Original Article

Low Level Quantification Of Potential Genotoxic Impurity In Daclatasvir Hydrochloride By RP-HPLC Method

Dumbre NG [1*], Chopade VV [2], Chaudhari PD [3]

[1,2,3] Department of Pharmaceutical chemistry, Modern college of pharmacy, Nigdi, Pune, Maharashtra, India.
DOI: 10.47750/pnr.2022.13.S07.268

Abstract

The goal of our research work was to develop new specific analytical method for the determination of 4 4'-bis(2-bromoacetyl) biphenyl at genotoxic level in Daclatasvir dihydrochloride drug substances. The method was developed by using Reverse phase High performance liquid chromatography technique (RP-HPLC). The comprehensive method development was done to accomplish right combination of chromatographic conditions and validation was completed as per ICH guidelines. The method utilizes waters X-select CSH C-18 (250 mm x 4.6 mm ID, 5.0 µm) HPLC column maintained at 45^0C temperature and detected by ultra violet detector at 210nm. The separation of 4'-bis(2-bromoacetyl) biphenyl was attained by gradient elution mode where mobile phase A was purified water pH-3 adjusted with Ortho-phosphoric acid and mobile phase B was acetonitrile. Flow rate was used 1.5 mL/min and injection volume was 20 µL. The method was entirely screened with all the key validation parameters likewise system suitability, specificity, linearity, sensitivity (LOD/LOQ), precision, accuracy, robustness, and solution stability. All the related substances of sample were specific with impurity and no blank interference was found. The achieved limit of detection (LOD) were 3 µg/ml and limit of quantitation (LOQ) 10 µg/ml with respect to sample. The calibration curve exhibited good linearity from LOQ to 150% level and the correlation coefficient was found 0.999. The accuracy in terms of % recovery of the added known amount found in the range of 95-102 %. Based on experimental result developed analytical method can be used for quantitation of 4,4'-bis(2-bromoacetyl) biphenyl genotoxic impurity in Daclatasvir dihydrochloride drug substances at low level.

Keywords: Daclatasvir, 4,4'-bis(2-bromoacetyl) biphenyl, genotoxic impurity, QSAR and RP-HPLC.

INTRODUCTION

Hepatitis C is a liver disease produced by the hepatitis C virus and can increase liver cirrhosis, liver failure, liver cancer and liver transplantation. The treatment for HCV is pegylated-interferon (Peg-IFN) and ribavirin (RBV) whoever these agents caused few side effects such as bacterial infections, anemia, hematological toxicity, neutropenia and anorectal symptoms [1, 2]. Telaprevir and boceprevir were the first generation direct-acting protease inhibitors that approved for the treatment of genotype I chronic hepatitis C However, they have to be co-administered with interferon and ribavirin,therefore they have side effects so their effectiveness were limited [3, 4].

Second-generation direct-acting antiviral drugs were developed with fewer undesirable side effects. i.e. Daclatasvir Dihydrochloride This medicines have effective antiviral activity and genotypic coverage [5, 6].

Daclatasvir, Methyl [(2*S*)-1-{(2*S*)-2-[4-(4'-{2-[(2*S*)-1-{(2*S*)-2-[(methoxycarbonyl) amino]-3-methylbutanoyl}-2-pyrrolidinyl]-1*H*-imidazol-4-yl}-4-biphenylyl)-1*H*imidazol-2-yl]-1-pyrrolidinyl}-3-methyl-1-oxo-2-butanyl] carbamate [7], is a nucleotide analogue NS5A polymerase inhibitor [8].

Litrature review reveals different techniques for the quantitative determination of Daclatasvir including different chromatographic methods (9-11), Chiral HPLC separation(12) , stability indicating HPLC study in bulk and formulations and with a Chitosan modified electrode, electrochemical detection (13) .

Genotoxicity prediction by QSAR model:

4,4'-bis(2-bromoacetyl) biphenyl was used as one of the raw material in the synthesis of Daclatasvir (Figure 1).

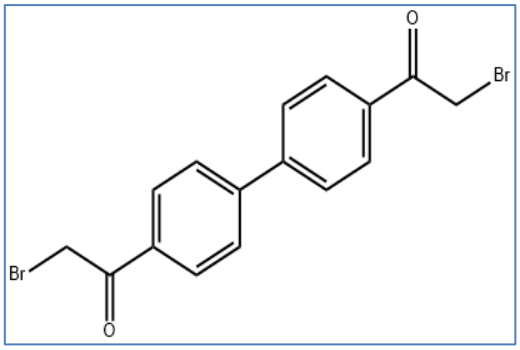

Figure:1: *4,4'-Bis(2-bromoacetyl)biphenyl structure*

The structural alert has been taken from Derek Nexus software for toxicity alert (Estimation of toxic hazards approach). The software prediction for 4,4'-Bis(2-bromoacetyl)biphenyl structure shows alerts (**Table1)**.

