RESEARCH PAPER

Stability-Indicating UPLC Method for Degradation Studies of Hydrocortisone, Neomycin, and Polymyxin B

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Abstract:
Hydrocortisone, Neomycin, and Polymyxin B combination of three antibiotics medicines. Forced degradation studies include the degradation of new drug substance and drug product at conditions more severe than accelerated conditions. 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
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Introduction:
The Combination drug contains neomycin and polymyxin, which are antibiotics that work by stopping the growth of bacteria. It also contains hydrocortisone, which is an anti-inflammatory corticosteroid that works by reducing swelling. This medication treats/prevents only bacterial eye infections. It will not work for other types of eye infections. Unnecessary use or overuse of any antibiotic can lead to its decreased effectiveness. Neomycin; Polymyxin B; Hydrocortisone is used to treat ear infections.

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.

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

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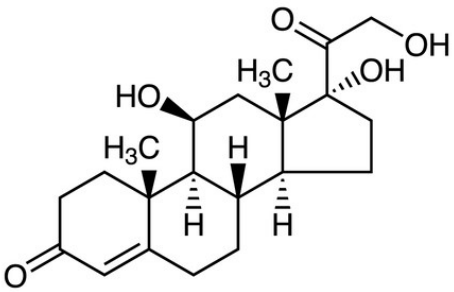
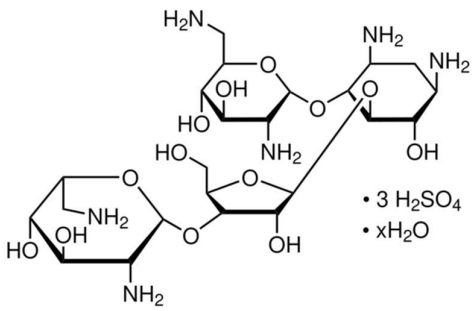
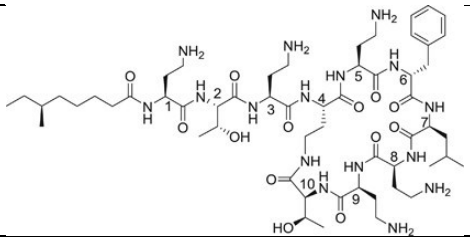
establishing specificity) also provide the following information: (1) determination of degradation pathways of drug substances and drug products; (2) discernment of degradation products in formulations that are related to drug substances versus those that are related to non-drug substances (eg, excipients); (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]. In India, a variety of NSAID combinations are available, often as over the counter products [7]. Non-steroidal anti-inflammatory drugs (NSAIDs) are medicines that are widely used to relieve pain, reduce inflammation, and bring down a high temperature. They're often used to relieve symptoms of: headaches. painful periods. These combinations are an easy way to sell two drugs when one (or even none) may be needed for the patient. The 'combined' pills are marketed with slogans like 'ibuprofen for pain and paracetamol for fever' and 'ibuprofen for peripheral action and paracetamol for central action'. It is indeed very unfortunate that the medical fraternity in India has fallen prey to such gimmicks. The gullible patient then has to pay for the doctor's complacence in terms of extra cost and extra adverse effects. There is no synergism when two drugs acting on the same enzyme are combined. Thus combining two NSAIDs does not and cannot improve the efficacy of treatment. It only adds to the cost of therapy and more importantly, to the adverse effects [8] and the 'muscle relaxants' in some of these combinations are of questionable efficacy.

Details of Drugs:

S.No	Name of the Drug	Structure	Molecular formula
1	**Hydrocortisone**		$C_{21}H_{30}O_5$
2	**Neomycin**		• $C_{23}H_{46}N_6O_{13}$
3	**Polymyxin B**		$C_{56}H_{100}N_{16}O_{17}S$

Chromatographic condition:

During the selection of chromatographic conditions, numbers of trails were carried out and the best trail was selected for optimized method.

Use Agilent1290 Infinity II LC System equipped with PDA- detector.

