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A CRITICAL STUDY ON ANALYTICAL TECHNIQUES FOR THE DETECTION OF SITAGLIPTIN AND ERTUGLIFLOZIN

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ABSTRACT

A key factor in ensuring a drug product's identification, safety, efficacy, purity, and quality is the pharmaceutical analyst. Regulatory restrictions are a major factor in the demand for pharmaceutical analysis. The development of compendia testing methods, formulation of standards, and method validation are typically the steps involved in the regularly used tests of pharmaceutical analysis. By giving accurate information on the identification, composition, and purity of the drug goods, analytical testing is one of the more engaging ways for scientists to contribute to the quality process. Pharmaceutical analysis is essential to the regulatory authorities' or the industry's statutory certification of drug compositions. In the business world, the quality assurance and quality control divisions are crucial to the development of a drug or dosage form that is both safe and efficient. The Food and Drug Administration (FDA) guidelines and current good manufacturing practises (CGMP) demand the implementation of reliable analytical techniques with higher sensitivity and repeatability. As a result, it's crucial to achieve selectivity, speed, low cost, simplicity, sensitivity, specificity, precision, and accuracy in drug estimation. This is due to the complexity of challenges encountered in pharmaceutical analysis.

KEY WORDS: *Analytical Techniques, Detection, Sitagliptin Ertugliflozin, Pharmaceutical analysis.*

INTRODUCTION

Pharmaceutical analysis is primarily utilised in the production of pharmaceutical chemicals. The pharmaceutical analyst is a key player in any research on the synthesis of novel chemicals. The analytical work that needs to be done includes everything from the standardised elemental analysis of organic molecules to the highly specialised chemical or instrumental functional group determination in complicated and medicinally significant compounds. The analyst's next responsibility is to provide techniques for process control for the analysis of the intermediates when a novel medicine has been created on a lab scale. Both the pilot plant stage and the full-scale production of the medicine must continue this process. The control division, which is organizationally separate from both

production and research, is present in most drug manufacturing companies. This team is in charge of certifying to the firm management that each manufactured lot of medications satisfies the relevant quality criteria prior to being made available for distribution.

One or more types of control actions (or a mix of both) are used to determine whether to release or reject a sample. Analytical information would be used to determine whether to release or reject the product if it is a single chemical entity with a high degree of purity that is amenable to precise chemical analysis. But many medicines are actually physical admixtures of a number of different ingredients, sometimes up to a dozen. In these situations, a thorough investigation after production typically either becomes impossible or unaffordable. The identification and purity of the raw materials and components are therefore verified by the central chemists. They personally verify that the components are charged into the blending vessel in the appropriate lots and that the blending is carried out in accordance with the formula card. Additionally, it is customary to add a particular analysis of the active ingredient to this batch certification, especially for patented medications.

SITAGLIPTIN

The molecular symbol for sitagliptin is (R).Four-oxo-4-[3- (trifluoromethyl) Dihydro-5,6 trazodone [pyrazin-7 (8H)-yl -1-(2,4,5-trifluorophenyl) Butan-2-amine has a molecular weight of 407.314 g/mol and the chemical formula C16H15F6N5O. It is used as an adjuvant in individuals with type-II diabetes mellitus in addition to diet and exercise to improve glycemic control. The dipeptidyl peptidase-4 (DPP-4) enzyme is competitively inhibited by sitagliptin. The GLP-1 and GIP incretins, hormones secreted in the gastrointestinal tract in response to meals, are broken down by the DPP-4 enzyme. Increase insulin secretion and decrease glucagon release by the pancreatic beta cells by suppressing GLP-1 and GIP activation. The body's blood glucose levels return to normal as a result of this procedure. In order to prevent a "overshoot" and subsequent low blood sugar levels as seen with some types of oral hypoglycemic medicines, the amount of insulin produced and inhibition of glucagon diminishes as blood glucose approaches normal level.

