DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY METHODS FOR ESTIMATION OF ANTI DIABETIC DRUGS

Ph.D. Synopsis

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by

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1. Thesis Title and Abstract

Thesis Title: Development and Validation of stability indicating assay methods for estimation of Anti diabetic drugs.

Abstract:

Stability testing of drugs is mandated by regulatory bodies and health agencies of various countries across the globe. According to various regulatory requirements, validated stability-indicating analytical methods should be applied for stability testing. Stability-indicating analytical methods are needed for assurance of quality, safety and efficacy of drugs and pharmaceuticals. This is the basis for the present research work.

Now a days, number of people having diabetes is dramatically increasing, therefore number of antidiabetic drugs are available in market. So, antidiabetic drugs viz., Alogliptin Benzoate, Teneligliptin Hydrobromide Hydrate, Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride are selected for method development.

In present work, stability indicating HPTLC method, alkaline degradation kinetic study and stability indicating HPLC method for Alogliptin Benzoate were developed and validated. An acid degradation product for Alogliptin Benzoate was isolated and characterized. Stability indicating HPTLC method was developed, validated and oxidative degradation kinetic study was performed for Teneligliptin Hydrobromide. Stability indicating HPTLC method was developed and validated for simultaneous estimation of Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride. Dual wavelength and first order derivative method for estimation of Teneligliptin Hydrobromide Hydrate and Metformin Hydrochloride in their combined dosage form were developed and validated.

A comprehensive study of the stress degradation behaviour of Alogliptin Benzoate was carried out in accordance with ICH guidelines. The degradation products of Alogliptin Benzoate were successfully separated by a developed simple, selective, and precise stability-indicating HPTLC method. It was validated and applied for determination of Alogliptin Benzoate in synthetic mixture. The validated proposed HPTLC method for Alogliptin Benzoate was extended for degradation kinetic study in alkaline medium. Alkaline degradation kinetic study of Alogliptin Benzoate in 0.1 N, 0.5 N and 1.0 N NaOH at 40°C, 50°C and 60°C by HPTLC reveals that it follows first order reaction. Degradation at 45°C and 55°C was predicted using Response surface methodology.

A simple, selective, precise and cost effective stability indicating RP-HPLC method for estimation of Alogliptin Benzoate was developed and validated. A comprehensive study of the stress degradation behaviour of Alogliptin Benzoate was carried out in accordance with ICH guidelines. The degradation products of Alogliptin Benzoate were successfully separated by a developed method.

Degradation product in acid hydrolytic condition was isolated and identified by NMR, mass and IR analysis. The chemical formula of degradation product is $C_{13}H_{11}N_3O_3$ and chemical name is 2-((3-methyl-2, 4, 6-trioxotetrahydropyrimidin-1-(2H)yl}methyl)benzonitrile.

A comprehensive study of the stress degradation behaviour of Teneligliptin Hydrobromide Hydrate was carried out in accordance with ICH guidelines. The degradation products of Teneligliptin Hydrobromide Hydrate were successfully separated by a developed simple, selective and precise stability-indicating HPTLC method. It was validated and applied for determination of Teneligliptin Hydrobromide Hydrate in marketed formulations.

Oxidative degradation kinetic study of Teneligliptin Hydrobromide Hydrate in 1%, 2% and 3% H_2O_2 at 40°C, 50°C and 60°C by HPTLC reveals that it follows first order reaction.

A stability indicating HPTLC method for simultaneous estimation of Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride in their combined dosage form. A comprehensive study of the stress degradation behaviour of Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride was carried out in accordance with ICH guidelines. The degradation products of Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride were successfully separated by a developed simple, selective and precise stability-indicating HPTLC method. It was validated and applied for determination of Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride in their combined marketed formulation.

Two simple and precise spectrophotometric methods (dual wavelength and first order derivative method) for estimation of Teneligliptin Hydrobromide Hydrate and Metformin Hydrochloride in their combined dosage form were developed and validated as per ICH guidelines. It was applied for determination of Teneligliptin Hydrobromide Hydrate and Metformin Hydrochloride in their combined dosage form.

2. **Brief description on the state of the art of the research topic:**

METHOD DEVELOPMENT:

The number of drugs introduced into the market are increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals.

