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Determination and Quantification of Carryover Genotoxic Impurities 2-Chloropyridine (2CP) and 4-Bromobenzyl Cyanide (PBBCN) by GCHS in Brompheniramine Maleate API

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ABSTRACT

The aim of this research work is to develop a suitable GCHS method for the quantitative determination of genotoxic impurities 2-Chloropyridine (2CP) and 4-Bromobenzyl Cyanide (pBBCN) as this compound is liquid in nature hence these carryover impurities are not easy to identify and quantify at ppm level present in Brompheniramine Maleate Active Pharmaceutical Ingredients with any other methods. Hence the GCHS method was developed on Shimadzu- GC2010 plus and Agilent – 7890B Gas Chromatograph with using FID detector. The GC was equipped with a capillary column (Gs-Tek, GsBP, 30 m x 0.32 mm ID x 0.5 µm). The Limit of Detection and the Limit of Quantitation for both impurities were established. Validation of the developed Gas Chromatography Head Space method was carried out as per International Conference on Harmonization requirements 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 GCHS method was found to be suitable to identify and quantify the genotoxic impurities 2-Chloropyridine (2CP) and 4-Bromobenzyl Cyanide (pBBCN) at ppm level present Brompheniramine Maleate.

INTRODUCTION

2-Chloropyridine (2CP) **[1]** and 4-Bromobenzyl Cyanide (pBBCN) are starting key material process Impurity of Brompheniramine Maleate (Fig.1) genotoxic in nature. 2-Chloropyridine and 4-Bromobenzyl Cyanide shows presence of structural alert for genotoxic mutagenicity and carcinogenicity. QSTR models predict the compound positive for genotoxicity, mutagenicity and carcinogenicity the compound is shown positive for mutagenicity in training set used for Ames mutagenicity model.

2-Chloropyridine **[2]** is reported to be irritating and toxic by ingestion (Lewis, 1993; Aldrich Chemical Co., Inc., 1996). Prepared by Technical Resources International, Inc. under contract No. NO2-CB-50511. The pathology caused by exposure to 2-Chloropyridine is essentially the same as that caused by exposure to pyridine. Exposures less than those required to produce overt clinical signs may cause varying degrees of liver damage with central lobular fatty degeneration, congestion, and cellular infiltration; repeated low-level exposures cause cirrhosis. The kidney is less sensitive to pyridine induced damage than is the liver. In general, pyridine and its derivatives cause local irritation on contact with the skin, mucous membranes, and cornea.

Bromobenzyl cyanide is a colorless organobromide compound. It is slightly soluble in water but readily soluble in organic solvents. Bromobenzyl cyanide **[3]** is resistant to the action of water and oxidizers; it decomposes upon heating above 120°C and also when exposed to the action of a number of metals, which are thereby intensely corroded. Bromobenzyl cyanide is obtained by the action of sodium cyanide or potassium cyanide on benzyl chloride with subsequent bromination of the benzyl cyanide that has been formed. Bromobenzyl cyanide acts powerfully on the mucous membranes of the eye, causing irritation and heavy lachrymation. It was proposed as a toxic lachrymatory agent at the end of World War I and an irritant gas for law enforcement. It is chemically and biologically similar to Benzyl cyanide. Benzyl cyanide and its derivatives are used in organic synthesis for dyes, perfumes, pesticides, pharmaceuticals, especially penicillin precursors.

Organic nitriles decompose into cyanide ions both *in vivo* and *in vitro*. Consequently, the primary mechanism of toxicity for organic nitriles is their production of toxic cyanide ions or hydrogen cyanide. Cyanide is an inhibitor of cytochrome c oxidase in the fourth complex of the electron transport chain (found in the membrane of the mitochondria of eukaryotic cells).

It complexes with the ferric iron atom in this enzyme. The binding of cyanide to this cytochrome prevents transport of electrons from cytochrome c oxidase to oxygen. As a result, the electron transport chain is disrupted and the cell can no longer aerobically produce ATP for energy. Tissues that mainly depend on aerobic respiration, such as the central nervous system and the heart, are particularly affected. Cyanide is also known produce some of its toxic effects by binding to catalase, glutathione peroxidase, methemoglobin, hydroxocobalamin, phosphatase, tyrosinase, ascorbic acid oxidase, xanthine oxidase, succinic dehydrogenase, and Cu/Zn superoxide dismutase. Cyanide binds to the ferric ion of methemoglobin to form inactive cyanmethemoglobin [3].

