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Quantification Of (4-Bromophenyl) {Pyridine-2-Yl} Acetonitrile Impurity (4-BPPA) By HPLC In Brompheniramine Maleate Active Pharmaceutical Ingredient

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Abstract: *The aim of this research work is to develop a suitable HPLC method for the quantitative determination of process impurity (4-Bromophenyl) {Pyridine-2-yl} Acetonitrile as this compound is organic cyano compound hence it is necessary to quantify at ppm level presence of Brompheniramine Maleate Active Pharmaceutical Ingredient. Hence the HPLC method was developed on Instrument Waters 717 plus Auto Sampler, Waters 2487 Dual _ Absorbance Detector, Waters 2695 Separation Module, Waters 996 PDA Detector with Empower 2 Software on HPLC column Varian C18, 150 x 4.6 mm, 5µ at a flow rate of 1.5 mL/min. The limit of detection and the limit of quantitation for the impurity were established. Validation of the new developed HPLC method was carried out as per requirements of ICH guideline and the data shows that the proposed method is specific, linear, accurate, precise and robust. This method has been tested in a number of Brompheniramine Maleate samples and used successfully for quantification of the impurity at ppm level. The developed HPLC method was found to be suitable to quantify this impurity (4-Bromophenyl) {Pyridine-2-yl} Acetonitrile at ppm level present Brompheniramine Maleate.*

Keywords: *High-performance liquid chromatography (HPLC), Process impurity; (4-Bromophenyl){Pyridine-2-yl} Acetonitrile (BPPA), HPLC Column, Brompheniramine Maleate, Threshold of Toxicological Concern (TTC).*

I. Introduction

(4-Bromophenyl) {Pyridine-2-yl} Acetonitrile is Process Impurity of Brompheniramine Maleate. Consumer ‘s protection depends on a products safety, characteristics, purity of the components. All these are regulated by The U.S. Food and Drug Administration (FDA)[1-2]. Small amount of impurity can change the efficacy, toxicity of any pharmaceutical compounds. International Conference on Harmonization said that impurities are unwanted chemicals that remain with the Active Pharmaceutical Ingredients (APIs) or develop during formulation or develop upon ageing of both APIs and formulated APIs [3-4]. The major challenge of any industry is to produce quality product and for that reason, it is necessary to conduct vigorous quality control checks in order to maintain the quality and purity of output from each industry. Raw materials, manufacturing method, crystallization and purification process play an important role to maintain the purity of any product. Analytical chemistry which is related to the developmental concepts in industry also changes with time [5-6]. Stringent limits of purity and impurity is specified by the various pharmacopoeias. Modern separation methods are advanced as these methods simultaneously separate and quantify the components to make the separation and characterization of impurities easier. As safety and quality of pharmaceutical products can be affected by the impurities present in the Active Pharmaceutical Ingredients (APIs) the impurity profile study of the API to be used in the manufacturing of drug substance. Thus, impurity profiling like identification, Isolation & characterization are done and their threshold values comply with the limits set and specified by official bodies. ―Issue related to impurities‖ addressing must be the same for each and every sector and there must be a unified system to ensure it. International Conference on Harmonization (ICH) has published guidelines for validation methods for analysis of impurities in new drug products, new drug substances, residual solvents & microbiological impurities [7-8] for registration of pharmaceuticals. ICH defines impurities as ―substance in the API itself. For pharmaceutical products, impurities are defined as ―substances in the product that are not the API itself or excipients used to manufacture it. i.e. impurities, are unwanted chemicals that remain within the formulation or API in small amounts which can influence QSE, thereby causing serious health Hazards.

II. Experimental

Chemicals and reagents: Samples of Brompheniramine Maleate and (4-Bromophenyl){Pyridine-2-yl} Acetonitrile (Figure 1) were collected from Supriya Lifescience Ltd., Maharashtra, India. HPLC grade Acetonitrile and Trifluoro Acetic Acid was procured from Advent, Mumbai, India.

Equipment: The HPLC method development and validation were done using Waters 717 plus Auto Sampler, Waters 2487 Dual _ Absorbance Detector, Waters 2695 Separation Module, Waters 996 PDA Detector and the data were collected using Empower 2 Software.

