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The Use of ACQUITY UPC² and the Waters SFC Investigator System for the Analysis, Separation, and Isolation of Canagliflozin and Two Isomeric Impurities

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APPLICATION 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
- Understand how SFC Investigator System is a versatile, cost effective system for pharmaceutical labs performing achiral and chiral analysis and small scale purifications

WATERS SOLUTIONS

ACQUITY® UPC²® System

ACQUITY UPC² Trefoil® Column

Empower® 3 Software

ACQUITY SQD Mass Spectrometer

SFC Investigator System

KEYWORDS

UPC² SFC Investigator System, SFC Prep 80q, isomeric mixture, chiral analysis, canagliflozin beta isomer, method transfer, scale up, MS detection

GOAL OF THE ANALYSIS

To develop a robust and effective method for the separation, isolation, and identification of Canagliflozin and its isomeric impurities by Waters ACQUITY UPC² SFC Investigator System, and MS detection.

INTRODUCTION

Canagliflozin is an anti-diabetic beta-isomeric drug used to improve glycemic control in people with Type 2 Diabetes. In development of generic drug for ANDA approval, synthesis of Canagliflozin often begins with materials containing sugar moieties having alpha and beta isomers in equimolar proportions. These facts serve as a potential source of undesired isomeric impurities in the final API, which must be separated and collected for their structural information. Fast turnaround time is essential for generic ANDA submissions. 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.

- ACQUITY UPC² renders feasibility for faster screening of a chromatographic method on the analytical scale with the goal of transferring the separation technique to the preparative scale
- A wide range of UPLC® Column chemistries are added benefit for a research based lab to save the method development time
- These benefits of UPC² were exploited for method screening and exploring the suitability of supercritical fluid chromatography technique for Canagliflozin impurity analysis

In addition, SFC Investigator System is deemed by many to be a cost effective preparative/semi-preparative chromatographic technique. Due to higher diffusivity and lower viscosity of supercritical fluid, SFC Investigator System provides a three- to eight-fold faster separation than normal and reverse phase LC, resulting in a measurable increase in productivity.

Canagliflozin and its isomeric impurities were analyzed by reverse phase (RP) chromatography. RP chromatography for such analysis takes a longer run time of 65 minutes and does not provide enough resolution for the targeted isomeric entities. With RP chromatography, it is extremely challenging to develop a method with enough separation of the conformational isomers that can be scaled up to a prep system. (Rapid) screening was performed using the ACQUITY UPC² which yielded a method that had sufficient resolution to separate the isomers. The method was then scaled up, transferred and further optimized to SFC with similar chiral chemistry to obtain a similar separation profile. The optimized method with a non-Waters chiral column of similar chemistry was used for method transfer to SFC Investigator System, achieving better resolution of the targeted peaks of interest. The transferred method with high resolution of the isomeric peaks can be used for scale up to prep SFC systems.

The SFC Investigator System can be utilized as both an analytical and semi-preparative instrument. Compared to liquid chromatography, SFC offers unique selectivity, less organic consumption and waste removal, smaller collection volume, and faster post purification dry down time. Hence, the method screened in UPC² was transferred to an analytical column of different chemistry of similar chiral affinity that could suit the higher flow rate of SFC Investigator System when transferred. Optimization of the method in UPC² with an analytical column saved time and higher solvent consumption before proceeding to direct method transfer to SFC investigator System.

ACQUITY UPC² method is further transferred to SFC Investigator and optimized with low flow rate to achieve the best possible resolution of the peaks of interests. This facilitated ease of transfer of the method to higher SFC prep instruments with higher loading.

This application note demonstrates the separation, purification, and subsequent identification of Canagliflozin and its isomeric impurities from its other impurities in a shorter runtime with improved separation.

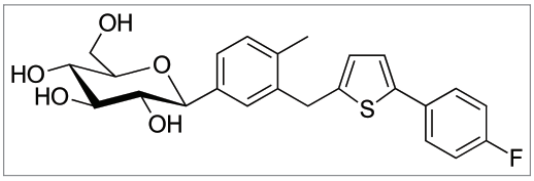

Figure 1. Canagliflozin.

