International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 96-104
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

A Simple and Sensitive High Performance Liquid Chromatographic Method for Determination of Trifluoroacetic Acid

Narasimha Rao Avupati*[1], Nageswara Rao Gollapalli[2] and Moses Babu[3]

[1, 2]Department of Chemistry, Andhra University, Visakhapatnam 530003, Andhra Pradesh, INDIA.
[1, 3]Dr. Reddy's Laboratories Ltd. Active Pharmaceutical Ingredients, IPDO, Bachupally, Hyderabad-500090, Andhra Pradesh, INDIA.

Received: 12 Jan 2019 / Accepted: 10 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: narasimharaoavupati@gmail.com

Abstract

A simple and sensitive High performance liquid chromatographic method for determination of Trifluoroacetic acid (TFA) in drug substances at pharmaceutical industry has been developed. The quantitative analysis of trifloroacetic acid was achieved on YMC Basic column (150mm x 4.6mm, 3.0µm) with gradient elution at a flow rate of 0.5 mL/min. Gradient elution containing mobile phase-A and mobile Phase-B, 0.05M KH2PO4 Solution used as mobile phase-A and mixture of Acetonitrile, water in the ration of 90:10 (v/v) solution used as mobile Phase-B. The elution of trifluoroacetic acid is monitored at 200nm, by using Ultra Visible / PDA detector. The high correlation coefficient (R2>0.9995) values indicated clear correlations between the investigated compound concentrations and their peak areas within the LOQ (limit of quantification) to 150% level. Advantage of the method is Trifluoroacetic acid content can be determined in **water soluble and water insoluble drug substance** (substances, which is soluble in Dichloromethane).

Keywords
Trifluoroacetic acid (TFA), Content, HPLC, water soluble, water insoluble, Drug substances.

*****

1. INTRODUCTION

Back ground:

Trifluoroacetic acid is common solvent which is using in the synthetic process of active pharmaceutical ingredient and peptides industry. Generally, Trifluoroacetic acid using in pharmaceutical industry mainly for acidification reaction or base neutralization reaction, for peptide industry is to separation of peptide or amino acid from resin.

2. Experimental

2.1. Chemicals, standards and impurities

Acetonitrile (HPLC grade, Merck, India), KH_2PO_4 Anhydrous (AR grade, Merck, India), High pure water is from Milli-Q water purification system from

Millipore, Trifluoroacetic acid (AR grade, Merck, India) and Dichloromethane (AR grade, Merck, India). Bivalirudin drug and Canagliflozen substance were obtained from Process Research department of Dr. Reddy's Laboratories, Hyderabad.

2.2. Equipment's

LC was carried out with Shimadzu HPLC with photodiode array detector.

The output signal was monitored and processed by using LC solution software.

2.3. Chromatographic Conditions

A new gradient method is developed for Trifluoroacetic acid in drug substances (water insoluble & water solution). The chromatographic method employs a mobile phase-A consisting of 0.05M mono potassium phosphate in water and a mobile Phase-B consisting acetonitrile and water in the ratio of 90:10. The method employs a gradient program (Time in min / % Mobile phase B) 0.01/0, 5/0, 10/30, 15/90, 20/0, 25/0. The method was developed using YMC Basic 150mm, 4.6mm and 3.0 µm column. The flow rate of the mobile phase was 0.5 mL/min. The column temperature was maintained at 25°C, sample cooling rack temperature was maintained at 15°C and the wavelength was monitored at 200 nm. The injection volume was 20 micro liter (µL). Diluent is **Dichloromethane or water** (Based on the solubility of drug substance).

2.4. Preparations of Blank solution

Preparation of blank solution was given in Table-1 for dichloromethane (water insoluble compounds) and water soluble compounds

Table-1: Preparation of Blank solution

For Dichloromethane soluble (water insoluble) compounds analysis	For water soluble compounds analysis
Mix 10 mL of Dichloromethane and 10 mL of water in a 25 mL of volumetric flask shake to mix well, allow to layer separation and collect the upper layer (water layer) for the blank analysis.	Use water for blank analysis.

