"STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT, VALIDATION AND ESTIMATION OF SOME ANTIDIABETIC DRUGS IN MARKETED FORMULATIONS"

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1. Title: Stability Indicating Analytical Method Development, Validation and Estimation of Some Antidiabetic Drugs in Marketed Formulations

2. Abstract:

Diabetes is a common disease that affects a large no. of the population. So, newer treatment options are available for it. In recent years, newer formulations have been available in the market which are approved by the FDA or CDSCO. These FDCs will reduce the cost of therapy as well as improve glycaemic control reducing insulin resistance within a short time which is advantageous over previously approved antidiabetic formulations. Analytical methods are useful for the identification of % of drugs present in the drug and also helpful for the estimation of degradants produced during different stress conditions like acid, base, oxidation, photolysis and temperature. Many analytical methods can be used for this like HPLC, UV, HPTLC, etc. Mostly RP-HPLC method is used due to its sensitivity, accuracy, precision, rapidity, and quantitative estimation of components that are present in low-concentration. The present research work reveals stability indicating RP-HPLC method development for the estimation of some anti-diabetic formulations that have been recently approved. These methods are also validated as per ICH guidelines.

3. Introduction:[1]

Diabetes is a condition in which a patient has a high blood sugar level. It is produced when the body is not reacting to the effects of insulin appropriately or when the pancreas produces very little or no insulin at all. Any age people can be affected by diabetes. This disease can be treated by medicine and /or lifestyle modifications, but mostly it is a chronic disease. Basically, anti-diabetic drugs can be categorized into two classes:

A. Oral anti-diabetic drugs: This includes the following classes: sulphonylureas and non-sulphonylureas (Glinides/Meglitinide), Biguanides, Thiazolidinediones, α-glucosidase inhibitors, Di-peptidyl Peptidase-4 (DPP-4) inhibitors/gliptins, Sodium-glucose co-transporter 2 (SGLT2) inhibitors

B. Injectable anti-diabetic drugs: Insulin preparations Glucagon-like peptide 1 (GLP1) agonists.

- Metformin hydrochloride is the medication of choice for all type-2 diabetes mellitus unless it is contraindicated or intolerable.

- Remogliflozin etabonate contains a specific inhibitory effect on SGLT-2 and decreases the amount of blood glucose level which is excreted through urine and insulin re-absorption and reduces blood glucose levels.
- Vildagliptin decreases blood glucose levels by selective inhibition of dipeptidyl peptidase-4 (DPP-4), an enzyme that rapidly inactivates GLP-2. It also reduces HbA1c and fasting glucose. It enhances glucose-dependent secretion and also sensitivity of alpha and beta cells to glucose increases by it. Postprandial and fasting glucose levels are reduced by it.
- Teneligliptin works by raising levels of incretin (GLP-1 and GIP), which blocks the release of glucagon. This reduces blood glucose, increases insulin secretion, and delays stomach emptying.
- Pioglitazone is a thiazolidinedione antidiabetic drug used to treat diabetes mellitus type-2. It can be combined with other anti-diabetic drugs also. It can reduce hyperinsulinemia, hypertriglyceridemia and hyperglycaemia.

4. Brief description on the state of the research topic

- Pharmaceutical analysis is a part of chemistry that involves a series of processes for the estimation, purification, identification and quantification of a compound, separation of different components in a mixture or the determination of the structure of chemical compounds.
- Probably the versatile and most powerful analytical method available is chromatography. It is defined as “one type of separation process achieved by the distribution of substances between two phases, a stationary phase and a mobile phase.” Solutes move rapidly if they are more distributed in the mobile as compared to the stationary phase. So, the solutes will elute based on their increasing distribution coefficient concerning the stationary phase.
- HPLC is the most widely used analytical separation method. Due to more reliable columns, new pumping methods and a variety of sensitive detectors HPLC can be used for the separation of most of drugs.
- Reverse phase HPLC is the most widely used mode of chromatography for the separation of almost 90% of all analytes of low molecular weight samples. It is more popular due to its ease of use in varying retention and selectivity, as well as its capacity to distinguish between extremely similar molecules.