Column : Shield RP 18 (50 x 1.0mm, 1.7µm)

Wavelength : 250 nm

Injection Volume : 5 µL
Column Temperature : Ambient
Flow rate : 0.5 ml/min
Run time : 3 min

METHOD VALIDATION SUMMARY:

FORCED DEGRADATION STUDIES:

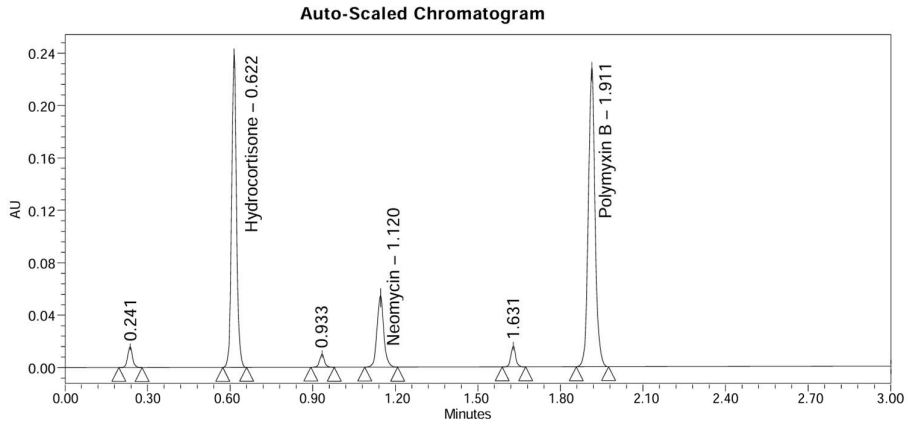
Preparation of Sample stock solution: Take 1 ml of Hydrocortisone, Neomycin and Polymyxin B sample and transferred into a 10ml volumetric flask add 7ml of diluent sonicate to dissolved and makeup to the mark.

1. Acid Degradation:

Procedure:

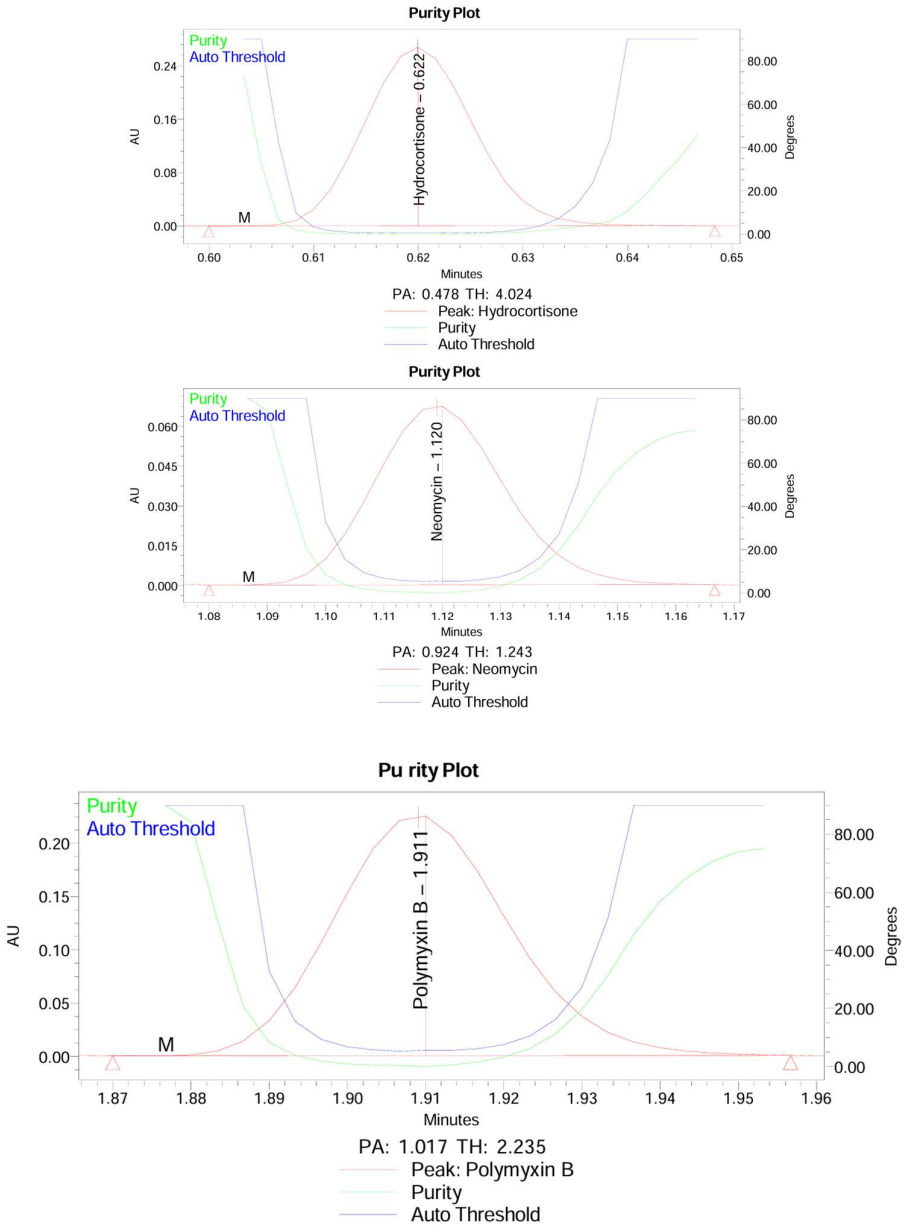
Take 1 ml of sample stock into a 10 ml volumetric flask and add 1ml of 1N HCl. Leave it for 15 min. After 15 min add 1ml of 1N NaOH to neutralize the solution and diluted to volume with diluent and mixed.

