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Development and validation of RP-HPLC method for quantitation of Aviptadil Acetate and its degradation products

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Abstract

Pulmonary arterial hypertension (PAH) is a disease of the pulmonary arteries and arterioles that increases the pulmonary vascular resistance and pulmonary artery pressure. Aviptadil, a vasoactive intestinal peptide (VIP), has been suggested as a novel drug for the treatment of idiopathic PAH. In this paper, we developed an HPLC method for quantitating aviptadil and monitoring acid and base catalyzed degradation of the drug. The method was validated according of ICH and US FDA guidelines of HPLC methods. The drug was separated in 4.5±0.1 minutes; the calibration curve was linear over a concentration range between 2 and 10µg/ml (r2 = 0.9996); the recovery was 99.65%; the relative standard deviation (RSD) for intra- and inter-day was < 1.0. Thus the method was simple, accurate, precise, reliable and reproducible. Degradation studies revealed that aviptadil is stable in neutral medium and unstable in both acidic as well as basic media with faster degradation in later.

Key words : Aviptadil Acetate, HPLC, development and validation method, Stability.

INTRODUCTION

In pulmonary arterial hypertension (PAH), elevated pulmonary vascular resistance (PVR) and pulmonary arterial pressure (PAP) results from the progressive narrowing of the pulmonary arterial lumens [1]. Although an array of anti-PAH drugs are currently available; PAH therapy still suffers from a number of limitations. Thus a series of small molecular weight drugs and peptides have been evaluated for their anti-PAH effects [2-3]. Aviptadil, the nonproprietary or generic name for VIP [4,5], is a synthetic 28-amino-acid VIP (Bachem, Bubendorf, Switzerland). Inhalation of aviptadil has been suggested as a novel approach for the treatment of idiopathic PAH [4]. Idiopathic PAH patients are deficient in VIP that triggers a compensatory up regulation of receptor expression in the pulmonary vasculature to counter-regulate VIP deficiency.

Aviptadil is well tolerated, acts as a weak pulmonary selective vasodilator, and alleviates right heart strain. Additionally, aviptadil tends to improve oxygenation in patients with chronic lung disease [6]. However, there is little or no report regarding the methods for quantitation of this peptide in dosage forms or biological samples. In fact, there is only one research paper that reports the development of sheathless capillary electrophoresis-mass spectroscopy CE-MS and nanoRP-HPLCMS for quantitation of this therapeutic peptide [7]. However, to our knowledge, there is no report concerning any RP-HPLC method for simultaneous estimation of aviptadil and its degradation products upon storage in neutral, acidic and alkaline media. In this study, we have developed and validated a new reverse phase HPLC method for the analysis of intact aviptadil acetate and its degradation products.

MATERIALS, INSTRUMENTS AND METHODS:

Materials

Aviptadil acetate was purchased from Xi'an Realin Biotechnology Company (Xi'an, China). Acetonitrile and HPLC grade water were purchased from Fisher Scientific UK (Leics, UK), Orthophosphoric acid (85%) was purchased from BDH (VWR International, UK), and potassium dihydrogen phosphate was purchased from BDH Chemicals Ltd (Poole, Dorset, UK). All chemicals and reagents used in the assay were of HPLC grade. HPLC instrument was a Water Breeze 2 HPLC system, consisting of binary pump series 1525, UV/VIS detector 2489, and auto sampler series 2707. The analytical column was a Thermo Scientific Hyprersil Gold column with a dimension of 250 x 4.6 mm, and particle size of 5 µm. (Thermo Fisher Scientific, Waltham, MA, USA).

The chromatographic conditions used

Mobile phase was prepared by mixing seven volumes 0.01M potassium dihydrogen phosphate (adjust with dilute phosphoric acid to a pH of 3.0 ± 0.2) and three volumes acetonitrile. The stock standard solution having a concentration of 0.1 mg/ml was prepared by dissolving pure drug of Aviptadil Acetate in HPLC water. Several factors were selected through a number of trials conducted as optimum conditions for the analysis of Aviptadil acetate.

System suitability testing was used to verify that the resolution and reproducibility of the system were adequate for the analysis to be performed (% RSD for retention time and for peak height of six replicates) were determined and depicted in Figure 1. Samples for calibration curve were prepared by diluting stock standard solution to get a concentration of 2, 4, 6, 8 and 10 µg/ml, Table 1 and figure 2.

