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A REVIEW ARTICLE ON ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF METFORMIN AND SITAGLIPTIN IN BULK AND FIXED DOSE COMBINATION

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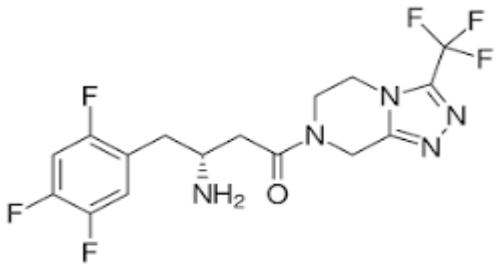
ABSTRACT
This article provides an overview of the simultaneous estimation of the combination drugs metformin and sitagliptin using the RP-HPLC method. Reverse phase chromatography is the most widely utilized separation technique in HPLC due to its simplicity, versatility, and capability to manage compounds with varying polarity and molecular weight. A solid understanding of the various types of mobile phases and their combinations is essential for developing a highly precise and accurate method. The retention time and linearity of metformin and sitagliptin were evaluated under different chromatographic conditions, which include factors such as column type, mobile phase, elution mode, flow rate, and the wavelength detected with a UV detector. In this article, we will review various methods that have been developed to estimate the combination of these drugs using RP-HPLC.

KEYWORDS: Metformin, Sitagliptin, UV spectrophotometry, Analytical method development, Simultaneous estimation and Validation, ICH guidelines.

INTRODUCTION

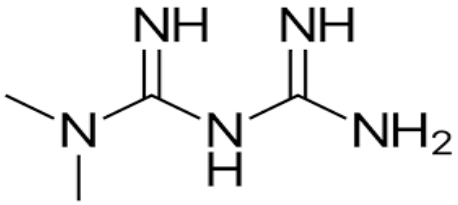
Sitagliptin (SITA) is chemically defined as (3R) -3-amino-1-[3- (trifluoromethyl)-6,8-dihydro-5h-[1,2,4] triazolo [3,4-c] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan-1-one (Figure 1) is an oral antidiabetic medication that inhibits DPP-4 function, utilized in the management of type 2 diabetes.[1,2] The DPP-4 enzyme degrades incretin hormones such as glucagon-like peptide-1(GLP-1) and glucose-dependent insulinotropic peptide (GIP). GLP-1 and GIP are hormones found in the gastrointestinal tract issued as a reaction to food consumption. By inhibiting the inactivation of GLP-1 and GIP, they can enhance the secretion of insulin and inhibit the secretion of glucagon from the pancreas. This helps to regulate blood glucose levels to a normal range.[3,4] The absolute bioavailability of SITA is around 87%. The combined intake of a high-fat meal with SITA does not have any impact regarding the pharmacokinetics. It can be taken with or without meals. Approximately 80% of SITA is eliminated remained the same in urine. The fecal pathway is responsible for 13% of excretion.[5] Various analytical techniques utilizing UV 10-12, Spectrofluorimetry12, RP-HPLC13-14, and LC-MS/MS15-17 have been documented for assessing sitagliptin phosphate.



Sitagliptin

Metformin is commonly employed for managing type 2 diabetes. It is a biguanide derived from galegine, which is a derivative of guanidine discovered in Galega officinalis.[6] Metformin is a hydrophilic base chemically (Figure 1), but it is typically found in oral dosage forms in the form of its hydrochloride salt. Metformin HCl exhibits acid dissociation constant values (pKa) of 2.8 and 11.5 and, as a result, is present in very high amounts as the hydrophilic cationic species at physiological pH

levels (>99.9%).[6] The The unionized species has low lipid solubility, indicated by its low water-oil partition coefficient value (logP=1.43).[7] This chemical characteristic shows low lipophilicity and, consequently, quick passive diffusion of metformin across cells membranes is improbable.[6] Based on these characteristics, metformin HCl is classified as class III (low permeability, high solubility) according to the Biopharmaceutics Classification System (BCS).[8] The oral administration of metformin is regarded as hydrochloride salt, yet all levels in biological fluids are represented as the free base.[9] Metformin is approved in IP 2014(21), USP 2012(22) and BP 2009(23) were estimated using potentiometric methods. Sitagliptin is not recognized officially in any Pharmacopoeia. The use of both drugs in combination is for treating non-insulin-dependent diabetes mellitus.



