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QUANTIFICATION OF LOW LEVEL ISOBUTYL BROMIDE AS A GENOTOXIC IMPURITY IN FEBUXOSTAT BY GAS CHROMATOGRAPHY

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Abstract:

A gas chromatographic method has been developed for the determination of Isobutyl bromide in Febuxostat drug substance. The method was optimized based on the basis of solubility of Isobutyl bromide. The method was validated as per ICH guideline in terms of LOD, LOQ, Method precision, accuracy and specificity. The LOD and LOQ values were found to be 1.9 ppm (1.9µg/mL) and 5.7 ppm (5.7 µg/mL) respectively.

Keywords: Development, validation, Isobutyl bromide, Febuxostat, genotoxic impurity, Gas chromatography.

Introduction:

Febuxostat is used to treat chronic gout and hyperuricemia. Chemically 2-(3-cyano-4-isobutoxyphenyl)-4-methyl- 1,3-thiazole-5-carboxylic acid. It has an empirical formula of $C_{16}H_{16}N_2O_3S$ and molecular weight of 316.37 g/mol. Febuxostat is used for Long-term treatment of high blood uric acid levels in patients with gout[1,2]. Febuxostat is a xanthine oxidase inhibitor. It works by blocking an enzyme in the body (xanthine oxidase), which lowers levels of uric acid in the blood. This helps to prevent gout flare-ups. Isobutyl bromide is an impurity during the synthesis of Febuxostat. The impurity is found to be genotoxic.

Literature survey reveals that few spectrometric methods [3, 4], HPLC [5-12], LC-MS [13] has been reported for the estimation of Febuxostat .However there is no method is available for the content of isobutyl bromide in Febuxostat by gas chromatography. In the present work we have developed a new, simple precise, accurate method for the determination of Isobutyl bromide in Febuxostat by gas chromatography in bulk drug. In synthesis of Febuxostat involves Isobutyl bromide is one of the impurity, which is an genotoxic alert. Hence evaluation of Isobutyl bromide is necessary.

Isobutyl bromide is Colorless to yellow liquid with ether like odor, chemical name is 1-bromo -2- methyl propane, it has an empirical formula C_4H_9Br and it has molecular weight of 137.02 g/mol. Isobutyl bromide is slightly soluble in water, miscible in dimethyl formamide. The toxicological assessment of these genotoxic impurities and the determination of acceptable limits for such impurities in active substances is a difficult issue and not addressed in sufficient detail in the existing International Conference on Harmonization (ICH) Q3X guidelines. The presence of trace level of the impurity in drug substance or drug product is of genotoxicity concern and has been closely scrutinized by regulatory agencies and pharmaceutical industries.

Experimental

Reagents and Chemicals

Isobutyl bromide was purchased from Aldrich chemicals; GC grade N,N-Dimethyl formamide was purchased from rankem. Samples of Febuxostat are received from local market.

Structure of Febuxostat and Isobutyl bromide are shown in figure.1 and figure.2.

Figure-1.

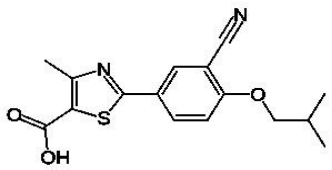
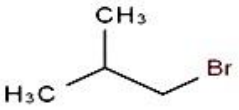


Figure-2.



Equipment

Analysis was carried on a GC system equipped with liquid auto sampler (Agilent 6890B gas

Chromatograph with auto sampler) with Flame ionisation detector and Nitrogen as a carrier gas. DB-624 (30 m x 0.32 mm I.D x 1.8 µm) was used for the analysis.

Standard Preparation:

Isobutyl bromide solution was prepared by diluting 18.75 mg of Isobutyl bromide to 100 mL with Dimethyl formamide, Further 1 mL of this solution was diluted up to 100 mL with dimethyl formamide. (equivalent to 18.75ppm with respect to sample concentration 100 mg per ml as per maximum daily dosage 80 mg i.e. TTC = 1.5/daily dosage grams).