2.4. Preparations of impurity (TFA) stock solution

Preparation of impurity (TFA) stock solution was given in Table-2 for dichloromethane (water insoluble compounds) and water soluble compounds

Table-2: Preparation of Trifluoroacetic acid stock solution

For Dichloromethane soluble (water insoluble) compounds analysis	For Water soluble compounds analysis
Accurately transfer 0.67ml/1000mg of trifluoroacetic acid in to 100ml of volumetric flask containing around 30 to 40 ml of **dichloromethane**, mix well and make up to the volume with dichloromethane. Further accurately dilute 1.0 ml of this solution to 100 ml of dichloromethane.	Accurately transfer 0.67ml/1000mg of trifluoroacetic acid in to 100ml of volumetric flask containing around 30 to 40 ml of water, mix well and make up to the volume with water. Further accurately dilute 1.0 ml of this solution to 100 ml of water.

2.5. Preparations of impurity (TFA) standard solution

Preparation of impurity (TFA) standard solution was given in Table-3 for dichloromethane (water insoluble compounds) and water soluble compounds

Table-3: Preparation of Trifluoroacetic acid standard solution

For Dichloromethane soluble (water insoluble) compounds analysis	For Water soluble compounds analysis
Accurately transfer 1.0 ml of Impurity (TFA) stock solution in to 25ml of volumetric flask containing 9.0 ml of **dichloromethane**, then added 10 ml of water in to the same volumetric flask mix well, allow to layer separation and collect the upper layer for standard analysis.	Accurately transfer 1.0 ml of Impurity (TFA) stock solution in to 10ml of volumetric flask containing around 3 to 4 ml of water, mix well and make up to the volume with water. Use this solution for standard analysis.

2.6. Preparations of test sample solution

Preparation of test sample solution was given in Table-4 for dichloromethane (water insoluble compounds) and water-soluble compounds.

Table-4: Preparation of test sample solution

For Dichloromethane soluble (water insoluble) compounds analysis	For Water soluble compounds analysis
Accurately weight and transfer 100mg of test sample in to 25ml of volumetric flask, add 10 ml of **dichloromethane,** dissolve and add 10 ml of water in to the same volumetric flask mix well, allow to layer separation and collect the upper layer for test sample analysis.	Accurately weight and transfer 100mg of test sample in to 10ml of volumetric flask and add 3 to 4 ml of water, dissolve and make up to the mark with water. Use this solution for test sample analysis.

2.7. Preparations of TFA spiked with test sample solution

Preparation of impurity (TFA) spiked with test sample solution was given in Table-5 for dichloromethane (water insoluble compounds) and water soluble compounds

Table-5: Preparation of Trifluoroacetic acid spiked test sample solution

For Dichloromethane soluble (water insoluble) compounds analysis	For Water soluble compounds analysis
Accurately weight and transfer 100mg of test sample in to 25ml of volumetric flask, add accurately 1.0 ml of Impurity (TFA) stock solution and 9.0 ml of dichloromethane, dissolve then add 10 ml of water in to the same volumetric flask mix well, allow to layer separation and collect the upper layer for TFA spiked with test sample analysis.	Accurately weight and transfer 100mg of test sample in to 10ml of volumetric flask and add 1.0 ml of Impurity (TFA) stock solution, dissolve and make up to the mark with water. Use this solution for TFA spiked with test sample analysis.

2.7. RESULTS AND DISCUSSION (METHOD VALIDATION)

2.7.1. Linearity

Linearity test solutions for the content method are prepared from trifluoroacetic acid stock solitons at five concentration levels from 50 to 150% of analyte concentration (50, 75, 100, 125 and 150%). The peak area versus concentration data is treated by least-squares linear regression analysis. Linearity solutions for the impurities method were prepared by diluting impurity stock solutions to the required concentrations. The solutions are prepared at different concentration levels from LOQ to 0.15%. The correlation coefficients of Trifluoroacetic acid was found 0.9996 for dichloromethane diluent and 0.9998 for water diluent.

