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Review on Strategies for Analysis of Some Structurally Related Pharmaceutical Compounds: Approach to Forced Degradation, Degradation Kinetics and Impurity Profiling of Drugs

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ABSTRACT

Structurally related compounds encounter a challenge in their determination due to similarity in chemical structure thus having remarkably close chemical and/or physical properties. This work presented a review of the analysis of some structurally related pharmaceutical compounds such as prodrugs in the presence of active and/or inactive metabolites in addition to the determination of active pharmaceutical ingredients in the existence of possible degradation compounds. This review also encompassed a simple approach to how to carry out forced degradation studies including hydrolysis and pH rate profiling of drugs, oxidative degradation, photo-degradation, and thermal degradation studies to develop a stability-indicating assay method (SIAM). The review considered degradation kinetics including the determination of degradation reaction order, reaction rate, Half-life ($t_{1/2}$), and shelf life ($t_{90\%}$) in addition to applying Arrhenius equation to calculate activation energy. The proposed review also justified the Relative Response Factor (RRF) application to study impurity profiling of drugs for analysis of pharmaceutical compounds in the presence of their reported impurities.

Keywords: Stability indicating assay method; degradation reaction order; Arrhenius equation; Relative Response Factor

1. INTRODUCTION

Structurally related compounds encounter a challenge in their determination due to their similar chemical structure, thus having remarkably close chemical and/or physical properties. Different classes of structurally related compounds include:

1.1. Simultaneous determination of prodrug in the presence of active metabolite and/or inactive metabolite

Prodrugs are inactive drug derivatives that are metabolized after administration into pharmacologically active metabolites, both prodrugs and their metabolites are structurally related where one form is active and the other requires activation. Prodrugs represent alternative forms of bioactive substances to improve physicochemical and biopharmaceutical properties.[1] Poor intestinal permeability and absorption represent the main challenges in low lipophilic oral drugs resulting in poor pharmacokinetic properties, poor solubility, and chemical instability.[2] The most common approach for prodrug development is through chemical modifications either by creating salts to improve dissolution rate or integrating alkyl moieties to increase lipophilicity.[3]

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Several prodrugs were developed through esterification reaction as dabigatran etexilate, sofosbuvir, aripiprazole lauroxil, and dimethyl fumarate.[4] Thus, ester hydrolysis is a crucial step for achieving optimum efficacy; ester activation can be achieved either chemically or enzymatically.[5] A vital issue with oral esterified prodrugs is whether they will be activated before or after absorption and reaching the systemic circulation, thus physicochemical properties and pH-stability profile of prodrugs are critical for the finished product formulation.

Several methods were reported for prodrugs determination such as:

Yuan et al.[6] reported an HPLC method to study the degradation kinetics of phosphonates prodrugs. An oxycarbonyl-methyl prodrug of phosphonates was developed to improve their oral bioavailability as phosphonates ionic character limits their permeability across the intestinal mucosa, resulting in low bioavailability. Kinetics of phosphonates prodrugs hydrolysis were studied as a function of pH and buffer concentration. Phosphonates prodrugs were found to have better chemical stability compared to phosphonates.

LC-MS/MS approach was reported for the estimation of aripiprazole lauroxil prodrug in the presence of its active and inactive metabolites. Aripiprazole lauroxil is a sustained-release esterified prodrug of aripiprazole typically administered monthly.[7]

An HPLC method with electrochemical detection was reported to determine L-DOPA and dopamine after oral administration of potential L-DOPA prodrugs. L-DOPA prodrugs were developed to extend L-DOPA pharmacological activity, improve absorption, and protect against metabolism.[8]

A Hydrophilic interaction liquid chromatography approach was described for metformin estimation in existence of its more lipophilic prodrugs formulated to improve permeability and oral absorption of metformin.[9]

An HPLC approach was reported for the estimation of curcumin with its prodrug (curcumin didecanoate).[10]

Habib et al. developed a SIAM using HPLC for the estimation of dimethyl fumarate in the existence of monomethyl fumarate (active metabolite) as well as fumaric acid **(Figure 1)**.[11]

Other reported methods for the determination of some prodrugs in the presence of their metabolites are summarized in **Table 1**.

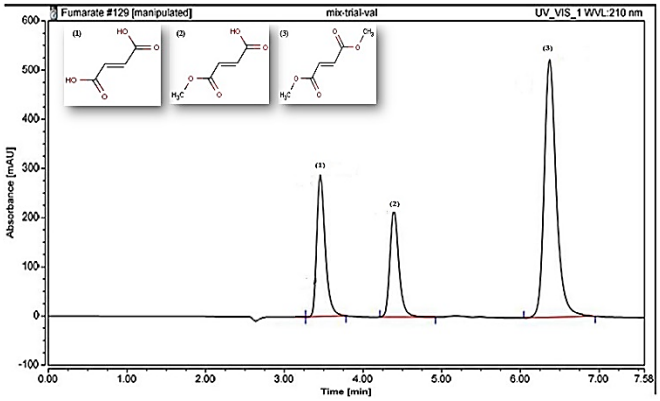

Figure 1. The obtained chromatogram of (1) fumaric acid, (2) monomethyl fumarate, and (3) dimethyl fumarate. Their chemical structures are given in the inset.[11]

Table 1. Some reported methods for the determination of some prodrugs in the presence of their metabolites