- A stability-indicating assay method (SIAM) is a "Quantitative analytical technique used to estimate a decrease in the concentration of the active pharmaceutical ingredient available due to the degradation."
- Why stability-indicating methods are required for the analysis of drugs?
  - When specificity is determined in SIAM, then it is also referred to as a forced degradation study which can be used to give data about degradation pathways and products that may be produced during storage.
  - API is stressed in solutions or solid form and produces the sample that contains the components most likely to form during storage, which is analyzed for SIAM.
  - The main reason for the SIAM is to get baseline resolution of all the resulting products like API and all degradants with no co-elutions.
  - Basically, the purpose of these studies is to degrade the API by 5-10%. Greater than this may destroy relevant compounds or irrelevant degradants may produce.

5. Definition of the problem:

- Safety and efficacy of any drug formulation are affected by the chemical stability of the molecule. Information about the selection of proper formulation, package, shelf life and storage condition can be obtained from the stability of a molecule. This data is also required for the regulatory documentation. A stability study should be performed before filing the registration dossier.
- International Conference on Harmonisation (ICH) guidelines, make it essential to organize the forced degradation studies and it is mandated to perform forced degradation of new drug products.
- The forced degradation study gives information to support the detection of potential degradants. It also illustrates the degradation pathway for pharmaceutically active components. Probable polymorphic or enantiomeric substances and variation between drug-related degradation and excipient interferences can also be evaluated by forced degradation studies. ICH guidelines are mandatory and require forced degradation studies under a range of conditions, like pH, light, oxidation, dry heat, etc. Moreover, it provides the separation of drugs from degradation products. The FDA and ICH guidance mandate the requirement of forced degradation to recognize how the quality of a drug substance and drug product varies with time and different environmental factors. [2-8]

- According to FDA guidance, phase III of the regulatory submission process is the prominent time for these studies. To establish the stability of the drug substance forced degradation studies should be done in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels. The forced degradation studies are conducted on a single batch. The outcomes should be summarized and submitted in an annual report.
- The developed and validated analytical method permits the analysis of each degradation product. Unfortunately, there is less guidance available to establish true selective forced degradation methods. Appropriate experimental conditions for forced degradation studies (temperatures, duration, and extent of degradation, etc.) are not specified properly. This research provides an overview of forced degradation under a variety of environmental factors for different recently approved anti-diabetic formulations.
- Outcomes of forced degradation studies

Forced degradation studies offer the following information:

a) Determination of likely degradants,
b) Determination of degradation pathways,
c) Determination of intrinsic stability of the drug molecule,
d) Determination of validated stability-indicating analytical methods.

6. Objective and scope of work: [7-18]

1] To develop a novel analytical technique for the simultaneous estimation of anti-diabetic medications in selected combinations and validate them according to ICH guidelines for insightful application.
2] To conduct stability analysis of drugs in selected formulations.
3] To develop stability indicating method for Remogliflozin etabonate and Metformin hydrochloride in single and tablet dosage form by RP-HPLC.
4] Development and validation of an analytical method for simultaneous estimation of Remogliflozin etabonate and metformin hydrochloride in bulk and tablet dosage form by RP-HPLC.
5] To develop a method for the study of the degradation behaviour of Remogliflozin etabonate, Vildagliptin and Metformin hydrochloride in single and tablet formulation by RP-HPLC.

6] Development and validation of an analytical technique for quantitative estimation of Remogliflozin etabonate, Vildagliptin and Metformin hydrochloride in bulk and tablet formulation by RP-HPLC.

7] To conduct stability analysis of Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride and Metformin hydrochloride in single and tablet formulation by RP-HPLC.

8] Development and validation of a quantitative method for simultaneous estimation of Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride and Metformin hydrochloride in bulk and tablet dosage form by RP-HPLC.

9] To perform a statistical comparison between the developed methodology and the previously developed method.

7. Original contribution by the thesis:

The complete research in this synopsis is original. A narrative review was done to check whether analytical methods were developed for selected formulations or not. The stability of selected drugs in their bulk form and their combined dosage form at different stress conditions have been performed and analyzed by an accurate, sensitive and inexpensive validated RP-HPLC method which can be useful for the estimation of another new formulation also.

8. Methodology of Research and Results/Comparisons:

8.1 "Stability-indicating analytical method development and validation for simultaneous estimation of Remogliflozin etabonate and Metformin hydrochloride in bulk and marketed formulations"

A simple efficient stability-indicating validated RP-HPLC method has been developed for the simultaneous determination of Metformin hydrochloride (MET) and Remogliflozin etabonate (REM) in bulk and was applied to marketed formulations. The mobile phase used for detection was Phosphate Buffer (pH 4.0): Acetonitrile (60:40%v/v). Drug peaks were detected by a UV detector at 226nm.

