ISSN: 0975-833X

Availableonlineathttp://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 05, pp.3796-3804, May, 2019

DOI: https://doi.org/10.24941/ijcr.34931.05.2019

INTERNATIONAL JOURNAL OFCURRENTRESEARCH

REVIEW ARTICLE

QUANTIFICATION OF NOVEL DPP4 INHIBITOR-SAXAGLIPTIN BY SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC TECHNIQUES: BRIEF REVIEW

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ARTICLEINFO

Article History:
Received 04th February, 2019
Received in revised form
24th March, 2019
Accepted 05th April, 2019
Published online 30th May, 2019

Key Words:
Saxagliptin, DPP-4,
Analyte, Clinical,
Diabetes.

ABSTRACT

A review is presented on different analytical techniques used for quantitative analysis of novel Dipeptidyl peptidase-4 inhibitor (DPP-4) - Saxagliptin. Efforts have been made to collate all the relevant references to the extent possible. The review discusses the advantages and disadvantages of the cited analytical techniques, which will help to give insights into the methods used for estimation of saxagliptin, from clinical isolates and from its dosage forms. The review highlights the basic as well as advanced techniques performed for estimating saxagliptin. The techniques illustrated here have been demonstrated to be useful for quantitative determination of saxagliptin and may find application in analyzing other related properties.

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***Citation: Chandrika Sailaja et al.*, 2019.** "Quantification of novel dpp4 inhibitor-saxagliptin by spectrophotometric and chromatographic techniques: brief review", *International Journal of Current Research*, 11, (05), 3796-3804.

INTRODUCTION

Saxagliptin, sold under the trade mark of Onglyza, is a novel oral hypoglycemic (anti-diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class and also available in combination- Kombiglyze consists of Saxagliptin with Metformin are the active constituents. Early development was solely by Bristol-Myers Squibb; in 2007 Astra Zeneca joined with Bristol-Myers Squibb to co-develop the final compound and collaborate on the marketing of the saxagliptin (https://en.wikipedia.org/wiki/Saxagliptin). It is chemically (1S, 3S,5S)-2-((2S)-Amino(3-hydroxytricyclo (3.3.1.13, 7)dec-1-yl) acetyl)-2 azabicyclo (3.1.0)hexane-3-carbonitrile (Fig.1) and CAS Number is 361442-04-8. Saxagliptin is used as monotherapy or in combination with other drugs for the treatment of type 2 diabetes. It does not appear to decrease the risk of heart attacks or strokes. Dipeptidyl-peptidase IV (DPP-4) inhibitors inhibit the degradation of the incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). The first available DPP-4 inhibitors are sitagliptin and vildagliptin. These compounds are orally active and have been shown to be efficacious and well tolerated. Two additional DPP-4 inhibitors are under review, and there are several others in clinical development. This article gives an overview on the mechanism of action of DPP-4 inhibitors and focuses on their development and their important physiological actions with regard to the treatment of type 2 diabetes.

Saxagliptin is used with a proper diet and exercise program to control high blood sugar in people with type 2 diabetes. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, loss of limbs, and sexual function problems. Proper control of diabetes may also lessen your risk. In this article we reviewed some analytical methods for the estimation of Saxagliptin in pure API, its available dosage forms, Combination with other drugs and also in biological fluids and tissue extracts.

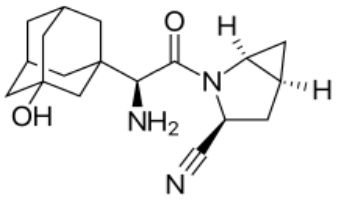

Figure 1. Structure of Saxagliptin

Quantitative analytical techniques for saxagliptin: Analytical techniques for quantitative analysis help an analyst to accurately determine the concentration of individual component in the test sample. The separation of analyte is often performed before analysis by classical methods or during analysis by instrumental methods. Wet analytical methods such as titrations, precipitations, and extractions are used to estimate a drug or an analyte. Instrumental methods for Quantification of analyte- use chromatography, electrophoresis, etc. for

separation and physical properties such as absorption, fluorescence, conductivity and light scattering are utilized to measure the analyte accurately and precisely. The Saxagliptin can be estimated quantitatively using different methodologies. There are innumerable publications based on this simple basic technique but a few were selected here.

Quantification of Saxagliptin by Spectrophotometric methods: It is impossible to envision any literature reference not taking advantage of this powerful technique in assaying Saxagliptin. Many studies have focused on this technique and have performed quantitative estimation of Saxagliptin as an active pharmaceutical ingredient or in its dosage form, either when formulated alone or with other active components. Spectrophotometric method used for the analysis of saxagliptin was cited by Marwa S. Moneeb published simple and sensitive spectrophotometric and spectrofluorimetric methods for the determination of saxagliptin and vildagliptin in bulk and pharmaceutical preparations. The spectrophotometric methods were based on derivatization of the investigated drugs with two reagents: 1, 2-naphthoquinone-4-sulfonic acid sodium salt (NQS) and 4-chloro-7-nitrobenzofurazan (NBD-Cl). For further increase in the sensitivity, the D1 spectra of the reactions products were also recorded. For NQS reaction, Beer's law was obeyed over the ranges of 5–30 and 7–45 μg mL1 for the absorbance readings; and 3–32 and 5–50 μg mL1 for the derivative readings of Saxagliptin and Vildagliptin, respectively. Pravin Cholke *et al.* (2018) developed simple, rapid and validated analytical method for estimation of Saxagliptin and Metformin in tablet dosage form. The optimum conditions for the analysis of the drug were established. The maximum wavelengths (λmax) of Saxagliptin were found to be 274nm and Metformin 231nm. The percentage recovery of Saxagliptin(API) 100.10% and Metformin(API) 99.98%. Beer's laws were obeyed in the concentration range 50-90 μg/ml for Saxagliptin and 2-10μg/ml for Metformin. Narendra Nyola and Govinda Samy Jeyabalan (Narendra Nyola, 2012) created an analytical method for estimation of Saxagliptin and Metformin in active pharmaceutical ingrident. The maximum wavelength (λ max) of Saxagliptin and Metformin were found to be 274 nm and 231 nm respectively.

