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Research Article

QUANTIFICATION OF GENOTOXIC IMPURITIES IN FEXOFENADINE HYDROCHLORIDE AN ANTIHISTAMINE PHARMACUITICAL DRUG

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ABSTRACT

The objective of the research work is to develop suitable analytical method for quantitative determination of Genotoxic impurities at ppm level in Fexofenadine hydrochloride. A single isocratic reversed phase HPLC method was developed on Zorbax RX C-8 column using the mobile phase consists of 0.01M potassium dihydrogen phosphate at Ph-3.0 and acetonitrile in the ratio of 40:60 (v/v) at a flow rate of 1.0 ml/min. The column temperature was maintained at 27°c and UV maximum is 250nm.The limit of detection and limit of quantification of the impurities are established. The method has been tested in number of fexofenadine samples and it is found that the method is suitable for the quantification of genotoxic impurities at ppm level (Below TTC).

Keywords: Fexofenadine hydrochloride, TTC (Threshold of toxicological concern), HPLC, ppm.

INTRODUCTION

Fexofenadine[1] (trade names Allegra, Fexidine, Telfast, Fastofen, Tilfur, Vifas, Telfexo, Allerfexo) is an antihistamine pharmaceutical drug used in the treatment of allergy symptoms, such as hay fever, nasal congestion, and urticaria. Fexofenadine is sometimes called a third-generation antihistamine because it is less able to pass the blood-brain barrier and cause sedation, compared to first-generation antihistamines.

Methyl-2-(4-(4-Chlorobutanoyl) phenyl) -2-methylpropanoate,4-chloro-1-(4-(1-hydroxy-2- Methyl propan-2-yl) phenyl) butan-1-one, 2-4-(4-Chlorobutanoyl) phenyl)-2-methyl propanoic acid is the three impurities arise during the synthesis of Fexofenadine hydrochloride and these impurities are found to be Genotoxic[2-6] based on the encountered search results.

The presence of drug-substance impurities[7] that are DNA-reactive, has posed significant problems for drug regulators and industry alike over the last decade or so. The principal concern relates to drug safety in that exposure, particularly if prolonged, to compounds that can alter (alkylate) DNA[8] may ultimately produce a carcinogenic response. A further practical issue is that the conventional testing procedures applied to drug substances for carcinogenicity and mutagenicity/Genotoxicity[9] (the property of being able to damage cellular DNA and induce genetic mutation) generally lack sufficient sensitivity to detect potentially adverse effects associated with DNA-reactive impurities (which are often present only at ppm levels). During the literature search we did not found any quantification methodology for the above impurities till date. so we felt that it is necessary to develop suitable analytical methodology for quantification of above three impurities in fexofenadine hydrochloride.

The toxicological assessment of these genotoxic impurities and the determination of acceptable limits for the above impurities in fexofenadine is a difficult issue.

A TTC[9]-based acceptable intake of a mutagenic impurity of 1.5 µg per person per day is considered to be associated with a negligible risk (theoretical excess cancer risk of <1 in 100,000 over a lifetime of exposure) and can in general be used for most

pharmaceuticals as a default to derive an acceptable limit for control (ICH M7: Assessment and control of DNA reactive (mutagenic) impurities in pharmaceutical to limit potential carcinogenic risk). As per ICH M7 the target impurities are comes under the category of Class-3 (Alerting structure, unrelated to the structure of the drug substance; no mutagenicity date) and the proposed action was to control at or below acceptable limits (appropriate TTC).

We need to control the target genotoxic impurities at 8.33 ppm level by using maximum daily dose of fexofenadine hydrochloride 180.0 mg.

Experimental

Chemicals and reagents

Samples of fexofenadine hydrochloride, Methyl-2-(4-(4-Chlorobutanoyl) phenyl)-2-methylpropanoate, 4-chloro-1-(4-(1-hydroxy-2-methyl propan-2-yl) phenyl) butan-1-one and 2-4-(4-Chlorobutanoyl) phenyl)-2-methyl propanoic acid were recieved from CTO-III, Dr.Reddys laboratories limited, Hyderabad, India. HPLC grade Potasium phosphate and acetonitrile was purchased from Merck, Mumbai, India. HPLC grade water purchased from Rankem, Haryana, India.

