

## A VALIDATED REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-CHARGED AEROSOL DETECTOR TECHNIQUE FOR THE SIMULTANEOUS ESTIMATION OF SITAGLIPTIN AND ERTUGLIFLOZIN IN PURE AND PHARMACEUTICAL DOSAGE FORMS

ANJANEYULU REDDY B<sup>1\*</sup>, RADHAKRISHNANAND P<sup>2</sup>, IRSHAD ALAM MD<sup>3</sup>, RAVI KIRAN P<sup>4</sup>

<sup>1</sup>Department of ???, Faculty of Science, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.

<sup>2</sup>Department of ???, Daicel Chiral Technologies, Hyderabad, Telangana, India. <sup>3</sup>Department of ???, Faculty of Science and Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India. <sup>4</sup>Department of Chemistry, BITS-Pilani, Medchal, Telangana, India. Email: anjireddy98@gmail. Com

Received: 02 February 2019, Revised and Accepted: 09 April 2019

### ABSTRACT

**Objective:** The main objective of the present work is to develop and validate a selective reverse-phase (RP) high-performance liquid (HPLC)-charged aerosol detection technique for the quantitation of the sitagliptin and ertugliflozin in dosage form to attain high degree of sensitivity.

**Materials and Methods:** In present HPLC technique, separation of drugs was achieved on Phenomenex C<sub>18</sub> column (250×4.6 mm, 5 μ) with a mobile phase composition of phosphate buffer (pH – 5.8), acetonitrile, and methanol in the proportion of 40:40:20%V/V. 1 ml/min flow rate and 256 nm wavelength detection were maintained for the elution of drugs in the chromatographic system. The retaining time of sitagliptin and ertugliflozin in column was found to be 4.2 and 2.4 min, respectively.

**Results:** The projected technique was successfully applied for the quantitation of sitagliptin and ertugliflozin as a single combined mixture. The linearity statistics for calibration curves shown a good linearity in the concentration range of 0.3125–10 μg/ml for sitagliptin and 0.0625–2.5 μg/ml for ertugliflozin. The average values of regression coefficient, slope, and intercept were 0.9998, 8688.2, and 1977.6 for sitagliptin and 0.9996, 33602, and 1852.6 for ertugliflozin. The technique was validated as per the International Council for Harmonization guidelines. The limit of detection and limit of quantification findings were 0.082 and 0.247 μg/ml for sitagliptin and 0.04 and 0.12 μg/ml for ertugliflozin.

**Conclusion:** The developed and validated RP-HPLC-charged aerosol detector technique of sitagliptin and ertugliflozin in dosage form showed that the method was accurate and selective with high degree of sensitivity.

**Keywords:** Ertugliflozin, Sitagliptin, Diabetes mellitus, Reverse-phase high-performance liquid chromatography-charged aerosol detector, Linearity and validation.

© 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2019.v12i5.32604>

### INTRODUCTION

The charged aerosol detector (CAD) is a universal detection system used to quantify the amount of chemical compounds present in a sample by the process of creating charged aerosol particles which were detected by an electrometer. Reverse-phase (RP) high-performance liquid chromatography (HPLC) with CAD detection system was used in this work to quantify the drugs with high degree of sensitivity. Sitagliptin chemically designated as (R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine with molecular formula C<sub>16</sub>H<sub>15</sub>F<sub>6</sub>N<sub>5</sub>O (Fig. 1) and molecular mass of 407.314 g/mol. It used as an adjunct along with exercise and diet to progress glycemic control in type-II diabetes mellitus patients [1-3]. Sitagliptin obstructs the dipeptidyl peptidase (DPP)-4 enzyme competitively. This DPP-4 enzyme breaks down the glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) incretins, hormones released in gastrointestinal in response to meal. **By inhibiting the activation of GLP-1 and GIP, increase the insulin secretion and decrease the glucagon release by the α-cells of pancreas.** This process leads to normal blood glucose levels in the body. When blood glucose approaches normal level, the insulin amount released, and suppression of glucagon diminishes, this nurture is to inhibit an “overshoot” and consequent low blood sugar levels as seen with some kind of oral hypoglycemic drugs [4,5].

