Original Research Article

COMPARATIVE EVALAUTION OF DIFRENT BRANDS OF NITROGLYCERINE CONTROLLED RELEASE TABLETS AVAILABLE IN INDIAN MARKET

Abstract

The nitroglycerine oral solid preparations are available in the international and Indian market as – Sublingual tablets and Controlled Release tablets since a very long time. However, the quality issues (like few of the mentioned below) are consistently there. Due to this poor stability issues, even the Pharmacopoeial monographs insists on wider Dosage content uniformity limits (75 – 135% of label claim) and relaxed assay limits (90 – 115% of label claim).

In the present study a comparison for the most critical quality parameters such as, Dissolution and Related substance among the In-House developed product and different brands of 2.6 mg Nitroglycerin Controlled Release tablet available in Market was carried out. It was observed that Impurity 1 MNG was not observed in any of the market product while Impurity 2 MNG was observed initially in two branded products but on stability this impurity observed in all branded products except In-house developed product. Initially, drug dissolution result of In-house developed product was comparable with the all branded product. On stability, Drug release profile of all branded product varies to a significant level from its initial value while the drug release profile of the In-house developed product remain unchanged.

Introduction

Globally Cardiovascular diseases (CVDs) are the major cause of mortality. To reduce the risk of CVDs, early identification of Angina is necessary. It is a clinical condition which is a result of constrictions developed in the arteries of the heart.[1]

Angina is a type of chest pain caused by reduced blood flow to the heart. Angina is a symptom of coronary artery disease. Angina is also called angina pectoris. Angina pain is often described as squeezing, pressure, heaviness, tightness or pain in the chest. It may feel like a heavy weight lying on the chest. Angina may be a new pain that needs to be checked by a health care provider, or recurring pain that goes away with treatment. Although angina is relatively common, it can still be hard to distinguish from other types of chest pain, such as the discomfort of indigestion.[2]

Angina pectoris is a clinical condition which is the result of constrictions developing in the arteries of the heart. When these constrictions develop, angina pectoris patients experience severe pains in the chest from time to time.

Angina pectoris results from an imbalance between the blood supply to the heart and the myocardial oxygen demand. The result is myocardial ischemia, which often manifests itself as chest pain. This ischemia is most commonly a direct result of poor coronary blood flow, and it induces the myocardial cells to use anaerobic metabolism as their primary energy source. Additionally, during ischemia, ATP is broken down into adenosine, which serves the role of dilating the arterioles and manifesting the pain of angina by stimulating the A1 receptors in the cardiac nerve endings.[3]

There are different types of angina. The type depends on the cause and whether rest or medication relieve symptoms.

Stable angina. Stable angina is the most common form of angina. It usually happens during activity (exertion) and goes away with rest or angina medication. For example, pain that comes on when you're walking uphill or in the cold weather may be angina. Stable angina pain is predictable and usually similar to previous episodes of chest pain. The chest pain typically lasts a short time, perhaps five minutes or less.

Unstable angina (a medical emergency). Unstable angina is unpredictable and occurs at rest. Or the angina pain is worsening and occurs with less physical effort. It's typically severe and lasts longer than stable angina, maybe 20 minutes or longer. The pain doesn't go away with rest or the usual angina medications. If the blood flow doesn't improve, the heart is starved of oxygen and a heart attack occurs. Unstable angina is dangerous and requires emergency treatment.

Variant angina (Prinzmetal angina). Variant angina, also called Prinzmetal angina, is not due to coronary artery disease. It's caused by a spasm in the heart's arteries that temporarily reduces blood flow. Severe chest pain is the main symptom of variant angina. It most often occurs in cycles, typically at rest and overnight. The pain may be relieved by angina medication.

Refractory angina. Angina episodes are frequent despite a combination of medications and lifestyle changes

Angina symptoms include chest pain and discomfort. The chest pain or discomfort may feel like: Burning, Fullness, Pressure, Squeezing. Pain may also be felt in the arms, neck, jaw, shoulder or back.

