Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 2423-2427 | ISSN: 2251-6727

Development and Validation of RP-HPLC method for Estimation of Olopatadine HCl in Rabbit Plasma: Application to Pharmacokinetic Studies

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(Received: 14 January 2024 Revised: 22 February 2024 Accepted: 31 March 2024)

KEYWORDS

Olopatadine HCl, RP-HPLC, plasma, accuracy, precision, pharmacokinetic studies.

ABSTRACT:

Anew, simple, sensitive, accurate, precise, and stable reverse phase liquid chromatographic method was developed for the estimation of olopatadine HCl in rabbit plasma, using a hypersil C18 (5 µm, 250×4.6 mm) column and a mobile phase comprising of methanol and water mixture in the ratio of 75:25 (%v/v). This mixture was adjusted to pH 3.0 with trifluor acetic acid. The detection wavelength and retention time for olopatadine HCl obtained were at 254 nm and 5.6 minutes respectively with the optimized chromatographic conditions and internal standard was not used as resolution is good enough for the study. Calibration curve was established for olopatadine HCl in the range of 1-30µg/mL. A good linear relationship was observed as indicated by correlation coefficient (r=0.9999) and the limit of detection was found to be 0.5 µg/mL. The percentage recovery of the suggested method for olopatadine HCl was found to be in the range of 100–101% with low %CV values (<2%). The precision of the method showed low %CV values (< 2%)at intra-day and inter-day estimation of olopatadine HCl. Solution stability studies indicated that solution was stable for 2 days at room temperature(~26ºC).The method developed for estimation of drug in rabbit plasma was found to be reproducible, accurate and precise with good solution stability. Hence, this method can be successfully used for estimation/quantification of olopatadine HCl in rabbit plasma, for pharmacokinetic studies in animal models.

1. INTRODUCTION

In recent years, the prevalence of the symptomatic allergic conditions are increasing due to the change in environment and change in the lifestyle. Allergic conditions, also called as allergic diseases, are resultant of hypersensitivity response of immune system. Most commonly seen allergic diseases are allergic rhinitis and conjunctivitis, eczema, choric urticaria and bronchial asthma[1].

The most common symptoms observed in the allergic rhinitis and conjunctivitis are red eyes, itchy rash, sneezing, runny nose, nasal obstruction and itching of nose and eyes. Allergic skin conditions are also extremely common and often manifest as hives or urticaria. In this allergic skin reaction, lower layer of the surface of the skin involves and shown as a localized swelling, produced by the release of serum into the tissue (wheals), and redness of the skin, resulting from the dilation of the blood vessels (flare), which are associated severe pruritis[1].

As per current statistics, common allergic diseases like allergic rhinitis and conjunctivitis are affecting approximately 10 to 30% of the adult population and up to 40% of children population in the world[2]. Surprisingly, approximately 40% of patients suffering with allergic rhinitis and conjunctivitis have asthma and approximately 80% of asthma patients have symptoms of allergic rhinitis and conjunctivitis[3].

In spite of having more drugs for treating these allergic conditions, majority of the anti-allergic drugs particularly antihistamines are permeable to the blood brain barrier and cause side effects like drowsiness, dizziness. Scientists are aiming at discovery of new chemical entities to provide safe, effective and preventive medication to the treatment of these allergic conditions without these side effects.

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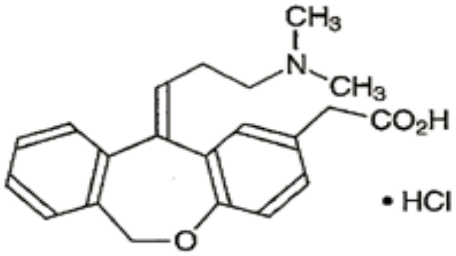
JCHR (2024) 14(2), 2423-2427 | ISSN: 2251-6727

As a part of discovery of new anti-allergic drugs, Kyowa Hakko Kogyo Co., Ltd. developed and evaluated a new anti-allergic agent olopatadine HCl, an orally active anti-histamine agent with low incidence of side effects of the central nerve system. The blood brain barrier is relatively impermeable to olopatadine and marketed under the brand name of Allelock® in Japan for the treatment of common allergic diseases. It is considered as a potent histamine H_1 receptor antagonist and administered twice a day to adults. It is soluble in water. The pharmacokinetic profile of olopatadine HCl is linear at doses from 5 to 80 mg with elimination half- life of 7-9 hours[4-5]. The main site of absorption was the duodenum to the jejunum [6-9].