Ertugliflozin chemically designated as (1S,2S,3S,4R,5S)-5-[4-Chloro-3-(4-ethoxybenzyl) phenyl]-1-(hydroxymethyl)-6,8-dioxabicyclo[3.2.1]

octane-2,3,4-triol with molecular formula C<sub>22</sub>H<sub>25</sub>ClO<sub>7</sub> (Fig. 2) and molecular weight of 436.89 g/mol [7-9]. The glucose reabsorption process is mediated by sodium-glucose cotransporters (SGLT), mainly the Type-II diabetes which is accountable for 90% of the reabsorbed glucose. It inhibits SGLT-2 and its activity raises the glucose elimination, decreases hyperglycemia without any requirement of insulin secretion. It progresses the control of glycemia in Type-II diabetes patients [10].

In this paper, we demonstrated the development of RP-HPLC technique using Corona CAD for the quantification of sitagliptin and ertugliflozin. CAD is a universal and highly sensitive detector (Fig. 3), which provides a consistent response for all analytes and has a suitable dynamic range for quantification of sitagliptin and ertugliflozin [11-14]. According to literature, there are no reports of RP-HPLC-CAD technique for the simultaneous quantitation of sitagliptin and ertugliflozin as of now with high sensitivity [15-22]. Few analytical techniques were available with less sensitivity [19,20,22] for the quantification of these drugs by HPLC with ultraviolet and photodiode array. Thus, there is a need for the development of highly sensitive method for the estimation of both drugs using CAD.

### MATERIALS AND METHODS

#### Reagents and chemicals

All the HPLC-grade solvents procured from Sigma-Aldrich (St. Louis Missouri, USA). HPLC-grade water obtained from Milli-Q system (Millipore, Billerica, MA). The reference standards of ertugliflozin and sitagliptin were

AQ1  
AQ1

AQ4

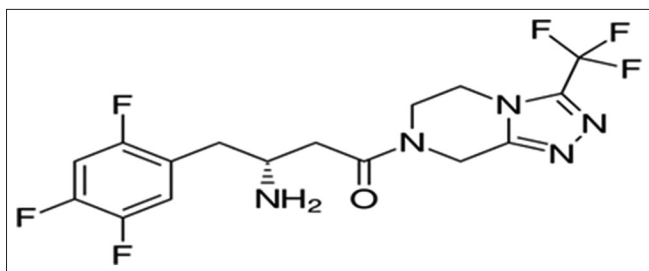


Fig. 1: Structure of sitagliptin

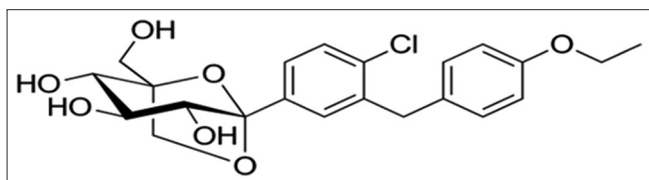


Fig. 2: Structure of ertugliflozin

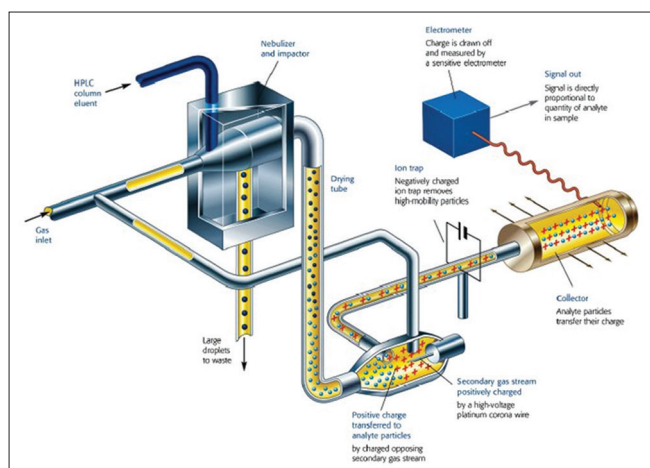


Fig. 3: Charged aerosol detector

gift samples supplied by MSN Laboratories, Hyderabad, India. Ertugliflozin and sitagliptin (15 mg/100 mg) marketed product (Steglujan tablets) bought from local pharmacy. All other chemicals of analytical grade were bought from Qualigens Fine Chemicals, Mumbai, India.

