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RESEARCH ARTICLE

Identification and synthesis of process related unspecified impurities of betahistine dihydrochloride.

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Manuscript Info *Abstract*

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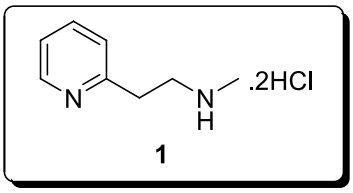
Herein, we describe the syntheses and characterization of process related impurities of anti-vertigo drug betahistine dihydrochloride. We have identified seven impurities during the synthesis of betahistine dihydrochloride from 2-pyridineethanol. Among that, four impurities such as *N*-acetyl betahistine, 2-(pyridine-2-yl)ethyl acetate, *N*-methyl betahistine and N-methyl-2-(pyridine-2-yl)prop-2-en-1-amine are not reported in the literature. In this article, we describe the origin, syntheses and characterization of three of these unspecified impurities.

Introduction:-

In organic chemistry, purity of the material is more important criteria to define quality of substance. But in the case of pharmaceutical industry, impurity profile of the Active Pharmaceutical Ingredients (API) is essential as per various regulatory authorities because impurities may be highly toxic to the human bodies. Impurity may have formed during the manufacturing process due to either undesired reaction or degradation of the active substances. Control and elimination of impurities during the development of API is very difficult task to accomplish.

Betahistine dihydrochloride (**1**) is a pyridine derived anti-vertigo drug that closely resembles the amino acid, histamine (Figure-1).[1]

Figure-1: Chemical structure ofBetahistine dihydrochloride

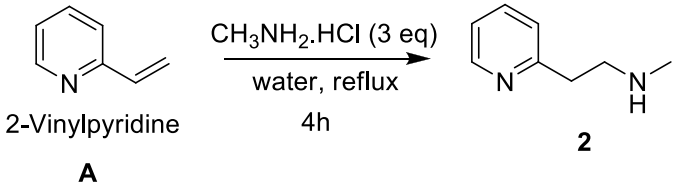


1

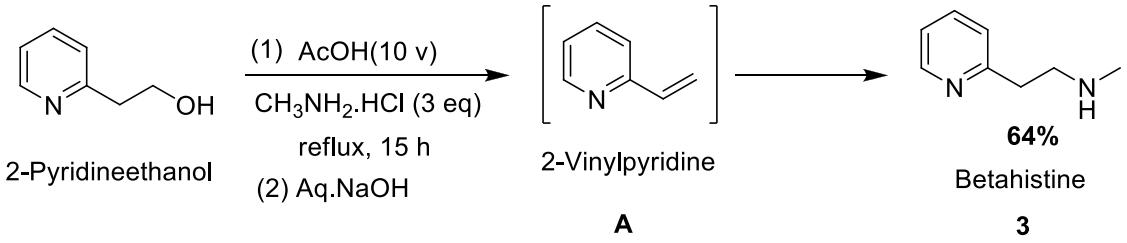
Betahistine dihydrochloride was approved by FDA (Food and Drug Administration) in 1960 and it was the first drug prescribed to treat vertigo associated with Meniere's disease in US and also commonly used for patients with balance disorders.[2] It has two modes of action – agonistic effect of H_1 receptors and antagonistic effect of H_3 receptors. The chemical synthesis of betahistine was studied extensively. Loffler first reported the synthesis of betahistine from 2-methylpyridine.[3] Subsequently, numbers of methods have been reported for the preparation of

betahistine and its salts.[4-8] Commercial production, kilogram scale, of betahistine dihydrochloride was executed by the reaction between 2-vinylpyridine and methylamine or methylamine hydrochloride followed by salt formations (Scheme-1). Ivano *et al*[9] described one-pot synthesis of betahistine from 2-pyridineethanol and methylamine hydrochloride. Here, this reaction involves *in-situ* preparation of 2-vinylpyridine from 2-pyridineethanol to minimize the polymerization of 2-vinylpyridine (Scheme-2). Unfortunately this method also gave moderate yield of betahistine, about 64%.

