Review Article

A Systematic Review on Analytical Method Development and Validation of Antidiabetic Drug by HPLC-MS Method

Deepika Dhomse[1], Swapnali R. Patil[2], Harshal Chavan[3], Ragini Kamlesh Kadvekar[4], Supriya Ravindra Bhamare[5]

[1,2,3]Assistant Professor, Sir Dr. M.S. Gosavi College of Pharmaceutical Education and Research, College Rd, Krishi Nagar, Nashik, Maharashtra, India.

[4,5]Assistant Professor, Department of Quality Assurance, Matoshri College of Pharmacy, Eklahare, Near Odhagaon Aurangabad Highway, Nashik, Maharashtra, India.

***Corresponding author's E-mail:** wdeepika.05@gmail.com

Received: 09-02-2026; **Revised:** 27-04-2026; **Accepted:** 04-04-2026; **Published online:** 20-05-2026.

ABSTRACT

The critical and comprehensive validation of bioanalytical approaches to the simultaneous quantitative measurement of antidiabetic drug combinations in human plasma was done later. Drugs were analysed from different methods of HPLC. The current review reports a developed HPLC technique and has been utilized to develop and validate a number of anti-diabetic drugs. The full information of development and validation of analysing chromatographic techniques such as a column / separation technique, Mobile phase or Solvents, Accuracy, Precision, Recovery and Internal standard is present. This review has been called research gold by many researchers because in this review highly sensitive biosensors will be reviewed extensively and critically that will be able to detect a precise quantity of antidiabetic drugs in human blood and urine. A comprehensive and careful analysis was performed on bioanalytical methods that were shown to be useful in measuring antidiabetic drugs combinations in human blood. This article was aimed at discussing the correctness of bioanalytical procedures and the quality of the reported results. International standards were used to evaluate the validation assays. Problems in the verification process are brought to light and elaborated on to assist the researchers. Select processes that are capable of being utilized effectively in their potential running and that are truly dependable. Due to this fact that these are among the most studied oral antidiabetic drugs, a combination of them was applied. In the literature, there are various medications and techniques. Also, this paper may be regarded as the validation process of bioanalytical methods.

Keywords: Method validation, bioanalytical procedures, diabetes medications, systematic.

INTRODUCTION

An extensive review of the existing literature was carried out to determine validated analytical methods to determine oral antidiabetic combination therapy in human blood. The systematic review obtained the inclusion and evaluation of all the relevant literature related to the subject matter. There were no such limits as to the nature of the method of analysis and the time of publication. Keywords such as the antidiabetic and antidiabetic along with the exact name of drugs like insulin, liraglutide, semaglutide, exenatide, metformin, glimepiride, gliclazide, chlorpropamide and tolbutamide (pu), tofogliflozin (purity 100%) canagliflozin (98%), ipragliflozin were included in this search approach to identify the literature; validation quantification therapeutics monitoring plasma blood serum human plasma human blood human serum and human urine words with different names METHODS OF analysis were also entered for comprehensive data base search.

All the data related to validation parameters such as selectivity, linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) matrix effects, carryover stabilities robustness recovery and system suitability were documented systematically. The above validation characteristics were evaluated critically on the backdrop of commonly accepted regulatory guidance on bioanalytical method validation guidelines such as the Guidance for Industry: Bioanalytical Method Validation issued by the U.S. Food and Drug Administration (FDA) and corresponding guidelines by European Medicines Agency (EMA).

Classification of diabetes medication drugs:

1) Insulins and their preparations.
2) GLP-1 receptor agonists: Albiglutide, Dulaglutide, Exenatide, Liraglutide and Semaglutide.
3) Amylin analogs: Pramlintide
4) Biguanides: Metformin
5) Sulfonylureas: Glimepiride, Gliclazide, Glyburide, Chlorpropamide, Tolazamide and Tolbutamide.
6) Megalitinides: Nateglinide and Repaglinide.
7) ADH: A way to remember the names of common SGLT-2 inhibitor from SLGT and AD as follows : SGLT - 2.
8) DPP-4 inhibitors: linagliptin, sitagliptan, saxagliptin and alogliptin.
9) Alpha- glucosidase inhibitors: Acarbose, Miglitol and Voglibose.
10) Thiazolidinedione: Pioglitazone and Rosiglitazone.