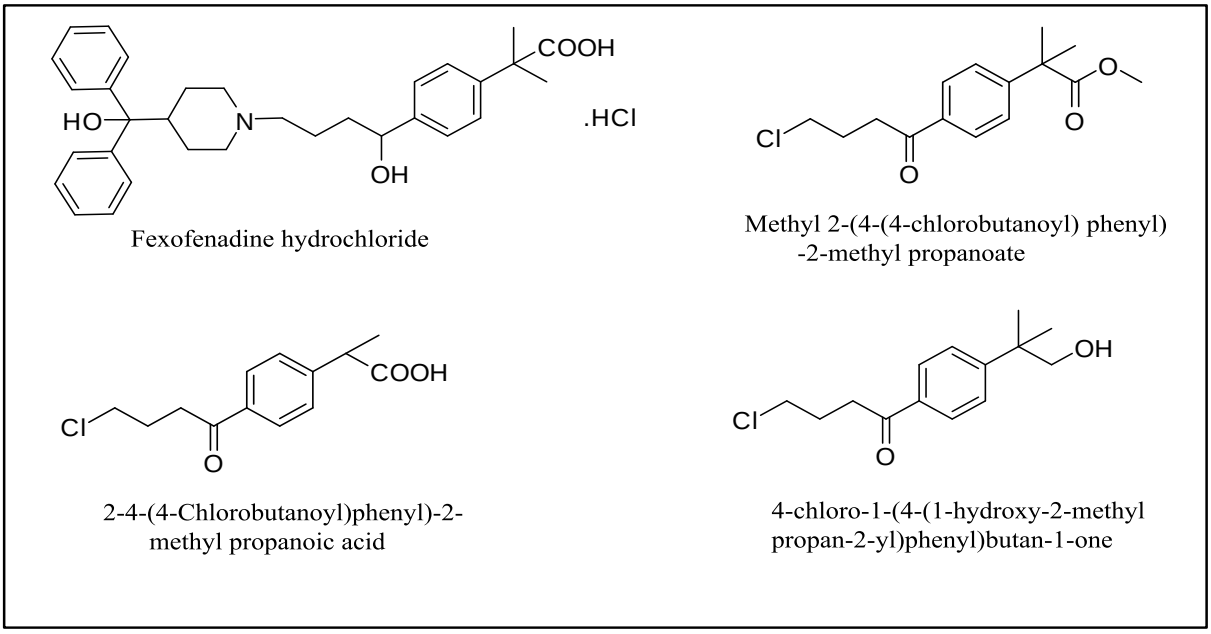

Fig. 1: Structures of Fexofenadine and Genotoxic impurities

Equipment

The HPLC method development and validation were done by using Waters e 2695 separation module equipped with 2998 PDA detector. The data were collected using Empower software.

Chromatographic conditions

HPLC chromatographic separation was achieved on Zorbax RX-C8 column (150 mm length x 4.6 mm ID and 5 µm particle size). The mobile phase was 0.01M Potassium dihydrogen phosphate, Ph adjusted to 3.0 with diluted ortho phosphoric acid in the ratio of 400:600 (v/v).

The flow rate of the mobile phase was 1.0 mL min^{-1}. The column temperature was maintained at 27°c and the detection wavelength was 250 nm. The injection volume was 40µL and mobile phase is used as diluent to dissolve the impurities as well as fexofenadine hydrochloride.

Preparation of impurity stock solutions and test solutions

The impurity sock solutions for the three genotoxic impurities are prepared at 1.0 mg mL^{-1} in diluent. LOD, LOQ and Linearity solutions are prepared by further diluting the stock solutions. The fexofenadine hydrochloride stock solutions are prepared at approximately 100 mg mL^{-1} in diluent and sonicated for 5 minutes.

RESULTS AND DISCUSSIONS

Optimization of chromatographic conditions

The main target of the research work is to develop a simple, specific and sensitive HPLC method for the quantification [10] of three Genotoxic impurities in Fexofenadine hydrochloride. The development was started with USP monograph method, we have injected all the three impurity solutions and

