Separation Of Voriconazole Enantiomer By Using Supercritical Fluid Chromatography

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ABSTRACT:

Voriconazole and its enantiomer were well separated with the resolution of 5.4 on an amylose derivative of the Chiralpak AD-H column (250x30 mm,5µ) within 10 minutes of runtime using supercritical fluid chromatography (SFC). It screened the separation of the aforementioned compounds under ten different polysaccharide derivatives of amylose and cellulose columns, solvents with a wide range of polarities as methanol, acetonitrile, 2-propanol, ethanol. The effect of both acidic (TFA) and basic (DEA) modifiers was also tried to achieve better separation. Elution times and enantio-selectivities of above mention conditions affected the separation of the drug and its enantiomer. Finally, the chromatographic conditions were fully optimised to achieve the excellent separation of those compounds. The developed method was applied for the separation of desired voriconazole and its enantiomer. Besides, it also used it as for the isolation and purification of voriconazole between mg to kg level. The developed method was robust, rapid and easy to apply from analytical scale to preparative scale.

Index terms*:* Voriconazole, SFC (Supercritical fluid chromatography), TFA (Trifluoroacetic acid), DEA (Diethanolamine)

I.INTRODUCTION:

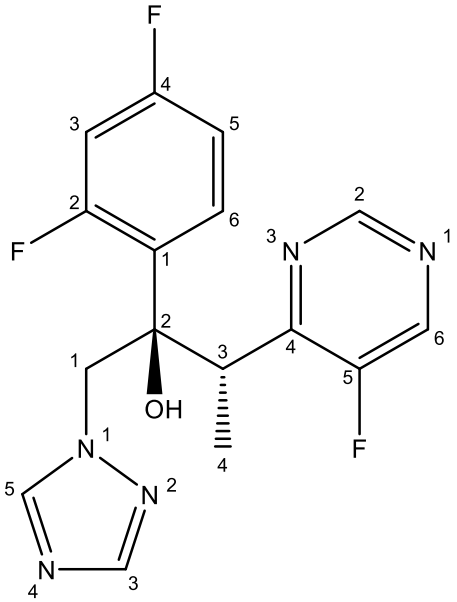
Most of the pharmaceutical industries make different enantiomeric compounds that contain one or more stereo genic centres that are mirror images (1,2,3). These compounds show different physiological effects, means one enantiomer is in a useful form, and others can be hurtful. Enantiomers have similar physical and chemical properties in achiral environs, but in chiral conditions, one enantiomer differs from their second enantiomer, which behaves different chemical and pharmacological effects (4,5). The R-enantiomer will not behave as S-enantiomer given to the patient. So, we must separate each enantiomer unless proven.

It was a known fact that I found stereoisomeric compounds to be tedious in its separation by typical HPLC methods using reversed-phase C18 for determining their relative composition. For their separation, chiral phases have to be used, which own modified cellulose (cellulose derivative) or amylose on their surface. In the past normal-phase, HPLC was the only choice used for the chiral analysis, though some problems like long-run times, unreproducible retention times, and usage of corrosive solvents pertain. Analytical methods (6,7,8) which have been used for enantiomers separation are depicted in Fig 1. Among them, SFC (supercritical fluid chromatography) (9,10) has proven to be one of the adequate separation techniques that came into light these days, which overcame all the mentioned problems.

