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Research Article

SEPARATION AND PURIFICATION OF VORICONAZOLE ENANTIOMER BY SUPERCRITICAL FLUID CHROMATOGRAPHY

Atchuta Ramakrishna Gudapati*[1], Srinivasa Rao Pakalapati[1], Chidananda Swamy Rumalla[1], Benarji Patrudu Tammina[2]

[1]*Department of Medicinal Chemistry, GVK Biosciences Pvt. Ltd, IDA Nacharam, Hyderabad, Telangana, India*

[2]*Department of Chemistry, Gitam School of Science, Rudraram, Patancheru Mandal, Hyderabad, Telangana, India*

**Corresponding author: atchutaramakrishnagudapati@gmail.com*

ABSTRACT

The present research work involves the development and purification of a novel supercritical fluid chromatography SFC) method for the separation of voriconazole and its enantiomer. The separation of the aforementioned compounds was examined using ten different polysaccharide derivatives of the amylose and cellulose columns, solvents having a broad range of polarities such as methanol, acetonitrile, 2-propanol, ethanol. The effect of both acidic (TFA) and basic (DEA) additives (0.1-0.5% v/v) was also tried to achieve better separation. Elution times and enantio-selectivities of above mentioned conditions affected the separation of the drug and its enantiomer. Finally, the chromatographic conditions have been fully optimized to achieve the excellent separation of these compounds. Voriconazole and its enantiomer were well separated with the resolution of 5.4 on an amylose derivative of the Chiralpak AD-H column (250x4.6 mm, 5µ), backpressure 100 bar, within 10 min of runtime using supercritical fluid chromatography (SFC). The method developed has been applied for separating the desired voriconazole and its enantiomer. In addition, it is also used for the isolation and purification of voriconazole between mg to g level. Thus, the method developed was quick and easy to apply from an analytical scale to a preparative scale.

Keywords: Voriconazole, Supercritical Fluid Chromatography (SFC), Amylose, Cellulose, Analytical scale, Preparative scale.

1. INTRODUCTION

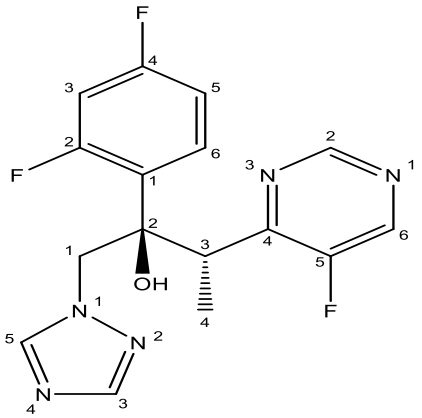
Most pharmaceutical industries manufacture different enantiomeric compounds that contain one or more stereogenic centres that are mirror images [1-3]. These compounds show different physiological effects, which means one enantiomer has preferential biological properties, where the other enantiomer is undesired due to different biological properties or side effects. Enantiomers have similar physical and chemical properties in achiral environs, but under chiral conditions, one enantiomer is different from their second enantiomer, which has 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. It was a known fact that found stereoisomeric compounds were difficult to separate by typical HPLC methods using reversed-phase C18 to determining their relative composition except polar organic mode. For their separation, chiral phases have to be used, which own modified cellulose (cellulose derivative) or amylose on their surface. Numerous other chiral stationary phases do not contain polysaccharides (*i.e.* Pirkle type, Cyclodextrin, antibiotics, etc). 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, preparative methods [6, 7] which have been used for enantiomers separation are depicted in Fig 1. Among them, SFC (supercritical fluid chromatography) [8, 9] has proven to be one of the adequate separation techniques that came into light these days, which overcame all the mentioned problems.

Some issues arise in their separation by using NP-HPLC, polar organic mode, such as longer retention times, more solvent consumption, and mostly reproducibility. In order to overcome all these problems, we developed a greener condition using the SFC technique, which has shown better results in comparison with existing methods.

