PREFACE

Most pharmaceutical compounds are subjected to degradation accompanied with partial, or even complete loss of pharmacological activity.

Stability indicating methods are developed for determination of the extent of drug degradation. A stability indicating method is defined as any method that affords selective determination of the intact drug in presence of its degradation product(s).

The object of the present work is to develop simple, efficient and selective methods for the quantitative determination of the intact molecules of the three studied drugs namely, aceclofenac (AC), clozapine (CL), diloxanide furoate (DF), which can be adopted for their stability studies.

The thesis comprises four parts:

PART I: GENERAL INTRODUCTION

In this part, a brief idea about sources of drugs degradation and the theoretical background for stability indicating methods.

PART II: Stability indicating methods for the determination of aceclofenac

This part comprises:

1- Literature review about structure, properties, pharmacology and the reported methods for analysis of aceclofenac.

2-Experimental study, dealing with the determination of AC in presence of its degradation product by spectrophotometric, densitometric and high-performance liquid chromatographic (HPLC) methods.

PART III:Stability indicating method for the determination of clozapine

This part comprises:

1- Literature review about structure, properties, pharmacology and the reported methods for analysis of clozapine.

2-Experimental study, concerned with the determination of CL in presence of its degradation product by spectrophotometric, densitometric and high-performance liquid chromatographic (HPLC) methods.

PART IV: Stability indicating method for the determination of diloxanide furoate

This part comprises:

1- Literature review about structure, properties, pharmacology and the reported methods for analysis of diloxanide furoate.

2-Experimental study, about the determination of DF in presence of its degradation products using spectrophotometric, densitometric and high-performance liquid chromatographic (HPLC) methods.

This thesis further comprises 122 references and ends with an arabic summary.

I.1. Concepts in drug stability

Most drugs are subject to some forms of decomposition, including physical, chemical and microbiological changes that may lead to partial or complete loss of the desired pharmacological activity or even to the production of harmful decomposition products [(1).]

Drug degradation may result either from hydrolysis or oxidation. Drugs may also lose their activity due to isomerisation or photochemical decomposition [(1-4)] .

I.2. Sources of drugs degradation

1- Temperature:

Thermal effects are involved in almost all chemical reactions. High temperature results in rapid degradation while cooling diminishes it. For example storing β-lactam penicillins in a refrigerator reduces the hydrolysis rate by 90% of that at room temperature [(3)] .

2- Hydrolysis:

The most likely cause of drug instability is hydrolysis, which is due to the presence of hydrolysable groups such as ester or amide groups. Procaine, atropine, aspirin, pilocarpine, penicillins, cephalosporins, benzodiazepines, and tetracyclines as well as the three studied drugs in this thesis AC, CL and DI are examples of drugs suffering from hydrolytic decomposition [(1-3,5)] .

3-Solvolysis:

When the reacting solvent is no longer water, then the breakdown is termed " solvolysis". Furthermore, the definition can be extended to include any change in solvent polarity and resulting into increased ionic strength.

For example, phenobarbitone is considerably more stable in preparations containing water-miscible solvents whereas acetylsalicylic acid, which undergoes extensive hydrolysis, is degraded further by aqueous solvents [3].

4-Oxidation:

Oxidative degradation is a major cause of drug instability. Phenolic compounds (e.g. morphine and phenyl ephedrine), catecholamines (e.g. dopamine and adrenaline), steroids (e.g. prednisolone and hydrocortisone), antibiotics (e.g. streptomycin and neomycin), vitamins (e.g. vitamins A, D and E) and other similar compounds are greatly affected by oxidation [1,2].

5- Photolysis:

Oxidation and, to some extent hydrolysis are often catalyzed by light. Photodegradation is greatly dependent on both the intensity and wavelength of light and is usually mediated by free radicals. Therefore, protection from light is essential in case of photosensitive drugs. Among these drugs are benzodiazepines, catecholamines, corticosteroides, phenothiazines and most of the vitamins [1,3] .

6-Isomerisation:

It is the process of conversion of a drug into its optical or geometrical isomers. Since various isomers of a drug are frequently of different activities, such conversion may be regarded as a form of degradation [1]. For example, the appreciable loss of activity of adrenaline solutions at lower pH has been attributed to racemization of the active (L)- form into its less active isomer [6]. Tetracyclines

undergo epimerization under acidic conditions to give 4-epi-tetracyclines which posses much less activities than the natural isomers [7].

I.3. Stability indicating methods of analysis

The method used to indicate the stability of a compound should be sufficiently sensitive and selective to determine quantitatively the intact drug in the presence of its degradation products [2,8]. During drug formulation, the active ingredient is diluted or dispersed in pharmacologically inert excipients which are not necessarily chemically inert. Thus the analytical problem is far more complex and stability is much more difficult to predict than in case of pure active ingredients [2].

A pharmaceutical analytical chemist has, therefore, to develop selective methods that, not only avoid interferences by other coformulated compounds, but also exclude degradation products.

The following approaches have been proposed for developing a stability indicating method:

[a] Functional group approach:

Functional group analysis is greatly concerned with stability indicating methods. A functional group assay is one that is selective for a particular structural feature in a molecule and is considered as a stability indicating one, if such a feature disappears after decomposition [9] , for example hydroxamic acid method is frequently used for the determination of penicillins via the β-lactam ring. Further, if the functional group dealt with is, at the same time, the "pharmacologically active group ", the chemical method would also determine the " activity " of the drug [10-12].

[b] Combination of separation and measurement operations:

In several cases a preliminary separation step is carried out before measurement. This is usually done either by using solvent extraction or chromatographic separation [2,14].