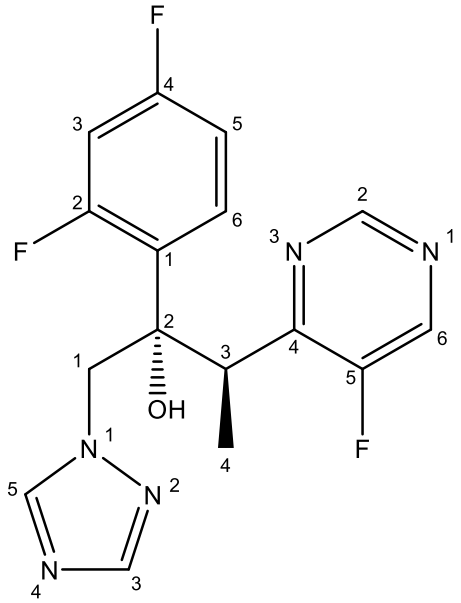
There are some issues arisen in their separation, such as longer retention times, solvent consumption, and mostly reproducibility. To overcome all these issues, we developed greener and a mild condition through the SFC technique, which has shown better results when compared with existing methods.

Fig1: Techniques used for the separation of enantiomers

Voriconazole ((2R, 3S)-2- (2,4-di fluorophenyl)-3- (5-fluoro-4-pyrimidinyl)-1- (1H-1,2,4-tri azol-1-yl)-2-butanol) is a derivative of fluconazole (11,12) and known to be an antifungal agent (Fig2). It possesses a triazole part replaced in with a fluoropyrimidine ring and a methyl group attached to the adjacent hydroxy. This change in structure resulted in the enhancement of its potency and broad-spectrum activity in vitro and a fungicidal activity studies against different mould species, beside Aspergillus. Voriconazole is available in the form of tablets and powder for the sake of oral suspension, along with sterile freeze-drying powder for intravenous infusion following reconstitution with proper dilutions.



(2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol



(2*S*,3*R*)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol

Fig2: Structure of Voriconazole and its enantiomer

The determination of voriconazole was reported through various chromatographic techniques such as HPLC, LC-MS, XRD, IR, DSC, and UV (13,14,15,16,17.18,19,20). "A coupled achiral-chiral LC method (21) was also described for the determination of the potential impurities of Voriconazole both qualitatively and quantitatively".

II.MATERIALS AND METHODS:

2.1 Chemical reagents and Sample: CO2 bought from Sicgil; ethanol, methanol, acetonitrile, DEA, and TFA from Merck; IPA from Rankem. All chemicals were of analytical grade. Voriconazole was gifted from a manufacturing unit in Hyderabad, India.

2.2 Equipment's: The chromatographic system from Thar SFC method station comprised a diode array detector (Waters 9908), a binary pump, an automatic injector, and a thermostatic column compartment was used for the development. I used the chrome scope software for data acquisition and system control. I attained separation of the compound on chiralpak AD-H (250X30 mm),5µ.

2.3 Chromatographic conditions: The mobile phase has a mixture of CO2, Acetonitrile, Methanol, Ethanol,2-propanol, acidic (TFA), and basic additive (DEA). The optimum mobile phase composed of (CO2: MeOH) (90:10) pumped at a fixed flow rate of 3.0 ml/min. I selected the temperature at 30 °C, and the volume of injection was 10ul. The analyte detection carried out photometrically at 260 nm. Resolution (Rs) was calculated by the retention times.

III.RESULTS AND DISCUSSION:

3.1 Method development and Optimisation:

A few of normal phase HPLC, DSC&NMR methods for the determination of voriconazole were well reported (22,23,24,25), but we demonstrated no SFC method for separation of voriconazole and its enantiomers in the racemic mixture till now to the best of our knowledge. Polysaccharide (chiralpak AD-H) stationary phase column with methanol as a modifier; these two enantiomers were separated by using the SFC technique. Methanol is an often-used mobile phase in SFC applications for both achiral and chiral molecules. In this study, the aptness of this position of methanol as the primary way is studied by performing all separation attempts with methanol, acetonitrile, 2-propanol, and ethanol as cosolvents.