There are three primary effects that Genotoxins can have on organisms by affecting their genetic information. Genotoxins can be carcinogens, or cancer-causing agents, mutagens, or mutation-causing agents, or teratogens, birth defect-causing agents [4-5]**.** 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 Q3X guidelines [5-7]. The presence of trace level of the Genotoxic Impurity in drug substance or drug product is of genotoxicity concern and has been closely monitored by regulatory agencies and pharmaceutical industries. The 'Threshold of Toxicological Concern' (TTC) of 1.5 µg/person/day "Guideline on the limits of genotoxic impurities" [8-9] and the Pharmaceutical Research and Manufacturers of America's (PhRMA) white paper. Based on the TTC, the concentration limits of genotoxic impurity in drug substances or drug products can then be derived based on the maximum daily dose: concentration limit (ppm) = [1.5 µg /day)] / [dose (g/day)] [10-11]. For a drug dosed at 1g per day, for example, 1.5 ppm would be the limit of a specific genotoxic impurity which would also be the 'Target Analyte Level' from an analytical perspective. Given such a low ppm concentration limit, besides the control challenges in process chemistry, developing sensitive and robust methodology for their detection poses a tremendous analytical challenge for the pharmaceutical industry [12-15]. Therefore potential genotoxins must be minimized during the synthesis the compounds and where there is difficulty achieving this, the method of manufacture should preferably be changed. As2-Chloropyridine and 4-Bromobenzyl Cyanide is a genotoxic compound, the regulators may require the toxin levels to be controlled to 37.5 ppm in the drug substance on the basis of Maximum Daily Dose of drug substance.

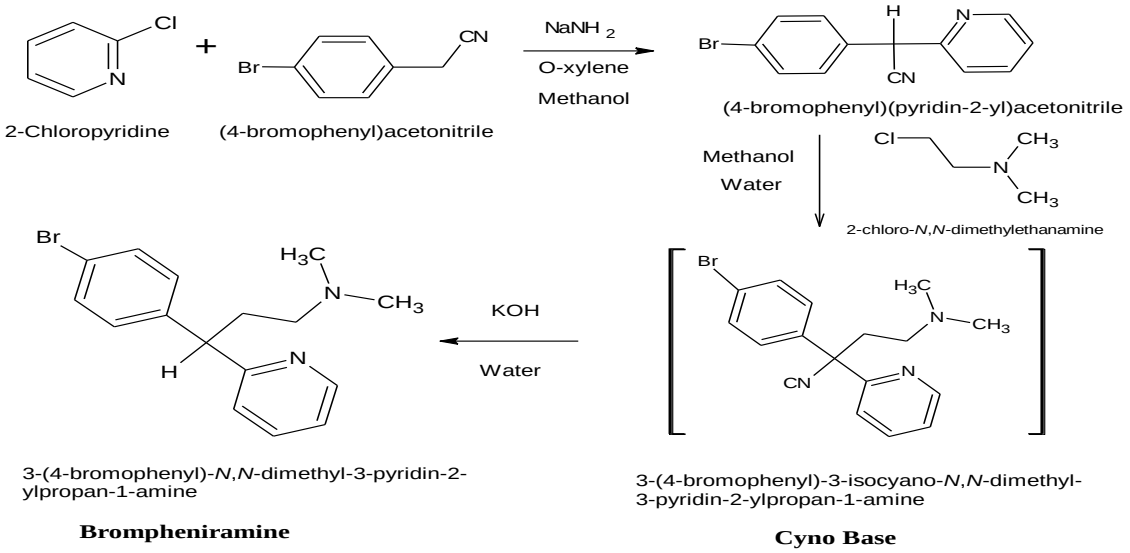


Fig. 1: Reaction Scheme of Brompheniramine Maleate

Quantification at such low level can be possible only by using GCHS or LC and also there is no method for the quantification of this impurity hence a high sensitive GCHS method developed for the identification and quantification of such genotoxic impurities 2-Chloropyridine and 4-Bromobenzyl Cyanide.