HPLC chromatographic conditions: The LC chromatographic separations were achieved on, 150 x 4.6 mm, 5µ Varian C18 column 150 mm length × 4.6 mm ID with 5 µm particle size using the isocratic mobile phase of mixture of Buffer (Mix 1ml Trifluoro acetic acid in 2 litres of water) and Acetonitrile in the ratio 60:40 (v/v) at a flow rate of 1.5mL/min at detector 203. The test concentration was about 10 ppm and the injection volume was 20 µL for a total run time of 18 minutes. Mobile phase was used as diluent during the standard and test samples preparations.

Preparation of impurity standard and test sample Solution: The stock solution of impurity standard prepared at approximately 0.01 mg/ml (10.0 ppm) in mobile phase. To calculate detection limit predication linearity, the stock solution impurity was diluted using mobile phase to give standards at 0.125, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 5.0, 7.5 and 1.0 ppm with respect to test concentration. The testing API samples were typically prepared at approximately 10 mg/mL in mobile phase[9].

III. Result And Discussions

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ values of (4-Bromophenyl) {Pyridine-2-yl} Acetonitrile were predicted from the predication linearity data. Each predicted concentration was verified for precision by preparing the solutions at about predicted concentration and injecting each solution six times on HPLC instrument and the predicted concentration for LOQ was 0.266 ppm and LOD was 0.126 ppm (Fig. 2 ABC &D) and the results are tabulated in table 1.

Linearity: The linearity of (4-Bromophenyl) {Pyridine-2-yl} Acetonitrile was satisfactorily done. A series of solutions were prepared using (4-Bromophenyl) {Pyridine-2-yl} Acetonitrile at concentration levels from around quantification level to 150% and the concentration levels are 0.12, 0.24, 0.48, 0.97, 1.45, 1.93, 2.42, 4.83, 7.25 and 9.67 ppm respectively. The peak area versus concentration data was done by linearity plot slope, intercept, and residual sum of squares analysis. The calibration curve was given based on response over the concentration range for (4-Bromophenyl) {Pyridine-2-yl} Acetonitrile. The correlation coefficient (4-Bromophenyl) {Pyridine-2-yl} Acetonitrile was 0.999 and the Linearity results are tabulated in table 2[10].

Precision: The precision of the developed method was checked by preparing solutions by spiking the impurity at 100% level with the drug substance for six times and injected each once also injected 100% spiked solution for 6 times to show the system precision. The % relative standard deviation (RSD) of the areas at each level 0.87% and 3.57% confirming developed that method is précised.

Accuracy: The accuracy of the method was conducted in sample solutions were prepared in triplicate by spiking (4-Bromophenyl) {Pyridine-2-yl} Acetonitrile at LOQ level, 50%, 100% and 150% with Brompheniramine Maleate and injected each solution in to HPLC as per methodology. The percentage of recovery for the impurity was calculated and the values are 98.6%, 103.0%, 104.0% and 104.1. At such low levels, these recoveries and % relative standard deviation (RSD) were satisfactory and the results are tabulated in table 3[11].

IV. Tables And Figures

Table 1: LOD and LOQ Precision

Injection	Area of LOD BPPA (0.126 ppm)	Area of LOQ BPPA (0.266 ppm)
1	9145	18624
2	7818	18978
3	7342	18484
4	7466	19317
5	8708	17807
6	8296	19195
Mean	**8129**	**18734**
SD	**714.29**	**556.04**
%RSD	**8.787**	**2.968**

Table 2: The regression analysis data for BPPA

Level	Conc. (ppm)	Mean Area
1	0.12	8361
2	0.24	18987
3	0.48	37380
4	0.97	78239
5	1.45	115354
6	1.93	147461
7	2.42	201119
8	4.83	413449
9	7.25	624584
10	9.67	829882
	Slope	**86404.66**
	Correlation	**0.9998**
	Intercept	**-5707.48**
	Residual sum of squares	**0.9996**

Table 3: % recoveries found for spiked (4-Bromophenyl) {Pyridine-2-yl} Acetonitrile in Brompheniramine Maleate

