



Review

Practical and regulatory considerations for stability-indicating methods for the assay of bulk drugs and drug formulations

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ABSTRACT

As part of the overall analytical control strategy, current regulations require stability-indicating methods (SIMs) to demonstrate product integrity until the re-test period of drug substances (DSs) or throughout the shelf life of the drug products (DPs). Accordingly, relevant topics related to SIMs (mainly for DSs but also for DPs) are critically reviewed and some recommendations are given.

The development of a SIM is a process that embraces three stages; these entail obtaining suitable samples, selecting the separation technique and choosing the right detection, which also comprises method development and optimization and, finally, validating the method.

The first stage yields proper knowledge of the required physicochemical properties of the DS and a deep understanding of its intrinsic stability; these are acquired through stress and accelerated testing, an approach that provides the most appropriate samples for developing SIMs.

For small organic molecules, HPLC is the first choice for undertaking the second stage, which entails developing powerful separations with stability-indicating properties. In case of biologics and certain combined products, achieving analytical methods with stability-indicating properties demands a series of methods based on different, orthogonal approaches. Adequate separation of the relevant degradation products from the main analytes requires optimization of a number of chromatographic factors, including column packing, mobile phase composition, the elution mode and other variables.

Evaluation of all the stability-related issues demands proper detection systems. Sophisticated hyphenated chromatographic methods are extremely useful for developing powerful SIMs; however, similar chromatographic methodologies with simpler detections are enough for routine use of the SIMs as analytical tools. Full method validation according to official guides, and demonstration of suitability of the SIM for monitoring those products actually formed, finally proves its true stability-indicating power.

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1. Introduction

Quality, safety and effectiveness are the most important attributes of pharmaceutical products. However, considering that active pharmaceutical ingredients (APIs) comprise a wide spectrum of compounds, ranging from small natural or synthetic molecules to large biologicals and biotechnologically-derived products, any approach towards controlling compliance with these attributes must be comprehensive, yet simple and feasible to be carried out.

Because of its key role in public health, the pharmaceutical industry has always been a comparatively highly regulated area. Up to the end of World War II, the quality of pharmaceutical products was determined mainly by assaying the content of their active ingredient(s). Since then, analytical instrumentation has undergone a fantastic revolution, successively enabling the resolution of increasingly complex samples and the detection of minor amounts of any kind of analyte contained therein.

This revolution facilitated the setting of an analytical chemistry-based regulatory framework to govern the development of pharmaceuticals. This paradigm has been continually evolving, especially in those aspects that define their standards of quality.

Since the early 1970s, it has become a concern that unstable drug products (DPs) may not be able to maintain their quality attributes after being stored over a period of time, so, in 1975, the United States Pharmacopeia (USP) included a clause regarding the drug-expiration-dating period. In addition, in 1984, the US Food and Drug Administration (FDA) issued the first stability guideline. Furthermore, in 1987, specific requirements on statistical design and analysis of stability studies for human drugs and biologics were published with the aim of establishing product requirements and appropriate expiration dates. Guidelines on the submission of stability information and data for applications to the FDA for Investigational New Drugs (INDs) and New Drug Applications (NDAs) were released at the same time.

In 1993, the International Conference on Harmonization (ICH) issued the Q1A guideline on stability [1], based on the strong industrial interest in harmonizing the requirements for international marketing in the European Union, Japan, and USA. Other guidelines dealing with drug stability followed shortly afterwards (Table 1) and the requirement for stability studies was also included in its GMP for APIs guideline Q7A [2]. Despite these efforts, stability practices are still not fully harmonized. The regulatory bodies of Japan, USA, the European Union [3], Canada [4], Australia

Table 1
ICH guidelines related to pharmaceutical stability and their requirement of SIMs

Subject	Code	Contents	Stability ^a	SIMs ^a
Stability	Q1A	Stability testing of new substances and products	X	X
	Q1B	Stability testing: Photostability testing of new substances and products	X	-
	Q1C	Stability testing for new dosage forms	X	-
	Q1D	Bracketing and matrixing designs for stability testing of drug substances and drug products	X	-
	Q1E	Evaluation of stability data	X	X
Analytical validation	Q2	Validation of analytical procedures. Text and methodology	-	-
Impurities	Q3A	Impurities testing in drug substances	X	-
	Q3B	Impurities in new drug products	X	-
	Q3C	Impurities: Guideline for residual solvents	-	-
	Q3D	Impurities: Guideline for metal impurities	-	-
Pharmacopeias	Q4A	Pharmacopoeial harmonization	-	-
	Q4B	Recommendation of Pharmacopoeial Texts for Use in the ICH Regions	-	-
Quality of biotechnological products	Q5A	Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin	-	-
	Q5B	Analysis of the expression construct in cells used for production of r-DNA derived protein products	-	-
	Q5C	Stability testing of biotechnological/biological products	X	X
	Q5D	Derivation and characterization of cell substrates used for production of biotechnological/biological products	-	-
	Q5E	Comparability of biotechnological/biological products subject to changes in their manufacturing process	X	-
Specifications	Q6A	Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: chemical substances	X	X
	Q6B	Specifications: Test procedures and acceptance criteria for biotechnological/ biological products	X	X
API	Q7A	GMP guide for active pharmaceutical ingredients	X	X
Development	Q8	Pharmaceutical development	X	X
Risk	Q9	Quality risk management	X	-
Quality systems	Q10	Pharmaceutical quality systems	X	-
Manufacturing processes	Q11	Development and manufacture of drug substances (chemical entities and biotechnological/biological entities)	X	X

^a The guideline contains explicit mention to drug/product stability or indication(s) that the analytical methods to be used should be stability-indicating.

and other countries have adopted and expanded the ICH texts, which now have the force of law. The World Health Organization (WHO) has also issued directives regarding the study of stability in pharmaceuticals [5,6].

As a consequence, ensuring regulatory compliance with regards to the integrity and/or content of the active ingredient(s) became essential, but not enough. Regulations currently require the full definition of the purity of pharmaceuticals and exhaustive tests and evaluations of the presence of impurities. These important tasks aim to ensure that the observed pharmacological and toxicological effects are not due to the impurities, but are truly those of the purported active principle.

From the point of view of impurities, an exhaustive detail of the commonly observed impurities and those likely to appear during the product shelf-life (or until re-test in case of APIs) as a result of degradation has therefore become mandatory for new items [7,8] and analytical methods able to carry out their determination are now essential [9].

