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Degradation Studies for Combination of Three Antidiabetic Medicines by UPLC

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Abstract:

Forced degradation studies include the degradation of new drug substance and drug product at conditions more severe than accelerated conditions. Dapagliflozin + Metformin + Vildagliptin is a combination of three antidiabetic medicines. These studies illustrate the chemical stability of the molecule which further facilitates the development of stable formulation with suitable storage conditions. ICH guidelines demonstrate certain degradation conditions like light, oxidation, dry heat, acidic, basic, hydrolysis etc.

Keywords*: Degradation; Drug substance; Stability; Safety; Testing; ICH guidelines, FDA*

INTRODUCTION:

A combination drug or a fixed-dose combination (FDC) is a medicine that includes two or more active ingredients combined in a single dosage form [1]. Terms like "combination drug" or "combination drug product" can be common shorthand for an FDC product (since most combination drug products are currently FDCs), although the latter is more precise if in fact referring to a mass-produced product having a predetermined combination of drugs and respective dosages (as opposed to customized polypharmacy via compounding [2]). And it should also be distinguished from the term "combination product" in medical contexts, which without further specification can refer to products that combine different types of medical products—such as device/drug combinations as opposed to drug/drug combinations [3].

According to an FDA guidance document, a stability-indicating method is "a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability-indicating method accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities [4].

The demonstration of specificity and the ability of the method to monitor a change in the chemical properties of the drug over time, invariably calls for a forced degradation (stress testing) study to be done on the drug substance and drug product. Forced degradation on the drug substance and product will (in addition to establishing specificity) also provide the following information: (1) determination of degradation pathways of drug substances and drug products; (2) discernment of degradation products in formulations that are related to drug substances versus those that are related to non-drug substances (eg, excipients); (3) structure elucidation of degradation products; (4) determination of the intrinsic stability of a drug substance molecule in solution and solid state; and (5) reveal the thermolytic, hydrolytic, oxidative, and photolytic degradation mechanism of the drug substance and drug product[5-6].

From the foregoing, it is obvious that forced degradation plays a key role not just in the development of stability-indicating methods, but also in providing useful information about the degradation pathways and degradation products that could form during storage. The information thus obtained will facilitate pharmaceutical development in areas such as formulation development, manufacturing, and packaging, where knowledge of chemical behaviour can be used to improve the quality of drug product. Despite the importance of forced degradation in pharmaceutical development, the current regulatory guidance documents governing forced

degradation studies are very general. One of the guidance documents, Q1A (R2) – Stability Testing of New Drug Substances and Products, states: "Stress testing is likely to be carried out on a single batch of the drug substance. Dapagliflozin + Metformin + Vildagliptin is a combination of three antidiabetic medicines. Dapagliflozin increases urinary glucose excretion and reduces blood glucose levels. Vildagliptin helps control blood sugar levels by increasing substances (incretins) in the body that make the pancreas release more insulin. It also signals the liver to stop producing sugar (glucose) when there is too much sugar in the blood. Metformin decreases the amount of glucose absorbed from your food and the amount of glucose made by the liver. It also increases the body's response to insulin, a natural substance that controls the amount of glucose in the blood.

Recent studies [7-8] evaluated the efficacy of these drugs as an adjuvant to metformin monotherapy in managing T2DM and provided inconsonant results. Lately, a network meta-analysis [9] showcased the improved efficacy of add-on vildagliptin and dapagliflozin to metformin monotherapy as compared to antidiabetic drugs in the long-term management of diabetes. Despite several antidiabetic medications, sustained glycemic control, complications, and compliance remain unresolved for decades. Hence, there remains a constant requirement for novel drugs to tackle these issues [10].

Experimental:

UPLC Simultaneous Method Development for Metformin, Dapagliflozin and Vildagliptin

Chromatographic condition:

Use suitable Ultra Performance Liquid Chromatographic equipped with PDA detector.