The prepared stock solutions were initially screened for chiral method development using liquid CO2 (90%) and methanol (10%) as the mobile phase with amylose-based columns(Fig3). I performed the solution through all the mentioned columns (amylose and cellulose) given in table 1. I observed it that in chiralpak AD-H, the resolution of the peaks was around 5.4 for the peaks got at 4.4 and 5.85 RT (Retention Time). Similarly, the same condition used for the

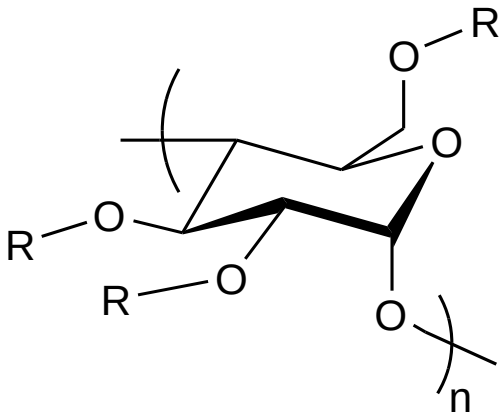
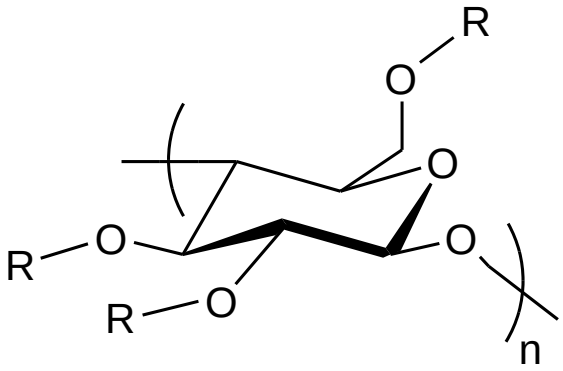
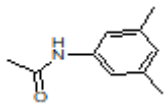
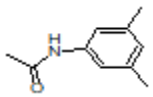
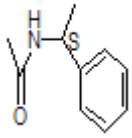
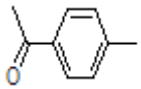
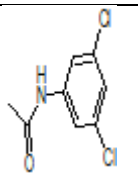
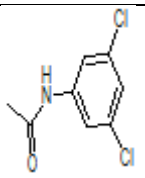
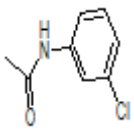
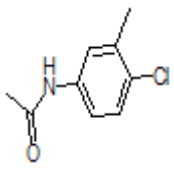
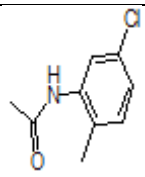
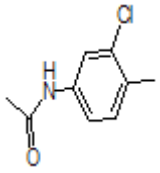
chiralpak AS-H, where the resolution was 0.49, with peak RTs at 1.68 and 1.78 min. Chirapak-IE showed a resolution of 1.8 with peak RTs 6.7 and 7.5 minutes under the same conditions. Chiralpak-ID displayed a resolution of 1.13 with 3.73 and 3.98 RT.

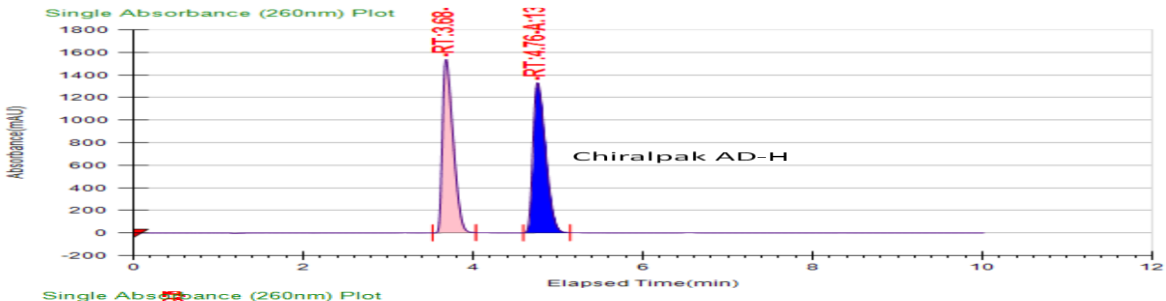
It was also tested with luxamylose-2 that produced 0.3 resolution for the peaks 2.56 and 2.62 RT. Among all the screening of the columns, it was observed that chiralpak AD-H had shown better resolution when compared with the other chiral polysaccharide amylose columns. The same screening was also carried out with 15-30% of methanol/CO2 mobile phases with all the mentioned columns in table 1. Among all the screenings, we observed a good resolution with 10% methanol/CO2, as mentioned above.

