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DEVELOPMENT AND VALIDATION OF A CHIRAL LIQUID CHROMATOGRAPHIC METHOD FOR QUANTITATIVE DETERMINATION OF (R)-I-BET762 IN (S)-I-BET762

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ABSTRACT

A simple, rapid and robust enantioselective method was developed and validated for the quantitation of I-BET762 enantiomers [(S)-I-BET762 and (R)-I-BET762] with ultra-performance liquid chromatography (UHPLC) as per ICH guidelines. The active **[**(S)-I-BET762] and inactive **[**(R)-I-BET762] enantiomers were resolved on a Chiralpak-IA column using 0.1% trifluoro acetic acid in water (v/v) and acetonitrile at a flow rate of 1.2 mL min^{-1}. The resolution between the enantiomers was found to be more than 3.4 in the optimized method. The developed method was extensively validated and proven to be robust. The calibration curve for (R)-I-BET762 showed excellent linearity over the concentration range of 5 to 100 µg mL^{-1}. The limit of detection and the limit on quantitation for (R)-I-BET762 were 2 and 5 µg/mL, respectively. The recovery for the (R)-I-BET762 ranged between 96.1 to 109.2% in the bulk drug sample. The proposed method was found to be suitable and accurate for the quantitative determination of (R)-I-BET762 in the bulk drug.

Keywords: I-BET762, Enantiomers, Liquid chromatography, Bulk drug.

INTRODUCTION

The inhibition of bromodomain and extra terminal (BET) domain protein is a growing and extensively studied epigenetic target for the development of novel anti-cancer agents [5]. (S)-I-BET762 (GSK525762) is a novel benzodiazepine derivative and selective BET inhibitor. Currently, (S)-I-BET762 is in Phase I clinical trials for NUT midline carcinoma and hematologic malignancies. (S)-I-BET762 works by mimicking acetylated histone residues thereby disrupting the formation of chromatin complexes essential for expression of inflammatory genes [3]. This compound showed BET proteins inhibition with IC_{50} values of ~35 nm and shown to suppress the pro-inflammatory protein production by macrophages, confer protection against endotoxic shock and bacterial sepsis [2].

For the development of a chiral drug, it is essential to know the pharmacological effect of each enantiomer, as it is well known that the enantiomers can differ in their pharmacological, pharmacokinetic and toxicological behavior [7,8]. Of late, pharmaceutical companies are giving greater importance on evaluating the stereoisomeric composition of drugs having a chiral center. It is therefore important to develop enantioselective separation methods for studies on stereoselective pharmacokinetics and metabolism [6]. Pure enantiomers often exhibit higher potency, bioavailability and reduced side effects when compared to racemates. It is reported that (-)-JQ-1 is inactive as it showed no significant interaction with any BET protein. However the other enantiomer, (+)-JQ-1 is a selective BET protein inhibitor [1].Similarly, (S)-I-BET762 has shown activity against various tumor cells in nano molar range, whereas (R)-I-BET762was found to be inactive (unpublished in house data). With this background we performed a literature search and found that there is no enantioselective method reported for quantification of I-BET762 enantiomers. During the synthesis of (S)-I-BET762 there is a possibility

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of carrying undesired (R)-I-BET762. Hence it is of paramount importance to develop an enantioselective method for quantification of I-BET762 enantiomers. In the present paper we report the development and validation of enantioselective ultra high-performance liquid chromatographic (UHPLC) method for quantification of I-BET762 enantiomers. The application of this method in determining the purity of (R)-I-BET762 in bulk drug is also being elucidated.

METERAILS AND METHODS

Chemicals and reagents

(±)-I-BET762 (50:50; racemic), (S)-I-BET762 (active enantiomer) and(R)-I-BET762 (inactive enantiomer) were synthesized by the Medicinal Chemistry group, Jubilant Biosys, Bangalore, India and were characterized using chromatographic (HPLC, LC-MS) and spectral techniques (Mass, ^{1}H and ^{13}C-NMR) by the Analytical Department, Jubilant Biosys. The purity of the synthesized compounds was >99.8%.HPLC grade of trifluoroacetic acid (TFA) and acetonitrile were purchased from Merck, Mumbai, India and the purity of all the reagents was>99.5%.