Other symptoms of angina includes: Dizziness, Fatigue, Nausea, Shortness of breath& Sweating.

Patients affected by the angina pectoris require an almost immediate response to the drug not only to get relieve from the pain in such an attack but also for the remission of the fright which often accompanies the pain due to the relationship with the heart.[4]

Glyceryl trinitrate, hereinafter referred to as nitroglycerin, is one of the most effective drughas been employed in the alleviation of the distress of angina pectoris for many decades.[5]

Nitroglycerine (Glyceryl Trinitrate), which a nitrate group of cardiovascular drug used for the relief of pain arising due to angina pectoris. Nitroglycerine is employed to dilate the cardiac arteries with the subsequent relief of the pain.

Nitroglycerin converts to nitric oxide (NO) in the body. NO then activates the enzyme guanylyl cyclase, which converts guanosine triphosphate (GTP) to guanosine 3',5'-monophosphate (cGMP) in vascular smooth muscle and other tissues. cGMP then activates many protein kinase-dependent phosphorylations, which enhances the reuptake of calcium into the sarcoplasmic reticulum, increases extracellular calcium, and opens the calcium-gated potassium channel. This ultimately results in the dephosphorylation of myosin light chains within smooth muscle fibers. This activity causes the relaxation of smooth muscle within blood vessels, resulting in the desired vasodilatory effect.[6,7]

Nitroglycerine is a liquid at normal temperatures and is a highly volatile substance at atmospheric vapor pressure also. Nitroglycerine is a violent explosive which is required to be handled with great care. A diluted form of nitroglycerine with Lactose or other inert substance, in the composition of 10% w/w is safe and effective. It is further diluted with other agents for the preparation of tablets.[8]

The quality of pharmaceuticals is a global concern; counterfeit medicines are increasingly detected worldwide. Quality of pharmaceutical product is the most essential for efficacy and safety of product. Quality of product defines to its confining to the standards pre-set to assure the desired purpose.

In order to ensure the requisite quality, drug manufacturers are required to test their products during and after manufacturing and at various intervals during the shelf life of the product. It is needed to ensure that branded drugs products are qualitative and it also necessity to choose one product from several generic drug products of the same active ingredients.

The nitroglycerine oral solid preparations are available in the international and Indian market as – Sublingual tablets and Controlled Release tablets since a very long time. However, the quality issues (like few of the mentioned below) are consistently there. These issues are widely described and available in no. of published literature so far –

- Assay content
- Incomparability with packaging materials
- Dosage Content Uniformity

- Stability over the shelf life
- Degradation product formation during storage

Due to this poor stability issues, even the Pharmacopoeial monographs insists on wider Dosage content uniformity limits (75 – 135% of label claim) and relaxed assay limits (90 – 115% of label claim). This is one of the very few classic example for such a wide limits for most critical quality tests. For sublingual tablets, Pharmacopeial monograph recommends an amber glass bottle packaging only and also restricts the no. of tablets per pack.[9]

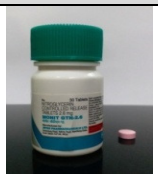
The available brands of controlled released tablets are available in varying quantity of pack sizes, packaging materials and using different compositions. The drug being a lifesaving product for emergency and long term usage of angina treatment, the assessment of variability among these tablets is important scientific requirement.

The aim of the present study was to develop a In –House product of Nitroglycerin 2.6 mg Controlled Release tablet and compared with the different brands available in Market. The most critical quality parameters such as Related substance and Dissolution were compared at initial and after 6 month accelerated stability conditions.

Experimental

Materials

Four Domestic multiple brands of bestselling Nitroglycerin Controlled Release tablets (B to E) were compared with the In-house formulated product (F-50). Details of each products are given in the Table 1.

Product Name	In-House developed Product (F-50)	Sample-B	Sample- C	Sample-D	Sample-E
Product Image					

List 1 : List of samples use for the study

Methods

Physical Evaluation

All Branded products were physically evaluated for their Primary Packaging and Label claims. Tablets of these brands were also evaluated for their physical appearance like color, shape and marking.

