HOW TO APPROACH
A FORCED DEGRADATION STUDY

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The stability of a drug product or a drug substance is a critical parameter which may affect purity, potency and safety. Changes in drug stability can risk patient safety by formation of a toxic degradation product(s) or deliver a lower dose than expected. Therefore it is essential to know the purity profile and behaviour of a drug substance under various environmental conditions.

Forced degradation studies are carried out for the following reasons:

- To develop and validate a stability indicating method
- To determine degradation pathways of drug substances and drug products (e.g., during development phase)
- To identify impurities related to drug substances or excipients
- To understand the drug molecule chemistry
- To generate more stable formulations
- To generate a degradation profile that mimics what would be observed in a formal stability study under ICH conditions
- To solve stability-related problems (e.g., mass balance)

In order to monitor possible changes to a product over time, the applied analytical method (in most cases a chromatographic method) must be stability-indicating.

The best case for testing the suitability of a method is using real-time stability samples containing all relevant degradation products that might occur. But due to product development timelines, process characteristics, excipients, and other environmental factors, a forced degradation study (stress test) can serve as an alternative.

In a typical study, relevant stress conditions are light, heat, humidity, hydrolysis (acid / base influence) and oxidation or even a combination of described parameters. If it is necessary to form degradation products, the strength of stress conditions can vary due to the chemical structure of the drug substance, the kind of drug product, and product specific storage requirements. An individual program has to be set up in order to reach a target degradation of 5 to 20%. A higher level of degradation will be out of the scope of product stability requirements and therefore unrealistic. The scope of the test is to generate degradation products in order to facilitate a method development for determination of the relevant products.

Therefore, samples will be stressed in a solid form and/or in solution. Typically, stress tests are carried out on one batch of material. For drug products the placebo should be stressed in a similar way in order to exclude those impurities that are not degradation products (e.g. impurities arising from excipients). Table 1 shows typical stress conditions of API and drug product.

Drugs that are poorly soluble in water can be conducted either in suspension or in solution using inert organic co-solvents (e.g., DMSO, acetic acid or propionic acid). It is important to avoid co-solvents that may be reactive with the drug or complicate analysis (e.g. by LC-MS).

TABLE 1: TYPICAL STRESS CONDITIONS OF API & DRUG PRODUCTS

STRESS CONDITION	EXAMPLES	API		DRUG PRODUCT	
		SOLID	SOLUTION / SUSPENSION	SOLID	SOLUTION / SUSPENSION
ACID/BASE	0.01 to 0.1N	–	X	–	X
OXIDATIVE	0.3% H_2O_2	–	X	–	X
LIGHT	1200 Lux h	X	X	X	X
TEMPERATURE	10°C to 70°C	X	X	X	X
TEMPERATURE / HUMIDITY	10°C to 70°C and 60 to 90 r.h.	X	–	X	–

X. recommended; (-) not tested

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A typical study design should be able to cover different stress conditions using different time periods in order to assess the degradation kinetics. The following table documents a typical stress design. Testing of samples can be performed at defined sampling points using the new method. The specificity of method will be documented and mass balance calculated. Specificity of method for the active ingredient can be established by peak purity experiments using LC-UV, LC-MS or other suitable methods. Investigation of mass balance in stress samples show the suitability of analytical method to examine for degradation products. Usually 100% should be reached by adding assay value and levels of degradation products. If 100%-level is not achieved, efforts to account for the missing mass should be made e.g. response factors, formation of volatiles or retained impurities. Depending on method precision,

a variation between 97 to 104 % can be acceptable.

Based upon the product, a specific design will be chosen. An example of a stress study performed on a semi-solid formulation containing a preservative and a hormone as active ingredient is outlined in Table 2. In this example, the active ingredient is specified within European Pharmacopoeia and six known impurities (A to F) are described.

The stress study was performed using the design described in Table 3. In addition to a batch of drug product, the stress test was also performed on a placebo (containing preservative), and the stressed samples were tested for assay and purity. Procedurally, the samples that are being stressed for the latest time period are analyzed first. Peak purity was checked (match >900), and results were

compared to a stressed placebo and an unstressed sample and relevant impurity selected. For a UV-match factor, the correlation of a spectrum at peak maximum and at beginning and end of a peak will be tested. In the case of a 100% match, a match factor of 1000 will be the result. For mass spectra, the distance between masses and the relative height of a mass peak will be tested. A match factor of 1000 documents a perfect correlation. The results of main components formed under the stressed conditions for both placebo and drug product are summarized in Table 4 and Table 5.

Based on the results of the stress test, a high number of impurities were formed in sample as well as in placebo solutions within the tested period of stress.

Testing of placebo containing preservative lead to a high number of different im-

TABLE 2: TYPICAL STRESS STUDY DESIGN

STRESS CONDITION	EXAMPLES	DRUG SUBSTANCE	DRUG PRODUCT	PRODUCT PLACEBO
ACID/BASE	0.01 to 0.1N	1-7 days	24 to 48h	24 to 48h
OXIDATIVE	0.3% H_2O_2	few hours to 7 days	24 to 48h	24 to 48h
LIGHT	1200 Lux h	>48h	>48h	>48h
TEMPERATURE	10°C to 70°C	up to 2 weeks	up to 3 weeks	up to 3 weeks
TEMPERATURE / HUMIDITY	10°C to 70°C and 60 to 90 r.h.	up to 2 weeks	up to 3 weeks	up to 3 weeks

TABLE 3: STRESS STUDY FOR HORMONE EXAMPLE

PARAMETER	24H / 1 DAY	3 DAYS	7 DAYS	14 DAYS	28 DAYS
LIGHT	X				
HEAT, 70°C		X	X	X	X
OXIDATION (3%)	X				
1 M HCL (pH 1.0)	X	X	X		
1 M NaOH (pH 12.0)	X	X	X		

purities regarding an unstressed placebo. The amount of preservative decreased mainly by influence of oxidation, light and acid. Mass balance of preservative shows almost 100%.