Prodrug	Principle	Application	Ref.
Exatecan and its cathepsin B-sensitive prodrug	HPLC-MS/MS	Assess the pharmacokinetics of exatecan and its prodrug	12
Leflunomide (prodrug metabolized to active metabolite teriflunomide)	LC-quadrupole TOF-MS	Quantitation of teriflunomide and leflunomide in human plasma	13
Lisdexamfetamine (prodrug that is converted to d-amphetamine in the body)	LC-MS/MS	Quantification of Lisdexamfetamine in human plasma	14
Three paclitaxel fatty acid esters	UPLC-MS/MS	Pharmacokinetics investigation in mouse plasma	15
Mycophenolic acid (a secondary metabolite of *Penicillium Brevicompactum)* and a chitosan-linked prodrug	HPTLC	Analysis in microsphere formulations	16
Icaritin and its novel 3-methylcarbamate prodrug	HPLC–MS/MS	Quantification in rat plasma and application to pharmacokinetic study	17

1.2. Estimation of the drug in the existence of its degradation compounds

SIAM is an estimation analytical route that is applied to determine the stability of an active pharmaceutical ingredient **(API)** varies over time. A SIAM should distinguish each API from possible degradation products to accurately measure API content. Stability studies provide indications of the potential liabilities of an API.

Stability studies are vital for manufacturing, packaging, and formulation development to estimate the optimum storage circumstances as well as drug shelf life.[18, 19]

Instability is usually encountered with substances having reactive chemical groups which may undergo hydrolysis or oxidation as well as photolysis.[20]

2. STRATEGY TO DEVELOP SIAM

Several ICH guidelines were reported about stability studies such as **ICH Q1A (R2)**[21] which is concerned with testing the stability of new API as well as its products, **ICH Q1B** [22] which is concerned with photo-stability testing of API and its products, **ICH Q3A (R2)**[23] which is concerned with impurities in new API and **ICH Q3B(R2)**[24] which is concerned with impurities in products of new drug. However, applied steps to be trailed during the development of **SIAM** are not reported in guidelines or pharmacopoeias. A strategy to be followed during developing **SIAM** was reported[19] and summarized in (**Figure 2).**

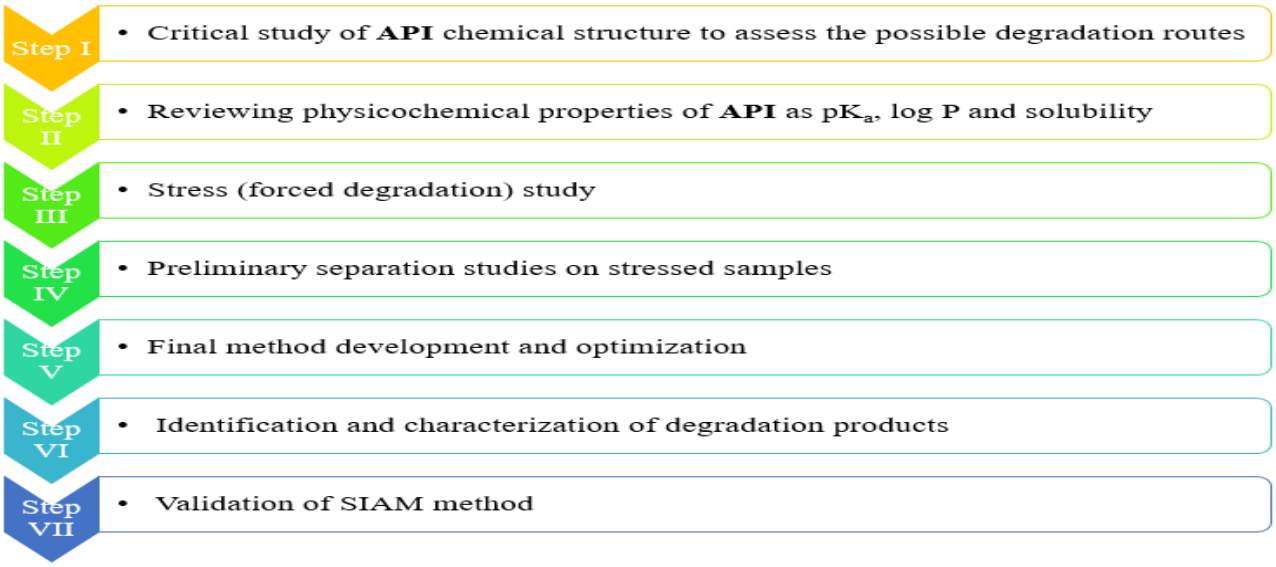

Figure 2: General strategy to perform stability-indicating assay methods (SIAM)

2.1. Forced degradation studies (stress testing)

Forced degradation is to expose **API** to conditions such as light, heat, humidity, and an extensive range of pH values, leading to the formation of all possible degradation products, thus allowing the determination of the stability profile of API.[25,26]

Forced degradation or stress testing is carried out when developing SIAM to confirm specificity, especially when possible degradation products aren't well recognized. Forced degradation also reveals the mechanism of degradation that may occur during storage.

Stress degradation is conducted to obtain an approximately 5–20% degradation of the drug[26]. Feasible maximum conditions achievable for API should be reported if API isn't degraded for given stress conditions. [27, 28]

2.1.1. How to carry out forced degradation procedures?

As mentioned by **ICH Q1A (R2)**, stress testing of the drug enables the detection of the possible degradation products to suggest the pathways for degradation as well as the intrinsic stability of the drug in addition to validation of SIAM [21]. The study of the relationship for any degradation governs whether the data can be represented graphically by a **linear** or **quadratic** or **cubic** function using either logarithmic or arithmetic scale".

Though several guidelines were reported about stability studies, the practical aspects related to stress testing are not clearly addressed by regulatory guidelines. Conditions to be employed to study a new API stress degradation aren't well identified.

Singh and *Bakshi* [27] have suggested decision trees to be followed to investigate stress conditions for a new API. Stress conditions for hydrolysis and oxidation such as degree of temperature and time of exposure as well as strength of the acid, the alkali, or % of hydrogen peroxide were varied until sufficient degradation is obtained.