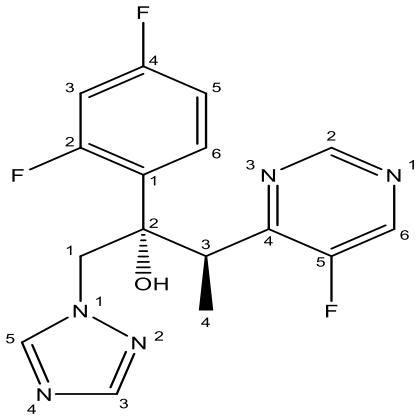
Fig 1: Techniques used for the separation and purification of enantiomers

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 [10, 11] and known to be an antifungal agent. It possesses a triazole part replaced with a fluoropyrimidine ring and a methyl group attached to the adjacent hydroxy (Fig. 2).This change in structure resulted in the enhancement of its potency and broad-spectrum activity in vitro and fungicidal activity studies against different mold species, besides 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.

The determination of voriconazole was reported through various chromatographic techniques such as HPLC, LC-MS, XRD, IR, DSC, and UV [12-19]. A coupled achiral-chiral LC method [20] was also described for the determination of the potential impurities of Voriconazole both qualitatively and quantitatively.



(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

Fig. 2: Structure of Voriconazole and its enantiomer

2. MATERIAL AND METHODS

2.1. Chemical reagents and Sample

CO_2 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. Equipments

The chromatographic system from the 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. The chrome scope software was used for data acquisition and system control. Purification was done in the Thar SFC-350 prep system. Separation of the compound was achieved on Chiralpak AD-H (250X4.6 mm) 5µ, and prep purification was done in Chiralpak AD-H (250X30 mm) 10 µ column. All the columns were procured from Diacel technologies except Lux amylose, Lux cellulose (Phenomenex).

2.3. Chromatographic conditions

The mobile phase has a mixture of CO_2, Acetonitrile, Methanol, Ethanol, 2-propanol, acidic (TFA), and basic additive (DEA). The optimum mobile phase composed of (CO_2: MeOH) (90:10) pumped at a fixed flow rate of 3.0 ml/min and backpressure 100 bar. The temperature was set at 35°C, and the volume of injection was 10ul. The analyte detection was carried out photometrically at 260 nm. Resolution (Rs) was calculated by the retention times.

3. RESULTS AND DISCUSSION

A few normal phase HPLC, the polar organic mode by HPLC, DSC&NMR methods for the determination of voriconazole were well reported (21-24) but so far, to the best of our knowledge, no SFC method for separation of voriconazole and its enantiomers in the racemic mixture. 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 CO_2 (90%) and methanol (10%) as the mobile phase with amylose-based columns. The solution was performed through all the mentioned columns (amylose and cellulose) given in table 1. It was observed 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 was 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 min under the same conditions. Chiralpak-ID displayed a resolution of 1.13 with 3.73 and 3.98 RT.

It was also tested with Lux amylose-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 (Fig. 3). The same screening was also carried out with 15-30% of methanol/CO_2 mobile phases with all the mentioned columns in Table 1. Among all the screenings, we observed a good resolution with 10% methanol/CO_2, as mentioned above.

Similarly, the same experiment was carried out in cellulose columns, as mentioned in table 1. It wasobserved that in (Fig. 4) 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 min. Keeping all these results (Table 2) in mind, Chiralpak AD-H was considered being an optimum column in 10 % methanol/CO_2 mobile phase that has shown better resolution in shorter runtime (Fig. 5). Our major interest in this experiment stayed in developing a convenient method that could produce better separation in a shorter run time. The developed method was also verified by using different modifiers such as ethanol, isopropyl alcohol (IPA), acetonitrile (ACN), and additives 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.

The developed method was successfully applied with minor modifications of the preparative scale using the Chiralpak AD-H column (250x30) mm, 10µ column, flow rate 100ml/min. Under these developed conditions 1gr of enantiomer mixture was injected by using stacked injections (Fig. 6). The two enantiomers were collected separately in separate containers and each fraction was injected under optimized analytical conditions. Each isomer was achieved more than 99% of ee. The yields of both enantiomers were 95% based on the total amount of both isomers. Also, the same purification has been performed in NP-PREP purification but more consumption of organic solvent, longer runtime, more workup time, too much fraction volume obtained than SFC purification details given in (Table 3).