Linearity results were provided in table-6, Linearity curve for Trifluoroacetic acid in dichloromethane diluent was given as Figure-1 and Trifluoroacetic acid curve for in water diluent was given as Figure-2. Overlay chromatograms of Trifluoroacetic acid of linearity was given as Figure-3.

Table-6: Linearity result of Trifluoroacetic acid

Linearity		Linearity of TFA for Dichloromethane soluble (water insoluble) compounds analysis	Linearity of TFA for Water soluble compounds analysis
Level	**concentration**	**Area of TFA**	**Area of TFA**
LOQ	0.016	3746	4099
50%	0.050	12584	14695
75%	0.075	20584	22645
100%	0.10	27810	30154
125%	0.125	34568	38562
150%	0.15	41625	45895
Slop		283951	309940

Intercept	-853.2	-507.2
Correlation	**0.9996**	**0.9998**

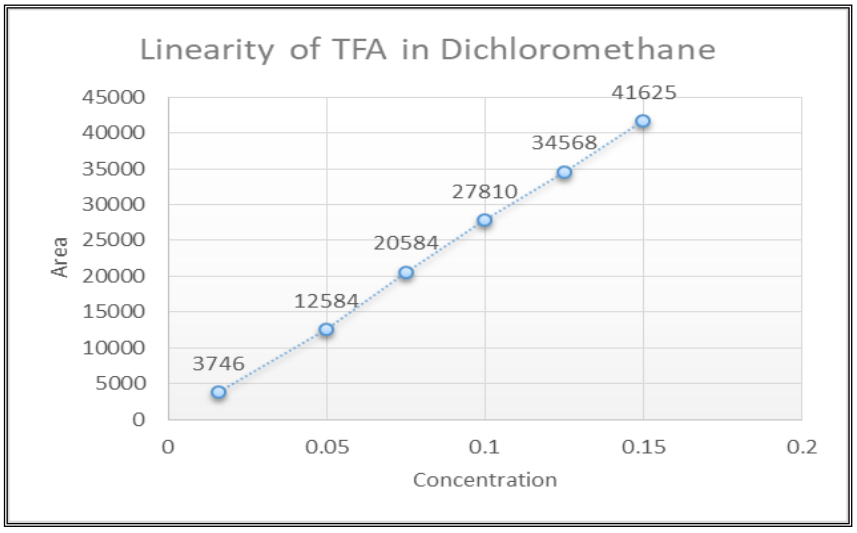

Figure-1: Linearity curve for TFA in Dichloromethane diluent

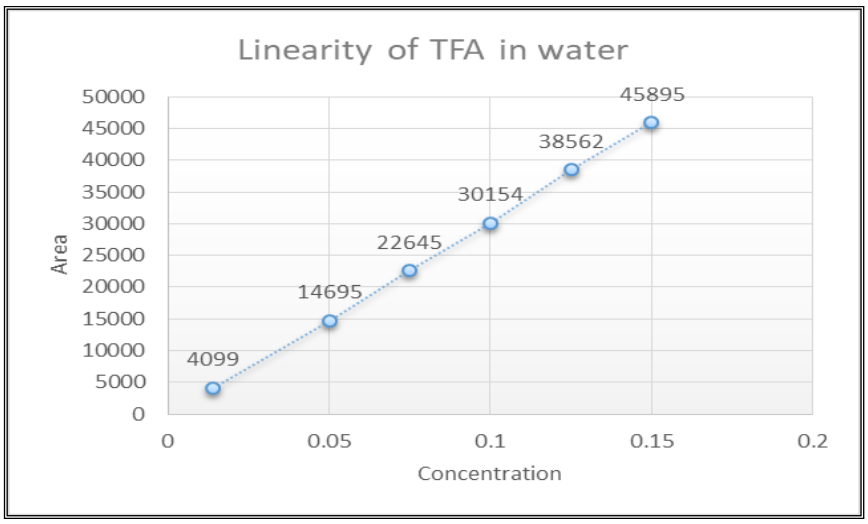

Figure-2: Linearity curve for TFA in water diluent

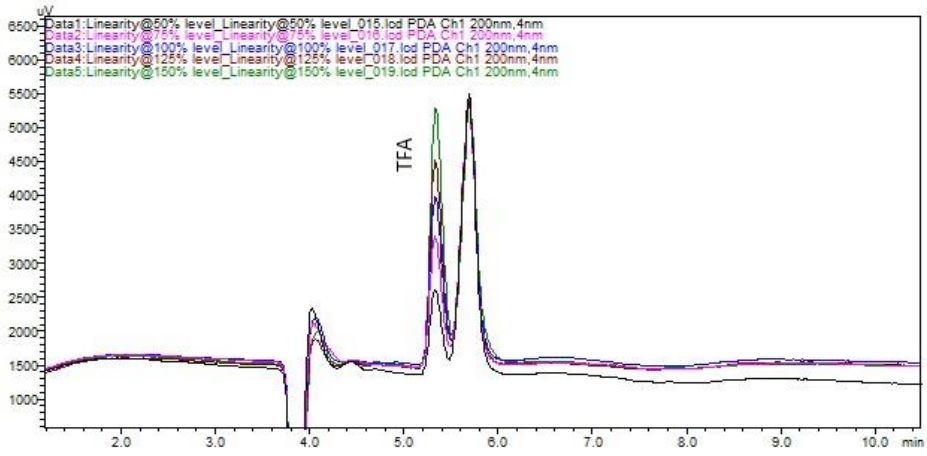

Figure-3: Overlay chromatogram of Linearity solution of Trifluoroacetic acid

2.7.2. Limits of detection (LOD) and quantification (LOQ)

Limit of detection(LOD) and limit of quantification (LOQ) solution preparation was given in table-7

Table-7: Preparation of Trifluoroacetic acid LOQ solution

For Dichloromethane soluble (water insoluble) compounds analysis	For Water soluble compounds analysis