MATERIAL AND METHODS

Chemicals and Reagents: Samples of Brompheniramine Maleate, 2-Chloropyridine and 4-Bromobenzyl Cyanide (Fig. 2) were collected from Supriya Lifescience Ltd., Maharashtra, India. GCHS grade Methylene Dichloride was purchased from Advent, Mumbai, India.

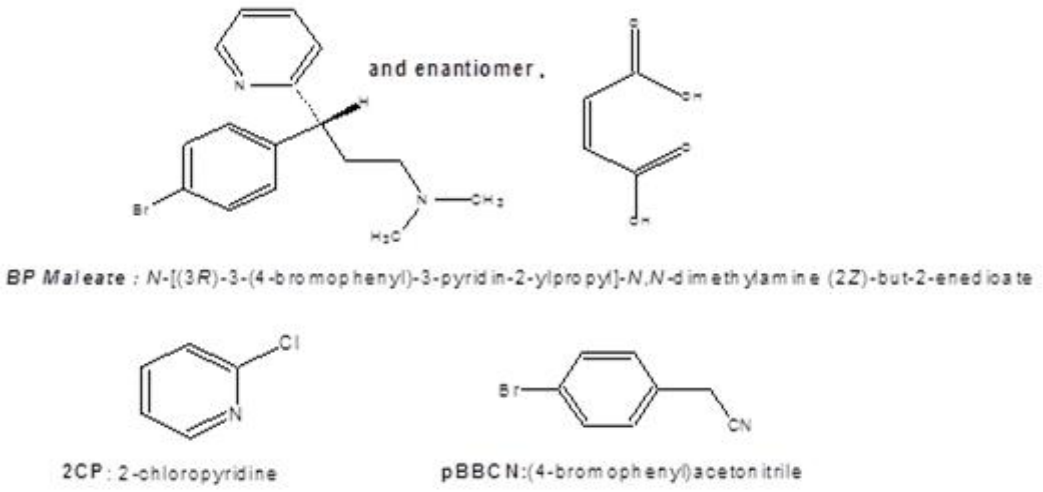


Fig. 2: Structures of Brompheniramine Maleate, 2-Chloropyridine and 4-Bromobenzyl Cyanide.

Equipment: The GCHS method development and validation were performed using Shimadzu –GC2010 plus and Agilent – 7890B using FID detector Connected with Head space system. The data were collected using GCsolution Chromatography Data System Software.

GCHS Chromatographic Conditions: The GC chromatographic separations were achieved on Shimadzu- GC2010 plus and Agilent – 7890B Gas Chromatograph with using FID detector. The GC was equipped with a capillary column (Gs-Tek, GsBP, 30 m x 0.32 mm ID x 0.5 µm). Nitrogen was employed as the carrier gas with split ratio 5:0, column flow 3.62 ml/min, purge flow 2.0ml/min. Injection volume 1 µl.The injector and detector temperature was 250ºC and the initial oven temperature was 70ºC, which was held for 2 minutes. The oven was ramped at 20ºC/min to 230ºC. The final temperature was held for 20 minutes for a total run time of 30.0 minutes. GCSolution Chromatography Data System Software was used to quantitatively analyze the samples [16].

Preparation of Genotoxic Impurity Standard and Test Sample Solution: The stock solution of impurities standard prepared at approximately 0.0375 mg/ml (37.5 ppm) in Methylene Dichloride. For limit of detection linearity, the stock solution impurity was diluted using diluent to give standards at 1.0, 2.0, 3.0, 4.0, 5.0 ppm with respect to test concentration. The testing API samples were typically prepared at approximately 10 mg/mL in Methylene Dichloride.

RESULT AND DISCUSSIONS

Linearity: The linearity of 2-Chloropyridine and 4-Bromobenzyl Cyanide was satisfactorily done. A series of solutions were prepared using2-Chloropyridine and 4-Bromobenzyl Cyanide at concentration levels from around quantification level to 150% and the concentration levels are 3.00, 18.75, 30.00, 37.50, 45.00, 56.25 ppm respectively. The peak area versus concentration data was done by linearity plot slope, intercept, and residual sum of squares analysis [17]. The calibration curve was given based on response over the concentration range for 2-Chloropyridine and 4-Bromobenzyl Cyanide. The correlation coefficient and 4-Bromobenzyl Cyanide was 0.9995 and 0.9994 respectively and the Linearity results are tabulated in table 1, Fig. 3 and 4.