Column : C18 (100x 2.1mm, 1.7µm)
Mobile phase ratio : Acetonitrile: 0.1% Perchloric acid (40:60)
Detection wavelength : 235 nm
Flow rate : 0.5ml/min
Injection volume : 5µl
Run time : 3min

a) Equipment:

Table No.1: List of Apparatus used in UPLC

S.No	Name	Model	Manufacturer
1.	UPLC	ACQUITY	Waters - Empower software2.0versions
2.	pH meter	-	Eutech
3.	Weighing balance	-	Sartouris
4.	UV/VIS spectrophotometer	-	UV-1700
5.	Pipettes, beakers and Burettes	-	Borosil
6.	Ultra sonicator	UCA 701	Unichrome
7.	Pump	Isocratic model	--

(b) Reagents & Chemicals

Table No.2: List of chemicals used in UPLC Method

S.No	Name	Grade	Manufacturer
1.	Acetonitrile	HPLC	Rankem
2.	Perchloric acid	HPLC	Analytical reagents
3.	Water	HPLC	Milli Q or equivalent

DEGRADATION STUDIES:

Preparation of stock: Accurately weigh 35.6 mg of Metformin, dapagliflozin and Vildagliptin sample and transferred into a 10ml volumetric flask add 7ml of diluent sonicate to dissolved and makeup to the mark.

Acid Degradation:

Take 1 ml of sample stock into a 10 ml volumetric flask and add 1ml of 1N HCl. Leave it for 15 min. After 15 min add 1ml of 1N NaOH to neutralize the solution and diluted to volume with diluent and mixed. The above solution is injected into UPLC system.

Alkali Degradation:

Take 1 ml of sample stock into a 10 ml volumetric flask and add 1ml of 1N NaOH. Leave it for 15 min. After 15 min add 1ml of 1N HCl to neutralize the solution and diluted to volume with diluent and mixed. The above solution is injected into UPLC system.

Peroxide Degradation:

Take 1 ml of sample stock into a 10 ml volumetric flask and add 1ml of 10% H_2O_2. Leave it for 15 min. After 15 min diluted to volume with diluent and mixed. The above solution is injected into UPLC system.

Reduction Degradation:

Take 1 ml of sample stock into a 10 ml volumetric flask and add 1ml of 10% Sodium bi sulphite solution. Leave it for 15 min. After 15 min diluted to volume with diluent and mixed. The above solution is injected into UPLC system.

Thermal Degradation:

100 mg of Metformin, Dapagliflozin and Vildagliptin standard was exposed at 105°C for 6 hrs and the exposed standard was analysed. 25mg of Metformin, 5mg of dapagliflozin and 5mg of Vildagliptin was transferred into 10ml volumetric flask and make up to the mark. Further dilute 1ml to 10ml with diluent. The above solution is injected into UPLC system.

Photolytic Degradation:

100mg of sample was placed in photo stability chamber for 3 hrs and the exposed sample was analysed. 35.6 mg of sample was transferred into 10ml volumetric flask and make up to the mark. Further dilute 1ml to 10ml with diluent. The above solution is injected into UPLC system.

RESULTS AND DISCUSSION

Table No.3: Forced degradation results of Metformin by UPLC

		Metformin						
	Sample Weight In mg	Area Counts / Injections	Mean Area Count	% Label Claim	Purity Angle	Purity Threshold	%Degradation	Pass/Fail
Control	35.6	2579769	2579769	100	1.875	6.328	0	Pass
Acid	35.6	2272340	2272340	88.1	1.822	6.364	11.9	Pass
Alkali	35.6	2241953	2241953	86.9	1.861	6.372	13.1	Pass
Peroxide	35.6	2197044	2197044	85.2	1.824	6.358	14.8	Pass
Reduction	35.6	2471639	2471639	95.8	1.839	6.395	4.2	Pass
Thermal	35.6	2305309	2305309	89.4	1.818	6.304	10.6	Pass
Photolytic	35.6	2500710	2500710	96.9	1.846	6.377	3.1	Pass
Hydrolysis	35.6	2512348	2512348	97.4	1.827	6.316	2.6	Pass