Level	Qty. Added (mg)	Mean Qty. Added (mg)	Qty. Recovered (mg)	Mean Qty. Recovered (mg)	% Recovery	Mean %Recovery
LOQ-1	0.00348	0.00348	0.0034	0.0034	97.7	98.6
LOQ-2	0.00348		0.0036		103.4	
LOQ-3	0.00348		0.0033		94.8	
50%-1	0.05801	0.11602	0.0588	0.0597	101.4	103.0
50%-2	0.05801		0.0598		103.1	
50%-3	0.05801		0.0606		104.5	
100%-1	0.11602	0.11602	0.1204	0.1206	103.8	104.0
100%-2	0.11602		0.1189		102.5	
100%-3	0.11602		0.1226		105.7	
150%-1	0.17404	0.17404	0.1862	0.1764	107.0	104.1
150%-2	0.17404		0.1810		104.0	
150%-3	0.17404		0.162		101.2	
					Mean	102.43
					SD	3.37
					% RSD	3.29

SD= Standard Deviation, RSD= Relative Standard Deviation

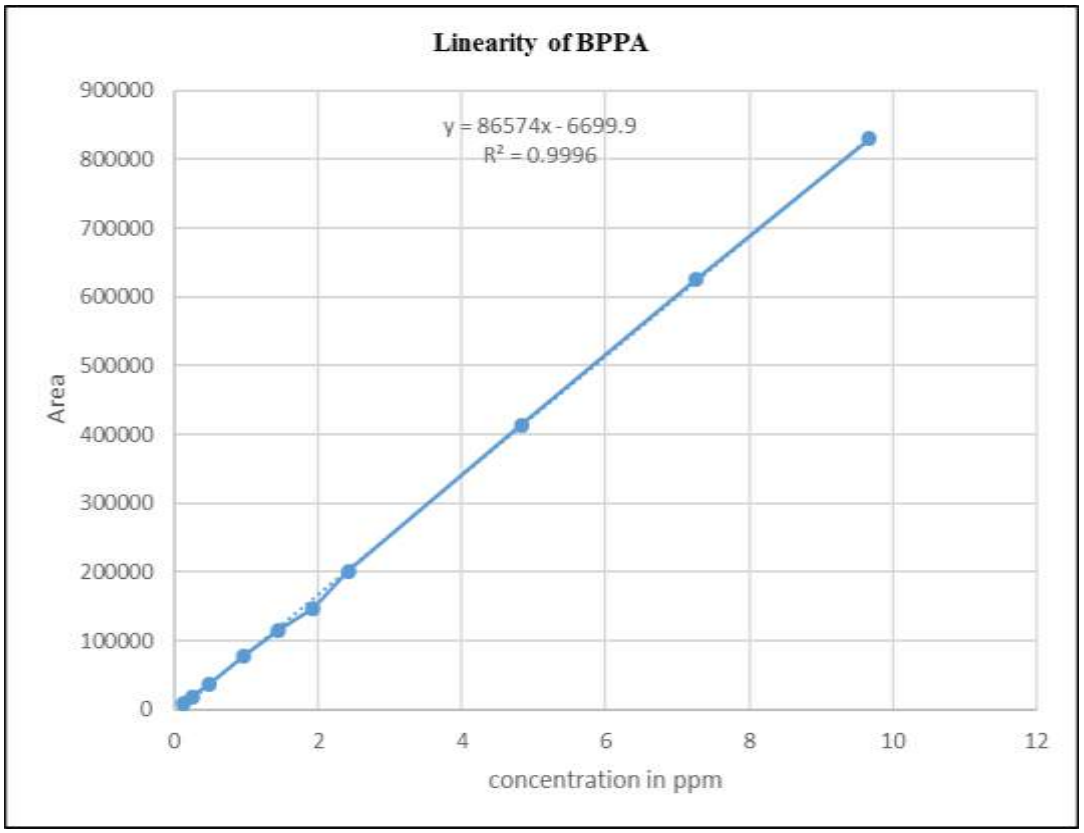

Fig.1: linearity of BPPA

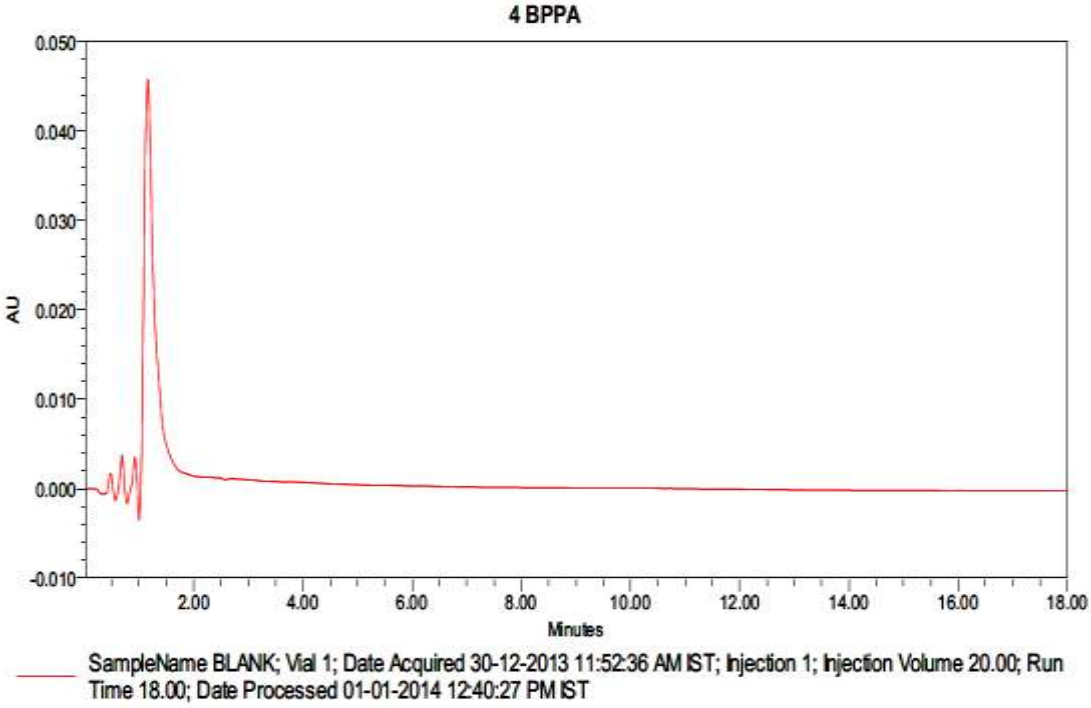

Fig. 2A: Typical HPLC Chromatographs of Blank

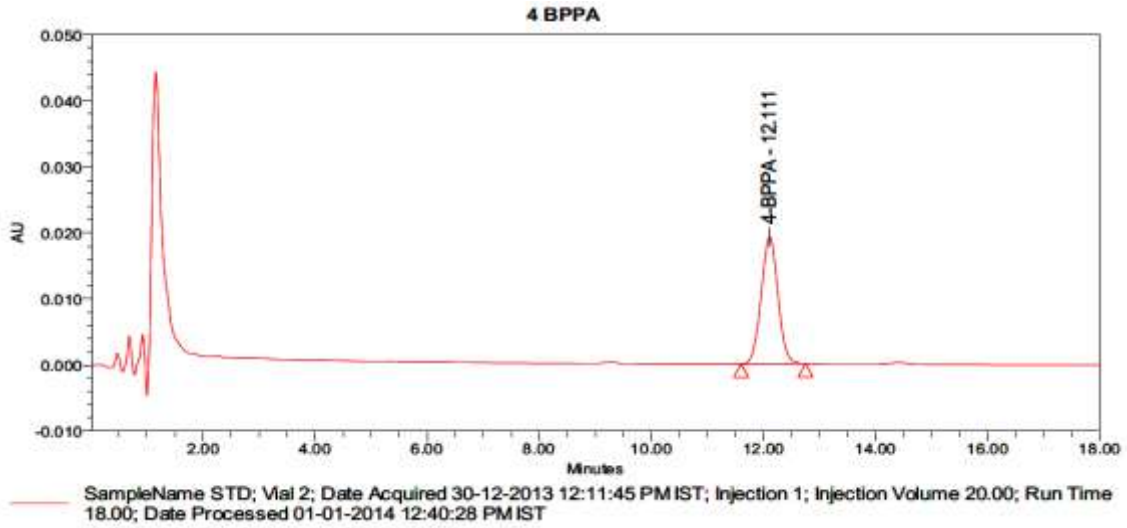

Fig. 2B: Typical HPLC Chromatographs of Standard.