PHARMACODYNAMICS

Sitagliptin is a medication that can be used orally and belongs to the new class of DPP-4 inhibitors. The advantage of this medication is anticipated to be its reduced risk of hypoglycemia's negative effects in the management of blood glucose levels. The medication reduces the effects of a protein or enzyme (by inhibiting this protein or enzyme) on the pancreas at the level of glucagon release (reduces its release) and at the level of insulin release

(increases its synthesis and release) until blood sugar levels are restored towards normal, at which point the protein/enzyme-enzyme inhibitor becomes less effective and the amounts of insulin released diminish, reducing the "overshoot" of hypoglycemia seen in the drug

ROUTE OF ELIMINATION

With metabolism being a minor mechanism of elimination, sitagliptin is eliminated in the urine approximately 79% of the time unaltered. Within a week of oral sitagliptin treatment to healthy subjects, 100% of the radioactivity was excreted in the faeces (13%) or urine (87%) of the patients. Sitagliptin is largely eliminated by the kidneys, and this process requires active tubular secretion.

ERTUGLIFLOZIN:

Chemically, ertugliflozin is denoted as (1S, 2S, 3S, 4R, and 5S).5 [4-Chloro-3[4-ethoxybenzyl] phenyl (Hydroxymethyl) -1 - 6, 8- dioxabicyclo octane -2, 3, 4 - triol, having a molecular weight of 436.89 g/mol and the chemical formula C22H25ClO7. Less than 1% of the glucose in human blood is normally removed in the urine because it is filtered out of the blood for elimination and reabsorbed in the glomerulus. The sodium dependent glucose co-transporter, or SGLT, mediates the process of reabsorption of glucose. Type II diabetes is primarily responsible for 90% of the reabsorption of glucose. It blocks SGLT-2, and as a result, increases glucose excretion and lowers hyperglycemia without the need for insulin secretion. It advances the management of glycaemia in people with type II diabetes.

PHARMACODYNAMICS

Ertugliflozin administration increases the excretion of glucose in the urine, which results in a negative balance and osmotic diuresis. Therefore, it has been noted that this antidiabetic drug greatly lowers the body weight and blood pressure of diabetic patients.

ABSORPTION

Ertugliflozin is well absorbed and has an oral bioavailability of 70–90%, according to preclinical investigations. Tmax was observed between 0.5 and 1.5 hours after dosing. The Cmax and AUC after oral treatment appeared to be dosage proportionate. Following administration of 15 mg, Cmax and AUC values were 268 ng/ml and 1193 ng h/ml, respectively.

METABOLISM

Studies conducted in vitro revealed that the reactions of monohydroxylation, O-demethylation, and glucuronidation shape the metabolic profile of ertugliflozin in liver microsomes and hepatocytes. According to a proposed model, eight distinct metabolites present in urine, faeces, and plasma contribute to the metabolism of ertugliflozin. Ertugliflozin's unaltered form was discovered to make up the majority of the injected dose in plasma. Six other minor metabolites were also found in circulating plasma.

METHOD OF EXCLUSION

Ertugliflozin's overall recovery rate was 91%, and its distribution throughout the elimination pathway was 50% urine and 41% faeces. After the initial treatment, the administered dose recovered roughly 168 hours later. Urine elimination happened quite quickly, and after 24 hours, 80% of the dosage that was recovered in urine was obtained. The unmodified form of ertugliflozin was just a minor metabolite in the urine-eliminated dosage, which was made up of seven separate main metabolites. The percentage of the dose that was evacuated in faeces varied based on each patient's bowel movements, but 98.5% of the dose was eliminated 168 hours after the original dose. Ertugliflozin that had not been altered and three other minor metabolites made up the majority of this removed dose 50% of life. Ertugliflozin has a terminal elimination half-life of 11 to 17 hours.

CLEARANCE

Ertugliflozin has an apparent total plasma clearance rate of 178.7 ml/min following oral dosing, and total systemic plasma clearance after intravenous injection is reported to be 187.2 ml/min.

In this study, we present the development of RP-HPLC technique for the quantification of sitagliptin and ertugliflozin using Corona charged aerosol detector (CAD). CAD is an all-purpose, highly sensitive detector with a good dynamic range for sitagliptin and ertugliflozin quantification. It responds consistently to all analytes. The technique of producing charged aerosol particles, which were then detected by an electrometer, is known as the charged aerosol detector (CAD), and it is a universal detection system used to quantify the number of chemical compounds contained in a sample. This study employed RP-HPLC with a CAD detection system to accurately and sensitively quantify the medications.