Table 1: The regression analysis data for2-Chloropyridine and 4-Bromobenzyl Cyanide

Levels	2-Chloropyridine		4-Bromo benzyl cyanide	
	Conc. (ppm)	Response Area	Conc. (ppm)	Response Area
LOQ	3.00	32486	3.00	43529
50%	18.75	298040	18.75	299751
80%	30.00	459363	30.00	460897
100%	37.50	589045	37.50	598626
120%	45.00	713075	45.00	718834
150%	56.25	883633	56.25	890844
Slope		15973.38		15954.38
Correlation		0.9997		0.9997
Intercept		-11214.46		-4471.30
Residual sum of squares		**0.9995**		**0.9994**

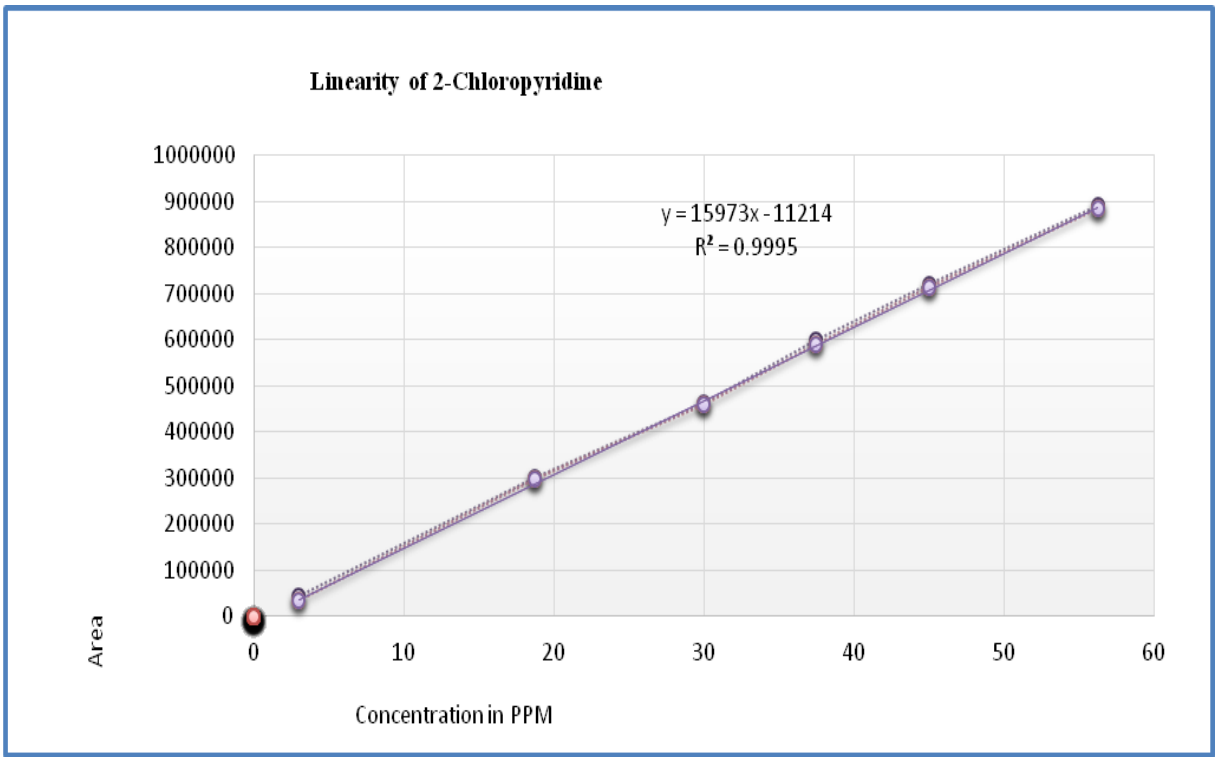


Fig. 3: Regression line of 2-Chloropyridine

