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Research Article

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Determination and Validation of Benzyl Chloride by HPLC Method in Posaconazole Drug Substance

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ABSTRACT

A simple and sensitive High performance liquid chromatographic method was developed for the determination of trace level Benzyl chloride in posazonazole drug substance. The separation was achieved using Waters X Bridge C18(250X4.6mm,3.5µm) using mobile phase containing10mm Ammonium acetate PH:5.5 .The flow rate was 0.8ml/min and column temperature was 25°c.Benzyl chloride was detected using UV detector at the wave length 220nm.The Retention time was 13.7 min is for Benzyl chloride .The optimized method was validated to prove its performance characteristic by demonstrating selectivity, sensitivity (Limit of detection and Limit of quantification), Linearity, precision and accuracy. The limit of detection and limit of quantification of Benzyl chloride was found to be 3ppm and 10ppm for 20µl injection volume. The linearity of the method is found to be 0.1µg/ml to 0.75 µg/ml. Relative standard deviation of system precision, method precision is found to be 0.50% and 0.16% respectively. The accuracy of the method is found to be 97.5% to 99.7%.

Keywords: HPLC; Gradient separation; Reverse phase; Benzyl chloride; Posaconazole; Validation

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INTRODUCTION

Posaconazole is a triazole antifungal agent containing 4 chiral centres. It is synthesised solely as the (R,R,S,S) enantiomer. Posaconazole, [1-5]. Chemical name 4-{4-[4-(4-{[(3R,5R)-5-(2,4-difluorophenyl)-5-(1H-1,2,4-triazol-1-ylmethyl)oxolan-3-yl]methoxy}phenyl)piperazin-1-yl]phenyl}-1-[(2S,3S)-2-hydroxypentan-3-yl]-4,5-dihydro-1H-1,2,4-triazol-5-one.one which is used as antifungal agent. The biological activity of chiral substances often depends up on their stereochemistry. A large percentage of commercial and investigational pharmaceutical compounds are enantiomers, and many of them show significant enantioselective difference in their pharmacokinetics and pharmacodynamics. Three polymorphic forms of posaconazole have been observed during development, but the synthetic process is designed to constantly produce form I. Moreover, the crystal form is controlled as part of the drug substance specification and there has been no evidence of polymorphic transition on storage as micronised powder, during manufacture or as formulated in the finished product. Posaconazole is lipophilic, highly permeable, and practically insoluble in water. Therefore, the rate of drug dissolution is likely to be important to the rate and possibly to the extent of absorption.

Literature survey show some work related to posacoanzole assay in biological fluids applying mainly chromatographic methods [6-10]. Considering, the analysis in bulk or pharmaceutical products, there is no work published and no monograph available in pharmacopoeias [10-12]. The objective of this work is to develop and validate the method. The developed High performance liquid chromatographic method was validated for the determination of trace level benzyl chloride in posaconazole drug substance. Hence no HPLC method is reported for the estimation of Benzyl chloride in Posaconazole drug substance. The present work, a successful attempt has been

made to estimate the Benzyl chloride in posaconazole drug substance. The posacoanzole empirical formula is $C_{37}H_{42}F_2N_8O_4$ and its molecular weight is 700.8 and the chemical structure is shown in (Figure 1)
HPLC is a conventional effective analytical technique. The optimized HPLC Reverse phase gradient method was validated according to ICH guidelines [13-17]. To prove its suitability and reliability for the determination Benzyl chloride (Figure 2) content in Posacoanzole drug substances during routine analysis.

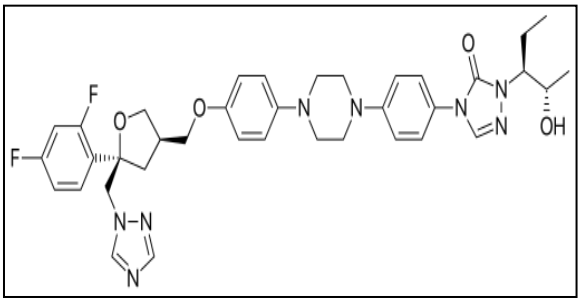

Figure 1: Molecular structure of Posaconazole

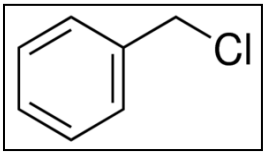

Figure 2: Molecular structure of Benzyl chloride

MATERIALS AND METHODS

Chemicals, Reagents and Samples

Analytical reagent grade of Ammonium acetate; Acetic acid and Methanol from Merck chemicals, India were used. Analytical reagent grade Benzyl chloride from Merck; Posaconzole, and related impurities are gift samples from reputed pharma company.

