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Note d'application

Analysis of Antidiabetic Drug Canagliflozin and Related Isomeric Impurities Using the ACQUITY UPC^2 System for Method Screening and Scale Up to Prep SFC System

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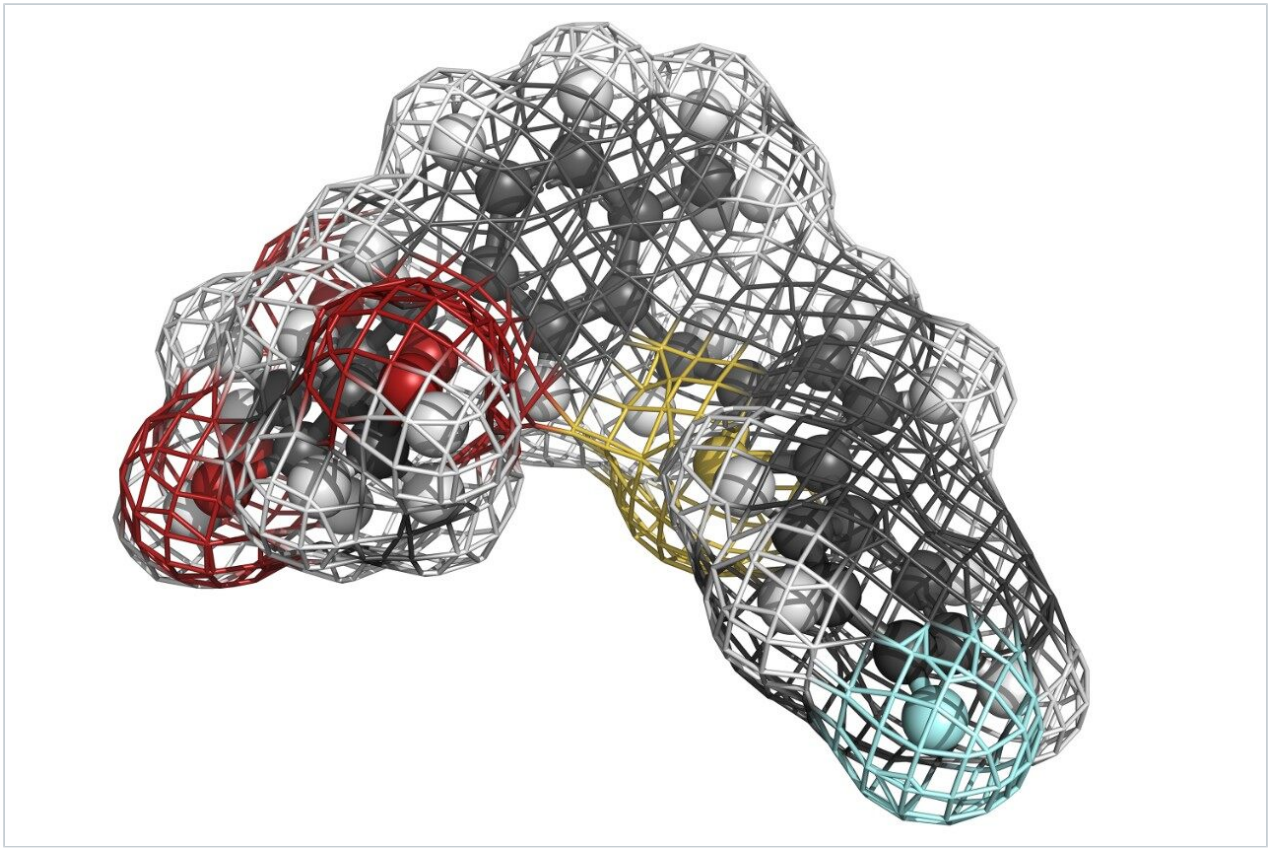

Abstract

This application note demonstrates the separation of Canagliflozin and its isomeric impurities from its other impurities.