Table -1 Details of Indian Market Products and In-House Developed Product

Product Name	Packaging	Label Claim	Description
In-House developed product (F-50)	White plastic bottle with white cap. (Qty.: 30 tab/bottle) Mfg. Date: 10/2021	Each uncoated tablet contains: Diluted Nitroglycerin IP eq. to Nitroglycerin 2.6 mg (In a controlled release system) Colour: Erythrosine	Pink, round, FFBE, uncoated tablets engraved with characteristic marking on both the facets
Sample-B	White plastic bottle with green cap. (Qty.: 30 tab/bottle) Mfg. Date: 08/2021 Expiry Date:07/2023	Each uncoated tablet contains: Diluted Nitroglycerin IP eq. to Nitroglycerin 2.6 mg (In controlled release form) Excipient Q.S. Colour: Lake of erythrosine	Light Pink, round, FFBE, uncoated tablets engraved with characteristic marking on both the facets
Sample-C	Pink plastic bottle with pink cap. (Qty.: 30 tab/bottle) Mfg. Date: 06/2021 Expiry Date:05/2023	Each uncoated tablet contains: Diluted Nitroglycerin IP eq. to Nitroglycerin 2.6 mg (In controlled release form) Colour: Erythrosine	Pink, round, FFBE, uncoated tablets with score line on one of the facet.
Sample-D	White plastic bottle with white cap. (Qty.: 30 tab/bottle) Mfg. Date: 07/2021 Expiry Date: 06/2023	Each uncoated tablet contains: Diluted Nitroglycerin IP eq. to Nitroglycerin 2.6 mg (In controlled release form) Colour: Erythrosine	Pink, round, FFBE, uncoated tablets with score line on one of the facet.
Sample-E	**Amber colored glass bottle** with white cap. (Qty.: 30 tab/bottle) Mfg. Date: 07/2021 Expiry Date: 06/2023	Each uncoated tablet contains: Diluted Glyceryl Trinitrate IP eq. to Glyceryl Trinitrate 2.6 mg (In a controlled release system) Excipient q.s. Colour: Sunset yellow FCF	Light orange, round, FFBE, uncoated tablets with score line on one of the facet.

Controlled release tablets of Nitroglycerine are not official in any of the pharmacopoeia. However, there is a monograph of the sublingual tablets of Nitroglycerine is available in US Pharmacopeia. There is an assay test available in USP, but Related Substance (RS) is not available. This RS is specified in the BP monograph of sublingual tablets.[10]Therefore, a suitable HPLC based validated In-House test method was developed. The developed test methods were subjected to ICH requirements for analytical method validation for its assay and related substance quantitative estimation. After due qualification to the norms as per ICH for specificity, repeatability, robustness, linearity and reproducibility, the method was used for estimation.[11]

Drug Dissolution

The in vitro drug release studies were carried out using USP type II dissolution apparatus at 50 rpm. The dissolution was done in a medium of 900 ml Purified water for 8 hrs. The temperature was maintained at 37±0.5 °C. Aliquot samples of 10 ml were withdrawn at pre scheduled intervals (1, 2, 4, 6, and 8 h) and replaced with an equal volume of fresh dissolution medium which was kept at 37±0.5 °C to maintain sink condition. Each filtered sample was analyzed by HPLC method as per the parameters mentioned in the related substance.

Related Substance

For Related Substances test purposes, Crushed 10 tablets to coarse powder and transferred accurately weighed amount of sample powder equivalent to about 12.5 mg of Nitroglycerin to 50 ml volumetric flask. Added 10 ml of Acetonitrile and sonicated for 10 minutes. Volume was made up with water and mixed well using Centrifuge for 10 minutes. Filtered the solution through Glass nylon filter.