Instrument and operating conditions

Agilent technologies LC1290 Infinity system equipped with 1290 binary pump (G4220A), 1290 sampler (G4226A), Infinity TCC (G1316C) and 1290 DAD (G4212A). The output signal was monitored and processed using Agilent Chemstation software. The mobile phase0.1% trifluoroacetic acid was prepared by dissolving 1.0 mL trifluoroacetic acid in 1000 mL of water and mixed with 1000 mL of acetonitrile and the same was filtered through a 0.45 µm membrane filter (XI5522050) (Millipore, USA or equivalent) and then degassed ultrasonically for 5 min. The chromatographic chiral stationary phase used was Chiralpak-IA [(Amylose tris (3,5-dimethylphenylcarbamate) immobilized on 5µm silica-gel] (250 x 4.6mm, 5 µm; Daicel Chemical Industries Ltd, Tokyo, Japan). The flow rate of the mobile phase was 1.2 mL min^{-1}. The column temperature was maintained at 35°C and the eluent was monitored at a wavelength of 230 nm.

Preparation of stock solutions

Stock solutions of (S)- and (R)- enantiomers of I-BET762 at 1.0 mg mL^{-1} were prepared separately by dissolving 10mg of each enantiomer in 10 mL of acetonitrile.

Validation procedures

The developed method was validated according to International Conference on Harmonization (ICH) guidelines for the quantitative determination of the (R)-I-BET762 in (S)-I-BET762 [4].

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The objective of this study was to separate the enantiomers of I-BET762 and accurately quantify the (R)-I-BET762. To develop a suitable chiral HPLC method for the separation of the enantiomers, different mobile phases and chiral stationary phases were employed. The different chiral stationary phases used were Chiralpak-IA, Chiralpak-IC, Chiralcel-OJ-H, Chiralcel-OD-H and Chiralcel OJ-RH. The separation of the enantiomers was realized only on the Chiralpak-IA in reverse phase mobile phase condition. To convert the Chiralpak-IA to reverse phase mode, the column was flushed with ethanol and isopropyl alcohol (1:1, v/v) with 0.7 $mLmin^{-1}$ flow-rate for 30 min, then the column can be used in the reverse phase mode with solvents like water, acetonitrile, methanol and in buffers by adjusting the appropriate pH as recommended by the manufacturer.

The effect of modifier in the mobile phase was checked and the separation remains same as shown in Fig. 2a and Fig. 2b. The effect of the temperature on the separation was observed and there is not much difference in the resolution between the enantiomers (Fig.3).The effect of the flow-rate of the mobile phase was also seen in the chromatogram. Fig. 4 shows that the separation factor remains unchanged. Better separation was achieved on the Chiralpak-IA column (resolution between enantiomers was found to be >3.4) using 0.1%TFA and acetonitrile as mobile phase with a very short chromatographic analysis total run time of 8.0min.

System suitability

The system suitability was determined by injecting a racemic mixture containing equal ratio of (R)-I-BET762 and (S)-I-BET762 under the optimized LC conditions. The specificity was determined by peak purity and it passes having the purity angle lesser than the purity threshold. The retention time of (S)-I-BET762 and (R)-I-BET762 are at 4.10 and 5.18 min, respectively as shown in Fig.5 and the retention time, selectivity, symmetry factor, plates are tabulated in Table 1.

VALIDATION RESULTS OF THE METHOD

Precision

As can be seen in Table 2, the method reproducibility was determined by measuring repeatability and intermediate precision (between-day precision) of retention times and peak areas for each enantiomer. In order to determine the repeatability of the method, replicate injections (n = 6) of a 1.0 mg mL^{-1}solution containing (S)-I-BET762 spiked with (S)-I-BET762 (0.5%) was carried out (Fig.6). In the precision study, the percentage relative standard deviation (RSD) was 0.01 and 0.17% for the retention times of (S)-I-BET762 and (R)-I-BET762, respectively. Peak area (RSD) 1.15% for (S)-I-

BET762 and 0.87% for (R)-I-BET762 as captured in the Table 2.