MATERIAL AND METHODS

In this review the parameters are classified in terms of classification of Antidiabetic drugs.

1. Insulin (2020):[1]

Zhai et al.[1] reported the construction and verification of the analytical method based on LC-MS to use in quantifying both liraglutide and insulin in rat plasma simultaneously. A protein precipitation method was used to process plasma samples which were then separation chromatographically on an Inert Sustain Bio C18 column. The mobile phase involved the use of gradient elution with a mixture of water, acetonitrile, and formic acid and Acetone and formic acid in the necessary volumetric proportions.

The technique exhibited an approved linear range of 1. 0 – 500 ng/mL of both analytes. The recovery of extraction was about 41.849.2 percent with liraglutide and 69.7 percent with insulin. Intra and inter-day precision of liraglutide was 3.5-9.4 and 8.4-9.8, respectively, whereas the accuracy was -12.6 to -1.3. In case of insulin, the intra and inter-day variability was between 5.2 and 13.6% and 11.8 to 19.1% respectively with an error range of -3.0 to 9.9. The verified procedure was effectively used in pharmacokinetic studies.

2) Liraglutide (2018)[2]:

A quantitative method allowing the determination of liraglutide in rat plasma by LC CMS, using human insulin as a component of an internal standard, was developed and validated. The procedure included a simple precipitation of the proteins by any two-dimensional precipitation procedure, then the chromatographic separation of the proteins was achieved with an InertSustain Bio C18 column. The mobile phase was made of acetonitrile and water with respective concentration of 0.1% formic acid.

Multi reaction monitoring (MRM) mode electrospray ionization was used to carry out detection. The procedure had excellent linearity, covering the concentration range of 0.5 to 250 ng/mL with correlation coefficients (r 2) more than 0.99. The intra intra-day and inter-day precision were 1.97763 and 5.2511.9 percent respectively. The accuracy (relative error) was between -8.79 to 11.4. Manufacturing matrices and recovery were also found to be acceptable. This technique was successfully used in the pharmacokinetic analysis of liraglutide after subcutaneous injection of the rats.

3) Semaglutide (2023)[3]:

The semaglutide concentration in biological samples was developed using a bioanalytical method that is based on an LCMSMS. Plasma samples were subjected to a simple protein precipitation method with methanol and the samples of the brain tissue were treated with the help of solid-phase extraction. The internal standard used was liraglutide. Gradient elution was performed in mobile phases of 0.1% formic acid in water followed by acetone, which was used to separate the chromatography.

The procedure showed a lower limit of quantification (LLOQ) of 0.5 ng/mL of plasma and brain samples. Intra- and inter-day accuracy ranged from 89.20% to 109.50% in plasma and 92.00% to 105.00% in brain tissue, with precision values of 8.92% and 7.94%, respectively. The Pharmacokinetic analysis involved Sprague Dawley rats as research subjects after they were given intravenous (0.02 mg/kg) and subcutaneous (0.1 and 0.2mg/kg) dosage. Plasma concentration profiles were exponential with an average half-life of between 7.22 and 9.26 hours. Semaglutide was reported to have a subcutaneous bioavailability ranging between 76.65 and 82.85. It was established that the brain-to-plasma modal of partition (Kp) is less than 0.01, which is a poor brain penetration factor.

4) Extended (2020)[4]:

Sauter et al. developed a very sensitive UPLCMS / MS / MS technique to quantify exenatide in plasma. The isolate was done through solid-phase extraction of the analyte using the anion-exchange sorbents. The detection was done by electrospray ionization with tandem mass spectrometry in the selected reaction monitoring mode.