Sitagliptin

Objectives

1. To study the UV absorption spectra of Metformin and Sitagliptin individually and in combination.
2. To select suitable analytical wavelength(s) (λmax) for estimation of both drugs.
3. To develop a UV spectrophotometric method (e.g., simultaneous equation method, absorbance ratio method, or derivative method) for simultaneous estimation.
4. To validate the developed method according to ICH Q2 (R1) guidelines with respect to: ➢ Linearity ➢ Range.

UV information

Ultraviolet radiation, commonly referred to as UV, is a type of electromagnetic radiation with wavelengths ranging from 10 to 400 nm, shorter than visible light yet longer than X-rays. UV radiation exists in sunlight and makes up roughly 10% of the overall electromagnetic radiation emitted by the Sun. It is likewise generated by electric arcs, Cherenkov radiation, and specific light sources, including mercury-vapor lamps, tanning lights, and black lights.

Ultraviolet photons possess more energy than visible light photons, ranging from approximately 3.1 to 12 electron volts, near the threshold needed for atom ionization. While long-wavelength ultraviolet is not classified as ionizing radiation due to insufficient photon energy, it can trigger chemical reactions and lead many materials to emit light or fluoresce. Numerous practical uses, such as chemical and biological impacts, originate from the interaction of UV radiation with organic molecules. These interactions may excite orbital electrons to elevated energy levels in molecules, potentially causing chemical bonds to break.

Visibility of UV

Humans typically are unable to utilize ultraviolet rays for seeing. The lens of the human eye and lenses implanted surgically since 1986 prevent most radiation in the near UV wavelength range of 300–400 nm; the cornea blocks shorter wavelengths. Humans do not have adaptations in colour receptors for ultraviolet light. The retina's photoreceptors are responsive to near-UV, but the lens fails to focus this light, resulting in UV light bulbs appearing blurry. Individuals without a lens (a condition termed aphakia) see near-UV as bluish-white or violetish-white. Near-UV light can be perceived by certain insects, mammals, and birds.

Evolutionary significance

Modern evolutionary theory attributes the development of early reproductive proteins and enzymes to ultraviolet radiation. UVB leads to adjacent thymine base pairs in genetic sequences linking together to form thymine dimers, disrupting the strand in a way that makes it uncapable by reproductive enzymes. This results in frameshifting in genetic replication and protein synthesis, often leading to cell death. Prior to the development of the UV-blocking ozone layer, when early prokaryotes reached the ocean's surface, they nearly always perished. The small number that endured created enzymes that checked the genetic material and eliminated thymine dimers through nucleotide excision repair enzymes. Numerous enzymes and proteins that play roles in contemporary mitosis and meiosis resemble repair enzymes and are thought to be evolved adaptations of those originally utilized to address DNA damage resulting from UV exposure.

METHODS

Different methods for simultaneous estimation of metformin and sitagliptin from literature review are as follows.

METHOD	CHROMATOGRAPHIC CONDITION	OBSERVATION	REFERENCE
1.	**Column**: C18 Monolithic column (100mm× 4.5mm id., 5µm) connected with an C18 guard cartridge (4mm×3mm id., 5µm). **Mobile Phase**: MeOH, ACN, 0.01mM	pH 3.5±0.5 adjusted with the weakened orthophosphoric acid answer and rate of flow 0.484 ml/min and a pH of 3.946. Apex ratio of the analyte's area was employed for the assessment of tests of pharmaceutical formulations. Complete chromatographic	[18]