8.1.1 Forced degradation study

Different Stress conditions of acid (0.1 N HCl- 2 mL for 2 hr at room temperature), base

(0.1 N NaOH- 2 mL for 30 mins at room temperature), oxidation (3% hydrogen peroxide- 2 mL for 3 hrs at room temperature), photolytic stress (UV chamber for 24 hrs), heat (exposed at 80°C for 5 hrs) were applied on solution containing 40 and 8 µg/mL MET and REM individually, in standard stock solution and sample stock solution. After completion of degradation, solutions were injected and the chromatograms were monitored under optimized conditions and % degradation was calculated shown in Table 1.

Table 1: Summary of % Degradation for Metformin hydrochloride (MET) and Remogliflozin etabonate (REM) in formulation

Type of Degradation	Conditions of Degradation	% Degradation	
		MET	REM
Acid	0.1 N HCl for 2 hrs at room temperature	20.89	21.39
Alkali	0.1 N NaOH for 30 mins at room temperature	22.45	18.43
Oxidative	3 % H_2O_2 for 3 hrs at room temperature	19.49	18.35
Thermal	80℃ for 5 hrs.	12.69	12.46
Photolytic	UV chamber for 24 hrs	13.31	11.25

8.1.2 Analytical method validation

It is performed as per ICH guidelines and different parameters like accuracy, Precision, Linearity, Range, LOD, LOQ, Robustness and Specificity are checked for the developed stability indicating method. The linearity of the developed method was found to be 20-60µg/mL for Metformin hydrochloride and 4-12µg/mL for Remogliflozin etabonate. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were found to be 0.085µg/mL and 0.258µg/mL for MET and 0.010µg/mL and 0.030µg/mL for REM respectively. The %R.S.D. value was found to be less than 2 in precision and robustness which proved that the developed method was precise and robust. The validation parameters were summarized in Table 2.

Table 2: Summary of validation parameters

Sr. No.	Parameters	MET	REM
1	**Linearity Range (µg/mL)**	20 - 60	4 - 12

2	**Regression Equation (y = mx + c)**	y = 126.32x - 0.4126	y = 514.11x – 1.2354
3	**Slope (m)**	126.32	514.11
4	**Intercept (c)**	- 0.4126	- 1.2354
5	**Correlation coefficient (R^2)**	0.9998	0.9997
6	**LOD (µg/mL)**	0.085	0.010
7	**LOQ (µg/mL)**	0.258	0.030
8	**Asymmetry ± RSD**	1.414 ± 0.114	1.260 ± 0.162
9	**Theoretical Plates ± RSD**	7211 ± 0.14	7849 ± 0.24
10	**Retention time ± RSD**	4.560 min ± 0.004	7.793 min ± 0.001
11	**Resolution ± RSD**	11.303 ± 0.14	
12	**Assay (% of label claim) Mean ± S. D.**	100.08 ± 0.123	100.12 ± 0.118

8.2 "Stability-indicating analytical method development and validation for simultaneous estimation of Metformin hydrochloride, Vildagliptin and Remogliflozin etabonate in bulk and marketed formulations"

A simple and efficient stability-indicating method has been developed and validated for the simultaneous estimation of vildagliptin, metformin hydrochloride and remogliflozin etabonate in bulk and was applied on marketed formulations. KH_2PO_4 buffer (10 mM): Acetonitrile (70:30% v/v) at pH 5 was used as a mobile phase. Detection of drug peaks was at 215 nm by UV detector.

8.2.1 Forced degradation study

Different Stress conditions of acid hydrolysis (5 mL of 0.1N HCl, 2 hour at 60℃,), Hydrolysis by base (5 mL of 0.1N NaOH, 1 hour at 70℃), Degradation by hydrogen peroxide (5 mL of 3% H_2O_2, 30 mins at room temp.), photodegradation (in UV chamber for 24 hours) and thermal degradation (at 70℃ for 3 hours) were applied on 500, 50 and 100 µg/ mL concentration of MET, VIL and REM individually, in sample stock solution and standard stock solution. After completion of degradation, solutions were injected and

the chromatograms were monitored under optimized conditions and % degradation was calculated shown in Table 3.