The percentage recovery of Saxagliptin and Metformin were 100.1 and 99.98 respectively. Beer's laws were obeyed in the concentration range 50-90μg/ml for Saxagliptin and 2-10μg/ml for Metformin. Asim M Suthar *et al.* (2018) studied simple, precise, and accurate method for simultaneous quantitative estimation of saxagliptin hydrochloride and dapagliflozin propendiol monohydrate in pharmaceutical tablet dosage form. The method was based on determination of saxagliptin hydrochloride at an absorbance difference between 214.40 nm - 220.0 nm and dapagliflozin propendiol monohydrate at an absorbance difference between 208.0 nm - 209.0 nm. The linearity was obtained in the concentration range of 4-16 μg/ml and 10-22 μg/ml for saxagliptin hydrochloride and dapagliflozin propendiol monohydrate respectively. R.L. Sawant and Mhaske (2014) established four simple, accurate and precise spectrophotometric methods have been developed for simultaneous determination of saxagliptin and methyldopa in a laboratory mixture. Simultaneous equation method (Method I) shows absorbance at 211 nm (λ1) and 280 nm (λ2) corresponding to the absorbance maxima of saxagliptin and methyldopa respectively. In absorbance ratio method (Method II) isobestic point is observed at 240 nm. Isobestic point (240 nm) is considered as λ1 and an absorbance maximum of methyldopa (280 nm) is considered as λ2. In area under curve method (Method III) measurement of area under curve in the range of 204-241nm (for saxagliptin) and 265-314 nm (for methyldopa) was carried out. In dual wavelength method (Method IV), saxagliptin and methyldopa were quantified using principle that absorbance difference between two points on mixture spectra was directly proportional to concentration of component of interest and independent of interfering component. All dilutions were prepared in distilled water. Linearity range was observed in the concentration range of solution 5-30 μg/ml for saxagliptin and 2-12 μg/ml for methyldopa. R. Kalaichelvi and E. Jayachandran (Kalaichelvi, 2011) developed Simple, sensitive and cost effective UV-spectrophotometric method for the estimation of saxagliptin in bulk and pharmaceutical formulations. Saxagliptin was estimated at 208 nm in methanol. Linearity range was found to be 5–40 μg/ml. Y= mx+c, r^2 = 0.999. Pranali *et al.* (2016) developed simple, accurate and precise spectroscopic method was developed for simultaneous estimation of Saxagliptin Hydrochloride and Glibenclamide in synthetic mixture using first order derivative zero-crossing method. Saxagliptin Hydrochloride showed zero crossing point at 315.00nm while Glibenclamide showed zero crossing point at 229.40nm.

The dA/dλ was measured at 229.40nm for Saxagliptin Hydrochloride and 315.00nm for Glibenclamide and calibration curves were plotted as dA/dλ versus concentration, respectively. Ramalingam Kalaichelvi and Ekambaram Jayachandran (Ramalingam, 2012) created two simple and sensitive ion-pairing spectrophotometric methods for the assay of saxagliptin in pure form and in tablet dosage form. The developed methods involve formation of yellow colored chloroform extractable ion-pair complexes of the drug with Bromocresol Green (BCG) and Bromothymol Blue (BTB) in acidic medium. The extracted complexes showed absorbance maxima at 420, 415 nm for BCG, BTB, respectively. Raveendra *et al.* (2018) developed for stability-indicating, simultaneous estimation of Saxagliptin and Dapagliflozin in rat serum by using UV spectroscopy. Saxagliptin detection wave length was at 222 nm with water is solvent and Dapagliflozin detection wave length was at 274 nm with phosphate buffer pH 6.8 is solvent. Both drugs are obeyed the beers-lamberts concentration range was founds to be 1-10 μg/mL. The present method was optimized and validated in spiked rat serum according to ICH guidelines. Abdel-Aziz O, Ayad MF and Tadros MM. (Abdel-Aziz, 2015) proposed simple, selective and reproducible spectrofluorimetric and spectrophotometric methods for the determination of vildagliptin and saxagliptin in bulk and their pharmaceutical dosage forms. The first proposed spectrofluorimetric method is based on the dansylation reaction of the amino group of vildagliptin with dansyl chloride to form a highly fluorescent product. The formed product was measured spectrofluorimetrically at 455 nm after excitation at 345 nm. The second proposed spectrophotometric method is based on the charge transfer complex of saxagliptin with tetrachloro-1, 4-benzoquinone (p-chloranil). The formed charge transfer complex was measured spectrophotometrically at 530 nm. The third proposed spectrophotometric method is based on the condensation reaction of the primary amino group of saxagliptin with formaldehyde and acetyl acetone to form a yellow colored product known as Hantzsch reaction, measured at 342.5 nm. All the variables were studied to optimize the reactions' conditions using factorial design. Ramzia *et al.* (2012) created simple, accurate and precise spectrophotometric

methods for the determination of saxagliptin in bulk and dosage forms. The proposed methods are based on the charge transfer complexes of saxagliptin with 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) and 7, 7, 8, 8-tetracyanoquinodimethane (TCNQ). Joseph Tresa *et al.* (2018) established simple, rapid, accurate, precise and improved analytical method for estimation of Saxagliptin and Metformin. The maximum wavelength (λmax) of Saxagliptin and Metformin were found to be 212nm and 233nm respectively. Sanjeev V. Deshpande, Madhumita A. Roy and Shubhangi C. Daswadkar developed three simple, precise and economical UV spectrophotometric methods for the estimation of Saxagliptin in bulk and pharmaceutical formulations. Saxagliptin is an antidiabetic drug belongs the chemical class is dipeptidyl peptidase-4 enzyme (DPP-4) inhibitor. Saxagliptin has absorbance maxima at 211 nm in zero order spectrum method (Method A), and in the first order derivative spectra, showed sharp peak at 204 nm when n = 1 (Method B).