The above solution is injected into UPLC system.



Peak Results

	Sample Name	Name	Area	USP Tailing	USP Plate count	USP Resolution
1	Acid deg		385647	0.99	3524	
2	Acid deg	Hydrocortisone	2645128	1.13	13627	7.14
3	Acid deg		112063	1.01	4251	6.59
4	Acid deg	Neomycin	918044	1.19	5568	3.67
5	Acid deg		387458	1.34	3974	9.48
6	Acid deg	Polymyxin B	2550970	1.22	13479	4.17

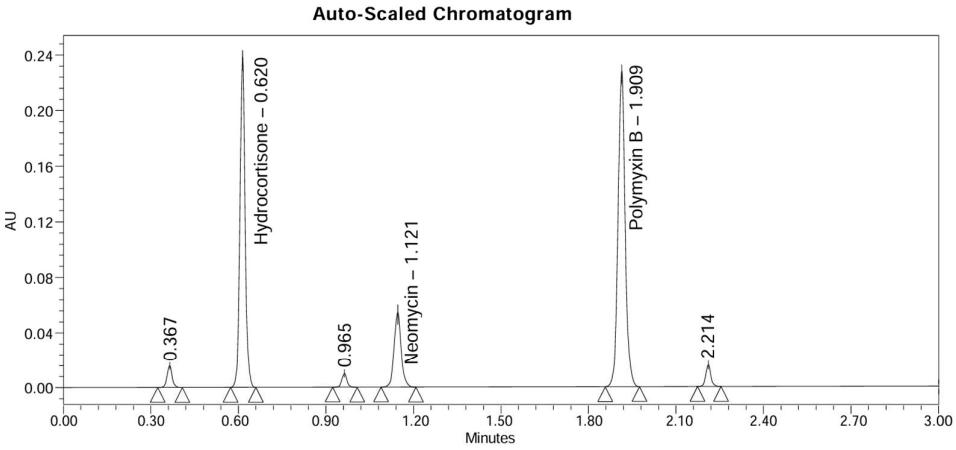


2. Alkali Degradation:

Procedure:

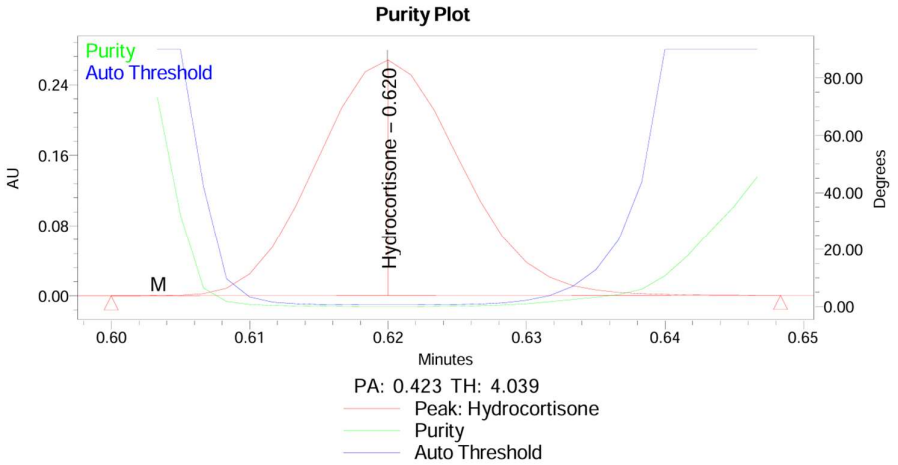
Take 1 ml of sample stock into a 10 ml volumetric flask and add 1ml of 1N NaOH. Leave it for 15 min. After 15 min add 1ml of 1N HCl to neutralize the solution and diluted to volume with diluent and mixed.

