WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 10, 635-643. Research Article ISSN 2277– 7105

SIMULTANEOUS ESTIMATION OF BENZYL CHLORIDE AND BENZYL BROMIDE IN ENTECAVIR BY USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Vidyagauri V. Lele*[1] and Uttam Prabhanand Dalvi[2]

[1,2]Department of Chemistry, N. G. Acharya and D. K. Marathe College, Chembur, Mumbai-400071, India.

Article Received on
26 July 2016,

Revised on 16 August 2016,
Accepted on 06 Sep. 2016

DOI: 10.20959/wjpr201610-7069

***Corresponding Author**
Dr. Vidyagauri V. Lele
Department of Chemistry, N. G. Acharya and D. K. Marathe College, Chembur, Mumbai-400071, India.

ABSTRACT

A highly sensitive analytical method for the simultaneous quantification of trace level impurities of Benzyl chloride and Benzyl bromide in the active pharmaceutical ingredient Entecavir has been developed. These genotoxic impurities have demonstrated to induce genetic mutation, chromosomal breaks or chromosomal rearrangement and have potential to cause cancer. The analysis was accomplished on an Inertsil poroshell C18 column (50mm x 4.6mm, 3µ) using phosphate buffer and acetonitrile as the organic solvent. The flow rate was set at 1.2 mL/ minute with a total analysis time of about 6 minutes. The method was validated for the analytical parameters such as system suitability, specificity, linearity and range, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, and solution stability. The limit of detection and limit of quantification were found to be 0.05µg/mL and 0.2µg/mL respectively with respect to Entecavir sample concentration (2µg/mL).

KEYWORD: Benzyl bromide, Benzyl chloride, Mutation, Genotoxic, Antiviral.

1. INTRODUCTION

Entecavir (Fig.1) is an antiviral drug used in the treatment of hepatitis B virus (HBV) infection. It is a reverse transcriptase inhibitor. It prevents the hepatitis B virus from multiplying in the body. Benzyl halides are the common reagents used as alkylating reagent in the synthesis of the Entecavir.[1-4] Most of the benzyl halides cause mutation and are considered to be genotoxic.[5] Genotoxic impurities have demonstrated to induce genetic mutation, chromosomal breaks or chromosomal rearrangement and have potential to cause

cancer.[6] Benzyl halide impurities in active pharmaceutical ingredients are responsible for deleterious action on a cell's genetic material affecting its integrity. Therefore, exposure to even low levels of such impurities in final active pharmaceutical ingredient (API) may be of significant toxicological concern. Hence it is necessary to control these impurities to the lowest ppm level in the API so that the API can be considered safe to the administered.[6, 7]

The potential presence of these genotoxins has attracted the attention of regulatory authorities. European Medicines Agency's (EMEA) Committee for Medicinal products for Human use (CHMP) has published guidelines regarding limits of genotoxic impurities.[6, 7] In 2008, US FDA has also come up with the draft guidelines on genotoxic and carcinogenic impurities in drug substances and products. These guidelines describe the potential lifetime cancer risk associated with patient's exposure to genotoxic and carcinogenic impurities and the ways to reduce them. Both the PhRMA white paper and EMEA guidelines recommend a maximum daily exposure target of 1.5 µg per day of genotoxic impurities in pharmaceuticals [acceptable Threshold of Toxicological Concern (TTC)].[6, 7] Benzyl bromide (Fig.2) and Benzyl chloride (Fig.3) are colorless liquids often used as alkylating reagent[1-3] for most of the pharmaceutical products have been notified as Potential Genotoxic Impurity (PGI). These molecules are highly reactive, widely used in chemical synthesis of intermediates and active pharmaceutical ingredients. These impurities are known carcinogens and can have an impact on product risk assessment if present in the final drug substance and drug product as an impurity.[6] Benzyl halides have been found at various hazardous waste sites of departments of defense in United States. They are designated as environmental contaminants causing adverse effects to public health and have been included in many National Priorities List (NPL) hazardous waste sites and federal facilities sites in the United States.[11]

