Application Derivatization by Means Perhydrolysis Reactions in Pharmaceutical Analysis

M.Ye. Blazheyevskiy

National University of Pharmacy, 4 Valentynivs'ka str., Kharkiv 61168, Ukraine; e-mail: blazejowski@ukr.net

Recieved: February 21, 2017; Accepted: May 08, 2017
DOI: 10.17721/moca.2017.31-54

This article describes derivatization chemistry used in conjugation with either spectrophotometry, fluorimetry or voltammetry methods to facilitate the determination of pharmaceuticals. Generally, derivatization is used in spectrophotometry, kinetic spectrophotometry, voltammetry and fluorimetry, principally to enhance detectability. The section considers derivatization of drugs such as antibiotics (penicillins and cephalosporins), esters and peroxides. For spectrophotometry, fluorimetry and voltammetry sections, data are included giving the structures of the more important for derivatization agents, the analytes, and the corresponding reaction products. Derivatization for spectrophotometry, fluorimetry or voltammetric methods is often directed toward esters and amides that are difficult to detect at low levels by absorbance, luminescence, or electrochemical means. On the oxidation by means of peroxy acids or oxone and/or perhydrolysis reactions of drugs was the primary focus in this article.
The spectrophotometric, fluorimetric, voltammetric and kinetic methods can be easily applied for indirect determination by means of derivatization by peroxy acid oxidation and/or perhydrolysis (with peroxy acid oxidation before alkali hydrolysis) of the investigated drugs (penicillins and cephalosporins, alkaloids, esters and etc) in pure and dosage forms that do not require elaborate treatment and tedious extraction of chromophore produced. The proposed methods with use peroxy acid and oxone as derivative agents for obtain of derivatives is sensitive enough to enable determination of lower amounts of drug, these advantages encourage the application of proposed method in routine quality control of investigated drugs in industrial laboratories. Finally, method provides advantages of improving selectivity and accuracy, avoiding interference of colored and/or turbidity background of samples because it measures the increase in analytical signal (absorbencies, emission) with time against blank treated similarly and at strong dilution.

Keywords: derivatization, phenothiazine, antibiotics (penicillins and cephalosporins) spectrophotometry, fluorimetry, voltammetry, kinetic method, peroxy acid, oxone, perhydrolysis

Застосування дериватизації за допомогою реакцій пергідролізу надкислотами у фармацевтичному аналізі

М.Є. Блажеєвський

Національний фармацевтичний університет, вул. Валентинівська, 4, м. Харків, Україна, 61168;
e-mail: blazejowski@ukr.net

Надійшла: 21 лютого 2017 р; Прийнята: 8 травня 2017 р
DOI: 10.17721/moca.2017.31-54

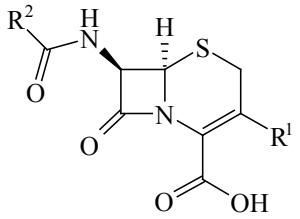
У даній статті описані процеси добування функціональних похідних лікарських препаратів (дериватизаційна хімія), використані у поєднанні з спектрофотометрією, флуориметрією та методом вольтамперометрії з метою полегшення здійснення фармацевтичного аналізу. Загалом, дериватизація використовується у спектрофотометрії, кінетико-спектрофотометричних методах, вольтамперометрії та флуориметричному аналізі, головним чином, щоб збільшити виявну здатність (чутливість). Розглянуто процеси дериватизації лікарських препаратів таких класів, як антибіотики (пеніциліни та цефалоспорини), естери та пероксиди. У підрозділах, що стосуються спектрофотометрії, флуориметричного аналізу та вольтамперометорії наведені дані стосовно структури продуктів реакції окиснення і застосовуваних окисників. У спектрофотометрії, флуориметрії та вольтамперометричних методах дериватизація переважно спрямована на перетворення естерів та амідів, які важко прямо визначати через слабке їх світлопоглинання або здатність до люмінесценції чи виявлення електрохімічними засобами. Цей огляд сфокусований саме на реакціях окиснення і/ або пергідролізу лікарських препаратів за посередництвом аліфатичних надкислот та оксону.

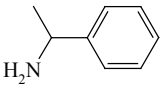
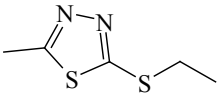
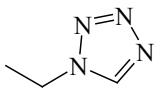
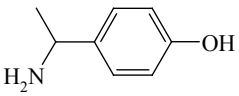
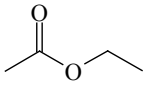
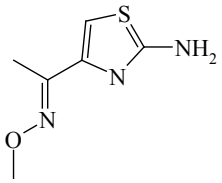
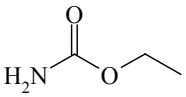
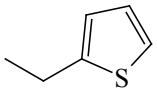
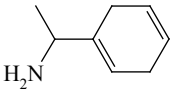
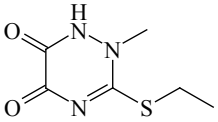
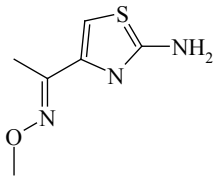
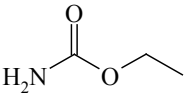
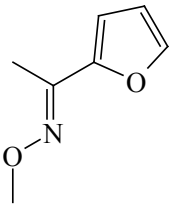
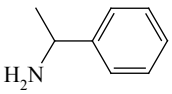
Ключові слова: дериватизація, антибіотики (пеніциліни та цефалоспорини), спектрофотометрія, флуориметрія, вольтамперометрія, кінетичні методи, пероксикислоти, оксон, окиснення, пергідроліз

Derivatization in conjugation with physical-chemical methods, generally, often prior to the determination and is used principially to enhance the detectability. Prederivatization is often directed toward esters or amides that are difficult to detect at low levels by absorbance, luminescence, or electrochemical means. The desirable conditions to be met for derivatization are the reaction stoichiometry and product structure should be known, the reaction should be reasonably fast and produced quantitatively or at least reproducibly, and the derivate should be stable. One advantage of the derivatization approach is that simple equipment is commercially available to allow for the reaction chemistry to be done in the batch mode. On the oxidation by means of peroxy acids or oxone and/or perhydrolysis reactions of drugs will be the primary focus in this article. A specific examples and an overview for each of these classes reactions will be described below.