In general, pharmaceutical items intended for the global pharmaceutical market are currently tested for stability under normal storage conditions for as long as 36 months [1], though, typically, regulatory agencies would initially assign only a 24-month conformance period, thereby providing an extra stability reserve [10]. This is achieved through exhaustive research and thorough understanding of the stability characteristics of the drug substances (DSs) and DPs, and a long-term testing program [1]. Meaningful product-expiration dates are obtained only after meticulous, scientifically-designed studies using specific stability-indicating assays, rigorous computer-assisted analysis of the resulting data, and appropriate statistics [11]. Thus, a satisfactory 3-month accelerated data submission may also permit granting a 24-month tentative expiry date, providing that the room-temperature data also meet specifications [10]. Hence, stability-indicating methods (SIMs) play a key role in current pharmaceutical regulation. This review presents and discusses the importance of SIMs in achieving regulatory compliance with regards to the stability of pharmaceutical items.

2. Scope and regulatory status of stability-indicating methods

According to the FDA, SIMs are “validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the DS and the DP, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference” [12]. From the regulatory perspective, a SIM must therefore “accurately measure the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities” [13].

It is implicit in the definition that SIMs must be quantitative, specific and reproducible methods, and able to monitor relevant chemical, physical and microbiological changes in DSs or DPs over time. Demonstration of these characteristics, which make the method suitable for its proposed use, must always be documented.

However, despite the official requirement to use SIMs, as emphasized in the British Pharmacopoeia, the USP and several ICH regulatory guidelines, none of these documents provides a definition of a SIM [1,2,14,15]. Moreover, the stability-indicating requirement is absent from several well-recognized Pharmacopoeias, such as the 15th Edition of the Japanese Pharmacopoeia. Furthermore, available official guidance concerning scope, timing and best practices for degradation studies, required for developing SIMs, is still very general.

SIMs are required for stability studies, and stability information is needed for regulatory submissions, such as INDs and NDAs, and to set expiration dates for APIs and DPs. SIMs are also required for

complying with other regulated events, such as API and DP release, toxicology dosing solutions, excipient-compatibility evaluation, pre-formulation and packaging studies, and line extension. They are also powerful tools for routine quality control (QC) and for investigating out-of-specification [16,17] and out-of-trend results [18].

Hong and Shah distinguished between a “stability-specific method”, as a method capable of quantifying the API in the sample matrix without previous separation and a “stability-indicating method”, where the drug is measured after separation from other analytes, including its degradation products. In both cases, the power of the method arises from its discriminating nature [19].

However, in their thorough collection of references to degradation studies of DPs and in-depth analysis of SIMs, Bakshi and Singh [20] distinguished the terms “specific stability-indicating method” (specific SIM) and “selective stability-indicating method” (selective SIM). According to these authors, a specific SIM is an analytical method suitable for unequivocally measuring the API in the presence of all of its degradation products, as well as excipients and additives, expected to be present in the formulation. However, a selective SIM is a method capable of unambiguously measuring the API and all of its degradation products in the presence of excipients and additives, expected to be present in the formulation.

Bakshi and Singh also demonstrated that the claims of stability-indicating ability were not always well founded. The approaches described for method development were very variable, and included conducting stress testing at only a few of the recommended conditions or performing no stress testing at all; therefore, they fell short of meeting the current regulations at that time. [20]. Fortunately, however, a better proportion of the methods published during the past decade results from samples subjected to various hydrolytic, oxidative, photolytic and thermal stress conditions, seemingly following the ICH guidelines more strictly.

3. Strategies and considerations for developing SIMs

Although different authors agree that there is no universal strategy for developing SIMs [10,20,21], three major stages are currently recognized, in agreement with the main process flow of analytical method development [22,23]. These broadly include:

- generating suitable samples for testing method selectivity;
- choosing a method and optimizing its selectivity and sensitivity; and, finally,
- validating the method.

Other multi-step approaches have been reported. For example, Dolan emphasized selecting a detector able to detect all the relevant entities (API and pertinent degradation products) as one of the three major stages of SIM development [24]. At this point, DAD is most suitable for HPLC methodology if the entities have chromophores. Dolan's proposal also considers method validation within the task of selecting the proper separation technique.

However, Bakshi and Singh [20] considered that the development of a SIM likely to meet regulatory requirements is a seven-step process that entails

- critical study of the drug structure to assess the likely decomposition route(s);
- collection of information on physicochemical properties;
- conducting stress (forced decomposition) studies;
- preliminary separation studies on stressed samples;
- final method development and optimization;
- identification and characterization of degradation products, and preparation of standards; and,
- validation of SIMs.

3.1. Stage 1: obtaining suitable samples

Obtaining suitable samples and analyzing them are crucial steps in establishing method specificity. They require thorough knowledge of the degradation chemistry and the physicochemical properties of the DS and its degradation products, as well as good scientific judgment to ensure that the samples truly contain all relevant degradation products.

The ability of a SIM to monitor changes in the chemical properties of the drug over time, determines the need to perform forced degradation studies (stress and accelerated tests) on the DS and the DP, so these tests constitute a convenient alternative to generating samples containing the analyte and its degradation products [25]. According to the available regulatory guidance, they provide valuable information, including the determination of the degradation pathways of DSs and DPs, revealing the intrinsic stability of the API in the solid state and in solution and its susceptibility to hydrolytic, oxidative, thermolytic, and photolytic degradation. Furthermore, the resulting structural elucidation of the degradation products enables the discernment of compounds in formulations related to the DS from those arising from the excipients.

Stress tests also fulfill the purpose of providing meaningful amounts of degradation products, which can be isolated and purified [26] for complete physicochemical characterization and acquisition of impurity standards before carrying out the method development and validation [27].

Alternatively, resorting to the high sensitivity and excellent separation capability of coupled methods, such as UPLC with MS detection (UPLC-MS), which also provide structural information, samples can be submitted to concomitant separation and spectroscopic analysis for peak deconvolution and structure elucidation [28]. In this case, no purified impurity standards are generated at this stage. Monitoring of degradation reactions is also helpful for better understanding of some important characteristics of the analytes (e.g., polarity and stability) and to decipher which degradation product is relevant and which is not. A relevant degradation product is considered that resulting from direct degradation; usually, it is formed in high concentration before the drug becomes degraded not more than 10–20%, is representative of the degradation under real conditions, or is generated under mild conditions and/or after short exposure time [29]. However, non-relevant degradation products are those resulting from non-purposeful degradation, usually obtained under exaggerated conditions [30].