Intermediate precision

In the intermediate precision study, the percentage RSD was 0.01 and 0.11% for the retention times of the (S)-I-BET762 and (R)-I-BET762, respectively. Peak area (RSD) 0.34% for (S)-I-BET762 and 1.29% for (R)-I-BET762 are given in the table. 2.

Linearity

The described method was linear in the range of 5 to 100 µg $mL^{-1}$for (R)-I-BET762. The calibration curve was drawn by plotting the peak area of (R)-I-BET762 versus its corresponding concentration with a correlation coefficient of 0.999 (Fig. 7). The equation of the calibration curve for (-)-enantiomer Y = 5.112x – 8.636. Linearity was checked for (R)-I-BET762 over the same concentration range for three consecutive days which was showing 0.996 and the developed method was very linear.

Recovery

Standard addition and recovery experiments were conducted to determine the accuracy of present method for the quantitation of the (R)-I-BET762 in bulk drug sample. The recovery for (R)-I-BET762 in bulk sample in triplicate at 40, 60 and 80 µgmL^{-1} of the analyte concentration 1000 µg and the percentage recovery was 96.1, 109.2 and 108.9%, respectively (Table 3). The %RSD for the area was 0.86, 0.31 and 2.0% for the above concentrations. Thus, the method was proved to be accurate for the quantitation of (R)-I-BET762.

Limit on detection and limit on quantitation

The LOD and LOQ concentration were estimated at 2 and 5 µg mL^{-1} for (R)-I-BET762. The precision of the developed enantioselective method for (R)-I-BET762 at LOQ was checked by analyzing six test solutions prepared at the LOQ level and calculating the percentage relative standard deviation of area, the precision was 1.05%.

(R)-I-BET762 stability

The % RSD for the area of (R)-I-BET762 content during solution stability study and mobile phase stability experiments were carried out for 48 h, the drug was stable and there was no formation of the other enantiomer. The %RSD in the solution stability study was less than 0.3 and the %RSD in the mobile phase stability study was less than 0.5. The sample solution and mobile phase were stable for at least 2 days.

Table 1. System suitability

	RT(min)	k'	Symmetry factor	Plates	Rs	Selectivity
(S)-I-BET762	4.100	0.97	0.67	5509	3.55	1.54
(R)-I-BET762	5.182	1.49	0.67	2853		

k': capacity factor; Rs: resolution

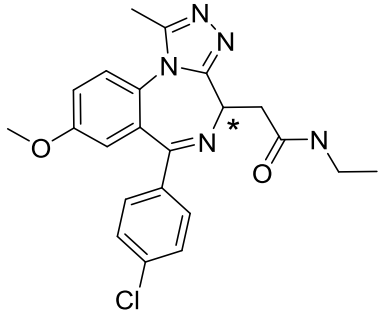
Table 2. Repeatability, intermediate precision, LOD, LOQ and Linearity

Validation parameters	Results	
Precision (*n*=6) (%RSD) (Lab. A)	**(S)-I-BET762**	**(R)-I-BET762**
Retention time (%RSD)	0.01	0.17
Peak area (%RSD)	1.15	0.87
Intermediate Precision (n=6) (Lab. B)		
Retention time (%RSD)	0.01	0.11
Peak area (%RSD)	0.34	1.29
Sensibility		
LOD (µg mL^{-1})	2	2
LOQ (µg mL^{-1})	5	5
Precision at LOD (%RSD) (*n*=6)	1.89	1.92
Precision at LOQ (%RSD) (*n*=6)	1.02	1.05

Table 3. Recovery study of (R)-I-BET762

Amount spiked (µg mL^{-1})	% Recovery	% RSD
40	96.1	0.86
60	109.2	0.31
80	108.9	2.00

Figure 1. Structural representation of (±)-I-BET762 (racemic)