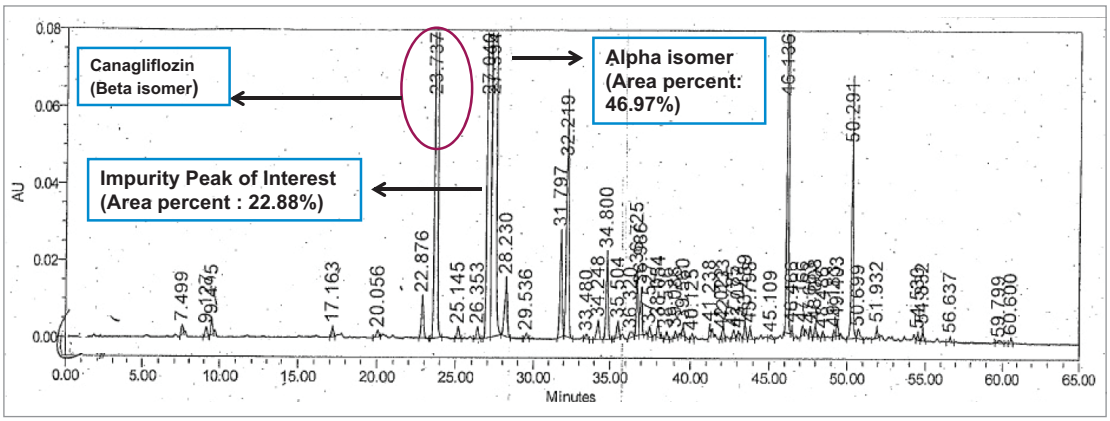

Figure 2. RP chromatography of Canagliflozin API with isomeric peaks.

EXPERIMENTAL

UPC² conditions with ACQUITY UPC² Trefoil Amylose Column

System:	ACQUITY UPC² with ACQUITY UPC² PDA Detector
Software:	Empower 3
Detection:	UV at 290 nm from UV range 200–430 nm (compensation reference 330–430 nm)
Column:	ACQUITY UPC² Trefoil AMY1, 3 x 150 mm, 2.5 µm
Column temp.:	45 °C
Sample temp.:	10 °C
Sample conc.:	250 ppm
Injection volume:	5 µL
Flow rate:	1.5 mL/min
Mobile Phase A:	Compressed CO_2
Mobile Phase B:	0.1% Trifluoro acetic acid in methanol: isopropyl alcohol (50:50)
Run time:	11 min
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
Polarity:	Positive
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