Quantification of Saxagliptin by Chromatographic methods: Today not a single molecule is analyzed without the use of powerful analytical tools like HPLC, UPLC and HPTLC. Salwa R. El-Shaboury *et al.* (2018) developed and validated a simple, sensitive and precise spectrodensitometric method for the simultaneous determination of sofosbuvir, ribavirin and saxagliptin in their pure and pharmaceutical dosage forms. The method employed TLC plates precoated with silica gel G 60 F254 as the stationary phase. The mobile phase consisting of acetonitrile-water (80:20, v/v) was used to give compact bands for all the studied drugs at 228 nm. They were resolved with retardation factor (Rf) values of 0.71, 0.36 and 0.21 for sofosbuvir, ribavirin and saxagliptin respectively. Thiyagarajan Deepan and Magharla Dasaratha Dhanaraju (Thiyagarajan Deepan, 2018) developed simple, fast, and highly selective RP-HPLC method for the determination of Dapagliflozin (DAP) and Saxagliptin (SAX) in API and tablet dosage form. The separation was done using an Xterra RP18 (4.6×150 mm, 5µm particle size) column with Acetonitrile: water (60:40). The isocratic elution mode at a flow rate of 1 mL/min, and the analytes were measured at 248 nm. The retention time for Dapagliflozin and Saxagliptin were about 2.091 and 3.249 min, respectively. R. Pravin Cumar, M.Vasudevan and Deecaraman (Pravin Cumar, 2012) proposed simple, economic, sensitive RP-HPLC method for the simultaneous estimation of metformin and saxagliptin in tablets. The method was carried outon C_{18} column (5µm, 25 cm x 4.6 mm, I.D) using phosphate buffer (pH 5.0), acetonitrile and methanol in the ratio 75:15: 10 respectively as a mobile phase at a flow rate of 1.5mL/min. The wavelength for metformin and saxagliptin at 225 nm was found to be appropriate. The retention time of metformin and saxagliptin was found to be 5.65 and 6.20 min, respectively. Hanan A. Merey *et al.* (2017) suggested two chromatographic methods for the simultaneous determination of a binary mixture containing Saxagliptin HCl and Metformin HCl. First method was RP-HPLC method. Chromatographic separation was done on Kinetex TM column–C_{18} (4.6x150 mm, 2.6mm) using mobile phase consisted of acetonitrile : phosphate buffer pH = 4.5 ± 0.1 adjusted with orthophosphoric acid (13:87, v/v). Isocratic elution at a flow rate 1.5 mL/min and UV detection at 220.0 nm was performed. Second method was spectro-densitometric method. Chromatographic separation was done on precoated silica gel aluminium plates 60 F254 as a stationary phase and developing system consisting of chloroform: methanol: formic acid (80:20:0.3, by volume). The density of the separated bands was measured by UV detector at 210.0 nm. Siva Krishna Nachaka *et al.* (2017) developed simple and sensitive gas chromatography method using a flame ionization detector, the method validated and applied for the determination of TEMPO in Saxagliptin monohydrate drug substance. The chromatography separation was achieved on capillary column (DB-FFAP (30m, 0.53mm, 1.5µm)) with fused silica coated with nitro terephthalic acid modified polyethylene glycol stationary phase. P. B. N. Prasad, K. Satyanaryana and G. Krishnamohan (Prasad, 2015) created simple, specific, sensitive, precise and accurate RP-HPLC for the simultaneous analysis of Metformin and Saxagliptin in active pharmaceutical ingredients (APIs) as well as in marketed tablet (combination) dosage forms. The method was achieved on Enable C_{18} G (250 × 4.6 mm; 5µm particle size) column using 0.05 M KH_2PO_4 buffer (pH 4.5):Methanol : Acetonitrile (60:20:20 %v/v) as a mobile phase at a flow rate of 0.6 mL/min and by employing UV detection at 220 nm wavelength. The retention time of Metformin and Saxagliptin were found to be 4.38 min and 6.92 min, respectively. Nyola Narendra and Govinda samy Jeyabalan (Nyola Narendra and Govindasamy Jeyabalan, 2012) developed a new simple, accurate, precise and reproducible RP-HPLC for the simultaneous estimation of Saxagliptin and Metformin in bulk drug form using C_{18} column (Phenomenex, 250 x 4.6 mm, 5µm) in isocratic mode. The mobile phase consisted of 0.02M Potassium dihydrogen phosphate (KH_2PO_4), Acetonitrile, Methanol in the ratio of 50:25:25 (v/v/v) at pH 4.3. The detection wavelength was carried out at 240 nm. R. Aswini, M. Mukkanti Eswarudu and P. Srinivasa Babu (Aswini, 2018) developed and validated a rapid, specific, accurate and precise RP-HPLC method for simultaneous determination of Dapagliflozin and Saxagliptin in bulk and pharmaceutical dosage form. Successful chromatographic separation of Dapagliflozin and Saxagliptin was carried out with Inertsil-ODS, C_{18} column (250 × 4.6 mm; 5µm) with mobile phase consisted of a mixture of Methanol and Potassium dihyrogen phosphate buffer in the ratio of 45:55 v/v delivered at a flow rate of 1.0 ml/min. The eluents are monitored by PDA detector and peaks values were measured at 210 nm. The retention times for Dapagliflozin and Saxagliptin were 4.707 min and 6.684 min respectively. Wael Abu Dayyih *et al.* (2015) developed a simple, validated a rapid chromatographic method for quantification of saxagliptin in rats serum in order to study saxagliptin pharmacokinetics parameters in sucralose fed rats simultaneously to detect any interaction possibility between saxagliptin and sucralose in rats. In this analysis, mobile phase was consisted of phosphate buffer (pH =4) and methanol (70:30) v/v at flow rate of 1 ml/min with UV detection at 230 nm., C_8 column of separation by using injection volume of 50µl, samples run time was 10 min, and sildenafil citrate was used as internal standard. Saxagliptin was given to rats orally of (2g/kg) dose while sucralose was given with (11 mg/kg/day) dose. Patil Prafulla Prakash *et al.* (2012) developed a new simple economical reverse phase high performance liquid chromatographic method for the determination of Metformin Hcl and Saxagliptin in bulk and dosage form. The separation was eluted on a Zodiac C_{18} column (150 mm x 4.6 mm; 5µ) using a mobile phase mixture of Phosphate buffer pH 6.8 and acetonitrile in a ratio of 94:6 v/v at a flow rate of 1.0ml/min. The detection was made at 248 nm. The retention times were 1.6min for Metformin and 4.1min for Saxagliptin. Asiya Begum *et al.* (2014) proposed a sensitive selective and precise stability indicating-high performance liquid chromatographic (HPLC) method for Saxagliptin and Metformin in Tablet