Accurately transfer 0.16 ml of Impurity (TFA) stock solution in to 25ml of volumetric flask containing 9.0 ml of **dichloromethane**, then added 10 ml of water in to the same volumetric flask mix well, allow to layer separation and collect the upper layer for standard analysis.	Accurately transfer 0.14 ml of Impurity (TFA) stock solution in to 10ml of volumetric flask containing around 3 to 4 ml of water, mix well and make up to the volume with water. Use this solution for standard analysis.

The LOD and LOQ for trifluoroacetic acid was estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively , by injecting a series of diluted solutions with known concentration. Precision study was also carried at the LOQ level by injecting six individual preparations of trifluoroacetic acid and calculating the % R.S.D. of the area. Accuracy at LOQ level was evaluated in triplicate for the trifluoroacetic acid by spiking at the estimated LOQ level to test solution.

Limit of Quantification (LOQ) was found 0.016% for dichloromethane diluent and 0.014 % for water diluent, Limit of Detection (LOD) was found 0.005% for both the diluents with respect to test concentration.

Relative standard deviation for Limit of Quantification (LOQ) found 4.60 % for dichloromethane diluent and 2.55 % for water diluent.

The limit of detection, limit of quantification and precision at LOQ values for Trifluoroacetic acid are shown in Table 8 and Table-9.

Overlay chromatograms of Trifluoroacetic acid at LOQ precision was showing in the Figure-4

Table-8: Concentration of LOD and LOQ for Trifluoroacetic acid in both diluent

Level (% with respect to test concentration)	Concentration of TFA for Dichloromethane soluble (water insoluble) compounds analysis	Concentration of TFA for Water soluble compounds analysis
LOD	0.005%	0.005%
LOQ	0.016%	0.014%