Table1: *Derek Nexus software prediction for 4,4'-Bis(2-bromoacetyl)biphenyl structure*

Structure	**Alert found**
alpha-Halo ketone	Irritation (of the eye) in mammal
alpha-Halo carbonyl compound	Chromosome damage in vitro in mammal
alpha-Halo ketone.	Lachrymation in mammal
Haloalkane	Skin sensitisation in mammal

The 4,4'-bis(2-bromoacetyl) biphenyl impurity has been considered as a genotoxic carcinogen as per structural alert. The genotoxic impurity limit is 25ppm calculated as per ICH guideline M7 considering maximum daily dose of 60mg for Daclatasvir.

In this work, we developed and validated, a sensitive and precise method for low level quantification RP-HPLC (Reverse phase High performance liquid chromatographic method) for determination of 4,4'-bis(2-bromoacetyl) biphenyl. There is no method available for quantification of this genotoxic impurity in literature. We demonstrated the method sensitivity, linearity, accuracy and robustness with intermediate precision.

MATERIAL AND METHODS

2.1 Reagents and Solvents:

HPLC grade methanol and Acetonitrile purchased from Fischer scientific; Orthophosphoric acid purchased from Merck (Germany), purified water generated through Milli-Q (Millipore, Bedford,MA,USA). Reference standard 4,4'-bis(2-bromoacetyl) biphenyl was obtained from Sigma Aldrich (Miliwaukee,WI) and Daclatasvir dihydrochloride drug substances obtained as gift sample from reputed pharmaceutical company.

2.2 Column selection and Optimization of Gradient Mobile Phase:

For adequate retension of 4,4'-bis(2-bromoacetyl) biphenyl on various columns like Inertsil C-18, Kromasil C-18 and Waters X-select CSH C-18 column of different dimension were tried. On Inertsil C-18 and Kromasil C-18 blank interference and early elution was observed. However, on Waters X-select CSH C-18 column dectector response for 4,4'-bis(2-bromoacetyl) biphenyl was found suitable with adequate separation from analyte.

Mobile phase composition using purified water adjusted to pH-3 with Orphophosphoric acid and Acetonitrile were evaluated. Good responses and separation were obtained using gradient mobile phase of purified water adjusted to pH-3 with Orphophosphoric acid and Acetonitrile was optimized at flow rate 1.5 ml per minute, injection volume 20 microlitres and detection at 210nm. Under this optimized condition retention of 4,4'-bis(2-bromoacetyl) biphenyl observed about 8.0 minutes.

2.3 Instrumentation:

Chromatography: The chromatography system used was Agilent 1200 series LC system (Agilent Technologies) consisting of an 1200 series pump with degasser, auto sampler and column compartment. The analytical column was a Waters X-select CSH C-18 (25cm X 4.6mm X 5um). The mobile phase consisted of mobile phase A (Purified water adjusted pH-3 with Ortho-phosphoric acid) and mobile phase-B (Acetonitrile). The flow rate was 1.5ml/minute and run time was 20minutes. Column oven temperature maintained at 45^0C. Injection volume was 20ul. Gradient was optimized as shown in table (**Table 2**). Diluent used as methanol, both sample and standard have high solubility.

Table 2: *Gradient composition*

Time (minute)	Mobile phase A	Mobile phase B
0.00	50	50
10.00	25	75
15.00	25	75
15.10	50	50
20.00	50	50

2.4 Standard and sample preparation:

2.4.1 Standard preparation:

Stock solution of 4,4'-bis(2-bromoacetyl) biphenyl standard was prepared in methanol to obtained concentration as 100.0 ug/ml. Final standard solution was prepared in methanol having concentration 0.25 ug/ml (Which is equivalent to 25 ug/ml with respect to sample concentration).

2.4.2 Sample preparation:

Weighted 200mg sample of Daclatasvir dihydrochloride drug substances in 20ml volumetric flask. Added diluents about half of volumetric flask, sonicated to dissolve and dilute up to the mark with diluents.