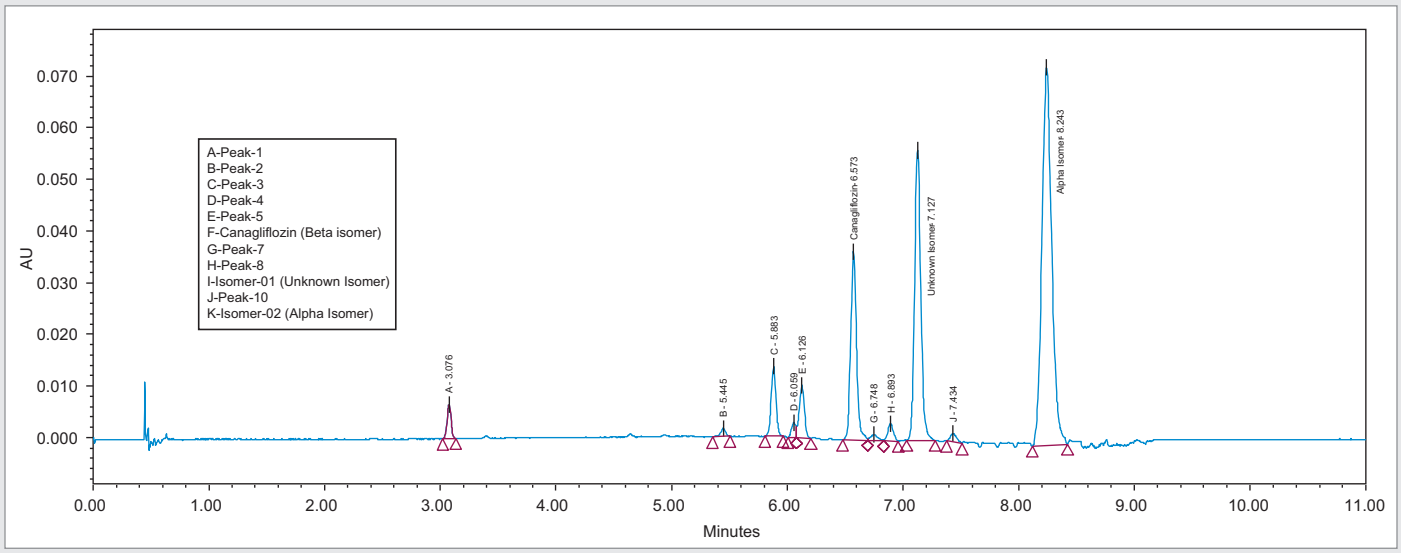

Figure 3. UV Chromatogram of sample in ACQUITY UPC² Trefoil Amylose Column

RESULTS AND DISCUSSION

As the data (Figure 4) suggests, a quick screening of all Trefoil Column chemistries in UPC², provided the choice for selection of the chiral chemistry for best selectivity. A quick screening with Trefoil chiral Column chemistries on UPC² system eliminates the necessity of screening with wide range of non-Waters chiral column chemistries on the SFC Investigator System which helps save time and solvent consumption. This allowed for the simultaneous development of a robust UPC² method that can be efficiently used for testing isomeric Canagliflozin compounds in the QC environment. The selected Trefoil chiral chemistry provided the choice of selection of Diacel chiral column chemistry for method optimization. Method optimization with Diacel chiral column in ACQUITY UPC² further provided a suitable chromatographic condition for Diacel column with lesser solvent consumption. The method was further transferred and finalized with minimum optimization in the SFC Investigator System suitable for semi prep level collection of the peaks of interest.

Peak Results

	Name	RT	Area	Height	%Area	USP Resolution
1	A	3.073	15720	6583	1.76	
2	B	5.449	4395	1548	0.49	35.92
3	C	5.885	40785	13854	4.56	5.93
4	D	6.059	8473	3155	0.95	2.21
5	E	6.128	32175	10344	3.60	0.85
6	F	6.575	130528	36484	14.60	5.02
7	G	6.744	5442	1224	0.61	1.32
8	H	6.893	11471	3355	1.28	1.18
9	I	7.127	211523	55760	23.65	2.47
10	J	7.438	6616	1646	0.74	2.97
11	K	8.242	427199	72101	47.77	5.93

A-Peak-1
B-Peak-2
C-Peak-3
D-Peak-4
E-Peak-5
F-Canagliflozin
G-Peak-7
H-Peak-8
I-Isomer-01
J-Peak-10
K-Isomer-02

Figure 4. Peak results table for UV chromatogram of Canagliflozin with ACQUITY UPC² Trefoil Amylose Column.

As Trefoil chiral chemistries are specific to UPC² and analytical dimension is not yet available commercially, scale up of the method needed prior optimization with Diacel column of similar chiral chemistry that could be used for direct method transfer to the SFC Investigator. This strategy of optimizing the method with analytical columns of similar chemistry in UPC² shortened the optimization time and solvent consumption. The method is further screened with similar column chemistry like AD-H and AD-3 analytical columns in UPC², which will suit the higher flow rate and serve the preparative approach upon method transfer. Diacel Chiralpak AD-3 (Figure 5) was selected as the column of choice from the obtained chromatographic data.