Determination Limit of Detection (LOD) and Limit of Quantitation (LOQ):

For the limit of detection (LOD and that of quantitation (LOQ) were be calculated using the following equations: $LOD = 3.3\sigma / S$ and $LOQ = 10\sigma / S$. Where: σ = Standard deviation of the response; S = Slope of the calibration curve (Table 4).

Table 1: Test results for calibration curve.

Concentration of Aviptadil (µg / ml)	Response (peak height)
2	34414.33
4	65148.67
6	98062.00
8	129460.67
10	160523.00

Table 2: Test results for accuracy studies (Recovery).

Run	% Amount addition	% Amount found	% Recovery	RSD
1	80	81.03	98.73	0.47
2	80	80.26	99.67	
3	80	80.64	99.21	
1	100	100.97	99.04	0.52
2	100	101.36	98.66	
3	100	100.32	99.68	
1	120	119.02	100.82	0.20
2	120	119.26	100.62	
3	120	119.52	100.40	
Accuracy = Mean overall recovery			99.65 %	

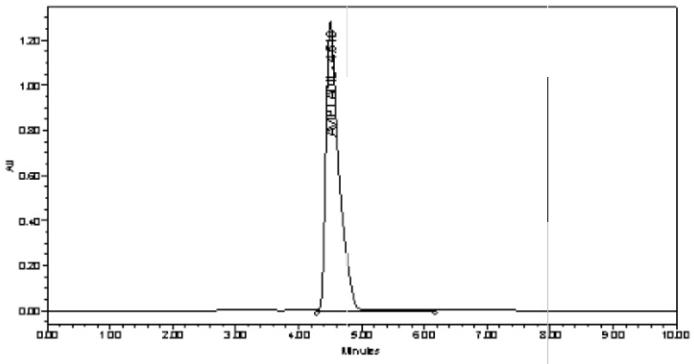

Figure 1: The chromatogram of aviptadil acetate standard sample.

Accuracy, Recovery and Precision Studies

Samples for accuracy and recovery studies were prepared by addition of standard drug solution to the sample at three different levels (80%, 100%, and 120%) of the test concentration (Table 2 and Figure 3). The precision of the method was checked by repeatability of intraday and interday assay. The intra-assay precision (repeatability) was studied by analyzing repeated six replicate concentration of 10 µg/ml of Aviptadil acetate on one laboratory on the same day, whereas intermediate precision was studied by carrying out repeated study by three different analyst (X, Y, and Z) over three days and the results were expressed as a relative standard deviation percentage (RSD%), Table 3.

Stability studies

***Sample preparation in acidic and alkaline media at room temperature*:**

For acidic medium, 10 mg of aviptadil acetate was dissolved in 100 ml of 0.1M HCl, 10 ml was transferred into a 100 ml volumetric flask. The volume was completed with water to the mark. Thirty µl of the sample and the reference standard were separately injected into the chromatographic column (Table 5, Figure 6). For alkaline medium, 10 mg of aviptadil acetate was dissolved in 100 ml of 0.1M NaOH, 10 ml was transferred into a 100 ml volumetric flask. The volume was completed with water to the mark. Thirty µl of the sample and the reference standard were separately injected into the chromatographic column (Table 5, Figure 6).

