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Pathology

DEVELOPMENT AND EVALUATION OF (3-BROMOPROPYL) TRIPHENYL PHOSPHONIUM BROMIDE AS A GENOTOXIC IMPURITY IN OLOPATADINE HYDROCHLORIDE DRUG SUBSTANCE BY USING UPLC

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ABSTRACT A simple and accurate reverse phase chromatography method was developed for the determination of (3-Bromopropyl) Triphenyl Phosphonium Bromide in the Olopatadine Hydrochloride bulk drug. Chromatographic separation between (3-Bromopropyl) Triphenyl Phosphonium Bromide and Olopatadine Hydrochloride was achieved on C18 column using mobile phase containing buffer and acetonitrile in gradient mode. The resolution between the between (3-Bromopropyl) Triphenyl Phosphonium Bromide and Olopatadine Hydrochloride was found to be more than 7.0. The limit of detection (LOD) and limit of quantification (LOQ) of the (3-Bromopropyl) Triphenyl Phosphonium Bromide was 0.08 and 0.17 µg mL-1, respectively. The percentage recoveries of the (3-Bromopropyl) Triphenyl Phosphonium Bromide ranged from 91.5 to 95.9 in the samples of Olopatadine Hydrochloride. The developed method was validated as per International Conference on Harmonization guidelines in terms of specificity, limit of detection, limit of quantification, precision, linearity, accuracy and ruggedness.

KEYWORDS : Development, Validation, Olopatadine Hydrochloride, (3-Bromopropyl) Triphenyl Phosphonium Bromide, Genotoxic, UPLC

INTRODUCTION

Olopatadine Hydrochloride [1,2] (trade name Patanol), 2-[(11Z)-11-[3-(dimethylamino)propylidene]-6H-benzo[c][1]benzoxepin-2-yl]acetic acid;hydrochloride (Figure 1), is a medication that used to treat itching of the eye caused by a condition known as allergic conjunctivitis (pink eye) [2]. It works by preventing the effects of certain inflammatory substances, which are produced by cells in your eyes and sometimes cause allergic reactions [2, 3].

(3-Bromopropyl)Triphenyl Phosphonium Bromide was one of the key intermediate used in the manufacturing process Olopatadine Hydrochloride. (3-Bromopropyl)Triphenyl Phosphonium Bromide is halogenated hydrocarbons having the structural alert for the genotoxicity.

The study is proposed and conducted for the method development and further validation of method for determination of (3-Bromopropyl) Triphenyl Phosphonium Bromide in Olopatadine Hydrochloride drug substance [4, 5]. Recommended maximum daily dose for Olopatadine Hydrochloride is 5.32 mg. Based on the genotoxic impurity guideline, daily dose and threshold of toxilogical concern (TTC) approach, the limit for the (3-Bromopropyl) Triphenyl Phosphonium Bromide is decided as 282 µg/mL [5, 6, 7]. In the present work we have developed a simple precise method for determination of (3-Bromopropyl) Triphenyl Phosphonium Bromide in Olopatadine Hydrochloride using C18 column by Ultra performance liquid chromatography. The developed method was validated according to International Conference on harmonization (ICH) guidelines [8].

Experimental Section

Chemicals and Reagents

Samples of Olopatadine Hydrochloride were obtained from R & D synthetic department of FDC Ltd, Mumbai, India. Orthophosphoric acid, Acetonitrile was procured from Merck, Darmstadt, Germany. (3-Bromopropyl) Triphenyl Phosphonium Bromide standard procured from Alfa Assar.

Instrumentation

Ultra performance liquid chromatography (UPLC) system used was Waters (Acquity, US) system equipped with auto sampler, quaternary pump, degasser and a UV Detector. The output signal was monitored and processed using Empower 3.0 software.

Chromatographic condition

The chromatographic column used was CSH C18 column (100 mm x 2.1 mm, 1.8µm), (Waters Ltd., USA). The mobile phase used was water, pH adjusted to 2.2 with diluted Orthophosphoric acid as a mobile phase A and acetonitrile was used as a mobile phase B. The gradient program time (minutes) % mobile phase B (T%B) was set as 0/25, 1/25, 6/50, 8/50, 9/25 and 15/25 respectively. The flow rate of the mobile phase was 0.3 mL/min. The column temperature was maintained at 25°C, and the eluent was monitored at a wavelength of 225 nm. The injection volume used was 3 µL. Initial composition of mobile phase (75:25 v/v) was used as diluent.