Table 3: Summary of % Degradation for Metformin hydrochloride (MET), Vildagliptin (VIL) and Remogliflozin etabonate (REM) in formulation

Types	Conditions of degradation	% of Degradation		
		MET	VIL	REM
Acid	0.1 N HCl for 2 hours at 60 °C	08.30	07.69	16.43
Alkali	0.1 N NaOH for 1 hour at 70 °C	18.22	08.82	15.41
Oxidation	3 % H_2O_2 for 30 min	15.54	13.82	16.55
Thermal	70°C for 3 hours.	08.41	11.35	20.32
Photolysis	UV chamber for 24 hours	09.93	17.38	11.92

8.2.2 Analytical method validation

It is performed as per ICH guidelines and different parameters like accuracy, Precision, Linearity, Range, LOD, LOQ, Robustness and Specificity are checked for the developed stability indicating method. The method was found to be linear in the concentration range of 2.5 to 7.5, 25 to 75 and 5 to 15 µg/mL for vildagliptin, metformin hydrochloride and remogliflozin etabonate, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.137 and 0.415 µg/mL for vildagliptin, 3.737 and 11.326 µg/mL for metformin hydrochloride and 0.348 and 1.055 µg/mL for Remogliflozin etabonate. Summary of validation parameters was given in Table 4. From the results, it was concluded that the developed method was successfully estimate degradants produced during stability study and also accurate, precise, robust, specific and sensitive for simultaneous estimation of Metformin hydrochloride (MET), Vildagliptin (VIL) and Remogliflozin etabonate (REM) in bulk and combined formulation.

Table 4: Summary of validation parameters

Sr. No.	Parameters	MET	VIL	REM
1	**Linearity Range (µg/mL)**	25-75	2.5-7.5	5 -15

2	**Regression Equation (y = mx + c)**	y = 3000000 x + 600000000	y = 7000000 x+ 514157	y = 20000000 x + 1000000
3	**Slope (m)**	3000000	7000000	20000000
4	**Intercept (c)**	600000000	514157	1000000
5	**Correlation coefficient (R^2)**	0.9995	0.9997	0.9995
6	**LOD (µg/mL)**	3.737	0.137	0.348
7	**LOQ (µg/mL)**	11.326	0.415	1.055
8	**Asymmetry ± RSD**	1.10 ± 0.114	0.98 ± 0.240	1.14 ± 0.162
9	**Theoretical Plates ± RSD**	14223 ± 0.141	36554 ± 0.714	73443 ± 0.246
10	**Retention time ± RSD**	2.980 min ± 0.001	6.458 min ± 0.027	18.540 min ± 0.008
11	**Resolution ± RSD**	NA	23.72± 0.14	29.13± 0.14
12	**Assay (% of label claim) Mean ± S. D.**	99.42 ± 0.114	99.09 ± 0.106	99.51 ± 0.162

8.3 "Stability-indicating analytical method development and validation for simultaneous estimation of Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride and Metformin hydrochloride in bulk and marketed formulations"

In accordance with ICH guidelines, a forced degradation study using the RP-HPLC-PDA method has been designed and validated for simultaneous estimation of Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride and Metformin hydrochloride in bulk and in recently approved triple fixed drug combination (FDC). Quantification was done by 0.025 M KH_2PO_4 Buffer: Methanol: Acetonitrile (50:25:25 % v/v/v) pH 3 at 225 nm wavelength and 1mL/min flow rate.

8.3.1 Forced Degradation Study

Different Stress conditions of acid (0.1 N HCl at 70°C for 1 hr), base (0.1N NaOH at 70°C for 1 hr), oxidation (3% H_2O_2 for 2 hr), photolytic (36 hrs at 254 nm) and thermal (3 hrs at 70°C) were applied on 4 µg/mL TEN, 3 µg/mL PIO and 100 µg/mL MET concentration individually, in sample stock solution and in standard stock solution. After completion of degradation, solutions were injected and the chromatograms were monitored under optimized conditions and % degradation was calculated shown in Table 5.

Table 5: Summary of % Degradation for Metformin hydrochloride (MET), Teneligliptin hydrobromide hydrate (TEN) and Pioglitazone hydrochloride (PIO) in formulation.

