Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2020, 9 (1): 116-122

(International Peer Reviewed Journal)

Comparative study of the Enantio Selective Separations of Pioglitazone and Pioglitazone Degradation Products by Ultra Performance Convergence Chromatography (UPC^2) and Supercritical Fluid Chromatography

Kumaraswamy Kasani[1, 2], Hima Bindu Gandham[2], V.V.S.R.N. Anji Karun Mutha[1, 2], Srinivasa Rao Pakalapati[1, 2], Ramulu Yanaka[1, 2], Muralidharan Kaliyaperumal[1] and Chidananda Swamy Rumalla[1]*

1 .Department of Medicinal Chemistry, GVK Biosciences Pvt. Ltd, IDA Mallapur, Hyderabad-500076, Telangana, **INDIA**

2. Department of Engineering Chemistry, Andhra University, Visakhapatnam-530003, A.P., **INDIA**

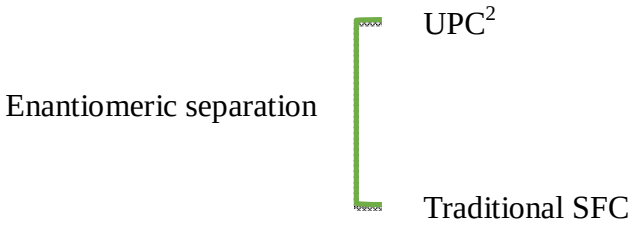
Email: chidanand_swamy@yahoo.co.in

Accepted on 25th December, 2019

ABSTRACT

Pioglitazone hydrochloride is thiazolidinedione class of drug and it is used to treat type 2 diabetes. Pioglitazone, Pioglitazone peroxide degradation product referred as DP-PIO-A, Pioglitazone base degradation product referred as DP-PIO-B possess stereogenic centres and rapid, reliable and precise enantiomeric ratio was determined by using Ultra Performance Convergence Chromatography (UPC^2).The effect of different co solvents such as 2-propanol, ethanol and methanol on the resolution and retention time was studied as well as the presence of additives in the mobile phase. All the compounds were enantiomerically well separated inUPC^2 with less than 5 min and method was compared with traditional Super Critical Fluid Chromatography (SFC).

Graphical Abstract



Keywords: Pioglitazone, Pioglitazone degradation products, Enantiomeric separation, UPC2 and Super Critical Fluid Chromatography (SFC).

INTRODUCTION

The enantiomeric separation is one of the most important task in analytical method development and it is well known that a pair of enantiomers can display different biological activity, toxicity profiles [**1, 2**]. The pharmacological evaluation of each enantiomer and the enantiomeric purity of a drug are

important assignment in drug development, the enantiomers should be analysed and regulated separately, according to their pharmacodynamic, pharmacokinetic and toxicological properties. For these reasons enantiomeric separations are necessary and the enantiomeric separation is quite interest not only in the pharmaceutical industry but also in the analytical research and development.

Primarily chiral separations have been carried out using HPLC on chiral stationary phase, more recently super critical fluid chromatography (SFC) was applied to chiral separations. SFC is usually a normal phase technique with significant advantages over normal phase HPLC such as extremely fast equilibration, excellent reproducibility and even aqueous-based samples can be injected. The physic-chemical properties of super critical fluidics allow to obtain separations with high efficiencies and the analysis of polar or thermo labile compounds is possible .The mobile phase is primarily CO_2 and organic solvents (co-solvent) can be used 5-50%. Ultra Performance Convergence chromatography (UPC^2) a category of separation science that provides an exceptional increase in selectivity to the chromatography laboratory. This technique has evolved to include the usage of co-solvents in sub-critical state. As it has been demonstrated that liquid CO_2-based chromatography can be performed in either supercritical or subcritical states, Convergence chromatography is the term used to cover this modern form of analytical and preparative techniques [**3-10**].