Sample preparation: Weighed about 500 mg of Febuxostat drug substance into 5 mL volumetric flak, and make up to mark with diluent (dimethyl formamide) for shake well to dissolve.

Chromatographic conditions: Analysis was done on DB-624 column of 30 m length, 0.32mm inner diameter and 1.8 µm film thickness. Injector temperature and detector temperature were kept as 200°C and 250°C respectively. After optimisation the split was decided as 2:1 for 3µL injection. Flow of carrier gas (Nitrogen) is kept at 1.0 ml/min at constant velocity mode. The GC oven temperature program is kept c as 80°C hold for 12 min after ramp rate at 20°C/min up to 240°C hold for 5 min.

Results and Discussion

Method development and optimization

Isobutyl bromide is a liquid with a boiling point of 91.5 ◦C. Solvents used for development were acetonitrile, dimethylsulfoxide and dimethyl formamide. Dimethyl formamide is finalised as the diluent as Isobutyl bromide shows good response and recovery. Isobutyl bromide is soluble in dimethyl formamide and Febuxostat is also soluble in dimethyl formamide. The experiment was initially carried out on DB-5 column but was replaced by DB-624 column for sharper peak. The effect of injection volume and split was observed and optimised up to 3 µL with a split of 2:1 for sample concentration of 100mg/mL.

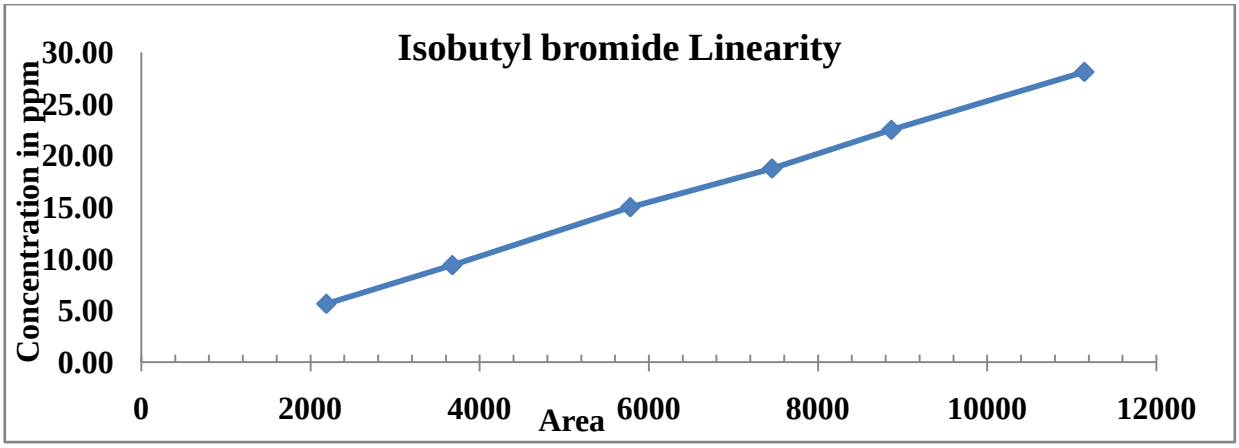
Method validation: The method validation work was conducted according to the ICH guidelines. The Validated method parameters include specificity, accuracy, sensitivity, precision, linearity, robustness, ruggedness and solution stability. LOD, LOQ values were obtained by preparing a series of known concentration solutions of increasing concentration and plotted a graph of Concentration against area of analyte. LOD and LOQ values were found to be 1.9 ppm and 5.7 ppm respectively for the sample concentration of 100 mg/mL. Linearity of the method was determined by preparing and analyzing a series of 7 standard solutions to cover the concentration range of LOQ to 150 % level for isobutyl bromide. The linearity correlation coefficient was found to be 0.9998. results are shown in Table 1 and figure-3.

Table-1: Linearity results.