Instrument

The HPLC System used for method development, method validation studies performed waters alliance 2695 separation module equipped with 2996 photodiode array detector with Empower handling system i.e. Empower 3 software (Waters Corporation, USA)

Chromatographic Conditions

Chromatography was carried out by using waters alliance 2695 separation module equipped with 2996 photodiode array detector, and the data was processed using Empower3 software, the chromatographic conditions were optimized using Reverse phase stationary phase, Waters X Bridge C18 (250X4.6mm), 3.5µm.The Mobile phase A Consists of ammonium acetate 10mm pH: 5.5 With diluted acetic acid) and Methanol combination in the ration of (90:10).and Mobile phase B Was methanol. The analysis was carried out on Waters X Bridge C18(Waters corporation Ltd)250mm long ,4.6mm ID and 3.5µm particle size column, thermo stated at 25°c.Mobile phase was flushed through the column at a flow rate 0.8ml/min and pump was in gradient mode and the program was as follows. Time (min)/A(v/v):B(V/V);$T_{0.01}$/45:55,$T_{15/}$40:60, $T_{25/}$20:80, $T_{35/}$20:80, $T_{40/}$45:55, $T_{50/}$45:55.The injection volume was used 20µl.The acquisition time for the standard and sample was 35 min. The Benzyl chloride was monitored at 220nm.The mixture of water and acetonitrile in the ratio of 20:80 v/v was used as a diluent. The retention time of Benzyl chloride was 13.8 min. The relative standard deviation for the peak areas of six replicate injections of benzyl chloride standard peak is not more than 5.0%

Standard and Sample Solutions

Preparation of standard solution:

Accurately weigh and transfer 51mg of Benzyl chloride standard into 100 ml volumetric flask .Add 50 ml of Diluent, Sonicate to dissolve and dilute to volume with the diluent. Pipette out 5ml of above solution to 50ml with diluent. Further dilute 1ml to 100 ml with diluent.(Concentration is 51 ppm with respect to sample concentration of 10 mg/ml). (Figure 3)

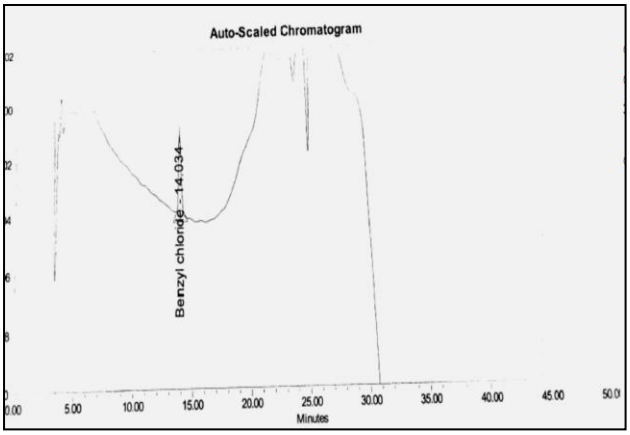

Figure 3: Benzyl chloride standard chromatogram

Preparation of sample solution:

Accurately weigh and transfer about 100 mg of posaconazole sample into 10 ml volumetric flask .Add 5 ml diluent and sonicate to dissolve ,make up to mark with the diluent.(Final sample concentration is 10mg/ml)

RESULTS AND DISCUSSIONS

Method Development and Optimization

The Objective of this work is to determine the low level concentration of Benzyl chloride in posaconazole drug substance with Reverse phase by using HPLC System. Method development was initiated with Benzyl chloride and drug solubility studies .Based on the experiments mixture of water and acetonitrile in the ratio of 20:80v/v was chosen as a diluent. (Figure 4) Preliminary experiment was carried out by using 0.1%v/v ortho phosphoric acid as Mobile phase-A and acetonitrile as Mobile phase-B .Gradient programme was run on symmetry C18,5µm(250x4.6mm) column. In this trail Benzyl chloride peak shape was not good .After that many trails were performed to getting good peak shape, known and unknown impurities are separated from benzyl chloride peak. Using diffrent stationary phases like Phenyl, C8 and C18 (among C18 we tried waters X Bridge, C18, 250X4.6mm, 5µm and 3.5µm) with different phosphate buffers and acetate buffers at various PH(2.0-7.0) by varying diffrent gradient programme.