Pioglitazone(5-(4-(2-(5-ethylpyridin-2-yl)ethoxy)benzyl)-5l3-thiazolidine-2,4-dione) Pioglitazone degradation products DP-PIO-A(2-(2-(4-(3-amino-3-oxo-2-sulfinopropyl)phenoxy)ethyl)-5-ethyl pyridine 1-oxide) and DP-PIO-B(3-(4-(2-(5-ethylpyridin-2-yl)ethoxy)phenyl)-2-mercaptopropanoic acid) possess an asymmetric carbon atom (n=1) with one set of enantiomers for each asymmetric carbon (Figure 1). The present study belongs to the rapid, reliable enantiomeric separation of Pioglitazone, DP-PIO-A, DP-PIO-B in Ultra performance convergence chromatography (UPC^2) and comparison between UPC^2 and traditional SFC technique.

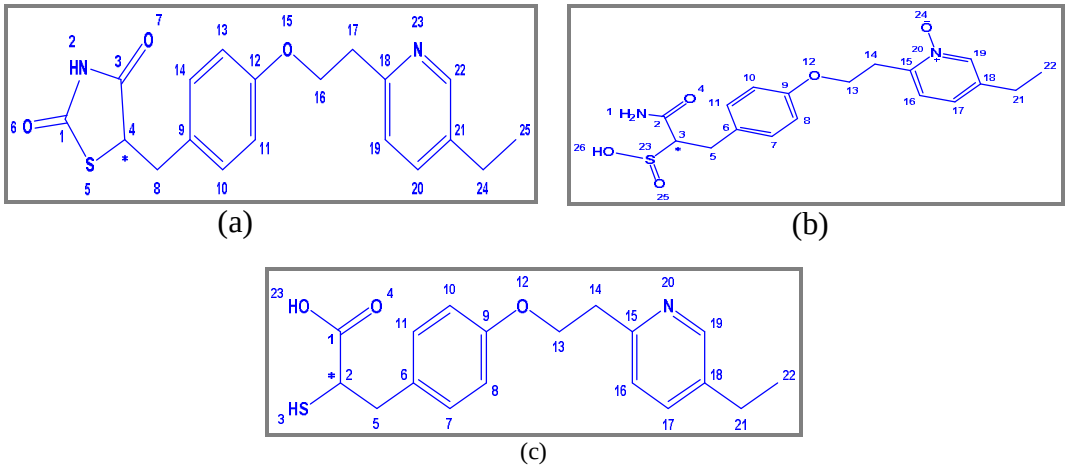


Figure 1. Structure of the compounds (a) Pioglitazone (b) DP-PIO-A and (c) DP-PIO-B.

MATERIALS AND METHODS

Chemicals and Reagents: Pioglitazone hydrochloride was a gifted sample from an API unit in Hyderabad. Solvents and chemicals used for the experiment were methanol from Sigma Aldrich, 2-propanol from Finar chemicals, absolute ethanol from sterling chemical and alcohols.

Super critical fluid chromatography (SFC): The SFC instrument was a Waters Analytical SFC method station X5 equipped with high pressure P-series pumps, a thermo stated oven for the column, stacked injection module, a variable wavelength 2489 UV detector and Automated back

pressure regulator. Automated back pressure regulator was set up 100bar and heater controller to maintain 35°C Carbon dioxide in super fluid state.

Ultra performance Convergence Chromatography (UPC2): UPC2 equipped with Waters Convergence manager which uses Compressed Liquid CO_2 as primary mobile phase and mixes to co-solvents. Column manager 30S screened different chiral column in very short period results maximum resolution between isomers. Column eluent connected to high strength silica lens photo diode array detector.

Sample preparation: Pioglitazone, DP-PIO-A, DP-PIO-B standards were prepared in methanol at a concentration of 1 mg mL^{-1}.