Effectiveness of preoperative SFC in comparison with preparative NP-HPLC

- The fraction volume on SFC is reduced by 4 times and therefore the solvent is easily removed during the post-purification process.
- Solvent use on SFC is 7 times lower than HPLC.
- The total cost of purification on SFC is lower than one fourth on purification by NP-HPLC.

Column: Chiralpak AD-H (250X30)mm,10µ ;Mobile phse (90:10) (CO_2 :MeoH);Flow rate:100ml/min; ABPR back pressure:100 bar;Temperature:35°C; PDA:Extracted wavelength at 260 nm.

Table 1: Different types of amylose and cellulose derivatives used for method development

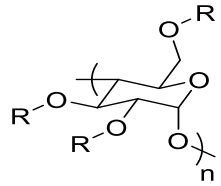
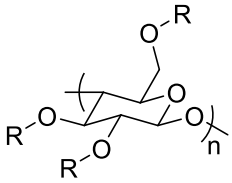
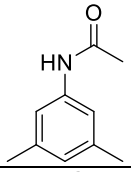
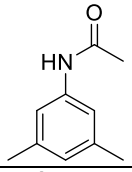
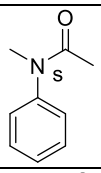
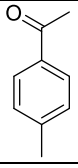
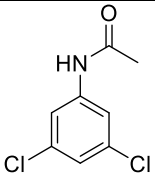
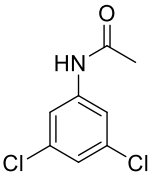
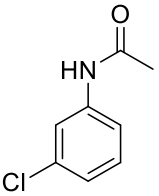
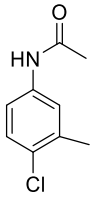
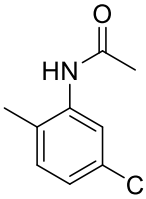
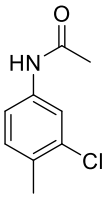
Modified Amylose1			Modified Cellulose 2		
					
Chiral stationary phase (CSP)	stationary phase (Nature)	R	Chiral stationary phase (CSP)	stationary phase (Nature)	R
Chiralpak AD-H	Coated		Chiralpak OD-H	Coated	
Chiralpak AS-H	Coated		Chiralcel OJ-H	Coated	
Chiralpak IE	Immobilized		Chiralpak IC	Immobilized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

Table 2: Resolution obtained using various columns

S. No	Column Type	Column	RT of peak 1	RT of peak 2	Resolution
1	Set-1 (Amylose derivatives)	Chiralpak AD-H	3.68	4.76	5.4
2		Chiralpak AS-H	1.68	1.78	0.49
3		Chiralpak IE	6.7	7.5	1.8
4		Chiralpak ID	3.73	3.98	1.13
5		Lux amylose-2	2.56	2.62	0.3
6	Set-2 (Cellulose derivatives)	Chiralcel OD-H	4.25	4.76	2.2
7		Chiralcel OJ-H	No separation	No separation	No separation
8		Chiralpak IC	15.9	27.07	7.4
9		Chiralcel OX-H	7.37	32.04	17.9
10		Lux cellulose-2	13.4	72.75	25.1