3.2. Stage 2: method selection and optimization

3.2.1. General questions

The task of developing a SIM is always a challenge, which includes:

- (a) method selection; and,
- (b) its optimization for selectivity and sensitivity to ensure that all the relevant analytes are separated and duly detected.

As previously noticed [20], approaches for method development are very variable and not every published method purported to be stability-indicating has this property nor is it suitable for its intended use.

In particular, most of the spectroscopic (UV, fluorescence) methods claimed as SIMs do not seem to be able to indicate stability. In their design, typically no real or virtual analyte-signal separation takes place and they often fail to comply with specificity, sensitivity and/or robustness issues. Other literature methods suffer from incomplete validation or inadequate assessment of robustness, and most lack proper selection of the relevant degradation products, or these were not completely and unequivocally identified.

Because multiple components need to be separated during analysis of stability samples, chromatographic methods have taken precedence over other methods of analysis. For low-molecular-weight organics, HPLC/UPLC [10,31,32] in reversed-phase mode has become the best choice for developing SIMs.

The speed, the resolution and the sensitivity of UPLC separations, when combined with the high-speed scan rates of UPLC-specific photodiode-array and MS detection, make the identification of degradation products more effective and the time required to develop the SIM is shortened. Not surprisingly, this technique is gaining pre-eminence among the options for studying the intrinsic stability of drugs and developing comprehensive SIMs.

Other LC modes, such as normal phase [33,34], ion exchange [35], ion-pairing [36,37] and hydrophilic interaction chromatography (HILIC) [38,39], in addition to alternative separation methodologies, such as capillary electrophoresis (CE) [40,41], have also been used, although to a lesser extent. CE stands out for its advantageous sensitivity, eco-friendliness, resolution and high efficiency; but it is not as precise as HPLC. However, spectrophotometric methods [42] have also been reported, but they seldom have truly stability-indicating properties, even when enhanced by the use of chemometrics methods [43].

Thin layer chromatography (TLC) has also been used for developing SIMs; however, its non-quantitative nature and high variability give methods that are unsuitable for current standards [44]. However, high-performance TLC (HPTLC), an advancement of TLC that has inspired a huge number of publications, appears to be a more valid option for developing SIMs, particularly when a few analytes must be separated. It is relatively inexpensive, reliable, fast and reasonably accurate for quantitative analysis. In comparison with HPLC, it allows simultaneous multiple runs and uses small volumes of mobile phase, resulting in an eco-friendly option [45,46]. However, the separation power of HPTLC is lower than that of HPLC.

There are very few reports on GC for the purpose of establishing SIMs [47]. The technique is not versatile enough, as the analytes may be non-volatile or thermally unstable, and any attempt to increase their volatility by increasing the temperature or performing derivatization may also lead to degradation.

HPLC is preferred for developing SIMs for low-molecular-weight organic molecules [48] for several reasons:

- (1) the compatibility of HPLC with aqueous and organic solutions;
- (2) high precision;
- (3) ability to handle thermally unstable and polar compounds;
- (4) the availability of highly sensitive detectors; and,
- (5) HPLC provides not only useful quantitative information on drug loss but also insights into the number of degradation products formed and their corresponding amounts.

Separation is effected by selecting appropriate chromatographic conditions [49–51].

Chiral drugs containing a single labile stereogenic center require an additional chiral method to establish their stereochemical purity and stability [52–54]. Analogously, drugs exhibiting polymorphism need an additional proof of polymorphic identity if instability at this level affects the performance of the DS or the DP [55].

Once the separation method is selected, literature precedents and knowledge of the chemical structures of the degradation products may offer some clues on how to set up the initial chromatographic conditions, which should aim to achieve a minimum separation among the analytes or at least to separate some impurities from the main peak. It is also very important to ensure that all relevant degradation products are in solution. When HPLC is

employed for developing a SIM, a 1:1 mixture of water and a miscible organic solvent compatible with the analytical technique chosen is a good starting point to dissolve the samples.

3.2.2. HPLC column selection

The column and the mobile phase define capacity factors (peak positions), method selectivity, the duration of the chromatography, and peak resolution. Most of the recorded SIMs have been developed employing reversed-phase HPLC, packed with chromatographically-active phases that are chemically bound to a silica-gel matrix. This matrix is compatible with the usual reversed-phase organic solvents and is stable under acid conditions, but slowly dissolves at pH above 7.0. Special columns, which resist alkaline conditions, are available if needed in these cases [56]. Alumina, graphitized carbon, synthetic polymers, zirconia and other matrices have also been used to a lesser extent. Mazzeo et al. have devised a systematic four-column approach to development of SIMs [57].

A survey of the recent literature indicated that the most commonly employed stationary phases are C8 and C18 [58,59], but packings containing other groups, such as aromatics (Ph) [60,61] have also been used. Specialized functionalities, such as cyano [62], amino [63], diol [64] and others [65,66], have found less use, and have been employed for more specific purposes. In addition, chiral columns have been occasionally used for method development [67].

Non-polar columns may collapse and further exhibit difficulties for re-equilibration when employed with mobile phases containing more than 90% water. Although some scattered, successful cases of SIMs with highly aqueous mobile phases and non-polar columns have been published [68,69], the use of stationary phases containing polar-embedded groups is indicated for these problems, and to solve selectivity issues [70].

Usually, 25-cm columns are employed, but shorter columns (15 cm or less) packed with spherical beads may be preferable if good resolutions are achieved, as they enable quicker separations. Particle size is relevant for separation efficiency, but it also determines the operating backpressure of the system. For HPLC, 5 μm is currently the most commonly-used particle size; however, particle sizes of 7 μm and 10 μm are still being employed [71], while columns packed with particles of 3 μm diameter or smaller can be employed for the generally more demanding UPLC-based separations [72,73].

Different manufacturers employ dissimilar technologies to prepare the stationary phases, which often result in wide inter-column variations and sometimes in small inter-lot differences for the same manufacturer and the same column [74]. This makes direct column exchange very difficult and often hinders method reproducibility; therefore, when developing a method, it is crucial for its future reproducibility to describe column characteristics fully.

3.2.3. HPLC mobile-phase selection

Most of the mobile phases employed in the SIMs recorded in the literature are mixtures (in widely different proportions) of water or aqueous buffer solutions (e.g., phosphate and acetate) with an organic solvent (mainly methanol or acetonitrile). Isopropanol [75], tetrahydrofuran [76], and other solvents [77,78], have also been used, albeit to a lesser extent. Additives {i.e., amines [79,80] or alkylsulfonates [81]} may also be added. However, volatile buffers or non-buffered mobile phases are required when MS or aerosol-based detectors are used [82]. In any instance, it is advisable to keep the composition of the mobile phase as simple as possible.