Benefits

- Faster analysis of isomeric impurities for Canagliflozin compared to reverse phase chromatography
- Orthogonal mass detection system providing confidence in analytical results and excellent chromatographic selectivity with better peak resolution and reproducibility

Introduction

Canagliflozin is an anti-diabetic beta-isomeric drug used to improve glycemic control in people with type 2 diabetes. In extensive clinical trials, canagliflozin produced a consistent dose-dependent decrease in HbA1c levels to 0.77% to 1.16% from initial HbA1c levels of 7.8% to 8.1% when administered either as monotherapy or in combination with metformin, sulfonylurea, pioglitazone, or in combination with insulin. When added to metformin, canagliflozin 100 mg daily was shown to be non-inferior to both sitagliptin 100 mg daily and glimepiride in reducing HbA1c levels at one year, whilst canagliflozin 300 mg successfully demonstrated statistical superiority over both sitagliptin and glimiperide in decreasing HbA1c levels. Secondary efficacy endpoints like higher reductions in weight and blood pressure (versus sitagliptin and glimiperide) were also observed in studies. Canagliflozin produces beneficial effects on HDL cholesterol whilst increasing LDL cholesterol to produce no change in total cholesterol.

In development of generic drug for ANDA approval, synthesis of Canagliflozin often starts with the starting materials containing sugar moieties having alpha and beta isomers in equimolar concentrations. These impurities of starting material serve as a potential source of undesired isomeric impurities in the final API, which must be separated and isolated for their structural information and enable faster turnaround time of analysis which are essential for generic ANDA submission. It is quite challenging to separate alpha and beta isomers in normal and reverse phase chromatography and hence it is difficult to purify these isomers.

UltraPerformance Convergence Chromatography (UPC^2) is a separation technique that leverages the unique properties of CO_2 at or near its supercritical state. When mixed with organic solvents, ACQUITY UPC^2 provides a higher separation efficiency, speed, and selectivity needed for a complex sample matrix which otherwise would be difficult to separate in normal or reversed-phase (RP) chromatography. This application note demonstrates the separation of Canagliflozin and its isomeric impurities from its other impurities in shorter runtime and thus enabling lesser consumption of organic solvent rendering true meaning to the “Go Green” tagline of convergence chromatography.

The Canagliflozin and its isomeric impurities were being analyzed by RP chromatography. Reversed-phase chromatography analysis time is about 65 minutes and does not provide enough resolution for the targeted isomeric entities. The challenge with this separation in reserved-phase chromatography was scale-up for Prep system. ACQUITY UPC^2 was effectively used for screening methods to achieve the desired resolution between the isomeric peaks and for scale up to Prep SFC systems. Furthermore, with increased column loading the ACQIUTY UPC^2 system can be used for analysis and accurate assay of the isomeric impurities as well.

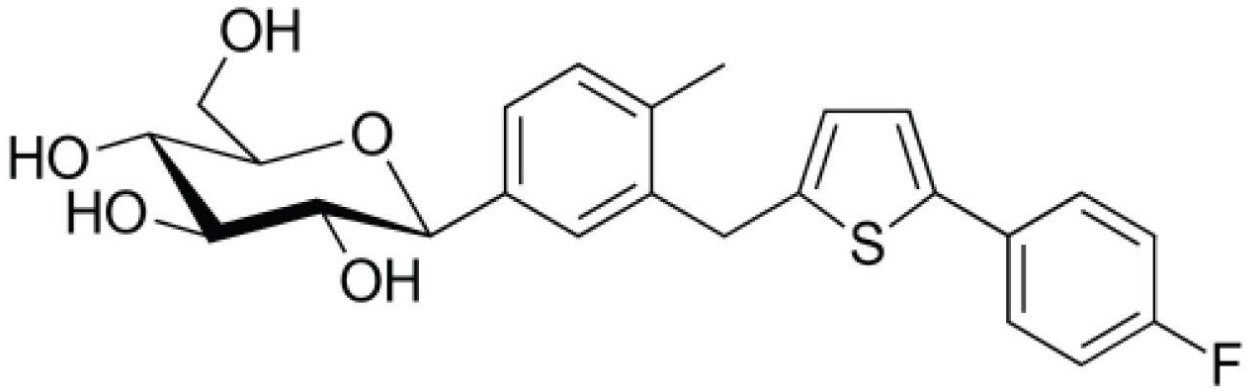

Figure 1. Canagliflozin.