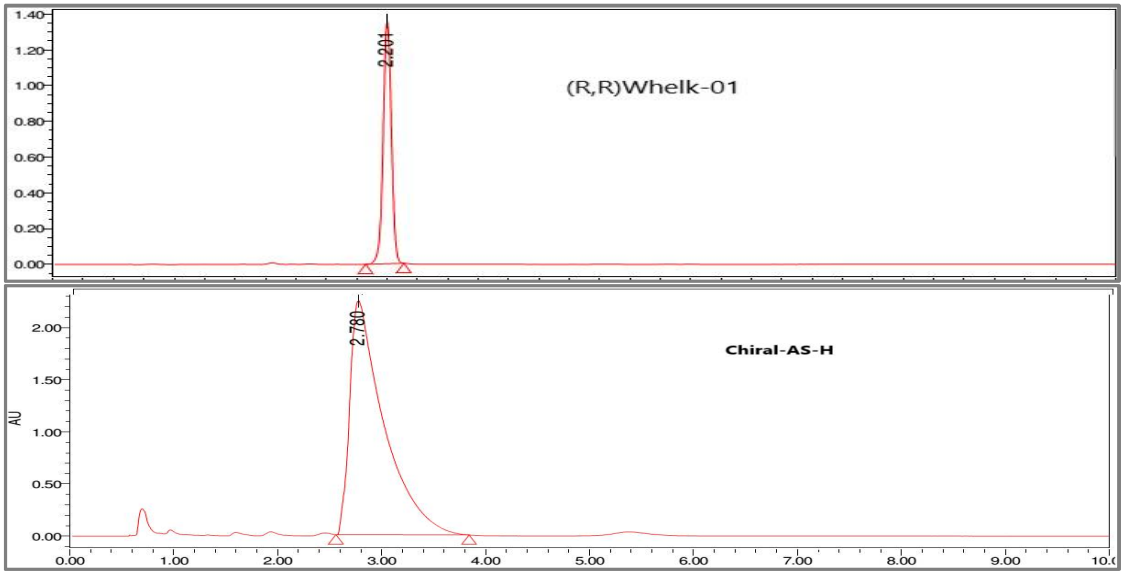
RESULTS AND DISCUSSION

Chiral method development often starts with screening different columns (chiral stationary phases), the three compounds separation was checked with 11 different chiral stationary phases. The primary mobile phase was CO_2 and different combination of additive, various co-solvents (methanol, ethanol, 2-propanol) were used in method development. The method screening details are shown in table 1.

Table 1. Chiral Method screening in different chiral stationary phases

S.No.	Column Name	Chiral Stationary Phases (CSP's)
1	(R,R) Whelk-01 4.6X150mm 3.5µm	1-(3,5-Dinitrobenzamido)-1,2,3,4,-tetrahydrophenanthrene
2	Chiralpak-AS-H 4.6X150mm 3µm	Amylose tris{(S)-alpha-methylbenzylcarbamate}
3	Chiralcel-OD-3 4.6X150mm 3µm	Cellulose tris(3,5-dimethylphenylcarbamate)
4	Lux-Amylose-2 4.6X250mm 5µm	Amylose tris(5-chloro-2-methylphenylcarbamate)
5	Chiralpak-IG 4.6X150mm 3µm	tris(3-chloro-5 methylphenylcarbamate
6	Chiralpak-AD-H 4.6X150mm 3µm	Amylose tris(3,5-dimethylphenylcarbamate)
7	Chiralcel-OD-H 4.6X150mm 3µm	Cellulose tris(3,5-dimethylphenylcarbamate)
8	Lux-Cellulose -2 4.6X250mm 5µm	Cellulose tris(3-chloro-4-methylphenylcarbamate)
9	Chiralcel-OX-H 4.6X150mm 3µm	Cellulose tris(4-chloro-3-methylphenylcarbamate)
10	Chiralpak-IC 4.6X150mm 3µm	Cellulose tris (3, 5-dichlorophenylcarbamate)
11	Chiralpak-AD-3 4.6X150mm 3µm	Amylose tris(3,5-dimethylphenylcarbamate)

Enantiomeric separation of Pioglitazone: Pioglitazone enantiomeric separation was checked in different chiral columns and co-solvents with UPC^2 instrument, the method development chromatograms were shown in figure 2.