The present research work is focused on development of a rapid, sensitive and accurate method for the determination of Benzyl bromide and Benzyl chloride in Pharmaceutical Products. Considering the allowable limit of 1.5 µg per day of benzyl halide, the concentration limit (ppm) of benzyl halide varies for different Pharmaceutical products based on the daily dose of the drug substance (g/day).[7] The method so developed was validated with respect to specificity, LOD, LOQ, linearity, precision, accuracy and solution stability. All these studies were performed in accordance with established ICH guidelines.[8, 9]

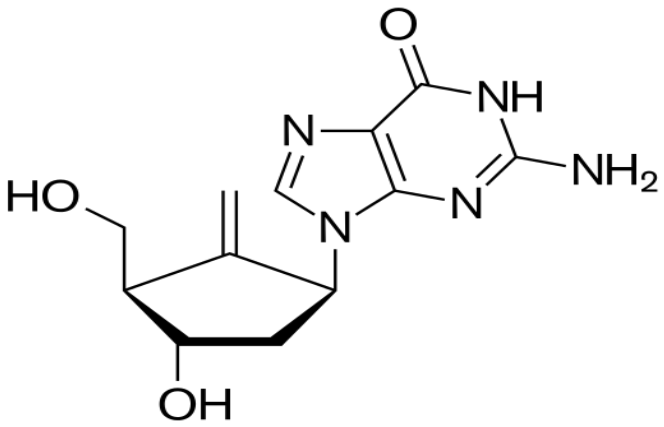

Fig. 1. Structure of Entecavir

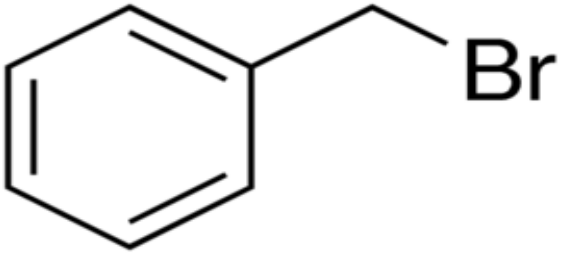

Fig. 2. Structure of benzyl bromide

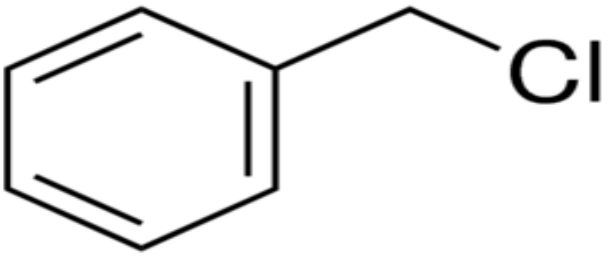

Fig. 3. Structure of benzyl chloride

2. EXPERIMENTAL

2.1 CHEMICALS AND REAGENTS

Entecavir (purity 99.2%) was sourced from Venus Laboratories. Benzyl bromide (A. R. grade) and Benzyl chloride (A. R. grade) were procured from s. d. Fine Laboratories. Acetonitrile (spectroscopic grade) was obtained from Fisher-Scientific. Sodium hydrogen phosphate monohydrate (A.R. grade) was procured from Merck and Water (spectroscopic grade) was used as received from Rankem.

2.2 INSTRUMENTATION

The analysis was performed on Agilent 1260 High Performance Liquid Chromatography (HPLC) system with an auto sampler and binary solvent system interfaced to an Agilent DAD detector and Chemstation Software. The detection was carried out at 210 nm using an Inertsil Poroshell C18 column (50mm x 3.5mm, 2.7µ).

2.3 CHROMATOGRAPHIC CONDITION

The HPLC method was developed with mobile phase consisting of Solution A (0.01M Sodium hydrogen phosphate) and Solvent B (Acetonitrile) as 55:45 v/v mixture, which was pumped at a flow rate of 1.2 mL/min. The temperature of the column was maintained at 25°C and the wavelength selected was 210 nm. The injection volume was 10 µl. Water: acetonitrile (1:1) v/v was used as diluent.