Basic criteria for new method development of drug analysis:

- The drug or drug combination may not be official in any pharmacopoeias,
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations,
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,
- Analytical methods for the quantitation of the drug in biological fluids may not be available,
- Analytical methods for a drug in combination with other drugs may not be available,
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable

METHOD VALIDATION:

Analytical method validation is carried out to demonstrate that it is suitable for its intended purpose. Validation is documented evidence, which provide a high degree of assurance for specific method. Variables like different elapsed assay times, different instruments, days, reagents lots, equipment, environmental conditions like temperature, humidity etc. may influence any developed method. So it is recommended that once the method has been developed and before it is communicated or transferred from one lab to the other, it is properly validated and the result of validity tests should be reported.

When Method validation required:

- A new method is being developed
- When established methods are used by different analysts and in different laboratories
- Comparison of methods

- Revision of established method
- When quality control indicates method changes

STRESS TESTING:

Degradation studies of the drug substance include both appropriate solution and solid-state stress conditions (e.g., acid/base hydrolysis, oxidation, heat, humidity, and light exposure in accordance with ICH guidelines).

Applied stress conditions should result in approximately 10–20 % degradation of the drug substance or represent a reasonable maximum condition achievable for the drug substance. In case, if no degradation is observed under the specified stress conditions, it is recommended to stop stress testing.

DEGRADATION KINETICS STUDY:

Principles of Kinetic are virtually important in stability study of drug substances and dosage form. For development of stable formulation, study of drug degradation kinetics is of greater importance. Establishment of expiration date for commercially available drug products requires knowledge of degradation kinetics.

degradation kinetic studies provides the information regarding the rate of process that generally leads to the inactivation of drug through either decomposition or loss of drug by conversion to a less favourable physical or chemical form. The kinetic studies and stability studies are not identical but they are different in following ways, chemical kinetics is studies through half-lives, while Stability studies down up to 85% of the initial strength. Stability study system contains relatively many components, while Chemical kinetics is carried out in pure system. The main purpose of stability study is to establish expiration date, where as that of chemical kinetics is to elucidate reaction mechanism.

3. Definition of the Problem

Literature review revealed few analytical methods i.e. HPTLC, HPLC, stability indicating HPTLC, stability indicating HPLC and UV spectrophotometric for an individual drug and in combination with other drugs. However, the reported analytical methods are not adequate to monitor degradation kinetics and to demonstrate the degradation pattern of the drugs in various stress conditions.

So it was thought to develop and validate stability indicating HPLC/ HPTLC methods estimate selected drugs (Alogliptin, Teneligliptin and Dapagliflozin-Metformin combination) and

validated as per ICH guidelines. The developed methods, will also extended to study degradation kinetics of drugs with different stress conditions.

Stability studies and degradation kinetics are integral parts of the quality control of a drug on an industrial scale. Degradation kinetics is the study of the rate at which degradation occur. It is useful to predict shelf life period of the medicine and it gives an insight into the mechanisms of changes involved. International Conference on Harmonization (ICH) guideline stipulates that the stability of active drug substances must be assessed. These facts are the base of this research work.

4. Research Objectives

Part- A: Alogliptin Benzoate

- To develop and validate Stability indicating HPTLC method for estimation of Alogliptin.
- To apply HPTLC method for estimation of Alogliptin in synthetic mixture.
- To study alkaline degradation kinetic of Alogliptin by Stability indicating HPTLC method.
- To develop and validate stability indicating HPLC method for estimation of Alogliptin.
- To apply HPLC method for estimation of Alogliptin in synthetic mixture.
- To Isolate and identify structure of a degradation product of acid hydrolysis of Alogliptin.

Part- B: Teneligliptin Hydrobromide Hydrate

- To develop and validate Stability indicating HPTLC method for estimation of Teneligliptin.
- To apply HPTLC method for estimation of Teneligliptin in pharmaceutical dosage form.
- To study oxidative degradation kinetic of Teneligliptin by stability indicating HPTLC method.

Part- C: Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride

- To develop and validate stability indicating HPTLC method for simultaneous determination of Dapagliflozin and Metformin.
- To apply HPTLC method for determination of Dapagliflozin and Metformin in their combined pharmaceutical dosage forms.