There are currently no reports of the RP-HPLC-CAD technique for the very sensitive simultaneous quantification of sitagliptin and ertugliflozin, according to the literature. For the quantification of these medications by HPLC with UV and PDA, there were only a few analytical techniques with lower sensitivity; the linearity ranges for sitagliptin and ertugliflozin were 25-150 g/ml and 3.75- 22.5 g/ml, respectively. Therefore, the development of a very sensitive approach is required for the CAD-based assessment of both medicines. The current publication covers the creation and validation of a precise and sensitive RP- HPLC-CAD quantification method for the combined dosage formulation of sitagliptin and ertugliflozin.

A new RP-HPLC technique for the quantitative determination of ertugliflozin and sitagliptin was created and validated in accordance with ICH recommendations. The medication was administered into a standard Azilent column (150 4.6 5 m), kept at room temperature, and the effluent was observed at 240 nm.Buffer (Potassium di hydrogen ortho phosphate): Acetonitrile (70:30 V/V) made up the mobile phase. The flow was kept constant at 1 ml/min. The 3.75-22.5 g/ml and 25-150 g/ml calibration curves for ertugliflozin and sitagliptin, respectively, were linear (r2 for ertugliflozin = 0.9992, r2 for sitagliptin = 0.9995). Ertugliflozin and Sitagliptin had retention times of 3.203 and 2.106 minutes, respectively. For both medicines, accuracy was in the range of 99.67-99.90%. Ertugliflozin and Sitagliptin's respective precision values were 0.1% and 0.2%; their respective LOD and LOQ values are 0.43 and 1.31 ng/ml and 0.74 and 2.24 ng/ml, respectively. The suggested approach was suitable for determining ertugliflozin and sitagliptin in bulk and pharmaceutical dose forms and was sensitive, repeatable, accurate, and exact. Drug content when used for tablet analysis was 99.18–99.13% of labelled substance. Studies on forced degradation demonstrated the method's applicability for stability investigations. A straightforward, accurate, and exact capillary zone electrophoresis method was created for the measurement of zolpidem tartrate in tablet dosage form. Separation was carried out utilising hydrodynamic injection for 10 s in normal polarity mode at 25 °C and 22 kV. A background electrolyte of 20 mM disodium hydrogen phosphate that had been pH-adjusted with phosphoric acid (85%) and detected at 254 nm was used to achieve separation. Complete determination utilising amiloride HCl as the internal standard took less than 3 minutes under the aforementioned optimised conditions. The approach had a correlation coefficient of 0.9999 and was linear over the range of 3-1000 g mL-1. For the forced degradation studies, zolpidem tartrate standard and pharmaceutical sample solutions were subjected to neutral (water), basic (0.1 M NaOH), acidic (0.1 M HCl), oxidative (10% H2O2), thermal (60°C in oven for 3 days), and photolytic (exposure to UV light at 254 nm for 2 hours) forced degradation conditions. The test can be regarded as stability-indicating because the stress studies' degradation products did not obstruct the detection of zolpidem tartrate.

A straightforward, exact, accurate, affordable, and trustworthy UV spectrophotometric approach was published. for the determination of sitagliptin phosphate in tablet dose form. The medication exhibits maximal absorption in water at 267 nm and follows Beer's law with an excellent correlation value (R2 = 0.9995) in the concentration range of 2 to 10 g/mL. Recovery studies supported the analysis's findings. The recovery ranged between 99.53 and 100.41. In every instance, the relative standard deviation was discovered to be 2.0%. The proposed spectrophotometric technique was approved in accordance with ICH Q2 (R1) recommendations. The suggested method can be used for routine analysis of pharmaceutical formulations as well as the accurate measurement of sitagliptin in bulk form.