Structure of Olopatadine HCl

Literature survey reported that, a liquid chromatography mass spectroscopy (LC-MS) method was developed for the estimation of olopatadine in human plasma[10]. There are no reported analytical methods available for the estimation of olopatadine in rabbit plasma. The aim of this study is to develop a simple, accurate, precise, stable and validated reverse phase high performance liquid chromatography (RP-HPLC) method for the estimation of olopatadine HCl in rabbit plasma for studying the pharmacokinetic parameters of different dosage forms developed for olopatadine HCl.

2. MATERIALS & METHODS

Materials and reagents

Olopatadine HCl (Gift sample from MSN Laboratories, Hyderabad, India) certified to contain 99.6% (w/w), on as is basis. Methanol, HPLC grade (Fischer Scientific), water, HPLC grade (Spectrochem) and trifluroaceticacid 99% extra pure (ACROS organics) and analytical reagent grades were used without further purification.

Instrument

Chromatography was performed with Waters, e2695 model HPLC-Empower software equipped with photodiode array detector for the detection of olopatadine HCl in rabbit plasma using below mentioned chromatographic conditions.

Preparation of mobile phase

The mobile phase was prepared by mixing methanol with water in the ratio of 75:25 (%v/v). This mixture was adjusted to pH 3.0 with trifluoracetic acid and degassed by sonication for 10 minutes and filtered through 0.22 µm membrane filter and used.

Preparation of parent stock solution

10 mg of olopatadine HCl was accurately weighed into 100 mL of volumetric flask. The drug was first dissolved in few mL of methanol. The volume was made up to 100 mL with methanol to get a concentration of 100µg/mL.

Preparation of standard solutions

Standard stock solutions of 1, 2, 3, 4, 6, 10, 15, 20, 25 and 30 µg/mL were prepared separately from the parent stock solution (100µg/mL) by appropriate dilutions with methanol.

Table 1: Chromatographic conditions

Stationary phase	Hypersil C18, 5 µm, 250×4.6 mm
Mobile phase	Methanol and water. Its pH was adjusted to 3.0 with trifluoracetic acid
Mobile phase ratio	75:25 V/V
Detection wavelength	254 nm
Flow rate	2.0 mL/minute
Injection volume	20 µL

Linearity

100 µL of rabbit plasma was spiked with 100 µL of individual standard stock solutions of concentration ranging from 1 to 30 µg/mL in a 2 mL centrifuge tube separately and vortexed for 2 minutes. 300 µL of chilled methanol was added to these solutions and vortexed for 2 minutes and finally cold centrifuged at 2000 rpm for 10 minutes. Supernatant was collected and 20 µL was injected to HPLC system. Blank was prepared in the similar manner without drug.

Accuracy

The accuracy of the drug content estimation was determined by calculating percentage recovery by adding 6, 15 and 30 µg/mL to the plasma. Three determinations were performed at each level and

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JCHR (2024) 14(2), 2423-2427 | ISSN: 2251-6727

average values were reported. The coefficient of variation was calculated to find out the accuracy.

Precision

The intra-day and inter-day precision was estimated by analyzing 15 µg/mL olopatadine HCl standard solution.

Solution stability

The solution stability studies were carried out on 15 µg/mL concentration. The solution was analyzed at initial, after 1st and 2nd day. The difference in % drug content at each time interval was calculated.

3. RESULTS AND DISCUSSION

HPLC method was developed for the estimation olopatadine HCl in rabbit plasma and accuracy, precision, solution stability studies were carried out for the method validation. Initially the flow rate and ratio of mobile phase were optimized by trial and error. The λ_{max} was determined and found to be 254 nm. In the present study methanol and water in the ratio of 75:25 was optimized as mobile phase. The detection wavelength for olopatadine HCl obtained at 254 nm and 5.6 minutes respectively with the optimized chromatographic conditions and internal standard was not used as resolution is good enough for the study. The calibration curve was constructed by plotting mean area against concentration of olopatadine HCl and shown in **Figure 1**. A good linear relationship was observed as indicated by correlation coefficient (r=0.9999) and the limit of detection was found to be 0.5 µg/mL. This calibration curve was used for the estimation of olopatadine HCl in plasma for *in vivo* studies.

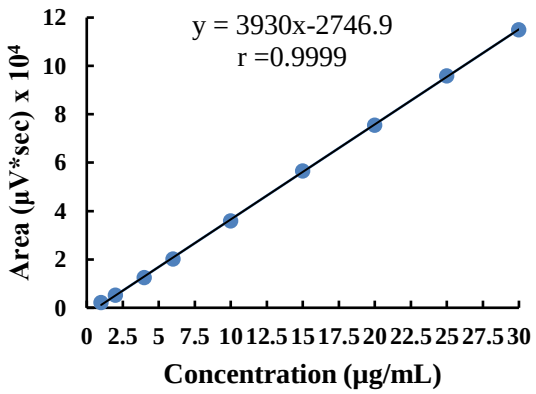

Figure 1: Calibration curve for the estimation of olopatadine HCl in rabbit plasma