checked the separation and found that the monograph method is not suitable for the quantification of these impurities at such low ppm concentrations. Trails were continued with different ratios of 0.01M potassium dihydrogen phosphate, Acetonitrile and methanol as mobile phase and Zorbax RX-C8 column (150 mm length x 4.6 mm ID and 5 µm particle size) as stationary phase. During the development [10] one peak is well separated where as the other two peaks are merged together and two merged peaks are confirmed as 2-4-(4-Chlorobutanoyl) phenyl)-2-methyl propanoic acid and 4-chloro-1-(4-(1-hydroxy-2-methyl propan-2-yl) phenyl) butan-1-one by injecting individual preparations into the chromatographic system. Because these two impurities are structurally very similar to each other so it took some trials to separate these two peaks. Tried to separate these two peaks by using different Ph study and found that are well separated at Ph 3.0.Studied the column temperature impact on separation by altering column temperature (High, Low) and concluded that ambient conditions are suitable for the good separation. Finally by comparing the UV spectra of all the three impurities found that all the peaks are having maximum absorbance at 250 nm and finalized 250 nm as wavelength maximum. Diluent selection is also played a crucial role in the development as we need to dissolve the API at 100 mg mL^{-1} to get desired limit of detection and limit of quantification [11] values of the target impurities. Tried different combinations of buffer, acetonitrile and methanol to dissolve the API and found that the buffer and acetonitrile at 40:60 (v/v) ratios has given desired solubility of the API. Injected all the Fexofenadine related impurities into the chromatographic system to ensure the separation of these impurities from the target Genotoxic impurities and found that related impurities are not merged with target impurities of concern.

Method validation

Limit of detection and Limit of Quantification

Prepared a series of dilutions of impurity stock solutions of Methyl 2-(4-(4-chlorobutanoyl) phenyl)-2-methyl propanoate, 2-4-(4-Chlorobutanoyl) phenyl)-2-methyl propanoic acid and 4-chloro-1-(4-(1-hydroxy-2-methyl propan-2-yl)phenyl)butan-1-one with diluent and injected into chromatographic system to get the signal to noise (S/N) ratio of 2 to 3 for LOD and 9 to 10 for LOQ. The results are tabulated below. (Table 1: LOD & LOQ results).

Precision

Precision of a method is the ability of a measurement to be consistently reproduced.

Precision was evaluated by preparing the Limit of quantification solutions for six times and injected into the chromatographic system and calculated. The % Relative standard deviation (RSD) of the areas of each impurity.**(Table 2: Precision results).**

Accuracy/Recovery

Accuracy or recovery of the method was evaluated by preparing the impurity spiked solutions to the test samples in triplicate and by injecting them into chromatographic system. Calculated the % recovery and found that % recovery values are not less than 95%. At such low levels the recovery values are very satisfactory and the results are tabulated below. (Table 3: Accuracy results).

Linearity

Linearity of the method was demonstrated by preparing Methyl 2-(4-(4-chlorobutanoyl) phenyl)-2-methyl propanoate impurity at 6,8,10,12,14,16 ppm (from LOQ to 200% level) with respect to test concentration and injected into chromatographic system. Linearity graph was plotted to area versus concentration and calculated correlation coefficient, residual sum of squares. (Table 4: Linearity of Methyl 2-(4-(4-chlorobutanoyl) phenyl)-2-methyl propanoate results).

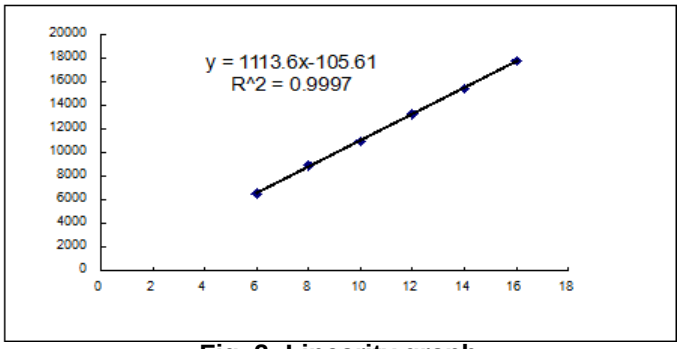

Fig. 2: Linearity graph

Linearity of 2-4-(4-Chlorobutanoyl) phenyl)-2-methyl propanoic acid impurity at 4, 6, 8,10,12,16 ppm (from LOQ to 150% level) with respect to test concentration and injected into chromatographic system. Linearity graph was plotted to area versus concentration and calculated correlation coefficient, residual sum of squares. (Table 5: Linearity of 2-4-(4-Chlorobutanoyl) phenyl)-2-methyl propanoic acid results).