Preparation of Standard Solutions

The stock solution of the (3-Bromopropyl) Triphenyl Phosphonium Bromide were prepared by dissolving an appropriate amount of the standard in diluent. For quantitation of (3-Bromopropyl) Triphenyl Phosphonium Bromide in Olopatadine Hydrochloride a solution of 0.56 µg/mL concentration was used. The target analyte concentration was fixed as 2.0 mg mL-1.

RESULTS AND DISCUSSION

Method Development

A solution of Olopatadine Hydrochloride and (3-Bromopropyl) Triphenyl Phosphonium Bromide (2 mg/mL & 0.56 µg/mL) prepared in diluent for method establishment. To develop a rugged and suitable UPLC method for the separation, different stationary phases and mobile phases were employed. Preliminary column screening involved different types of C18, C8, Cyano, Amino and Phenyl columns were employed.

On CSH C18 (100 mm x 2.1 mm, 1.8µm) column provided selectivity between the Olopatadine Hydrochloride peak and the (3-Bromopropyl) Triphenyl Phosphonium Bromide peak using a mobile phase consisting of buffer-methanol (75:25, v/v), but the retention times of Olopatadine Hydrochloride, known impurities and (3-Bromopropyl) Triphenyl Phosphonium Bromide were both longer than 30 min and the peaks were broad. We continued to select the best mobile phases that would give optimum resolution and selectivity for the (3-Bromopropyl) Triphenyl Phosphonium Bromide and Olopatadine Hydrochloride. Good separation was achieved on C18 column and buffer-acetonitrile (75:25 v/v) as the mobile phase. There was less separation when phosphate buffer of (pH 5.0) and methanol

used as mobile phase on C18 column.

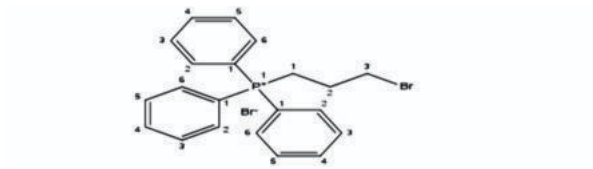
Optimized Chromatographic Conditions

Due to the better chromatographic results obtained on the C18 column, further method optimization and quantification of the (3-Bromopropyl) Triphenyl Phosphonium Bromide were carried out on this column. Based on the data obtained from method development and optimization activities, the CSH C18 column (100m x 2.1 mm x 1.8 µm) with the mobile phase of buffer-acetonitrile in gradient was selected for the final method. The flow rate of the experimental method was 0.3 mL/min with an injection volume of 3µL. The column temperature was 25°C, and the detection wavelength was 225 nm. Under these conditions, (3-Bromopropyl) Triphenyl Phosphonium Bromide and Olopatadine Hydrochloride and known impurities were well separated. In the optimized method, the typical retention times of the Olopatadine Hydrochloride and (3-Bromopropyl) Triphenyl Phosphonium Bromide were approximately 1.95 and 3.99 min, respectively. Baseline separation of Olopatadine Hydrochloride and (3-Bromopropyl) Triphenyl Phosphonium Bromide was obtained with a total run time of 15 min. The system suitability results were given in Table 1.

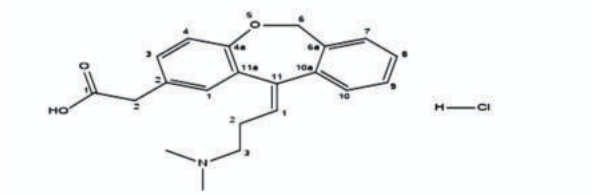
Table 1: System suitability criteria

Component	Retention time (min)	Relative retention time (min)	Resolution	Tailing factor
Olopatadin Hydrochloride	1.95	1.00	--	---
(3Bromopropyl)Triphenyl Phosphonium Bromide	3.99	2.04	9.48	0.98

The structure of (3-Bromopropyl) Triphenyl Phosphonium Bromide and Olopatadine Hydrochloride are displayed in Fig. 1. The typical chromatogram of the Olopatadine Hydrochloride spiked with (3-Bromopropyl) Triphenyl Phosphonium Bromide displayed in Fig. 2.