Scheme-1: General method of manufacturing of betahistine:-

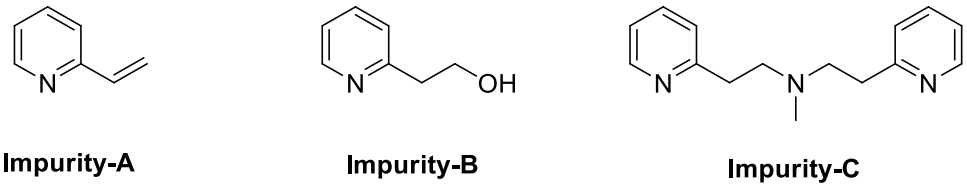


Scheme-2: Synthesis of betahistine by Ivano method:-



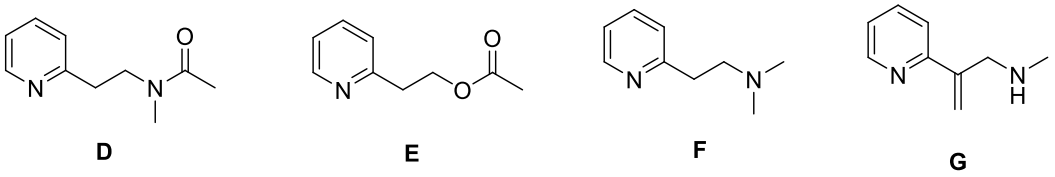
Further, Ivano *et al*did not discuss about the impurity profile of this process. However, Ivano's process could be convenient than other reported methods. So Ivano strategy could be modified to improve the yield and quality of the product. Thus, we planned to re-optimize the Ivano condition. The Ivano condition was modified by varying the temperature, duration, mol. improved from 64% to 85% with >99.9% purity. The modified reaction conditions are not presented here but we confirmed the yield and purity of betahistine dihydrochloride in 50 kg scale. During the process optimization we have found the formation of four process related impurities which are not listed in the pharmacopoeial monograph of betahistine dihydrochloride. Generally, impurity profiling of the drug substance is essential as per regulatory requirements and the allowed limits of unspecified impurities are ≤0.10%. The specified impurities of Betahistine dihydrochloride, listed in the pharmacopoeial monograph (European, US and IP) are 2-vinylpyridine (Impurity-A, limit: ≤0.2%), 2-pyridineethanol (Impurity-B, limit: ≤0.2%) and *N*-methyl-2-(pyridine-2-yl)-*N*-[(pyridin-2-yl)ethyl]ethanamine (Impurity-C, limit: ≤0.2%)[10] (Figure-2).

Figure-2: Pharmacopoeia listed impurities of betahistine dihydrochloride



In this reaction condition, we identified the formation of four new impurities (Impurity-**D**, Impurity-**E**, Impurity-**F** and Impurity-**G**) (Figure-3) in addition to the specified impurities (Impurity-**A**, Impurity-**B** and Impurity-**C**).

Figure-3: Unspecified impurities of betahistine dihydrochloride



In this article, we wish to present the origin, syntheses and characterization of these unspecified impurities (Impurity D to F).

Materials and methods:-

All reagents and solvents employed were of commercial grade and were used as such, unless otherwise specified. TLC was performed on Kieselgel 60 F254 silica-coated aluminium plates (Merck) and visualized by UV light (λ = 254 nm). The IR spectra were obtained on a PerkinElmer L1600300 Spectrum. HPLC analysis performed on Agilent Technologies, 1260 Infinity/waters 2695 separation module. NMR spectra were recorded with a Varian 400 MHz Mercury plus Spectrometer at 400 MHz. Chemical shift values are given in ppm relative to tetramethylsilane (TMS). LCMS were recorded on Waters Quattro premier XE triple quadrupole spectrometer using electron spray ionisation (ESI).

