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A Selective and Sensitive Method for Simultaneous Quantification of Genotoxic Impurities in Penciclovir drug substance and its dosage forms using UPLC-MS/MS

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ABSTRACT

A selective, rapid and sensitive method was developed for the determination of genotoxic impurities (2-Amino-6-chloro purine and Bromo compound) in Penciclovir drug substance using RPUPLC-MS/MS. The chromatographic separation was performed on Kromasil C8 column (150 mm x 4.6 mm, 5 μm) maintained at 45°C using 0.1%formic acids in water as buffer and acetonitrile through gradient programme. The flow rate was maintained at 0.5mL/min with an injection volume of 10 μL. For the quantification of genotoxic impurities, positive-electrospray ionisation (ESI) mode was selected. Penciclovir and its impurities were well separated within the shortest run time of 16min. The chromatographic method was developed, and the results of all validation parameters showed that the technique is well confined to the limits of ICH guidelines. The method has high sensitivity, and the limit of detection was found to be as low as 0.15 and 0.30 ppm for 2-Amino-6-chloro purine and Bromo compound. The recovery of 2-Amino-6-chloro purine and Bromo compound are found in the range of 80-120%. The linearity of peak area versus concentration was demonstrated in the range of LOQ - 150% level of impurities with a correlation coefficient of 0.9999. The method has proved too robust by introducing minuscule changes in the chromatographic parameters. The method was successfully validated and applied for Penciclovir drug substances and their dosage forms to determine the mentioned genotoxic impurities.

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INTRODUCTION

Penciclovir is chemically known as 2-[2-(2-amino-6-hydroxy-9*H*-purin-9-yl) ethyl]propane-1,3-diol ($C_{10}H_{15}N_5O_3$) with a molecular weight of 253.26. Penciclovir is a synthetic acyclic guanine derivative, nucleoside analogue with antiviral activity used for the treatment of various herpes simplex virus (HSV) infections. It has low toxicity and good selectivity (Vanessa N. Richardson *et al.*, 2013). During the preparation of drug substances, some of the critical starting materials, reagents, intermediates, solvents and by-products inevitably end up in the finished products as impurities in new commercial active pharmaceutical ingredients (APIs). Method for the analysis of penciclovir and

its related impurity in bulk drug as well as, pharmaceutical dosage forms (Ganji and Satyavati, 2016) and spectroscopic method for determination of Penciclovir and Entecavir (Elzaher *et al.*, 2016) are available in the literature. But no method suitable method was found for the quantification of genotoxic impurities present in penciclovir. The cause of cancer in human beings is due to the potential genotoxic impurities (GTIs) which induce genetic mutations due to chromosomal and chromosomal breaks, among all the impurities (Liu *et al.*, 2009). To control this, the analyst has to identify genotoxic impurities early in the synthetic process through new analytical method (Elder *et al.*, 2008). To control the genotoxic impurities, separate guidelines published by European Medicine Agency (EMEA)

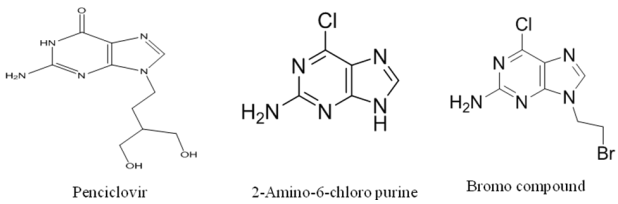

Figure 1: Structures of penciclovir and two genotoxic impurities

and US FDA for active pharmaceutical ingredients with the limit of a threshold of toxicological concern (TTC) 1.5 μg/day (USFDA, 2008). Generally, the alkylating agent is considered genotoxic impurities if they are absorbed by tissues (Smith and Webb, 2007). For the synthesis of penciclovir, 2-Amino-6-chloro purine and Bromo compound are used as necessary starting materials, which were identified as genotoxic impurities Figure 1. As per the Penciclovir maximum daily dosage, 2-Amino-6-chloro purine and Bromo compound are required to be controlled with the permitted limit of 5.0 μg/g (ppm) (Yuabova *et al.*, 2008).

