

CONCLUSION

The proposed method for the assay of Atvimopin n capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

REFEENCES

1. Neary P, Delaney P (2005). "Alvimopan". *Expert Opin Investig Drugs* 14 (4): 479–88.
2. Schmidt WK (2001). "Alvimopan* (ADL 8-2698) is a novel peripheral opioid antagonist". *Am J Surg* 182 (5A Supp): 27S–38S.
3. FDA press release - FDA Approves Entereg to Help Restore Bowel Function Following Surgery
4. Alvimopan Product Label as approved by the FDA on May 20, 2008.
5. Napa Delhi raj et al, Validated hptlc method for the estimation of antihypertensive drugs in pharmaceutical combined dosage forms IJRRPAS, 2012, 2(5).959-964,
6. Nallagatla. Vijaya Bhaskar Rao et al , A novel RP HPLC method for the quantification of Sitagliptin in formulations. The Experiment, December. 2012 , Vol. .5(5). 315-323