Table No.4: Forced degradation results of Dapagliflozin by UPLC

		Dapagliflozin						
	Sample Weight In mg	Area Counts / Injections	Mean Area Count	% Label Claim	Purity Angle	Purity Threshold	%Degradation	Pass/Fail
Control	35.6	51474	51474	100	0.792	2.866	0	Pass
Acid	35.6	50298	50298	97.7	0.737	2.821	2.3	Pass
Alkali	35.6	50126	50126	97.4	0.754	2.828	2.6	Pass
Peroxide	35.6	44711	44711	86.9	0.709	2.846	13.1	Pass
Reduction	35.6	49873	49873	96.9	0.776	2.874	3.1	Pass
Thermal	35.6	45812	45812	89	0.712	2.893	11	Pass
Photolytic	35.6	50525	50525	98.2	0.746	2.899	1.8	Pass
Hydrolysis	35.6	50986	50986	99.1	0.735	2.862	0.9	Pass

Table No.5: Forced degradation results of Vildagliptin by UPLC

		Vildagliptin						
	Sample Weight In mg	Area Counts / Injections	Mean Area Count	% Label Claim	Purity Angle	Purity Threshold	%Degradation	Pass/Fail
Control	35.6	515472	515472	100	5.024	11.385	0	Pass
Acid	35.6	452439	452439	87.7	5.063	11.312	12.3	Pass
Alkali	35.6	506458	506458	98.2	5.055	11.342	1.8	Pass

Peroxide	35.6	444745	444745	86.2	5.041	11.369	13.8	Pass
Reduction	35.6	500367	500367	97	5.024	11.337	3	Pass
Thermal	35.6	501994	501994	97.4	5.006	11.321	2.6	Pass
Photolytic	35.6	468211	468211	90.8	5.089	11.374	9.2	Pass
Hydrolysis	35.6	512078	512078	99.3	5.072	11.386	0.7	Pass