3.2.4. HPLC Elution mode: isocratic vs. gradient

For initial method development and screening of conditions, the use of broad gradients is the most appropriate approach for maxi-

mizing the separation of early-eluting peaks, while increasing the chances of detecting more retained peaks [32,83]. However, for the final method, it is desirable that the chromatographic separation be as short and as efficient as possible [84], generally no longer than 2.5 times the retention time of the main analyte. The isocratic mode avoids column stress due to variations in mobile-phase composition and internal pressure, as well as spending extra time for re-equilibration; however, when facing complex separations of a large number (i.e. >10) of compounds [85,86] with widely different polarities [the capacity factor (k') of the last eluting peak exceeds 5], use of a gradient is recommended [87,88].

The decision between isocratic and gradient modes can also be made by running the separation in the gradient mode and calculating the ratio between the gradient time required for elution of the analytes (∇_a) and the total gradient time (∇_t). Isocratic elution should be preferred when $\nabla_a/\nabla_t < 0.10$; however, for $0.10 \leq \nabla_a/\nabla_t \leq 0.40$, either elution technique could be used, while samples containing peaks that occupy more than 40% of the separation space certainly require gradient elution [89].

3.2.5. HPLC column temperature and flow-rate

Temperature changes may affect the retention times ($\Delta k' \approx -1\%/^\circ\text{C}$) of the analytes. This effect is common to all HPLC separations and impacts on resolution, selectivity and method repeatability, especially when gradients are run [90–92]. Thermostatting the column, usually in the 25–35°C range, is therefore desirable. Higher temperatures may be sometimes be used to advantage, since they reduce solvent viscosity, lowering the backpressure and allowing operation at higher flow-rates [93–95], albeit at the expense of some shortening of the useful life of the column. However, flow-rates should be commensurate with reasonable backpressure and chromatographic run times.

3.2.6. Detection of the analytes

The peak responses of all analytes of interest should fall within the linear range of the detector, and peaks corresponding to degradation products need to be resolved from the DS. However, all degradation products do not always need to be resolved from each other, so detection is usually oriented towards conditions that guarantee optimum detectability of the relevant degradation products.

Also, a SIM should be able to measure all the relevant impurities formed at low levels, commonly at their expected or required LOQ (down to $\sim 0.05\%$ of the main analyte).

There is no universal detector that can respond equally to all compounds and variable-wavelength UV detectors are the most used; however, developing SIMs requiring highly discriminating separations is likely to require the use of coupled techniques, such as LC-DAD [96] and HPLC/UPLC-MS [97,98].

DAD and MS detectors [99] are also able to detect spectral non-homogeneities in the chromatograms, ensuring the absence of masked co-eluting compounds. The algorithms for peak-purity determination [100,101] are employed to confirm method selectivity. For that purpose, the analytes and the detected interferences must have different spectra and some degree of separation. MS detection may face difficulties with co-eluting diastereomers (same molecular weight), and if the ionization of the degradation product is suppressed by the co-eluting component.

LC-NMR, which provides additional useful information for identification and characterization of the degradation products [59,102–104], is employed to a lesser extent in SIM development due to the higher analyte concentration required for routine use. More universal detectors, such as the charged aerosol detector (CAD) and the evaporative light scattering detector (ELSD), are finding increasing use in the development of SIMs, especially

where the DS or relevant degradation products do not have good chromophores [105,106].

However, less sensitive detectors, such as the refractive index detector [107], and more specific ones, such as amperometric [108] or fluorometric detectors [109], find less use because of their own shortcomings {e.g., the need for derivatization in fluorometric detectors [110,111]}.

Despite some rational strategies having been long available [112], optimization of the detection conditions is still mostly carried out by spectral observation or taking into account prior knowledge of the properties of the analytes of interest [113,114].

3.2.7. Method optimization

The initial chromatographic conditions must be developed with a focus on separating all the analytes of interest, and further optimized in order to obtain the most convenient separation. Unfortunately, despite this as an important goal, not all the published methods are fully optimized.

The most suitable separation can be achieved by the systematic optimization of all the chromatography variables. For HPLC-based methodologies, optimization of the separation should include selection of:

- (1) an acceptable column and chromatographic mode;
- (2) an adequate mobile phase;
- (3) suitable detection conditions;
- (4) a convenient column temperature; and,
- (5) an appropriate solvent flow.

This can be carried out by adjusting one variable at a time or through the use of experimental design strategies, where several variables are jointly optimized within a restricted experimental domain, employing statistical and graphic methodologies. Experimental designs are able to furnish proper conditions with a minimal number of experiments, while also providing the practitioner with better understanding of the effects of modifying different variables on the performance of the chromatographic separation [115,116]. As optimization of many variables may be impractical, the optimization problem can be simplified by establishing conditions for the less critical factors (e.g., injection volume, column temperature, flow-rate, components of the mobile phase, and detection conditions), before submitting the most crucial variables (e.g., column type, composition of the mobile phase, including pH, ionic strength, additives, and modifiers) to closer examination. When experimental design strategies are employed, the less critical factors are identified after subjecting the system to an initial screening phase.

Although not so widely employed, the approach involving experimental designs is smart, less time-consuming and more fit to the current Quality by Design (QbD) paradigm (see below) [117].

In all cases, the optimization stage should aim to achieve optimal separation of the critical pair(s), in the shortest possible time and under robust conditions [118]. It may also include paying due attention to obtaining good peak shapes (control of tailing), and the highest sensitivity and minimum difference in k' values between the earliest and the last eluting peak.

3.3. Stage 3: validation of the SIM

Full validation is the last step of SIM development [119], which should start only after the manufacturing process has been optimized, the formulation established, all the test procedures developed and qualified, and the method optimized and considered robust. This is because every change in the manufacturing process, product formulation or chromatographic conditions may require a modification of the SIM and its re-validation. Method validation must have a written, approved protocol prior to use [120,121].

Table 2

Classification of analytical methods according to the USP. Analytical performance characteristics required for validation

Analytical performance characteristic	Category I		Category II		Category III	Category IV
	Quantitative	Limit tests	Quantitative	Limit tests		
Accuracy	Yes	Yes	a	a	No	No
Precision	Yes	Yes	No	Yes	a	No
Specificity	Yes	Yes	Yes	a	Yes	Yes
Detection Limit	No	No	Yes	a	No	No
Quantitation Limit	No	Yes	No	a	No	No
Linearity	Yes	Yes	No	a	No	No
Range	Yes	Yes	a	a	No	No

^a Characteristic may be required, depending on the nature of the specific test.

