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The Review Article on Analytical Method Development and Validation of RP-HPLC for Determination of Antidiabetic Drug

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ABSTRACT:
This study reports the method development and validation for antidiabetic drugs. A novel, simple, rapid, specific, accurate, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed and validated according to ICH guidelines. The validation encompassed assessments of linearity, accuracy, precision, limits of detection (LOD) and quantitation (LOQ), selectivity, range, stability, and robustness. Diabetes mellitus represents a major global health concern and frequently co-occurs with other conditions, necessitating comprehensive management. Pharmaceutical analysis plays a crucial role in drug development, manufacturing, and therapeutic application. Various techniques, including UV spectrophotometry and HPLC, are employed for the simultaneous quantification of multiple drugs in dosage forms. These methods offer advantages such as high specificity, accuracy, linearity, and speed, making them powerful and reliable tools. The pharmaceutical industry relies heavily on quantitative chemical analysis to ensure that both raw materials and finished products meet stringent quality specifications.
Keywords: Validation, RP-HPLC method, anti-diabetic drug, analytical method.

I. INTRODUCTION:

Diabetes mellitus is characterized by persistent high blood glucose levels .This condition arises from defects in insulin secretion, insulin actionleading to disruptions in the metabolism of carbohydrates, fats, and proteins. Consequently, individuals with diabetes mellitus may experience symptoms such as excessive thirst (polydipsia), frequent urination (polyuria), increased hunger (polyphagia), general fatigue, and weakness.

Elevated blood sugar, or hyperglycemia, resulting from issues with insulin production, insulin utilization, or both, defines the long-term metabolic condition known as diabetes mellitus, which presents a substantial challenge to global health. The increasing occurrence of diabetes demands efficient treatment approaches, largely centered on the use of diverse medications designed to lower blood sugar. Precise and dependable measurement of these therapeutic substances in different sample types, such as drug products and biological specimens, is vital for assuring medication quality, how well the body absorbs the drug, and its effectiveness in treating the condition. This is also important for studying how the drug moves through and affects the body.

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) has become a primary tool in analysis due to its adaptability to detect small amounts compounds, and consistent performance . RP-HPLC is effective at separating a variety of substances, including the chemically diverse group of antidiabetic drugs.

1. INTRODUCTION TO PHARMACEUTICAL ANALYSIS :

The primary goal of pharmaceutical analysis is to assure drug quality. It is well known that quality cannot be tested into a product; however, well-planned testing with suitable methodology and instrumentation can help build quality into a drug product. It is essential to understand potential degradation reactions that may occur in the formulated product under various stress conditions that might be encountered during storage and in shipment of the final package. The combined dosage forms are complex in nature. During the process of estimation, it is important to confirm that one component does not interfere with the estimation of the other. There is a plethora of analysis of such formulations without prior separation. For the estimation of multi-component formulation, the instrumental techniques that are commonly employed are spectrophotometry, GLC, HPTLC, HPLC, etc. These methods are based upon the measurement of specific and nonspecific physical properties of the substances.

2. Chromatography:

Chromatography, in its fundamental essence, pertains to a method of separation, where resolution is attained through the strategic

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distribution of a mixture's components between two distinct phases - the stationary phase and the mobile phase. The components with a stronger attraction towards the stationary phase persist within the system for a prolonged duration relative to those more inclined towards the mobile phase. Consequently, the solutes are successively extracted from the system, in accordance with their varying distribution coefficients with respect to the stationary phase. This sequential extraction leads to the separation of the components.

2.1.High-Performance Liquid Chromatography:

The technique of high-performance liquid chromatography is so called because of its improved performance in terms of rapidity, specificity, sensitivity, accuracy, convenience, ease of automation, and the cost of analysis when compared to classical column chromatography.

3. Modes of Separation in HPLC:

There are two modes of separation in HPLC. They are normal phase mode and reversed phase mode.

3.1. Normal Phase Mode:

The nature of the stationary phase is polar, and the mobile phase is non-polar. In this technique, non-polar compounds travel faster and are eluted first because of the lower affinity between the non-polar compounds and the stationary phase. Polar compounds are retained for longer times and take more time to elute because of their higher affinity with the stationary phase. Normal phase mode of separation is not generally used for pharmaceutical applications because most of the drug molecules are polar in nature and hence take a longer time to elute.