Part- D: Teneligliptin Hydrobromide Hydrate and Metformin Hydrochloride

- To develop and validate dual wavelength method for estimation of Teneligliptin and Metformin.
- To develop and validate first order derivative method for estimation of Teneligliptin and Metformin.
- To apply dual wavelength and first order derivative method for estimation of Teneligliptin and Metformin in pharmaceutical dosage form.

5. Scope of work

Pharmaceutical research in the last few decades has resulted in the launch of numerous drugs. The advanced and extremely potent drugs have found their applicability in treating various types of diseases. Stability of drug substances is influenced by a variety of environmental factors. Stability testing provides information about potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and excipient in the drug product. Stability studies should include testing of those attributes of the drug substance that are susceptible to change during storage and are likely to influence quality, safety and/or efficacy. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. Stability-indicating analytical methods are needed for assurance of quality, safety and efficacy of drugs and pharmaceuticals.

In present work stability indicating methods for testing of anti-diabetic drugs were developed and validated. The developed methods are also extended to study degradation kinetics of drugs with different stress conditions.

6. Original contribution by the thesis.

This research is unique and original since similar work has not been carried out previously. This research will:

- Provide stability indicating methods for Alogliptin, which can be used for analysis of marketed formulations to ensure that marketed formulations are stable with in expiry date period.
- From alkaline degradation kinetic study of Alogliptin, degradation rate constant, half-life and shelf-life for Alogliptin was calculated.
- Degradation product from acid degradation condition was isolated and identified.

- Provide stability indicating method for Teneligliptin, which can be used for analysis of marketed formulations to ensure that marketed formulations are stable with in expiry date period.
- From oxidative degradation kinetic study of Teneligliptin, degradation rate constant, half-life and shelf-life for Teneligliptin calculated.
- Provide stability indicating method for simultaneous estimation of Dapagliflozin and Metformin, which can be used for analysis of marketed formulations to ensure that marketed formulations are stable with in expiry date period.
- Provide spectrophotometric methods for simultaneous estimation of Teneligliptin and Metformin in their marketed formulations.

7. Methodology of Research, Results / Comparisons

Part A: Alogliptin Benzoate

Section I: Development and validation of Stability indicating HPTLC method for estimation of Alogliptin

Optimized Chromatographic Conditions are as follows:

Stationary phase	Pre-coated silica gel G60 – F_{254} aluminium sheet (E. Merck, Germany) (100×100 mm, thickness layer 0.2 mm)
Mobile phase	Acetic acid : water : n-butanol (1:2:7) v/v/v
Chamber saturation time	15 min at room temperature (25 ± 2°C)
Distance run	8.0 cm
Detection	233 nm
Measurement mode	Absorbance
Source lamp	Deuterium
Slit dimension	6 mm × 0.45 mm
Syringe capacity	100 µL
Band width	6 mm
Distance from the plate edge	15 mm
Distance from the bottom of the plate	15 mm

Forced degradation of Alogliptin was carried out under acidic, alkaline, oxidative, photolytic, dry heat and neutral conditions. All the degradant peaks were well separated from the main

peak. The linear regression analysis data for the calibration plots showed a good linear relationship with R^2 =0.9941 in the concentration range of 200-600 ng/spot for Alogliptin. The developed procedure was evaluated for the specificity, linearity, accuracy, precision, limit of detection and limit of quantification. It was proved that method is specific, linear, precise, accurate and stability indicating. Developed Method was successfully applied for estimation of Alogliptin in synthetic mixture. Hence, the method is useful for routine quality control analysis and also for determination of stability.

Section II: Alkaline degradation kinetic study of Alogliptin Benzoate by stability indicating HPTLC method

With use of developed HPTLC method, Alkaline Degradation kinetic study of Alogliptin was performed in 3 different strengths of NaOH at different temperature conditions.

1. In 0.1 N, 0.5 N and 1.0 N NaOH at 40 ± 2°C for 150 mins.
2. In 0.1 N, 0.5 N and 1.0 N NaOH at 50 ± 2 °C for 150 mins.
3. In 0.1 N, 0.5 N and 1.0 N NaOH at 60 ± 2 °C for 150 mins.