On ten different chiral stationary phases, created a direct chiral separation method for sitagliptin and its (S)-enantiomer. The Chirobiotic R (Ristocetin A), Chirobiotic V (Vancomycin), Chirobiotic T (Teicoplanin), Chirobiotic TAG (Teicoplanin aglycone), OJ-H, and OJ-RH belonging to tris (4-methylbenzoate) of cellulose were among the chiral stationary phases that were examined. On polysaccharide-based CSP columns, such as Chiralpak IA-3, Chiralpak IC-3, which represent the tris-(3,5-dimethylphenyl carbamate) of amylose, tris-(3,5-dichlorophenyl carbamate), of cellulose, and OD-H (tris-3,5-dimethylphenylcarbamate), respectively, chiral selectivity was observed. On a cellulose tris-(3,5-dichlorophenyl carbamate) column (Chiralpak IC-3), better enantioselective separation has been attained utilising IPA and n-hexane as the mobile phases, both of which contain 0.05% ethylene diamine, and a flow rate of 0.5 mL/min. The column was kept at 35°C and the detection was done at 266nm using a PDA detector. The method's precision, accuracy, linearity, and robustness were all validated. The method's benefits include quick equilibration and decreased solvent use as a result of the short column length. Due to the small particle size of 3 m, there is effective enantio separation (Resolution 3.38). Therefore, sitagliptin and its (S)-enantiomer can be determined to be chiral pure using this method.

A selective HPLC approach was created by Shang et al. to determine the examined pharmaceuticals in either single or combination form. For the separation and determination of sitagliptin phosphate, metformin hydrochloride, and atorvastatin calcium in pure form and in pharmaceutical formulations with quetiapine as an internal standard, the approach is quick, precise, accurate, and specific. The three medicines are separated using HPLC on a HyperSil GOL column (150x4.6mm, 5%) in this procedure. The buffer (containing 1% of concentrated nitric acid solution at 65% and 2% of concentrated ammonia solution at 28-32%, pH 8.5) and methanol in the ratio 30:70 was combined to create the mobile phase-A. With isocratic elution and UV detection at 254 nm, the flow rate is 1 ml/min. The calibration plots' data from linear regression analysis revealed a solid linear connection for the concentration ranges of atorvastatin (0.3125-10 g/ml), metformin (0.625-25 g/ml), and

sitagliptin (3.125-100 g/ml). For sitagliptin, the mean values of the correlation coefficient, slope, and intercept were 0.9976, 96.92, and +274.21; for metformin, they were 0.9995, 218.82, and +14.97; and for atorvastatin, they were 0.9994, 828.87, and +81.95. The technique was approved in accordance with ICH standards. For sitagliptin, the limit of detection (LOD) and limit of quantification (LOQ) were respectively 0.82 and 2.46 g/ml, 0.4 and 1.2 g/ml, and 0.09 and 0.27 g/ml. An accurate, straightforward, novel, precise, robust, and stability indicating approach for the simultaneous determination of sitagliptin and metformin HCl in tablets was devised. The system that was created was quick and efficient. Using ammonium acetate buffer (pH 5.0 adjusted with glacial acetic acid): MeOH (60:40 v/v), the chromatographic separation was accomplished isocratically on a C-18 column. As an ion pair agent, sodium salt of octane-1-sulfonic acid was utilised. Sitagliptin and metformin were detected with a flow rate of 1 mL/min using different UV wavelengths (265 nm and 225 nm, respectively). Metformin and sitagliptin have respective retention durations of 2.398 and 17.113 minutes. The devised technique was precise and effectively isolated from metformin and sitagliptin contaminants. Both metformin and sitaglitpin are linear in the manner from 50% to 150%. For sitagliptin and metformin, the correlation coefficient was found to be r2= 0.9997 and 0.9998, respectively. Both the standard and test solutions demonstrated 48-hour stability. A forced deterioration investigation revealed that the strategy indicates stability. Sitaglitpin and metformin fixed dose combinations can be routinely analysed using the described approach.

MATERIALS AND MTHODS

REAGENTS AND CHEMICALS

All of the solvents for HPLC were purchased from Sigma-Aldrich in St. Louis, Missouri, USA. From the Milli-Q system (Millipore, Billerica, MA), HPLC-grade water was collected.The gift samples used as the ertugliflozin and sitagliptin reference standards were provided by MSN labs in Hyderabad, India. Purchased from a neighbourhood pharmacy, the marketed medication, Steglujan pills, contains ertugliflozin and sitagliptin (15mg/100mg). All additional analytical-grade chemicals were purchased from Qualigens Chemicals in Mumbai, India.