Chromatographic Parameters

Table 2 Test Method parameters for Related Substance in Nitroglycerine CR tablets

Column	Intersil ODS-3, 3 µm (150 mm x 4.6 mm)
Mobile phase	Mobile phase A: Water Mobile phase B: Acetonitrile
Diluent	Water: Acetonitrile:: 80:20
Column oven Temperature	$35^0C \pm 5^0C$
Sample Tray Temperature	$25^0C \pm 5^0C$ (Room Temperature)
Detector	UV at 210 nm
Run time	50 minutes
Injection volume	20 µl

Mobile Phase - Gradient

Time	Flow rate (mL/Minute)	Mobile phase A (%)	Mobile phase B (%)
Initial	0.8	95	5
7	0.8	95	5
10	1.0	85	15
24	1.0	85	15
25	1.0	60	40
42	1.0	60	40
43	0.8	95	5
50	0.8	95	5

The order of elution are given as per table below

Table 3

S.No.	Name of Component	RRTs	Origin	RF
1	2-Mono Nitroglycerin Impurity	0.11	P @	NA
2	1-Mono Nitroglycerin Impurity	0.13	P @	NA
3	1,3 Dinitroglycerin Impurity	0.50	D	0.99
4	1,2 Dinitroglycerin Impurity	0.58	D	1.09
5	Nitroglycerin	-	-	-

P = Process impurity, D = Degradation impurity

@ = Process impurities are for information only and need not be reported and calculated

NA = Not Applicable, RF = Response Factor

Result and Discussion

All the samples used for the study were within their shelf life at the time of investigation.

Drug Dissolution:

The results of In- house formulation was comparable with the marketed products.

Related Substance:

The Related Substance test is a measure of the Impurities which appears in the finished product. Theses impurities may be Product impurities or Process impurities or both. Product impurities are the impurities which are already present in the Raw material i.e. Drug & Excipient. Process impurities are the impurities which are generated during product manufacturing and appear in the finished product.

Primarily four impurities are observed in the finished drug product containing nitroglycerine. These Impurities are-

- 2-Mononitroglycerin (2 MNG),
- 1-Mononitroglycerin (1 MNG),
- 1,3-Dinitroglycerin (1,3 DNG), and
- 1,2-Dinitroglycerin (1,2 DNG).

Since, Controlled release tablet of Nitroglycerin is not official, so the Specification limit for the Related Substance was decided based upon the ICH guideline and the Related Substance limit mentioned for sublingual tablet in British Pharmacopoeia.

As per the data it can be concluded that the impurity level in the In-House formulation is minimum as compared to other branded products.

Table-4 Drug Dissolution Results of Developed and Different Brand Products (Initial)

Time	Developed Product [F-50]	[Sample-B]	[Sample-C]	[Sample-D]	[Sample-E]
	Observations (n=12)				
After 1st Hr.	31.9	33.4	28.2	46.2	32.3
After 2nd Hr.	49.4	49.3	43.7	63.9	49.6
After 4th Hr.	74.4	69.7	65.8	82.4	74.1
After 6th Hr.	90.7	82.7	81.7	88.0	91.4
After 8th Hr.	101.3	91.2	91.5	89.1	103.0

Table-5 Drug Dissolution Results of Developed and Different Brand Products (After 6month/40^{0}C/75%RH)

Time	Developed Product [F-50]	[Sample-B]	[Sample-C]	[Sample-D]	[Sample-E]
	Observations (n=12)				
After 1st Hr.	30.5	33.2	22.5	25.1	33.1
After 2nd Hr.	46.4	47.2	34.7	36.2	49.8
After 4th Hr.	70.4	64.2	51.9	47.0	73.9
After 6th Hr.	90.1	74.7	63.6	51.5	90.3
After 8th Hr.	102.6	81.9	70.8	52.4	101.4

Table-6 Results of Different Brand Products of Nitroglycerin Controlled Release Tablet (Initial)