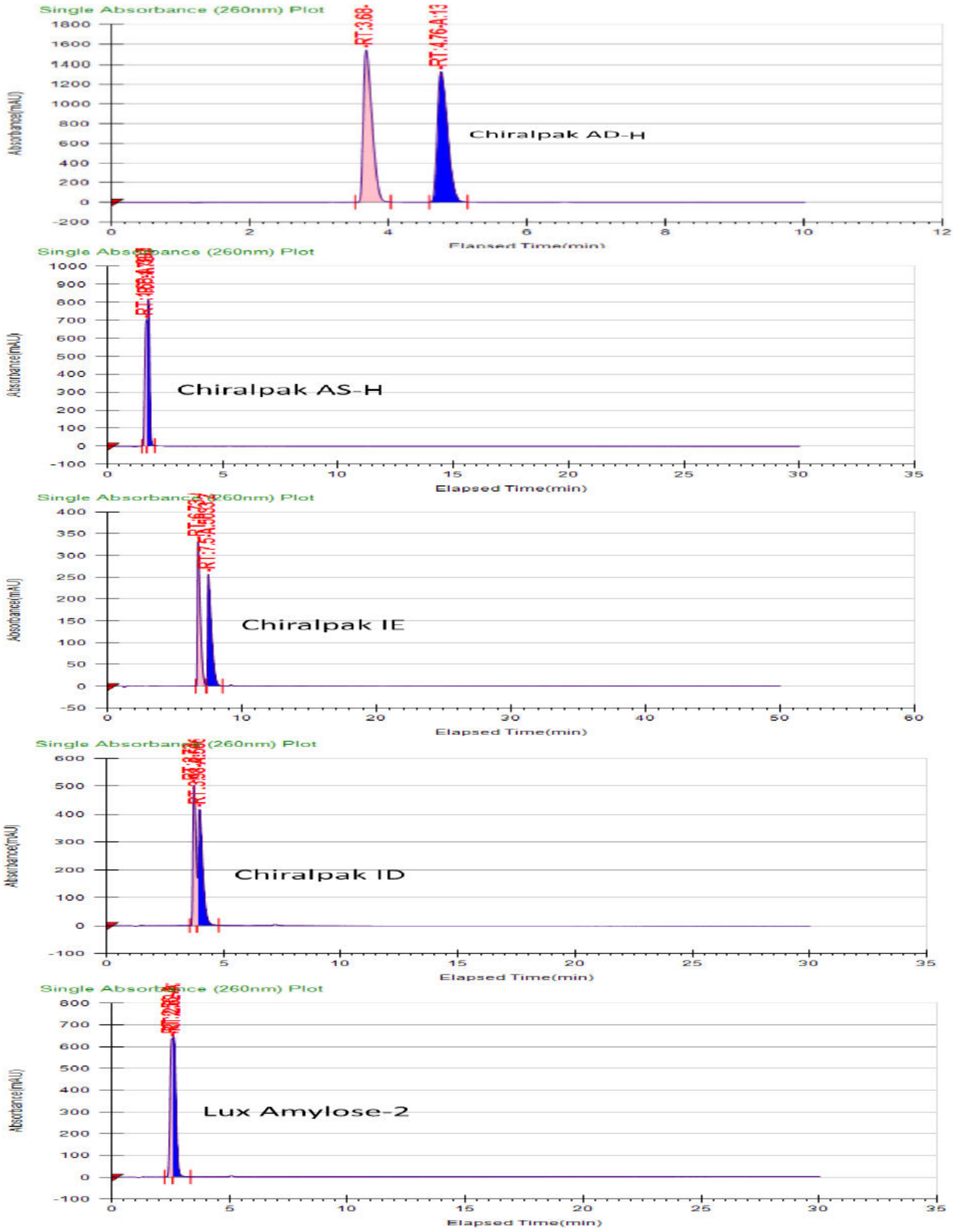


Fig. 3: Separation chromatograms of voriconazole and its enantiomer using amylose derivative columns