SIMs should be validated in agreement with the ICH Q2 guide [122], Pharmacopeia or other related guidelines [13,123] found in current regulations. The USP [124] and the ICH Q2 guide classify methods in four categories:

- I analytical procedures employed for the quantitation of the major components of bulk DSs and DPs, including preservatives;
- II analytical procedures (quantitative assays and limit tests) used for the determination of impurities in bulk DSs or degradation compounds in DPs;
- III analytical procedures employed for the determination of performance characteristics of DPs; and, finally,
- IV involves the identification tests.

The USP specifies the analytical information needed for each category (Table 2).

Thus, validations of analytical methods for bulk APIs and their degradation products have highly similar requirements, demanding documented proof of method linearity in the working range, accuracy and precision, and compliance with quantitation limits, when applied.

Specificity is very important, so peak-purity analysis of the main peak, to ensure the absence of co-eluting analytes, is a mandatory aspect of validation. Baseline separation of relevant peaks is also very important. However, mass balance is helpful to assess the appropriateness of the analytical method as a SIM and to determine roughly whether all major degradation products have been accounted for [1,32,130,131].

When the SIM is extended from DP to formulations, the emphasis should be placed on proving the pertinence of the validation results (particularly regarding specificity), due to the presence of other formulation constituents, including excipients. At this stage, forced degradation studies may help in the assessment of specificity, challenging the stability-indicating power of the method.

The corresponding acceptance criteria should be pre-established and, in the case of failure with compliance, the method should be modified accordingly and suitably re-validated. The method development and validation stages end with the corresponding reports. These provide future users with useful historical information and may serve as starting points for related developments, future developments and re-validation. Table 3 depicts typical acceptance criteria for an HPLC-based SIM.

4. SIMs and the QbD paradigm

QbD is a systematic process of building desirable quality in the end product by careful evaluation of all the attributes that go into characterizing quality, from the inception of a product to its end

Table 3
Typical acceptance criteria for HPLC-based SIMs^a

Parameter	Active ingredient	Limit of related/degradation compounds
Specificity (Peak purity on stressed samples, DAD, MS)	>0.999	>0.999
Linearity	r: > 0.99 (minimum 5 concentrations) y ₀ : ±2% Residues ~ N (0, σ ²) Visually linear	r: > 0.98 (minimum 5 concentrations) y ₀ : ±15.0% Residues ~ N (0, σ ²) Visually linear
Range (% of expected concentration)	DS and DP: 80–120 DP: 70–130 (also used for testing content uniformity)	LOQ-120 or Reporting level-120
Accuracy (Mean recovery, %)	Expected concentration: 98.0–102.0 Whole range: 96.0–104.0	0.05% ≤ x < 0.1%: 50.0–150.0 0.1% ≤ x < 0.5%: 70.0–130.0 0.5% ≤ x < 1.0%: 80.0–120.0 x ≥ 1.0%: 90.0–110.0
Precision (RSD, %)	Injection repeatability: ≤1.0 Analysis repeatability: ≤2.0 Intermediate precision: ≤3.0	x < 1.0%: 25.0 x < 0.5%: 15.0 x < 1.0%: 10.0 x ≥ 1.0%: 5.0
LOQ (Concentration Units)	-	Below lower level of range

^a Based on [14,122,125–129].

use, thus ensuring at any time that it meets patient needs [132]. This initiative was introduced by the FDA in 2002 and is being promoted within the pharmaceutical industry with the aims of increasing regulatory flexibility and creating an easier path for the manufacturers to introduce process and product improvements.

In this context, since product stability depends on DS properties, formulation design, the container-closure system and the manufacturing process, the QbD paradigm should be employed to obtain better understanding of the effect of these factors on product stability in order to ensure the product stability through to the expiry date.

Implementing QbD requires gathering sufficient product information during development, setting up suitable SIMs, analyzing changes in process/formulation related to stability, and assessing the risk on product stability during the product life cycle, based on critical quality attributes. In this context, stability tests can be regarded as stability-indicating measures over time of critical product-quality attributes.

Although the QbD philosophy does not modify directly general practice and stability-study guidances, it impacts on the design, the implementation and the reporting of stability testing, mainly as a consequence of its effect on formulation design. However, thorough understanding of the stability of the DS and the DP gained during the development of stability studies is important to the task of “building the quality in” the product, as it is also influential in developing analytical methods and setting up or vindicating specifications [133]. Stability testing should therefore continue to be part of a regulatory submission, but we expect that implementation of the QbD paradigm will change the amount of stability data required. Interestingly, applications for generics are submitted and subjected to revision under the Quality by Review (QbR) paradigm, for which stability tests are also a regulatory requirement [10].

The QbD concept is also being implemented for analytical method-development activities [134–138]. Application of statistical design of experiments is currently encouraged by the regulatory agencies, sometimes together with the use of chromatographic, modeling and optimization software, among them DryLab [139], LC Simulator [140], ChromSword [141–143], Fusion AE [144], and Design Expert [145]. This allows systematic assessment of the critical parameters of the SIM, since method sensitivity, specificity, and

robustness [146,147] are properties especially addressed by this approach with a few experiments and relatively small laboratory effort [148].

For example, upon submission to stress conditions, solutions of pridinol mesylate (PRI) were demonstrated to be stable under basic and neutral hydrolytic conditions, yielding a degradation product (ELI) when exposed to acid hydrolysis, long-wavelength UV or visible light. However, oxidation conditions furnished another degradation product (NOX), while short wavelength UV light and exaggerated exposure to H₂O₂ as oxidant yielded irrelevant degradation (which would not be observed during accelerated or long-term storage conditions) [119]. The impurities were isolated and unequivocally characterized employing infrared and nuclear magnetic resonance spectroscopy. Then, they were also synthetically prepared, as shown in Fig. 1, and kinetic parameters for the acid degradation of the DS were obtained [149].

In the same work, with standards of the impurities at hand, a SIM was rationally developed, employing HPLC. The detection wavelength was selected after examination of the UV spectra of PRI and its degradation products, while selection of the optimum mobile phase was carried out employing an experimental design strategy coupled to a response-surface methodology (RSM) study.

The effects of pH and proportion of the aqueous phase on the retention time of PRI, the resolution between each degradation product and PRI, and the run time of the separation were thus simultaneously evaluated. Method robustness was also demonstrated with the aid of RSM tools before validation, which was carried out according to the ICH guidelines, employing a diode-array detector to assess method specificity. System-suitability conditions were also determined, according to Pharmacopeial indications. The SIM was different from an alternative developed for controlling the synthesis of the drug [150].