Drugs are classified into six categories depending on conditions that cause sufficient degradation as summarized in **Table 2**.

Table 2. Classification system for acidic, alkaline, neutral hydrolysis, and oxidative degradation[27]

Category	Acidic or alkaline hydrolysis	Neutral hydrolysis	Oxidative degradation
Class I **Extremely labile**	0.01N acid or alkali 25˚C	25˚C-2hrs	1% H_2O_2 30 min
Class II **Very labile**	0.01N acid or alkali 40˚C	40˚C-8hrs	1% H_2O_2 3hrs
Class III **Labile**	0.1N acid or alkali Refluxing	Refluxing 12hrs	3% H_2O_2 6hr
Class IV **Stable**	1N acid or alkali Refluxing	Refluxing 1day	3% H_2O_2 24hrs
Class V **Very stable**	2N acid or alkali Refluxing	Refluxing 2days	10% H_2O_2 24hrs
Class VI **Practically stable**	5N acid or alkali Refluxing	Refluxing 5days	30% H_2O_2 48hrs

Another strategy for selecting degradation conditions in forced degradation studies was reported [29], where initial trials aim to find the conditions that cause degradation of nearly 10% of API. Conditions commonly used for forced degradation as reported [29] are summarized in **Table 3**.

Table 3. Conditions mostly applied for forced degradation studies.

Degradation reaction	Conditions
Hydrolysis	**Acidic**: 0.1M HCl at 40˚C or 60˚C **Alkaline**: 0.1M NaOH at 40˚C or 60˚C **pH 2,4,6,8** at 40˚C or 60˚C
Oxidation	**3% H_2O_2** at 25˚C or 60˚C **Azobisisobutyronitrile(AIBN)** at 40˚C or 60˚C
Photolytic	**1.2 million lux hr** **6 million lux hr**
Thermal	**Heat chamber** at 60˚C or 80˚C

2.1.2. How to carry out hydrolysis and pH rate profiling of drugs?

Many APIs contain ester or amide functional group that is readily hydrolyzed in solution. The most common pathways for drug degradation are hydrolytic reactions.

The type and concentration of acid or alkali used in the stress degradation study are chosen depending on the **liability** of the **API**.

Frequently used reagents for hydrolysis are HCl (0.1–1M) for acid hydrolysis and NaOH (0.1–1M) for alkalin hydrolysis. Stress degradation using neutral conditions is usually carried out by the refluxing of API in water.

Kinetic-pH profiling is to study the effect of pH on the hydrolysis reaction kinetics of API to find the pH value showing maximal stability. The pH-rate profile is used to quantify how quickly degradation will occur at different pH ranges.

2.1.3. How to carry out oxidative Degradation?

Oxidative degradation of API can occur by the reaction with molecular oxygen or with oxidizing agents existing in the dosage form such as hydrogen peroxide.

Concentrations as well as the type of an oxidizing agent are selected depending on the stability of the API. Most oxidative degradation stress studies incorporate H2O2 in the concentration range of 3–30%.

2.1.4. How to carry out photo-degradation?

Photo-stability testing of API must be performed to ensure that there is no undesirable change occurred due to light exposure. The free radical mechanism is the most common in photo-degradation.

ICH guidelines **Q1B** recommend exposure to UV/Vis light of at least 1.2 million lux hours and energy of at least 200-watt hours/square meter. The maximum illumination of 6 million lux hours is also recommended.

Also according to **ICH Q1B**, different exposure conditions can be applied to study the photo-degradation of API according to its photosensitivity and light intensity. "The purpose of photo-degradation study is to identify the overall photosensitivity of the substance for degradation pathway elucidation.[22]

2.1.5. How to carry out thermal degradation

Thermal degradation is performed at more vigorous conditions compared to that of accelerated testing suggested by **ICHQ1A (R2)**. Usually, the study of thermal degradation can be performed at 40–80˚C. The temperature effect on the thermal degradation of an API is investigated by Arrhenius equation.[30]

Some reported SIAM methods for the determination of **API** in the presence of their degradation products are summarized in **Table 4**.

Table 4. Some reported SIAM methods for the determination of API in the presence of their degradation products

Principle	Technique	Application	Ref.
Stability indicating assay method for mitapivat	HPLC/quadrupole-time of flight mass spectrometry	Seven novel hydrolytic, photolytic, and oxidative degradation products were identified	31
Stability study of two short universal cancer peptides derived from telomerase	RP-HPLC	First report including the lightness and chromaticity measurements in a peptide vaccine	32
Environmentally benign and stability-indicating assay of metronidazole	RP-HPLC	Assess metronidazole-related process and degradation impurities in finished drug formulation	33
Stability indicating assay for concurrent quantification of linagliptin and dapagliflozin	RP-HPLC/ LC-MS/MS	Linagliptin has shown major degradation products under acidic, alkaline, and oxidative environments	34
Stability Indicating Determination of Idelalisib	RP-HPLC	Forced degradation to separate and quantify idelalisib with its degradation products	35
Stability Method for Quantification of Amoxapine	HPLC/quadrupole-time of flight mass spectrometry	Three hydrolytic degradation products and one oxidative degradation product were formed	36
Identification and characterization of two new oxidation degradation impurities in cinnarizine	LC-HRMS/MS and ^{1}H-NMR	Cinnarizine was labile to oxidative conditions and stable to acidic, alkaline hydrolytic, photolytic, and thermal conditions	37
Stability-indicating chromatographic methods for the simultaneous determination of Phenylephrine and Tropicamide	HPTLC HPLC	Phenylephrine degraded under oxidative conditions. Tropicamide yielded tropicamide degradation and tropicamide impurity using acidic and basic hydrolysis	38

2.2. Degradation kinetics

Degradation kinetic is defined as the investigation of the rate of drug degradation. Its information can be utilized to understand the mechanism of drug degradation. Its target is the prediction of the intrinsic stability of API to anticipate problems that may occur throughout development as the rate at which API degrades varies dramatically[39].