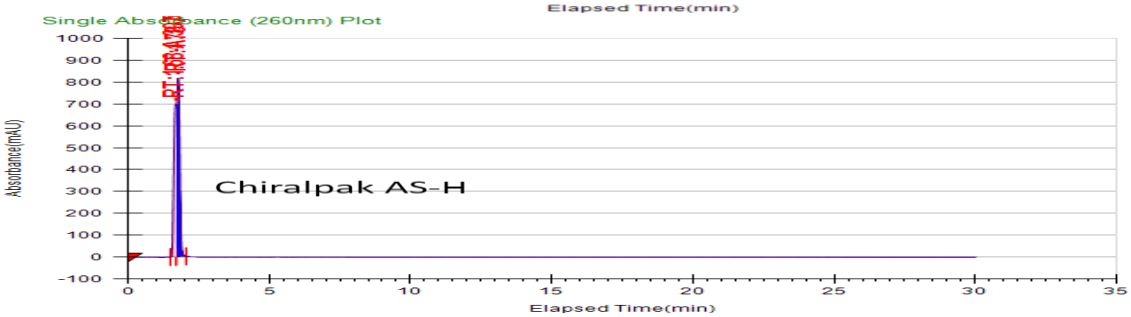
Similarly, the same experiment was carried out in cellulose columns, as mentioned in table 1. I observed it that in (Fig4) chiralcel OD-H; the resolution was around 2.2, with peaks appearing at 4.25 and 4.76 RT. Whereas in chiralcel OJ-H, no separation occurred for the voriconazole solution. Chiralpak-IC displayed a better resolution with 7.4, with the peaks appearing at 15.9 and 27.07 RT. A better resolution even occurred when the samples are run through chiralcel OX-H and luxcellulose-2 with 17.9 and 25.1. Though the latter three columns displayed a good resolution, we observed their runtime was also over 30 minutes. Keeping all these results in mind, chiralpak AD-H was considered being an optimum column in 10% methanol/CO2 mobile phase that has shown better resolution in shorter runtime (Fig5). Our major interest in this experiment stayed in developing a convenient method that could produce better separation in the shorter run time.

The developed method was also verified by using different modifiers such as ethanol, isopropyl alcohol (IPA), acetonitrile (ACN), diethylamine (DEA), and Trifluoroacetic acid (TFA). Unfortunately, in none of the cases, a good resolution was not observed, and in most cases, a proper separation also did not observe.

Table1: Different amylose and cellulose derivative columns

Modified Amylose1			Modified Cellulose2		
					
Chiral stationary phase (CSP)	Nature of the stationary phase	R	Chiral stationary phase (CSP)	Nature of the stationary phase	R
Chiralpak AD-H	Coated		Chiralcel OD-H	Coated	
Chiralpak AS-H	Coated		Chiralcel OJ-H	Coated	
Chiralpak IE	Immobilised		Chiralpak IC	Immobilised	
Chiralpak ID	Immobilised		Chiralcel OX-H	Coated	
Lux Amylose 2	Coated		Lux Cellulose 2	Coated	