The Ph. Eur., USP and In-house HPLC chromatographic conditions are tabulated below,

Table-1: HPLC chromatographic condition

<table>
<tr><th></th><th>In-house</th><th>Ph. Eur.</th><th>USP</th></tr>
<tr><td>Column</td><td>Zorbax SB-Phenyl, 250x4.6mm, 5µ or equivalent</td><td>Eclipse XDB-C18, 150x3.0mm, 5µ or equivalent</td><td>Eclipse XDB-C18, 150x3.0mm, 5µ or equivalent</td></tr>
<tr><td>Flow rate</td><td>1.2 mL/min.</td><td>1 mL/min.</td><td>0.5 mL/min.</td></tr>
<tr><td>Injection volume</td><td>20 µL</td><td>20 µL</td><td>10µL</td></tr>
<tr><td>Diluent</td><td>Mobile phase</td><td>Mobile phase</td><td>Mobile phase</td></tr>
<tr><td>Test concentration</td><td>0.5 mg/ml</td><td>1.0 mg/ml</td><td>0.4 mg/ml</td></tr>
<tr><td>λ max</td><td>260 nm</td><td>260 nm</td><td>254 nm</td></tr>
<tr><td>Run time</td><td>40 min.</td><td>50 min.</td><td>50 min.</td></tr>
<tr><td>Preparation of Buffer</td><td>Preparation of Buffer
Dissolve about 0.69 g of ammonium acetate (0.01M) in 1000 ml of water, adjust pH 4.7 with glacial acetic acid.</td><td>Preparation of Buffer Dissolve 2.0 gr ofsodiumdodecyl sulfate in a mixture of 15 ml of a 10% v/v solution of sulfuric acid and 35 mL of a 595 mg solution of Tetrabutylammonium hydrogen sulfate and 650 mL of water,adjust to PH 3.3 using dilute 1N sodium hydroxide solution</td><td>Preparation of Buffer:
Dissolve about 0.69 g of ammonium acetate in 1000ml of water.Adjust with glacial acetic acid to a pH of 4.7.
.</td></tr>
<tr><td>Preparation of Mobilephase-A</td><td>To above prepared 650 ml of ammonium acetate add 2.88 gr of sodium lauryl sulphate and 350 mL of acetonitrile Isocratic) mix well and degas.</td><td>Use Buffer as solution Mobilephase-A</td><td>To above 650 ml of ammonium acetate add 2.88 gr of sodium lauryl sulphate.</td></tr>
<tr><td>Preparation of Mobilephase-B</td><td colspan="2">Use Acetonitrile as Mobilephase-B</td><td>Use Acetonitrile as Mobilephase-B</td></tr>
<tr><td>Preparation of Mobilephase</td><td>Gradient :
<table><tr><th>Time (min.)</th><th>Mobile phase A (%)</th><th>Mobile phase B (%)</th></tr><tr><td>0</td><td>100</td><td>0</td></tr><tr><td>22</td><td>100</td><td>0</td></tr><tr><td>25</td><td>25</td><td>75</td></tr><tr><td>32</td><td>25</td><td>75</td></tr><tr><td>35</td><td>100</td><td>0</td></tr><tr><td>40</td><td>100</td><td>0</td></tr></table></td><td>Isocratic :
Mix Mobilephase-A and Mobilephase-B in the ratio of 700:300 v/v degass and filter through 0.45µ</td><td>Isocratic :
Mix Mobilephase-A and Mobilephase-B in the ratio of 650:350 v/v degass and filter through 0.45µ</td></tr>
</table>