Samples were withdrawn at every 15 min. interval up to 150 mins. an appreciable amount of Alogliptin was degraded. From the data of degradation kinetic study, degradation rate constant, half-life and shelf-life for Alogliptin was calculated. The data obtained at various time intervals fits in to the equation of first order rate kinetics.

i.e. $k = [2.303 \times \log (C_0/C)]/t$

Where, t = Time

C_0 = Initial concentration

C = Concentration remaining after time interval

3^2 level experimental design (2 factor- 3 level) were constructed for the response surface prediction to obtain degradation data at different temperatures and NaOH strengths using the Design expert software.

Degradation rate constant, half-life, Shelf life for Alogliptin were predicted at 45°C and 55°C from contour plots.

Section III: Development and validation of Stability indicating HPLC method for estimation of Alogliptin Benzoate

Optimized Chromatographic Conditions are as follows:

Stationary phase C18, MG-CAPCELL PAK 250mm x 4.6mm, 5 µm

Mobile phase	water: acetonitrile (80:20 v/v)
pH	adjusted to 4.5 using 2% o-phosphoric acid
Flow rate	1.0 ml/min
Temperature	25 ± 2°C
Wavelength	277 nm
Total run time	8 minutes

Forced degradation of Alogliptin was carried out under acidic, alkaline, oxidative, photolytic, dry heat and neutral conditions. All the degradant peaks were well separated from the main peak. The linear regression analysis data for the calibration plots showed a good linear relationship with R^2 =0.9991 in the concentration range of 10-60 µg/ml for Alogliptin. The developed procedure was evaluated for the linearity, accuracy, precision, limit of detection and limit of quantification. It was proved that method is specific, linear, precise, accurate and stability indicating. Developed Method was successfully applied for estimation of Alogliptin in synthetic mixture. Hence, the method is useful for routine quality control analysis and also for determination of stability.

Section IV: Isolation and structural identification of a degradation product of acid hydrolysis of Alogliptin Benzoate

Degradation product of Alogliptin from acid stress condition was isolated with use of preparative TLC plates. The collected degradation product was studied for NMR, Mass and IR characterization. From the data of NMR, Mass and IR analysis, it was found that Alogliptin is probably hydrolysed in acidic condition and converted in to 2-(3-methyl-2, 4, 6-trioxotetrahydropyrimidin-1-(2H)yl}methyl) benzonitrile, with chemical formula $C_{13}H_{11}N_3O_3$ and molecular weight 257.25.

Possible mechanism of acid degradation of Alogliptin and structure of acid degradation product is shown in below figure. 1.