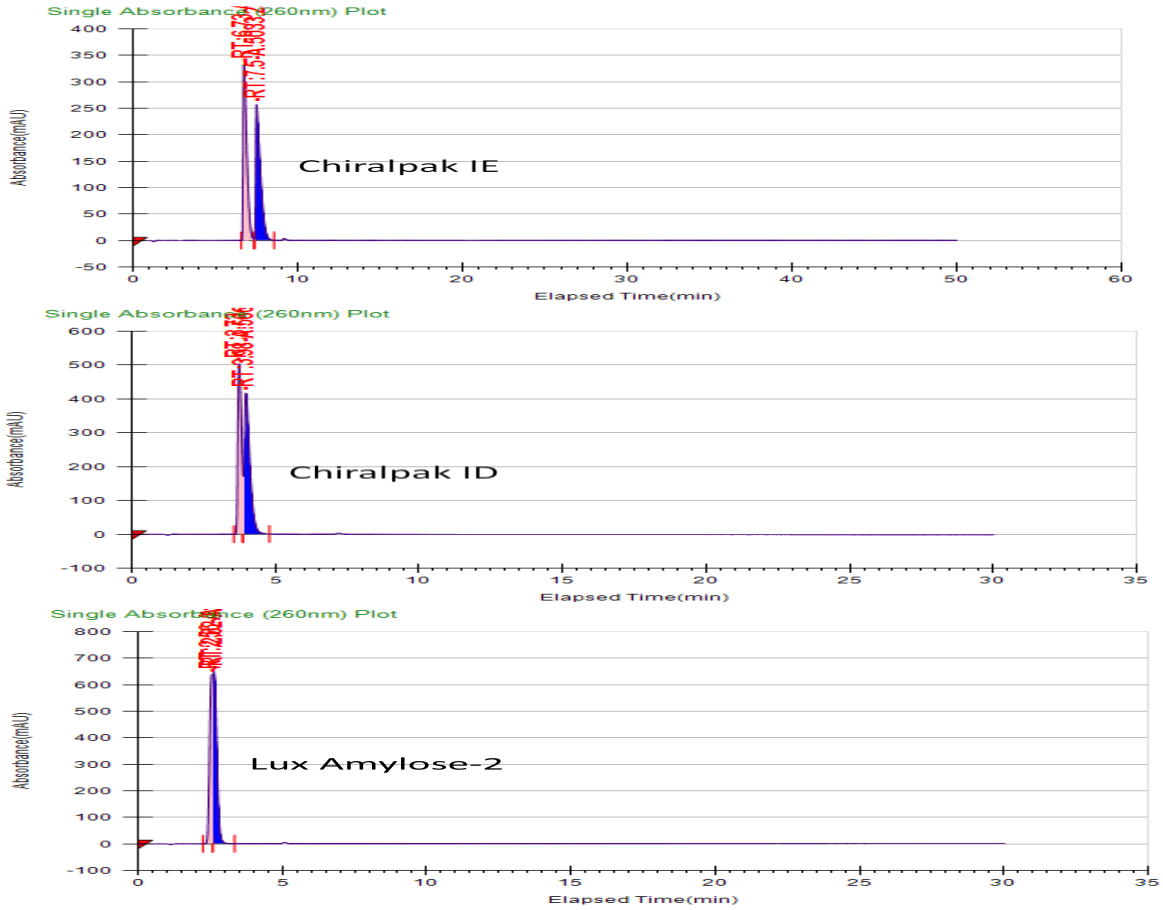