Assay had a lower limit of quantification (LLOQ) of 5 pg/mL (1.2 pM) with a plasma volume of 200 µL. The range of the calibration was 5-10000 pg/mL and the correlation coefficient was higher than 0.99. The intra- and inter-day accuracy was between 97.5 percent and 105.4 percent with a value of precision less than 10.9. The accuracy was scored between 93.0-102.5 at the LLOQ level with the precision of 15.9. The technique also showed good performance of diluted samples making them able to be quantified to 100, 000 pg/mL. Its relevance was established by conducting pharmacokinetic studies by measuring plasma concentration of exenatide in intravenous and intranasal administration of beagle dogs.

5) Metformin(2019):

El-Zaher et al.[5]used bioanalytical LC-MS/MS to simultaneously measure the concentration of dapagliflozin, saxagliptin, and metformin in human plasma. The separation was done by chromatography with a Zorbax C18 column (50 × 4.6 mm, 5 µm) with a mobile phase of acetonitrile and 0.1% formic acid (45:55, v/v). The detection was done by electrospray ionization (ESI) in positive ion mode.

In order to accommodate the disparity in the physical character of the analytes, the method was used as a combination of liquid–liquid extraction and protein precipitation. The experiment revealed that the method was well linear over the concentration range of 5-500ng/mL in dapagliftozin, 2-50ng/mL in saxagliptin, and 10400ng/mL metformin respectively. Inter-day precision Coefficients of variation%. 8.27 9.77 7.60 Intra-day accuracy Accuracy [Range from lowest to highest values] 101.46–105.18 97.23–105.75 98.82–107.52.The methodology is appropriate to use in the future in pharmacokinetic research.

6) Glimipiride (2007):

Rabbaa-Khabbaz et al.[6]developed a simple and sensitive high-performance liquid chromatography (HPLC) assay that was used in measuring glimepiride in human serum. The sample preparation entailed a one-step liquid to liquid extraction with acidified serum with dichloromethane. Glibenclamide was utilized as a standard. The detection was done at 228 nm, and it was able to quantify glimepiride below 10 ng/mL.

This procedure indicated a good linearity to 10-1000 -range of concentrations. The reliability of the method was established by validation studies which involved precision, accuracy, and stability studies. It was effectively used in the analysis of serum samples taken out of 41 Lebanese males who had a single oral dose of glimepiride of 3 mg. The approach was appropriate to be used in pharmacokinetic, pharmacodynamic, and bioavailability/ bioequivalence research.

7) Glimipiride (2010):

Samala et al.[7]came up with a simple and sensitive reverse-phase high-performance liquid chromatography (RP-HPLC) technique to quantify glimepiride in the rat serum. The extraction was performed using a one-step liquid-liquid method that used a single extra component of the system, which was methanol, and the internal standard that was gliclazide.

The chromatographic separation took place in a C18 column using a mobile phase that consisted of 10 mM phosphate buffer (80:20, v/v) using a mix of methanol and phosphate buffer, with a pH that was adjusted to pH 3.0 using orthophosphoric acid. Flow rate was kept at 1.0 mL/min and ultraviolet spectroscopy was used to detect it at wavelength of 230 nm. The retention times of glimepiride and gliclazide were estimated to be 5.5 and 4.0 minutes respectively with 100 percent separation realized within 10 minutes.

This method was linear and has satisfactory linearity within the 0.5 0500 µg/mL concentration, with good accuracy and validation parameters. It was actually used to analyse serum samples in pharmacodynamics, pharmacokinetic and bioavailability/bioequivalence studies proving to be fit in more pharmacodynamics analysis.

8) Chlorpropamide (2020):

Lam et al.[8]An Agilent Zorbax Eclipse XDB C8 column (inner diameter 150 mm × 4.6 mm, particle size 5 µm) was used to separate chromatographically on Agilent 1200 series, LC system "Santa Clara, CA, USA. The mobile phase is made of 2 mM ammonium format and 0.2% formic acid in purified water (mobile phase A, MPA) and 2 mM ammonium format and 0.2% formic acid in ACN (mobile phase B, MPB). LOD of chlorpropamide ranges between LOD 200-500 ng/mL. This method has 1 hundred percent reproducibility and repeatability. Relative standard deviation on the retention time is below 0.5 percent.