The active ingredient remains almost stable within tested period. Some known impurities rose to a level of about 2% and one unknown component was detected at a higher level. Consequently, this component was the reason that mass balance rises to a level above 105%. Identification of peaks was performed using LC-MS and the unknown component was identified to be a degra-

TABLE 4: RESULTS - DRUG PRODUCT

NAME	DRUG PRODUCT UNSTRESSED [%]	DRUG PRODUCT OXIDATION 24H [%]	DRUG PRODUCT LIGHT STRESS 24H [%]	DRUG PRODUCT 70°C 28 DAYS [%]	DRUG PRODUCT 0.1N NaOH 7 DAYS [%]	DRUG PRODUCT 0.1N HCL 7 DAYS [%]
API	100	102	102	100	96	94
UNKNOWN 1*	–	7	2	4	1.4	0.4
IMPURITY A	0.2	–	–	1	0.2	0.1
IMPURITY F	0.2	0.3	2	–	0.1	0.1
IMPURITY D	0.2	0.3	0.2	0.2	0.2	0.2
SUM OF OTHER UNKNOWN PEAKS (<10%)	–	–	2	5	3	1
MASS BALANCE API	101	**103**	106	106	100	96

* Peak was related to be a degradation product of preservative.

TABLE 5: RESULTS - PLACEBO

NAME	DRUG PRODUCT UNSTRESSED [%]	DRUG PRODUCT OXIDATION 24H [%]	DRUG PRODUCT LIGHT STRESS 24H [%]	DRUG PRODUCT 70°C 28 DAYS [%]	DRUG PRODUCT 0.1N NaOH 7 DAYS [%]	DRUG PRODUCT 0.1n HCL 7 DAYS [%]
PRESERVATIVE	99	33	39	84	96	57
UNKNOWN P 1	–	16	–	–	–	–
UNKNOWN P2	–	17	–	–	–	–
UNKNOWN P3	–	–	10	–	–	–
UNKNOWN P4	–	–	–	–	–	37
UNKNOWN P5	–	–	–	–	–	–
TOTAL UNKNOWN OTHER (<10%)	–	35	45	17	4	4
MASS BALANCE PRESERVATIVE	99	101	104	101	100	98

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dation product of preservative. An overlay of a light stress experiment is shown Figure 1. An unstressed sample is shown in chromatogram 1, a sample stored for 24 h in a sun-tester (1.2 million lux hours) in chromatogram 2, and a placebo stored under the same conditions as the sample in chromatogram 3. As demonstrated, the main degradation products were related to placebo containing preservative. In order to decrease high mass balance, level response factors should be evaluated. This additional test was not part of our work.

FIGURE 1: OVERLAY LIGHT STRESS
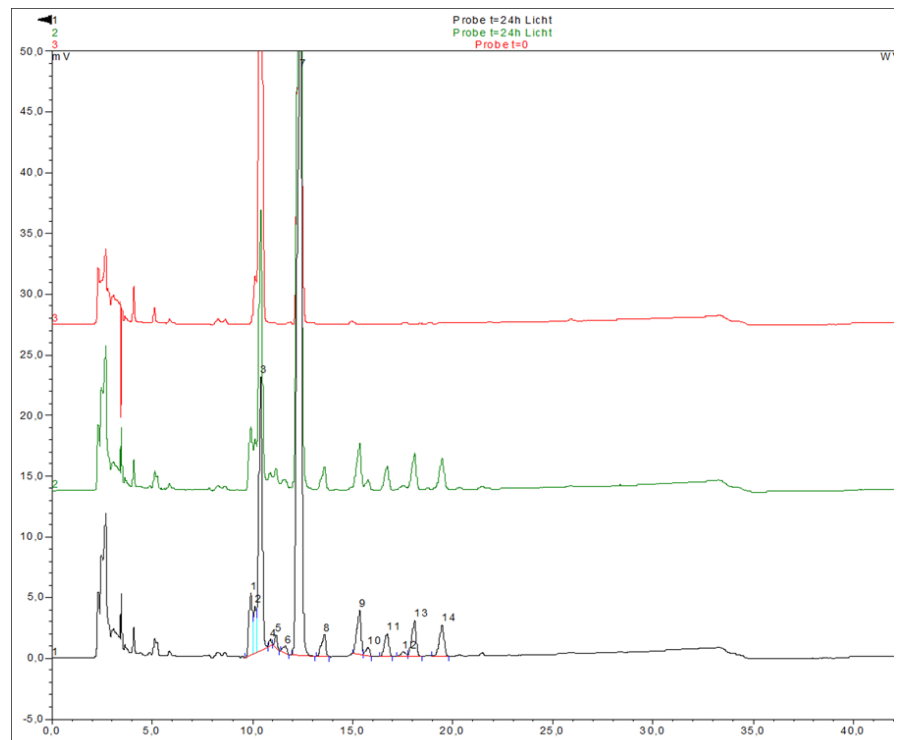
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