Level	Corrected concentration (ppm)	Area obtained
LOQ	5.63	2189
50%	9.38	3678
80%	15.00	5781

100%	18.75	7456
120%	22.50	8867
150%	28.13	11148
Slope		398.450
Y-intercept		-79.501
correlation coefficient		0.9998

Figure-3.



The method is precise which is indicated by the low % relative standard deviation of six replicate Standards, which was 0.39%, results are shown in table-2.

Table-2: Precision results.

Inj. No.	Isobutyl bromide (ppm)
1	18.76
2	18.79
3	18.81
4	18.61
5	18.69
6	18.71
Mean	18.73
SD	0.0739
% RSD	0.39

The accuracy of method was determined by spiking the samples at 50 %, 100 % and 150 % level. Method also validated for solution stability at room temperature. Standard solution of Febuxostat at concentration of 18.75 ppm was injected at regular intervals. The recovery was in the range of 95-105 %.Typical figures of chromatograms of blank, standard, sample and spiked sample are shown in fig 4-7.

Figure 4: Typical chromatograms of Blank

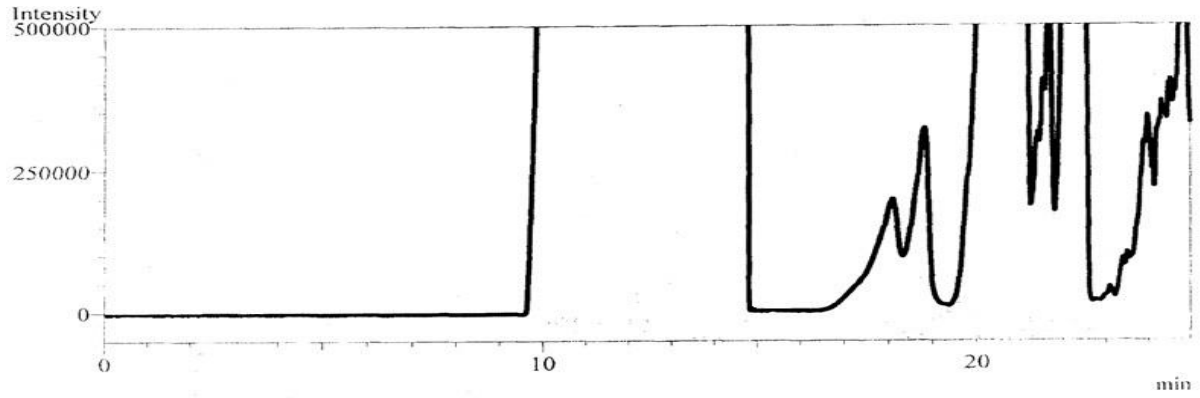


Figure 5: Typical chromatograms of Standard

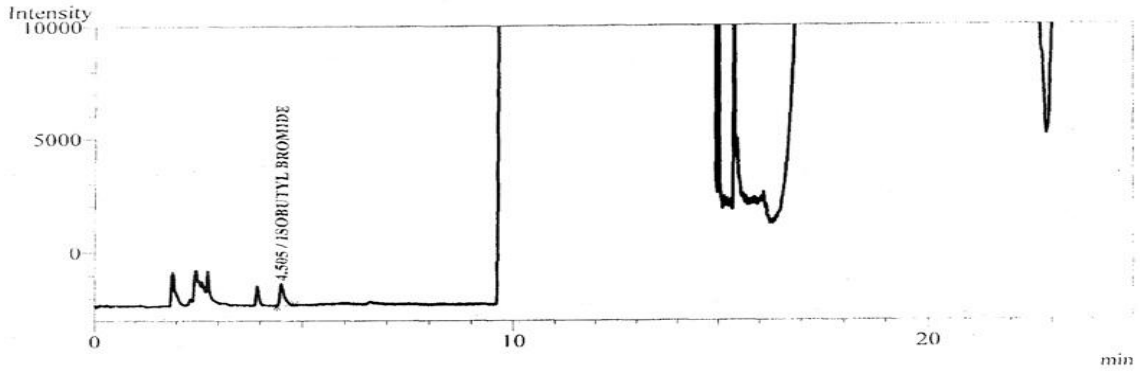


Figure 6: Typical chromatograms of Sample.