Preparation of N-acetyl betahistine (D):-

Acetic anhydride was added (22.5 g, 220 mmol) to a mixture of Betahistine (20 g, 147 mmol) and triethylamine (29.8 g, 293.5 mmol) in dichloromethane (100 mL) at 0-5 ^{o}C. The resulting mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with water (40 mL), separated the organic layer and concentrated to get crude compound. The crude compound was purified by column chromatography using 1% methanol in dichloromethane to give N-acetyl betahistine (D) pure product as light yellow oil. Yield 24.5 g (94%); Purity 99.2% by HPLC; ^{1}H NMR (400 MHz, DMSO) δ 1.81 (s, 3H), 1.94 (s, 3H), 2.77 (m, 3H); 2.86-2.89 (m, 6H), 2.99-2.96 (m, 3H), 3.57-3.65 (m, 6H), 7.30-7.22 (s, 4H); 7.69-7.71 (m, 2H); 8.5 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 20.73, 21.59, 22.38, 25.36, 32.34, 35.28, 35.84, 36.06, 38.87, 39.08, 39.29, 39.50, 39.71, 39.91, 40.13, 46.82, 49.72, 121.37, 121.622, 123.05, 123.47, 136.36, 136.48, 148.93, 149.09, 158.48, 159.04, 169.17, 169.39; IR (neat) 3013, 2935, 2855, 1633, 1570, 1476, 1435, 1404, 1301, 1261, 1168, 1152, 1128, 1095, 1036, 1008, 850, 782, 576, 510 cm^{-1};ESI MS (m/z) 179.20 (M+1).

Preparation of 2-(Pyridin-2-yl)ethyl acetate (E):-

Acetic anhydride was added (2.5 g, 24.3 mmol) to a mixture of 2-pyridineethanol (2 g, 16.2 mmol) and triethylamine (3.3 g, 32.4 mmol) in dichloromethane (20 mL) at 15-20 ^{o}C. The resulting mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with ethyl acetate (150 mL), washed with water (3 x 100 mL) and concentrated to get crude compound. The crude compound was purified by column chromatography using 1% methanol in dichloromethane to give 2-(Pyridin-2-yl)ethyl acetate (E) pure product as light yellow oil. Yield 2.3 g (86%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3H), 3.09 – 3.12 (t, 2H), 4.44 – 4.47 (t, 2H); 7.11-7.19 (m, 2H); 7.58-7.62 (td, 1H); 8.5 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.25, 29.13, 36.69, 62.96, 121.10, 122.87, 135.90, 148.80, 157.46, 170.20; IR (neat) 1739, 1593, 1571, 1475, 1437, 1365, 1241 cm^{-1}; ESI MS (m/z) 166.1 (M+1).

Synthesis of N-methyl betahistine (F):-

2-(2-Hydroxyethyl)pyridine (20 g, 0.162 mmol) was added to a mixture of dimethylamine hydrochloride (40 g, 0.487 mmol) in acetic acid (240 ml) at room temperature. The mixture was heated to reflux at 120-125 ^{o}C for 15 hours. The resulting solution was cooled to 70 ^{o}C and concentrated under reduced pressure. The residue was dissolved in water; pH was adjusted to 11.0 using aqueous sodium hydroxide solution and extracted with dichloromethane (4x100 mL). The combined dichloromethane layer concentrated under vacuum. The crude compound was purified by column chromatography using 5% methanol in dichloromethane to give N-methyl betahistine (F) as yellow oil. It was converted into its dihydrochloride salt by treating with 16% ethanolic HCl solution to obtain off-white powder with purity 99.47% by HPLC. ^{1}H NMR (400 MHz, D_2O) δ 3.07 (s, 6H); 3.71-3.64 (m, 4H), 8.07-8.03 (m, 2H), 8.64-8.60 (m, 1H), 8.79-8.78 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 28.22, 43.13, 54.99, 126.14, 127.71, 141.77, 147.59, 150.45; IR (KBr) 3038, 2961, 2706, 2466, 1622, 1542, 1475, 1416, 1306, 1244, 1169, 1050, 964 787, 766, 627 cm^{-1}; ESI MS (m/z) 151.05 (M+1).