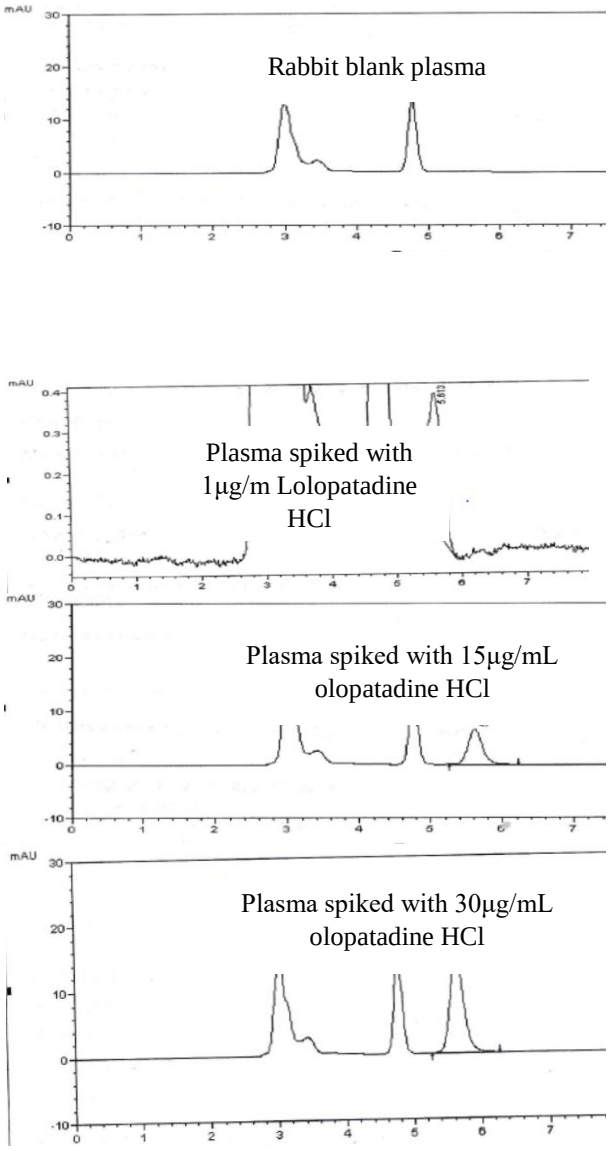

Figure 2: HPLC chromatograms

Accuracy

The results from the accuracy study are mentioned in Table 2. The low %CV values (<2%) indicated the accuracy of the method.

Table 2: Accuracy of the estimation of olopatadine HCl in plasma

Concentration (µg/mL)	Trial	% Recovery	Mean (%)	%CV
6 µg/mL	1	101	101	1.08
	2	100		
	3	101		
15 µg/mL	1	100	100	1.58

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JCHR (2024) 14(2), 2423-2427 | ISSN: 2251-6727

	2	98		
	3	102		
30 µg/mL	1	101	100	1.21
	2	101		
	3	99		

Precision

The results of the precision of percent drug in plasma are mentioned in **Table 3.** The precision of the study was confirmed with low %CV values (<2%) both at intra-day and inter-day estimation of olopatadine HCl, indicating that the method was precise. Hence this method is used for the estimation of olopatadine HCl in plasma during the *in vivo* studies.

Table 3: Precision of the estimation of olopatadine HCl in plasma (n=6)

Sample	Intra-day	Inter-day
1	100.6	101.6
2	99.8	99.8
3	100.7	98.6
4	99.5	100.2
5	101.7	102.6
6	98.2	99.2
Mean	100.0	100.6
%CV	1.12	1.51

Solution stability

The solution stability studies indicated that, standard solution was stable for 2 days at room temperature (~26ºC).

Table 4: Solution stability testing

Time (Day)	Room Temperature		
	% Drug content	Difference in the percent drug content from the initial	%CV
Initial	100.7	-	1.6
1	99.8	0.9	1.3
2	99.0	1.8	1.4

4. CONCLUSION

Olopatadine HCl was estimated by using validated HPLC method for the *in vitro* estimation of olopatadine HCl in the formulation and in dissolution. The *in vitro* method obeyed Beer's law in the concentration ranges studied for both calibration curves with high accuracy and precision. The solution stability studies indicated that the solutions were stable for two days at room temperature.

The *in vivo* method developed for estimation of drug in rabbit plasma was found to be reproducible with high accuracy and precision with good solution stability. Hence, this method can be used for the estimation of olopatadine HCl in rabbit plasma during Pharmacokinetic studies.

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JCHR (2024) 14(2), 2423-2427 | ISSN: 2251-6727

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