RESULTS

The mobile phase containing 0.01M potassium dihydrogen

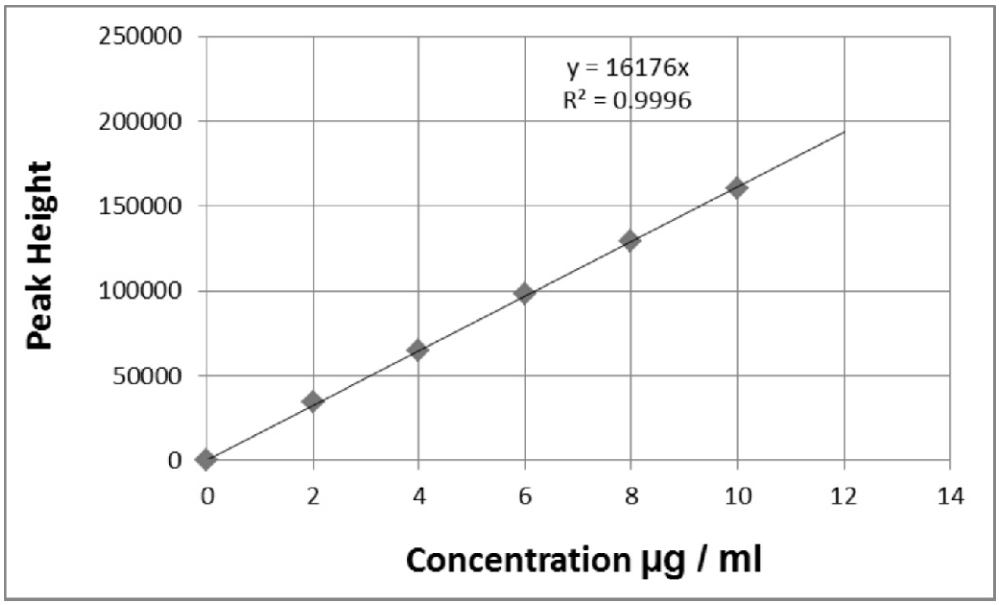

Figure 2: The calibration curve for aviptadil acetate standard.

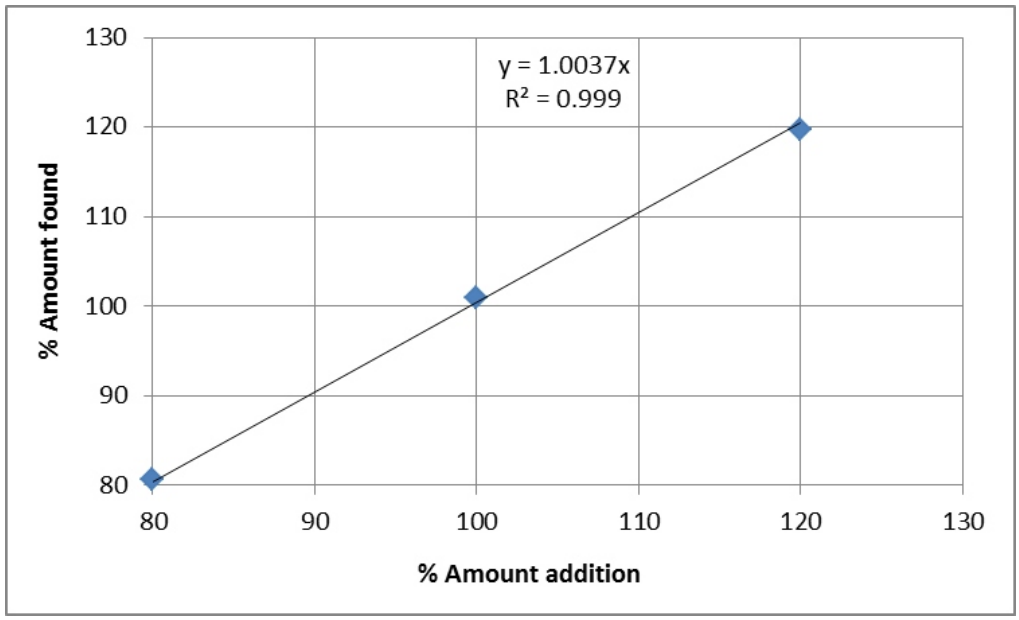

Figure 3: The calibration curve of aviptadil acetate.

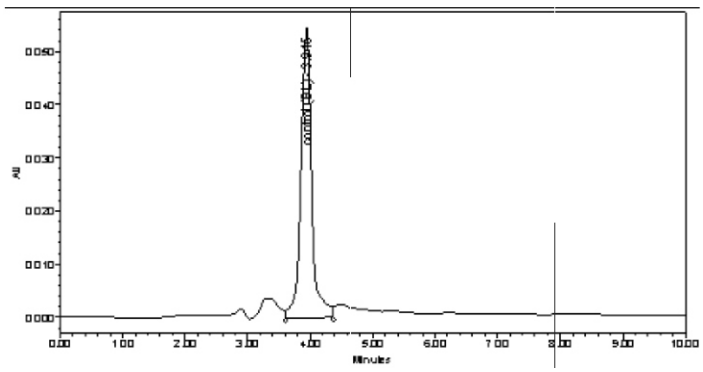

Figure 4: The chromatogram of poly-lactic-glycolic-acid (PLGA).