Betahistine dihydrochloride (1):-

White crystalline solid; mp 151-153 ^{o}C; ^{1}H NMR (400 MHz, DMSO) δ 2.56 (s, 3H); 3.41-3.53 (m, 4H), 7.90-8.03 (m, 2H), 8.47 (br s, 1H), 8.81 (br s, 1H), 9.64 (br s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 29.11, 32.14, 46.28, 125.29, 127.45, 142.03, 145.52, 152.27; IR (KBr) 3423, 2959, 2771, 2043, 1621, 1544,1471, 1307, 1249, 1167, 1051, 771cm^{-1}; ESI MS (m/z) 137.0 (M+1).

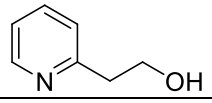
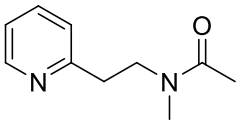
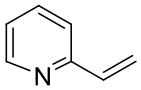
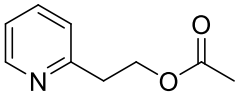
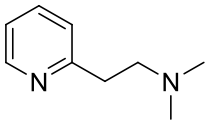
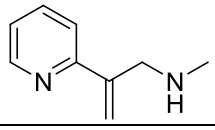
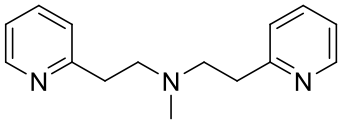
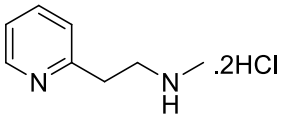
Results and discussion:-

Initially we have performed the reaction for the preparation of betahistine as described by Ivano *et al*. According to that, a mixture of 2-pyridineethanol, methylamine hydrochloride and acetic acid was refluxed at 120 ^{o}C for 15 hours. The conversion of the reaction was monitored by HPLC. The reaction mass was analyzed by both EP and USP pharmacopoeial methods. Analyses by both methods showed the product formation about 77%. However it differs completely in impurity profile. In Ph. Eur. HPLC method, the area of 2-pyridineethanol (A) is 6.71% (RRT 0.21) whereas USP method shows about 0.67% (RRT 0.15) but it shows another impurity very close to 2-pyridineethanol at RRT (Relative Retention Time) 0.16 is 4.59%. RRT value of 2-pyridineethanol should be 0.16 and RRT value of another impurity very close to 2-pyridineethanol should be 0.15 according to the USP method. To understand this discrepancy, we analyze the sample with another HPLC method which was developed by our laboratory. The HPLC condition and mobile phase gradient are tabulated in the experimental section (Table-1).

In this HPLC condition, all the peaks are resolved including the peaks which are not resolved in pharmacopoeial HPLC conditions. In this condition, 2-pyridineethanol content is 0.62% (RRT 0.37). It showed that, 2-pyridineethanol was merged with an impurity in Ph. Eur. HPLC condition.

ESI-MS analysis of the crude reaction mixture shows the abundant peaks at 106.1, 124.1, 136.9, 149.1, 151.1, 166.1, 179.1 and 242.1. Based on the LC-MS report, possible structures of these impurities were derived (Table-2). In our optimized reaction condition, the amount of formation of these impurities are lesser than the Ivano condition and also the formation of betahistine is increased from 77% to 89% (Table-2, entry-8). These impurities were prepared and its structure was further confirmed by NMR, IR and Mass spectroscopy.