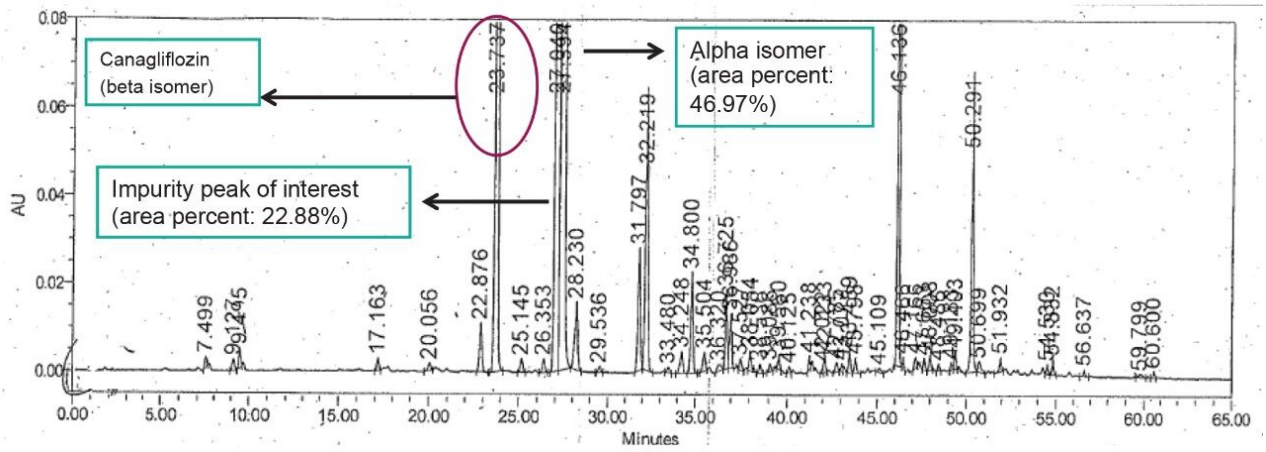

Figure 2. Reversed-phase chromatography results of Canagliflozin API with isomeric peaks.

Experimental

ACQUITY UPC2 conditions

System:	ACQUITY UPC^2 with ACQUITY UPC^2 PDA Detector
Software:	Empower 3

Detection:	UV at 290 nm from UV range 200–430 nm (compensation reference 330–430 nm)
Column:	ACQUITY UPC^2 Trefoil AMY1 2.5 µm, 3 mm x 150 mm (P/N 186007460)
Column temp.:	45° C
Sample temp.:	10° C
Sample concentration:	250 ppm
Injection volume:	5 µL
Flow rate:	1.5 mL/min
Mobile phase A:	Compressed CO_2
Mobile phase B:	0.1% Trifluoroacetic acid in methanol: isopropyl alcohol (50:50)
Run time:	11 minutes
ABPR pressure:	2000 psi
Gradient:	5% B for 0.8 minute, ramp to 50% of B in 7 minutes, hold at 50% B for 1 minute, and return to 5% B in 0.5 minutes to equilibrate up to 11 minutes
Diluent:	Acetonitrile

MS conditions

System:	ACQUITY SQ Detector

Software:	Empower 3
Cone voltage:	30 V
Capillary voltage:	3 KV
Desolvation temp.:	300 °C
Desolvation gas:	550 L/Hr
Cone gas:	20 L/Hr
Make up solvent:	Methanol with 0.1% Acetic acid
Make up solvent flow:	0.3 mL/min

Results and Discussion

The API and its isomeric impurities were separated and identified based on the area percent of the peaks observed in UV and corresponding MS spectral data generated orthogonally with ACQUITY SQ Detector (Figures 3, 4, 5, and 6). The retention time of beta, unknown isomeric impurities, and alpha were 6.5 min, 7.1 min, and 8.2 min respectively. USP resolution of all the desired compounds was within acceptable range (5.10, 2.47, and 6.01 for Canagliflozin beta isomer, unknown isomer, and alpha isomer respectively) [Table 1].