#### Chromatographic system and equipment

Waters-2590 series LC system with Thermo (ESA-corona) CAD detector.

The equipments utilized in the work were Sigma-200 electronic balance, PCI-3.5 L sonicator, Universal hot air oven, and Unilab Digital pH Meter.

#### Preparation of phosphate buffer pH - 5.8

Transfer the mixture containing 8.5 ml of 1 M K<sub>2</sub>HPO<sub>4</sub> and 91.5 ml of 1 M KH<sub>2</sub>PO<sub>4</sub> into a 1000 ml volumetric flask and make up the volume to mark with HPLC-grade water and sonicate the resulting solution for 10 min.

#### Preparation of mobile phase

Preparation of mobile phase done by mixing methanol, acetonitrile, and phosphate buffer (pH - 5.8) in the proportion of 20:40:40% V/V. The resultant mobile phase was degasified by sonication and vacuum filtration 0.45-micron nylon filter.

#### Preparation of standard stock solutions

Standard stock methanolic solutions of the drugs were prepared in concentrations of 1 mg/ml of each of sitagliptin and ertugliflozin [22-25]. Solutions were processed by transferring 100 mg of sitagliptin and ertugliflozin into separate 100 ml volumetric flasks containing 40 ml of methanol, sonication for 5 min. The final volume was made by methanol. The processed stocks were kept at 2–8°C.

#### Preparation of sample solution

Ten tablets of each of studied drug were accurately weighed, transferred to a clean, dry mortar, and ground to fine powder. A powder equivalent to 100 mg sitagliptin and 15 mg ertugliflozin was transferred into a separate 100 ml volumetric flask, 40 ml methanol was added, sonication for 10 min and diluted to the volume with methanol. The resultant solution filtered through 0.45 µm pore size nylon filter membrane.

#### Chromatographic conditions

Drugs were resolved in the liquid chromatographic system consisting Phenomenex C<sub>18</sub> column (250×4.6 mm, 5 µ) with a mobile phase mixture of methanol, acetonitrile, and phosphate buffer (pH 5.8) in the proportion of 20:40:40% V/V. Flow rate of 1 ml/min and

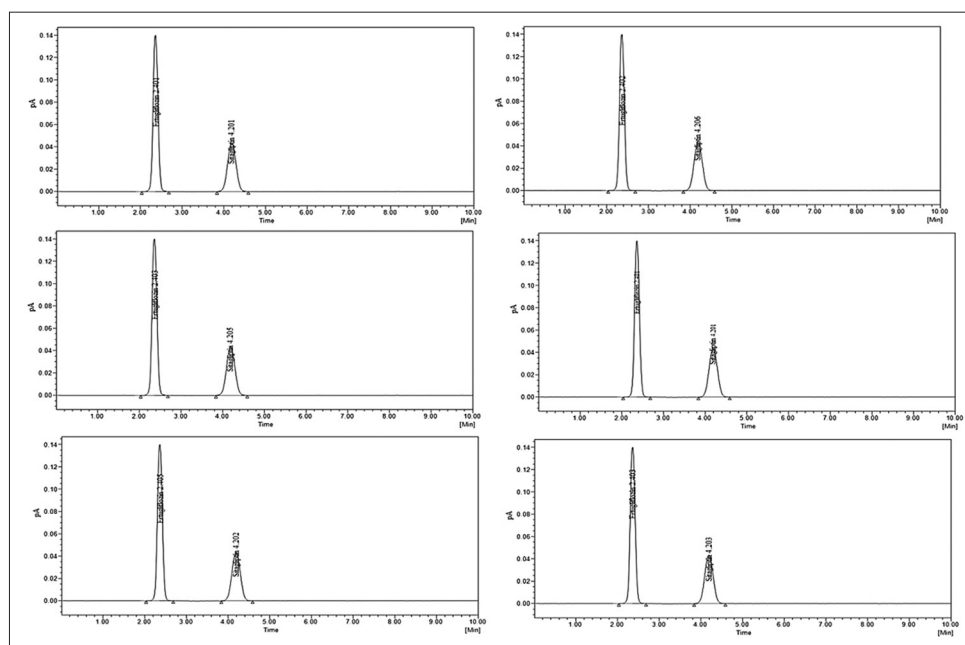


Fig. 4: System precision chromatograms of sitagliptin and ertugliflozin

Corona charged aerosol detection was used for the elution of drugs in the chromatographic system. The retention time of sitagliptin and ertugliflozin was found to be at 4.2 and 2.4 min, respectively.