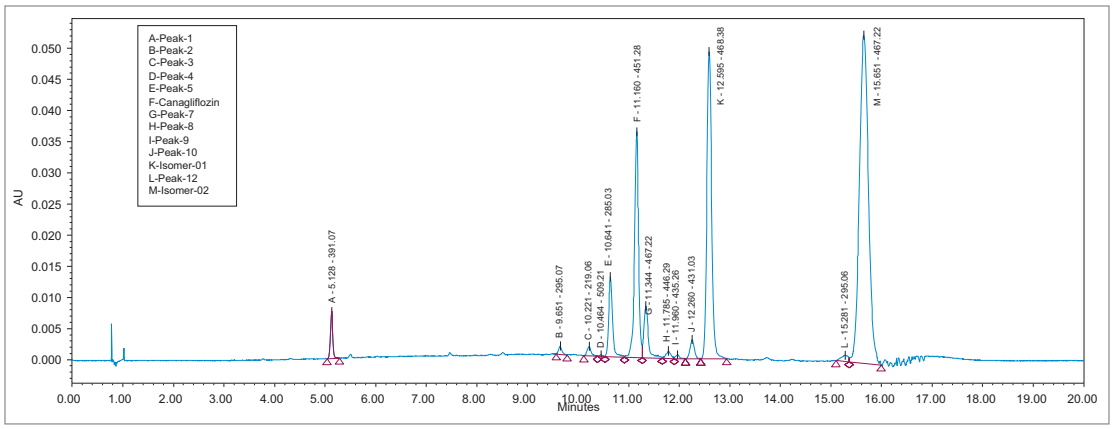

*Figure 5. UV chromatogram of sample in Diacel ChiralPak AD-3 (4.6*150 mm, 3 µ) column from ACQUITY UPC².*