(±)-I-BET762

Figure 2a. Effect of modifier on the separation of I-BET762 enantiomers

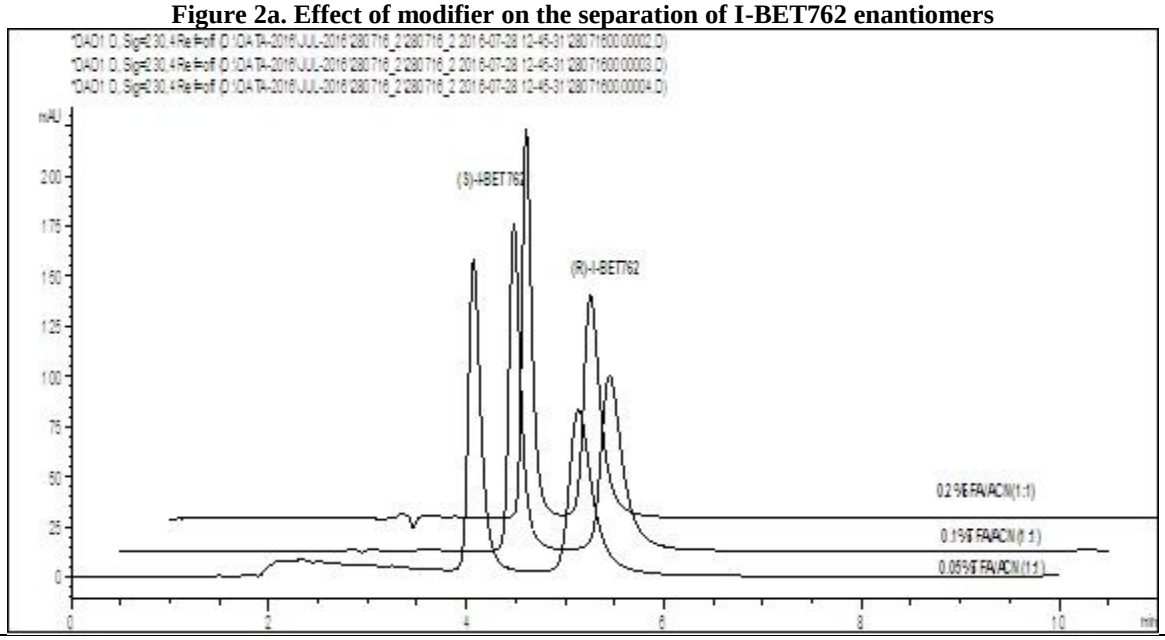


Figure 2b. Effect on the separation of I-BET762 enantiomers without modifier

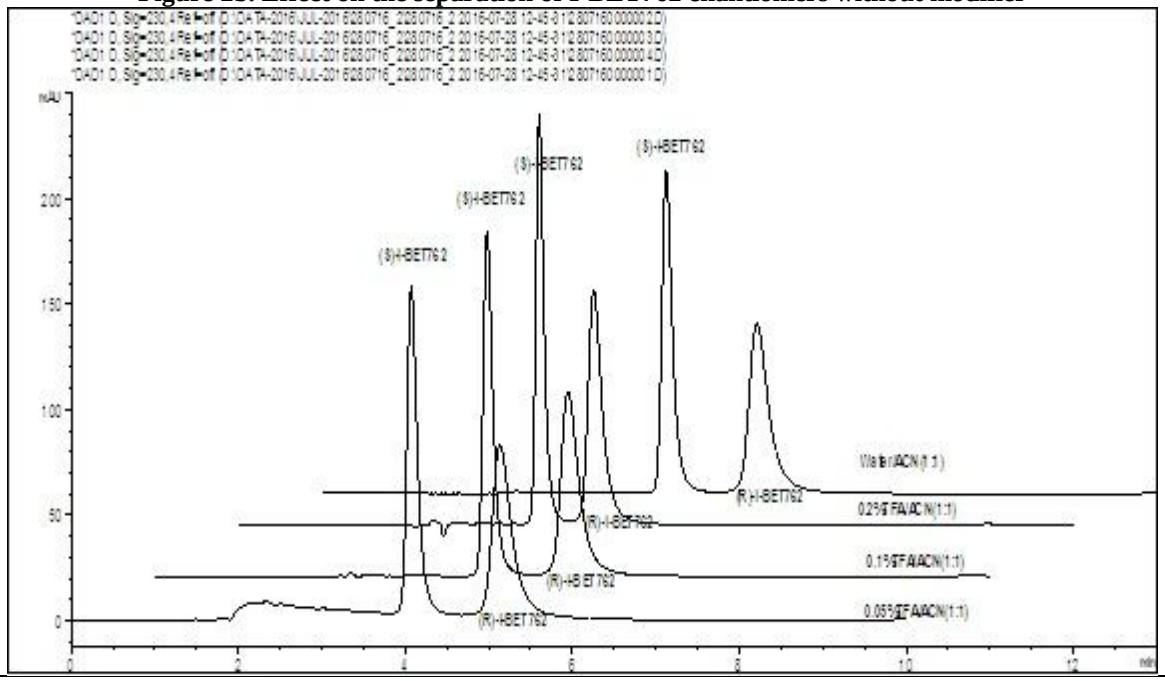


Figure 3.Effect of column temperature(30°C, 35°C and 40°C) on the separation of I-BET762 enantiomers