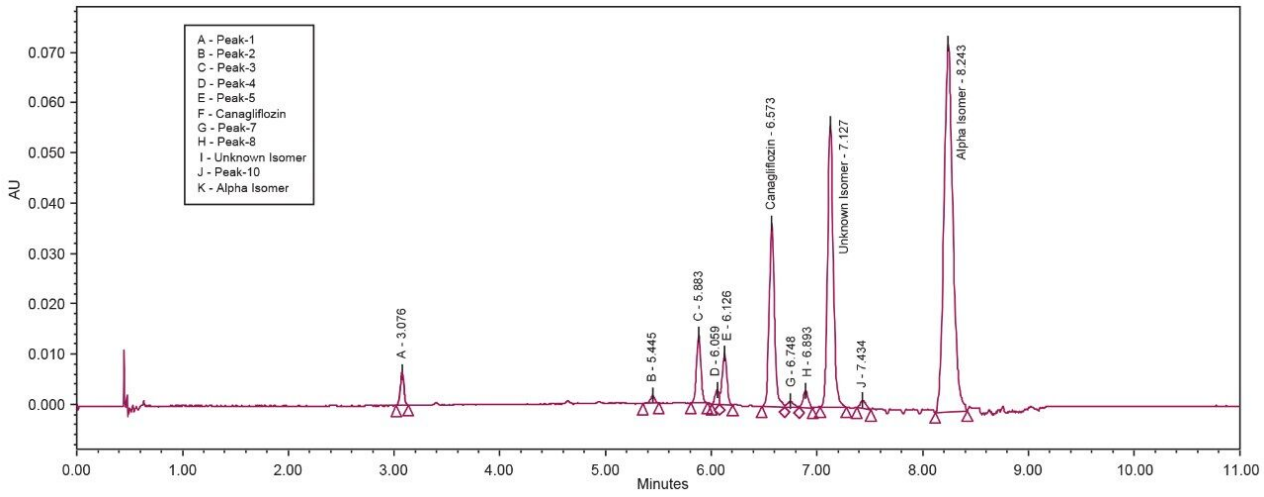

Figure 3. UV chromatogram of sample at 290 nm.

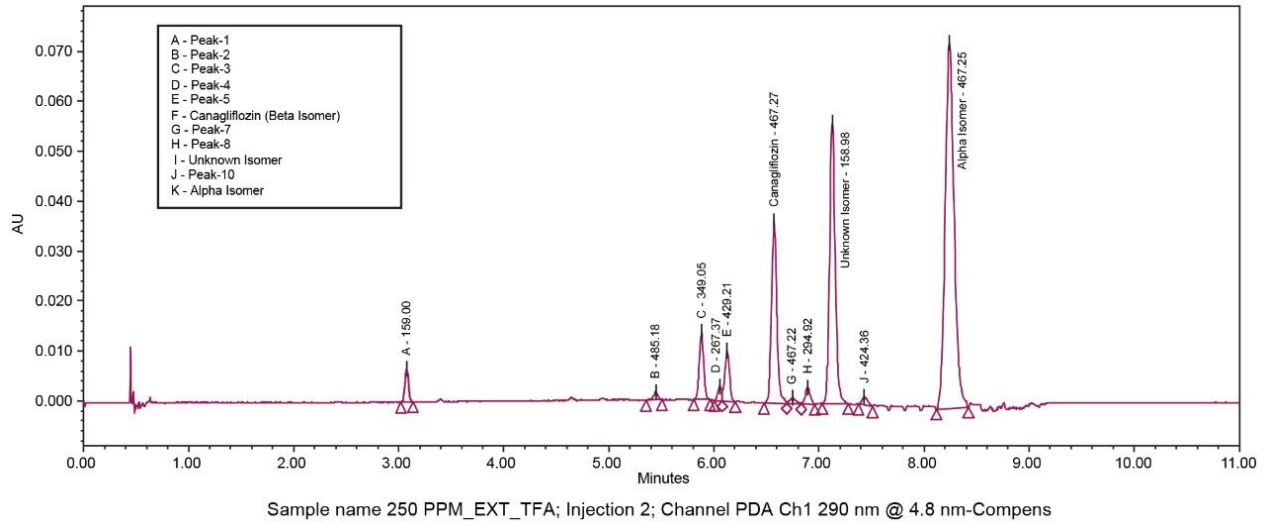

Figure 4. UV chromatogram of Sample at 290 nm with base peak mass value (m/z) in ESI positive ionization mode.