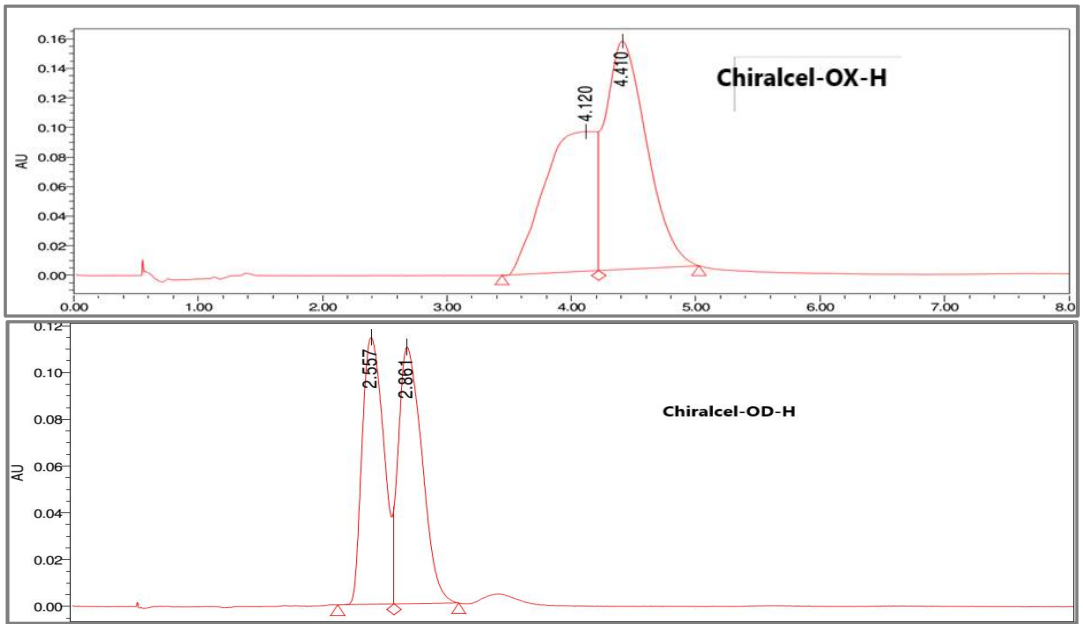


Figure 2. Pioglitazone UPC^2 chromatograms in different chiral columns and co-solvents.

After extensive screening of Pioglitazone, CHIRALCEL OD-3(4.6*150 mm) 3 µm Column and methanol were selected for further optimization and 0.5% dimethyl amine in methanol was used as a co-solvent to get sharp peak shapes. The temperature was increased from 30°C to 60°C, the solvent strength of supercritical CO_2 decreased and retention time became longer. The final UPC^2 method conditions were liquid CO_2, 40 % of 0.5% Diethyl amine in methanol as a co-solvent, total flow 3 g min^{-1}, ABPR pressure 15000 psi , column temp 60°C and the total analysis time was 4 min. The retention time was peak-1 at 2.04 min and peak-2 at 2.21 min, resolution was 1.63. The pioglitazone enantiomeric separation was achieved in traditional SFC at 5.06 min, 7.16 min and analysis time was 10 min. The comparison chromatogram between UPC^2 and traditional SFC was shown in figure 3.