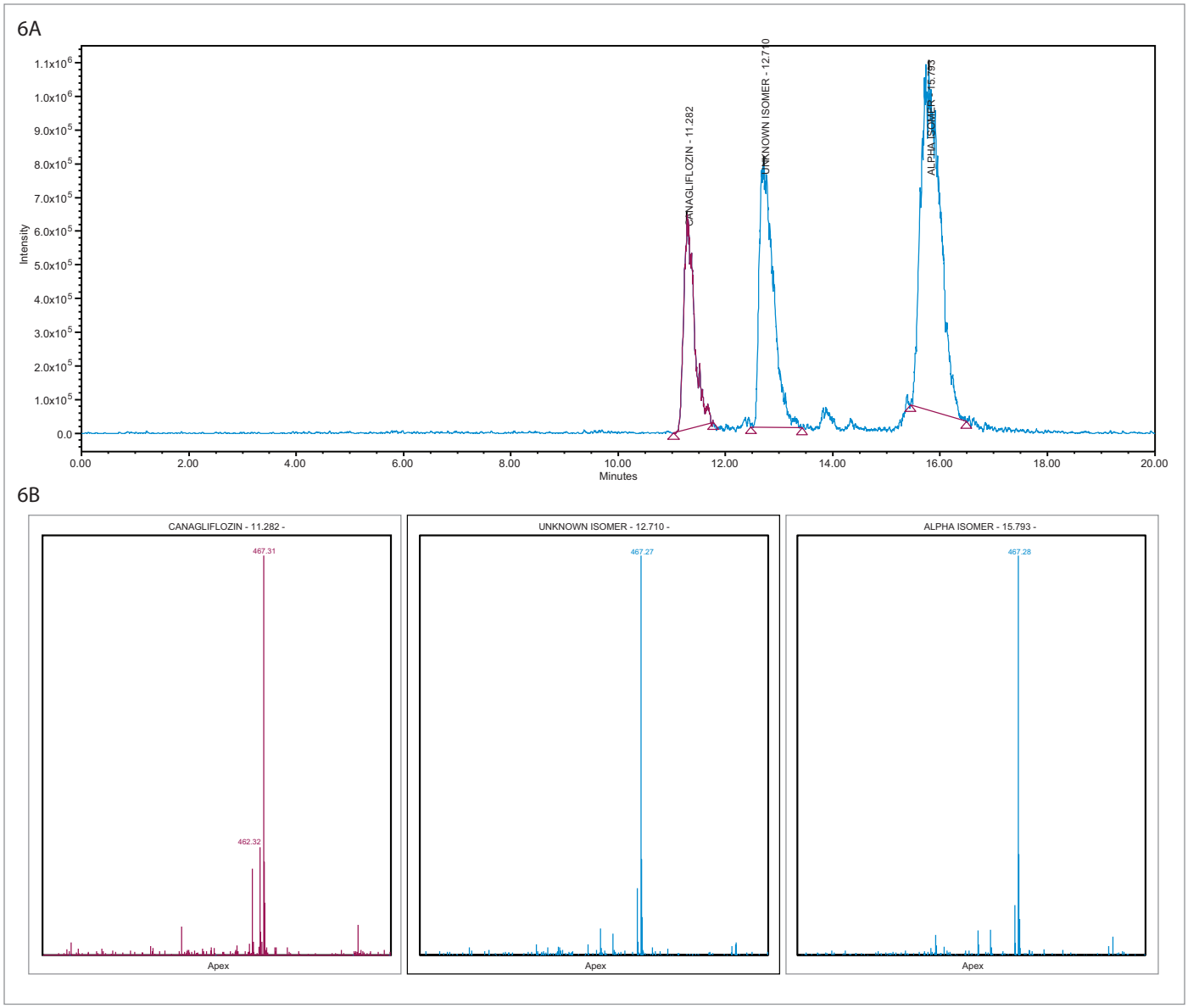

Figure 6A. MS spectra and XIC of 467 m/z in Diacel ChiralPak column from ACQUITY UPC² interfaced with SQD.
Figure 6B. Mass Analysis plot in Diacel ChiralPak column from ACQUITY UPC² interfaced with SQD.

Based on the chromatographic results of UPC² with the analytical column, the method transfer strategy is undertaken by SFC Investigator System. Keeping the chromatographic parameters the same, optimization of the method was done to achieve a lesser runtime of 15 minutes in SFC Investigator. Method transfer calculations were for the system volume difference of SFC Investigator and UPC² System and the flowrate was optimized. Flow rate was kept as low as 5 mL/min as that would serve the purpose of easy scale up to newer SFC Prep Systems like SFC Prep 80 or SFC Prep 100 with higher loading and flow rate optimization.

Fraction collection for peaks of interests was performed using the SFC Investigator System. The three individual collected fractions of interest were confirmed by area percent, retention time profile, and compared to UPC² data. Recovery was found to be more than 90% when calculated based on dilution and area counts of the collected fractions (Figure 10, 11, 12).

All three peaks showed *m/z* 467 [M+Na] and *m/z* 462 [M+ NH_4] (Figure 7) when analyzed by orthogonal mass detection technique in UPC² analysis and by infusion in tandem quadrupole mass spectrometer confirming a molecular weight of 444 Da for all three isolated fractions from the SFC Investigator System.

Peak Results

	Name	RT	Area	Height	%Area	USP Resolution
1	A	5.128	24656	7571	1.79	
2	B	9.651	6072	1328	0.44	43.78
3	C	10.221	8548	1523	0.62	4.26
4	D	10.464	978	241	0.07	1.89
5	E	10.641	66181	12836	4.82	1.47
6	F	11.160	203867	36235	14.84	3.76
7	G	11.344	50173	8317	3.65	1.25
8	H	11.785	7864	1207	0.57	2.57
9	I	11.960	3510	604	0.26	1.00
10	J	12.260	18513	3106	1.35	1.89
11	K	12.595	320598	49316	23.34	2.02
12	L	15.281	9519	974	0.69	15.83
13	M	15.651	653412	52665	47.56	1.48

A-Peak-1
B-Peak-2
C-Peak-3
D-Peak-4
E-Peak-5
F-Canagliflozin
G-Peak-7
H-Peak-8
I-Peak-9
J-Peak-10
K-Isomer-01
L-Peak-12
M-Isomer-02

Figure 7. Peak results table for UV chromatogram of Canagliflozin in ACQUITY UPC² with Diacel ChiralPak column.