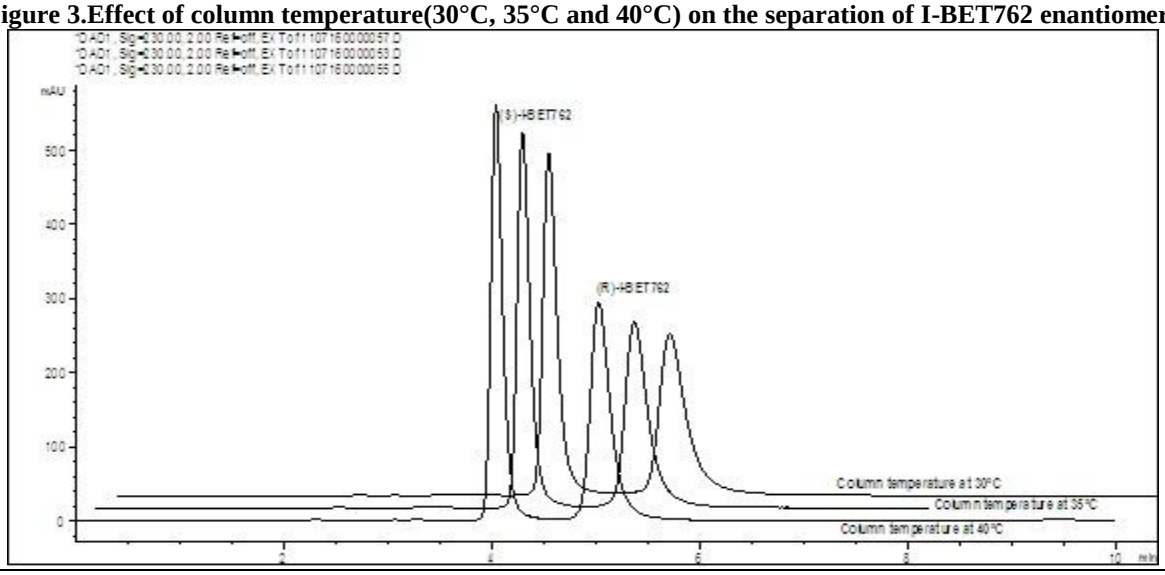


Figure 4.Effect of flow rate on the enantiomeric separation of I-BET762

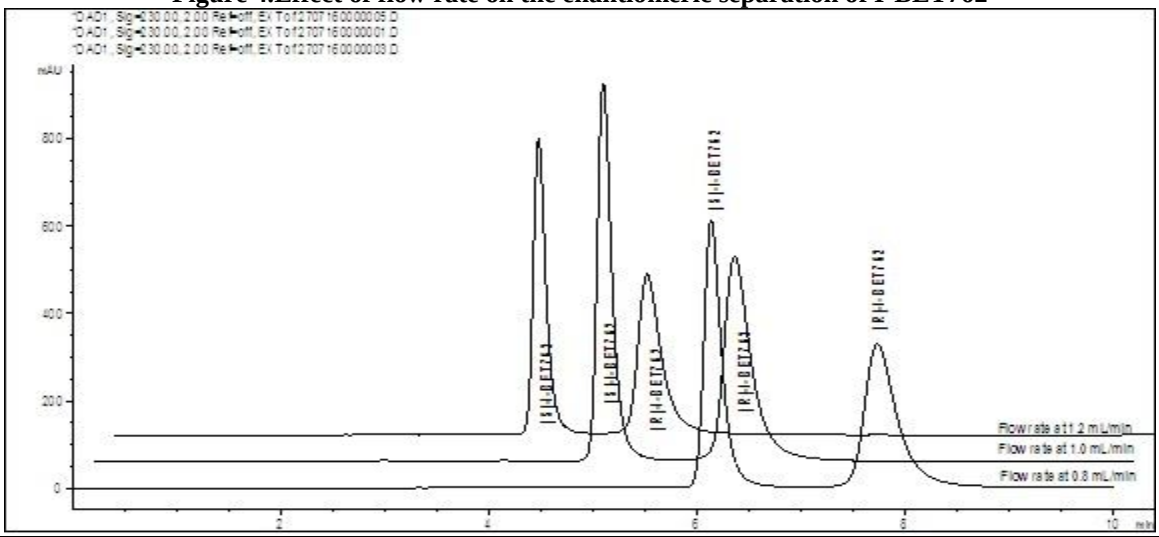


Figure 5. Enantiomeric resolution