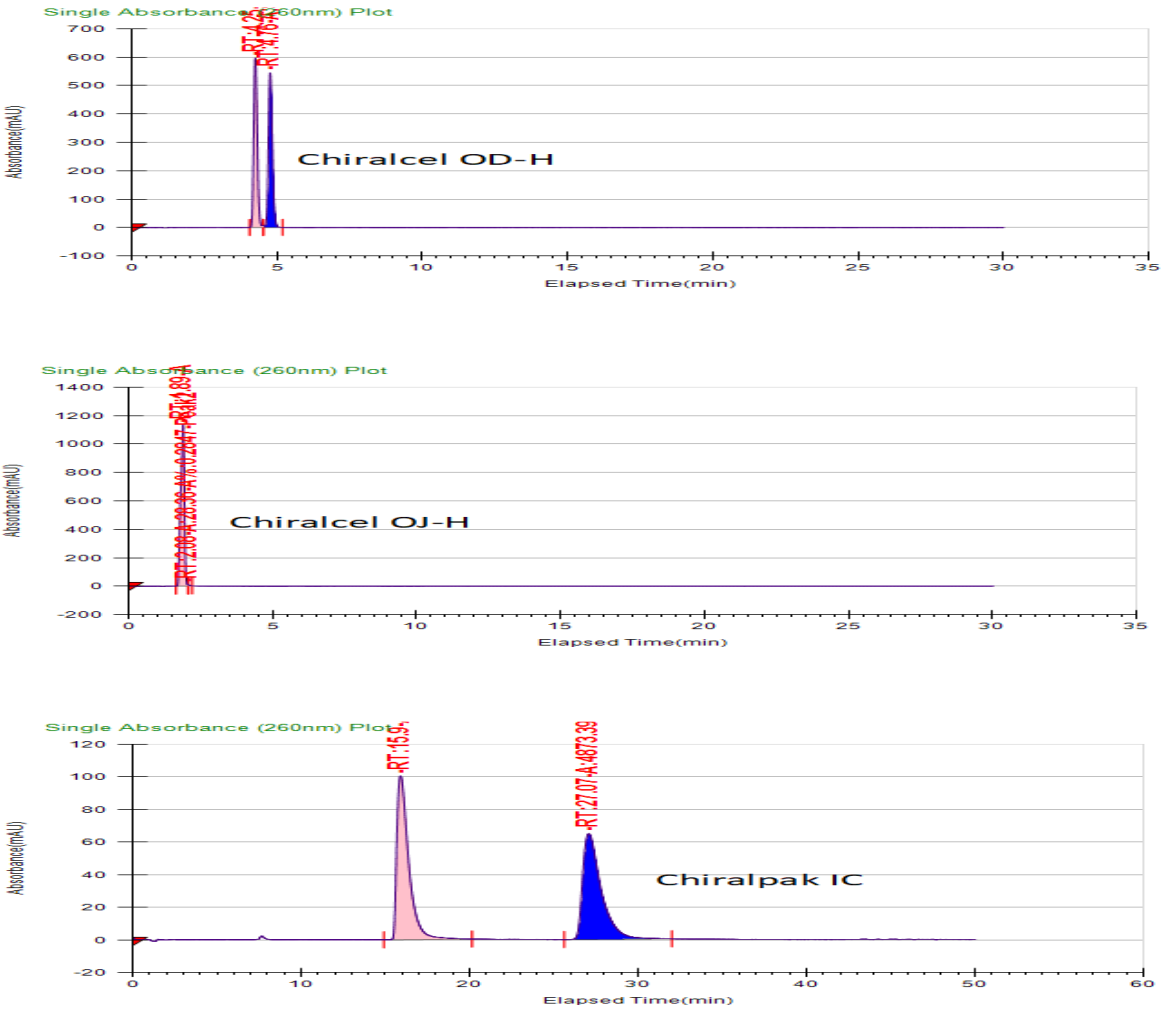