[b-1] Solvent extraction:

The selectivity of direct spectrophotometry has been improved through preliminary separation techniques, such as organic solvent extraction of the amine formed by the hydrolysis of atropine in alkaline medium, followed by UV measurement in acid
medium [2] . Also, the direct spectrophotometric determination of Clorazepate dipotassium via N-desmethyldiazepam [15], or via its final degradation products [16], were accomplished through preliminary organic solvent extractions.

[b-2] Chromatographic separation:

Separation was also affected by TLC followed by UV spectrophotometric determination of chloramphenicol and its palmitate in presence of their degradates [17] and in studying their hydrolysis, kinetics [18]. Similarly, the colorimetric determinations of streptomycin and its dihydride [19,20] in pharmaceutical preparations were carried out after chromatographic separations. Also secnidazole was determined in presence of its degradates using densitometry after separation on a TLC plate [21] .

[C] Determination of the intact drug in presence of its degradate without separation

This can be done by using suitable and selective methods, including:

[C.1] Spectroscopic methods:

C.1.a. UV-Visible spectrophotometric methods – Direct UV-Visible spectrophotometry has been widely used in pharmaceutical analysis to study decomposition mechanisms but, generally, it lacks sensitivity. However, Amer et al [22] used the orthogonal functions to determine intact nystatin selectively in presence of its degradation products, by direct UV-measurements.

Several stability indicating colorimetric methods have been reported. For example, the micro-colorimetric determination of thiamine through its precipitation with a special iodobismuthic acid reagent without any interference from its hydrolytic products [23]. Different reagents were also used to quantitate some H_2-antagonists in presence of their decomposition products by colorimetric procedures [24]. In addition, the acid-dye technique

has been reported for the determination of nizatidine in presence of its degradate [25]. Also the basic dye "neutral red " was used for the determination of ibuprofen in presence of its degradation product [26] .

Derivative spectrophotometry has become a well established technique for the analysis of drugs in mixtures and formulations. The main advantage of this technique is the improvement of resolution of the overlapping absorption bands and, consequently, the accuracy and precision of absorption spectra methods. Clorazepate dipotassium [16], nizatidine [24] , carbamazepine [27] , secnidazole [21] and some cephalosporins [28] were determined in presence of their degradation products by derivative spectrophotometry.

A new spectrophotometric method for resolving binary mixtures was proposed by Salinas et al [29], based on the simultaneous use of the first derivatives of the ratio spectra of substances with overlapping spectra and is termed ratio-spectra first derivative spectrophotometry (RSD_1). An RSD_1 method for the determination of noramidopyrine methane sulfonate sodium salt, pitophenon hydrochloride and fenpiverine bromide in pharmaceuticals was developed by Morelli [30]. Nevin et al [31] described an RSD_1 method for the simultaneous determination of hydrochlorothiazide and amiloride hydrochloride in sugar coated tablets. Also, Özkan et al [32] used RSD_1 spectrophotometry for the simultaneous determination of two-component mixtures in pharmaceutical formulations containing chlordiazepoxide.

Direct UV spectrophotometry is particularly useful in case of compounds whose absorption spectra are affected by changing pH .The decomposition of iodoxuridine [33] and phenobarbitone [34] have been determined by measuring

their absorbances at different pH. Difference spectrophotometry was also used for the determination of azathioprine in presence of its degradation product [35].

C.1.b. Fluorimetric methods **-** Spectrofluorimetric methods are used when great sensitivity is required. Tetracycline and its degradate, (anhydrotetracycline) were separately determined using fluorimetry [36].

C.1.c.NMR-spectroscopic methods **-**Although NMR-spectroscopic methods
was applied either qualitatively or quantitatively as it offers specificity along with simplicity of operation, yet, it lacks sensitivity and precision. It has few applications in stability evaluation such as the determination of clonazepam in presence of its degradation products [37].

[C.2] <u>Electrochemical methods:</u>

Polarographic techniques have been adopted for the analysis of ethacrynic acid in presence of its decomposition products [38] .A polarographic investigation was used to follow the acid-base hydrolysis and β-lactamase degradation of several cephalosporins [39]. Other electrochemical methods include amperometric titration of ampicillin through its hydrolysate, penicillamine [40], and voltametric assay of bromazepam degradate, (2-amino-5-bromobenzoylpyridine) [41].

[C.3] <u>Titrimetric methods:</u>

A simple, titremetric, stability indicating method was adopted for the determination of nitromin via its pharmacologically active functional group [10]. Said et al [23] developed a titrimetric method for the determination of thiamine in presence of its hydrolytic product.

[C.4] Chromatographic methods:

Due to their high separation power, different chromatographic techniques were widely applied in stability indicating procedures, including paper [42] , TLC [43,44] , column [45,46] and gas liquid chromatography [47,48] . High-performance liquid chromatography (HPLC) combines the excellent separation properties of column chromatography with spectrophotometry and is considered one of the most useful and applicable selective analytical techniques for the determination of numerous drugs in presence of their degradation products [49-52].

In this thesis five different selective methods, namely, Derivative spectrophotometry, Ratio-spectra first derivative spectrophotometry, pH-induced difference spectrophotometry, Densitometry and High performance liquid chromatography (HPLC), were adopted for the determination of intact aceclofenac, clozapine and diloxanide furoate in presence of their degradation products.

The suggested methods are simple, accurate, selective and sensitive. Application of the proposed methods to the analysis of the aforementioned three drugs in their pharmaceutical formulations shows that neither the excipients usually formulated in these market preparations nor the degradation products interfere with the determinations.