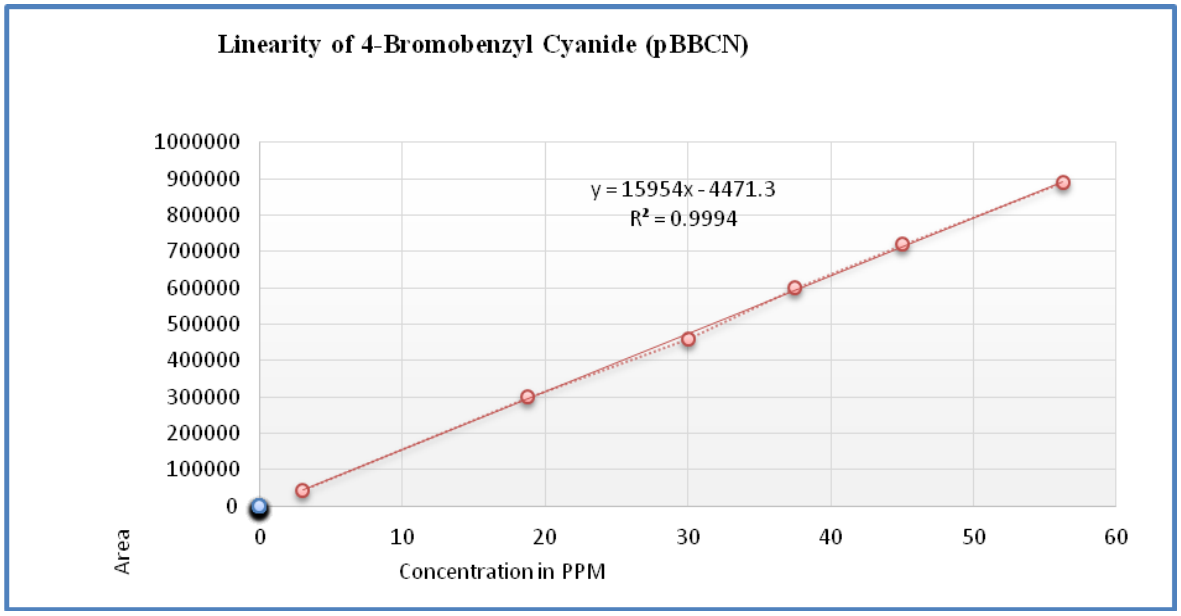
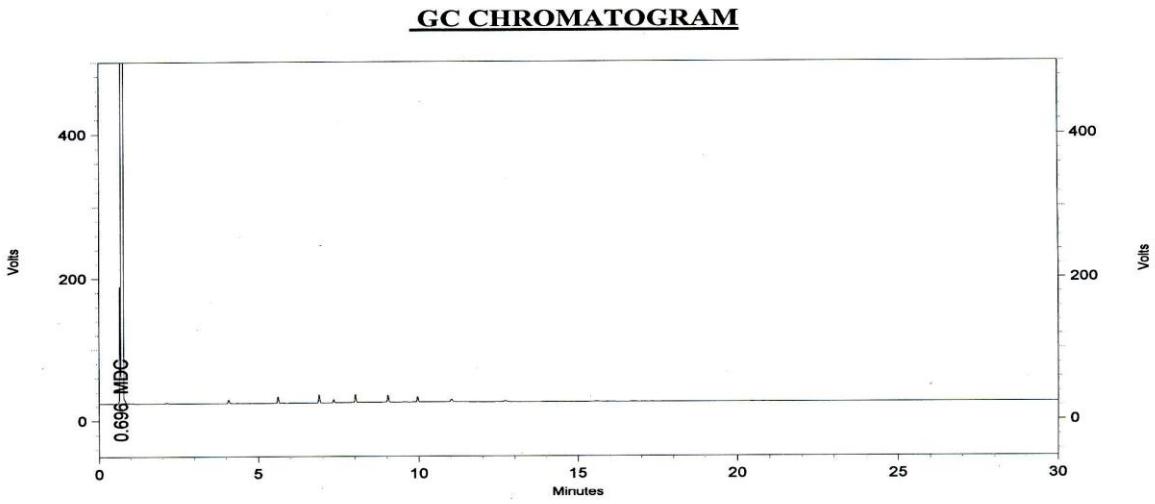


Fig. 4: Regression line of 4-Bromobenzyl Cyanide (pBBCN)

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ values of 2-Chloropyridine and 4-Bromobenzyl Cyanide were predicted from the predication linearity data [18]. Each predicted concentration was verified for precision by preparing the solutions at about predicted concentration and injecting each solution six times for GCHS study and the predicted concentration for LOQ was 3.0 ppm and LOD was 1.0 ppm respectively (Fig. 5 & 6) and the results are tabulated in table 2.