Types	Conditions of degradation	% of Degradation		
		MET	TEN	PIO
Acid	0.1 N HCl at 70°C,1 hr	19.16	13.59	11.66
Alkali	0.1 N NaOH at 70°C, 1 hr	17.57	19.93	18.49
Oxidation	3% H_2O_2, 2 hr	15.07	17.67	18.33
Thermal	3 hrs at 70°C	10.20	14.59	11.98
Photolysis	36 hrs at 254 nm	13.98	08.90	12.85

8.3.2 Analytical method validation

It is performed as per ICH guidelines and different parameters like accuracy, Precision, Linearity, Range, LOD, LOQ, Robustness and Specificity are checked for the developed stability indicating method. Linearity and range obtained were 2-6 µg/mL for Teneligliptin hydrobromide hydrate, 1.5- 4.5 µg/mL for Pioglitazone hydrochloride and 50-150 µg/mL for Metformin hydrochloride. % Relative standard deviation was found to be less than 2% for precision. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.021 and 1.159 µg/mL for Teneligliptin hydrobromide hydrate, 4.718 and 14.298 µg/mL for metformin hydrochloride and 0.417 and 1.189 µg/mL for Pioglitazone hydrochloride. Summary of validation parameters was shown in Table 6. From the results, it was concluded that the developed method was successfully estimate degradants

produced during stability study and also accurate, precise, robust, specific, sensitive, easy to perform and cost effective for simultaneous estimation of Metformin hydrochloride (MET), Teneligliptin hydrobromide hydrate (TEN) and Pioglitazone hydrochloride (PIO) in bulk and formulation.

Table 6: Summary of validation parameters

Sr. No.	Parameters	MET	TEN	PIO
1	**Linearity Range (µg/mL)**	50-150	2.0-6.0	1.0 – 4.5
2	**Regression Equation (y = mx + c)**	y = 4447.3 x + 5240.6	y = 935 x+ 226.6	y = 3351.5 x -327.4
3	**Slope (m)**	4447.3	935	3351.5
4	**Intercept (c)**	5240.6	226.6	-327.4
5	**Correlation coefficient (R^2)**	0.9997	0.9993	0.9998
6	**LOD (µg/mL)**	4.718	0.021	0.417
7	**LOQ (µg/mL)**	14.298	1.159	1.189
8	**Asymmetry ± RSD**	1.17 ± 0.114	1.24 ± 0.240	1.05 ± 0.162
9	**Theoretical Plates ± RSD**	3549 ± 0.12	4905 ± 0.86	12644 ± 0.27
10	**Retention time ± RSD**	2.186 min ± 0.002	2.992 min ± 0.003	11.483 min ± 0.007
11	**Resolution ± RSD**	NA	2.36 ± 0.14	19.13 ± 0.14
12	**Assay (% of label claim) Mean ± S. D.**	99.23 ± 0.14	99.21 ± 0.19	99.15 ± 0.49

9. Achievements with respect to objectives:

Different types of solvents were screened and analyzed for the proper selection of the mobile phase in RP-HPLC method development. Optimized chromatographic conditions

were determined which can easily estimate degradants. Different types of stress studies were employed for stress testing on Metformin hydrochloride, Remogliflozin etabonate formulation, Metformin hydrochloride, Vildagliptin and Remogliflozin etabonate triple drug formulation and Teneligliptin hydrobromide hydrate, Pioglitazone hydrochloride and Metformin hydrochloride triple drug formulation. The developed RP-HPLC method can accurately separate different degradants produced during the forced degradation study and validate them as per ICH guidelines.

10. Conclusion:

The current research presents the development of a stability-indicating RP-HPLC assay method. Optimization of buffer solution pH, mobile phase solvent selection and its concentration to improve peak shape was a critical part of the method. Good resolution between all drugs was best key part of the developed method. Specificity, simplicity, sensitivity and cost-effectiveness forced degradation approach were added to set methods for routine analysis of drug samples in Analytical development laboratories and Quality control laboratories suitable for simultaneous determination in the presence of degradants. This method has complied with ICH guidelines. These methods were also less time-consuming and easy to perform. Thus, they can be applied for regular analysis of the simultaneous determination of MET and REM formulation, VIL, MET and REM formulation and TEN, PIO and MET formulation.

11. List of Publications:

1. Kagarana CS, Patel KN, Patel AB, (2024), Forced Degradation Method Development and Validation for Simultaneous Determination of Vildagliptin, Metformin hydrochloride and Remogliflozin etabonate in Bulk and its Formulation by RP-HPLC, International Journal of Pharmaceutical Quality Assurance, 15(01), pp.119-123, ISSN 0975-9506 (SCOPUS indexing)
2. Chhayaben S. Kagarana, Kunal N. Patel, Advaita B. Patel, (2024), Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Metformin Hydrochloride and Remogliflozin Etabonate in Bulk and Tablet Formulation, 17(5), pp.2025-30, ISSN 0974-3618 (Print), 0974-360X (Online). (SCOPUS indexing)

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