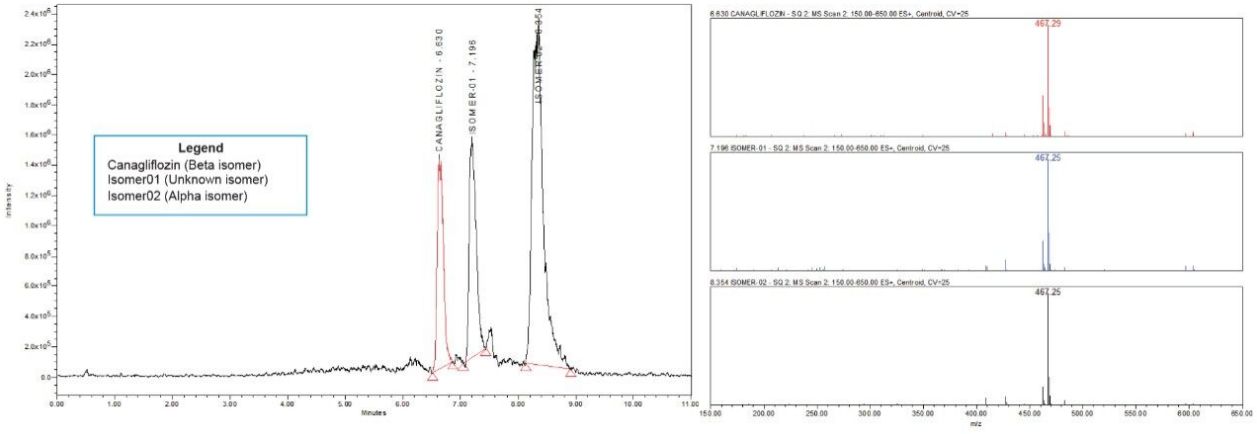

Figure 5. MS trace (XIC) and spectra at corresponding retention time of peaks of Canagliflozin sample.

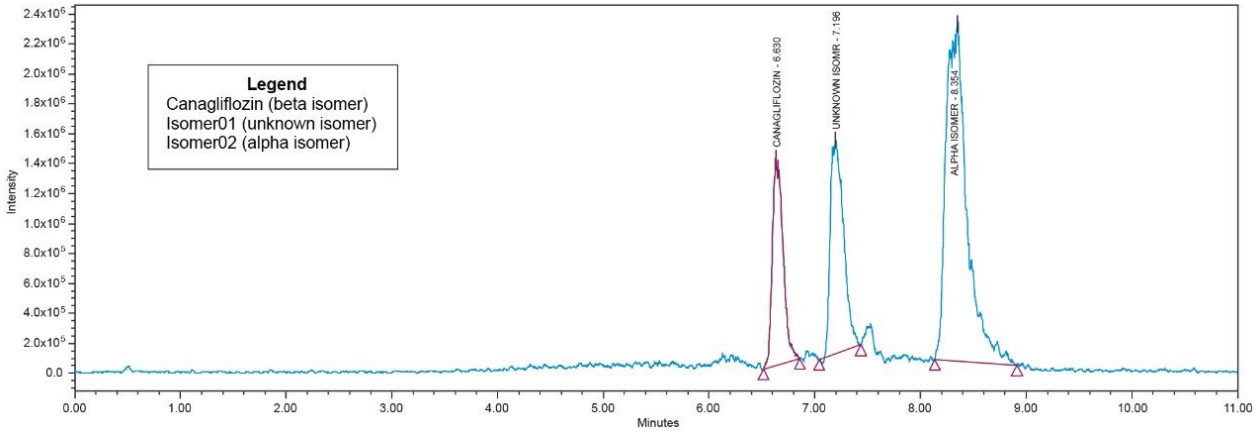

Figure 6. XIC of 467 m/z with corresponding retention time of peaks of Canagliflozin sample.

Peak Results

	Name	RT	Area	Height	%Area	USP Resolution
1	A	3.076	15798	6594	1.77	
2	B	5.445	4656	1572	0.52	32.48
3	C	5.883	39395	13489	4.42	5.49
4	D	6.059	7683	2925	0.86	2.26
5	E	6.126	31478	10237	3.53	0.83
6	Canagliflozin	6.573	127999	36488	14.36	5.10
7	G	6.748	4977	1111	0.56	1.59
8	H	6.893	11206	3327	1.26	1.33
9	Unknown Isomer	7.127	212163	56135	23.80	2.47
10	J	7.434	6399	1653	0.72	2.96
11	Alpha Isomer	8.243	429626	73195	48.20	6.01

Table 1. Peak results table for UV chromatogram of the sample.