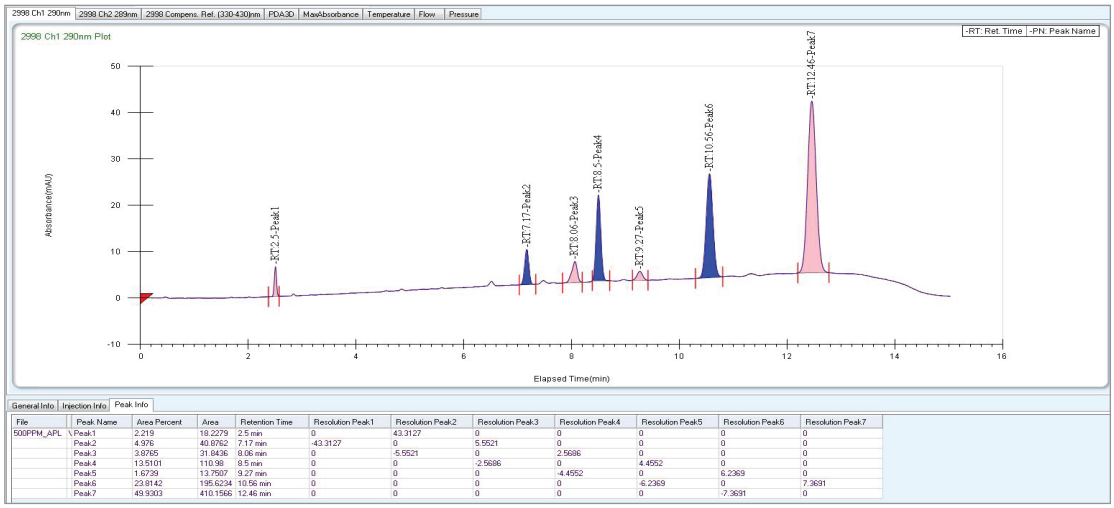

Figure 8. UV Trace of Canagliflozin sample in SFC Investigator at 290 nm with Diacel ChiralPak column.

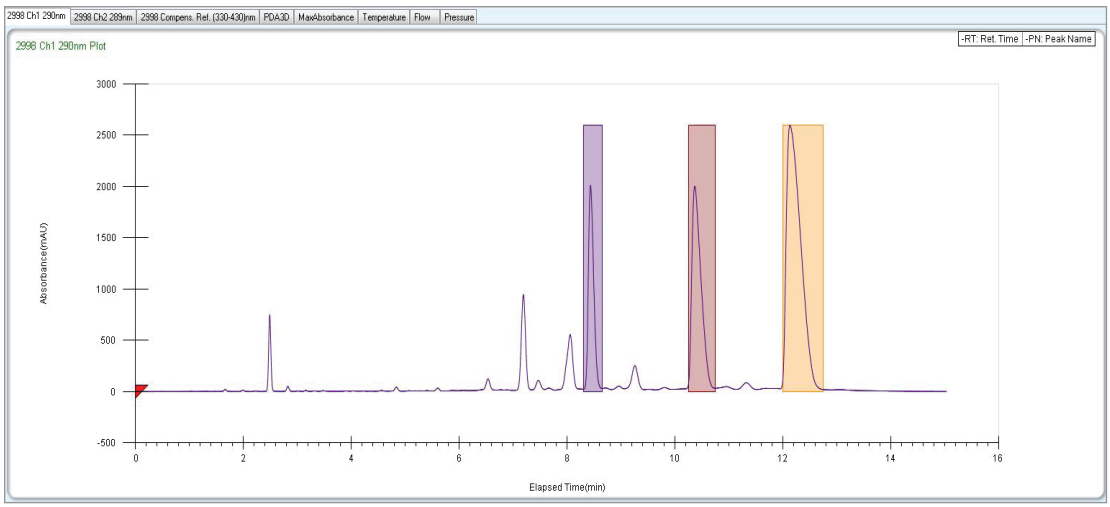

Figure 9. Collected fraction of Canagliflozin sample in SFC Investigator with Diacel ChiralPak column.

UPC² CONDITIONS WITH DIACEL CHIRALPAK AD-3 COLUMN (4.6 x 150 mm, 3 µm)

System:	ACQUITY UPC² with ACQUITY UPC² PDA Detector
Software:	Empower 3
Detection:	UV at 290 nm from UV range 200–430 nm (compensation reference 330–430 nm)
Column:	Diacel ChiralPak AD-3 (4.6 x 150 mm column, 3 µm)
Column temp.:	45 °C
Sample temp.:	10 °C
Sample conc.:	500 ppm
Injection volume:	5 µL
Flow rate:	1.5 mL/min
Mobile phase A:	Compressed CO_2
Mobile phase B:	Ethanol: Isopropyl Alcohol (50:50)
Run time:	20 min
ABPR pressure:	2000 psi
Gradient:	5% B for 1.2 minute, ramp to 50% of B in 11 min, Hold at 50% B for 2 min, and return to 5% B in 2 min to equilibrate up to 20 min
Diluent:	Acetonitrile