CHROMATOGRAPHIC SYSTEM AND EQUIPMENT

Thermo(ESA-corona) CAD (charged aerosol detector) detector with a Waters-2590 series LC system. Sigma-200 electronic balance, PCi-3.5L sonicator, Universal-hot air oven, and Uni-lab digital pH metre were the tools used in the experiment.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Drugs were separated using a liquid chromatographic system with a phenomenex C18column (250 x 4.6 mm, 5 m) and a mobile phase made up of a 20:40:40%V/V combination of methanol, acetonitrile, and phosphate buffer (pH 5.8). The chromatographic system used a flow rate of 1 ml/min with corona charged aerosol detection to elute the medicines. Both sitagliptin and ertugliflozin had retention times of 4.2 and 2.4 minutes, respectively.

PH-5.8 PHOSPHATE BUFFER PREPARATION

Transfer the 8.5 ml of 1 M K2HPO4 and 91.5 ml of 1 M KH2PO4 combination into a 1000 ml volumetric flask, add the necessary amount of HPLC grade water, and sonicate the resultant solution for 10 minutes.

MAKING READY FOR MOBILE PHASE

Methanol, acetonitrile, and phosphate buffer (pH-5.7) are combined in a ratio of 20:40:40%V/V to create the mobile phase. By vacuum filtration through a 0.45-micron nylon filter and sonication, the resulting mobile phase was degasified.

STANDARD STOCK SOLUTION PREPARATION

Sitagliptin and ertugliflozin were produced as standard stock methanolic solutions in quantities of 1 mg/ml each. Processing involved adding 100 mg each of sitagliptin and ertugliflozin into separate 100 ml volumetric flasks containing 40 ml of methanol, followed by 5 minutes of sonication. Methanol was used to create the final volume. The treated stocks were stored between 2 and 8 °C.

SOLUTION SAMPLE PREPARATION

Each medicine under study had ten pills, which were carefully weighed, transferred to a dry, clean mortar, and then ground into a fine powder. 100 mg of sitagliptin and 15 mg of ertugliflozin powder were put into a separate 100 ml volumetric flask, 40 ml of methanol was added, and the mixture was sonicated for 10 minutes before being diluted to the required volume with methanol. The resulting mixture was filtered through a nylon filter membrane with a pore size of 0.45 m.

RESULTS AND DISCUSSIONS

OPTIMIZATION OF RP-HPLC-CAD METHOD

Both medications were subjected to chromatographic processes utilising several mobile phases with various pH values, columns, and flow rates. With adjustments in the mobile phase, columns, flow rate, and pH, it was possible to observe variations in drug retention periods, selectivity, and sensitivity. Initially, several ratios of acetonitrile to water were utilised, but poor peak separation was noticed. Next, different ratios of methanol to water and methanol to buffer at various pH levels were tested, but low sensitivity and peak splitting were noticed.Later, buffers with various pH levels and methanol were tested. The best results were obtained using a mixture of 20:40:40%V/V methanol, acetonitrile, and phosphate buffer (pH 5.8) at a flow rate of 1ml/min on a phenomenex C18-column (250x 4.6mm, 5).

APPROACH VALIDATION

The enhanced RP-HPLC-CAD method was validated in accordance with the ICH validation criteria.

PRECISION

System precision and intermediate precision supported the technique precision. System precision is used to assess the health of the HPLC system. Six standard solutions were infused into the system to establish system precision, and then the peak response was used to calculate the percent RSD. Three distinct concentrations on various days were examined to assess intermediate precision, and the % RSD values were calculated based on the results.

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	NA	More than 2
LOD	0.040	0.082
LOQ	0.120 µg/ml	0.247 µg/ml

TABLE -2: PRECISION STUDY OF SITAGLIPTIN AND ERTUGLIFLOZIN

Drugs	ncentration(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

ACCURACY

By examining recovery at three different concentrations of sitagliptin and ertugliflozin using triplicate analysis (n=3), the accuracy of the developed approach was evaluated. The findings from the accuracy assessment were expressed as a percentage recovery and were displayed in Table.