5. SIMs and pharmacopeial monographs

All modern Pharmacopeias include DS monographs; however, not all of them contain DP monographs and not every known API and DP is included. The Pharmacopeias also have general chapters directly or indirectly devoted to stability assessment of DSs and DPs.

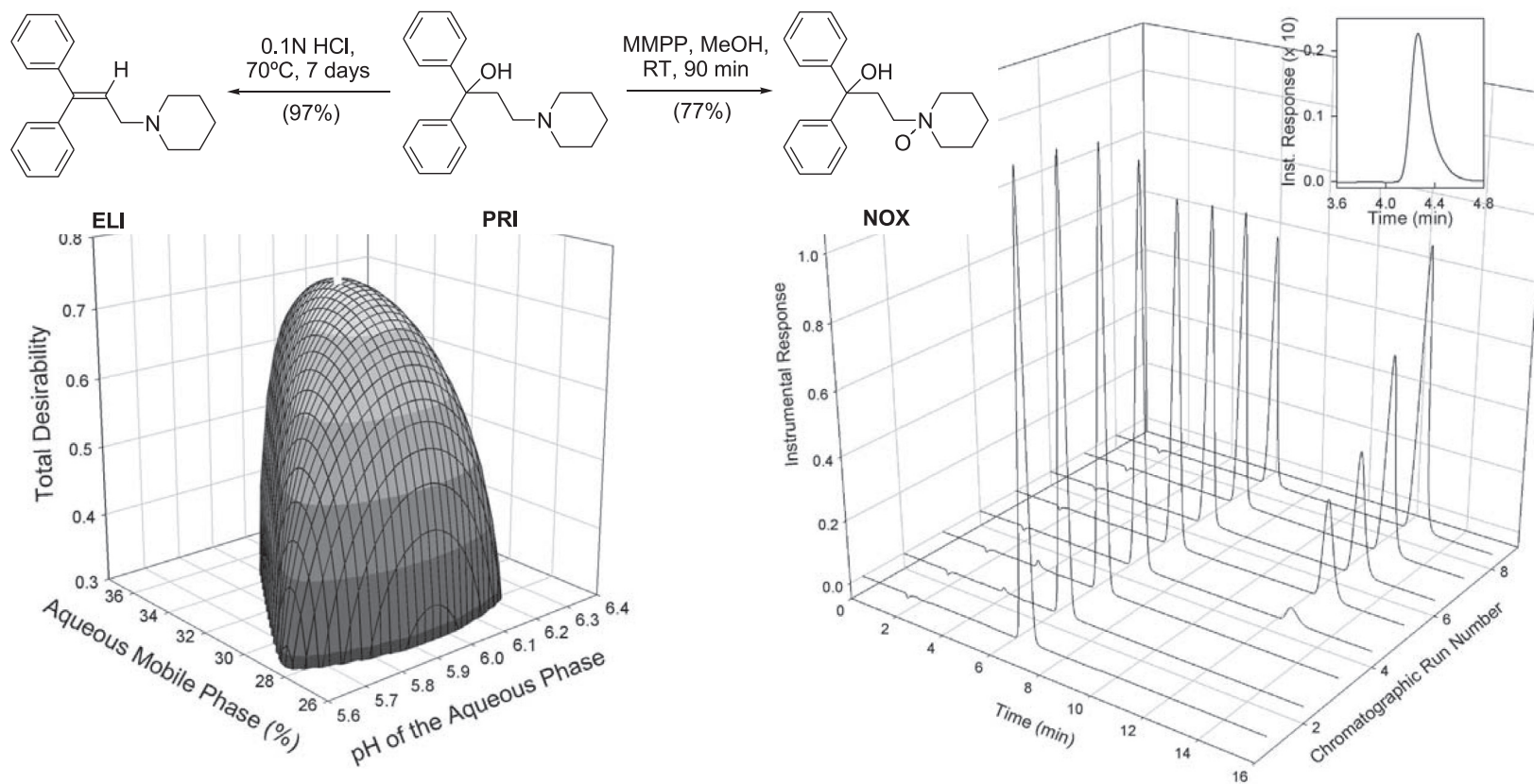


Fig. 1. Top left: Synthesis of degradants of pridinol (PRI). Bottom left: Response surface plot of the optimization of the mobile-phase composition for the separation of PRI from its relevant degradants. The white dot atop of the surface represents the most desirable conditions. Bottom right: Typical HPLC chromatograms of PRI and its degradation products under the optimized chromatographic conditions: (run 1) unstressed sample; (runs 2 and 3) degradation under oxidizing conditions; (runs 4–8) degradation under acid conditions, at different times. [Reprinted from [149]].

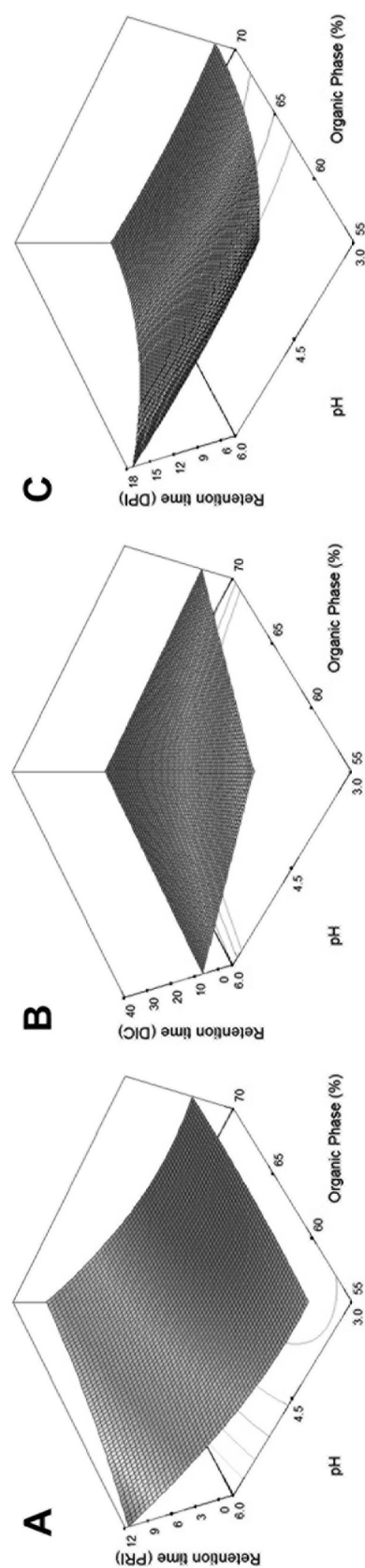


Fig. 2. Modification of the retention times of (A) pridnol (PRI), (B) diclofenac (DIC), and (C) diclofenac-related compound A (DPI) with the composition of the mobile phase. [Reprinted from [155]].