Table-9: Precision Limit of Quantification results for Trifluoroacetic acid

LOQ	TFA for Dichloromethane soluble (water insoluble) compounds analysis	TFA for Water soluble compounds analysis
Preparation	**Area of TFA**	**Area of TFA**
Preparation-1	3786	4251
Preparation-2	3983	4013
Preparation-3	3906	3954
Preparation-4	3617	4125
Preparation-5	3606	4098
Preparation-6	3577	4150
Mean	3746	4099
SD	172	105
% RSD	**4.60**	**2.55**

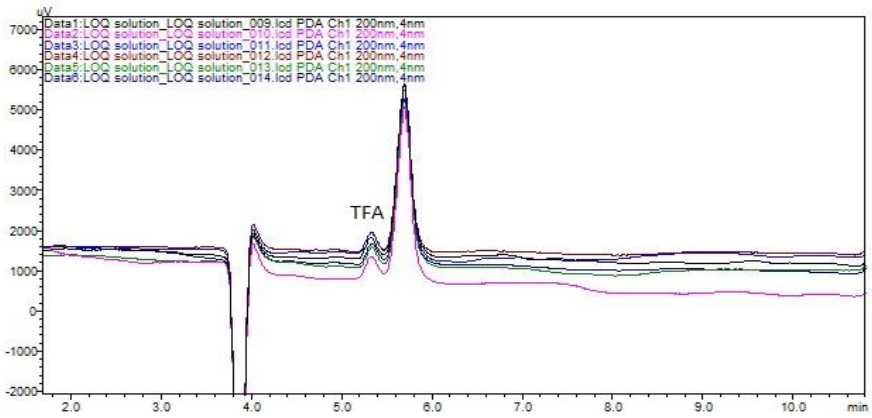

Figure-4: Overlay chromatogram of Trifluoroacetic acid for Precision at LOQ

2.7.3. Accuracy

The accuracy of the trifluoroacetic acid content method is evaluated in triplicate at three concentration levels, i.e. 50, 100 and 150% of the specification concentration. The recovery is calculated against 100 mg/ml of test concentration. Recovery study of the trifluoroacetic acid was performed in both the diluents (Dichloromethane and Water) at 0.05%, 0.10% and 0.15% levels and found that accuracy of the method falls in the range of 88% to 98%. Accuracy data is shown in the Table-10 and Table-11. The Accuracy study was performed with API samples of Canagliflozen (Dichloromethane as diluent) and Bivalirudin (water as diluent).

Accuracy result for Trifluoroacetic acid in dichloromethane diluent was given in the table-10 and Accuracy result for Trifluoroacetic acid in water diluent was given in the Table-11.

Table-10: Accuracy result for Trifluoroacetic acid in Dichloromethane diluent

Accuracy levels	Preparation	TFA Area	TFA spiked (%)	TFA Obtained (%)	% Recovery	Avg % Recovery
	Preparation-1	3897	0.0160	0.0139	87.13	
At LOQ	Preparation-2	4001	0.0160	0.0143	89.41	88.3
	Preparation-3	3956	0.0160	0.0141	88.34	
	Preparation-1	13678	0.0499	0.0489	97.95	
At 50%	Preparation-2	13564	0.0499	0.0486	97.41	96.4
	Preparation-3	13112	0.0499	0.0469	93.99	
	Preparation-1	26587	0.0999	0.0950	95.21	
At 100%	Preparation-2	26354	0.0999	0.0942	94.34	94.2
	Preparation-3	26123	0.0999	0.0930	93.17	
At 150%	Preparation-1	39567	0.1498	0.1418	94.76	94.8