## RESULTS AND DISCUSSION

### Optimization of RP-HPLC-CAD method

Both drugs were exposed to chromatographic conditions using different mobile phases of different pH values, different columns, and flow rates. The variation in retention times, selectivity and sensitivity of drugs were observed with changes in the mobile phase, columns, flow rate, and pH. Initially, acetonitrile:water in different proportions was used, but poor separation of peaks was detected; then, methanol:water and methanol:buffer in different proportions and different pH values were tried, but low sensitivity and splitting of peaks were observed. Later, methanol, acetonitrile, and buffer at different pH were tried. Best results were given on methanol, acetonitrile, and phosphate buffer (pH 5.8) in the proportion of 20:40:40% V/V with 1 ml/min flow rate on Phenomenex C<sub>18</sub> column (250×4.6 mm, 5 μ).

### Validation

Optimized RP-HPLC-CAD technique was validated as per the International Council for Harmonization validation parameters [26-30].

### Precision

The method precision was confirmed by system precision and intermediate precision. System precision carried out to determine the HPLC system condition. System precision was calculated by infusing six standard solutions, and finally, the percentage relative

standard deviation (RSD) was calculated from the peak response [28]. Intermediate precision was evaluated by the examination of three dissimilar concentrations on different days and percentage RSD values were determined by calculating from resultant findings. Results for precision are shown in Tables 1 and 2.

### Accuracy

Developed method accuracy was processed by studying recovery at three dissimilar concentrations of sitagliptin and ertugliflozin by triplicate analysis (n=3) [23]. The results found from the determination of accuracy were expressed in the form of percentage recovery and finding is shown in Table 3.

### Limit of detection (LOD) and limit of quantification (LOQ)

These were determined separately on the basis of standard calibration curve (Figs. 5 and 6). The residual standard deviation of the regression line or the standard deviation of y-intercepts of regression lines was utilized to calculate LOD and LOQ [22]. Sensitivity of the proposed technique was determined in terms of LOD and LOQ using the following formulae.

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

$$\text{LOQ} = 10 \times \frac{\sigma}{S}$$

Where, σ=Standard deviation determined from the calibration curve.  
S=Slope from calibration curve.

The findings are tabulated in Table 1.

**Table 1: Sitagliptin and ertugliflozin system suitability parameters**

| Parameters     | Ertugliflozin    | Sitagliptin     |
|----------------|------------------|-----------------|
| Linearity      | 0.0625–2.5 μg/ml | 0.3125–10 μg/ml |
| Retention time | 2.4 min          | 4.2 min         |
| Resolution     | -                | More than 2     |
| LOD            | 0.040            | 0.082           |
| LOQ            | 0.120 μg/ml      | 0.247 μg/ml     |

LOD: Limit of detection, LOQ: Limit of quantification

### Linearity

Linearity of the technique was analyzed by infusing six replicates of standard solution containing drugs in the concentration range of 0.3125–10 μg/ml for sitagliptin and 0.0625–2.5 μg/ml for ertugliflozin in triplicate (n=3) into the chromatographic system with constant infusion volume. The linearity graph plotted for peak area of drugs against the related concentrations (Figs. 5 and 6). The average of correlation coefficient (R<sup>2</sup>) values was found to be >0.998 during the progress of validation [19].

**Table 2: Precision study of sitagliptin and ertugliflozin**

| Drugs         | Concentration (μg/ml) | Repeatability (n=6) % RSD | Intermediate precision (n=6) % RSD |
|---------------|-----------------------|---------------------------|------------------------------------|
| Sitagliptin   | 3                     | 0.294                     | 1.740                              |
|               | 6                     | 0.242                     | 1.145                              |
|               | 9                     | 1.010                     | 1.014                              |
| Ertugliflozin | 0.45                  | 1.414                     | 1.641                              |
|               | 0.9                   | 0.566                     | 1.232                              |
|               | 1.35                  | 0.426                     | 1.002                              |

