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A Sensitive and Selective GC-MS Analysis of Process Related Genotoxic Impurities of Nebivolol Hydrochloride

VIJAYA LAKSHMI MADDALA*, P.C. RAY, K. VENUGOPAL and K.M.V.N. RAO

Inogent Laboratories Private Limited, Plot No. 28 A, Street No. 15, IDA Nacharam, Hyderabad-500 076, India

*Corresponding author: E-mail: vijaya_chem@yahoo.com

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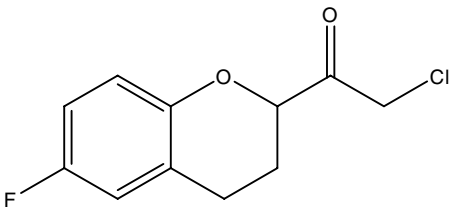
A sensitive GC-MS method has been developed and validated for the estimation of genotoxic impurities namely 2-chloro-1-(6-fluorochroman 2-yl)ethanone (**1**), 2-chloro-1-(6-fluorochroman 2-yl)ethanol (**2**) and 6-fluoro2-(oxiran2-yl)chroman (**3**) in nebivolol hydrochloride drug substance. Under optimized conditions of the proposed method is specific, precise, linear, accurate and rugged. The response was linear in the concentration range of limit of quantification to 0.07 mg g^{-1} with correlation coefficients of > 0.99 for each impurity. Under optimum conditions used for the sample preparation the recovery of each impurity is in between 70-130 %.

Keywords: Genotoxic impurities, Nebivolol, GC-MS.

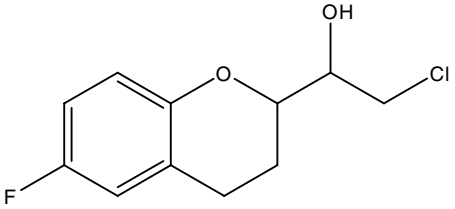
INTRODUCTION

Nebivolol [1-3] is used for the treatment of hypertension through vascular endothelial nitric oxide releasing capabilities and β1-antagonist action. It is highly cardio selective under certain circumstances. Nebivolol is an unique as a β-blocker. Nebivolol [2] lowers blood pressure (BP) by reducing peripheral vascular resistance and significantly increases stroke volume with preservation of cardiac output. The net hemodynamic effect of nebivolol is the result of a balance between the depressant effects of β-blockade and an action that maintains cardiac output. Antihypertensive responses were significantly higher with nebivolol [4] than with placebo in trials enrolling patient groups considered representative of the U.S. hypertensive population, in Black patients and in those receiving concurrent treatment with other antihypertensive drugs.

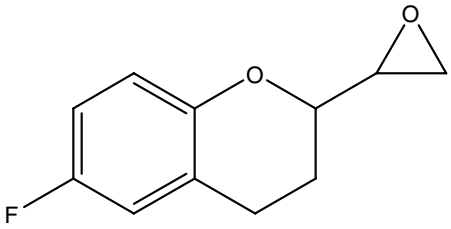
According to the current regulatory guidelines, it is important that the genotoxic impurities [5] have the potential damage of the DNA at very low level of exposure. Genotoxic substances are the chemicals that harm an organism by damaging its genetic material (DNA). There are three primary effects that genotoxins can have on organisms by effecting their genetic information. Genotoxins can be carcinogens, or cancer causing agents, mutagens or mutations- causing agents or teratogens, birth defect causing agents. Thus, potential impurities most likely arise during synthesis, purification and storage should be identified. These potential presence of these genotoxins has attracted by the regulator agencies like European medicines agencies (EMEA) committee [5] for the medicinal products for human use, USFDA [6] has published the guidelines regarding the limits of Genotoxic impurities. Both EMEA guidelines and a phRMA propose a maximum of daily exposure target of 1.5 µg per day Genotoxic impurities [7,8] in pharmaceuticals is recommended in these guidelines. Based on the threshold of toxicological concern (TTC), the concentration limits [9] of the impurity in a drug substances or drug products can be derived based on the maximum daily dose: Concentration (ppm) = [1.5 µg/day]/[dose (g/day)]. For a drug dosed at 1 g per day, for example, 1.5 ppm would be the limit of that specific genotoxic impurity.



2-chloro-1-(6-fluorochroman-2-yl)ethanone



2-chloro-1-(6-fluorochroman-2-yl)ethanol



6-fluoro-2-(oxiran-2-yl)chroman

Based on the structure, these are the three genotoxic impurities present in the nebivolol hydrochloride. An approach based on GC-MS [10] is feasible within the limits of time, ease of application and sensitivity, cost also. Despite the importance of the issue, based on literature survey there is no method to estimate all these genotoxic impurities. These impurities were prepared in Inogent laboratories private limited, Nacharam, Hyderabad, India.

EXPERIMENTAL

Active pharmaceutical ingredient standards and samples were supplied by Inogent Laboratories private Limited, Hyderabad, India. The HPLC grade Toluene was purchased from Merck, Darmstadt, Germany.