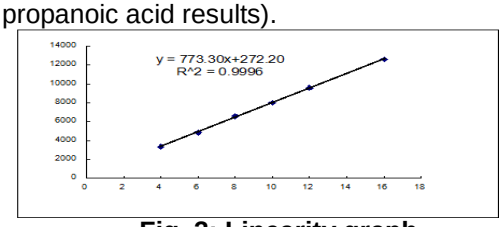

Fig. 3: Linearity graph

Linearity of 4-chloro-1-(4-(1-hydroxy-2-methylpropan-2-yl) phenyl) butan-1-one impurity at 4,6,8,10,12,16 ppm (from LOQ to 150% level) with respect to test concentration and injected into chromatographic system. Linearity graph was plotted to area versus concentration and calculated correlation coefficient, residual sum of squares. (Table 6: Linearity of 4-chloro-1-(4-(1-hydroxy-2-methylpropan-2-yl) phenyl) butan-1-one).

Table 1: LOD & LOQ results

S.No	Name of Impurity	Concentration (ppm)		Signal to noise (S/N) ratio	
		LOD	LOQ	LOD	LOQ
1	Methyl 2-(4-(4-chlorobutanoyl) Phenyl)-2-methyl propanoate	2	6	2.4	10.2
2	2-4-(4-Chlorobutanoyl) phenyl) -2- Methyl propanoic acid	1.3	4	2.5	9.7
3	4-chloro-1-(4-(1-hydroxy-2-methyl propan-2-yl) phenyl) butan-1-one	1.3	4	2.9	10.3

Table 2: Precision results

S.No	Name of Impurity	Standard deviation	% RSD
1	Methyl 2-(4-(4-chlorobutanoyl) phenyl)-2-methyl propanoate	84.36	0.94
2	2-4-(4-Chlorobutanoyl) phenyl)-2-Methyl propanoic acid	84.23	2.45
3	4-chloro-1-(4-(1-hydroxy-2-methyl propan-2-yl) phenyl) butan-1-one	78.55	1.55

Table 3: Accuracy results

S.No	Name of Impurity	% Recovery
1	Methyl 2-(4-(4-chlorobutanoyl) phenyl)-2-methyl propanoate	96.4
2	2-4-(4-Chlorobutanoyl) phenyl)-2- Methyl propanoic acid	98.0
3	4-chloro-1-(4-(1-hydroxy-2-methyl propan-2-yl) phenyl) butan-1-one	96.9

Table 4: Linearity of Methyl 2-(4-(4-chlorobutanoyl) phenyl)-2-methyl propanoate results

Concentration (ppm)	6	8	10	12	14	16
Area	6547	8916	10952	13259	15409	17780

Table 5: Linearity of 2-4-(4-Chlorobutanoyl) phenyl)-2-methyl propanoic acid results

Concentration (ppm)	6	8	10	12	14	16
Area	3332	4855	6569	7995	9589	12598

Table 6: Linearity of 4-chloro-1-(4-(1-hydroxy-2-methylpropan-2-yl) phenyl) butan-1-one

Concentration (ppm)	6	8	10	12	14	16
Area	1268	1790	2401	3059	3659	5162

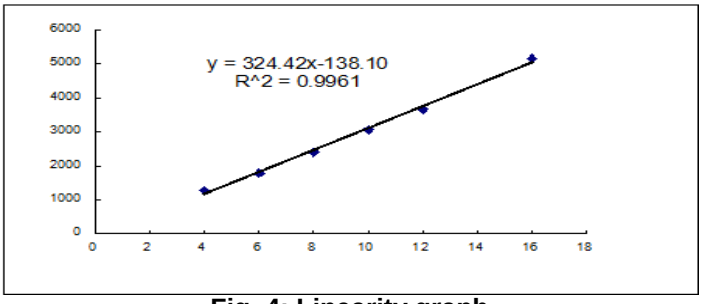

Fig. 4: Linearity graph

Batch analysis

Prepared standard solution containing the three target impurities at 8 ppm level and the test preparations at a concentration of 10 mg mL^{-1} in diluent and injected into chromatographic system. It is found that all the three impurities are not detected in all the fexofenadine plant batches.

CONCLUSION

A simple sensitive RP HPLC method was developed for the quantification of Genotoxic imputies at ppm level in Fexofenadine hydrochloride and the method is proven to be sensitive, accurate and linear to analyze the API samples of Fexofenadine hydrochloride. The method is very sensitive so that the impurities can be quantified at below their TTC limit.

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