Degradation kinetics is an important feature in pre-formulation studies, estimation of shelf life as well as drug stabilization against degradation.

2.2.1. Significance of the study degradation kinetics:

- Development of optimum formulation
- Determine the optimal conditions for storage
- Choosing the appropriate container used for dispensing
- API shelf life estimation
- Expecting the interactions between the drug and the excipients.

2.2.2. Strategy to carry out degradation kinetics studies (Figure3):

First evaluate the vulnerability of the drug to be degraded by various conditions such as hydrolytic conditions involving acid, base, and neutral conditions, photolytic conditions, and oxidative stress conditions by carrying out a stress degradation study of targeted API.

The next crucial step is to select the conditions for degradation kinetic study as it can give useful data about the rate-limiting parameters that control degradation reaction.

Various variables such as pH and temperature as well as light exposure should be optimized to investigate the degradation kinetics of API by hydrolytic conditions. Such optimization is achieved by one factor at a time approach as the choice of multiple factors simultaneously to investigate degradation kinetics can give false data due to overlapping effects for multiple factors at a time.

Obtained data is plotted as the percentage of remaining drugs versus time. The remaining drug percentage is plotted as (C_t/C_0), ln(C_t/C_0), and 1/(C_t/C_0); according to the highest coefficient of determination (r^2), the reaction order is determined from the best-fitted line.

Where: C_t is remaining the concentration of the drug after the time (t) and C_0 is the initial concentration of the drug.

Different kinetic parameters such as degradation rate constant (k), as well as half-life (t1/2) in addition to shelf life (t90%), can be calculated.

Arrhenius equation is applied by determining degradation rate constant at different temperature values, and activation energy (E_a) is calculated for API. If the structure of the degradation product isn't recognized, different techniques for structure elucidation should be considered as IR, LC-MS/MS. The degradation mechanism and pathway for degradation product formation are suggested.

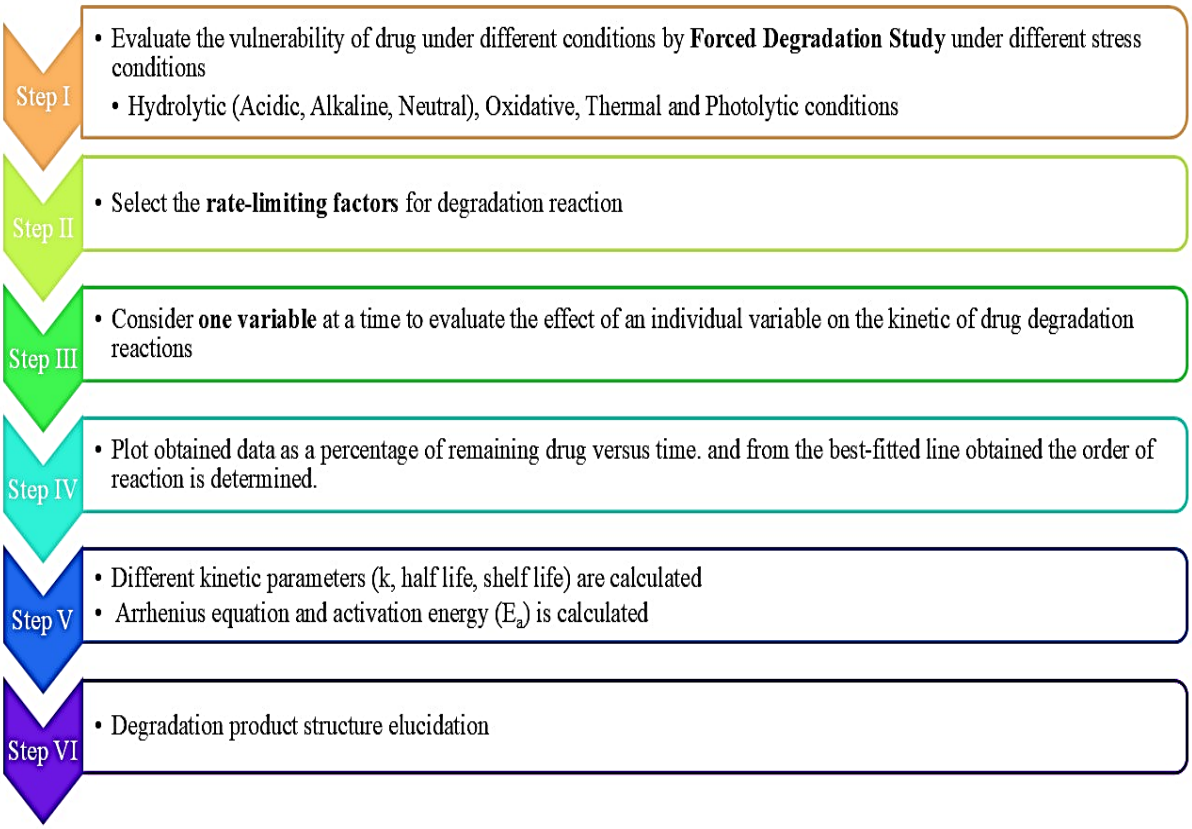

Figure 3: Guideline to perform degradation kinetics study

2.2.3. Determination of degradation reaction order:

The order of the reactions indicates how changing the reactant concentration affects the speed of the reaction so its determination is vital **(Figure 4)**.