2.4.3 Optimization of sample preparation:

Sample preparation is a challenging part of the genotoxic impurity analysis. As evaluation was required to be done at low levels, so that sample concentration was need to be increased in order to achieve accurate detection at trace level. Selection of diluents for solubility of sample with higher concentration required various solubility trials. Methanol found as a suitable diluents with no precipitation of sample after 24 hr, keeping as such sample on table top.

RESULT AND DISCUSSION

The developed RP-HPLC method for evaluation of 4,4'-bis(2-bromoacetyl) biphenyl in Daclatasvir dihydrochloride was validated as per ICH guidelines. The linearity was assessed by preparing and analyzing six levels of 4,4'-bis(2-bromoacetyl) biphenyl in concentration rage of 0.1-0.375 ug/ml. The regression coefficient, slope and intercept were determined by least square method. Method precision and system precision performed by injecting six replicated injections of sample and standard preparation.

The limit of quantitation (LOQ) was obtained on the basis of the lowest concentration of 4,4'-bis(2-bromoacetyl) biphenyl that gives signal to noise ratio not less than (NLT) 10. The precision and accuracy evaluated by spiking 4,4'-bis(2-bromoacetyl) biphenyl and determing %RSD < 5%. Solution stability studied for 24hrs at 25^0C.Ruggedeness study (intermediate precision) was performed on different day by different analyst using different column by spiking analytes and determining %RSD <10%.

For application of developed method for commercial products,validation was necessary. The developed method for 4,4'-bis(2-bromoacetyl) biphenyl in Daclatasvir dihydrochloride was validated. The linearity was established by plotting peak area counts versus concentration of genotoxic impurity 4,4'-bis(2-bromoacetyl) biphenyl in the concentration range of 0.1ug/ml to 0.375ug/ml. Data shown in **Table 3**. The correlation coefficient, slope and % Y intercept were determined as shown in **figure 2**. The R square obtained as 0.999 indicates the linearity correlation.

The LOD was found 0.03 ug/ml and LOQ was found 0.10 ug/ml. The content of 4,4'-bis(2-bromoacetyl) biphenyl in Daclatasvir dihydrochloride was not found so spiking study was performed for method precision and accuracy experiments and determined the % RSD. Recovery of the spiked amounts were carried out (**Figure 3**) and the mean percentage recovery obtained in the range of 90%-110% for 4,4'-bis(2-bromoacetyl) biphenyl, data shown in **Table 4**.Solution stability checked for 24 hrs at 25^0C and solution found stable during this period.

Ruggedness study was performed on different day by different analyst, %RSD was observed 2.9% for 4,4'-bis(2-bromoacetyl) biphenyl. Robustness study performed by change of pH by ±0.2 and change of column temperature $\pm2^0$C. The derived results indicated good sensitivity and reproducibility of the method.

Table 3: *Linearity of 4,4'-bis(2-bromoacetyl) biphenyl (ug/ml) at different level.*

Level	As such concentration of 4,4'-bis(2-bromoacetyl) biphenyl (ug/ml)	Sample concentration (ug/ml)	Concentration of 4,4'-bis(2-bromoacetyl) biphenyl (ug/ml) with respect to sample.
LEVEL 1(LOQ)	0.10	10000	10
LEVEL 2 (50%)	0.125	10000	12.5
LEVEL 3 (80%)	0.20	10000	20
LEVEL 4 (100%) (Working Level)	0.25	10000	25
LEVEL 5 (120%)	0.30	10000	30