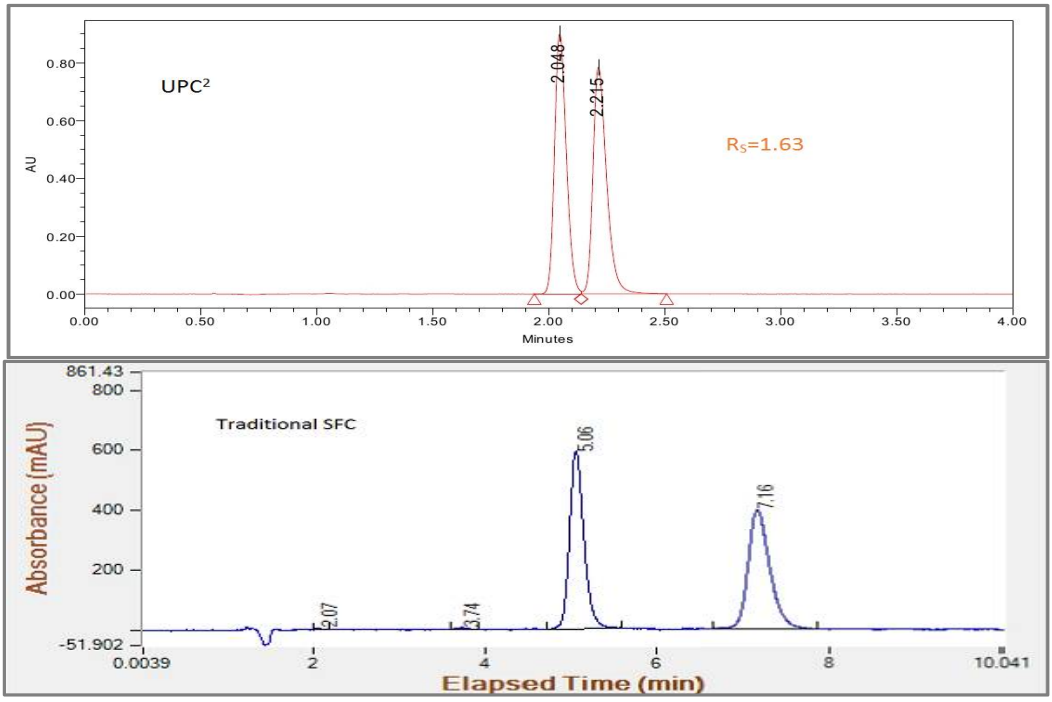


Figure 3. UPC2 and traditional SFC comparison chromatograms of Pioglitazone.

Enantiomeric separation of DP-PIO-A: DP-PIO-A enantiomeric separation was checked in different chiral columns with UPC^2 instrument, the method development chromatograms were shown in **figure 4**.