of racemic I-BET762 on Chiralpak IA

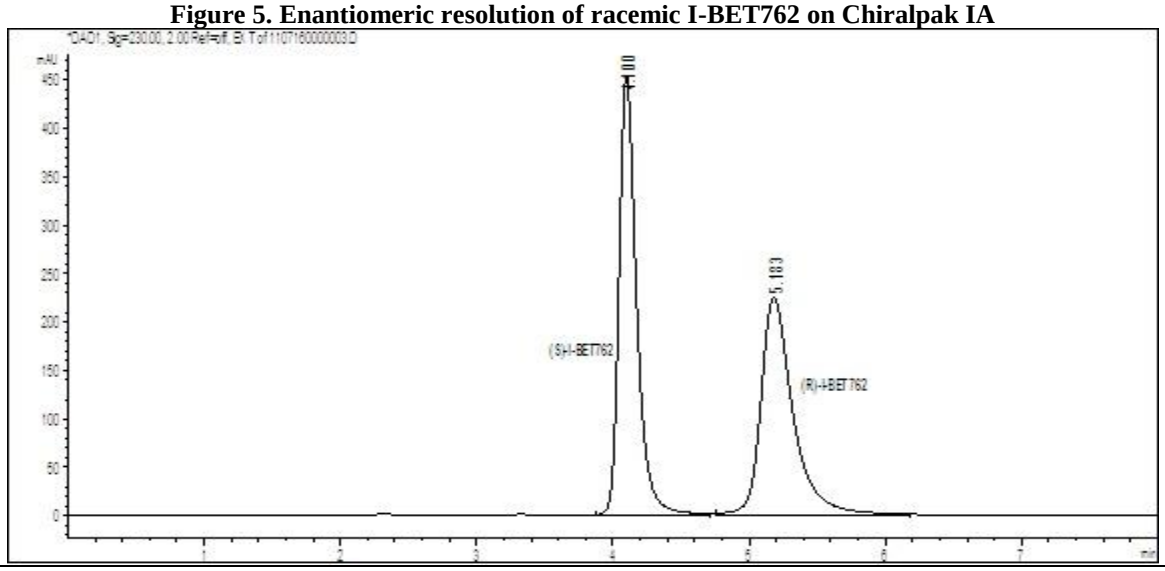


Figure 6. HPLC chromatogram of the (S)-I-BET762bulk samples (1.0 mg mL^{-1}) spiked with (R)-I-BET762 (0.5%).