Fig3: Separation chromatograms of voriconazole and its enantiomer using amylose derivative columns



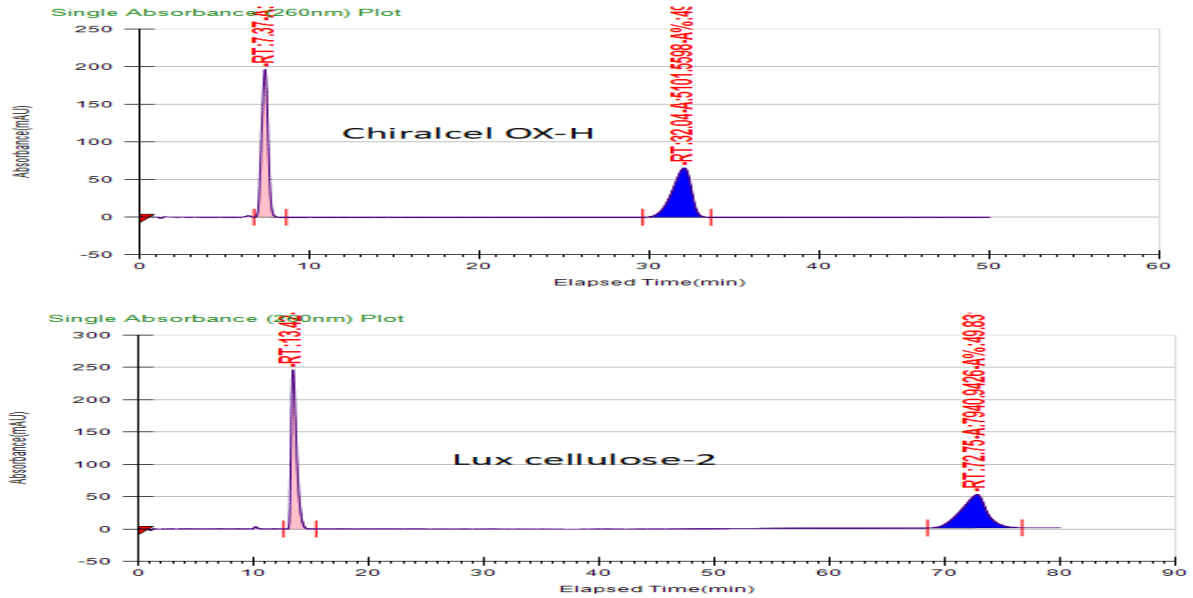


Fig4: Separation chromatograms of voriconazole and its enantiomer using cellulose derivative columns

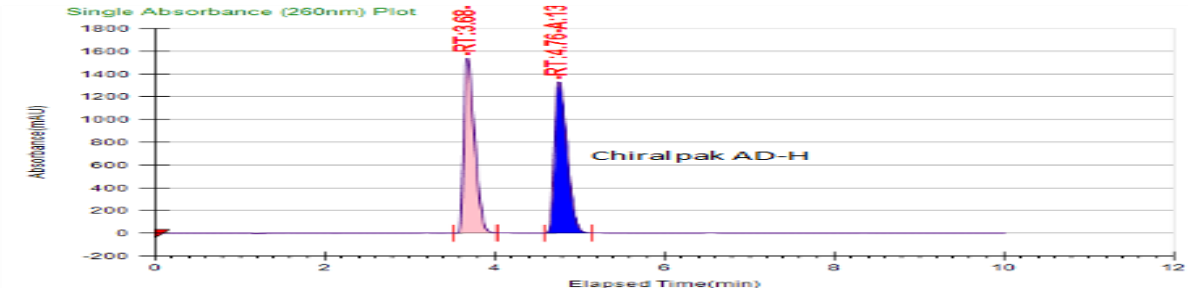


Fig5: Chromatogram of optimized condition.

IV.CONCLUSION:

A facile, rapid Supercritical fluid chromatographic method for the enantiomeric separation of voriconazole has been developed. Different stationary phases tested among them; an amylose derivative of the chiralpak AD-H column was suitable for the separation of the enantiomers of voriconazole. Use of chiralpak AD-H column with Supercritical fluid liquid (CO2) and methanol (90:10%, v/v) as the mobile phase was found to be a suitable condition for the separation of enantiomers present in voriconazole. The advantage of enantiomeric separations by SFC is its cost-effective, less time, and environmentally benign than normal phase liquid chromatographic methods. We can also use this method for the isolation of undesired isomer by using the preparative SFC system with lesser time and low cost.

V.ACKNOWLEDGMENTS

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