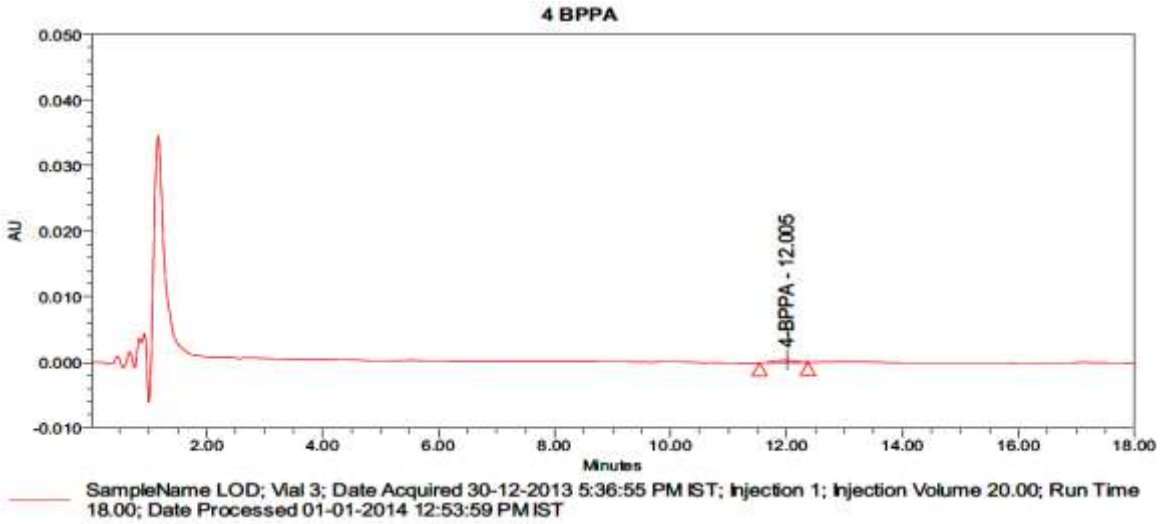

Fig. 2C: Typical HPLC Chromatographs of LOD.

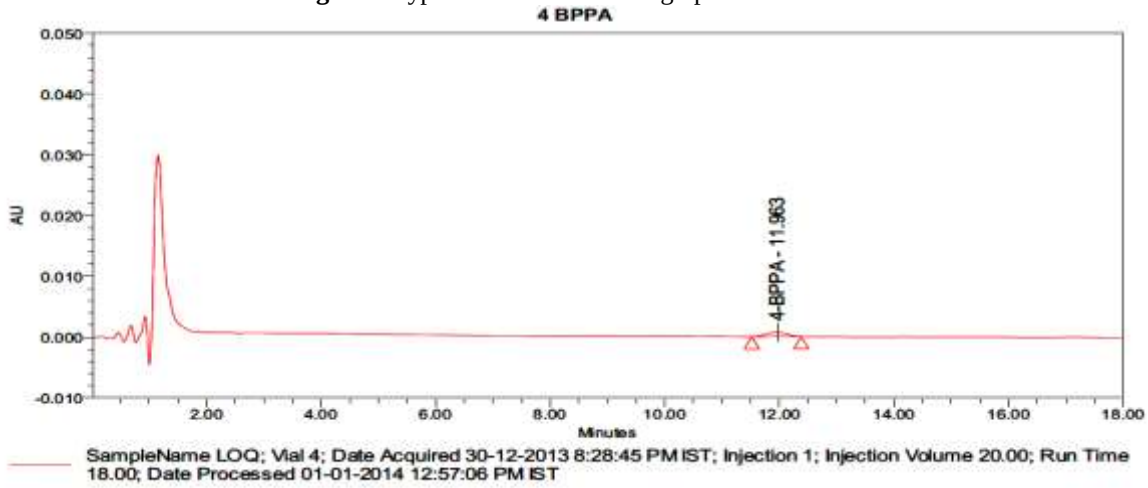

Fig. 2D: Typical HPLC Chromatographs of LOQ.

V. Conclusions

On the basis of above study conducted, reported method is sensitive specific, accurate, and précised validated and well defined HPLC method for the Quantification of impurity (4-Bromophenyl){Pyridine-2-yl} Acetonitrile (BPPA) at ppm level in Brompheniramine Maleate. The detection limit and quantification limit found to be 0.126 ppm and 0.266 ppm respectively. The described method is highly reliable technique for the quantification of the process impurity present in the Brompheniramine Maleate during quality control testing.

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