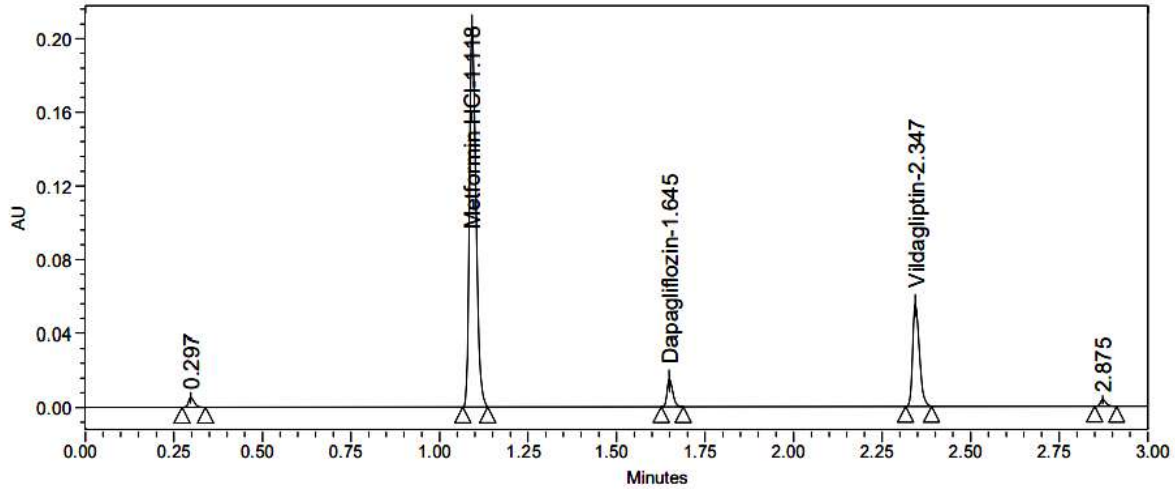

Fig No.56: chromatogram for Acid degradation

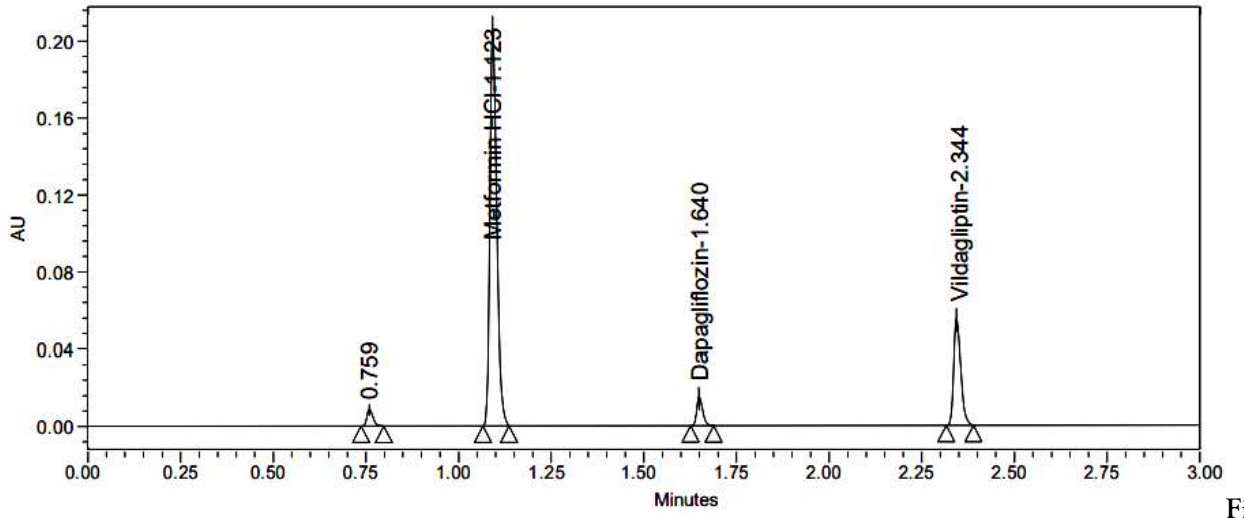

Fig No.57: chromatogram for Alkali degradation