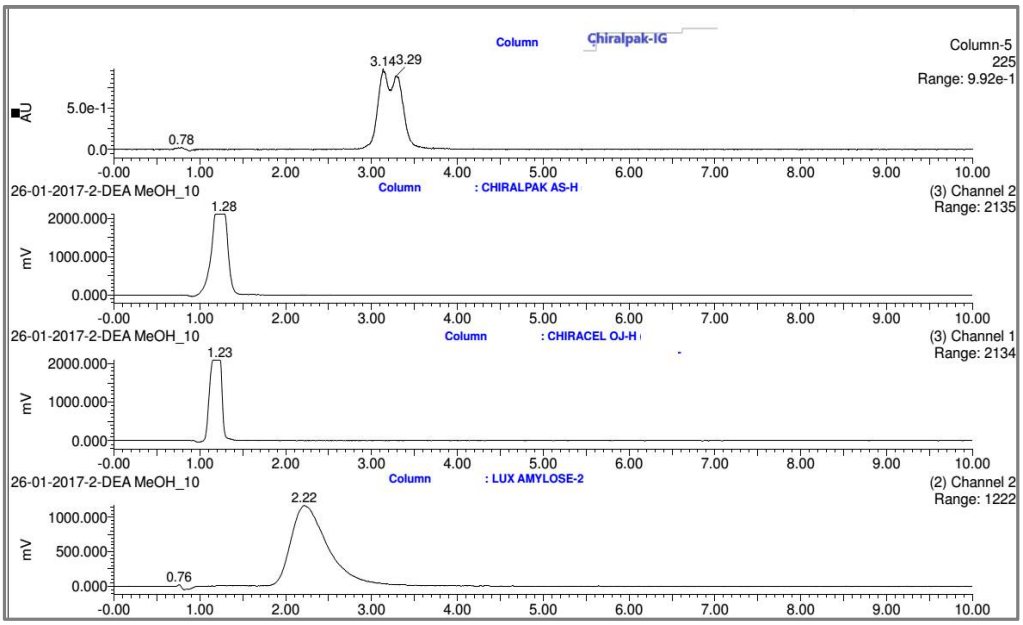


Figure 4. DP-PIO-A UPC^2 chromatograms in different chiral columns and co-solvents.

After chiral method development of DP-PIO-A, (R, R) WHELK-01(4.6*150 mm) 3.5 µm column, methanol were selected for further optimization. The methanol percentage was increased 0 to 40% and 0.5% diethyl amine was added to enhance solute peak shape. The final UPC^2 method conditions were liquid CO_2, 40% of 0.5% DEA in methanol as a co-solvent, total flow 3 g min^{-1}, ABPR pressure 15000 psi, column temp 30°C and the retention time of first elution peak at 1.32 min and second elution peak was observed at 1.54 min, resolution was 3.17 and the total UPC^2 runtime was 4.0 minutes. The DP-PIO-A separation was achieved in traditional SFC with Peak-1 at 8.22 min, Peak-2 at 9.81 min and the total analysis time was 15 min and the comparison chromatogram was shown in figure 5.

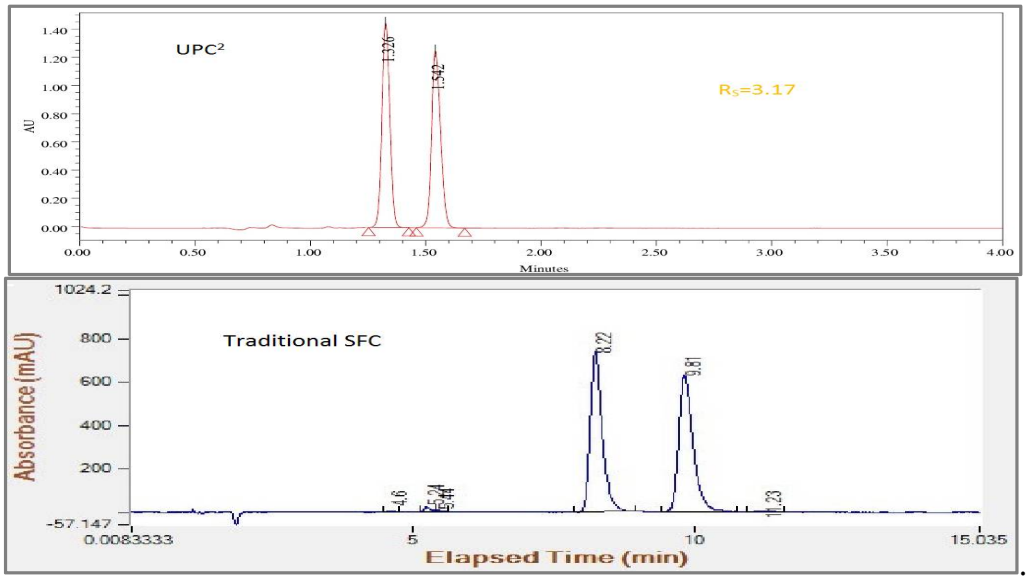


Figure 5. UPC2 and traditional SFC comparison chromatograms of DP-PIO-A.

Enantiomeric separation of DP-PIO-B: DP-PIO-B enantiomeric separation was checked in different chiral columns with UPC^2 instrument, the method development chromatograms were shown in figure 6.