LEVEL 6 (150%)	0.375	10000	37.5

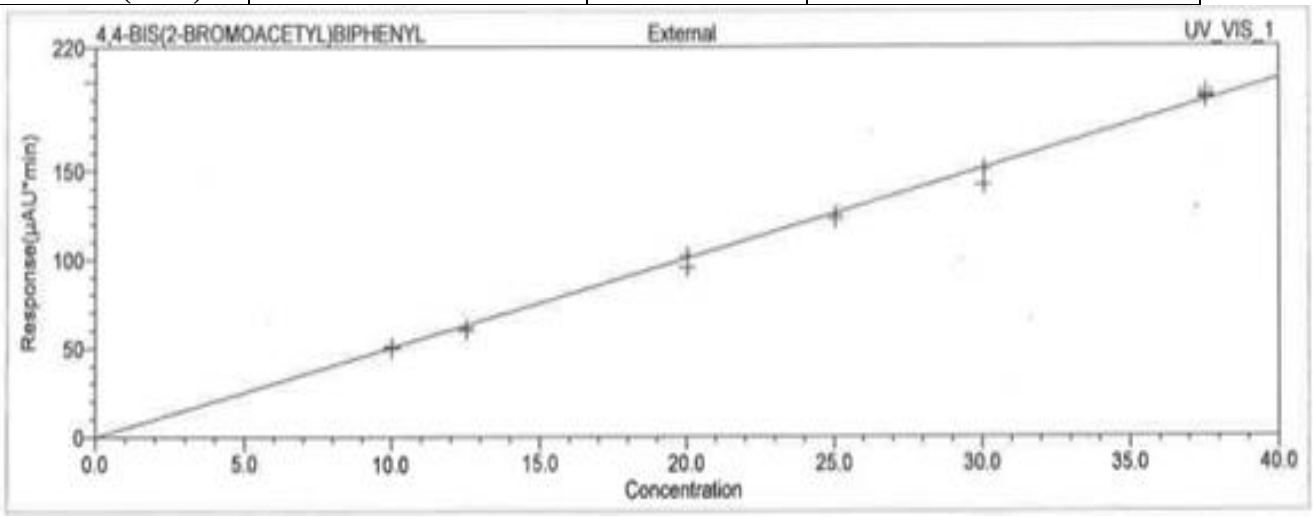

Figure2: *Linearity plot of 4,4'-bis(2-bromoacetyl) biphenyl in the concentration range of 0.1ug/ml to 0.375 ug/ml.*

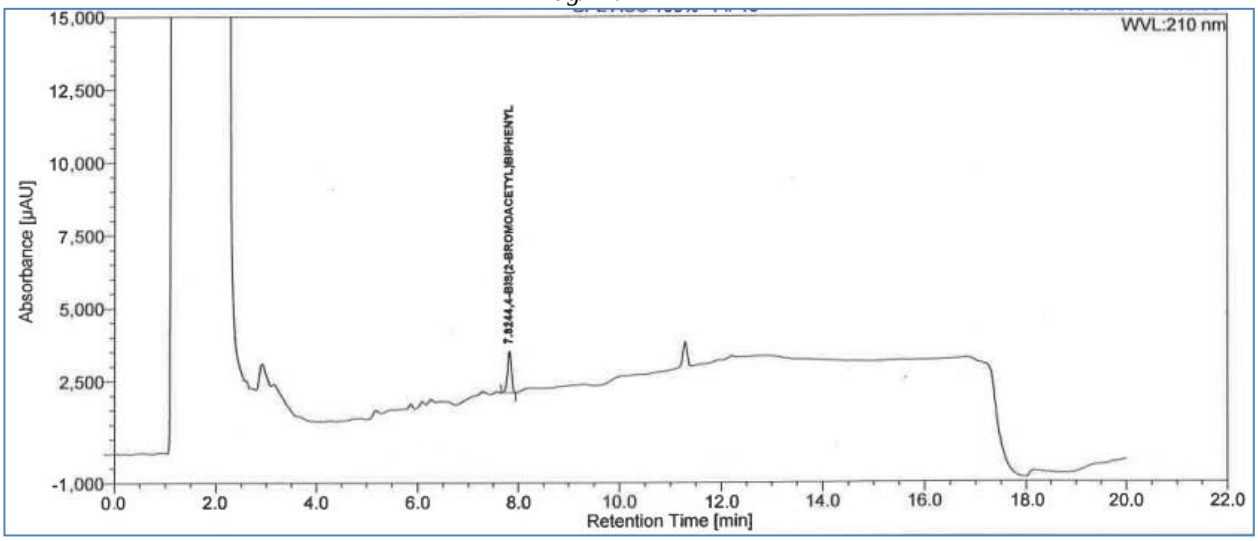

Figure 3: *Accuracy of 4,4'-bis(2-bromoacetyl) biphenyl in Daclatasvir dihydrochloride sample at Working level.*

Table 4: *Accuracy of 4,4'-bis(2-bromoacetyl) biphenyl at different level.*

LEVEL	Amount of 4,4'-bis(2-bromoacetyl) biphenyl present in analysed sample	Amount added with respect to sample ug/ml	Amount found with respect to sample ug/ml	% Recovery
LOQ Recovery	Nil	0.099	0.094	95
50% Recovery	Nil	0.123	0.121	98
100% Recovery	Nil	0.247	0.251	102
150% Recovery	Nil	0.370	0.367	99

CONCLUSION

A method on RP-HPLC was employed for quantification and screening of 4,4'-bis(2-bromoacetyl) biphenyl in Daclatasvir dihydrochloride. This RP-HPLC method was cost effective, sensitive, specific, accurate and reproducible for detection of 4,4'-bis(2-bromoacetyl) biphenyl in Daclatasvir dihydrochloride. The high level of 4,4'-bis(2-bromoacetyl) biphenyl in Daclatasvir dihydrochloride is hazardous, if commercial product not tested properly by Quality control laboratories. This method presents a reliable technique for rapid quantification of genotoxic impurities in Daclatasvir dihydrochloride drug substances with accuracy and precision.