dosage form. An isocratic separation was carried out using Zorbax C_{18} (250 x 4.6 mm, 5µm) column and Potassium dihydrogen Phosphate: Methanol (60:40 v/v) as mobile phase.With quantification carried out at a wavelength of 248nm. Vinutha Kommineni, K.P.R.Chowdary and S.V.U.M. Prasad (Vinutha Kommineni, 2017) created and validated a new stability indicating RP HPLC method for simultaneous estimation of Saxagliptin and Dapagliflozin in bulk and dosage forms. The method involves separation on XTerra C_{18} column (150mm x 4.6mm x 5µm particle size). The optimized mobile phase consists of phoaphate buffer (pH 4) and Acetonitrile (50:50v/v) with a flow rate of 1ml/min and UV detection at 225mn. Retention time was 2.1min (Saxagliptin), 2.8min (Dapagliflozin). Shubhangi C. Daswadkar *et al.* (2016) developed and demonstrated an integrated multivariate approach to develop and quantify the constituent concentrations of saxagliptin drug in its pure and formulated forms. The method was developed using a mobile phase acetonitrile: water (pH-3), (20:80 v/v) on an Agilent, TC C_{18} (250 × 4.6 mm) 5µm column and flow rate 1.0 ml/min which was optimized with help of design expert software and validated according to ICH Q2 guideline and application of this method to different stress condition of saxagliptin. Vaishali V. Karkhanis and Anandkumari D. Captain (Vaishali, 2013) developed and validated an accurate, sensitive and precise RP-HPLC method for the estimation of Saxagliptin from bulk drug and Pharmaceutical Dosage form. The separation was achieved by Hypersil C_{18} column (250mm X 4.6mm, 5µm) in isocratic mode, with mobile phase comprises of Acetonitrile : Buffer in proportion of 30:70v/v, buffer was 0.02M Potassium Di-hydrogen Phosphate (pH 4.5 adjusted with Ortho Phosphoric Acid). The flow rate of mobile phase was 1.0ml/min and employing UV detection with 220nm wavelengths. The retention time of Saxagliptin was 3.487 min. ACK. Prasanna and Kanuri Priyanka (Prasanna and Kanuri Priyanka, 2015) developed an RP-HPLC method for the simultaneous determination of metformin and saxagliptin in marketed formulation. This method is based on RP-HPLC separation of the two drugs on the Inspire C_{18} column (250 mm × 4.6 mm, 5.0µ); and mobile phase containing Buffer: Methanol in a ratio of 55:45 v/v at a flow rate of 1ml/min, using UV detection at 208 nm. This method has been applied to formulation without any interference of excipients of formulation. B. Reddy Padmaja *et al.* (2018) developed a simple, accurate, precise method for the simultaneous estimation of Dapagliflozin and Saxagliptin in Tablet dosage form. Chromatogram was run through Standard BDS C8 column (50 × 4.6 mm, 5µ) The Mobile phase containing Potassium dihydrogen phosphate: Acetonitrile in the ratio 55:45, pH was adjusted to 3.8 with dilute orthophosphoric acid. The solution was pumped through the column at a flow rate of 1ml/min. Optimized wavelength selected was 210 nm. Retention time of Dapagliflozin and Saxagliptin were found to be 2.266 min and 2.805 min. M.Sarat, P. Murali krishna and C Rambabu (2018) developed and validated a simple, selective, accurate HPLC method for the analysis of Saxagliptin and Pioglitazone. Chromatographic separation achieved isocratically on a C_{18} column (Use Inertsil C_{18}, 5m , 150 mm x 4.6mm) utilizing a mobile phase of acetonitrile/phosphate buffer (60:40, v/v, pH 7.0) at a flow rate of 0.8 ml/min with UV detection at 260nm.Aceclofenac was used as an internal standard. The retention time of Saxagliptin, pioglitazone and aceclofenac was 2.48, 4.45 and 6.34 min respectively. Imran A. Sheikh, Manoj Charde and Abhilasha Mittal (2018) established a novel and simple reversed-phase liquid chromatographic method for the determination of saxagliptin and metformin HCl. The proposed work was performed on Young Lin (S.K) isocratic System UV Detector C_{18} column (150 mm × 4.6 mm). A mixture of potassium phosphate, mobile phase in this method with flow rate of 0.7 mL/min with UV detection at 203 nm. Laís Engroff Scheeren *et al.* (2015) developed and validated a liquid chromatography method to quantify the saxagliptin drug in tablets. This method was based on the isocratic elution of saxagliptin, using a mobile phase consisting of 0.1% phosphoric acid at pH 3.0 – methanol (70: 30, v/v) at a flow rate of 1 $mL.min^{-1}$ with UV detection at 225 nm. The chromatographic separation was achieved in 8 minutes on a Waters XBridge C_{18} column -250 mm x 4.6 mm, 5µm. Gandla. Kumara Swamy, S. Shruthi, M. Rajkumar and D. Sudheer Kumar (2017) developed and validated a simple, rapid, precise, accurate and robust stability-indicating RP-HPLC method to estimate Saxagliptin hydrochloride and Dapagliflozin in bulk and in tablet form. The samples were isocratically eluted using a C_{18} (250 cm x 4.6cm, 5µ) Primesil ODS column with mobile phase Potassium dihydrogren phosphate Buffer (pH 6.0): Acetonitrile (45:55 v/v) at wavelength 247 nm. Salvala Srividya, Ettireddy Swetha and Ciddi Veeresham (Salvala Srividya, 2015) developed a simple, specific, accurate and precise HPTLC method for the quantitative analysis of Saxagliptin in active pharmaceutical ingredients (APIs) and pharmaceutical dosage forms. The method was achieved using silica gel aluminum plate 60 F254 (10 × 10 cm) as stationary phase and Methanol:Chloroform (6:4 v/v) as mobile phase.