Understanding the order of drug degradation can assist in determining the shelf life and optimal storage conditions for drug substances and drug products.

Zero order reaction:

The rate of zero order reaction is independent of the reactant concentration. Its rate is expressed by the following equation1:

$$\text{Rate of reaction} = -d[C]/dt = k \qquad \textbf{(Equation 1)}$$

where: [C] is the remaining concentration of reactants & k is the rate constant.

Integrating the rate equation between initial concentration C_0 at t_0 & C_t is the remaining reactant concentration after time = t, we get the following:

$$C_t = C_0 - kt \quad \textbf{(Equation 2)}$$

$$\textbf{Half life } t_{1/2} = \frac{C_0}{2k} \quad \textbf{(Equation 3)}$$

$$\textbf{Shelf life } t_{90\%} = \frac{C_0}{10k} \quad \textbf{(Equation 4)}$$

When this linear relationship is plotted (Ct versus time), the slope of the obtained line **(Figure 5)** is equal to *–k*

Zero-order reactions imply rearrangement or radical-intermediated cleavage of chemical bonds under conditions of photolytic or oxidative tension. For example, the ultrasonic degradation of diclofenac under oxidative conditions follows zero-order kinetics. Similarly, the degradation kinetics of ascorbic acid, influenced by water activity and temperature, also adhere to a zero-order kinetic model.[40]

First order reaction:

The reaction rate depends on the concentration of a single reactant, and the rate expression for the chemical reaction is given by equation 5:

$$\textbf{- } d[C]/dt = k[A] \quad \textbf{(Equation 5)}$$

By integrating the rate equation from the initial concentration C_0 to the concentration C_t, after (t_{time}), the following equations were obtained:

$$\ln \frac{C_t}{C_0} = -kt \quad \textbf{(Equation 6)}$$

$$\textbf{Half life } t_{1/2} = \frac{-0.693}{k} \quad \textbf{(Equation 7)}$$

$$\textbf{Shelf life } t_{90\%} = \frac{-0.105}{k} \quad \textbf{(Equation 8)}$$

When this equation is plotted with persisting concentration, either as $\ln(C_t/C_0)$ or $\log(C_t/C_0)$ against time, the slope is **–k** ***or*** $\frac{-k}{2.303}$, respectively as shown in (**Figure 6)**.

Various pharmaceutical products degrade through first-order kinetics. For instance, the degradation of imidapril hydrochloride under hydrolytic (acid/base) stress conditions obeys first-order kinetics.[41]

Second order reaction:

The reaction rate depends on the concentrations of two reactants. There are two scenarios to consider, either

$$A + B \longrightarrow \text{products}$$
$$\text{or } 2A \longrightarrow \text{products}$$

then, $\textbf{- } d[C]/dt = k[A]^2$ **or** $k[A][B]$ (**Equation 9)**

by integrating the rate equation from the initial concentration C_0 to the concentration C_t, after (t_{time}),

$$\frac{1}{C_t} = kt + \frac{1}{C_0} \quad \textbf{(Equation 10)}$$

$$\textbf{Half-life } t_{1/2} = \frac{1}{k[C_0]} \quad \textbf{(Equation 11)}$$

$$\textbf{Shelf life } t_{90\%} = \frac{0.11}{k[C_0]} \quad \textbf{(Equation 12)}$$

When this equation is plotted, the slope of the obtained line is equal to k as shown in (**Figure 7)**.

Oxidative degradation kinetics of posaconazole under different concentrations of H_2O_2 and temperature values were found to follow second-order kinetics. Different initial and final degradation rate constants were observed. The half-life and shelf life of posaconazole under all conditions were determined.[42]

Thermal degradation of 7,8-dimethyl-10-formyl-methyl-isoalloxazine in acid solution was found to comprehend a **second-order** reaction[43].

Also, cefaclor was found to undergo self-aminolysis in solution via a nucleophilic attack that followed a **second-order** degradation rate.[44]

Pseudo order reactions

Reactions that are inherently of higher order but are manipulated to behave like lower-order reactions.

Pseudo zero order reaction:

When degradation is minimal, it becomes challenging to differentiate between zero and first-order reactions. Although it is essentially a first-order reaction, it behaves like a zero-order reaction. This condition is known as a pseudo-zero-order reaction.[45]

A study on the degradation kinetics of dacarbazine in an aqueous solution under photolytic and hydrolytic conditions revealed that its photolysis follows pseudo-zero-order kinetics. Conversely, in the dark, dacarbazine undergoes hydrolysis following pseudo-first-order kinetics. The pH-rate profile of dacarbazine indicated that both photolytic and hydrolytic reactions depend on the molecule's ionization state [46]

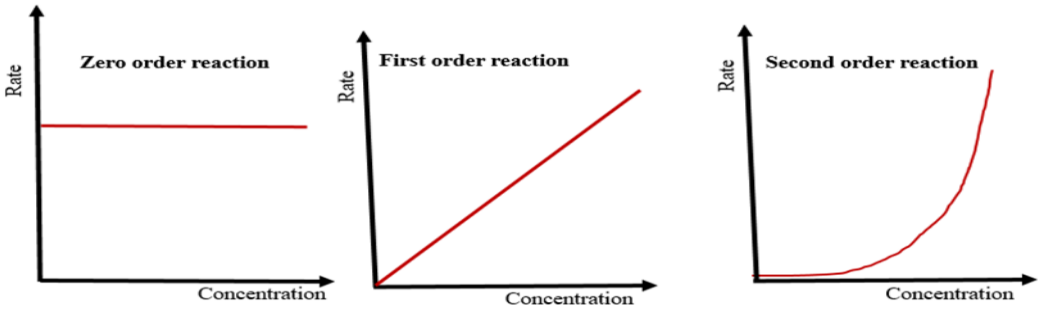

Figure 4: Relationship between the rate of a chemical reaction and concentration of reactants (for different orders of reactions)