3.2 Reversed Phase Mode:

Reversed-phase mode is the most popular mode for analytical and preparative separations of compounds of interest in chemical, biological, pharmaceutical, food, and biomedical sciences. In this mode, the stationary phase is non-polar hydrophobic packing with an octyl or octadecyl functional group bonded to silica gel, and the mobile phase is a polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing, and complexation) to control retention and selectivity. The polar compound gets eluted first in this mode, and non-polar compounds are retained for a longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for a longer time and hence elute faster. The different columns used are octadecyl silane (ODS or C18), octyl silane C8, butyl silane C4, etc. (in the order of increasing polarity of the stationary phase).

Instrumentation:

essence, HPLC is made up of the following parts:

➤ Degassing system and solvent reservoir. Temperature, flow, and pressure.

➤ Sample injection system and pumps. Columns.

➤ Sensors.

➤ Recorder for strip charts.

➤ Microprocessor and data handling device control**.**

Solvents must be degassed to eliminate the formation of bubbles. The pumps provide a steady high pressure with no pulsating and can be programmed to vary the composition of the solvent during the course of the separation. Detectors rely on a change in refractive index, UV-VIS absorption, or fluorescence after excitation with a suitable wavelength.

Schematic of an HPLC instrument:

HPLC BASIC INSTRUMENTATION:

Liquid chromatography has advanced significantly in terms of the development of adsorbents with various geometries and surface chemistry, the theoretical comprehension of the various mechanisms underlying analyte retention, and the practical development of HPLC equipment.

HPLC is a key analytical tool used at every stage of drug development, production, and discovery in the contemporary pharmaceutical industry. A solid grasp of HPLC theory, instrumentation, and principles facilitates the quick and efficient development of robust analytical HPLC methods.In HPLC, a liquid sample, or a solid sample dissolved in a suitable solvent, is carried through a chromatographic column by a liquid mobile phase

DRUG PROFILE

SITAGLIPTIN:

The powder is white to off-white and is known 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. Molecular weight 407.320, molecular formula C16H15F6N5O, solubility in ethanol, DMSO, and water, and classification Sitagliptin belongs to a class of drugs known as dipeptidyl peptidase-4

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(DPP-4) inhibitors. It functions by raising the concentrations of specific organic compounds that reduce elevated blood sugar.

Mechanism of Action:

Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing the breakdown of GLP-1 and GIP, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas. This drives blood glucose levels towards normal. As the blood glucose level approaches normal, the amounts of insulin released and glucagon suppressed diminish, thus tending to prevent an "overshoot" and subsequent low blood sugar, which is seen with some other oral hypoglycaemic agents.

Pharmacodynamics :

Sitagliptin inhibits DPP-4, which results in a stronger insulin response to glucose, lower levels of glucagon, and increased levels of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1

Absorption:

Sitagliptin has an oral bioavailability of 87%, and its pharmacokinetics are unaffected by whether it is taken with or without food. In two hours, sitagliptin's plasma concentration reaches its maximum.

Metabolism:

Sitagliptin is mostly not metabolized, with 79% of the dose excreted in the urine as the unchanged parent compound. Minor metabolic pathways are mediated mainly by cytochrome p450 (CYP) 3A4 and, to a lesser extent, by CYP2C8. After 18 hours, 81% of the dose has remained unchanged, while 2% has been N-sulfated to the M1 metabolite, 6% has been oxidatively desaturated and cyclized to the M2 metabolite, <1% has been glucuronidated at an unknown site to the M3 metabolite, and <1% has been carbamoylated and glucuronidated to the M4 metabolite, 6% has been oxidatively saturated and cyclized to the M5 metabolite, and 2% has been hydroxylated at an unknown site to the M6 metabolite. The M2 metabolite is the cis isomer, while the M5 metabolite is the trans isomer of the same metabolite.

Route of elimination:

Approximately 79% of sitagliptin is excreted in the urine as the unchanged parent compound. 87% of the dose is eliminated in the urine and 13% in the feces.

Adverse effects :

Sitagliptin side effects are comparable to those of a placebo, with the exception of uncommon nausea, common cold-like symptoms, and photosensitivity. The risk of diarrhoea is not increased by it. There is no discernible difference in the incidence of hypoglycaemia between sitagliptin and a placebo. Sulphonyl urea users are more likely to experience low blood sugar. The US prescribing information mentions the occurrence of infrequent case reports of kidney failure and hypersensitivity reactions, but it has not been proven that sitagliptin is the cause.