REFERENCES

1. Berenguer M. Systematic review of the treatment of established recurrent hepatitis C with pegylated interferon in combination with ribavirin. J Hepatol49 (2008): 274-287.
2. Wang CS, Ko HH, Yoshida EM, Marra CA. Richardson K. Interferon-based combination anti-viral therapy for hepatitis C virus after liver transplantation: A review and quantitative analysis. Am J Transplant 6 (2006): 1586-1599.
3. Coilly A, Roche B, Dumortier J, Leroy V, Botta Fridlund D, Radenne S. et al. Safety and efficacy of protease inhibitors to treat hepatitis C after liver transplantation: A multicenter experience. J Hepatol60 (2014):78-86.
4. Sundaram V, Kowdley KV. Dual daclatasvir and sofosbuvir for treatment of genotype 3 chronic hepatitis C virus infection. Expert Rev GastroenterolHepatol10 (2016):13-20.
5. Liao H, Tan P, Zhu Z, Yan X, Huang J. Sofosbuvir in combination with daclatasvir in liver transplant recipients with HCV infection: A systematic review and meta-analysis. Clin Res HepatolGastroenterol41 (2017): 262-271.
6. Shaadmin N shaik and Manjusri P.Dabhade, Development and validation of RPHPLC method for quantitative analysis of Sofosbuvir in pure and pharmaceutical formulation, World Journal of Pharmacy and Pharmaceutical Sciences, 6(8), 2017, 2249-2258.
7. R.M. Nemade, M.N. Dole, Dr.S.D.Sawant, Development and validation of stability indicating RP-HPLC method for the estimation of sofosbuvir by forced degradation studies, World Journal of Pharmacy and Pharmaceutical Sciences, 6(4), 2017, 1503-1512.
8. V. Ravikumar, C.V.S. Subramanyam, G. Veerabhadram, Estimation and validation of sofosbuvir in bulk and tablet dosage form by RP-HPLC, International Journal of Pharmacy, 6(2), 2016, 121-127.
9. Rezk MR, Bendas ER, Basalious EB, Karim IA. Development and validation of sensitive and rapid UPLC–MS/MS method for quantitative determination of daclatasvir in human plasma: Application to a bioequivalence study. Journal of Pharmaceutical and Biomedical Analysis. 2016; 128:61-65.
10. Jiang H, Kandoussi H, Zeng J, Wang J, Demers R, Eley T, et al. Multiplexed LC-MS/MS method for the simultaneous quantitation of three novel hepatitis C antivirals, Daclatasvir, Asunaprevir, and Beclabuvir in human plasma. Journal of Pharmaceutical and Biomedical Analysis. 2015; 107:409-418.
11. Nannetti G, Messa L, Celegato M, Pagni S, Basso M, Parisi SG, et al. Development and validation of a simple and robust HPLC method with UV detection for quantification of the hepatitis C virus inhibitor Daclatasvir in human plasma. Journal of Pharmaceutical and Biomedical Analysis. 2017; 134:275-281.
12. Srinivasu G, Kumar KN, Thirupathi C, Narayana CL, Murthy CP. Development and validation of the chiral HPLC method for Daclatasvir in gradient elution mode on amylose-based immobilized chiral stationary phase. Chromatographia. 2016; 79(21-22): 1457-1467.
13. Azab SM, Fekry AM. Electrochemical design of a new nanosensor based on cobalt nanoparticles, chitosan and MWCNT for the determination of Daclatasvir: a hepatitis C antiviral drug. RSC Advances. 2017;7(2):1118-1126.
14. ICH, Q2 (R1) validation of analytical procedures: text and methodology, Proceeding of The International Conference on Harmonization, Geneva, 2005.
15. European Medicine Agency (EMeA), Committee for Medicinal Products For Human Use (CHMP) Guidline on the limit of genotoxic impurities.2006.
16. M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals To Limit Potential Carcinogenic Risk ,Guidance for Industry,2018.