Chromatographic conditions and equipment: Mutagenic impurities [5,6] are separated by GC-MS on DB-5 ms 30 m × 0.25 mm, 0.25 µ column, Samples and standards are prepared in toluene in the system Agilent 7890B Gas chromatograph equipped with 5977A mass selective detector. The oven programme consists of Initial the oven is programmed at 100 °C for 3 min, then increased at the rate of 8 °C/min upto 140 °C, hold the temperature at 140 °C for 5 min, then increased at the rate of 10 °C/min upto 160 °C, hold the temperature at 160 °C for 5 min, increased at the rate of 15 °C/min upto 250 °C, hold the temperature at 250 °C for 2 min, injector temperatue is put at 200 °C, auxiliary temperature at 280 °C. The carrier gas is helium (high purity 99.999 %), at a constant flow rate of 1.4 mL/min and the split ratio is 1:10. having the injection volume 2.0 µL, the total runtime of 28 min.

The ion source in the mass spectrophotometer is the Electron impact ionization, in which the acquisition mode is selective ion monitoring (SIM), having the source temperature at 230 °C and quadrapole temperature at 150 °C, solvent delay time is put for 13.3 min. The selective ion monitoring parameters are group id is 1, high resolution and the ions/dwell time are 149/100, 151/100, 194/100, 228/100, 230/100.

Preparation of the stock solutions: An individual stock solution (100 µg mL^{-1}) of all impurities was prepared in diluent (toluene). Sample solution was prepared (10 mg mL^{-1}) in diluent. Limit of detection and limit of quantification for the impurities were evaluate by preparing the impurities in the level from 5 to 25 µg mL^{-1}. Linearity of each impurity is determined from LOQ level to 70 µg mL^{-1}.

RESULTS AND DISCUSSION

Method development was planned for the estimation of the above mentioned genotoxic impurities in nebivolol hydrochloride by using gas chromatographic technique with mass spectrophotometer as detector, as evaluation limit was as low as 5 µg mL^{-1}. Different columns with different stationary phases and dimensions were used like DB-624, DB-1, RTX-624 and RTX-1301 for the analysis. Finally separation is achieved on DB-5 column, mid polar column with 5 % phenyl and 95 % dimethyl polysiloxane as a stationary phase. During the method development using this column, appropriate separation is achieved between the impurities.

Method validation: Method validation activity was conducted with necessary parameters as per the analytical method validation guidelines of International conference on Harmonization. As FCE-02, FCE-03 are stereo isomers, will appear as two peaks.

Specificity: Specificity is done satisfactorily by checking the all impurities individually and with spiked solution, confirmed that there is no interference of any impurity.

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection and limit of quantification values for each impurity were predicted by using the series of solutions from 5, 10, 15, 20 and 25 ppm respectively by using standard deviation of the response and slope method. The limit of detection and limit of quantification results are shown in Table-1.

TABLE-1
LOD/LOQ

Concentration (ppm)	FCE-01 peak area	FCE-02 peak area	FCE-03 peak area
5	15876	14013	19160
10	33163	30277	37468
15	49188	42862	54636
20	63375	55292	72392
25	76942	68703	85571
STEYX	1441.74	1252.56	1687.13
SLOPE	3046.88	2687.90	3354.92
LOD	1.6	1.5	1.7
LOQ	4.7	4.7	5.0

Linearity: Linearity of the three impurities were satisfactorily done. A series of solutions were prepared at test concentration levels from around limit of quantification level to 70 ppm level. The peak area *versus* concentration data was done by linearity plot slope, intercept and residual sum of squares analysis. The calibration curve given based on response over the concentration range of the impurities. The results are tabulated in Table-2.

TABLE-2
LINEARITY

Concentration (ppm)	Average area		
	FCE01	FCE02	FCE03
4.7	10465	9607	13035
20	43596	39806	51322
30	63387	57895	71388
40	86536	78200	97487
50	107983	102179	123335
60	139132	125964	158903
70	166227	151980	189039
Correlation coefficient	1.00	1.00	1.00
Slope	2368.33	2168.79	2678.33
Intercept	-4750.56	137.53	776.29

Precision: The precision of the developed method was checked by preparing the solutions by spiking the impurities at 100 % level with the drug substance for six times and injected each once also injected 100 % spiked solution six times to show that the system is precise. The %Relative standard deviation of the areas at each level is less than 8 % confirming the good precision.

Accuracy: The accuracy of the method was evaluated in sample solutions were prepared in triplicate by spiking all impurities at LOQ level, 20, 35 and 60 ppm. The % recoveries of the impurities are in Table-3.

TABLE-3
ACCURACY

Accuracy level	Recovery (%)		
	FCE-01	FCE-02	FCE-03
4.7 ppm (LOQ)	100.0	100.5	100.5
20 ppm	99.9	100.7	101.6
35 ppm	100.8	101.8	105.4
60 ppm	101.2	100.4	102.3

Conclusion

In this paper a sensitive specific, accurate, validated and well defined GC-MS method for the quantification of genotoxic impurities in nebivolol hydrochloride at ppm level was described. The described method is highly reliable technique for the quantification of impurities present in the nebivolol hydrochloride.

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