The developed plate was scanned densitometrically using UV 222nm wavelength. The Rf value of Saxagliptin was found to be 0.50 ± 0.02. P. B. N. Prasad, K. Satyanaryana and G. Krishnamohan developed simple, specific, sensitive, precise and accurate RP-HPLC method for the simultaneous analysis of Metformin and Saxagliptin in active pharmaceutical ingredients (APIs) as well as in marketed tablet (combination) dosage forms. The method was achieved on Enable C_{18} G (250 × 4.6 mm; 5 µm particle size) column using 0.05 M KH_2PO_4 buffer (pH 4.5):Methanol:Acetonitrile (60:20:20 %v/v) as a mobile phase at a flow rate of 0.6 mL/min and by employing UV detection at 220nm wavelength. The retention time of Metformin and Saxagliptin were found to be 4.38 min and 6.92 min, respectively. Sayali S. More *et al.* (2018) developed and validated simple, accurate, precise and selective RP-HPLC method for simultaneous estimation of Saxagliptin and Dapagliflozin in tablet dosage form. Both drugs were separated on Phenomenex Hyperclone C_{18} column (250×4.6 mm, 5µ) using methanol: 20 mM phosphate buffer (pH3.0) (70:30, v/v) in an isocratic mode at flow rate of 1 mL/min. Chromatographic determination was carried out at wavelength of 225nm. Saxagliptin and Dapagliflozin were eluted at 4.70 and 6.45min, respectively. Md. Saiful Islam *et al.* developed a sensitive, accurate, rapid, cost effective and robust HPLC method for the quantification of Saxagliptin Hydrochloride with PDA detection. In this method; a reversed-phase C_{18} (250 × 4.6 mm internal diameter and 5 µm particle size) column with a mobile phase of phosphate buffer: acetonitrile (80:20; v/v) containing orthophosphoric acid (pH 2.70) at 1.0 mL/min flow rate was used to separate Saxagliptin with a detection of 210 nm. Advaita B. Patel, Deepa R. Patel and Zarna Shah developed and validated a simple, rapid, precise, accurate and robust stability-indicating RP-HPLC method to estimate Saxagliptin hydrochloride and Dapagliflozin in bulk and in tablet form. The samples were isocratically eluted using a C_{18}

(25cm x 0.46 cm) Inertsil ODS column with mobile phase Potassium dihydrogren phosphate Buffer (pH 6.0): Acetonitrile (45:55 v/v) at wavelength 220nm. Charmy P. Desai, Ankit B. Chaudhary and Bhoomi D. Patel (2018) described a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of saxagliptin and dapagliflozin in synthetic mixture. Chromatography was performed on a ODS inertsil C_{18} (250 mm x 4.6 mm i.d., 5µm particle size) column with mobile phase containing 0.05M Potassium dihydrogen phosphate (pH4.5±0.1 using orthophosphoric acid): Methanol (35: 65). The flow rate was 1.0 ml/min and the eluent was monitored at 227nm. The selected chromatographic conditions were found to effectively separate Saxagliptin (Rt- 2.68min) and dapagliflozin(Rt- 5.8 min). Phani RSCH, Prasad KRS and Useni Reddy Mallu developed a simple, precise and stability-indicating RP-HPLC method for simultaneous quantification of Dapagliflozin and Saxagliptin in combined dosage form.