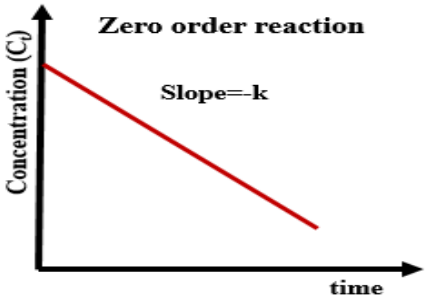

Figure 5: Zero order reaction plot

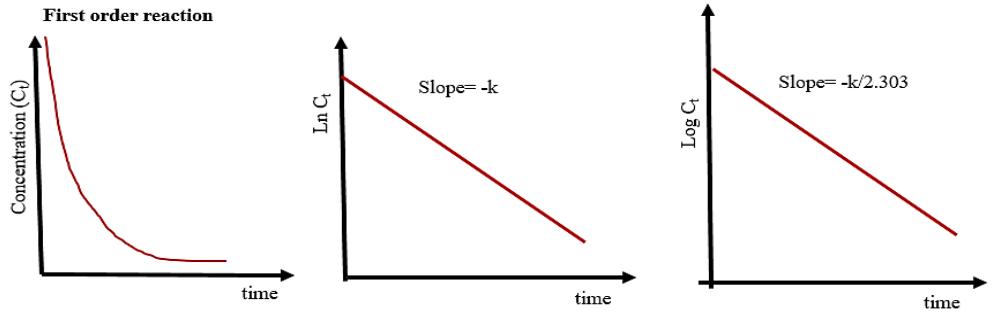

Figure 6: First order reaction plots

Figure 7: Second order reaction plots

Pseudo-first-order reaction:

A pseudo-first-order reaction can be described as a second-order reaction that behaves like a first-order one. This occurs when one reactant is in a much higher concentration or is kept constant compared to another substance, making the reaction rate dependent on only one reactant. Many reactions fall into this category, with hydrolysis being a common example that typically follows pseudo-first-order kinetics. While hydrolysis is generally considered undesirable, it is an intentional feature in the case of prodrugs.

Habib et al. [11]reported degradation kinetics and pH profile of dimethyl fumarate. Dimethyl fumarate followed a pseudo-first-order kinetics and showed highest stability at pH 7 and 5.

Kinetics, pH profile, and Arrhenius plots of disodium phosphate ester of phenytoin as a promising prodrug for parenteral administration of phenytoin showed hydrolysis rate following pseudo-first-order kinetics. [47]

The degradation of ascorbic acid follows a pseudo-first-order. Storage temperature and water activity were considered, finding that the rate constants and water activities are related by a second-order polynomial equation. The temperature's effect on the rate constant adheres to the Arrhenius relationship, and the activation energy was calculated [48].

Linagliptin was found to follow pseudo-first-order kinetics. Additionally, Arrhenius plots were created to estimate linagliptin's half-life at room temperature [49].

Carvedilol follows first-order kinetics under both acidic and alkaline conditions. The kinetic data were then fitted to the Arrhenius equation to calculate the activation energy [50].

A study on the degradation kinetics of dapagliflozin in the presence of metformin revealed that its alkaline degradation follows pseudo-first-order kinetics [51].

An eco-friendly SIAM to study the degradation kinetics of the antiviral prodrug baloxavir marboxil was reported. Kinetic studies of acidic, alkaline hydrolysis, and oxidative degradation of baloxavir revealed a pseudo-first-order reaction rate under all conditions. Main degradation product structures were revealed utilizing LC-MS. [52]

Amer et al. reported the degradation kinetics and pH profile of methylcobalamin. [53] It followed pseudo-first-order kinetics under acidic and alkaline degradation. It showed the highest stability at pH 5.

2.2.4. Determination of reaction rate (k):

The rate of a chemical depends on the product of the molar concentrations of the reactants, with each concentration raised to a power corresponding to the stoichiometric coefficients of the reacting substances. Therefore, the reaction rate is described as the rate at which reactants are consumed or products are formed.

If a**A** + b**B** $\longrightarrow$ Product, Rate of reaction can be explained as = $-d[C]/dt = k[\mathbf{A}]^a[\mathbf{B}]^b$

In this context, (C) represents the concentration of the species being studied. The concentrations of reactants (A) and (B) are indicated by brackets, such as ([A]) and ([B]). The symbol (k) stands for the rate constant of the reaction. Chemical stability is typically expressed through the rate constant ((k)), which indicates either the degradation of a drug or the formation of degradation products. To determine rate constants from experimental data at a specific temperature, the linear least squares method is commonly used. This involves plotting the remaining drug percentage as (C_t/C_0), the natural logarithm of the remaining drug percentage $\ln(C_t/C_0)$ and reciprocal of the remaining drug percentage $1/(C_t/C_0)$ against time; the reaction order is then identified based on the highest r^2 value and the best-fitting line.

2.2.5. Half-life ($t_{1/2}$) determination:

The half-life of a reaction is the time it takes for a reactant to decrease to half of its initial concentration. In a first-order reaction, the half-life remains constant and does not depend on concentration. Conversely, for zero-order and second-order reactions, the half-life is influenced by both the initial concentration and the rate constant.

2.2.6. Shelf life ($t_{90\%}$) determination:

Shelf life refers to the duration during which a pharmaceutical product, when stored correctly, is expected to remain within its specified quality parameters as established by stability studies. This period is used to determine the product's expiry date. Essentially, shelf life is the time required for 10% of the material to degrade, meaning the concentration of the product decreases to 90% of its original amount.