2.4 PREPARATION OF STANDARD AND ENTECAVIR SAMPLE

STANDARD PREPARATION

Standard solution contained 2µg/mL of Benzyl bromide as well as Benzyl chloride and was prepared in diluent.

SAMPLE PREPARATION

Sample Solution was prepared by dissolving 2 mg of entecavir per mL of diluent.

3. RESULT AND DISCUSSION

3.1. METHOD DEVELOPEMENT

Selection of the HPLC column has played a critical role in achieving the separation of Benzyl bromide, Benzyl chloride and Entecavir. Method development was initiated by using water and acetonitrile (1:1 v/v) at a flow rate of 1.0 mL/min. The column used was prontosil C18, 150 mm in length having internal diameter 4.6 mm and 5 µm particle sized Stationary phase.[8] The peak shapes of Benzyl bromide and Benzyl chloride were not appreciable. To get optimum resolution and improved peak shape various mobile phases and columns were tried. Finally phosphate buffer and poroshell RP-18 column were selected.

Typical chromatogram of standard Benzyl chloride and Benzyl bromide, Entecavir sample and Entecavir with spiked impurities (Benzyl bromide and Benzyl chloride) is shown in (Fig. 4, Fig. 5 and Fig. 6) respectively. System suitability data is as shown in Table 1.