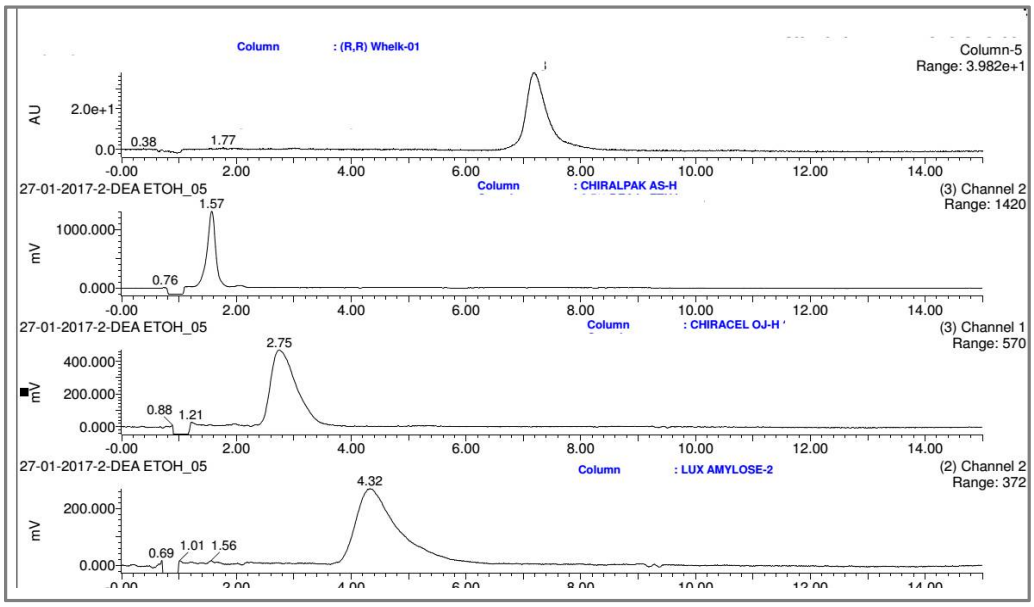


Figure 6. DP-PIO-B UPC^2 chromatograms in different chiral columns and co-solvents.

After method screening of DP-PIO-B, enantiomer separation was achieved in CHIRALPAKAD-3(4.6*150 mm) 3 µm Column and 0.5% diethyl amine in methanol. The final method conditions were liquid CO_2, 40% 0.5% diethyl amine in methanol as a co-solvent and the total flow 3g min^{-1}, ABPR pressure 15000 psi, column temp 30°C. The first elution peak at 1.09 min, second elution peak at 1.36 min, resolution was 4.2. The DP-PIO-B SFC analysis time was 15 min whereas 2.5 min in UPC^2 and comparison chromatogram with traditional SFC was shown in figure 7.