n=6: Number of replicates; RSD=RSD, RSD: Relative standard deviation

**Table 3: Accuracy of sitagliptin and ertugliflozin**

| % recovery statistical analysis |         |             |                       |         |              |
|---------------------------------|---------|-------------|-----------------------|---------|--------------|
| Ertugliflozin (0.45 μg/ml)      |         |             | Sitagliptin (3 μg/ml) |         |              |
| 98.00±101.00                    | Mean±SD | 99.33±1.247 | 97.80±99.00           | Mean±SD | 97.60±1.2328 |
| 99.00                           | % RSD   | 1.255       | 96.00                 | % RSD   | 1.2622       |
| Ertugliflozin (0.9 μg/ml)       |         |             | Sitagliptin (6 μg/ml) |         |              |
| 96.00±99.00                     | Mean±SD | 97.88±1.339 | 99.00±99.76           | Mean±SD | 99.08±0.5180 |
| 98.65                           | % RSD   | 1.386       | 98.50                 | % RSD   | 0.5227       |
| Ertugliflozin (1.35 μg/ml)      |         |             | Sitagliptin (9 μg/ml) |         |              |
| 96.00±97.00                     | Mean±SD | 97.33±1.247 | 98.00±97.75           | Mean±SD | 98.08±0.3118 |
| 99.00                           | % RSD   | 1.281       | 98.50                 | % RSD   | 0.3180       |

RSD: Relative standard deviation, SD: Standard deviation

AQ6

**Specificity**

Method specificity was evaluated from the chromatograms (Figs. 7-10) where complete separation of sitagliptin and ertugliflozin was attained [17]. The peak responses found were well separated with good baseline as shown in Fig. 7-10 and the resolution for all peaks was more than 2 as documented in Table 1.

**Robustness**

The robustness of the proposed HPLC method was assessed by the ability to remain unaffected by small changes in experimental conditions [24,30]. Change in flow rate by ±0.1 ml and small changes

in mobile phase organic strength by ±1 ml have no significant effect on chromatographic resolution. Results are presented in Table 4.

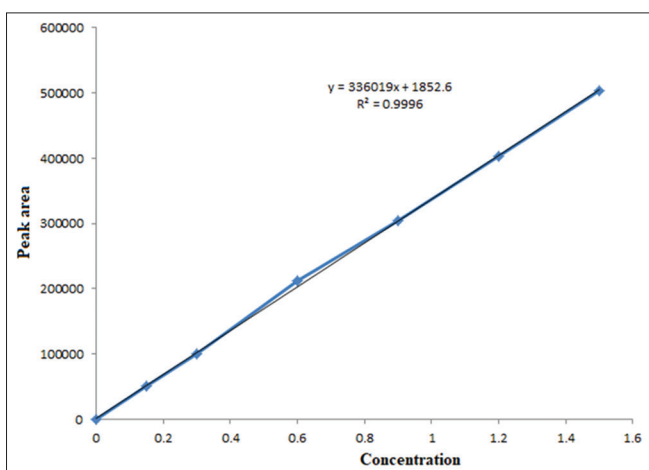
**Application of the method**

Steglujan marketed tablets were estimated by infusing sample solution into LC system under optimized chromatographic conditions. Steglujan each tablet contains 15 mg of ertugliflozin and 100 mg of sitagliptin. The amount of drugs present in the formulation was determined from the calibration curve method. The results of the assay method are shown in Table 5.

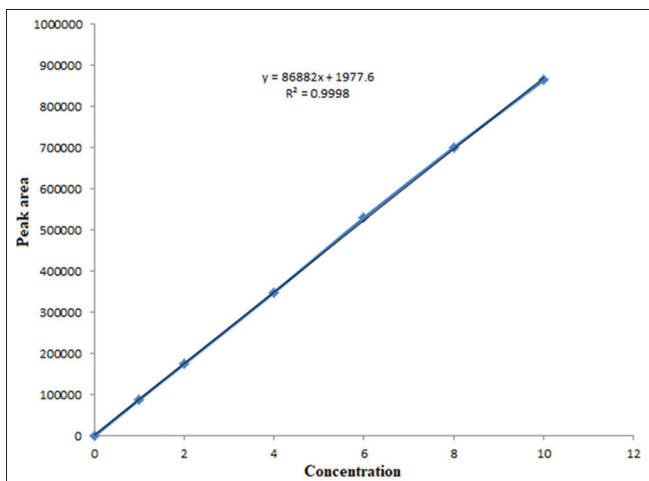
**Table 4: Robustness of ertugliflozin and sitagliptin**

| Parameter  | Variation                         | % RSD         |             |
|------------|-----------------------------------|---------------|-------------|
|            |                                   | Ertugliflozin | Sitagliptin |
| Robustness | Change in flow rate (+0.1 ml/min) | 0.92          | 0.45        |
|            | Change in mobile phase (+1 ml)    | 0.56          | 0.86        |