Product Name	Related Substance				
	2 MNG (NMT 1.0 % w/w)	1 MNG (NMT 1.0 % w/w)	1,3 DNG (NMT 1.0 % w/w)	1,2 DNG (NMT 1.0 % w/w)	Total Known Imp. (NMT 3.0 % w/w)
In-House Product [F-50]	0.000	0.000	0.101	0.063	0.164
Sample-B	0.000	0.000	0.306	0.200	0.506
Sample-C	0.297	0.000	0.707	0.517	1.521
Sample-D	0.080	0.000	6.314	1.813	8.207
Sample-E	0.026	0.000	0.511	0.348	0.885

Table-7 Results of Different Brand Products of Nitroglycerin Controlled Release Tablet (After 6 month/40^{0}C/75%RH)

Product Name	Related Substance				
	2 MNG (NMT 1.0 % w/w)	1 MNG (NMT 1.0 % w/w)	1,3 DNG (NMT 1.0 % w/w)	1,2 DNG (NMT 1.0 % w/w)	Total Known Imp. (NMT 3.0 % w/w)
In-House Product [F-50]	0.000	0.000	0.264	0.384	0.648
Sample-B	0.201	0.000	0.525	0.378	1.104
Sample-C	0.317	0.000	2.143	3.279	5.739
Sample-D	0.092	0.515	11.652	4.540	16.799
Sample-E	0.055	0.000	0.763	0.731	1.549

Where,

2 MNG = 2-Mononitroglycerin; 1 MNG = 1-Mononitroglycerin

1,3 DNG = 1,3-Dinitroglycerin; 1,2 DNG = 1,2-Dinitroglycerin

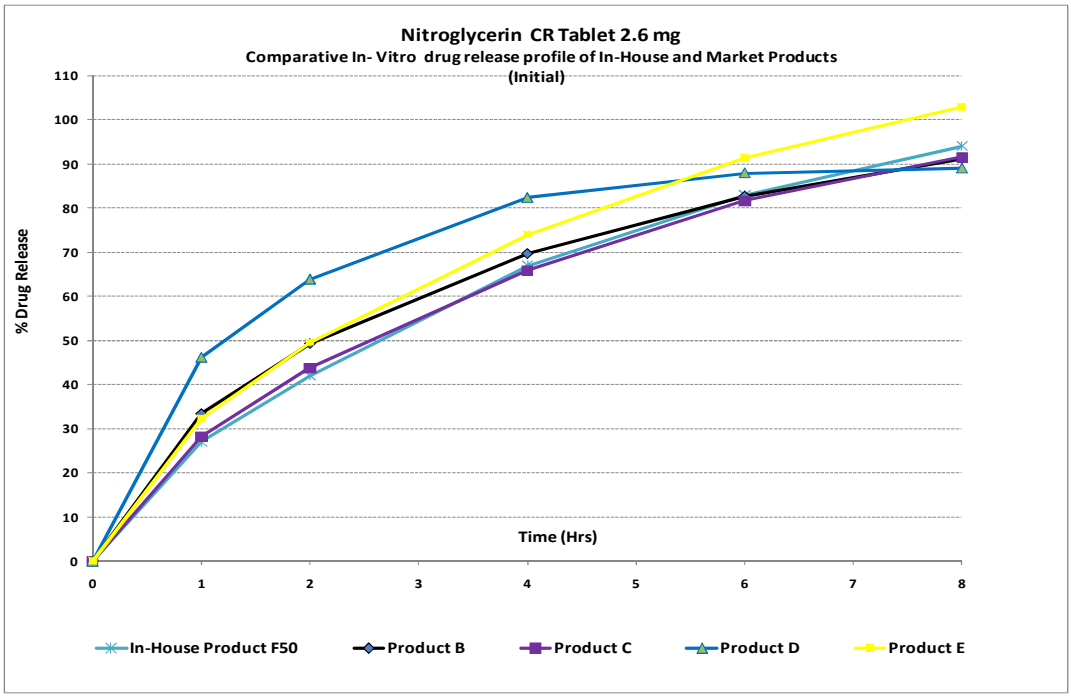


Fig.-1: Initial Drug Dissolution Results of In-House Developed Product vs Different Brand Products