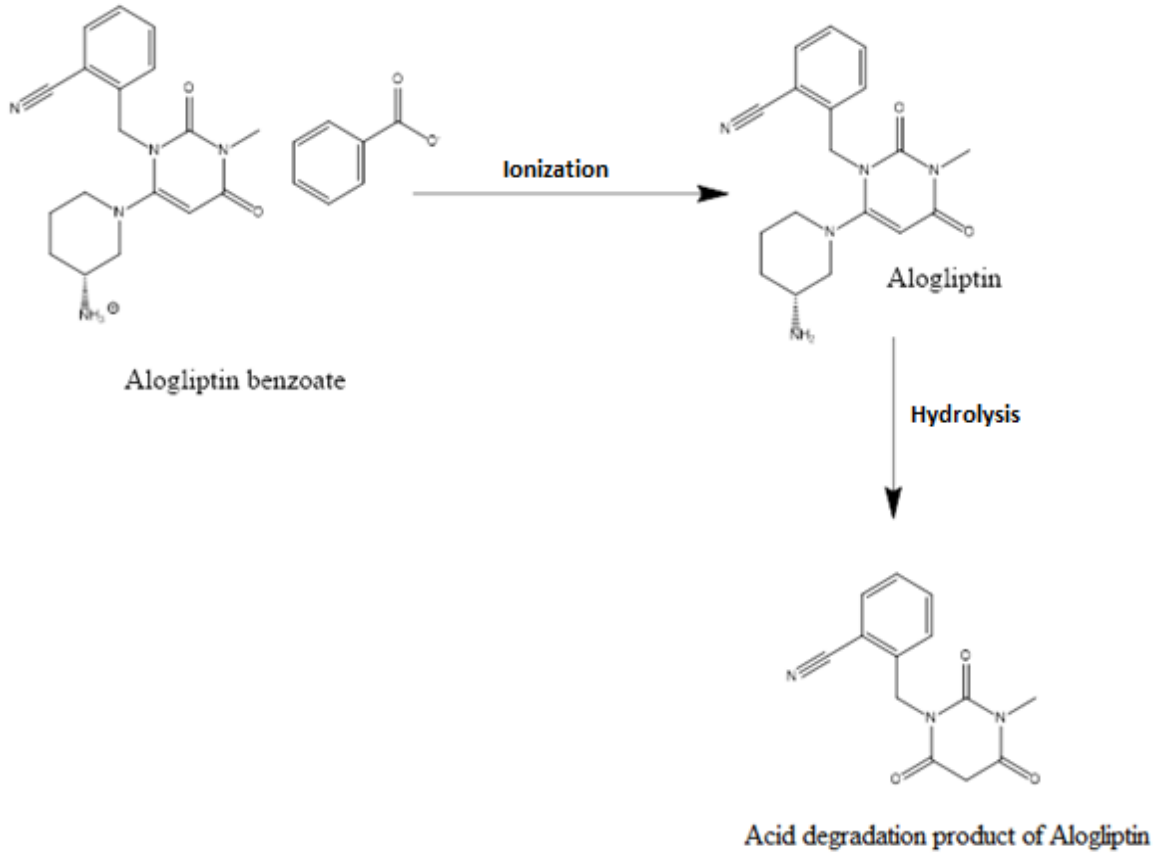

Figure 1: Possible mechanism of acid degradation of ALO

Part- B: Teneligliptin Hydrobromide Hydrate

Section I: Development and validation of stability indicating HPTLC method for estimation of Teneligliptin Hydrobromide Hydrate

Optimized Chromatographic Conditions are as follows:

Stationary phase	Pre-coated silica gel G60 – F_{254} aluminium sheet (E. Merck, Germany) (100×100 mm, thickness layer 0.2 mm)
Mobile phase	toluene : ethanol : diethyl amine (6:3:1) v/v/v
Chamber saturation time	20 min at room temperature (25 ± 2 °C)
Distance run	8.0 cm
Detection	244 nm
Measurement mode	Absorbance
Source lamp	Deuterium

Slit dimension	6 mm × 0.45 mm
Syringe capacity	100 µL
Band width	6 mm
Distance from the plate edge	15 mm
Distance from the bottom of the plate	15 mm

Forced degradation of Teneligliptin was carried out under acidic, alkaline, oxidative, photolytic, dry heat and neutral conditions. All the degradant peaks were well separated from the main peak. The linear regression analysis data for the calibration plots showed a good linear relationship with $R^2 = 0.9988$ in the concentration range of 200-1200 ng/spot for Teneligliptin. The developed procedure was evaluated for the specificity, linearity, accuracy, precision, limit of detection and limit of quantification. It was proved that method is specific, linear, precise, accurate and stability indicating. Developed Method was successfully applied for estimation of Teneligliptin in marketed formulations. Hence, the method is useful for routine quality control analysis and also for determination of stability.

Section II: Oxidative degradation kinetic study of Teneligliptin Hydrobromide Hydrate by stability indicating HPTLC method

With use of developed HPTLC method, Oxidative degradation kinetic study of Teneligliptin was performed in three different strengths of hydrogen peroxide (for oxidative degradation) each at 40°C, 50°C and 60°C.

In 1%, 2% and 3% H_2O_2 at 40 ± 2°C for 60 mins

In 1%, 2% and 3% H_2O_2 at 50 ± 2°C for 60 mins

In 1%, 2% and 3% H_2O_2 at 60 ± 2°C for 60 mins

Samples were withdrawn at every 10 min. interval up to 60 mins an appreciable amount of Teneligliptin was degraded. From the data of degradation kinetic study, degradation rate constant, half-life and shelf-life for Teneligliptin was calculated. The data obtained at various time intervals fits in to the equation of first order rate kinetics.

i.e. $k = [2.303 \times \log (C_0/C)]/t$

Where, t = Time

C_0 = Initial concentration

C = Concentration remaining after time interval

Part- C: Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride

Section: I Development and validation of stability indicating HPTLC method for simultaneous estimation of Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride

Optimized Chromatographic Conditions are as follows:

Stationary phase	Pre-coated silica gel G60 – F_{254} aluminium sheet (E. Merck, Germany) (100×100 mm, thickness layer 0.2 mm)
Mobile phase	Methanol: ethyl acetate: ammonium acetate (6: 4: 0.1 v/v/v)
Chamber saturation time	15 min at room temperature (25 ± 2°C)
Distance run	8.0 cm
Detection	220 nm
Measurement mode	Absorbance
Source lamp	Deuterium
Slit dimension	6 mm × 0.45 mm
Syringe capacity	100 µl
Band width	6 mm
Distance from the plate edge	15 mm
Distance from the bottom of the plate	15 mm

Forced degradation of Dapagliflozin and Metformin was carried out under acidic, alkaline, oxidative, photolytic, dry heat and neutral conditions. All the degradant peaks were well separated from the main peaks. The linear regression analysis data for the calibration plots showed a good linear relationship with $R^2 = 0.9985$ in the concentration range of 20-100 ng/spot for Dapagliflozin and $R^2 = 0.9984$ in the concentration range of 500-2500 ng/spot for Metformin. The developed procedure was evaluated for the specificity, linearity, accuracy, precision, limit of detection and limit of quantification. It was proved that method is specific, linear, precise, accurate and stability indicating. Developed Method was successfully applied for estimation of Dapagliflozin and Metformin in marketed formulations. Hence, the method is useful for routine quality control analysis and also for determination of stability.

Part- D: Teneligliptin Hydrobromide Hydrate and Metformin Hydrochloride

Section: I Development and validation of dual wavelength method for estimation of Teneligliptin Hydrobromide Hydrate and Metformin Hydrochloride in combined dosage form.

Developed Spectrophotometer parameters are as under:

Detection:	230.5 nm (λ_1) and 236 nm (λ_2) (for estimation of Teneligliptin)
	238 nm (λ_3) and 249 nm (λ_4) (for estimation of Metformin)
Cell path length:	1 cm
Diluent:	methanol
Conc. range:	5- 25 µg/ml for Teneligliptin
	2-10 µg/ml for Metformin

Dual wavelength method for estimation of Teneligliptin and Metformin in combined dosage form was developed and validated.

Teneligliptin and Metformin were detected at 230.5 nm and 236 nm respectively. The linear regression analysis data for the calibration plots showed a good linear relationship with R^2 =0.9929 in the concentration range of 5-25 µg/ml for Teneligliptin and R^2 =0.9998 in the concentration range of 2-10 µg/ml for Metformin. The developed procedure was evaluated for the specificity, linearity, accuracy, precision, limit of detection and limit of quantification. It was proved that the method is specific, linear, precise, and accurate. Developed method was successfully applied for estimation of Teneligliptin and Metformin in marketed formulation. Hence, the method is useful for routine quality control analysis.

Section: II Development and validation of first order derivative method for estimation of Teneligliptin Hydrobromide Hydrate and Metformin Hydrochloride in combined dosage form.

Developed Spectrophotometer parameters are as under:

Detection:	216 nm (for estimation of Teneligliptin)
	243.5 nm (for estimation of Metformin)
Cell path length:	1 cm
Diluent:	methanol
Conc. range:	5- 25 µg/ml for Teneligliptin
	2-10 µg/ml for Metformin

First order derivative method for estimation of Teneligliptin and Metformin in combined dosage form was developed and validated**.** Teneligliptin and Metformin were detected at 216 nm and 243.5 nm respectively. The linear regression analysis data for the calibration plots showed a good linear relationship with R^2 =0.9951 in the concentration range of 5 -25 µg/ml for Teneligliptin and R^2 =0.9993 in the concentration range of 2-10 µg/ml for Metformin. The developed procedure was evaluated for the specificity, linearity, accuracy, precision, limit of detection and limit of quantification. It was proved that method is specific, linear, precise, and accurate. Developed method was successfully applied for estimation of Teneligliptin and Metformin in marketed formulation. Hence, the method is useful for routine quality control analysis.