As selectivity of mass detectors is less prone to interference compared to UV and other non-specific detectors, UPLC-MS/MS method was selected for the determination of both impurities. HPLC method is available for the determination of penciclovir in plasma sample (Kumar *et al.*, 2010). Bioequivalence study of penciclovir is reported in the literature (Pan *et al.*, 2008). HPLC, coupled with mass spectrometry, was also used for the determination penciclovir in plasma sample (Lee *et al.*, 2007). Simultaneous determination of aciclovir, ganciclovir, and penciclovir in human plasma by high-performance liquid chromatography with fluorescence detection was also reported in the literature (jun Dao *et al.*, 2008). But no analytical method had been reported in the literature. The UPLC-MS/MS method presented here can detect the impurities with a low limit of detection and quantification limit as per ICH guidelines.

Table 1: Specificity of penciclovir and its genotoxic impurities

Name of the Component	Retention time (minutes)	Observed mass (M+H)+
2-Amino-6-Chloropurine	5.75	170
Bromo Compound	7.08	276
Penciclovir	3.45	254

MATERIALS AND METHODS

Chemicals

Reference substances of penciclovir, 2-Amino-6-chloro purine and Bromo compounds were received from Spectra Laboratories Limited, Hyderabad, India. Acetonitrile (HPLC grade) and Formic acid (AR grade) were procured from Merck (Mumbai, India). Milli-Q system from Millipore (Bedford, MA, USA) is used for purified water.

Instrumentation

Acquity UPLC (H-Class) consisting of an autosampler with a PDA detector coupled with a mass spectrometer (XEVO TQ-S, Waters, USA) used for the quantification of impurities. MassLynx software was operated for data the collection of analysis.

Preparation of mobile phase, standard and sample preparation

Buffer preparation

Prepared 1.0 mL of Formic acid in 1000 ml of water and filtered through 0.22mm filter and degassed.

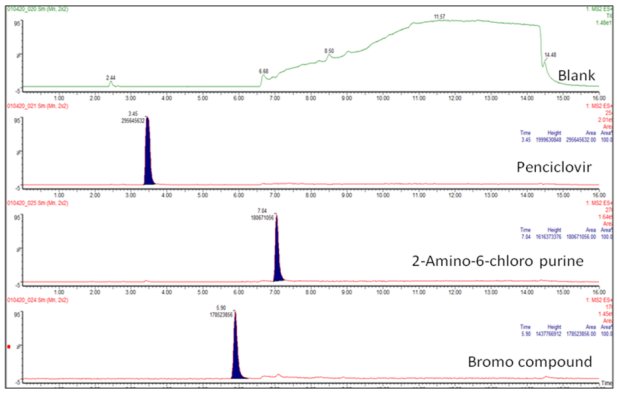

Figure 2: Specificity chromatograms of blank, penciclovir, 2-amino-6-chloro purine and bromo compound

Table 2: Results of linearity of penciclovir genotoxic impurities

Component	Slope	Intercept	Correlation coefficient (R)	R2
2-Amino-6-Chloropurine	22670.6990	1309.6242	0.9998	0.9996
Bromo Compound	28706.5029	4429.3177	0.9998	0.9996

Mobile Phase

Buffer used as solvent-A and acetonitrile was used as solvent B.

Diluent preparation

It is prepared by taking solvent A and solvent B in 80:20 % *v/v* ratio.

Standard solutions

Accurately weighed and transferred an appropriate amount of impurities (2-Amino-6-chloro purine and Bromo compound) to get a concentration of 2.5 μg/mL solution, which is equivalent to 5.0 ppm for the drug substance. The Linearity solutions were prepared at 0.5, 1.0, 1.25, 2.5, 3.75, 5.0, 6.25 and 7.5 ppm level concentrations with respect to the drug substance.

Sample Solution

Prepared the sample solution containing 0.5 mg/mL of penciclovir in a volumetric flask and diluted with diluent.

Spiked Sample Solution

Prepared the spiked sample solution containing 0.5 mg/mL of penciclovir in a volumetric flask and diluted with a standard solution.

Table 3: Results of accuracy study of amino chloro and bromo impurities

% Level	% Recovery	
	2-Amino-6-chloropurine	Bromo compound
LOQ	96.8	104.2
	98.2	103.9
	97.7	102.9
50	97.8	103.7
	98.2	104.1
	96.7	101.3
100	98.2	103.4
	97.9	103.9
	98.6	103.9
150	98.5	103.7
	96.9	103.9
	98.4	104.0

Table 4: Method precision

Sample id	2-Amino-6-Chloropurine (ppm)	Bromo Compound (ppm)
Preparation 1	4.83	4.94
Preparation 2	4.98	5.03
Preparation 3	4.94	5.09
Preparation 4	4.85	4.96
Preparation 5	4.92	4.91
Preparation 6	4.84	5.10
Average	4.8933	5.0050
%RSD	1.3	1.6

Chromatographic Parameters

The separation was performed using Kromasil C8 (150 x 4.6 mm, 5μm) analytical column by gradient elution of the mobile phase with 0.5 mL/min flow rate. Injection volume was taken as 10 μL.