For example, USP Chapter <1150> on Pharmaceutical Stability indicates that “the monograph specifications of identity, strength, quality, and purity apply throughout the shelf life” of the product. However, stability of a dosage form includes the chemical and physical integrity of the dosage unit and its content, and, when appropriate, its ability to maintain protection against microbiological contamination, while remaining fully functional and exhibiting no increased toxicity.

In general, the last item of a typical Pharmacopeial monograph for a DS is an assay intended to determine the content of the API. Since most modern assays are chromatographic (generally HPLC), they may provide an indication of the chemical stability of the corresponding item. There are cases, however, where regulatory and compendial expectations do not seem to be the same. For example, there are many DS items whose assays are based on spectrophotometric or titrimetric methods, but are devoid of stability-indicating power. In such cases, however, the complementary limitation of impurities, degradation products and other contaminants in related substances or chromatographic purity tests may confer some stability-indicating properties to the monograph as a whole [151].

In addition, the stability-indicating properties of a DS assay should be carefully considered before using the official procedure for testing DPs. However, official DP assays or assays developed for a specific dosage form cannot be assumed to be stability-indicating or valid for a different dosage form, because the same DP can be formulated with different excipients and drug-excipient interactions, and interferences due to the excipients themselves may not have been taken into account when the assays were developed. The need of method revalidation should therefore be taken into account before its use in a different scenario from the one originally validated.

6. SIMs for combination products

Combination products are therapeutic and diagnostic products that combine drugs (low-molecular-weight organics), devices, and/or biological products. In addition to the well-known fixed dose combinations, this group embraces a highly complex, increasingly growing set of products, such as pre-filled syringes (with small-molecule drugs or biologics), drug-eluting coronary stents [152], needleless injectors for use with drugs or biologics and separately packaged, and cross-labeled products, such as surgical kits associated with specific drugs.

Different regulatory bodies [FDA, CDER (Center for Drug Evaluation and Research), CDRH (Center for Devices and Radiological Health)] approach QC issues of this heterogeneous group of pharmaceuticals with different philosophies (in-depth assessment vs. risk assessment). However, SIMs for their control often comprise a set of orthogonal methods aiming to evaluate widely different aspects of the combined product stability. This array of methods includes means for evaluating the *in-vivo* and *in-vitro* release rate of the drug component and methods for assessing the stability of inactive ingredients (e.g., integrity of coating polymers). In the case of devices, the methods also entail means for monitoring fatigue, corrosion and durability of the device part, assessing features that are critical to product performance. Drug-device interactions, as well as the effects of the sterilization process on the whole product, should also be tested [153].

For pharmaceutical combinations of two or more APIs, SIMs should be oriented mainly to assess degradation products produced by the separate drugs [154]. For example, a method for the simultaneous determination of diclofenac (DIC) and pridnol (PRI) in their combined pharmaceutical dosage form was recently reported, which limited the presence of diclofenac-related compound A (DPI) [155].

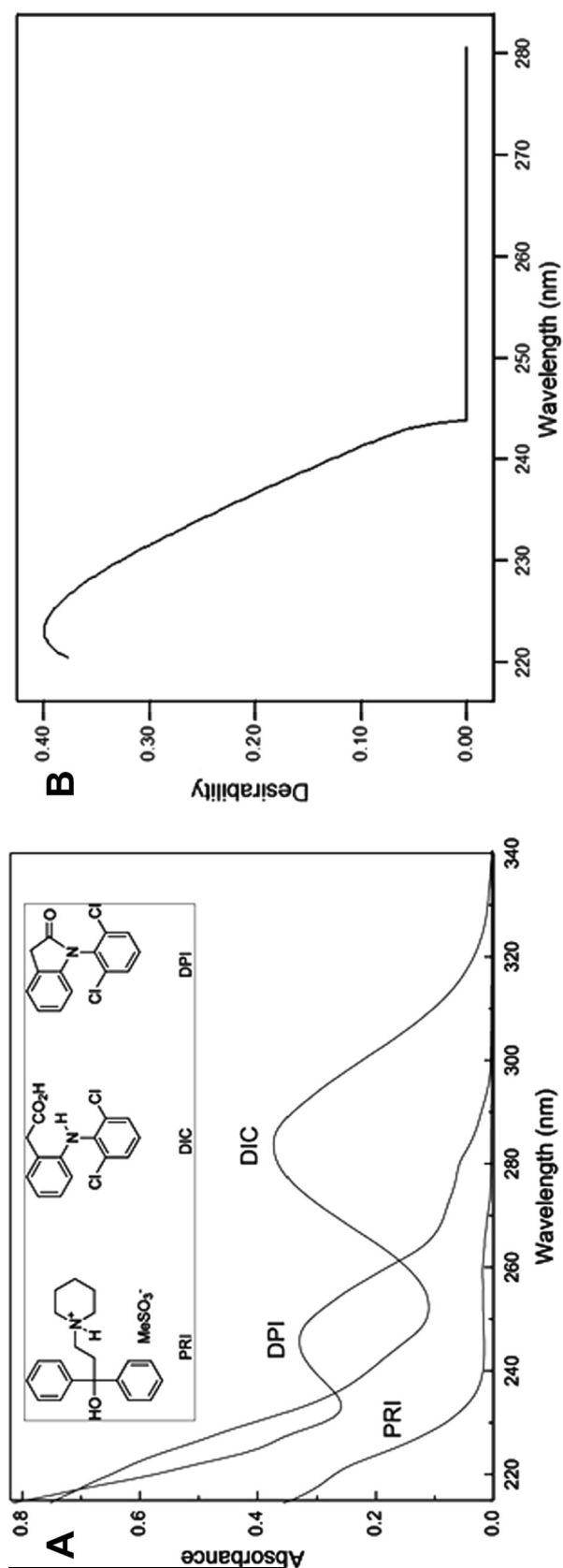


Fig. 3. (A) UV spectra of pridinol (PRI), diclofenac (DIC) and diclofenac-related compound A (DPI); (B) Desirability plot for the optimization of the detection wavelength. (Reprinted from [155]).