9) Toulbutamide (2010):

Zhang et al.[9]An effective and sensitive liquid chromatographic mass spectrometry (LC-MS) technique of measuring tolbutamide and its metabolite, hydroxy tolbutamide in the plasma of a rat has been established and validated. The liquid-liquid extraction of the omeprazole analyte and internal standard in plasma was followed by chromatographic separation by the use of the Zorbax SB-C18 column (2.1 mm 50 mm, 3.5 ml) through gradient elution where the mobile agent phase was a solution of acetonitrile and formic acid 0.1%. Quantification of Tolbutamide and its metabolite hydroxy tolbutamide was done in a SIM mode using an electron spray ionization (ESI) source in positive ion mode. Linearity 10- 20,000 ng=mL tolbutamide and hydroxy tolbutamide; LLOQ 10 ng=mL and 5 ng=mL, respectively. The accuracy of the dates is 87.8-109.6 with less than 12 between the dates and less than 12 points between the dates. The method developed has been effectively used to determine tolbutamide and its metabolite hydroxy tolbutamide in rat plasma to conduct pharmacokinetics studies.

10) Tofogliflozin (2016)[10]:

Japanese Journal of Medicine 2005, 51, pp. 1017-1018.

Kobuchi et al.[10]developed a bioanalytical LC-MS/MS technique to measure tofogliflozin, tolbutamide and hydroxy tolbutamide as metabolite of tolbutamide in biological samples. The Quicksorb ODS column (2.1 mm 150 mm, 5 mm) was used as the stationary phase in chromatography with an isocratic flow of the mobile phase being acetonitrile and 10 mM ammonium acetate (50:50, v/v), with a flow of 0.2 mL/min could be used. This run time was approximately 4 minutes.

The technique had shown a lower limit of quantification (LLOQ) of 0.5 ng/mL of tofogliflozin and with good specificity, precision, and accuracy. Intra-day and inter-day performance was measured by use of replicate quality control (QC) samples where validation was done. Five replicates were determined within one day as intra-day precision whereas inter-day precision was conducted over three days with several replicates. The technique was found to be acceptable in terms of accuracy and precision (less than 15 per cent).

In the case of tolbutamide and its metabolite hydroxy tolbutamide, the calibration curves were linear over the concentration of 5 to 800 ng/mL. Tolbutamide and hydroxy tolbutamide were found to have an LLOQ of 10 ng/mL and 5 ng/mL respectively. It ranged between 88.8% and 109.7 in terms of accuracy, and the values of precision were lower than 12. The developed technique was also effectively used to determine tolbutamide and hydroxy tolbutamide in rat plasma which justified its use in pharmacokinetic research.

11) Canagliflozin (2016):

Kobuchi et al.[11]created a validated LC-MS/MS procedure of quantitative determination of canagliflozin in low-volume rat plasma (0.1 mL) and used it successfully in

pharmacokinetic research. Tert-butyl methyl ether liquid liquid extraction was interred as a sample preparation. A chromatographic separation was realized on a Quicksorb ODS column (2.1 mm 150 mm, 5 µm) of mobile phase, which was acetonitrile and 0.1% formic acid (90:10, v/v) at a flow rate of 0.2 mL/min.

An API 3200 triple quadrupole mass spectrometer conducting in positive electrospray ionization mode was used to detect the bacteria products of the bioreactor. The quantification was carried out on the measurements of the reaction monitoring transitions m/z 462.0 191.0 and the internal standard m/z 451.2 71.0 of canagliflozin and empagliflozin, respectively.

The approach exhibited a reasonable level of specificity, precision and accuracy, that is per the requirements of validation. Canagliflozin was observed to stand up to the conditions of analysis applied. Intra and inter-day precisions (CV% were 6.9 percent and 9.4 percent respectively) and accuracy were between 91.1 percent and 106.1 percent respectively. The proven procedure was effectively employed in the pharmacokinetic analysis of canagliflozin on rat plasma.