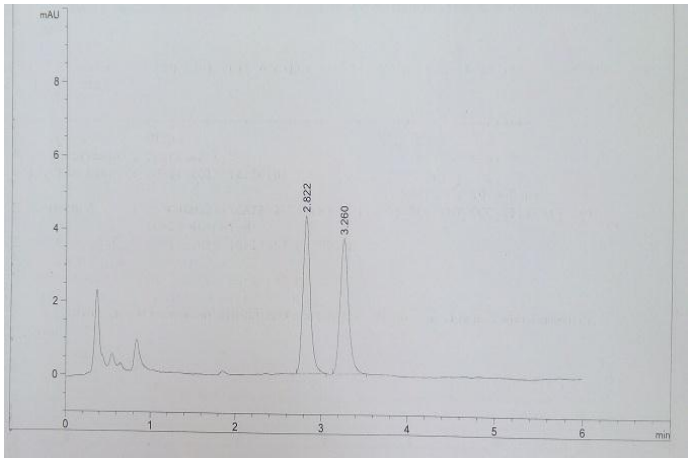

Fig. 4 Standard chromatogram of Benzyl chloride and Benzyl bromide

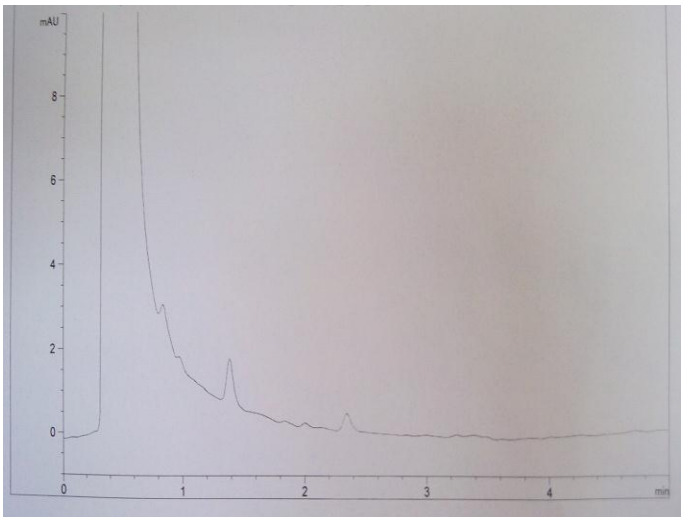

Fig. 5 Sample chromatogram of Entecavir

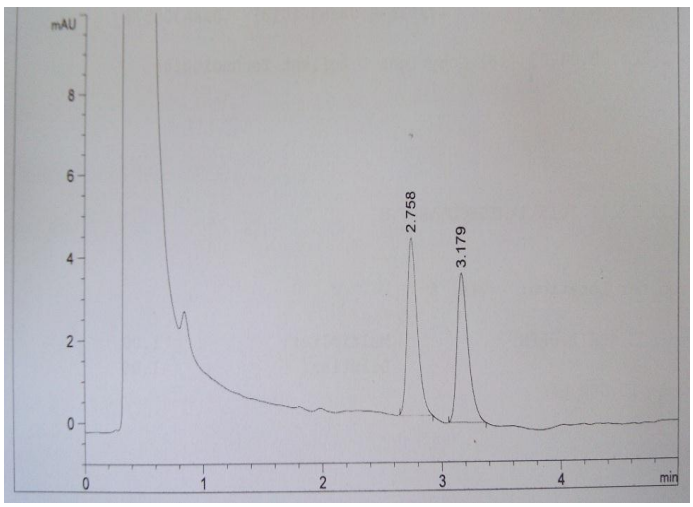

Fig. 6 Standard spike chromatogram of Entecavir

Table 1 System Suitability data

Parameter	Limit	Benzyl Chloride	Benzyl Bromide
Theoretical plate	2000	4980	5427
Symmetry	0.8-2.0	1.1	1.2
Resolution	NLT 1.5	-	2.5

NLT: Not less than.

3.2 VALIDATION OF METHOD

3.2.1. SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of component which may be expected to be present. Typically these might include impurities, degradants, matrix etc.[9] The retention times of Benzyl bromide and Benzyl chloride in the standard solution were compared with the ones in the sample solution. Moreover, the diluent was injected to see whether there were any interferences at the retention time of Benzyl bromide and Benzyl chloride.

3.2.2. LINEARITY

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.[9] Linearity of the method was checked by determining mean responses for each impurity at different dilutions. Hence solutions of each impurity ranging from of 0.2 ppm to 10.0 ppm were prepared. Each solution was injected three times. The mean responses for Benzyl bromide and Benzyl chloride were plotted against Concentration. The Correlation Coefficient was found to be 0.999, which indicates good linearity (Table 2).

Table 2 Linearity data

Peak Name	Slope	Y intercept	Correlation Coefficient
Benzyl chloride	26.15	-0.13	0.999
Benzyl bromide	25.23	-1.22	0.999

3.2.3. ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found.[9] Entecavir sample solutions of 2 mg/mL were spiked with Benzyl bromide and Benzyl chloride at different concentration i.e. 0.2µg/mL, 0.5µg/mL, 1.0µg/mL and 1.5µg/mL. Each solution was injected in duplicate. The recovery percentage was calculated. Results of

recovery are shown in Table 3 and Table 4 for Benzyl chloride and Benzyl bromide respectively. % Recovery for solutions of all concentrations was found between 90 to 110.

Table 3 Accuracy results (Benzyl chloride)

Amount added (µg/mL)	Amount obtained (µg/mL)	Recovery (%)
0.204	0.192	94.12
0.509	0.497	97.64
1.019	1.017	99.80
1.528	1.525	99.80

Table 4 Accuracy results (Benzyl bromide)

Amount added (µg/mL)	Amount obtained (µg/mL)	Recovery (%)
0.211	0.201	95.26
0.528	0.521	98.67
1.055	1.029	97.54
1.583	1.592	100.57

3.2.4. LIMIT OF DETECTION (LOD)

The Detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.[9] A signal to noise (S/N) ratio between 3 to 10 is generally considered to be acceptable for estimating the detection limit. S/N ratios of the individual peaks were determined at different concentrations to estimate LOD. Based on S/N ratio data obtained, the LOD estimated for both the impurities is 0.05µg/mL. The results are shown in the Table 5.