Uses:

Type 2 diabetes is treated with the medication sitagliptin. A person with type 2 diabetes has either insufficient insulin production or improperly functioning insulin

II. CONCLUSION:

The anti-diabetic drug is approved by the USFDA and follows guidelines. The above study gives the analytical methods for analysis of anti-diabetic drugs. These methods are reported for the development and validation of various drugs. Analysis of drugs plays a significant role during formulation to identify the drug and its metabolites.

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REFERENCE:

[1]. Nusrat K. Shaikh, Rakeshkumar Jat, and Jitendra O. Bhangle, " Development and validation of stability indicating RP-HPLC and UV method for simultaneous quantitation of sitagliptin and sitagliptin phosphate in combination," American Journal of Pharmacy Research 2020, 10(6): 2249-338.

[2]. S. Adsul, J.S. Bidkar, Sunil Harer, and G.Y. Dama, "RP-HPLC Method Development and Validation for Simultaneous Estimation for Metformin and Sitagliptin in Bulk and Tablet

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Formulation," International Journal of Chem Tech Research 2018, (11) 11, 2455-9555.

[3]. Priya Sadapha, Kavita Dhamak, "High-Performance Liquid Chromatography (HPLC) Method Development and Validation" International Journal of Pharmaceutical Sciences Review and Research, Research,2022(2)74, 23-29

[4]. Rushikesh Bacchav, Piyush Bacchav, Mayur Bhamre, Ruchita Bacchav, Ganesh Sonawane, Kajal Pansare, and Dhananjay Patil, "Review of High-Performance Liquid Chromatography and its Applications," Journal of Pharmacy and Pharmaceutical Sciences, 2023, 12(3), 30-44.

[5]. J. Anudeepa, R. Manavalan, R. Venkata, "Development of UV Spectrophotometric Method for Sitagliptin in Bulk and Pharmaceutical Formulation," International Journal of Science, Engineering, and Technology, 2015, 3(5): 1352-1354.

[6]. C. BalaSekaran& A. Prameela Rani, "Development and validation of a spectrophotometric method for the determination of DPP-4 inhibitor, sitagliptin, in its pharmaceutical preparations," EcleticaQuimica, vol. 35, no. 3, São Paulo, Sept. 2010.

[7]. Jain Pritam, Chaudhari Amar, Desai Bhargav, Patel Shani, Patel Santsara, Shimpi Hiren, ShimpiHiren,"Development and validation of first-order derivative UV-spectrophotometric method for determination of sitagliptin in bulk and formulation," International Journal of Drug Development and Research, Int. J. Drug Dev. & Res., Oct-Dec 2011, 3(4): 195

[8]. N. S. Disha and Dr. B. M. Gurupadhayya, "Spectrophotometric determination of Sitagliptin Phosphate in bulk and pharmaceutical formulations," IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 2, Issue 7, July 2015, 702-709.

[9]. Ramakrishna Nirogi, VishwottamKandikere, Koteshwara Mudigonda, Prashanth Komarneni, RaghupathSialeti, and Rajeshkumar Boggavarapu "Sensitive liquid chromatography-tandem mass spectrometry method for the quantification of sitagliptin, a DPP-4 inhibitor, in human plasma using liquid–liquid extraction," Biomed. Chromatography., (2008) 22: 214–222.

[10]. Wei Zeng, Yang Xu, Marvin Constanzer, and Eric J. Woolf, "Determination of sitagliptin in human plasma using protein precipitation and tandem mass spectrometry," Journal of Chromatography , Volume 878, Issue 21, 1 July 2010, Pages 181–182.

[11]. N. N.Monila, Ravi Pratap Pulla, Harshini Shabad, V. V.Swathi, Rajasekhar, A. Ramesh, B. Koti Reddy & B. Umashankar, New Extractive Method Development of Sitagliptin Phosphate in API and Its Unit Dosage Forms by Spectrophotometry," IOSR Journal of Pharmacy and Biological Sciences, Volume 1, Issue 6 (July-August 2012), PP 37-40.

[12]. Hiren Havelikar, Naitik Soni, Purvi Shah, Kirti Patel, Kalpana Patel, and Tejal Gandhi, "Spectrofluorimetric Method for Determination of Sitagliptin Phosphate in Formulation and Spiked Human Urine," International Journal of Analytical and Bioanalytical Chemistry, received 31 August 2012; accepted 17 October 2012.