All the isomeric peaks were identified by orthogonal mass detection technique (the retention time of Canagliflozin beta isomer, unknown isomeric impurity, and alpha isomer were 6.6 min, 7.2 min, and 8.4 min respectively) as the sodium adduct in ESI positive mode (467 *m/z* for 444 Da of Molecular weight) [Figure 7].

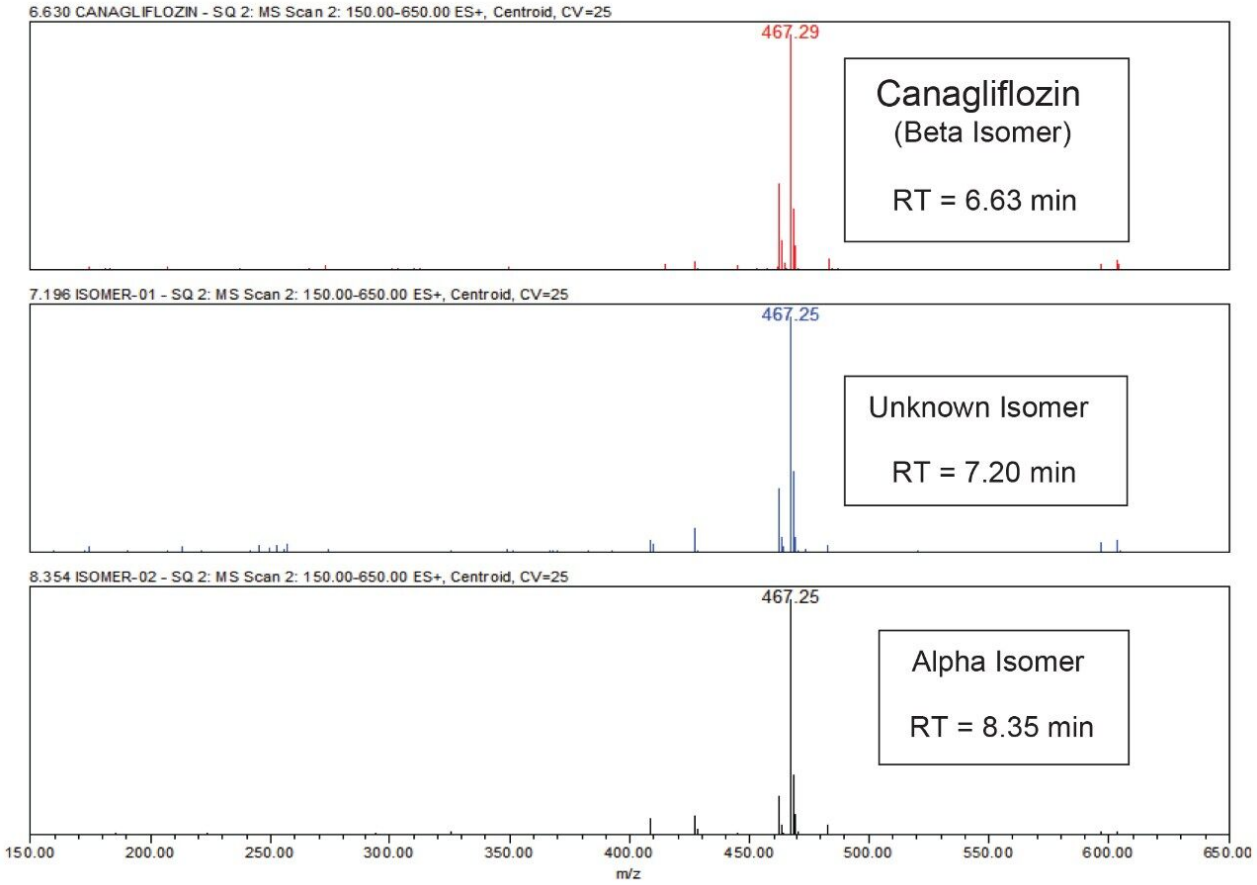

Figure 7. Match plot spectra at retention time of Canagliflozin (Beta Isomer), Unknown Isomer, and Alpha Isomer.