The method has been developed with ammonium dihydrogen phosphate buffer (pH 6.8) and methanol in a ratio of 65:35 v/v as mobile phase at a flow rate of 1.5 ml/min over Intersil ODS C_{18} column (250 mm × 4.6 mm × 5µ). The UV detection wavelength was fixed at 280 nm. Vanita P Rode, Sonali G Thorat and Madhukar R Tajne (Vanita, 2017) developed a simple, precise, and accurate stability-indicating normal-phase HPTLC method for estimation of Saxagliptin in the bulk drug and tablet formulation. Chromatography was performed on the plates precoated with silica gel 60F254 using 1% methanolic ammonium acetate: toluene 5:5 (v/v) as a mobile phase. Densitometric quantification was performed at 215nm by reflectance scanning. The retardation factor of Saxagliptine was 0.48 ± 0.02. Mohammad Yunoos and D. Gowri Sankar developed and validated a simple and precise stability indicating RP-HPLC method was for the simultaneous determination of Metformin Hydrochloride and Saxagliptin in pure drug and combined tablet dosage form. Chromatography was carried out on Hypersil ODS C_{18} (250x4.6mm, 5µ particle size) analytical column using a mobile phase of Phosphate buffer (KH_2PO_4) adjusted to pH 5.0 with dilute orthophosphoric acid, acetonitrile and methanol in the ratio of 25:50:25 % v/v/v at a flow rate of 1.0 ml/min. The analyte was monitored using PDA detector at 211nm. The retention time was found to be 2.246 min and 4.516 min for Metformin Hydrochloride and Saxagliptin respectively. Eman I. El-Kimary *et al.* (2016) developed a single, simple, selective and validated HPTLC method was for the determination of either linagliptin, saxagliptin or vildagliptin in their binary mixtures with metformin in pharmaceutical preparations using environmentally preferable green mobile phase system. Separation was carried out on Merck HPTLC aluminum sheets of silica gel 60 F254 using methanol–0.5% w/v aqueous ammonium sulfate (8: 2, v/v) as mobile phase. Densitometric measurement of the spots was performed at 225nm for Linagliptin/Metformin mixture and at 208 nm for both Saxagliptin/Metformin and Vildagliptin/Metformin mixtures. Nageswara Rao M *et al.* (2017) developed and optimized a sensitive ion chromatgraphic method for the determination of methanesulfonic acid and trifluroacetic acid in saxagliptin drug substance. These organic acids in lower limits act as potential impurities and causes undesirable by products. The method was developed to enhance the detection by this technique and minimizing the acquisition time by using 30 min. Sena Caglar and Ali Rahmi Alp (2016) developed a simple, precise and rapid high performance liquid chromatography method with UV detection for the determination of saxagliptin and metformin in bulk. An Agilent, Zorbax CN (250 × 4.6 mm I.D., 5 µm) column was used with a mobile phase mixture of methanol-50mM phosphate buffer (pH 2.7) in a gradient elution mode at a flow rate of 1.0 ml min^{-1}. The analytes were detected at 225nm and total run time for the method was 7 min. N. Singh, P. Bansal, M. Maithani and Y. Chauhan developed and validated a simple and precise stability indicating RP-HPLC method for the simultaneous estimation of dapagliflozin and saxagliptin in combined tablet dosage form. The chromatographic separation of the drugs was achieved with an Xterra C_{18} analytical column (150 mm × 4.6 mm i.d., particle size 3.5µ) using buffer and acetonitrile (53: 47 v/v) as the mobile phase. The buffer used in mobile phase contained 20mM sodium dihydrogen phosphate and its pH was adjusted to 5.5 ± 0.02 with orthophosphoric acid. The instrument was set at a flow rate of 1.2 mL min^{-1} at ambient temperature and the wavelength of the UV-visible detector was 230nm. B. Thangabalan, P.Srisowmya and S. Manohar Babu (Thangabalan, 2014) developed and validated a RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride and Saxaagliptin in pure and pharmaceutical dosage form. Chromatography was carried on Phenominex C_{18} (250 mm × 4.6 mm, 5 µm) column with mobile phase comprising of phosphate buffer and acetonitrile in the ratio (60:40) v/v. The flow rate was adjusted to 0.7 ml/min with UV detection at 242 nm. The retention times of Metformin and Saxagliptin were found to be 1.7 min, 2.9 min respectively. Patel PD and Pandya SS (Patel, 2018) developed and validated a new, precise, rapid, accurate RP – HPLC method was for simultaneous estimation of Dapagliflozin and Saxagliptin Hydrochloride in bulk and in tablet dosage form. The samples were isocratically eluted by using Hypersil BDS C18 (250 mm × 4.6 mm) 5µm column with a mobile phase mixture of Phosphate buffer (pH 4.5):

Methanol in the ratio of 85:15 v/v at a flow rate of 1 ml/min and detection wavelength of 222 nm. The retention time of Dapagliflozin and Saxagliptin HCl found to be 4.080 min and 5.343 min. Bolagani Sarada, K Narendra Kumar Reddy and Bada Pragati Kumar (Bolagani Sarada, 2007) developed an Analytical method by using RP-HPLC for the determination of saxagliptin and pioglitazone. Potassium dihydrogen phosphate buffer: methanol (80:20 v/v) was used as mobile phase. The detection wavelength was 272nm, flow rate of 1ml/min and retention time of Saxagliptin was 2.998 minutes and Pioglitazone was 4.310 minutes. N.V.M.S. Bhagavanji *et al.* developed isocratic, reversed phase-liquid-chromatographic method for the quantitative determination of Metformin and Saxagliptin in combined-dosage form. A thermo hypersil BDS C_8 (250 x 4.6 x 5µ) column with mobile phase containing water pH 3.0 adjusted with ortho phosphoric acid: methanol in the ratio of (70: 30, v/v) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 241 nm. The retention times of Metformin and Saxagliptin were 2.956min and 4.573 min, respectively. Ramesh J and Senthil Kumar N (Ramesh et al., 2016) developed and validated a Stability indicating RP-HPLC method for the simultaneous estimation of Saxagliptin and Metformin in bulk and pharmaceutical dosage form. The separation was carried on kromasil- C_{18} column (4.5 x 250 mm; 5µm) column with mobile phase consisting of 50mM sodium dihydrogen phosphate buffer -pH adjusted to 2.7 using orthophosphoric acid: methanol in the ratio of 80:20 v/v with a flow rate of 0.9 ml/min and PDA detection at 242nm. Rohini

Surisetti and K. Nagaraju Separated Metformin and Saxagliptin were successfully. It is achieved on thermo, C_{18} 250 X 4.6mm, 5µm, or equivalent in an isocratic mode utilizing 0.1M KH_2PO_4: Methanol (65:35) at a flow rate of 1.0ml/min and elute was monitored at 256nm, with a retention time of 2.787 and 3.436 minutes for Metformin and Saxagliptin respectively. Sarif Niroush Konari and Jane T. Jacob (2015) developed a simple and quick approach of stability indicating RP-HPLC technique for the determination of Metformin, Saxagliptin and Sitagliptin in bulk and pharmaceutical dosage forms. This projected methodology is apt for the multicomponent estimation of 2 totally different commercially existing combinations in pharmaceutical market used for the treatment of type II diabetes mellitus viz.