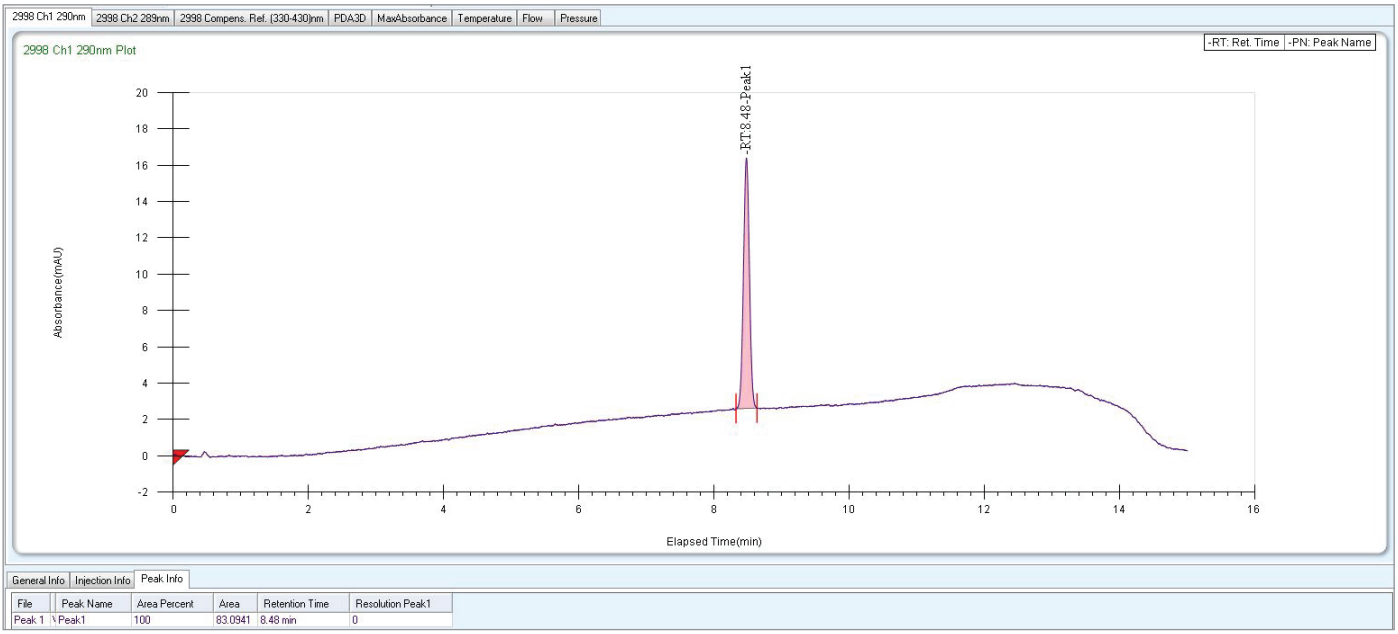

Figure 10. Confirmation of purity and recovery from collected fraction of peak-01 in SFC Investigator.