Interestingly, a three-level central composite experimental design allowed demonstration of method linearity, range and repeatability with data taken from the same experiment, while use of RSM allowed better understanding of the variation of the retention time of the analytes with the composition of the mobile phase (Fig. 2). However, selection of the most appropriate detection wavelength was performed (Fig. 3B) by applying Derringer's desirability function [156] to the spectral data of the analytes (Fig. 3A), taking into account their relative concentrations.

In addition, SIMs for fixed-dose pharmaceutical combinations should also take into account drug–drug and drug–excipient interactions [157]. Unfortunately, literature analysis indicated that in these cases drug–drug compatibility was seldom evaluated [158,159].

7. SIMs and the drug-development process

Regulations demand that the stability profile of new DSs must be acquired during drug development. Analogously, the profile of DPs must be established during their development, and analytical method development should meet different goals during this process [160]. The main purposes of a pharmaceutical product in its early phases of development are to deliver a known and bioavailable dose range of its active ingredient(s) during Phase I trials and to be efficacious and safe up to the end of Phase II trials.

Therefore, an analytical method in this stage (Phase I) should be designed to ensure potency control, useful for assessing drug efficacy. It should also be able to separate accompanying impurities in the DS and the DP, which relates to verification of the safety profile of the drug, and should permit evaluation of key dosage form characteristics, such as drug release and uniformity, because these properties may affect bioavailability of its active principle(s).

Later in the drug-development process, when the aim is to identify a robust, stable formulation for industrial manufacturing and preparing bioequivalent lots for Phase II (and also Phase III) trials, the method should have stability-indicating properties, being capable of measuring the effect of key manufacturing parameters on its quality and performance, to help ensure that the DS and the DP are produced consistently.

At this time, carrying out formal stability studies, due to be started during Phase III trials, forced degradation samples are used to develop the stability-indicating method. Here, the samples are analyzed for impurities co-eluting with the API (peak purity), as well as method specificity and mass balance. Usually, no further method-development activities are expected, unless process changes in the API and/or formulation demand additional work. It is also worth noting that, if a single method is intended to be used for QC and stability of an API, it should also be able to separate process-related impurities, as well as degradation products.

From Phase III onwards, the efforts aim to confirm product safety and prove its efficacy, while optimizing and scaling up the manufacturing process. At this level, the SIM is already optimized to ensure that the final method is not only robust, cost effective, transferable, accurate and precise for specification setting, but also useful for assessing DP stability and suitable for approving the marketed product. Thus, besides validation, the ability of the final method to detect instability or to assess stability is one of the main requirements that should be considered among its attributes [25,160].

8. Limitations to the nature and the number of the analytes in a SIM

Stress testing is performed with the purpose of delineating the degradation chemistry of the DS. Drug degradation under these conditions may lead to a large number of degradation products, because

the drug is subjected to a wide range of intense stimuli and all degradation products need to be separated and identified. The simultaneous consideration of the array of degradation products formed under all stress conditions may hinder achievement of method selectivity, thus impeding the development of a suitable SIM.

For example, oxidative and thermal degradation of ibuprofen generated 13 degradation products, the detection of all of which required two HPLC methods and a GC-MS technique [161]. However, an HPLC method was recently reported to be capable of assessing stability of corticosteroids and 30 of their impurities and degradation products [159,162].

However, since most of the degradation products obtained under stress conditions are never observed in stability samples [20], in order to facilitate the development of suitable SIMs, the ICH Q1A guideline suggests reducing the number of relevant analytes to those formed under more real life-like conditions. The guideline states that “it may not be necessary to examine specifically for certain degradation products if it has been demonstrated that they are not formed under accelerated or long-term storage conditions”. Under this approach, a simpler method can be developed and successfully replace the otherwise more comprehensive option, producing great savings in the long time.

9. SIMs and setting drug-product specifications

In order to develop stability-testing strategies and set specifications, the target product profile and specification aims must be considered. For testing in stability studies, the criteria must be stability indicating in nature. They must also have the ability to detect details that may compromise DP manufacture or performance, or patient safety.

The acceptance criteria for setting shelf-lives of DPs should be derived mainly from available stability information [163]. When or where release specifications apply, the shelf-life specification of a DP should allow acceptable, justifiable deviation from them, based on its stability evaluation and the changes observed upon storage. If applicable, it should include specific upper limits for degradation products, the justification for which should be influenced by the levels observed in material used in preclinical studies and clinical trials [15].

Limits proposed for certain other tests, such as particle size and dissolution rate, may be justified with reference to the results observed for batches used in bioavailability, bioequivalence and/or clinical studies, and not from stability experiments. Analogously, preservative-efficacy testing should support any differences between the release and shelf-life specification for antimicrobial preservatives.

However, due to the special nature of the biologics, good stability data on several batches of product are required to set their specifications. Clinical experience, as well as knowledge of process consistency and analytical variability, are also useful for that purpose.

10. Conclusions

From a regulatory perspective, the combined use of stress testing and accelerated degradation studies with an optimized separation technique and validation procedures is a useful approach to achieve a comprehensive understanding of DS and DP stability, with regards to the nature of the relevant degradation products, within a reasonable timeframe. This combination also allows the development of comprehensive separations that may be used as SIMs.

Not every degradation product found in the stress tests will be observed under natural degradation conditions, so, as suggested by the Q1A guideline, simplified SIMs should be designed that allow separation of the pertinent degradation products from the main component, while still being capable of unveiling the effects of

instability. However, since there is no single valid approach to development of SIMs, more than one option can fulfill the conditions of a SIM for a given DS or DP.

SIMs need to be optimized and require validation. Experimental design strategies save development and validation time, and constitute a rational approach towards better understanding of the variables influencing method performance.

SIMs are the most suitable tools for assaying DSs and DPs; however, a single method is not always able to achieve the goal of comprehensively assessing the stability. In biologics and combination products, regulations encourage the use of orthogonal, product-specific methods in order to approach understanding of DS stability from various, complementary perspectives. However, separative methods can be complemented by the use of specific tests for dosage form (dissolution/release rate, leachables/extractables, particle size, turbidity and preservatives) in order to assess DP stability.

Finally, despite the regulatory call for using more discriminating, often increasingly sophisticated, approaches, Pharmacopeias do not always adopt such complex methods, but resort to simpler, more cost-effective solutions, seeking to confer SIM properties to the entire official monographs. Hence, it can be anticipated that stability testing and development of SIMs will remain a continually evolving field during the next decade.

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