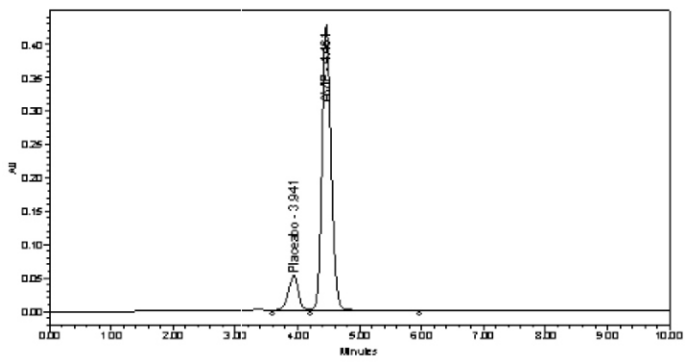

Figure 5: The chromatographs of aviptadil acetate and PLGA in their mixture.

Table 3: Test results for method precision.

PRECISION TYPES	RSD
Repeatability	0.60%
intermediate precision method (carried out byX-analyst)	0.26%
intermediate precision method (carried out by Y-analyst)	0.67%
intermediate precision method (carried out by Z-analyst)	0.06%
Overall RSD	0.40 %

Table 5: The effect of different media on the stability of Aviptadil acetate.

Sr.NO	Parameters	Acceptance criteria	Results
1	Linearity R^2	(R^2) = 0.99	0.9996
2	Precision Repeatability Intermediate	System precision = 1.0 % (%RSD) = 2.0 % = 3.0 %	0.60 % 0.60 % 0.26 %, 0.67 %, 0.06 %
3	Accuracy	3X3 concentration Mean recovery 98-102 %	99.65%
4	Limit of detection (LOD)	-	0.07 µg / ml
5	Limit of quantitation (LOQ)	-	0.22 µg / ml
6	Range	-	2 - 10µg / ml

Table 3: Validation parameters.

Peak name	Aviptadil in different media		
	R.T (minute) Water	R.T 0.1M HCl	R.T 0.1M NaOH
Aviptadil acetate	4.61	4.61	-
Decomposed 1	0	2.98	3.08
Decomposed 2	0	0	3.83
Decomposed 3	0	0	5.15
Decomposed 4	0	0	6.07

phosphate solution (pH= 3±0.2, adjusted by diluted orthophpspharic acid) and acetonitrile at a ratio of 7:3 and C18 column (Thermo, hypersil gold, 250x4.6 mm, 5µm) were optimal for resolution and well-defined peak for aviptadil acetate (Figure 1). The UV absorption spectra of aviptadil acetate in the range 200-400 nm showed maximum absorbance at 220 nm, which was used as detection wavelength. The mobile phase was run at a flow rate of 1.0 ml/min for about 4.5 minutes. The system suitability testing used to verify that the resolution and reproducibility of the system were adequate for the analysis (%RSD for retention time and for peak height of six were < 1.00). The calibration curve for the analysis of aviptadil acetate by HPLC method over the range 2-10 µg/ml (Figure 2) yielded a correlation coefficient of 0.9996.

DISCUSSION

As per ICH guidelines[8,9], the accuracy ranged from 98.66-100.82 % as shown in Table 2 and Figure 3, thus indicating very good reproducibility. The study for precision was carried out as

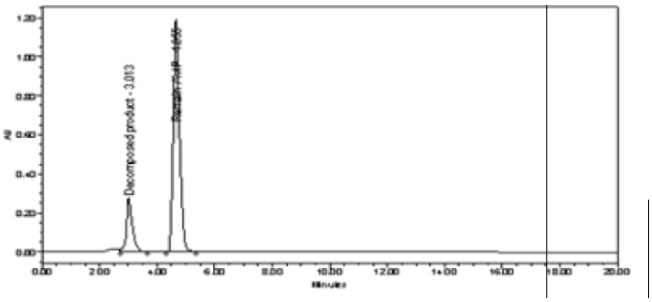

Figure 6: The chromatogram of degradation product of aviptadil acetate in an acidic medium.