Chromatography shows excellent reproducibility with three replicate injections in terms of %RSD of area and retention time (Figure 8 and Table 2 & 3).

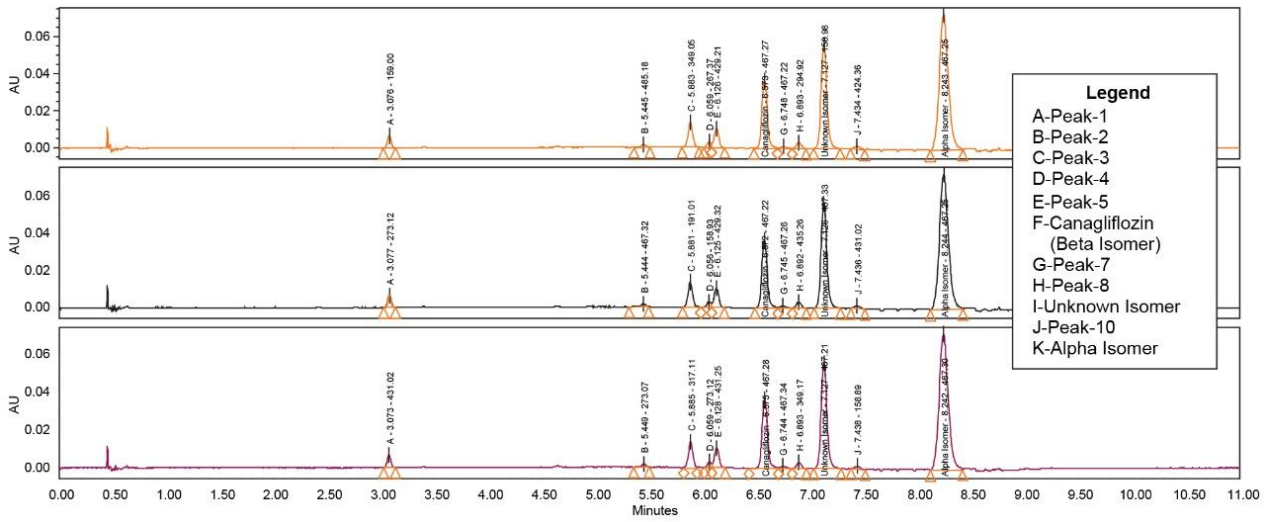

Figure 8. Overlay of three injections from UV trace at 290 nm.

Component Summary For Retention Time

	SampleName	Inj	Canagliflozin (min)	ISOMER_01 (min)	ISOMER_02 (min)
1	250PPM_EXT_TFA	1	6.575	7.127	8.242
2	250PPM_EXT_TFA	2	6.573	7.127	8.243
3	250PPM_EXT_TFA	3	6.572	7.126	8.244
Mean			6.57	7.13	8.24
Std. Dev.			0.001	0.000	0.001
%RSD			0.02	0.01	0.01

F-Canagliflozin
I-Unknown isomer
K-Alpha isomer

Table 2. Summary of experimental result for retention time for peaks of interest.

Component Summary For Area

	SampleName	Inj	Canagliflozin (µV*sec)	ISOMER_01 (µV*sec)	ISOMER_02 (µV*sec)
1	250PPM_EXT_TFA	1	130528	211523	427199
2	250PPM_EXT_TFA	2	127999	212163	429626
3	250PPM_EXT_TFA	3	128143	212415	430252
Mean			128890	212034	429026
Std. Dev.			1420.335	459.676	1612.280
%RSD			1.10	0.22	0.38

F-Canagliflozin
I-Unknown isomer
K-Alpha isomer

Table 3. Summary of experimental result for area count for peaks of interest.

Conclusion

ACQUITY UPC^2 technology enabled rapid and faster separation of isomeric impurities of Canagliflozin with a short run time of 11 minutes when compared to longer reverse phase chromatography of 65 minutes. The method provides excellent selectivity for the targeted impurity peaks, separation, and fast turnaround time with much lesser consumption of solvent system when compared to conventional normal or reserve phase methods. The ACQUITY UPC^2 method can potentially be used for the chiral analysis of Canagliflozin and its isomeric impurities during product development stage and can be used as a tool for faster screening of method that will serve the purpose of easy scale up to Prep system for isolation and collection of complex isomeric entities.

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