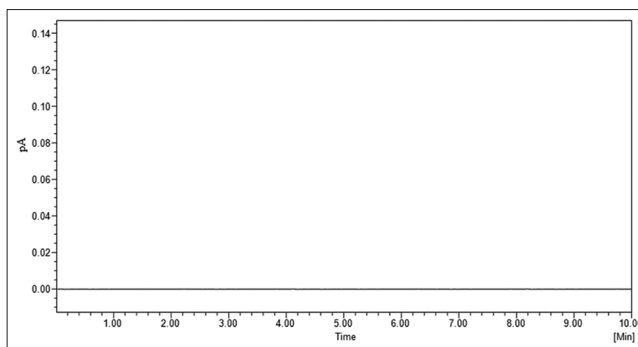
RSD: Relative standard deviation



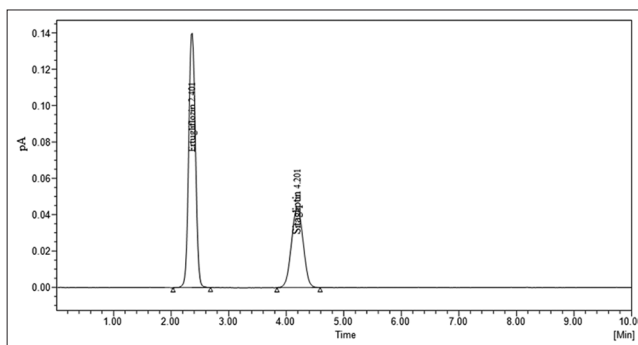
**Fig. 5: Linearity of ertugliflozin**



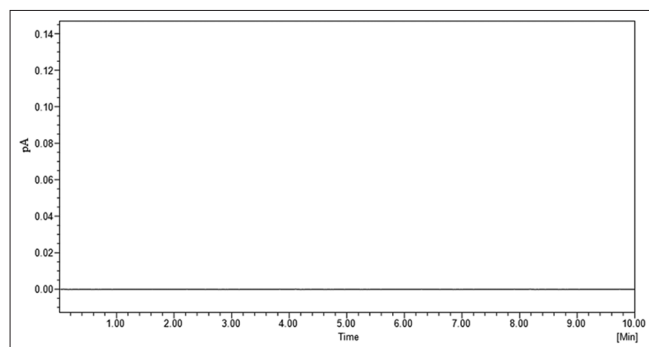
**Fig. 6: Linearity of sitagliptin**



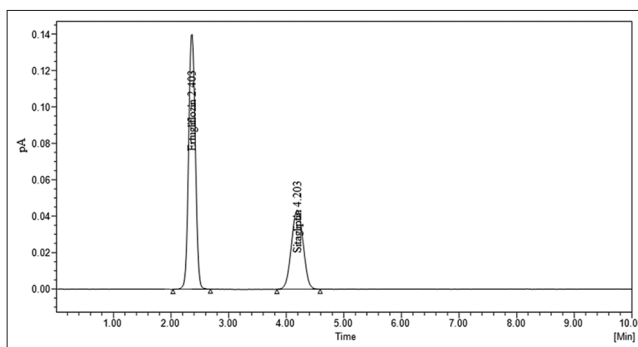
**Fig. 8: Blank chromatogram of ertugliflozin and sitagliptin**



**Fig. 9: Standard chromatogram of ertugliflozin and sitagliptin**



**Fig. 7: Placebo chromatogram of sitagliptin and ertugliflozin**



**Fig. 10: Sample chromatogram of ertugliflozin and sitagliptin**

AQ5

AQ5

**Table 5: Application of developed RP-HPLC-CAD method to tablet formulation**

| Dosage form of drug | Labeled amount in mg/tab | Recovered amount in mg Mean±SD | % CV  | % assay |
|---------------------|--------------------------|--------------------------------|-------|---------|
| Steglujan tablets   |                          |                                |       |         |
| Ertugliflozin       | 15                       | 14.96±0.620                    | 0.626 | 99.79   |
| Sitagliptin         | 100                      | 99.75±0.154                    | 0.613 | 99.75   |