	KH2 PO4 (pH 3.5±0.5), adjusted with freshly prepared 10% orthophosphoric acid **Wavelength**: 210 nm **Injection Volume**: 20µl.	assessment duration for each sample was around 4.33 minutes with metformin and sitagliptin releasing with retention durations of 3.3 and 4.4 min respectively.	
2.	**Column** : column C18 (Phenomenex, 250 x 4.6 mm, 5 mm **Mobile Phase**: 0.02M potassium solution dihydrogen phosphate (K2HPO4) and acetonitrile at a ratio of 55:45 (v/v) with a pH 4.3. **Elution Mode**: isocratic mode **Flow Rate**: 1 mL/min, **Volume of Injection**: 20 µl **Duration**: 10 minutes **Sensor**: 252nm	The retrieval of Sitagliptin and Metformin was discovered to be 100.27% and 100.73% correspondingly. The method's validation was executed following ICH guidelines. The HPLC outlined approach was effectively utilized for the examination of drug formulations featuring a combined dosage formulation.	[19]
3.	**Column**: Phenomenex Luna C18 A 100 C18Column (250mm X 4.6 mm i.d.,5µ) **Mobile Phase**: 0.02M Potassium Solution dihydrogen phosphate pH (4.0): Acetonitrile **Elution Mode**: Isocratic **Flow Rate**: 1.0 mL/min **Volume of Injection**: 20 µl **Temperature**: 25 degrees Celsius **Sensor**: 252 nm	Metformin Hydrochloride Sitagliptin Phosphate was eluted at 2.718 and 1.925 minutes. The identification was performed at a wavelength 252 nanometers. The approach was confirmed for system appropriateness, linear relationship, precision, accuracy and strength of example answer. The straight ranges for Metformin Sitagliptin and Hydrochloride Phosphate was 20-120 µg/mL, 2- 12µ g/mL correspondingly with favourable recoveries: 99.4% to 101.35%.	[20]
4.	**Column**: Zorbax Eclipse XDB C18 (150 mm × 4.6 mm) **Mobile Phase**: 0.01M **Phosphate solution**: methanol in a 50:50 % v/v ratio at pH 2.5 modified with 0.2% orthophosphoric acid **Flow Rate**: 0.7 mL/min **Volume of Injection**: 10 µl. Temperature: surrounding Detector: PDA identification at 267 nm	The analysis of Sitagliptin was discovered to reach 99.89 %. The outcomes of the research indicated that the suggested. The RP-HPLC technique is straightforward, swift, exact, dependable, correct and cost-effective which is beneficial for the regular assessment of Sitagliptin phosphate in quantity and its medicinal delivery method.	[24]
5.	**Column**: Agilent C8 (250 mm x 4.6 mm x 5 µm) **Mobile Phase**: Water: Methanol (75:25) v/v **Elution Mode**: Isocratic. **Flow Rate**: 1.0 DetectorPDA266	The retention times were determined as 3.227 and 15.760 for Sitagliptin and Simvastatin correspondingly. Validation criteria such as Exactness, Correctness, Durability and parameters for system suitability were identified and analyzed by utilizing verified parameters.	[25]
6.	**Column**: Hi-Q Sil C18 (250 x 4.6 mm x 5 µ) **Mobile Phase**: ACN: **Methanol**: 10 mM PB (65:25:10) v/ **Flow Rate**: 1.2 **Detector**: PDA 250		[26]
7.	**Column**: Phenomenex C18 (250 x 4.6 mm x 5µ) **Mobile Phase**: 0.02M KH2PO4: CAN (55:45) v/v **Flow Rate**: 1.0 Detector UV 252		[27]

CONCLUSION

The development of analytical methods with RPHPLC is considered to be very precise, particular, straightforward, and economical. Among the methods discussed, method 5 exhibits the shortest retention time of 1.773 minutes for metformin and 3.696 minutes for sitagliptin, respectively,

in which 50% Methanol and 50% Potassium dihydrogen phosphate buffer were utilized as mobile phases for elution, and a peak was detected at 260nm in a UV detector. Subsequently, method 4 illustrates the second shortest retention times are 2.35 min for metformin and 3.04 min for sitagliptin, with a composition of 20% methanol and 80% of Ortho Phosphoric Acid buffer served as mobile phases for elution, and the peak was detected at 210nm in UV detector. Ultimately, method 9 demonstrated a retention time of 3.3 minutes for metformin and 4.4 minutes for sitagliptin, resulting in 42.135%. 10% of Acetonitrile, 47.865% of Potassium dihydrogen orthophosphate buffer, and Methanol were utilized as the mobile phase elution phase and peak detected at 210nm in UV detector. This evaluation provides a general understanding of various mixture of mobile phases suitable for the concurrent assessment of Metformin and Sitagliptin.

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