Table 2: Table for LOQ Precision

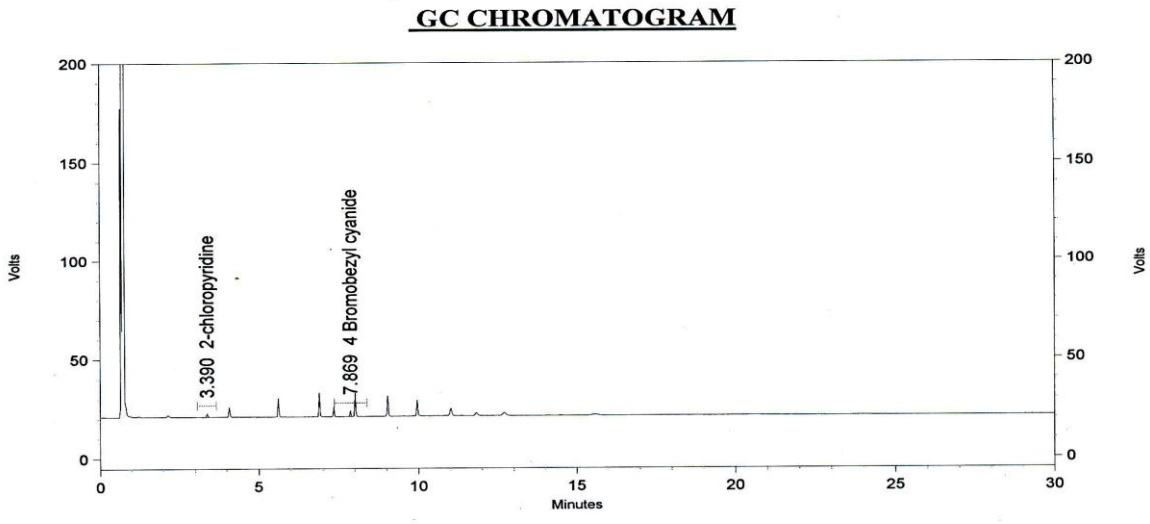
Injection No	Area of 2-Chloropyridine (3.0 ppm)	Area of 4-Bromobenzyl Cyanide (3.0 ppm)
1	33921	43900
2	32074	44562
3	33858	44522
4	34259	44210
5	34019	45257
6	33932	45526
Mean	**33677.17**	**44662.8**
SD	**797.824**	**618.9**
%RSD	**2.37**	**1.39**



Back Signal Results

Name	Retention Time	Area	Area Percent
MDC	0.70	4905871455	100.00
Totals		4905871455	100.00