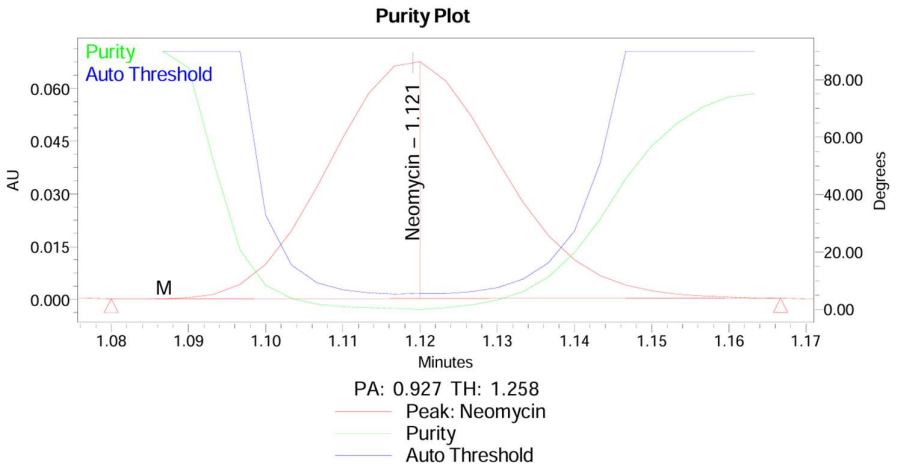
The above solution is injected into UPLC system.

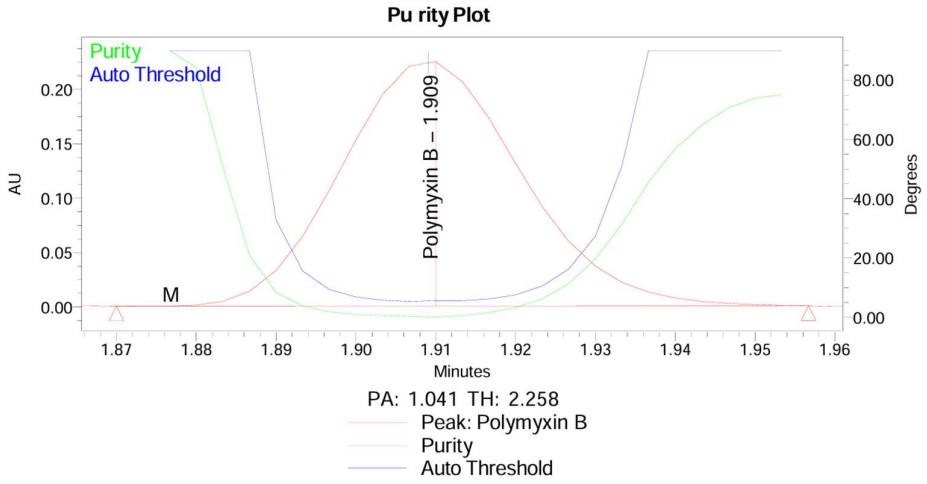


Peak Results

	Sample Name	Name	Area	USP Tailing	USP Plate count	USP Resolution
1	Alkali deg		352041	0.95	3387	
2	Alkali deg	Hydrocortisone	2680214	1.19	13655	3.86
3	Alkali deg		128567	1.26	4351	6.17
4	Alkali deg	Neomycin	900263	1.12	5596	3.23
5	Alkali deg	Polymyxin B	2561995	1.15	13463	12.48
6	Alkali deg		376582	1.34	4047	4.75





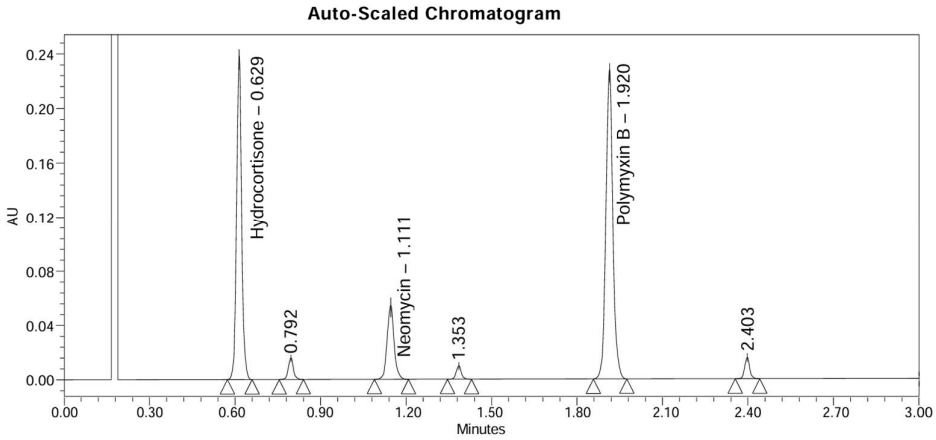


3. Peroxide Degradation:

Procedure:

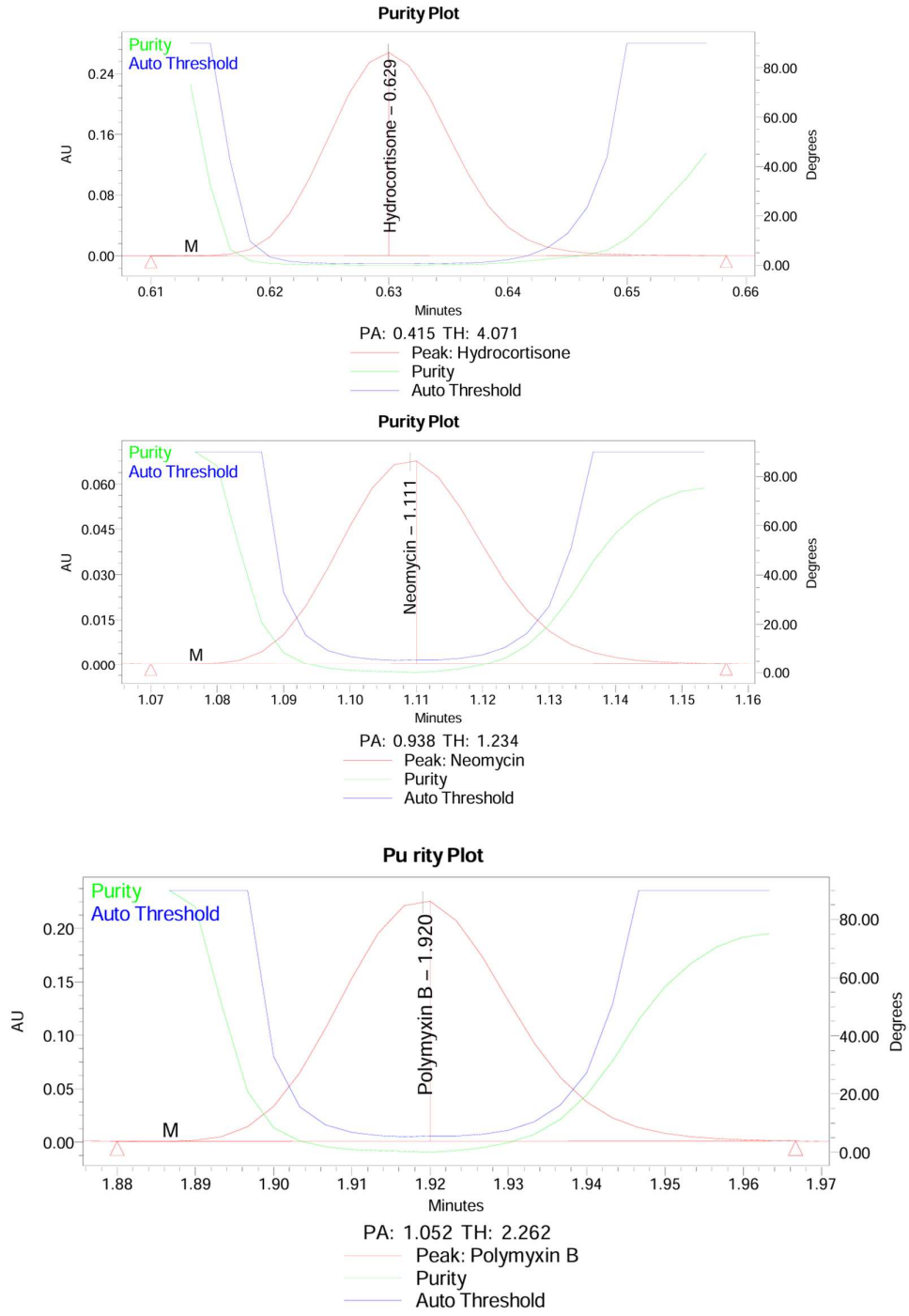
Take 1 ml of sample stock into a 10 ml volumetric flask and add 1ml of 10% H_2O_2. Leave it for 15 min. After 15 min diluted to volume with diluent and mixed.

The above solution is injected into UPLC system.



Peak Results

	Sample Name	Name	Area	USP Tailing	USP Plate count	USP Resolution
1	Peroxide deg	Hydrocortisone	2598647	1.19	13684	
2	Peroxide deg		438564	1.26	3856	2.98
3	Peroxide deg	Neomycin	895113	1.12	5536	4.75
4	Peroxide deg		134758	0.86	4527	3.41
5	Peroxide deg	Polymyxin B	2506507	1.15	13423	7.49
6	Peroxide deg		438565	1.34	6320	6.88



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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