[13]. Karimulla S K, Vasanth P M, Ramesh T, Ramesh M, "Method development and validation of sitagliptin and metformin using reverse-phase HPLC method in bulk and tablet dosage form," Der Pharmacia Lettre, 2013, 5(5): 168-174.

[14]. Sachin L. Patil, Jayant R. Bhinge, and Chetan M. Bhagat, "Development and Validation of Stability Indicating RP HPLC Method for Simultaneous Determination of Sitagliptin and Metformin in Tablet Dosage Form," International Journal of Research in Pharmaceutical and Biomedical Sciences (IJRPBS), Apr-Jun 2013; Vol. Vol.4(2): 590–596.

[15]. YaravedaYaravedaLathareddy& Naidu Srinivasa Rao, "Stability-indicating RP-HPLC method and its validation for analysis of metformin & sitagliptin in bulk and pharmaceutical dosage form, form"World Journal of Pharmacy and Pharmaceutical Sciences, Volume 2, Issue 5, 3691-3709.

[16]. Srinivasa Reddy, Imran Ahmed, Iqbal Ahmad, Arindam ArindamMukhopadhyay and Saral Thangam, "Development and Validation of a Method for Simultaneous Estimation of Metformin and Sitagliptin in Human Plasma by LC-MS-MS and Its Application in a Bioequivalence Study, Study",Journal of Chromatographic Science 2015;1–8.
[17]. Kumar R, Saha P, Kumar Y, Sahana S, Dubey A, Prakash O. A Review on Diabetes Mellitus: Type 1 & Type 2. World Journal of Pharmacy and Pharmaceutical Sciences. (2020)
[18]. Arneth B, Arneth R, Shams M. Metabolomics of type 1 and type 2 diabetes. International Journal of Molecular Sciences. (2019) May 18;20(10):2467.
[19]. Diabetes statistics 2023 [Internet]. Available from https://www.singlecare.com/blog/news/diabetes-statistics/. (2023)
[20]. Sadaphal P, Dhamak K. Review article on high-performance liquid chromatography (HPLC) method development and validation. Int J Pharm Sci Rev Res [Internet]. (2022); 23–9
[21]. Sandeep Pawar et al., International Journal of Pharmaceutical Sciences & Medicine (IJPSM), Vol. 7, Issue 10, October 2022, pp. 6-29.
[22]. International Journal of Pharmaceutical Sciences & Medicine (IJPSM), CDER Reviewer Guidance: Validation of Chromatographic Methods, Nov. 1994.
[23]. Kumar R, Saha P, Kumar Y, Sahana S, Dubey A, Prakash O. A Review on Diabetes Mellitus: Type 1 & Type 2. World Journal of Pharmacy and Pharmaceutical Sciences. (2020)
[24]. Arneth B, Arneth R, Shams M. Metabolomics of type 1 and type 2 diabetes. International Journal of Molecular Sciences. (2019) May 18;20(10):2467.
[25]. Diabetes statistics 2023 [Internet]. Available from https://www.singlecare.com/blog/news/diabetes-statistics/. (2023)
[26]. Daniel Ducker, Chris Easley, and Peter Kirkpatrick (2007). Sitagliptin. Nat. Rev. Drug. Discov. 6:109-110. doi.org/10.1038/nrd2245
[27]. Prashant BM, Rishikesh VA, Rajesh JO. Antidiabetic Drugs: An Overview. Int J Ph and Ch Sc. 2012;1(1):301-6.
[28]. Balamurugan K, Kirtimaya M, Suresh R. Optimization of the simultaneous determination of sitagliptin and metformin in human plasma by a rapid HPLC method. J Glob Pharma Technol. 2018;10(12):7-12.
[29]. Ramalingam P, Udaya BV, Padmanabha RY, Vinod KK. Stability-indicating RP-HPLC method for the simultaneous determination of sitagliptin and simvastatin in tablets. Ind J Pharm Sci. 2014;76(5):407.
[30]. Manasa S, Dhanalakshmi K, Reddy NG, Sreenivasa S. Method development and validation of dapagliflozin in API by RP-HPLC and UV-spectroscopy. Int J Pharm Sci Drug Res 2014;6(3):250-2.
[31]. Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin, a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. Diabetes Obes Metab 2013;15(9):853-62.
The combination of these studies underscores the importance of advanced analytical techniques in pharmacology. As new medications continue to emerge, the development and validation of reliable methods for their quantification remain critical for ensuring safety and efficacy in patient care.