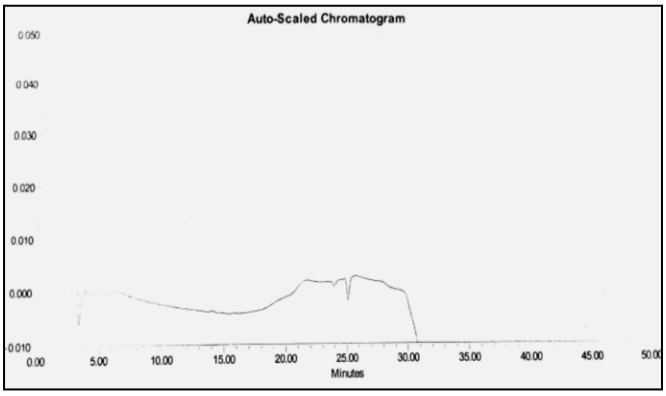

Figure 4: Blank chromatogram

Finally satisfactory results with better peak shape using Waters X Bridge,C18,250X4.6mm,3.5µm ,acetate buffer and Organic modified was methanol at ambient temperature was achieved on chromatographic conditions which have been mentioned in HPLC, was used for validation study to evaluate its performance characteristics.

Method Validation

In order to determine the content of Benzyl chloride in posaconazole drug substance ,the method was validated as per ICH Guidelines, individually in terms of Specificity, limit of detection, limit of quantification, linearity, accuracy, precision(System precision and method precision)

Specificity

Specificity is the ability of the method to determine the analyte in presence of other related substances of drug substance. For specificity determination, diluent, all the related substances of posaconazole were prepared individually and injected into HPLC as per the methodology to confirm the retention times. After that solutions of posaconazole drug substance, posaconazole drug substance spiked with all related substances of posaconazole including Benzyl chloride (all spiked sample solution)were prepared and injected in to HPLC As per the methodology to confirm any co-elution with analyte peaks from respective diluent, any of related substance peaks and the peak homogeneity was verified for each analyte using waters empower software and found to be pure(purity angel should be less than purity threshold) (Figure 5) (Table 1)

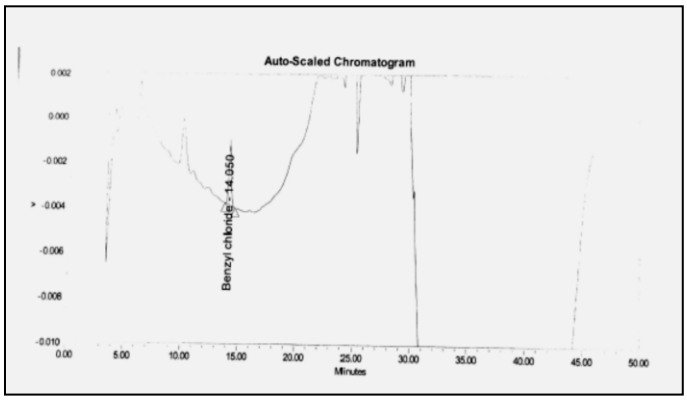

Figure 5: Benzyl chloride spiked chromatogram

Table 1: Specifity

Compound	Purity angle	Purity threshold	Peak purity
Spiked sample (Benzyl chloride)	0.236	0.528	Pass

Sensitivity

The Limit of detection (LOD) (Figure 4) and Limit of quantification (LOQ) (Figure 5) were determined based on the response of Benzyl chloride at that particular wavelength. Based on the response of benzyl chloride Limit of detection is 3ppm and Limit of Quantification is 10ppm.Same concentration of Limit of detection and Limit of quantification were prepared and injected to check the relative standard deviation (Tables 2 and 3)

Table 2: Limit of detection for Benzyl chloride

Compound	Benzyl chloride area
Injection-1	7264
Injection-2	7226
Injection-3	7427
Injection-4	7403
Injection-5	7425
Injection-6	7234
Mean	7330
SD^	98.1
%RSD*	1.34

Table 3: Limit of quantification of Benzyl chloride

Compound	Benzyl chloride area
Injection-1	12889
Injection-2	12789
Injection-3	12211
Injection-4	11876
Injection-5	12570
Injection-6	12638
Mean	12496
SD^	382.5
%RSD*	3.06

^Standard deviation,* Relative standard deviation

Linearity

Good Linearity of Benzyl chloride was evaluated over 6 levels of Benzyl chloride solutions from 0.1µg/ml to 0.75µg/ml. With the linear regression equation Y = mx+c, Where x is concentration in µg/ml and y is corresponding peak area of Benzyl chloride. We observed linear results with respect to concentration of Benzyl chloride VS area of Benzyl chloride (Table 4) (Figures 6 and 7).

The correlation coefficients were greater than 0.999, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 0.1-0.75 µg/ml.