3.2.5. LIMIT OF QUANTIFICATION (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.[9] S/N ratio of more than 10 is generally considered to be acceptable for estimating the limit of quantification, however the S/N ratio obtained in present study were more than 25 for both the impurities. The LOQ is calculated is 0.2µg/ml for Benzyl chloride as well as Benzyl bromide. The results are listed Table 5.

Table 5: LOD and LOQ data

Parameters	Conc (µg/mL)	S/N ratio	
		Benzyl chloride	Benzyl bromide
LOD	0.05	8	6
LOQ	0.20	30	26

3.2.6. PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple sampling of the same homogeneous sample under the prescribed condition.[9] The system for Benzyl chloride and Benzyl bromide impurity was checked for repeatability.[10] In order to determine System precision, the sample was prepared by spiking Entecavir with the Benzyl chloride(0.05%) and Benzyl bromide (0.05%) hence making the total impurity concentration of 0.10% of target analyte concentration[7] and injected six times. The %RSD (Relative Standard Deviation) so found out was less than 5.0% indicating good system precision.

To determine the Method Precision, six independent solutions of Entacavir were prepared by spiking with the impurities as mentioned for system precision. Each solution was injected once. The variation in the results for the Benzyl chloride and Benzyl bromide are expressed in terms of % RSD. The values calculated are found to be below 5.0%, indicating satisfactory Method Precision.

3.2.7. SOLUTION STABILITY

A solution of Entecavir containing impurities was prepared and stored at ambient temperature. This solution was injected at intervals of 0, 4, 8 and 12 hr. Areas of Benzyl bromide and Benzyl chloride were nearly identical to that obtained at 0 h and absence of additional peaks indicate good solution stability.

4. CONCLUSION

This study describes a trace-level method for simultaneous determination of Benzyl chloride and Benzyl bromide which are potential genotoxic impurities in bulk drug. The analytical method described in present study is cost effective, simple, accurate, linear and precise convenient quality control tool for simultaneous determination of these impurities in Entecavir. The advantage of this method lies in its improved sensitivity, shorter run-time and simple sample preparation technique. The method is validated as per requirements of ICH guidelines.

REFERENCES

1. Andrushko V, Andrushko N. Stereoselective Synthesis of Drugs and Natural Products: Vol 1, Germany; Wiley-Interscience, 1976; 202-207.

2. Mijin DZ, Jankovic VD, Petrovic SD. Alkylation of N-substituted 2-phenylacetamides: Benzylation of N-(4-chlorophenyl)-2-phenylacetamide. J Serb Chem Soc, 2004; 69(2): 85-92.
3. Starks CM, Liotta C. Phase Transfer Catalysis. New York; Academic Press Inc. Ltd. 1978; 98-220.
4. PENDRI et al. European Patent Specification EP1644384B1, 2004. https://docs.google.com/viewer?url=patentimages.storage.googleapis.com/pdfs/a22ba676d dc7fde88e42/EP1644384B1.pdf.
5. New jersey department of health and senior services occupational health services. Dec. 2000. http://nj.gov/health/eoh/rtkweb/documents/fs/0216.pdf.
6. Jagu P, Krishna kumar MA, Navaneeswari R. Generic approach for low level determination of 2-hydroxy ethyl hydrazine in pharmaceutical ingredients by GC-MS using chemical derivatization. World J of Pharm Research, 2014; 3: 864-878.
7. EMEA/CHMP/QWP/251344/2006. European Medicines Agency Evaluation of Medicines for Human Use. London, 28 June 2006.
8. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. 3rd Ed., Canada; Wiley, 2010.
9. CPMP/ICH/381/95, 1994. Q2 (R1) Note for guidance on validation of analytical procedures: text and methodology. CPMP adopted November 1994.
10. Mawazi SM, Chandra sekar reddy GNV. Method Development and Validation for Simultaneous Estimation of Atorvastatin and Ezetimibe in Pharmaceutical Dosage Form by HPLC. World J of Pharm Sci, 2014; 866-870.
11. United States Environmental Protection Agency. National centre for environmental assessment. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0393_summary.pdf.