	Preparation-2	39875	0.1498	0.1426	95.31
	Preparation-3	39498	0.1498	0.1411	94.35

Table-11: Accuracy result for Trifluoroacetic acid in water diluent

Accuracy levels	Preparation	TFA Area	TFA spiked (%)	TFA Obtained (%)	% Recovery	Avg % Recovery
	Preparation-1	3950	0.0140	0.0129	92.2	
At LOQ	Preparation-2	3912	0.0140	0.0128	91.2	92.3
	Preparation-3	4012	0.0140	0.0131	93.5	
	Preparation-1	14123	0.0499	0.0461	92.3	
At 50%	Preparation-2	14386	0.0499	0.0471	94.3	93.4
	Preparation-3	14263	0.0499	0.0465	93.3	
	Preparation-1	29857	0.0998	0.0974	97.6	
At 100%	Preparation-2	29953	0.0998	0.0977	97.9	97.4
	Preparation-3	29763	0.0998	0.0968	97.0	
	Preparation-1	44879	0.1496	0.1468	98.1	
At 150%	Preparation-2	44646	0.1496	0.1458	97.4	97.6
	Preparation-3	44776	0.1496	0.1461	97.6	

2.7.4. Precision

The precision of the method is evaluated by analyzing six test samples of spiked with trifluoroacetic acid at 0.10% level. The Relative standard deviation is found to be 1.51% for trifluoroacetic acid in Dichloromethane and 1.07% in water. Precision data is shown in Table-12. Overlay chromatograms of Trifluoroacetic acid for precision shown in Figure-5.

Table-12: Precision result for Trifluoroacetic acid

Precision	TFA for Dichloromethane soluble (water insoluble) compounds analysis	TFA for Water soluble compounds analysis
Injections	**Area of TFA**	**Area of TFA**
Injection-1	28318	30125
Injection-2	27941	30564
Injection-3	27813	30054
Injection-4	28136	30891
Injection-5	27403	30687
Injection-6	27232	30425
Mean	27807	30458
SD	420	324
% RSD	**1.51**	**1.07**

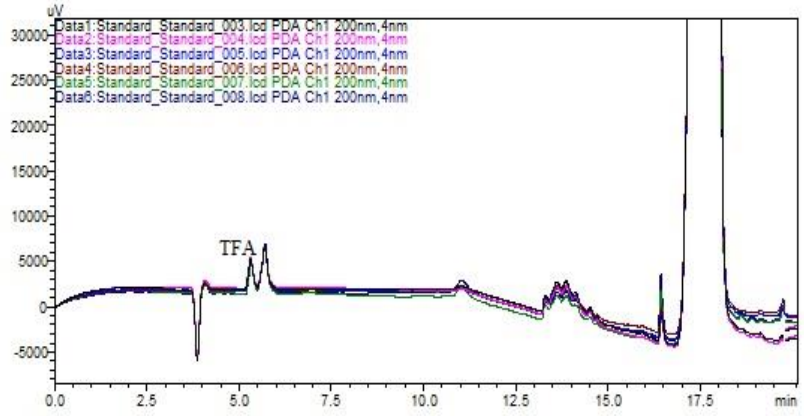

Figure-5: Overlay chromatogram of Trifluoroacetic acid for Precision.

3. Method development and optimization

3.1. Development strategy

Bivalirudin drug and Canagliflozen substance were obtained from Process Research department of Dr. Reddy's Laboratories. The main target of the chromatographic method is to get the accurate quantification for Trifluoroacetic acid. After achieving the method with better peak shape and resolution with the main peak, to confirm the separation, peak purity was ensured by using PDA detector.

3.2 Selection of detector & Basis for initial wavelength selection

Initial wavelength selection for Trifluoroacetic acid is decided by the literature search and found 210nm is the cut-off wavelength for Trifluoroacetic acid and 200nm is better wavelength for Trifluoroacetic acid as per reference from publication *Chem. Anal. (Warsaw),* 50, 387 (2005).

3.3 Buffer selection:

Trifluoroacetic acid is very strong acid and its pK_a value is 0.23 (as per literature) hence Trifluoroacetic acid elute at dead volume in normal chromatographic condition. As a preliminary guide to the selection of mobile phase was prepared with ion pair reagent based on the literature evidence of *Chem. Anal. (Warsaw),* 50, 387 (2005). Same conditions were applied the elution of target peak but the peak shape was not good as good (fronting peak observed). Based on this experiment concluded that the ion pair reagent influences the target peak to retains more hence peak shape was not good. Buffer changed to potassium hydrogen phosphate, peak shape was improved but the retention time was early when compare with TBAHS, hence mobile phase fixed for potassium hydrogen phosphate.