SD: Standard deviation, RP: Reverse phase, HPLC: High-performance liquid chromatography, CAD: Charged aerosol detector, CV: Curriculum vitae

## CONCLUSION

A simple and sensitive RP-HPLC-CAD technique was developed successfully for the simultaneous analysis of sitagliptin and ertugliflozin with good resolution value between the drugs. The CAD detection system was used in this work to give high degree of sensitivity to analytical method for the detection of sitagliptin and ertugliflozin when compared with the existing methods. Sitagliptin and ertugliflozin were linear in the concentration range of 0.3125–10 µg/ml and 0.0625–2.5 µg/ml, respectively. The average values of the correlation coefficient, slope, and intercept were 0.9998, 8688.2, and 1977.6 for sitagliptin and 0.9996, 33602, and 1852.6 for ertugliflozin. The analytical method shows high degree of precision and accuracy with not more than 2% of RSD values. The present analytical method was simple, rapid, accurate, and precise and can easily applicable for routine quantification of the two drugs in bulk and formulations such as capsules, tablets, and powders.

## AUTHORS' CONTRIBUTIONS

All authors contribute equally to this manuscript.

## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## AQ3

## REFERENCES

- Gallwitz B. Sitagliptin: Profile of a novel DPP-4 inhibitor for the treatment of Type 2 diabetes. *Drugs Today (Barc)* 2007;43:13-25.
- American Diabetes Association. Standards of medical care for patients with diabetes mellitus (Position Statement). *Diabetes care* 2003;26 Suppl 1:S33-50.
- Goodarzi MO, Breyer-Ash M. Ertugliflozin revisited: Re-evaluation of its properties and role in the pharmacopeia of modern antidiabetic agents. *Diabetes Obes Metab* 2005;7:654-65.
- Pritam J, Amar C, Bhargav D, Shani P, Santsaran P, Hiren S. Development and validation of first order derivative UV-spectrophotometric method for determination of sitagliptin in bulk and in formulation. *Int J Drug Dev Res* 2011;3:194-9.
- Barnard K, Cox ME, Green JB. Clinical utility of fixed combinations of sitagliptin-metformin in treatment of Type 2 diabetes. *Diabetes Metab Syndr Obes* 2010;3:363-72.
- O'Neil MJ, Heckelman PE, Koch CB, Roman KJ. *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*. 14<sup>th</sup> ed. Whitehouse Station NJ, USA: Merck and Co, Inc.; 2012.
- AbuRuz S, Millership J, McElnay J. Determination of metformin in plasma using a new ion pair solid phase extraction technique and ion pair liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003;798:203-9.
- Zarghi A, Foroutan SM, Shafaati A, Khoddam A. Rapid determination of metformin in human plasma using ion-pair HPLC. *J Pharm Biomed Anal* 2003;31:197-200.
- Koseki N, Kawashita H, Niina M, Nagae Y, Masuda N. Development and validation for high selective quantitative determination of metformin in human plasma by cation exchanging with normal-phase LC/MS/MS. *J Pharm Biomed Anal* 2005;36:1063-72.
- Wang Y, Tang Y, Gu J, Fawcett JP, Bai X. Rapid and sensitive liquid chromatography-tandem mass spectrometric method for the