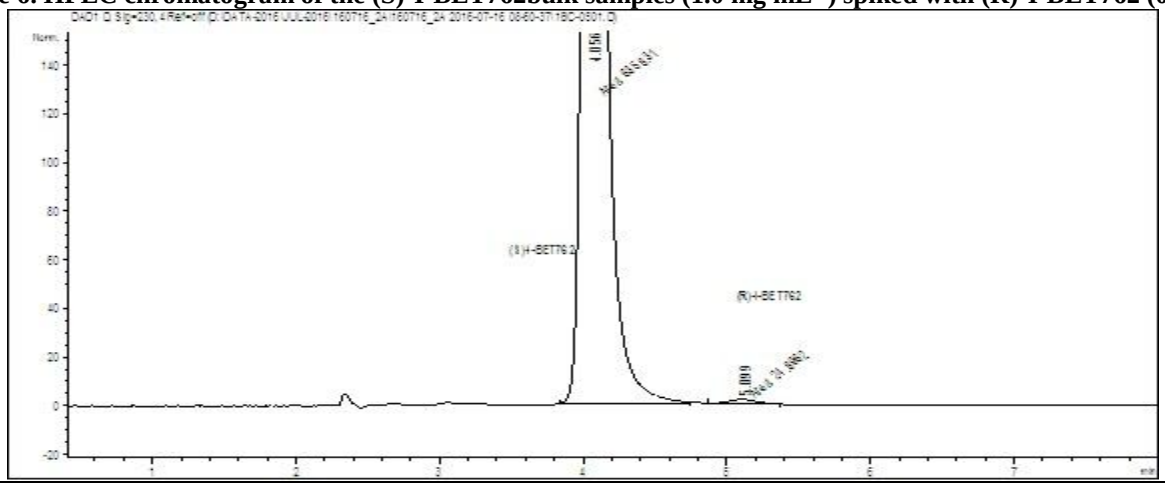
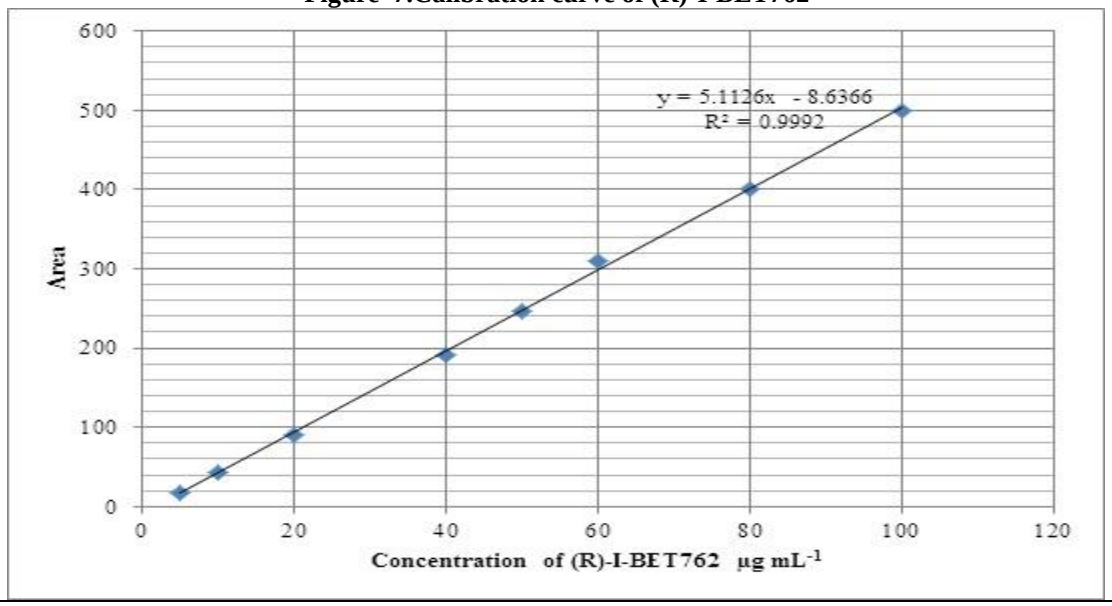


Figure 7.Calibration curve of (R)-I-BET762



CONCLUSION

A simple, rapid and selective chiral UHPLC method has been developed and validated as per ICH guidelines for the enantiomeric separation of I-BET762 using Chiralpak-IA having a resolution greater than 3.4 between the enantiomers within 8 min. The validated method can be conveniently used by the quality control department for the quantitative determination of (R)-I-BET762 in the bulk drug [(S)-I-BET762].

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