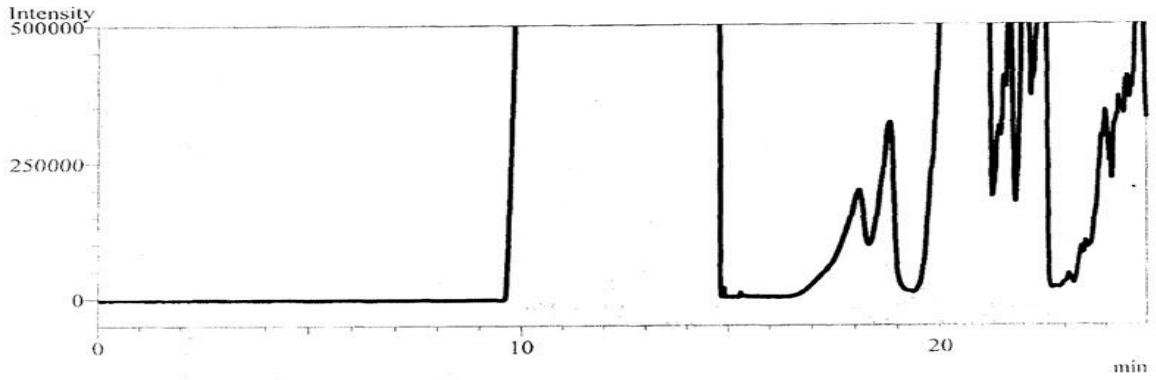
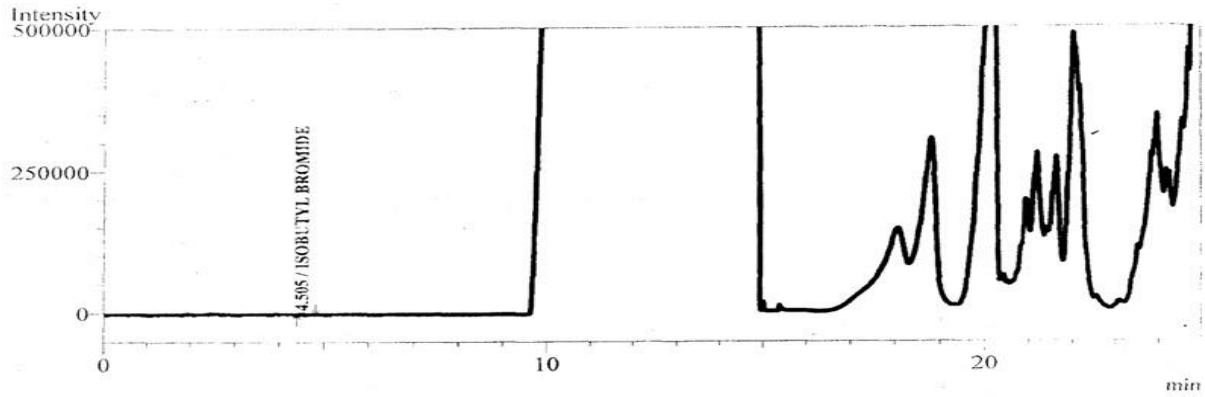


Figure 7: Typical chromatograms of spiked sample.



Conclusions

A simple and sensitive GC method has been developed and validated for the trace analysis of Isobutyl bromide in pharmaceuticals. The validation has been conducted according to ICH guidelines. Compared with the previously reported methodologies, this method utilizes a FID detector, which is readily available in most of the testing laboratories in the pharmaceutical industry and relatively simple to use. This method is sensitive enough to detect 1.9 ppm and quantify 5.7 ppm level of Isobutyl bromide in pharmaceutical drug substances.

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