12) Canagliflozin, dapagliflozin empagliflozin (2020)[12]:

We describe the simple and Powerful parallel liquid chromatography and mass spectrometry (LC-MS/MS) techniques of simultaneous measurement of the three SGLT2i (canagliflozin, dapagliflozin and empagliflozin) in human serum and urine and a 1-minute run time using methanol as one protein precipitate. Separation was done by chromatography using a Waters equipment. ACQUITY UPLC HSS T3 1.8m; 2.1 x 50 mm column using Waters ACQUITY UPLC Front column Vanguard HSS T3 1.8m; 2.1 x 5 mm, by gradient elution using ammonium's.

Acetate (4mole/L, pH 5) and acetone as a mobile phase with a flow rate of 0.8 ml/min Observe 200 7hg/L, 1 -500mu g/L linear. Canagliflozin, dapagliflozin, empagliflozin and corresponding stable isotopes are labelled internal standard (IS) [13C6]-canagliflozin, [13C6]- dapagliflozin and [13C6]- empagliflozin was used.

13) Ipragliflozin (2015):

Kobuchi et al.[13]developed a rapid and sensitive LC-MS/MS method for the quantitative determination of ipragliflozin in the rat plasma and later used in the pharmacokinetic analysis of the ipragliftozin in the animal models. The internal standard was empagliflozin and the extraction of the sample was performed by using liquid-liquid extraction with tert-butyl methyl ether.

Separations were accomplished on a Quicksorb ODS column (2.1 mm × 150 mm, 5 mm) using 0.1% formic acid and acetonitrile (90:10, v/v) as a mobile phase and a flow rate of 0.2 mL/min. Detection was done on an API 3200 triple quadrupole mass spectrometer operating in positive elect The transition of m/z 422.0 into 151.0 with ipragliflozin and m/z 451.2 into 71.0 with the internal standard were monitored.

Within bioanalytical validation criteria, the method showed good results with regard to accuracy and precision of within 15 percent. This confirmed technique was successfully used to pharmacokinetic trials of ipragliflozin in rats and may be efficiently applied to assess novel SGLT2 inhibitors with animal models.

14) Sitagliptin (2020):

Loh et al.[14]A quick, basic, delicate and repeatable LC system based on an oral dosage of sitagliptin was created. The internal standard was the use of diphenhydramine hydrochloride. The Agilent poroshell 120 ec-C18 Fast LC column (100 2.1 mm, 2.7 µm) was used to carry out the chromatographic separation accompanied by a UHPLC guard column of the identical stationary phase.

The mobile phase was composed of 0.1% formic acid and methanol (45:55, v/v); it was applied at the flow rate of 0.45 mL/min to use it as a protein-precipitating solvent, which created cleaner plasma extracts. Also, polytetrafluoroethylene (PTFE) membrane filters were used over nylon filters because they yielded clearer samples.

The procedure was linear in concentration with the range of 5 to 500 ng/mL. Intra-day precision ranged from 0.53% to 7.12%, with accuracy between 87.09% and 105.05%. The range of inter-day precision ranged between 4.74 percent and 11.68 percent and the standard accuracies were 95.02 percent and 97.36 percent. The sensitivity of the method was established through extended validation that revealed accuracy and precision of the method of 93.18-95.82 and 3.606.88 respectively, indicating that the method may be applied in bioanalytical work.

15) Alogliptin & pioglitazone (2017):

Abdel-Ghany et al.[15]The determination of alogliptin and pioglitazone in human plasma was developed by the use of a new rapid LC-MS/MS technique. The linearity of alogliptin is 10 to 400 ng mL-1 and that of pioglitazone is 25 to2000 ng mL-1, which will be suitable in bioanalyses such as Cmin and Cmax.g. Direct precipitation technique is used to successful isolation of drugs from human plasma samples. Pharmacokinetic studies involving volunteers have been done with method validates. Alogliptin and pioglitazone conversion tracking torque m/z 340.18 to 116.15 and 357.10 to 134.00 m/z conversion is done in active mode. The method has been confirmed to be correct and suitable for other clinical applications and possible bioequivalence studies. Waters Acquit UPLCH Xevo TQD system (USA) coupled with a Waters Quattro Premier XE triple quadrupole mass spectrometer and BEH C18 column with dimensions (50 mm × 2.1 mm, 1.7 µm) were used. A mixture of 0.1% aqueous formic acid and acetonitrile in the ratio of (40:60, v/v) was used keeping column temperature at 25◦ C, using 10 µL as the injection volume and 0.3 mL min-1 as the flow rate