Table 4: Linearity of Benzyl chloride

n	Concentraion of Benzyl chloride (µg/ml)	Benzyl chloride Area
1	0.1	10682
2	0.25	22045
3	0.4	33931
4	0.5	40809
5	0.6	49005
6	0.75	59983
CC	1	
R^2	1	
SLOPE	75973.52	
Y-Intercept	3155.4141	
STEYX	0.0039969	

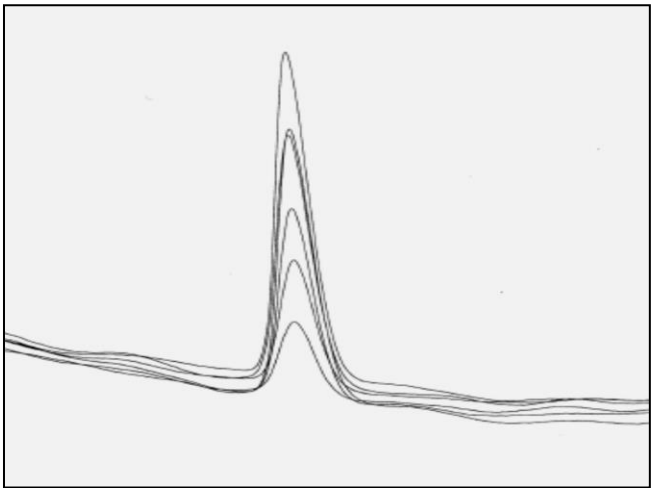

Figure 6: Linearity overlay of Benzyl chloride

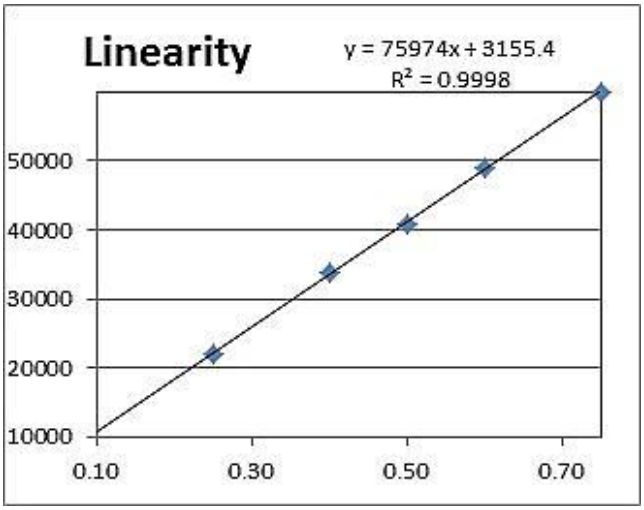

Figure 7: Linearity curve of Benzyl chloride

Precision

System precision:

System precision was the study of the method using repeatability .The performance of the method was evaluated with replicated injections of standard solutions, which indicates the acceptable reproducibility and thare by the precision of the system. The relative standard deviation (RSD) should be less than 5% (Table 5).

Table 5: System precision of Benzyl chloride

n	Area of Benzyl chloride
1	40578
2	40918
3	40751
4	46582
5	40961
6	40523
Mean	40718
SD^	188.206
%RSD*	0.5

^Standard deviation,* Relative standard deviation

Method Precision

Repeatability of the test method (method precision) was demonstrated by analysing six separate sample solutions were prepared using single batch of posaconazole drug substance with known amount of benzyl chloride spiked in sample solution and the percentage relative standard deviation of Benzyl chloride was found to be less than 5.0% (Table 6) (Figure 6)

Table 6: Method precision results of Benzyl chloride (51 ppm) spiked in Posaconazole

n	Benzyl chloride content (ppm)
1	49.94
2	49.83
3	50
4	50.05
5	49.98
6	49.88
Mean	49.95
SD^	0.08091
%RSD*	0.16

^Standard deviation,* Relative standard deviation

Accuracy

The accuracy of the test method was demonstrated by preparing sample solution spiked with known amount of benzyl chloride at three different levels from LOQ, 100% and 150% of specification level and calculates the Benzyl chloride content. (Table 7) The recovery value of enantiomer ranged from 96.7 to 98.1 and the average recovery of three levels (nine determinations) the accepted limits of recovery are 97.5% - 99.7% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 7: Recovery results from spiking of Posaconazole with Benzyl chloride

Accuracy	Level-I	Level-II	Level-III
(Average of triplicates)	(LOQ)	-100%	-150%
Amount added(µg/ml)	0.1	0.499	0.749
Amount found (µg/ml)	0.099	0.487	0.739
Recovery (%)	99.5	97.5	98.7
RSD (%)	0.18	0.48	0.48

CONCLUSION

A simple and sensitive reverse phase HPLC method has developed and validated for the determination of benzyl chloride content in posaconazole drug substance. The results of various validation parameters demonstrated that the method is specific, sensitive, linear, precise and accurate. Hence the proposed method is simple and user-friendly, for the determination of Benzyl chloride in posaconazole drug substance.

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Conflicts of interest: The author declares no conflicts of interest regarding the publication of this paper.

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