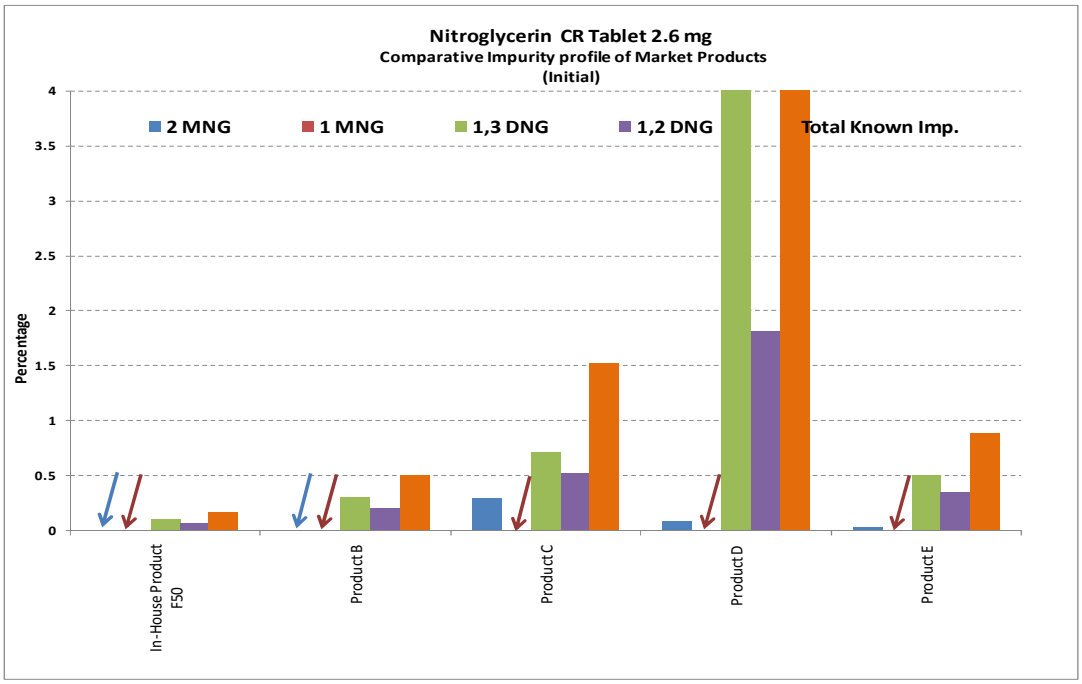


Fig.-2: Impurity profile of In-House Developed Product vs Different Brand Products (Initial)

As per graphical presentation, Initial Drug Dissolution results of Developed and marketed products are comparable.

Impurity 1 MNG was not observed in any of the product while Impurity 2 MNG was observed in three market products except the Sample – B & In-house developed products. Other Impurities i.e. 1,3 DNG &1,2 DNG are observed in all products.