16) Rosiglitazone (2018):

Rashid et al.[16]developed and validated a fast, sensitive, and specific method of LC-MS/MS. for the simultaneous

estimation of sildenafil and rosiglitazone in rat plasma. Pharmacokinetic studies made using the validated method were performed after intravenous administration of sildenafil and sildenafil plus rosiglitazone in adult male Sprague Dawley rats.

Methanol was used to precipitate the sample protein and the internal standard was octadeuterated sildenafil. Chromatographic separation was performed by use of gradient elution on a C18 column with acidic conditions of 0.1% (v/v) formic acid, at 0.25 mL/min, consisting of methanol and water as the mobile phases.

The method was confirmed using a Waters Quattro premier XE triple quadrupole mass spectrometer with BEH C18 column (50 mm × 2.1 mm, 1.7 µm) combined with Xevo TQD system. A solution of 0.1% aqueous formic acid and acetonitrile (40:60, v/v) was used at 25°C, 10 µL injection volume and 0.3 mL/min flow rate. Kinetic analysis indicated that when sildenafil was co-administered with another substance, plasma concentration rose and elimination half-life was prolonged compared to administration alone.

17) Pioglitzone (2019):

Kelani et al.[17]A simple and rapid sample preparation procedure based on protein precipitation using acetonitrile was employed. Chromatographic separation was carried out on a Kinetex® C8 analytical column (50 × 4.6 mm, 5 µm) using a gradient elution system with methanol and 0.1% formic acid as the mobile phase components. The flow rate was maintained at 0.7 mL/min, with an injection volume of 8 µL.

Detection was performed using a quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive ion mode under multiple reaction monitoring (MRM) conditions. The monitored transitions were m/z 357.2 → 119.1 for pioglitazone, 373.1 → 150.1 for its hydroxyl metabolite, 340.3 → 116.1 for alogliptin, 361.1 → 138.1 for pioglitazone-d4 (internal standard 1), and 343.2 → 116.1 for alogliptin-d3 (internal standard 2).

The analysis was performed in 4 minutes on linear concentration range of 10-3000ng/mL, 5-2000 ng/mL and 3 -300 ng/mL, for pioglitazone, hydroxyl pioglitazone and alogliptin, respectively

18) Glipizide (2014): [18]

In this study, a simple, fast and sensitive high performance fluid Parallel chromatography-mass spectrometry method is described for the determination of glipizide in human plasma samples using carbamazepine as internal standard (IS) for bioequivalence experiment. Sample preparation was performed by precipitating proteins with methanol and chromatographic separation was performed on an Acquit BEH C18 column (2.1 mm 3.50 mm, 1.7 mm). with a degraded profile at a flow rate of 0.4 mL/min. Mass spectrometry analysis was performed using a QTrap5500 mass spectrometer. combined with an ionizing source that sprays electricity in positive ions fashion. m/z 446.1's multi-reaction tracking transition! 321.0 and m/z 237.1! 194.2 was used to quantify glipizide and is. The linearity of this method was found to range from 10 to 1500 ng/mL for glipizide in human plasma. It only takes 1.0 minutes to scan. This method is applicable to the bioequivalence study of two glipizide-containing medicinal products in human plasma samples.

19) Repaglinide (2012):

Zhang et al.[19]developed a rapid and sensitive LC-MS/MS method for the quantification of repaglinide in human plasma. The analyte and internal standard (diazepam) were extracted from plasma samples (25 µL) using a liquid–liquid extraction technique with a mixture of diethyl ether and dichloromethane (60:40, v/v). Chromatographic separation was performed on an XDB-C18 column using a mobile phase composed of acetonitrile and ammonium acetate buffer (0.01 mol/L, pH 6.8).