TABLE-3: ACCURACY OF SITAGLIPTIN AND ERTUGLIFLOZIN

%Recovery	Statistical Analysis		%Recovery	Statistical Analysis	
Ertugliflozin (0.45µg/ml)			**Sitagliptin (3µg/ml)**		
98.00	Mean	99.33	97.80	Mean	97.60
101.00	SD	1.247	99.00	SD	1.2328
99.00	%RSD	1.255	96.00	%RSD	1.2622
Ertugliflozin (0.9µg/ml)			**Sitagliptin (6µg/ml)**		
96.00	Mean	97.88	99.00	Mean	99.08
99.00	SD	1.339	99.76	SD	0.5180

98.65	%RSD	1.386	98.50	%RSD	0.5227
Ertugliflozin (1.35µg/ml)			**Sitagliptin (9µg/ml)**		
96.00	Mean	97.33	98.00	Mean	98.08
97.00	SD	1.247	97.75	SD	0.3118
99.00	%RSD	1.281	98.50	%RSD	0.3180

LIMIT OF DETECTION (LOQ) AND LIMIT OF QUANTIFICATION (LOQ)

On the basis of the standard calibration curve, these were each independently determined. LOD and LOQ were calculated using the residual standard deviation of the regression line or the standard deviation of the y-intercepts of regression lines. The following formulae were used to determine the suggested technique's sensitivity in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ).

LINEARITY

By injecting six replicates of a standard solution comprising pharmaceuticals at concentrations between 0.0625 and 2.5 g/ml for ertugliflozin and 0.3125 to 10 g/ml for sitagliptin into the chromatographic apparatus in triplicate (n = 3), linearity of the method was examined. The linearity graph was plotted for the drug peak area versus the corresponding concentrations. During the validation process, an average correlation coefficient (R2) value of >0.998 was discovered.

The chromatograms showing the complete separation of sitagliptin and ertugliflozin were used to assess the method's specificity. The resolution for each peak was greater than 2, as indicated in Table, and the peak responses were all clearly separated from one another and had good baselines.

ROBUSTNESS

The proposed HPLC method's robustness was evaluated by its capacity to stay unaffected by minor modifications to the experimental setup. Chromatographic resolution is unaffected by modest variations in flow rate (0.1 ml) and mobile phase organic strength (1 ml).

TABLE 4: ROBUSTNESS OF ERTUGLIFLOZIN AND SITAGLIPTIN

Parameter	Variation	% RSD	
		Ertugliflozin	Sitagliptin
Robustness	i. Change in flow rate (± 0.1 ml/min)	0.92	0.45
	ii. Change in mobile phase (± 1ml)	0.56	0.86

APPLICATION OF THE METHOD

By infusing sample solution into LC system under optimal chromatographic conditions, the dosage of Steglujan-marketed tablets was calculated. Each pill of Steglujan includes 100 mg of sitagliptin and 15 mg of ertugliflozin. The calibration curve approach was used to calculate the amount of medicines included in the formulation.

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	15mg	14.96±0.620	0.626	99.79
Sitagliptin	100 mg	99.75±0.154	0.613	99.75

CONCLUSION

For the successful simultaneous analysis of sitagliptin and ertugliflozin with good resolution value between the medicines, a straightforward and sensitive RP-HPLC-CAD technique was devised. When compared to the currently available approaches, the CAD detection system was employed in this study to deliver high degree of sensitivity to analytical method for the detection of sitagliptin and ertugliflozin. In the concentration range of 0.3125-10 g/ml and 0.0625-2.5 g/ml, respectively, sitagliptin and ertugliflozin were linear. For sitagliptin and ertugliflozin, respectively, the average values of the correlation coefficient, slope, and intercept were 0.9998, 8688.2, and 1977.6. With no more than 2% RSD values, the analytical approach exhibits a high degree of

precision and accuracy. The current analytical technique proved straightforward, quick, accurate, and exact. It may be used to routinely quantify the two medications in bulk as well as in formulations like capsules, tablets, and powders.

A quick and accurate RP-HPLC-CAD technique for the simultaneous analysis of sitagliptin and ertugliflozin with good resolution value between the medications has been developed in Chapter 4. When compared to the currently available approaches, the CAD detection system was employed in this study to deliver high degree of sensitivity to analytical method for the detection of sitagliptin and ertugliflozin. In the concentration range of 0.3125-10 g/ml and 0.0625-2.5 g/ml, respectively, sitagliptin and ertugliflozin were linear. For sitagliptin and ertugliflozin, respectively, the average values of the correlation coefficient, slope, and intercept were 0.9998, 8688.2, and 1977.6. With no more than 2% RSD values, the analytical approach exhibits a high degree of precision and accuracy. The current analytical technique proved straightforward, quick, accurate, and exact. It may be used to routinely quantify the two medications in bulk as well as in formulations like capsules, tablets, and powders.