Sample cooler temperature and column temperature were maintained at 10°C and 45°C, respectively. At the beginning (0 minutes) buffer and acetonitrile were maintained in 90:10 ratio. At 4.5 minutes it changed to 20:80. At 8.5 minutes, it became 10:90, and the same ratio was maintained up to 11 minutes. At 12.5 minutes, the ratio was resumed to its initial composition, i.e. 90:10 and hold up to 16 minutes.

Mass Spectrometer

The MS/MS system was operated in positive polarity, The mass spectrometer operating parameters are as follows as scan range 50-1000 m/z, capillary 3.0 kV, cone 30 V, desolvation gas 1000 L/hr, cone gas at 150 L/hr, nebuliser gas flow 7.0 bars and dwell time was 200 ms.

Desolvation temperature and source temperature were maintained at 500°C and 150°C respectively. MRM (Multiple Reaction Monitoring) modes were selected for the determination of 2-Amino-6-chloro purine at parent ion 170.02 m/z, daughter ion 134.01 m/z and Bromo compound at parent ion 275.94 m/z, daughter ion 106.7 m/z, respectively.

Table 5: Robustness study with respect to flow and column oven temperature

	2-Amino-6-Chloropurine		Bromo Compound	
Parameter condition	RT(min)	ppm	RT(min)	ppm
Actual (Flow: 0.5 mL/min; Temperature 45°C)	5.73	4.88	7.04	4.87
Flow rate: 0.45 mL/min (Low flow)	6.22	4.71	7.45	4.82
Flow rate: 0.55 mL/min (High flow)	5.19	4.71	6.67	4.94
Column oven temperature 43°C (Low)	5.78	4.73	7.04	4.88
Column oven temperature 47°C (High)	5.66	4.71	7.02	4.78

Validation Study

The proposed method was developed and validated as per the ICH guidelines (Q3A (R2), 2006). The system precision of mass spectrophotometer was established by injecting standard solution six times.

The method precision was evaluated by preparing six spiked sample solutions at the specified limit of impurities and determined the %RSD. The linearity of 2-Amino-6-chloro purine and Bromo compound were evaluated by preparing and analysing seven-point calibration of 1.0 to 7.5 ppm and 0.5-7.5 ppm, respectively. LOD and LOQ were established by calculating the signal–to–noise ratios of 3:1 and 10:1, respectively (USFDA, 1996).

The accuracy of the method was evaluated at LOQ, 2.5 ppm, 5.0 ppm and 7.5 ppm in triplicate. The method has proved to be robust by introducing minuscule changes (Flow and Temperature) in the chromatographic conditions. Solution stability was verified by injecting standard, sample and spiked sample solution at specified time intervals at 24-26°C (room temperature) and 2-8°C (refrigerator). The method was successfully validated and applied for the penciclovir drug substance.

RESULTS AND DISCUSSION

Specificity

Penciclovir and each impurity solutions were prepared at a standard concentration and solution of penciclovir spiked with impurities were also prepared and injected into the chromatographic system. Retention time and observed mass values were represented in Table 1.

The typical chromatograms of blank, standard and impurities were presented in Figure 2.

Linearity

The linearity study of genotoxic impurities was performed by taking seven points between 1.0 ppm to 7.5 ppm for 2-amino-6-chloro purine and 0.5 ppm to 7.5 ppm for Bromo compound. The data were subjected to the least square method. The calibration curve was found to be linear with the correlation coefficient (R) of 0.9996, and the corresponding values are shown in Table 2.

Limit of Quantification and Limit of Detection

Limit of detection (LOD) and limit of quantification (LOQ) of amino and Bromo compounds were determined by calculating the signal-to-noise ratios of 3:1 and 10:1, respectively. The LOD of amino chloro impurity and Bromo compound was found to be 0.30 ppm, and 0.15 ppm and LOQ was found as 1.0 ppm and 0.5 ppm respectively.

Accuracy

Recovery study of penciclovir spiked with known impurities was carried out in triplicate at LOQ, 25%, 50%, 100% and 150% level of standard solution concentration. The percentage recovery of impurities has been calculated and found to be within the range of 80 to 120%. The results of the accuracy of amino chloro impurity and Bromo compound were represented in Table 3.