2.2.7. Arrhenius equation:

The Arrhenius regression equation is commonly used to evaluate the stability of drugs during various stability studies.

It establishes a relationship between temperature and rate constant, which helps estimate the shelf life of a drug substance during its development phase. Most active pharmaceutical ingredient (API) degradation studies follow Arrhenius kinetics. Reaction rates typically depend on: The fraction of molecules with energy equal to or greater than the activation energy (E_a), the number of collisions per second, and the percentage of molecules with the correct orientation.

Arrhenius equation describes a linear relationship between the natural logarithm of the reaction rate constant k) and the inverse of the absolute temperature (T). This relationship is expressed by the following equation 13:

$$k = Ae^{-Ea/RT} \quad \textbf{(Equation 13)}$$

where: k is rate constants, A is the pre-exponential factor or frequency factor, E_a is the activation energy in KJ mol^{-1} or KCal mol^{-1}, R is the gas constant and T is the absolute temperature in Kelvin. Thus, by experimentally determining the rate constant ((k)) at various temperatures and plotting **ln**k against 1/T is plotted, the activation energy (Ea) can be derived from the slope of the resulting line.

Another approach to determine activation energy (Ea) is by solving the following equation 14:

$$\ln\frac{k2}{k1} = -\frac{Ea}{R}\left(\frac{1}{T2} - \frac{1}{T1}\right) \quad \textbf{(Equation 14)}$$

where: k_2 & k_1 represents rate constants at temperature T2 & T1, respectively.

The findings from Arrhenius analysis are useful for forecasting how changes in storage conditions might affect the expiration of a drug product. For instance, Amer *et al.* applied the Arrhenius equation to determine the activation energy of rupatadine fumarate in a study on its oxidative degradation kinetics[54] . Some reported degradation Kinetics studies of API in the presence of their degradation products are summarized in **Table 5.**

3. DETERMINATION OF DRUGS IN THE PRESENCE OF POSSIBLE IMPURITIES (IMPURITY PROFILING)

According to **ICHQ3A(R2)** guidelines, an impurity profile is the indentation of the known and unknown impurities existing in a new API. Impurity profiling regularly starts with the detection of impurities, followed by their isolation and characterization.[23] An impurity is described as any constituent other than API or an excipient in dosage form.

Identification of impurities is a serious procedure in **API** determination to know the chemical structures of unknown impurities existing above the specified threshold. Classification of impurities by ICH guidelines [23, 24] is summarized in (**Figure 8).**

Optimally, official USP Reference Standards for impurities should be considered while performing impurity tests [63].

However, official USP Reference Standards for impurities may not be available. When an appropriate reference standard is not available, a USP relies on instrumental techniques to control the impurities depending on the Relative Response Factor (RRF). [63] RRF value, which is specific to each experimental circumstances, compares the instrumental responses of the impurity and the API reference standard to correct for differences in detector response.[64–66]

RRF is the ratio between the response of the impurity and that of API under the same chromatographic conditions. The following equation 15 is used to determine RRF:

$$\textbf{Relative Response Factor (RRF)} = \frac{\textbf{slope of impurity}}{\textbf{slope of API}}$$

(Equation 15)

However, in some formulas for the calculation of RRF, the slope of impurity is in the numerator, and in others, it is in the denominator.[64–68]

Some monographs use the term *Response Factor*, some use the term *Relative Response Factor*, and others use *Correction Factor*. British Pharmacopoeia referred to RRF as *a correction factor* in the recent update of BP (2020).[69] The *correction factor* is not used if it is < 0.2 or > 5. If this happens, different methods of determination are used by changing the detection wavelength (λ) or using different methods for visualization.

Several methods were reported to determine different APIs in the presence of process-related impurities such as:

A method for characterization and determination of RRF of process-related impurity in naproxen was reported. [70]

Karthikeyan *et al.* reported HPLC with UV detection method to estimate RRF of process-related impurities of meprobamate. A RRF value of 0.038 was obtained for olefin impurity due to its very high response obtained by the UV detector.[71]

Table 5. Some reported degradation Kinetics studies of API in the presence of their degradation products

Principle	Technique	Degradation Kinetics study	Ref.
Forced Degradation Study of Ritlecitinib under acidic, basic, oxidative, thermal, and photolytic conditions revealed four novel degradation products	UPLC with Diode Array Detector and Tandem Mass Spectrometry	Basic degradation followed second-order kinetics, while oxidative degradation followed zero-order kinetics	55
Compare the degradation kinetics of three antihypertensive drugs, perindopril tert-butylamine, amlodipine besylate, and indapamide.	RP-HPLC	Perindopril is most unstable under basic conditions. Amlodipine is most affected by basic conditions and oxidation. Indapamide undergoes extreme photolysis	56
Quantification of florfenicol in the presence of its different degradation products	First and second derivative synchronous spectrofluorimetry	The reaction rate order for acidic, alkaline, oxidative, and photolytic degradation proved to be first order	57
Electrochemical degradation of acetaminophen in urine matrices	Electrochemical oxidation with a dimensional-stable anode	Acetaminophen degradation kinetics followed a pseudo-first-order reaction	58
Stability indicating high-performance liquid chromatography method for determination of cenobamate	HPLC	Kinetic study of basic degradation indicated first-order kinetics	59
Discerning the stability behaviour of mavacamten	Liquid chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy	Degradation kinetics of mavacamten under 1 N acidic condition followed zero-order kinetics, and it was degraded completely within 6 h	60
Separation and characterization of hydrolytic degradation product of deucravacitinib	Liquid chromatography-tandem mass spectrometry	Characterize degradation products formed under hydrolytic, oxidative, thermal, and photolytic stress conditions and study the kinetics of the drug's degradation	61
Stability-indicating for the analysis of vilanterol degradation products in human urine	UPLC mass spectrometry	Degradation kinetics under acidic, basic, and oxidative stress conditions. Kinetics parameters, K, half-life time, and shelf-life time were assessed, and the degradation followed first order reaction	62