Sitagliptin and metformin, saxagliptin and metformin in 8 min. A chromatographic separation of the three drugs was attained with a Inertsil C_{18} (4.6 X 250mm, 5 mm) analytical column using a buffer potassium dihydrogen phosphate adjusted pH 4 with orthophosphoric acid: methanol: acetonitrile (70:10:20%v/v) in isocratic mode at a flow rate of mL/min, column at ambient temperature and detection of each 3 drugs were monitored at 215 nm using a DAD detector. Srikanth Inturi, Ravikanth Inturi, Israel kumar Tagaram developed a simple, sensitive and precise reverse phase high performance liquid chromatographic method for the estimation of Saxagliptin in pharmaceutical dosage forms. The mobile phase consist of buffer (0.02M sodium dihydrogen phosphate, pH-3 adjusted with ortho phosphoric acid): methanol: acetonitrile in the ratio of 45:20:35v/v delivered at a flow rate of 1.0 ml / min and wavelength of detection at 220 nm. The retention time of Saxagliptin was 8.20 min. C. Quantification of Saxagliptin by Spectrophotometry combined with Chromatographic techniques and its applicability to Pharmacokinetic, dynamic, bioavailability, bioequivalence and drug metabolism-disposition studies. Tangudu Nagabhusana Rao, Guntuku Girija Sankar and Lade Jyothi Rani (Tangudu Nagabhusana Rao, 2016) developed and validated High-throughput Liquid chromatography–mass spectrometry method for the quantification of Saxagliptin in rat plasma using Sitagliptin as internal standard (ISTD).

Following protein precipitation in 96 well plate format, the analytes and ISTD were run on ACE C_{18} 4.6 X 75 mm (5.0µm) using an isocratic mobile phase consisting of 2mM Ammonium Formate with 0.1% Formic Acid and Acetonitrile (50:50 v/v). The precursor and product ions of the drugs were monitored on a triple quadrupole instrument operated in the positive ionization mode. Shruti Surendran *et al.* (2019) reported a highly sensitive, selective and specific LC-MS/MS method for the estimation of a novel anti-diabetic combination of saxagliptin and dapagliflozin in rat plasma. An Agilent Eclipse Plus C_{18} column (150 mm × 4.6 mm, 3.6 µm) with gradient elution using 0.01% ammonia solution and acetonitrile as the mobile phase was used for the chromatographic separation. The ion transitions were quantified in positive and negative polarity using a polarity switching approach. A solid phase extraction process was used as the sample preparation approach. Shantikumar *et al.* (2015) developed and validated a simple and sensitive LC-QTOF/MS method to measure the human plasma concentrations of vildagliptin, saxagliptin, sitagliptin, linagliptin and teneligliptin, using pioglitazone as an internal standard. Chromatographic separation of five gliptins was achieved on a Zorbax Eclipse Plus C_{18} column Rapid Resolution HD (50 × 2.1 mm, 1.8 µm) using a mobile phase consisting of 20mM ammonium formate and acetonitrile in gradient mode. Detection was performed with positive ion electrospray ionization mass spectrometry using target ions in selective ion mode. Simpler protein precipitation was employed for sample extraction from human plasma. Maha F. Abdel-Ghany *et al.* (2015) developed and subsequently validated a new, simple, selective, reproducible and sensitive stability-indicating liquid chromatographic method for the determination of saxagliptin. It was subjected to oxidation, thermal, acid hydrolysis, alkali hydrolysis and photodegradation according to ICH guidelines. The major degradation products were separated from the pure drug and the proposed structures' elucidation was performed, using an LC–MS technique. Isocratic chromatographic elution was achieved on a Symmetry C_{18} column (150 x 4.6 mm, 5 mm), using a mobile phase of potassium dihydrogen phosphate buffer (pH 4.6)–acetonitrile–methanol (40: 30: 30, v/v/v) at a flow rate of 1 mL/ min21 with UV detection at 208nm. David W. Boulton *et al.* (2012) determined the absolute oral bioavailability ($F_{p.o.}$) of saxagliptin and dapagliflozin using simultaneous intravenous ^{14}C-microdose/therapeutic oral dosing (i.v.micro + oraltherap).

The $F_{p.o.}$ Values of saxagliptin and dapagliflozin were determined in healthy subjects (n = 7 and 8, respectively) following the concomitant administration of single i.v. micro doses with unlabelled oraltherap doses. Accelerator mass spectrometry and liquid chromatography-tandem mass spectrometry were used to quantify the labelled and unlabelled drug, respectively. Fura A, Khanna A *et al.* (2009) evaluated the pharmacokinetics of saxagliptin in rats, dogs, and monkeys and used to predict its human pharmacokinetics. Saxagliptin was rapidly absorbed and had good bioavailability (50-75%) in the species tested. The plasma clearance of saxagliptin was higher in rats (115 ml/min/kg) than in dogs (9.3 ml/min/kg) and monkeys (14.5 ml/min/kg) and was predicted to be low to moderate in humans. The plasma elimination half-life was between 2.1 and 4.4 h in rats, dogs, and monkeys, and both metabolism and renal excretion contributed to the overall elimination. The primary metabolic clearance pathway involved the formation of a significant circulating, pharmacologically active hydroxylated metabolite, M2. The volume of distribution values observed in rats, dogs, and monkeys (1.3-5.2 l/kg) and predicted for humans (2.7 l/kg) were greater than those for total body water, indicating extravascular distribution. The in vitro serum protein binding was low (< or =30%) in rats, dogs, monkeys, and humans. After intra-arterial administration of saxagliptin to Sprague-Dawley and Zucker diabetic fatty rats, higher levels of saxagliptin and M2 were observed in the intestine (a proposed major site of drug action) relative to that in plasma. Saxagliptin has prolonged pharmacodynamic properties relative to its plasma pharmacokinetic profile, presumably due to additional contributions from M2, distribution of saxagliptin and M2 to the intestinal tissue, and prolonged dissociation of both saxagliptin and M2 from DPP4. Katherine Esposito *et al.* (2015) developed a nomogram for estimating the glycated haemoglobin (HbA1c) response to different dipeptidyl peptidase-4 (DPP-4) inhibitors in type 2 diabetes. A systematic review and meta-analysis of randomised controlled trials (RCTs) of DPP-4 inhibitors (vildagliptin, sitagliptin, saxagliptin, linagliptin and alogliptin) on HbA1c were conducted. Electronic searches were carried out up to December 2013. Trials were included if they were carried out on participants with type 2 diabetes, lasted at least 12 weeks,