Table-2: Possible impurities derived from LC-MS

Entry	Content of Ivano condition (by HPLC)	Content of our condition (by HPLC)	Mol. wt. (LC-MS)[¶]	Chemical structure
1	0.62%	0.75%	124.1	
2	5.12%	6.0%	179.1	
3	6.04%	0.65%	106.1	
4	1.80%	0.48%	166.0	
5	0.06%	0.07%	151.2	
6	0.09%	0.28%	149.2	
7	8.76%	2.46%	242.3	
8	77.19%	89.2%	136.1	

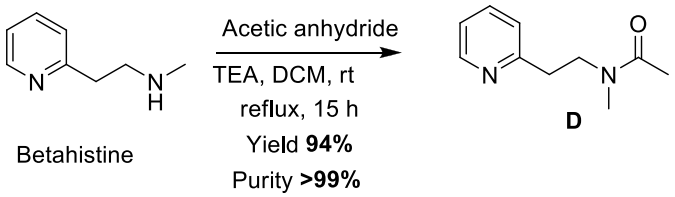
[¶]Ionisation mode – positive

The specified impurities 2-vinylpyridine (Impurity-**A**, entry-3), 2-pyridineethanol (Impurity-**B**, entry-1) and *N*-methyl-2-(pyridine-2-yl)-*N*-[(pyridin-2-yl)ethyl]ethanamine (Impurity-**C**, entry-7) were prepared or isolated from the reaction mixture. Its structure was confirmed by NMR spectroscopy and also matched with RT values of their reference material in HPLC.

All the other impurities were synthesized and structures were elucidated by using NMR, mass and IR spectroscopy.

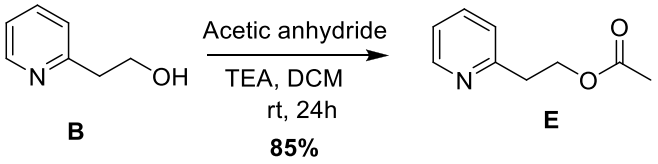
The impurity **D** (*N*-acetyl betahistine) was prepared from Betahistine as described in scheme-3. Betahistine was treated with acetic anhydride in the presence of triethylamine, which yielded (*N*-acetyl betahistine) **D** in 94 % yield. The impurity **D** is formed from betahistine by N-acetylation reaction with acetic acid during the preparation of betahistine.

Scheme-3: Isolation of *N*-acetyl betahistine (D):-



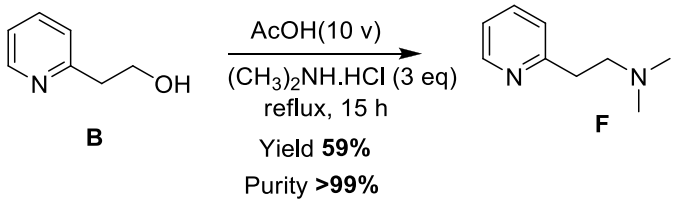
The impurity **E** (2-[pyridine-2-yl]ethyl acetate) was prepared from 2-pyridineethanol as described in scheme-4. Pyridine-2-ethanol was treated with acetic anhydride in the presence of triethylamine, which yielded 2-[pyridine-2-yl]ethyl acetate (**E**) in 85% yield. The impurity **E** is formed from 2-pyridineethanol by O-acetylation reaction with acetic acid during the preparation of betahistine

Scheme-4: Synthesis of 2-(pyridine-2-yl)ethyl acetate (E):-



The impurity **F** (*N*-methyl betahistine) was prepared from the reaction of 2-pyridineethanol and dimethylamine hydrochloride. The product **F** was isolated in 59% yield and >99% purity which shown in scheme-5.

Scheme-5: Synthesis of *N*-methyl betahistine (F):-



The contamination of dimethylamine hydrochloride in methylamine hydrochloride leads to the formation of N-methyl betahistine, **F**. According to the literature, dimethylamine hydrochloride is formed as a impurity by-product during the synthesis of methylamine hydrochloride from formaldehyde and ammonium chloride.[11] We checked the dimethylamine hydrochloride content in methylamine hydrochloride from three different sources (Commercial and LR grade) and the results are shown in Table-3. Both commercial and LR grade material of methylamine hydrochloride contains approximately 0.1% of dimethylamine hydrochloride.