REFERENCES

1. Kowalczuk D. Simultaneous high-performance thin-layer chromatography densitometric assay of trandolapril and verapamil in the combination preparation, Journal of AOAC International **(2005),** 88 (5): 1525–1529.
2. Gibbons J.A., Ouatas T., Krauwinkel W., Ohtsu Y., Vanderwalt J.S., Beddo V., Devries M., Mordenti J. Clinical pharmacokinetic studies of enzalutamide. Clin Pharmacokinet**. (2015),** 54 (10):1043–1055.
3. Kim T.H., Jeong J.W., Song J.H., Lee K.R., Ahn S, Ahn S.H., Kim S., Koo T.S.,Pharmacokinetics of enzalutamide, an anti-prostate cancer drug, in rats. Archives of Pharmacal Research. **(2015),** 38 (11): 2076–2082.
4. Benoist G.E., Hendriks R.J., Mulders P.F., Gerritsen W.R., Somford D.M., Schalken J.A., Vanoort I.M., Burger D.M., Vanerp N.P., Pharmacokinetic aspects of the two novel oral drugs used for metastatic castration-resistant prostate cancer: Abiraterone acetate and enzalutamide. ClinPharmacokinet. **(2016),** 55 (11): 1369–1380.
5. Saad F., Heinrich D., New Therapeutic Options for Castration-resistant Prostate Cancer. The Journal of Oncopathology. **(2013),** 1 (4): 23–32.
6. Scher H.I., Fizazi K., Saad F., Increased survival with enzalutamide in prostate cancer after chemotherapy. The New England Journal of Medicine. **(2012),**367 (13):1187–1197.

7. Emmanuel S.Antonarakis., Enzalutamide: the emperor of all anti- androgens. Translational Andrology and Urology. **(2013),**2 (2): 119–120.
8. Payton S., Prostate cancer: enzalutamide impresses in European studies. Nature Reviews. Urology. **(2014),** 11(5): 243.
9. Tombal B,, Borre M., Long-term Efficacy and Safety of EnzalutamideMonotherapy in Hormone-naïve Prostate Cancer: 1- and 2-Year Open-label Follow-up Results. European Urology. **(2015),** 68 (5): 787–794.
10. Neraj Kumar Saini, Suresh P. Sulochana, Mohd Zainuddin, Ramesh Mullangi , Development and validation of a novel method for simultaneous quantificationof enzalutamide, darolutamide and their active metabolites in mice dried blood spots using LC-MS/MS: application to pharmacokinetic study in mice. ADMET& DMPK. **(2018),** 6(3):242-257.
11. Dharmendra J Prajapati1, Usmangani K. Chhalotiya1, Minesh D. Prajapati1, Jalpa U. Patel1 and Harsha V. Patel. Quantification of newer anticancer drug enzalutamide by RP-LC method and UV-visible spectroscopic method in bulk and synthetic mixture.J Mol Oncol Res.. **(2017),**1(2):65-73.
12. Benoist M., Meulen E.V., Vanoort I., Development and validation of abioanalytical assay on LC/MS/MS to quantify enzalutamide and N- desmethylenzalutamide in human plasma. Journal of clinical oncology. **(2017),**34:330.
13. Puszkiel A., Ple A., Huillard O., Noe G., A simple HPLC-UV method for quantification of enzalutamide and its active metabolite N- desmethylenzalutamide in patients with metastatic castration-resistant prostate cancer. J Chromatogr B AnalytTEchnol Biomed Life Sci.. **(2017),** 15(1058):102-107.
14. Hamdana H., BaniJaber A.K., Abushoffa A.M., 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.
15. Zhai H., Wu Y., Huang B., Huang Q., Yang B., Chen Z., Rapid determination of ertugliflozin hydrochloride in ertugliflozin hydrochloride tablets by capillary electrophoresis. HuaxneYanjiu Yu Yingvong. **(2008),** 20 (7): 923- 926.
16. Polagani S. R., Pilli N. R., Gagula R., Gandu V., Simultaneous determination of atorvastatin, ertugliflozin and glimpiride in human plasma by LC- MS/MS andits application to human pharmacokinetic study. Journal of Pharmaceutical Analysis **(2013),** 3:9-19.