Precision

The system precision was performed by analysing six injections of a standard solution. Results of per cent standard deviations are in the range of 0.9 - 4.4%. The method precision was performed by analysing six sample preparations of penciclovir spiked with both impurities at standard concentration. The % relative standard deviation values of method precision were found in the range of 1.3 to 1.6%. The results of system precision and method

precision were represented in Table 4.

Robustness

Impact of the change of conditions was observed on the chromatographic performance. The method has proved to be robust by introducing minuscule changes in the chromatographic parameters. The results of the robustness study of retention time and recovery of impurities are summarised in Table 5.

Stability of Solution

A solution of penciclovir spiked with both the impurities at ICH specification level concentration and standard solutions were kept at 24-26°C (room temperature) and 2-8°C (refrigerator). The stability of solution was verified at 0 hours (initial), 24 hours and 48 hours intervals and the results indicate that the impurities, standard solution and sample solutions are stable up to 48 hours at 2-8°C as well as at room temperature (25°C).

CONCLUSIONS

In this study, the genotoxic impurities in penciclovir drug substances were quantified by using UPLC-MS/MS method. It was a sensitive, rapid, selective, accurate, linear, precise, robust and cost-effective method. The UPLC-MS/MS method is well confined to the limits of ICH guidelines for the quantification of genotoxic impurities. The values of the limit of detection indicate the sensitivity of approach. This method also used for the quantification of 2-Amino-6-chloro purine and Bromo compound in drug substance and dosage forms of penciclovir.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES

Elder, D. P., Lipczynski, A. M., Teasdale, A. 2008. Control and analysis of alkyl and benzyl halides and other related reactive organohalides as potential genotoxic impurities in active pharmaceutical ingredients (APIs). *Journal of Pharmaceutical and Biomedical Analysis*, 48(3):497–507.

Elzaher, A. A., Fouad, M. A., Elhoussini, O. M., Behery, Y. E.-E. 2016. Validated spectrometric determination of penciclovir and entecavir in bulk and in pharmaceutical preparations. *Bulletin of Faculty of Pharmacy, Cairo University*, 54(2):175–179.

Ganji, S., Satyavati, D. 2016. Method development and validation for the analysis of penciclovir and related impurity in bulk and pharmaceutical dosage forms by RP HPLC. *International Journal of Pharmacy*, 6(1):137–148.

jun Dao, Y., Jiao, Z., kang Zhong, M. 2008. Simultaneous determination of aciclovir, ganciclovir, and penciclovir in human plasma by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography B*, 867(2):270–276.

Kumar, C. H. V., Kumar, D. A., Rao, J. V. L. N. S. 2010. A New Validated RP- HPLC Method for the Determination of Nevirapine in Human Plasma. *E-Journal of Chemistry*, 7(3):821–826.

Lee, H. W., Seo, J. H., Lee, K. T. 2007. Development and validation of a high-performance liquid chromatography-tandem mass spectrometry for the determination of penciclovir in human plasma: Application to a bioequivalence study. *Journal of Chromatography B*, 852(1-2):382–388.

Liu, D. Q., Chen, T. K., McGuire, M. A., Kord, A. S. 2009. Analytical control of genotoxic impurities in the pazopanib hydrochloride manufacturing process. *Journal of Pharmaceutical and Biomedical Analysis*, 50(2):144–150.

Pan, Y., Gao, X., Guo, N., Li, J., Guo, X. 2008. HPLC Method for the Determination of Penciclovir in Human Plasma and Application to a Bioequivalence Study. *Journal of Chromatographic Science*, 46(9):819–822.

Smith, R. J., Webb, M. 2007. synthesis of penciclovir, 2-Amino-6-chloro purine and Bromo compound. *Analysis of Drug Impurities*, 82.

USFDA 1996. Q2B Validation of Analytical Procedures. Guidance for Industry.

USFDA 2008. Genotoxic and carcinogenic impurities in drug substances and products: recommended approaches. Guidance for Industry.

Vanessa N. Richardson, Davis, S. A., Gustafson, C. J., West, C. E., Feldman, S. R. 2013. Patterns of disease and treatment of cold sores. *Journal of Dermatological Treatment*, 24(6):439–443.

Yuabova, Z. Y., Holschlag, D. R., Rodriguez, S. A., Qin, C., Papov, V. V., Qiu, F., McCaffrey, J. F., Norwood, D. L. 2008. Genotoxic Impurities: A Quantitative Approach. *Journal of Liquid Chromatography and Related Technologies*, 31(15):2318–2330.