Table-3: Analytical results of methylamine hydrochloride from different sources

S.No	Source	Methylamine hydrochloride assay	Dimethylamine hydrochloride content by Ion chromatography
1	Source-1	99.02%	0.10%
2	Source-2	99.10%	0.12%
3	Source-3	99.05%	0.11%

We have procured three different lots of 2-pyridineethanol from the commercial supplier and its quality was studied by GC. We have found an impurity with 0.6% and GC-MS analysis showed that molecular weight of 134.1 (ionization mode: negative). The quality data of these materials was shown in table-4

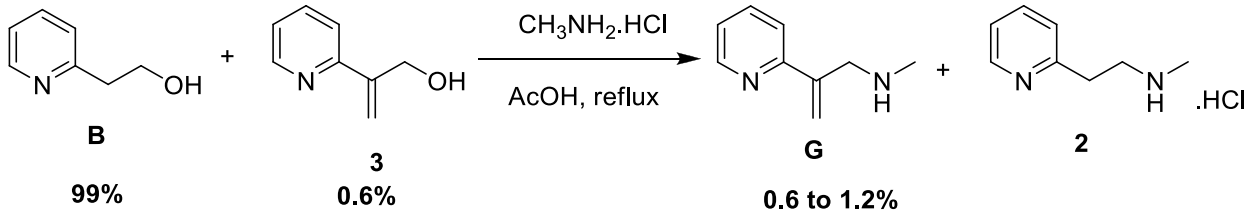
Table-4: GC analysis of 2-Pyridineethanol

S. No.	Lot #	Purity by GC	Unknown impurity[¶] (GC: RRT 1.05)
1	Lot-1	99.04	0.63
2	Lot-2	99.21	0.53
3	Lot-3	99.21	0.55

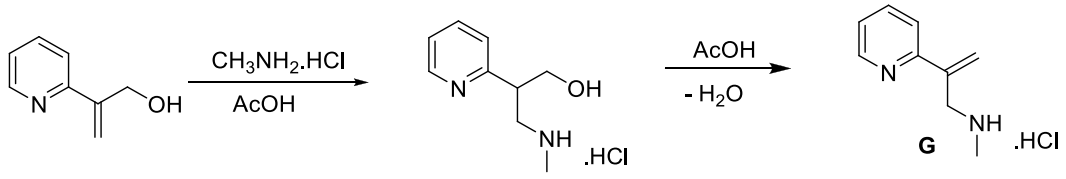
[¶]2-(pyridine-2-yl)prop-2-en-1-ol

The preparation of Betahistine was carried out with the modified process which provided 0.6 to 1.2% of a new unspecified impurity (impurity **G**). LC-MS analysis of the reaction mixture showed the molecular weight of this impurity is 149.1. By correlating these two molecular weights, we concluded that the impurity present in starting material leads to the formation of new unspecified impurity during the synthesis of Betahistine. Both the impurity structures (starting material and reaction mixture) were proposed based on the route of synthesis of starting material, 2-pyridineethanol, and molecular weight (from GC-MS & LC-MS). The possible impurity present in the starting material (2-pyridineethanol) is 2-(pyridine-2-yl)prop-2-en-1-ol (**3**) which leads to the formation of impurity **G** during the synthesis of **2**. The probable reaction sequence and mechanism are proposed in scheme-6 and scheme-**7** respectively

Scheme-6: Formation of 2-(1-methylazetidin-3-yl)pyridine (G):-



Scheme-7: Mechanism of formation of G:-



Conclusion:-

In conclusion, we have identified the four different process related non-identified impurities of anti-vertigo drug Betahistine dihydrochloride synthesized from 2-pyridineethanol. These impurities are synthesized and their structures are fully characterized by spectroscopic methods. Also, the origins of these impurities are studied.

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