included at least 30 participants and had a final assessment of HbA1c. A random effect model was used to pool data. A nomogram was used to represent results of the metaregression model. Swapna Goday, Abdul Rahaman Shaik and Prameelarani Avula developed a new, rapid and sensitive LC-ESI –MS/MS method for the simultaneous estimation of Dapagliflozin and saxagliptin in human K_2EDTA plasma by Liquid –liquid Extraction method (LLE) using deuterated internal standards dapagliflozin (DGd2) and saxagliptin (SGd5). The Chromatographic separation was carried out on a reverse phase hypersil Gold C_{18} (50mm x 3.0mm, 5µm) column using mixture of 10 mM Ammonium acetate and methanol (20:80, v/v) at a flow rate of 0.5ml/min in isocratic mode. Quantification was achieved using an electro spray ion interface operating in positive mode, under multiple reaction monitoring (MRM) conditions. L. Sridhar *et al.* (2014) studied and identified the degradation behaviour of saxagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, under hydrolytic (acidic, alkaline, and neutral), oxidative, photolytic, and thermal stress conditions guidelines. The drug was found to be labile under hydrolytic and oxidative stress conditions, whereas it was stable under photolytic and thermolytic stress conditions.

A total of seven degradation products were identified, and their chromatographic separation was accomplished on a C_{18} column (100 × 4.6 mm; 5µm) using a mobile phase consisting of 10 mM ammonium formate and methanol in a gradient elution mode. All of the stressed samples were subjected to LC-MS, LC-MS/MS, and ESI-Q-TOF-MS/MS analysis. Saxagliptin and its Degradation Products were characterized based on elemental composition and isotopic distribution information from full scan mode and fragmentation patterns obtained from MS/MS and HRMS experiments. Structural elucidation of Degradation Products was achieved by comparing their fragmentation patterns with that of Saxagliptin. Shah PA *et al.* (2017) proposed a specific and rapid liquid chromatography-tandem mass spectrometry method for the simultaneous determination of metformin, saxagliptin and its active metabolite, 5-hydroxy saxagliptin in human plasma. Sample preparation was accomplished from 50 µL plasma sample by solid-phase extraction using sodium dodecyl sulfate as an ion-pair reagent. Reversed-phase chromatographic resolution of analytes was possible within 3.5 min on ACE 5CN (150 × 4.6 mm, 5 µm) column using acetonitrile and10.0 mm ammonium formate buffer, pH 5.0 (80:20, v/v) as the mobile phase. Triple quadrupole mass spectrometric detection was performed using electrospray ionization in the positive ionization mode. Blisse Vakkalagadda *et al.* (2015) compared the bioequivalence of saxagliptin/dapagliflozin 2.5/5 mg and 5/10 mg fixed-dose combination (FDC) tablets with coadministration of the individual tablets and the food effect on both strengths of saxagliptin/dapagliflozin FDCs were evaluated in this open-label, randomized, single-dose crossover study.

Healthy subjects were randomized to saxagliptin 2.5 mg + dapagliflozin 5 mg fasted, 2.5/5 mg FDC fasted, 2.5/5 mg FDC fed (Cohort 1) or saxagliptin 5 mg + dapagliflozin 10 mg fasted, 5/10 mg FDC fasted, 5/10 mg FDC fed (Cohort 2). Serial blood samples for pharmacokinetics of saxagliptin and dapagliflozin were obtained predose and up to 60 h postdose. Bioequivalence of FDC tablets versus individual components was concluded if the 90% CIs for FDC to individual component geometric mean ratios of C_{max}, AUC_{0-T}, and AUC_{inf} of both analytes were between 0.80 and 1.25. Seventy-two subjects were randomized; 71 (98.6%) completed the study. Saxagliptin/dapagliflozin 2.5/5 mg and 5/10 mg FDC tablets were bioequivalent to the individual tablets administered concomitantly. Food had no clinically meaningful effect on saxagliptin or dapagliflozin overall systemic exposure. Saxagliptin/dapagliflozin FDC tablets were bioequivalent to coadministration of the individual components in healthy subjects under fasted conditions and food had no clinically meaningful effect on bioavailability.

Conclusion

Most of the techniques like Spectrophotometric and chromatography- HPLC, HPTLC, GC and Liquid chromatography coupled with mass spectrometry methods, which were application to pharmacokinetic, dynamic, bioavailability, drug metabolism-disposition and bioequivalence studies were reported. The above cited methods for the determination of Saxagliptin in pure API, its marketed formulations like alone or combinations and also in biological matrices followed by various tissue extractions, the drug Saxagliptin was quantified. As far as we know there is not a single research paper which refers to the quantification of saxagliptin for the applicability of chemometric assisted method and quality by design (QBD). Hence there is still scope for the development of new selective and specific analytical techniques for quantisation of saxagliptin.

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