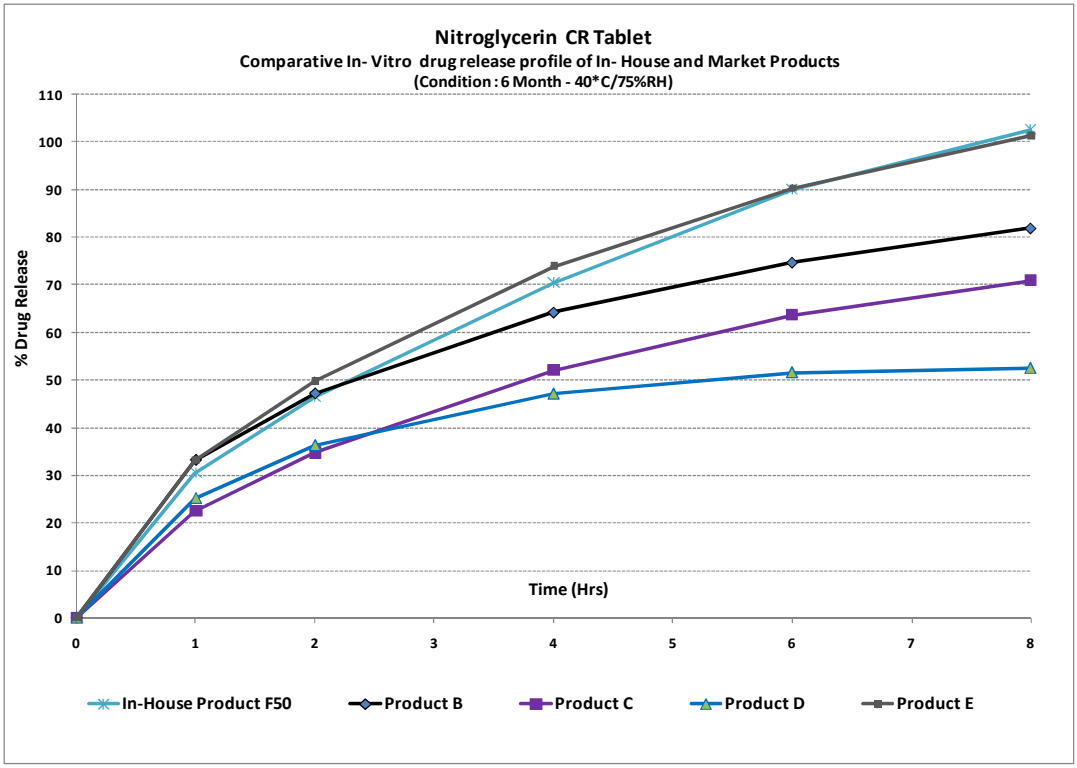


Fig.-3: Drug Dissolution Results of In-House Developed Product vs Different Brand Products (After 6 Month)

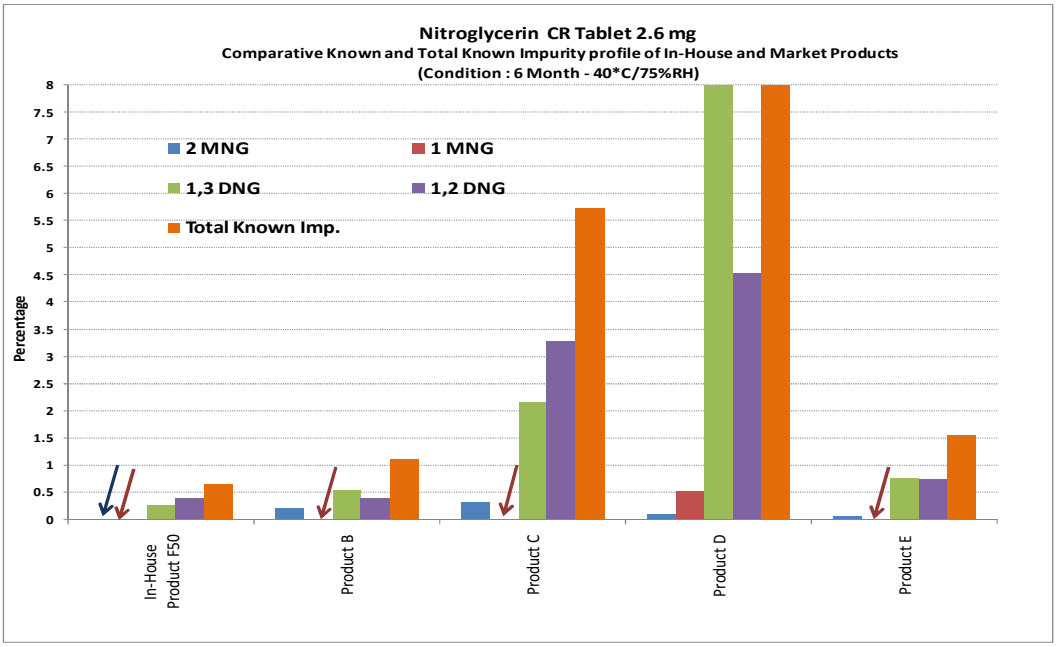


Fig.-4: Impurity profile of In-House Developed Product vs Different Brand Products (After 6 Month)

On Stability, for all branded products, Drug release profile varies drastically from their initial results. while the Impurity 2 MNG appears in all branded products and increases to a significant level. In Product C & D Total Known Impurity level increases to the level of more than 5%.