The retention times for repaglinide and the internal standard were approximately 1.95 and 2.35 minutes, respectively. Detection was carried out using an API 4000 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operating in multiple reaction monitoring (MRM) mode.

The method exhibited good linearity over the concentration range of 0.05–50 ng/mL, with a limit of detection (LOD) of 0.01 ng/mL. Intra- and inter-day precision, expressed as relative standard deviation (RSD), were within 5.07% and 11.2%, respectively, demonstrating the reliability of the method for quantitative bioanalysis.

20) Glibenclamide (2018):

Alam et al.[20]There is a need for quick and specific bioanalytical methods of drug analysis in biological fluids. A specific UPLC-MS/MS method simple, rapid, sensitive and A Glibenclamide was determined in plasma using a simple, rapid, sensitive and A specific UPLC-MS/MS method. The plasma Samples were treated by Protein precipitation method. The internal standard (IS) was Glimepiride. Procedures: the acquisition of glibenclamide and glimepiride was done on column C18 (Acquity UPLCBEH). The mobile phase was a mixture of acetonitrile (0.1% formic acid) and Water (0.1% formic acid) at a fixed flow rate in binary gradient mode during a 150 L/min total run time. Glibenclamide and IS eluted at the same time of about 1.0 minutes (elution time). Glibenclamide and IS were measured in MRM mode by mass spectrometer (three quadrupoles) in the positive electrospray ionization mode [M + Na] + sodium adder. The level of 10 to 1280 ng/mL generates a linear calibration and the regression equation is Y = 0.0076 X -0.0165, where the linear regression coefficient r 2 = 0.999. The lower quantification was 10 ng/mL. Accuracy of method at the levels of LQC, MQC and HQC is 109.7 % (±6.7), 93.6% (±0.4) and 93.6% (±0.4). respectively 99.3% (± 1.9). Accuracy was less than 6% coefficient of variation at any concentration of QC. The RR at LLQC,MQC and HQC were significant in order of 104.2% (±4.9), 100.6% (±0.9) and 102.9% (±5.8). The established

method has been applied for pharmacokinetic study (data on profiles).

21) Sexagliptin (2014):

Xu et al.[21] established and confirmed a liquid chromatography tandem mass spectrometric (LC 5 -hydroxy saxagliptin in the presence of the two active metabolites, 5-hydroxy saxagliptin) to determine saxagliptin concentrations in a clinical study to be used in pharmacokinetic analysis as a countermeasure. Saxagliptin and 5-hydroxy. saxagliptin have assay dynamic ranges of 0.1-50 ng/mL and 0.2-100 ng/mL respectively. Extract Method The extraction of the analyte was done by protein precipitation (PPT) with acetonitrile followed by a background injection into an Atlantis 1 extraction column LC-MS/MS (50 mm × 2.1 mm, 5 µm) column. THE sample pretreatment is carefully controlled in order to avoid certain and non-specific binding of DPP4. Lower concentrations were observed to be bound. The recoveries in both analytes were above 90%. The test was both selective, robust and reproducible; Storage stability (more than 401 days at -20C) have been established. Under these conditions, the saxagliptin and 5-hydroxy saxagliptin isomers can be separated chromatographically. Supported clinical trials and regulatory approvals used the test.

CONCLUSION

The width of this literature review gave an overview of all the documented bioanalytical technologies available to screen, identify and detect any pharmacological active drug mixture in human plasma/serum matrix. It varied greatly with the validation process as reflected by recent papers. Despite the fact that all the modern technologies, as of date, can be used now but the wrong assessment of analytical parameters needed to be satisfied could lead to the gap in theoretical vs. actual concentration that would generate vast amounts of useless data. Similarly to the methods described in some of the articles being intuitively free, going through the web was different and not checked with what was being prescribed. To achieve a valid and correct procedure, various confounding factors occurring in bioanalytical analysis should be taken into account.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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