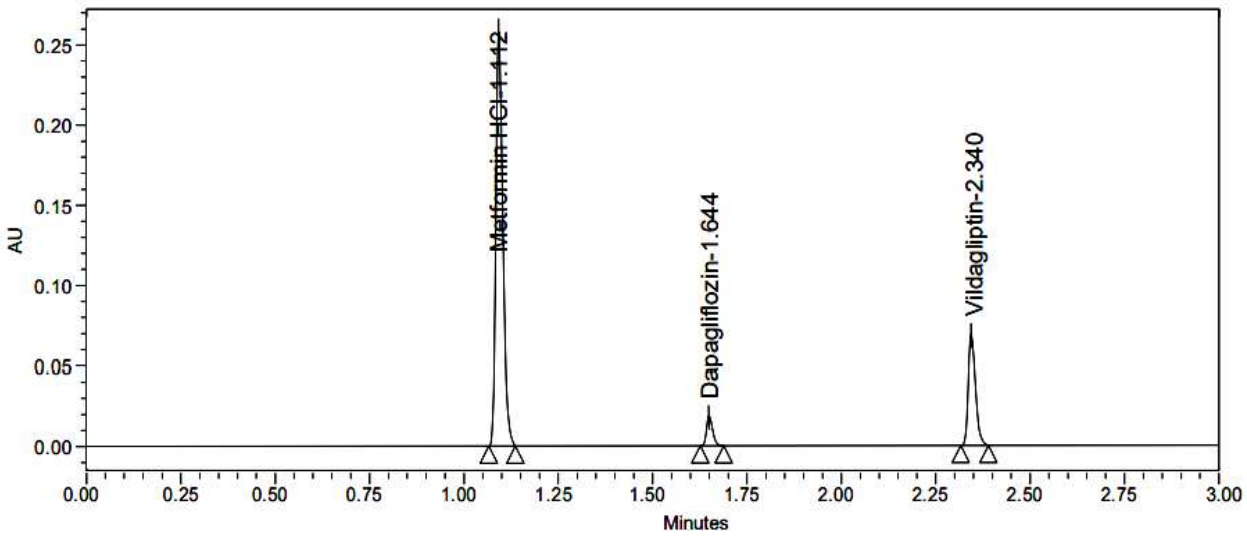

Fig No.58: chromatogram for Control degradation

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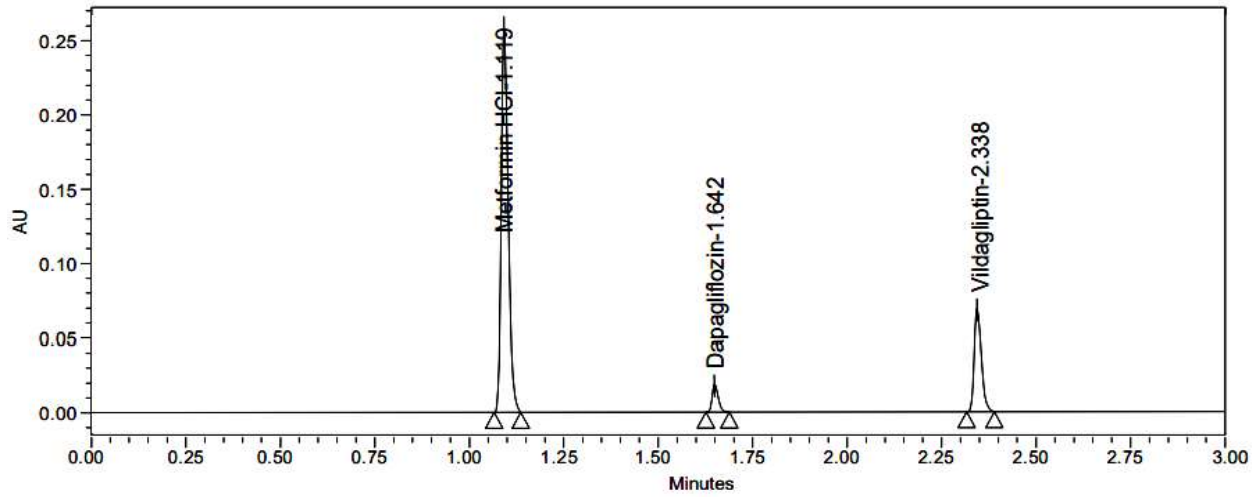