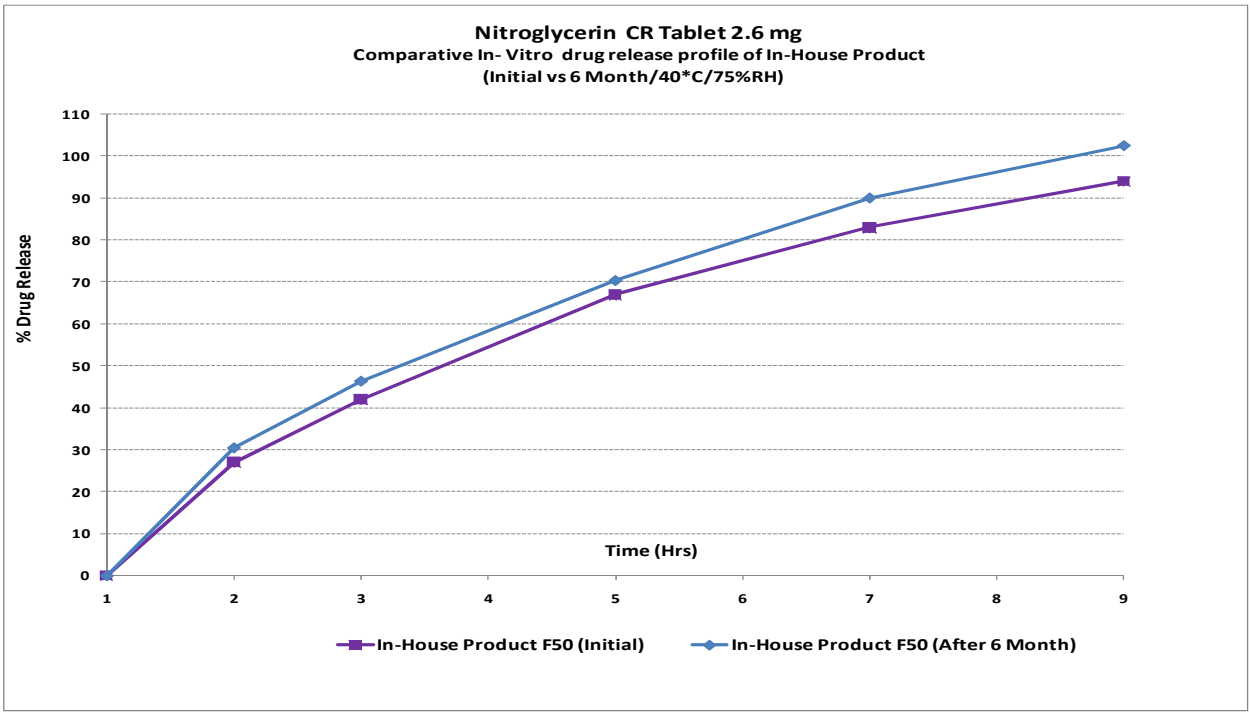


Fig.-5: Drug Dissolution Results of In-House Developed Product Initial vs After 6 Month

The In-House Developed product has Constant drug release profile without any significant variation from the initial results. Similarly, there is non-significant changes in the Impurity profile of when compared with the Initial values.

Conclusion:

As per the study, Drug dissolution of all 4 branded products and In-house product was comparable.

1 –Mono-Nitroglycerin impurity was not observed in any of the 4 market products but 2 –Mono-Nitroglycerin impurity was observed in sample-C, D and E. On stability this impurity level increases in all products but highest increase observed in product D.

As per the Graphical presentation of related substance it was observed that the impurity level of In-House product was lowest among the all 4 marketed products. Also, the Drug dissolution was comparable with the marketed products.

Overall, In-House formulated product has best quality parameters compliances.

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