(3-Bromopropyl) Triphenyl Phosphonium Bromide



Olopatadine Hydrochloride

Fig.1 Chemical structures of (3-Bromopropyl) Triphenyl Phosphonium Bromide and Olopatadine Hydrochloride

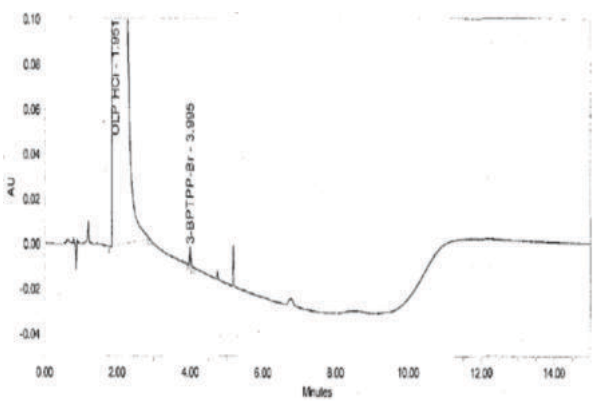


Fig. 2 A typical UPLC chromatogram of Olopatadine Hydrochloride spiked with (3-Bromopropyl) Triphenyl Phosphonium Bromide at specification level

Method Validation

Precision

The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions. The system and method precision for the (3-Bromopropyl) Triphenyl Phosphonium Bromide were checked at its specification level (i.e. 0.56 µg/ml with respect to analyte concentration, 2.0 mg mL-1). The percentage RSD of method repeatability and system repeatability for the (3-Bromopropyl) Triphenyl Phosphonium Bromide were found to be 1.09% and 0.55%, respectively, which confirms good precision of the method.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. The linearity of the method for the (3-Bromopropyl)Triphenyl Phosphonium Bromide was checked at six concentration levels, i.e. from limit of quantitation (LOQ (30%), to 150% of the (3-Bromopropyl)Triphenyl Phosphonium Bromide specification level (0.56 µg/ml), which is with respect to Olopatadine Hydrochloride test concentration. The coefficient of regression of the calibration curve was found to be 0.9998, thus confirming the excellent correlation between the peak area and concentration of the (3-Bromopropyl) Triphenyl Phosphonium Bromide.

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantification were achieved by injecting a series of dilutions of (3-Bromopropyl) Triphenyl Phosphonium Bromide [8]. The precision of the developed method for (3-Bromopropyl) Triphenyl Phosphonium Bromide at LOD and LOQ was checked by analyzing standard solutions prepared at the LOD and LOQ level and calculating the percentage relative standard deviation of area. The limit of detection and quantification for (3-Bromopropyl) Triphenyl Phosphonium Bromide was found to be 0.08 µg mL-1 and 0.17 µg mL-1 respectively.

Ruggedness and Robustness

The ruggedness [8] of a method was defined as degree of reproducibility of results obtained by analysis of the same sample under a variety of normal test conditions such as different analysts, different instruments and different days. The recovery experiments carried out for the (3-Bromopropyl) Triphenyl Phosphonium Bromide in Olopatadine Hydrochloride samples at the same concentration levels tested.

The data obtained from both the experiment was well in agreement with each other, thus proving the method ruggedness. The robustness [8] of an analytical procedure is measured by its capability to remain unaffected through small, but deliberate, variations in method parameters and provide an indication of its reliability during normal usage. In the varied chromatographic conditions like flow rate, mobile phase ratio and pH, the resolution between the peaks of (3-Bromopropyl) Triphenyl Phosphonium Bromide and Olopatadine Hydrochloride was found to be >7.0 illustrating the robustness of the method.

Recovery of (3-Bromopropyl) Triphenyl Phosphonium Bromide

The standard addition and recovery experiments were conducted for the (3-Bromopropyl) Triphenyl Phosphonium Bromide in bulk samples of Olopatadine Hydrochloride in triplicate at LOQ (0.17 µg/mL), 50% (0.28 µg/mL), 100% (0.56 µg/mL) and 150% (0.84 µg/mL) with respect to test concentration. The percentage recovery ranged from 91.5% to 95.9 % (Table 2).

Table 2 Summary of method validation data

Parameter	µg mL-1	r	% Mean recovery	% RSD
LOD	0.08	-	-	3.43
LOD	0.18	-	-	1.23
Linearity (LOQ to 150%)	-	0.9989	-	-
Accuracy LOQ % spiking	-	-	91.5	0.91
50% spiking	-	-	93.3	0.46
100 % spiking	-	-	95.9	0.99

150 % spiking	-	-	95.1	1.04
Precision System precision	-	-	-	0.55
Method precision	-	-	-	1.09
Intermediate pre (Ruggedness)	-	-	-	2.91

CONCLUSION

A simple, rapid and accurate Ultra Performance Liquid Chromatography (UPLC) method developed in ordered to separate (3-Bromopropyl) Triphenyl Phosphonium Bromide and Olopatadine Hydrochloride. Method validation was carried out using a CSH C18 column due to the better chromatographic results achieved on the column. The validated method was demonstrated to be specific, accurate, precise, sensitive and rugged. The developed and validated method can be implemented for the determination and quantitation of (3-Bromopropyl) Triphenyl Phosphonium Bromide in Olopatadine Hydrochloride bulk drug.

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