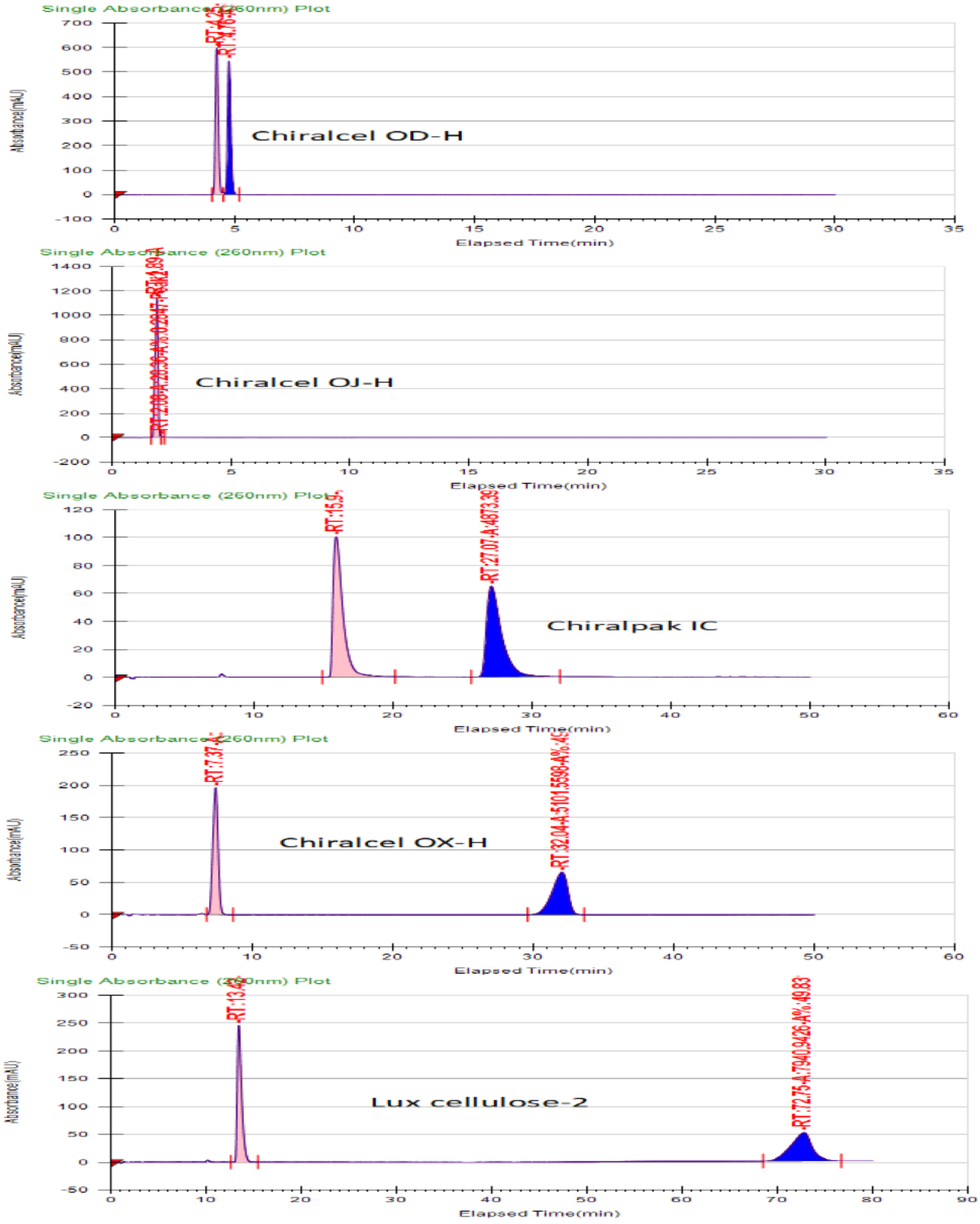


Fig. 4: Separation chromatograms of voriconazole and its enantiomer using cellulose derivative columns