8. Achievements with respect to objectives

A stability indicating HPTLC method for estimation of Alogliptin was developed and validated. From stress testing of Alogliptin, it was found to be degrading in acidic and alkaline stress conditions. The method was extended for degradation kinetic study of Alogliptin in alkaline medium. Also, a stability indicating HPLC method for estimation of Alogliptin developed and validated. From stress testing of Alogliptin, it was found to be degrading in acidic and alkaline stress conditions. Alogliptin is found to hydrolysed in acidic condition and converted in to 2-(3-methyl-2, 4, 6-trioxotetrahydropyrimidin-1-(2H)yl}methyl) benzonitrile, with chemical formula $C_{13}H_{11}N_3O_3$ and molecular weight 257.25.

A stability indicating HPTLC method for estimation of Teneligliptin was developed and validated. From stress testing of Teneligliptin, it was found to be degrading significantly in alkaline, oxidative, photolytic and thermal stress conditions. The method was extended for degradation kinetic study of Teneligliptin in oxidative medium.

A stability indicating HPTLC method for simultaneous estimation of Dapagliflozin and Metformin was developed and validated. From stress testing, Dapagliflozin was found to be significantly degrading in acid, alkaline, oxidative, photolytic and thermal degradation conditions, while Metformin was found to be significantly degrading in acid and alkaline degradation conditions.

Two simple, specific, accurate and precise spectrophotometric methods (Dual wavelength method and first order derivative method) were developed and validated for estimation of Teneligliptin and Metformin in their combined dosage form.

9. Conclusion

Part- A: Alogliptin Benzoate:

A simple, specific, accurate and precise stability indicating HPTLC method for estimation of Alogliptin was developed and validated. The method was able to estimate Alogliptin accurately in presence of its degradation products. The method was validated as per ICH guidelines. The validated proposed HPTLC method for Alogliptin was extended for degradation kinetic study of Alogliptin in alkaline medium. Alkaline degradation of Alogliptin follows first order kinetics. Degradation rate of Alogliptin increases as strength of NaOH and temperature or both increases.A simple, specific, accurate and precise stability indicating HPLC method for estimation of Alogliptin was developed and validated. The method was able to estimate Alogliptin accurately in presence of its degradation products. The method was validated as per ICH guidelines. The acid degradation product of Alogliptin is probably 2-(3-methyl-2, 4, 6-trioxotetrahydropyrimidin-1-(2H)yl}methyl) benzonitrile, with chemical formula $C_{13}H_{11}N_3O_3$ and molecular weight 257.25.

Part- B: Teneligliptin Hydrobromide Hydrate

A simple, specific, accurate and precise stability indicating HPTLC method for estimation of Teneligliptin was developed and validated. The method was able to estimate Teneligliptin accurately in presence of its degradation products. The method was validated as per ICH guidelines. The validated proposed HPTLC method for Teneligliptin was extended for degradation kinetic study of Teneligliptin in oxidative medium. Oxidative degradation of Teneligliptin follows first order kinetics. Degradation rate of Teneligliptin increases as strength of Hydrogen Peroxide and temperature or both increases.

Part- C: Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride

A simple, specific, accurate and precise stability indicating HPTLC method for simultaneous estimation of Dapagliflozin and Metformin was developed and validated. The method was able to estimate Dapagliflozin and Metformin accurately in presence of their degradation products. The method was validated as per ICH guidelines.

Part- D: Teneligliptin Hydrobromide Hydrate and Metformin Hydrochloride

Two simple, specific, accurate and precise spectrophotometric methods (Dual wavelength method and first order derivative method) were developed and validated for estimate Teneligliptin and Metformin in their combined dosage form. The method was validated as per ICH guidelines.

10. List of published papers

Sr. No.	Title of Paper	Details of Journal / Conference Proceeding	ISSN / ISBN No.	Month & Year of Publication
1	Study of Degradation Behavior of Alogliptin Benzoate by Stability Indicating High Performance Thin Layer Chromatographic Method	Asian journal of chemistry	0975-427X	May- 2017
2	Development and validation of dual wavelength UV spectrophotometric method for estimation of Teneligliptin Hydrobromide Hydrate and Metformin Hydrochloride in combined dosage form	Pharma Science Monitor	0976-7908	Apr-Jun 2018

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