Fig No.59: chromatogram for Hydrolysis degradation

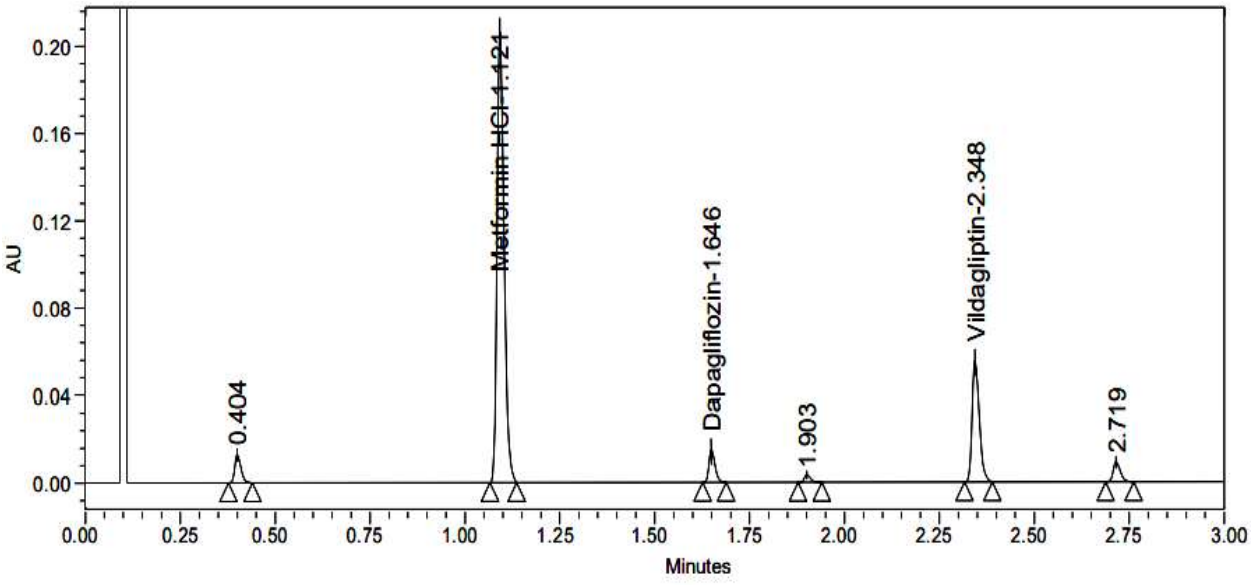

Fig No.60: chromatogram for Peroxide degradation

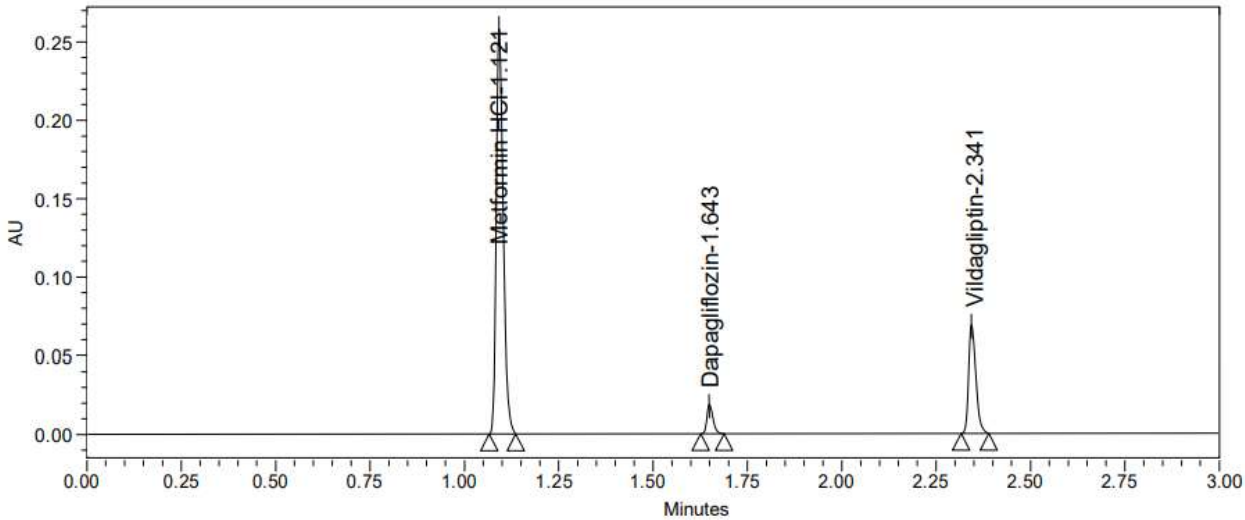

Fig No.61: chromatogram for Reduction degradation

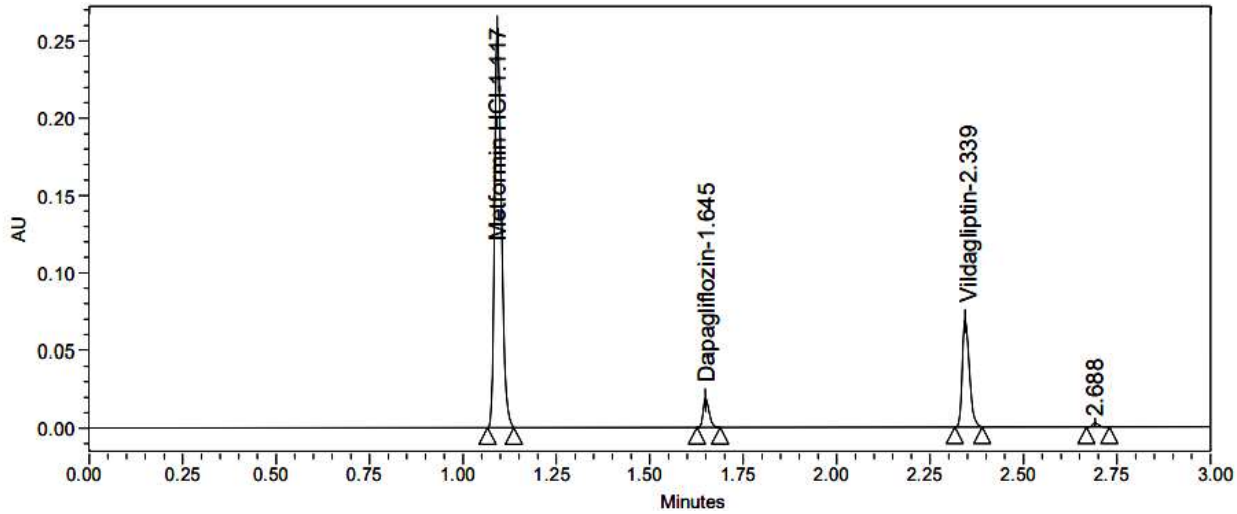

Fig No.62: chromatogram for Photolytic degradation

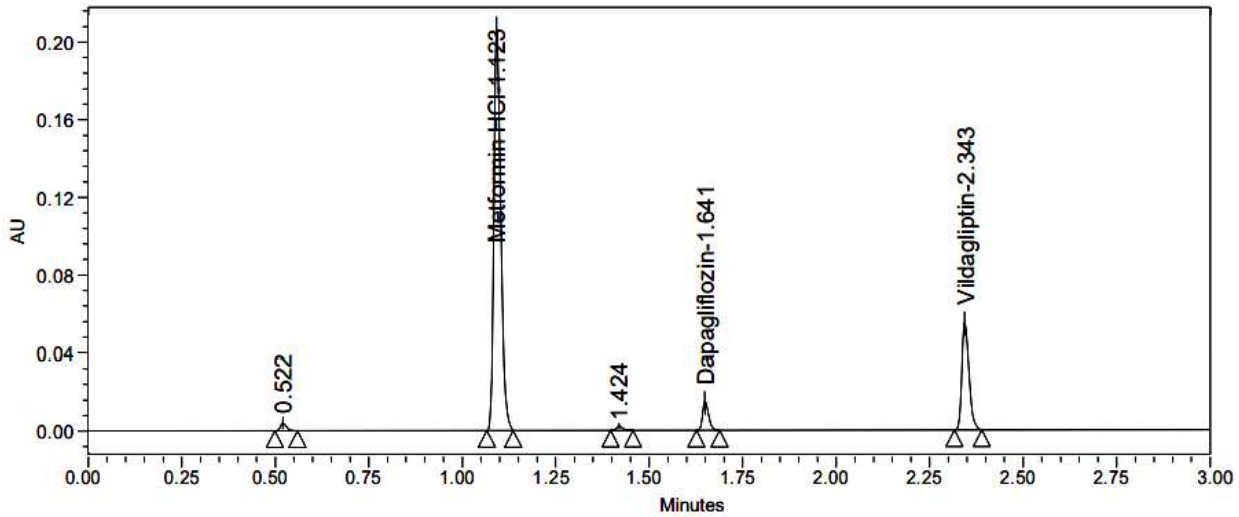

Fig No.63: chromatogram for Thermal degradation

SUMMARY

Forced degradation studies are indispensable in the development of stability-indicating and degradant-monitoring methods as part of a validation protocol. Forced degradation studies also provide invaluable insight in investigating degradation products and pathways of drug substances and products. Given that no specific set of conditions will be applicable to all drug substances and products, the pharmaceutical scientist should ensure the stress conditions are consistent with product decomposition under normal manufacturing, storage, and intended use conditions.

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