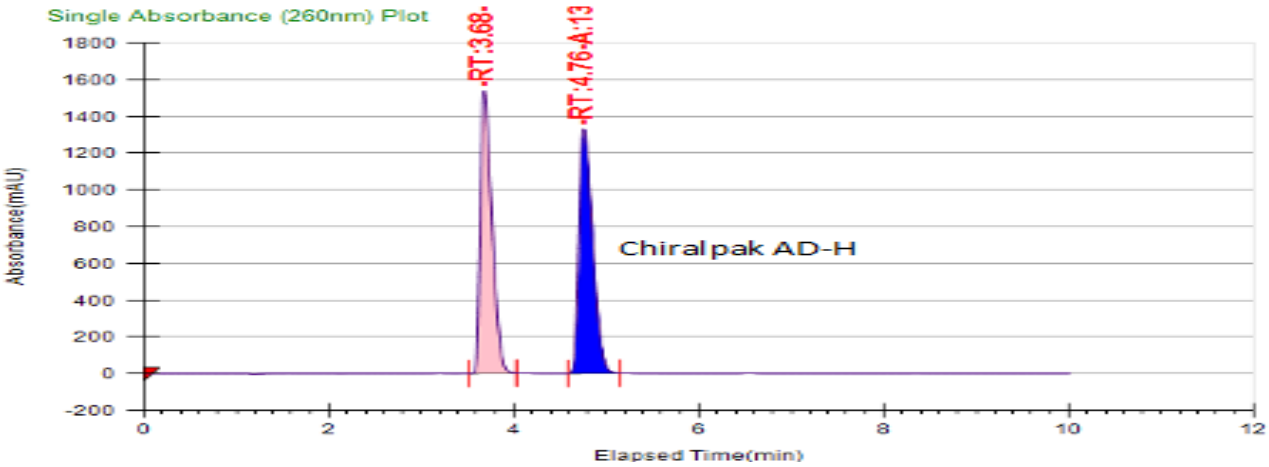


Fig. 5: Chromatogram of the optimized method

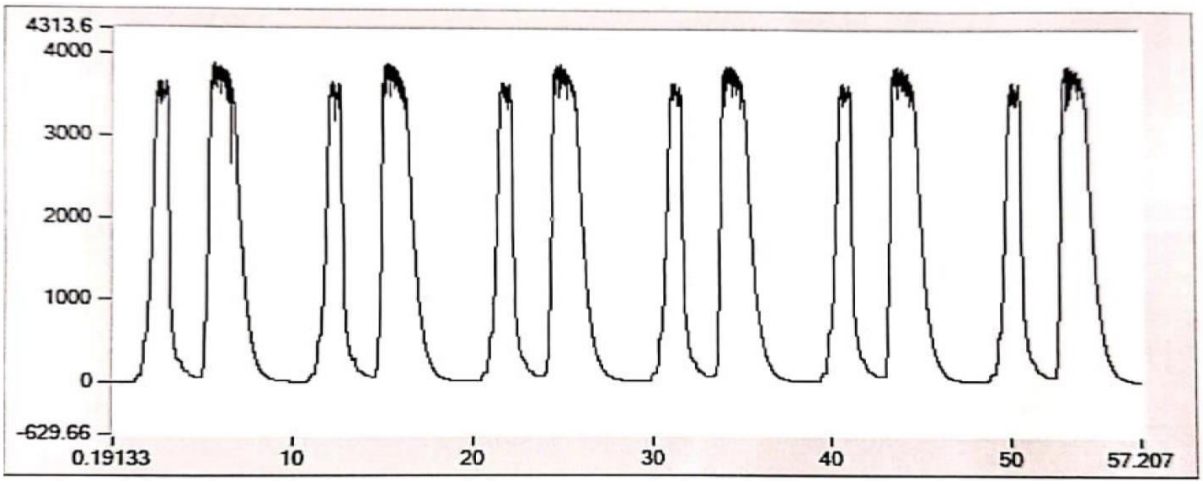


Fig. 6: Chromatogram of voriconazole enantiomers by stacked injections Chiral SFC Purification of (R, S) voriconazole details

Table 3: Comparison of preparative SFC vs Preparative NP-HPLC

Description	SFC		NP-HPLC	
Column ID :(250x30 mm),10µ	Fraction 1	Fraction 2	Fraction 1	Fraction 2
Percentage of enantiomeric excess (Enantiomeric purity)	>99.9	99.8	>99.8	99.6
Recovery	95.4	95.5	94.7	92.6
Fraction volume in liters	0.39	0.57	1.15	2.88
Purification time	5h		42h	
Consumption organic solvent in liters	6		42	
Time-consuming for solvent Workup	1h		7h	
Recovery(both fractions)	95%		92%	

4. CONCLUSION

An easy and fast supercritical fluid chromatographic method for the enantiomer separation of voriconazole was developed. Various stationary phases were tested among them; an amylose derivative of the Chiralpak AD-H column was suitable for the separation of the voriconazole enantiomers. Using the Chiralpak AD-H column with Supercritical fluid (CO_2) and methanol (90:10%, v/v) as a mobile phase proved to be a suitable condition for separating the enantiomers present in

voriconazole. The benefit of enantiomeric separations by SFC is cost-effective, less time-consuming, and environmentally friendly than normal phase liquid chromatographic methods. This method can also be used to isolate the undesirable isomer using the preparative SFC system with less time and at a low cost.

5. ACKNOWLEDGEMENTS

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List of abbreviations

SFC: Supercritical Fluid Chromatography; HPLC: High-Performance Liquid Chromatography; NP-HPLC: Normal Phase-High Performance Liquid Chromatography; RT:Retention time; ACN; Acetonitrile; MeOH: Methanol; TFA: Trifluoroacetic acid; DEA: Diethylamine;CO2: Carbon dioxide

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