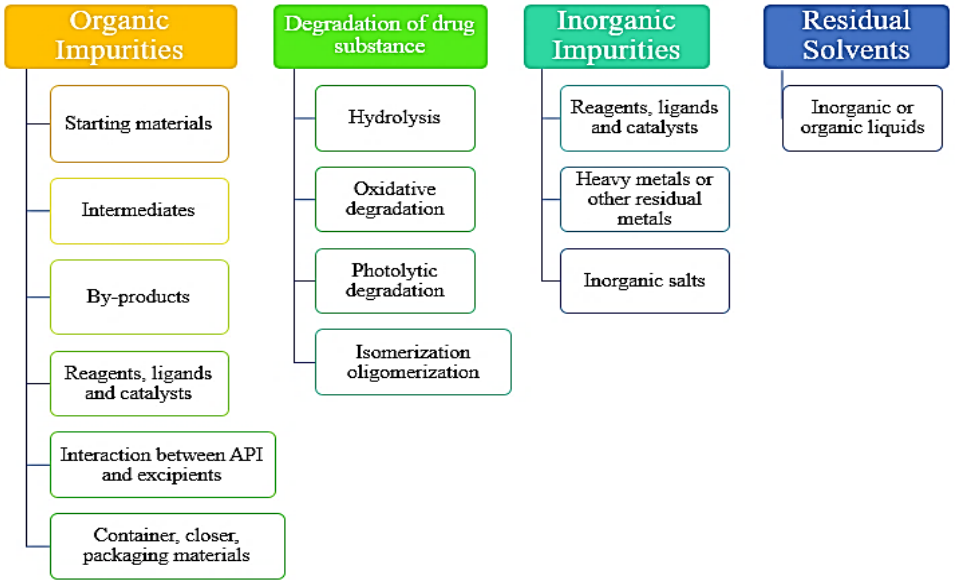

Figure 8: Impurities classification based on ICH guidelines[23,24]

A RP-HPLC method was developed to determine bicalutamide process-related impurities as well as degradation products, impurity profile study of bicalutamide was reported.[72]

A RP-HPLC SIAM was reported for levofloxacin determination in the presence of degradation products as well as process-related impurities was reported.[73]

An HPLC method for the estimation of process-related impurities of anticholinergic drug pridinol mesylate was reported. Synthetic impurities were used as standards.[74]

A study of the impact of changing the chromatographic conditions of HPLC (column type and temperature as well as buffer pH &concentration, also flow rate and detector wavelength) on RRF was reported. The study determines RRF for two impurities of imatinib mesylate using different chromatographic conditions. The study has revealed that any variations in chromatographic conditions greatly affect RRF values.[75]

A new approach for RRF determination applied for paclitaxel impurities was reported. Conventionally, RRF is estimated by analysis of API and the impurity under identical detection conditions. However, this is not achievable for unknown impurities, an alternative way to determine RRF through using two detectors in tandem as UV with a universal detector was reported.[76]

Amer *et al.* reported SIAM using HPLC for the estimation of rupatadine fumarate in the existence of its main impurity; desloratadine which was determined using the impurity relative response factor.[54]

Other reported methods for the determination of API in the presence of related impurities are summarized in **Table 6.**

Table 6. Some reported methods for the determination of API in the presence of related impurities

Principle	Technique	Ref.
Determination of oxytetracycline and lidocaine in the presence of toxic lidocaine impurity	Spectrophotometry univariate versus multivariate analysis	77
Determination of etomidate and its structural analogues	Electrospray ionization quadrupole time-of-flight mass spectrometry	78
Stability indicating the determination of process and degradation impurities of ivabradine including two diastereomeric N-oxide impurities which are major oxidative degradation impurities	HPLC method with QDa and PDA detectors	79
Quantitative Determination of Five Process Relevant Impurities in Menatetrenone	GC-flame ionization detector (GC-FID)	80
Determination of three nitrosamines in losartan API and assessment of nitrosamines formation	LC-MS/MS	81
Detection of N-nitrosochlordiazepoxide as a potential genotoxic impurity	LC-MS/MS	82
Stability-indicating assay for impurity profiling of rupatadine, given that desloratadine is a known degradation product.	chemometrics-assisted spectrophotometric methods	83
Determination of up to 15 small molecule nitrosamine impurities in pharmaceutical drug substances	LC-MS/MS	84
Spectrophotometric analysis of Fluconazole and its two toxic official impurities	Sequential Dual Amplitude Difference (SDAD) technique	85
Simultaneous determination of olopatadine hydrochloride and its related substances in eye drop	RP-HPLC	86
Assessment of pralsetinib impurities and degradation products	HPLC-MS/MS	87

4. CONCLUSION

This review involved strategies for analysis of some structurally related pharmaceutical compounds such as analysis of prodrugs in the presence of active metabolite and/or inactive metabolite as well as active pharmaceutical ingredients in the presence of possible degradation products. This review also included a simple strategy to perform a degradation kinetics study including the determination of degradation reaction order, reaction rate, Half-life ($t_{1/2}$), and

shelf life ($t_{90\%}$) in addition to the application of Arrhenius equation to calculate the activation energy. The review also demonstrated how to apply Relative Response Factor (RRF) to study impurity profiling of drugs.

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