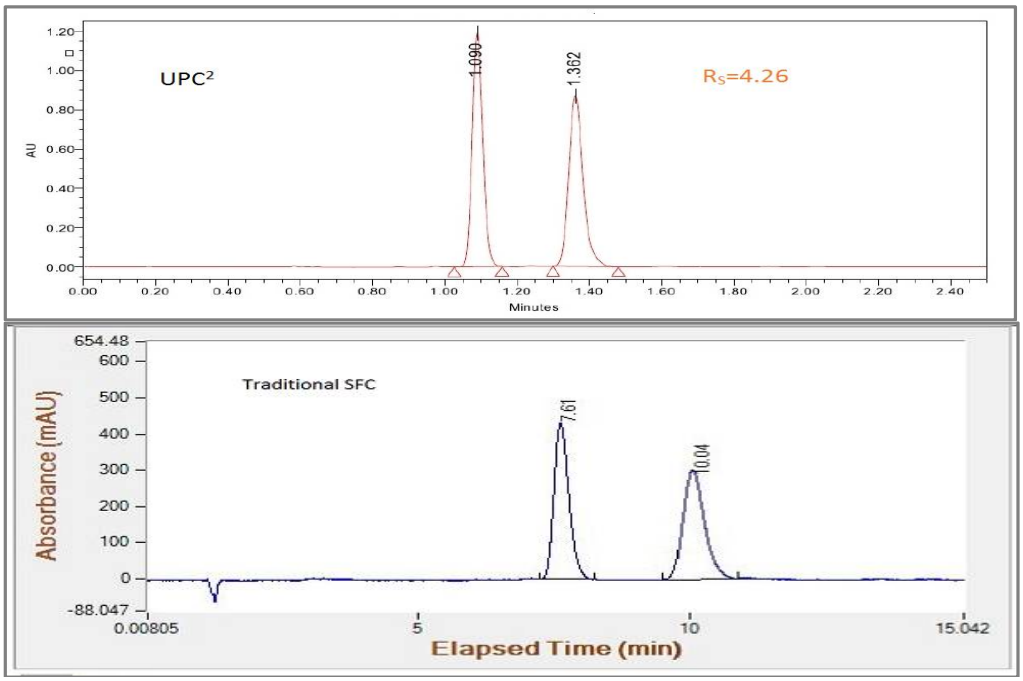


Figure 7. DP-PIO-B UPC2 and traditional SFC comparison chromatograms.

APPLICATION

We demonstrated some challenging chiral separations of pioglitazone and its degradation products with ultra performance convergence chromatography. The UPC^2 method is faster than tradition method of analysis and UPC^2 system eliminate the significant time and cost.

CONCLUSION

Pioglitazone, DP-PIO-A, DP-PIO-B enantiomeric separation was performed by Ultra performance convergence chromatography. UPC^2 enables faster, superior and more efficient enantiomeric separation of pioglitazone and its degradation products compared to traditional supercritical fluid chromatography.

ACKNOWLEDGEMENT

The Authors would like to thank the management of GVK Biosciences Pvt Ltd., Hyderabad for their supporting the present research work.

Conflict of interest: All authors declare that they have no conflict of interest.

REFERENCES

[1]. O. Gyllenhaal, A. Karlsson, J. Vessman, Applications of packed column supercritical fluid chromatography in the development of drugs. In: K. Anton, C. Berger, editors. Supercritical Fluid Chromatography with Packed Columns: Techniques and Applications, New York: Marcel Dekker, **1997**. p. 273–300.
[2]. T. Wang, Y. W. Chen, A. Vailaya, *J. Chromatogr. A*, **2000**, 902, 345.
[3]. A. Medvedovici, P. Sandra, L. Toribio, F. David, *J.Chromatogr.A*, **1997**, 785,159.
[4]. M. Garzotti, M. Hamdan, *J.Chromatogr.B*, **2002**, 770, 53
[5]. G. Terfloth, *J. Chromatogr. A*, **2001**, 902, 301
[6]. E. Yashima, *J. Chromatogr. A*, **2001**, 906, 105.
[7]. I. Kartozia, M. Kanyonyo, T. Happaerts, D. M. Lambert, G. K. E. Scriba, B. Chankvetadze, *J. Pharm. Biomed. Anal.* **2002**, 27, 457.
[8]. V. Perez-Fernandez, M. A. Garcia, M. L. Marina, Chiral separation of agricultural fungicides, *J Chrom A.* **2011**,1218: 6561–6582.
[9]. L. Toribo, del M. J. Noza, J. L. Bernal, J. J. Jimenez, C. Alonso. Chiral separation of some triazole pesticides by supercritical fluid chromatography, *J Chrom A.*, **2004** 1046, 249–253.
[10]. J. P. McCauley, L. Subbarao, R. Chen, Enantiomeric and Diastereomeric Separations of Pyrethroids Using UPC^2, *Waters Application note No.* 720004530en, December, **2012**.