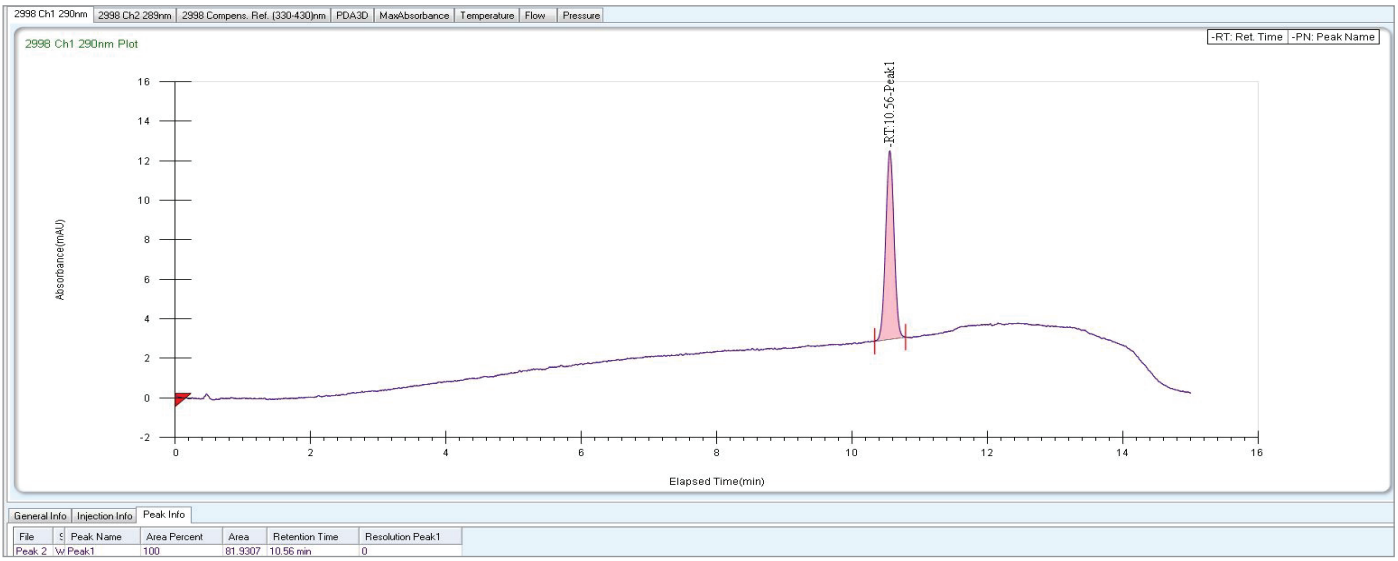

Figure 11. Confirmation of purity and recovery from collected fraction of peak-02 in SFC Investigator.

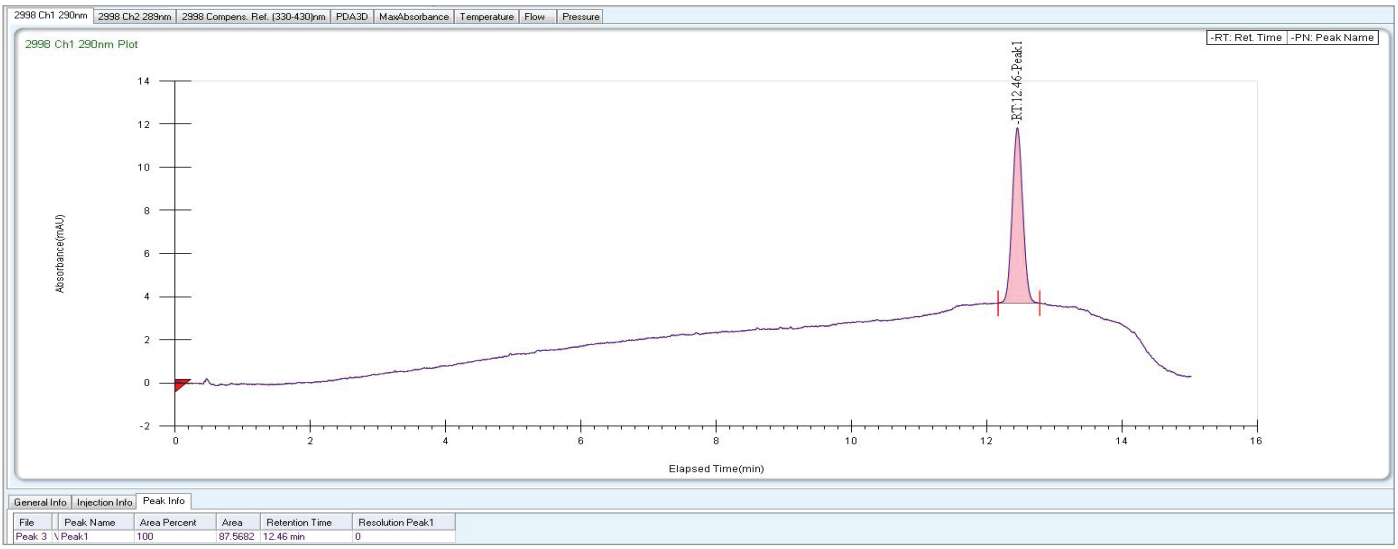

Figure 12. Confirmation of purity and recovery from collected fraction of peak-03 in SFC Investigator.

CONCLUSIONS

- With UPC² technology rapid separation of isomeric entities of Canagliflozin was achieved within 11 minutes, compared to 65 minutes run time with reverse phase chromatography
- Screening compounds with the ACQUITY UPC² System provided a good, simultaneous analytical method for the QC environment; the SFC Investigator System also helped minimize solvent and time consumption
- Based on the chromatographic parameters with the UPC² System, transferring the method to the SFC Investigator System was done easily using the same column chemistry, rendering high resolution of the isomeric fractions in less time
- Better chromatography with higher resolution and peak separation – without compromising the recovery – helps achieve higher recovery and purity of the targeted peaks during scale up to the SFC Prep System

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