3.4 Column and gradient Selection:

Initially Phenomenex Luna RP18 250mm, 4.6mm and 5µm column was used for development but the retention time early with potassium phosphate buffer, later trails conducted with different columns like phenyl and basic. Good peak shape and better retention time obtained with basic column, basic column was good for acid compounds due the more interaction between acid and basic nature of column hence peak was retained more time in the column.

YMC basic column was selected for the quantification of Trifluoroacetic acid in the analysis of Canagliflozen and Bivalirudin.

Key Benefits and features of YMC Basic 150mm, 4.6mm and 3.0 µm are

a) Virtually free of trace metal contaminants which can cause peak tailing.
b) Good peak shape for acids.
c) Physical and chemical durability provides long column life.

Different gradient trails, different organic compositions and solution stability trails were studied.

Observation:

Based on the final results, YMC Basic 150mm, 4.6mm with 3.0 µm particle size and mobile phase-A consisting of 0.05M mono potassium phosphate in water and a mobile Phase-B consisting acetonitrile and water in the ratio of 90:10 with a gradient program (Time in min / % Mobile phase B) 0.01/0, 5/0, 10/30, 15/90, 20/0, 25/0 at detection wavelength 200 nm was confirmed for the quantification of Trifluoroacetic acid in the analyte.

3.5 Diluent Selection:

Diluent section is the major achievement for this method to determine the Trifluoroacetic acid. Due to the low response of the target impurity (TFA) even at 200nm, sample concentration was increased up to 100mg / mL for better limit of quantification and limit of detection value. As per the literature water is diluent for quantification of Trifluoroacetic acid, but Canagliflozen was insoluble in water. Solubility was checked with Acetonitrile, Methanol, Dimethyl sulphoxide, Dichloromethane etc.

Identified that Dichloromethane is suitable solvent for Canagliflozen at the level of 100mg/mL, hence Dichloromethane was selected as diluent for this method. Advantage of the method is determination of Trifluoroacetic acid content can be done for drug substances with water and dichloromethane (i.e. water soluble and water insoluble drug substance)

4. CONCLUSION

A simple, sensitive and accurate method was developed for the quantitation of Trifluoroacetic acid in pharmaceutical drug substances using two different diluents like Dichloromethane and water for the benefit of both water soluble and water insoluble compounds. Selection of diluent (Dichloromethane) is the key step for the analytical approach which dissolves the water insoluble compounds and meets the specific requirement of analytical strategy. Method was developed with the wave length of 200nm is the other major step to get high response for Trifluoroacetic acid. YMC basic column was used for the purpose of retain the impurity (TFA) in the column and good peak (Gaussian peak). This study was demonstrated the method was Linear accurate and precise at specification level and Quantification level.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Reddy's laboratories limited for support extended for the research work. The cooperation from other colleagues is also highly appreciated.

REFERENCES

[1] *Chem. Anal. (Warsaw),* 50, 387 (2005)
[2] Glód B.K., Neurochem. Res., 22, 1237 (1997).
[3] Liang X., Li S.Z. and Li W.L., Progr. Biochem. Biophys., 26, 75 (1999).
[4] Mafé S., Ramirez P., Tanioka A. and Pellier J., J. Phys. Chem. B., 101, 1851 (1997).
[5] G³ód B.K., Alexander P.W., Haddad P.R. and Chen Z.L., J. Chromatogr. A, 699, 31 (1995).
[6] Bruzzoniti M.C., Mentasti E. and Sarzanini C., J. Chromatogr. A, 770, 51 (1997).
[7] Dunn B.M. and Pennington M.W., Methods in Molecular Biology 36, Peptide analysis Protocols, Humana Press 1994
[8] Patthy M., J. Chromatogr. A, 660, 17 (1994).
[9] Cecchi T., J. Chromatogr. A, 958, 51 (2002).