- quantitation of metformin in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004;808:215-9.
- Vehovec T, Obreza A. Review of operating principle and applications of the charged aerosol detector. *J Chromatogr A* 2010;1217:1549-56.
- Soliven A, Haidar Ahmad IA, Tam J, Kadrichu N, Challoner P, Markovich R, et al. A simplified guide for charged aerosol detection of non-chromophoric compounds-analytical method development and validation for the HPLC assay of aerosol particle size distribution for amikacin. *J Pharm Biomed Anal* 2017;143:68-76.
- Ligor M, Studzińska S, Horna A, Buszewski B. Corona charged aerosol detection: An analytical approach. *Crit Rev Anal Chem* 2013;43:64-78.
- Ragham PK, Kothapalli BC. Development and validation of a stability-indicating RP-HPLC-CAD method for gabapentin and its related impurities in presence of degradation products. *J Pharm Biomed Anal* 2016;125:122-9.
- Hamdana H, BaniJaber AK, Abushoffa AM. Development and validation of a stability indicating capillary electrophoresis method for the determination of ertugliflozin hydrochloride in tablets. *J Pharm Biomed Anal* 2010;53:1254-7.
- Zhai H, Wu Y, Huang B, Huang Q, Yang B, Chen Z. Rapid determination of ertugliflozin hydrochloride in ertugliflozin hydrochloride tablets by capillary electrophoresis. *Huaxue Yanjiu Yu Yingyong* 2008;20:923-6.
- Polagani SR, Pilli NR, Gagula R, Gandu V. Simultaneous determination of atorvastatin, ertugliflozin and glimepiride in human plasma by LC-MS/MS and its application to human pharmacokinetic study. *J Pharm Anal* 2013;3:9-19.
- Pathade P, Imran M, Bairagi V, Ahire Y. Development and validation stability indicating UV spectrophotometric method for the estimation of sitagliptin phosphate in bulk and tablet dosage form. *J Pharm Res* 2011;4:871-3.
- Reddy BV, Raman N, Kumar BS, Rambabu C. Chiral separation of sitagliptin phosphate enantiomer by HPLC using amylose based chiral stationary phase. *J Pharm Res* 2013;7:46-50.
- Jiu X-F, Shang D, Chen Y, Wang Y, Huang L, Peng D, et al. A high performance liquid chromatography method for quantitative determination assay of sitagliptin in rat plasma and its application in pharmacokinetic study. *J Chin Pharm Sci* 2011;20:63-9.
- Bodar JD, Kumar S, Yadav YC, Sahoo U. Development of the spectrophotometric method for the simultaneous estimation of pioglitazone and ertugliflozin. *Int J Pharm Sci* 2011;2:236-43.
- Rao PV, Rao AL, Prasad SV. A new stability indicating RP-HPLC method for simultaneous estimation of ertugliflozin and sitagliptin in bulk and pharmaceutical dosage form its validation as per ICH guidelines. *Indo Am J Pharm Sci* 2018;5:2616-27.
- Suneetha A, Kathirvela S, Ramachandrika G. A validated RP-HPLC method for simultaneous estimation of lopinavir and ritonavir in combined dosage form. *Int J Pharm Pharm Sci* 2011;3:49-51.
- Nashwahgadallah M. Validated HPLC method for simultaneous determination of sitagliptin, metformine and atorvastatin in pure form and in pharmaceutical formulations. *Int J Pharm Pharm Sci* 2014; 5:665-70.
- Brahmaiah B, Sujana K, Rani AP. Development and validation of RP-HPLC method for simultaneous determination of ramipril and valsartan in bulk and pharmaceutical dosage forms. *Asian J Pharm Clin Res* 2012;6:23-5.
- Abdel-Ghany MF, Abdel-Aziz O, Ayad MF, Tadros MM. Validation of different spectrophotometric methods for determination of vildagliptin and metformin in binary mixture. *Spectrochim Acta A Mol Biomol Spectrosc* 2014;125:175-82.
- Mohd M, Rahaman SA, Yadav BR, Battu R. Development and validation of a reverse phase HPLC method for simultaneous estimation of rosuvastatin calcium and fenofibrate in tablet dosage form. *Int J Pharm Pharm Sci* 2011;4:150-4.
- Charde MS, Welankiwar AS, Chakole RD. Development of validated RP-HPLC method for the simultaneous estimation of atenolol and chlorthalidone in combine tablet dosage form. *Int J Adv Pharm* 2014;3:6-18.
- Aburuz S, Millership J, McElnay J. The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimepiride in plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005; 817:277-86.
- Validation of Analytical Procedure, Text and Methodology. International Conference on Harmonisation ICH Q2 (R1), 2005.

Author Queries???

AQ1: Kindly provide the department

AQ2: Kindly cite Figure 4 in the text part

AQ3: Kindly cite reference 6 in the text part

AQ4: Kindly review the sentence.

AQ5: Kindly check the figure 7 and 8 seems to be same

AQ6: Kindly check the table format