Fig. 5: Typical HPLC Chromatograph of Blank

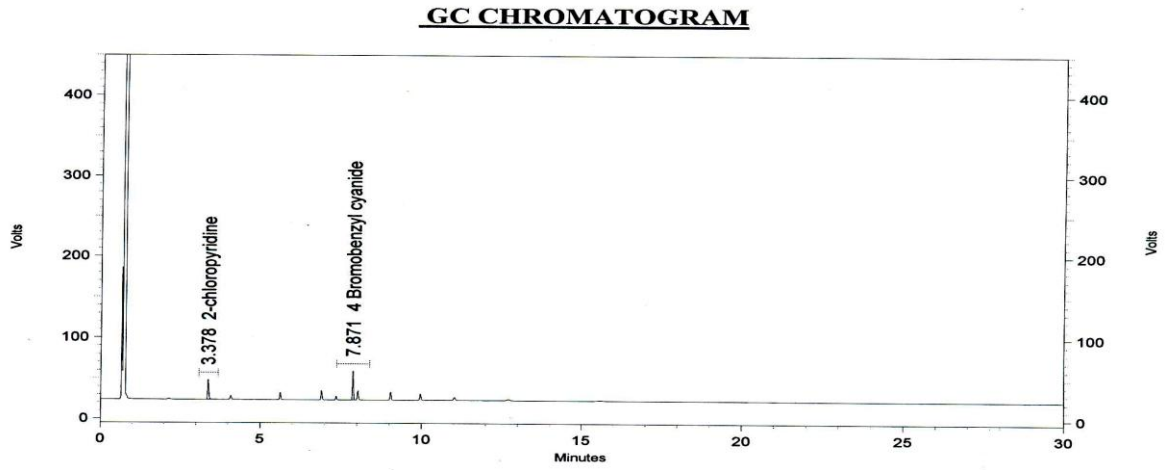


Back Signal Results

Name	Retention Time	Area	Area Percent
2-chloropyridine	3.39	33921	43.59
4 Bromobezyl cyanide	7.87	43900	56.41
Totals		77821	100.00

Fig. 6: Typical HPLC Chromatograph of LOQ

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 (**Fig. 7)** to show the system precision [19]. The % Relative Standard Deviation of the areas at each level for 2-chloropyridine is 2.44% and 1.93% and 4-Bromobenzyl Cyanide is 2.70% and 1.79% confirming developed that method is précised.



Back Signal Results

Name	Retention Time	Area	Area Percent	Asymmetry	Resolution (USP)
2-chloropyridine	3.38	465148	45.12	1.32	0.00
4 Bromobenzyl cyanide	7.87	565829	54.88	1.04	74.66
Totals		1030977	100.00		

Fig.7: Typical HPLC Chromatograph Standard

Accuracy: The accuracy of the method was evaluated in sample solutions were prepared in triplicate by spiking2-Chloropyridine and 4-Bromobenzyl Cyanide at LOQ level, 50%, 100% and 150% with Brompheniramine Maleate and injected each solution into GCHS as per methodology. The percentage of recovery for 2-Chloropyridine was calculated and the values are 101.35%, 108.23%, 106.03% and the percentage of recovery for 4-Bromobenzyl Cyanide was calculated and the values are 101.83%, 108.17%, 105.07%. At such low level, these recoveries and % Relative Standard Deviation Were satisfactory and the results are tabulated in table 3 and 4.

Table 3: % recoveries found for spiked 2-Chloropyridine (2CP) in Brompheniramine Maleate

Level	Qty. Added (ppm)	Qty. Recovered (ppm)	% Recovery	Mean % Recovery
80%-1	1.887	1.900	100.70	**101.35**
80%-2	1.887	1.915	101.50	
80%-3	1.887	1.910	101.20	
100%-1	3.775	4.058	107.50	**108.23**
100%-2	3.775	4.093	108.40	
100%-3	3.775	4.107	108.80	
120%-1	5.662	5.839	103.10	**106.03**
120%-2	5.662	6.094	107.60	
120%-3	5.662	6.083	107.40	
			Mean	**105.20**
			SD	**2.869**
			% RSD	**2.727**

Table 4: % recoveries found for spiked 4-Bromobenzyl Cyanide (pBBCN) in Brompheniramine Maleate

Level	Qty. Added (ppm)	Qty. Recovered (ppm)	% Recovery	Mean % Recovery
80%-1	1.887	1.90777	101.10	**101.83**
80%-2	1.887	1.92353	101.90	
80%-3	1.887	1.93431	102.50	
100%-1	3.774	4.06437	107.70	**108.17**
100%-2	3.774	4.07227	107.90	
100%-3	3.774	4.11088	108.90	
120%-1	5.660	5.72978	101.20	**105.07**
120%-2	5.660	5.99100	105.80	
120%-3	5.660	6.12529	108.20	
			Mean	**105.02**
			SD	**2.586**
			% RSD	**2.462**

SD= Standard Deviation, RSD= Relative Standard Deviation

CONCLUSION

On the basis of above study conducted, reported method is sensitive specific, accurate, validated and well defined GCHS method for the Identification and Quantification of genotoxic impurities 2-Chloropyridine and 4-Bromobenzyl Cyanide simultaneously in single method at ppm level in Brompheniramine Maleate. The detection limit and quantification limit found to be 1.0 ppm and 3.0 ppm respectively. The described method is highly reliable technique for the identification and quantification of the carryover key starting material genotoxic impurities present in the Brompheniramine Maleate during quality control testing.

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