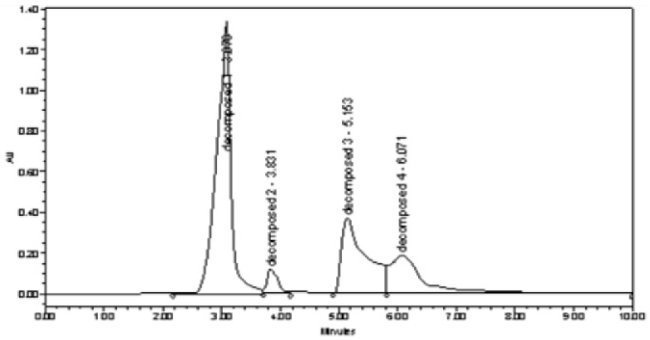

Figure 7: The chromatograms of base catalyzed degradation products of aviptadil acetate.

per ICH guidelines [8,9]; the precision was determined at one concentration with six replicates for intra-precision assay (repeatability), and one concentration level with 3 replicates at each level as intermediate precision (Table 3). For all three concentration levels % RSDs obtained were less than 1.0%. The results obtained indicated that the proposed method was precise. Table 4 contains a summary of the process of confirming the validation of the proposed analytical method that meets the standard outlines in ICH and U.S FDA guidelines [10].

The peak for excipient of poly-lactic-co-glycolic acid (PLGA) was observed in 3.9 minute; there was no overlap between peak of PLGA and that of the drug, PLGA. [Figures 4 and 5]. The drug underwent little or no degradation in the acetate in acidic media, but the drug completely degraded in the alkaline media Aviptadil acetate [Figures 6 and 7].

Overall, the method was developed and validated according to ICH and FDA guidelines. The standard deviation and coefficient of variation observed in precision studies were satisfactory; the recovery studies ranging from 98.68- 100.62 % demonstrated that the accuracy and precision our method were similar to that of published study**.**

CONCLUSION

The method reported in this study can be used quantificant of aviptadil acetate and monitoring stability of the drug. The method was simple, sensitive, accurate, precise, reproducible, and relatively inexpensive. The method exhibited acceptable recovery of the analyst and can distinguish the drug substance from its degradation products and excipients in the formulation. This RP-HPLC method can be used for quantitation aviptadil acetate and its degradation products.

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REFERENCES

1. Tuder RM, Marecki JC, Richter A, Fijalkowska I, Flores S. Pathology of pulmonary hypertension. *Clin Chest Med*. 2007;28(1):23-42.

2. Hoeper MM, Rubin LJ. Update in Pulmonary Hypertension 2005. *Am J Respir Crit Care Med*. 2006:499-505.

3. Rubin LJ, Galiè N. Pulmonary arterial hypertension: a look to the future. *J Am Coll Cardiol*. 2004;43(12 Suppl S):89S - 90S.

4. Petkov V, Mosgoeller W, Ziesche R, et al. Vasoactive intestinal peptide as a new drug for treatment of primary pulmonary hypertension. *J Clin Invest*. 2003;111(9):1339-1346.

5. World Health Organization. Proposed International Nonproprietary Names List 78. WHO Drug Information Vol. 11. Geneva. In: *World Health Organization*.; 1997:265-297.

6. Leuchte HH, Baezner C, Baumgartner R a., et al. Inhalation of vasoactive intestinal peptide in pulmonary hypertension. *Eur Respir J*. 2008; 32(5):1289-1294.

7. Gross PC, Burkart SC, Müller R. Analytics of the therapeutic peptide aviptadil by sheathless CE-MS and comparison with nanoRP-HPLCMS. *J Pharm Biomed Anal*. 2014;88:477-482.

8. Guideline IHT. Validation of Analytical Procedures: Text and Methodology Q2 (R1), (November 2005) International Conference on Harmonization. In: Geneva, Switzerland.

9. Guideline IHT. Q2B: Validation of Analytical Procedures: Methodology, May 1997.

10. U.S. FDA. Guidance for Industry Analytical Procedures and Methods Validation for Drugs and Biologics, February 2014.