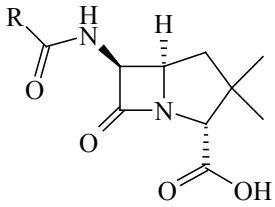
Lactams are cyclic amides and the most important class of drugs based on lactams are probably *β*-lactam antibiotics which include the families of *penicillins* and *cephalosporins*. Penicillins contain a β-lactam ring fused to a five-membered thiazolidine ring, where one of the atoms in the fully saturated ring is sulfur. On the other hand, the *β*-lactam in *cephalosporins* is fused to a six-membered rings. Chemical formula of some brand names of cephalosporins and penicillins are summarized in Table 1 and Table 2.

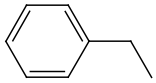
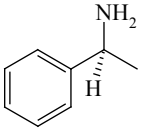
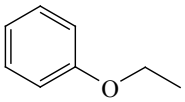
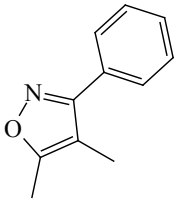
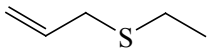
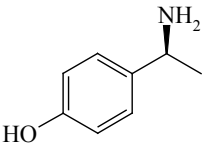
Tabl. 1. Chemical formula of some of cephalosporins.



Name	R^1	R^2	Name	R^1	R^2
Cefaclor	Cl–		Cefazolin		
Cefadroxil	CH_3–		Cefotaxime		
Cefoxitin			Cefradine	CH_3–	
Ceftriaxone			Cefuroxime		
Cephalexin	CH_3–				

Tabl. 2. Chemical formula of some penicillins.



Name	R	Name	R
Benzylpenicillin		Ampicillin	
Phenoxymethylpenicillin (Penicillin V)		Oxacillin	
Penicillin O		Amoxicillin	

Hydrolysis and perhydrolysis (reactions by means perhydroxyl anions and/or anions of peroxy acid) reactions

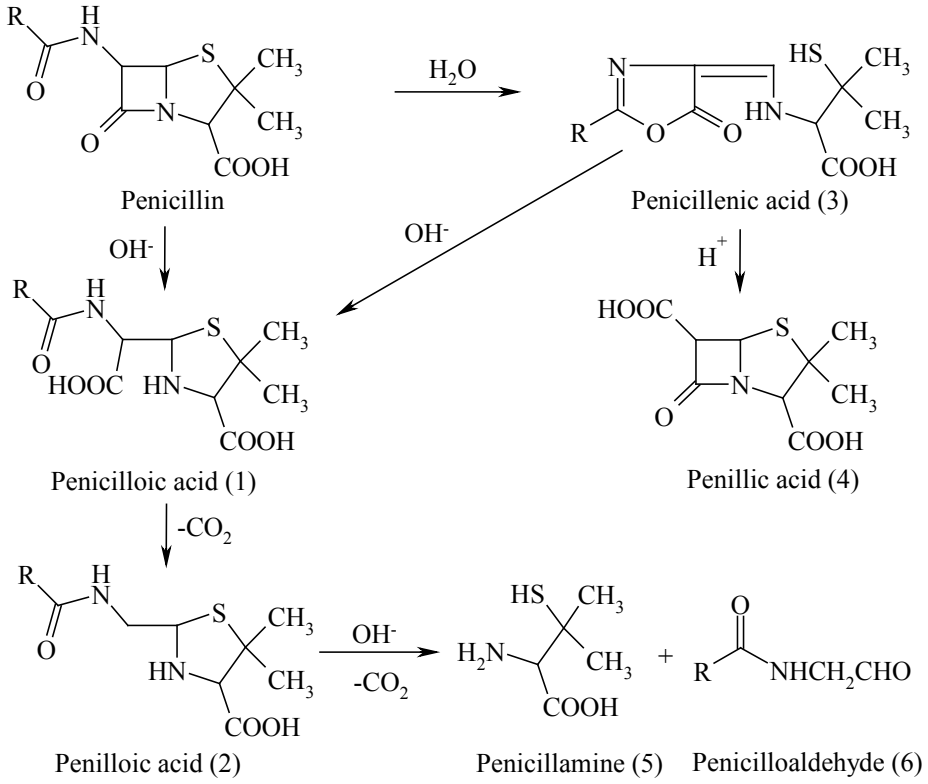
As is known β-Lactam antibiotics are suicide inhibitors of bacterial peptidases. They inhibit the enzymes by acylating the hydroxyl group of the active site serine residue. The acylation occurs *via* a nucleophilic attack by the serine hydroxyl group, resulting in the opening of the four-membered lactam ring. A similar process also occurs during the *hydrolytic degradation* of *β*-lactam antibiotics both *in vitro* and *in vivo*, in which water becomes the nucleophile. It was found the rate-limiting step is not the formation of the tetrahedron intermediate, but rather the subsequent ring opening step. In the latter step water acts as a general acid providing a proton for the leaving amine group. In aqueous solution on penicillins are generally stable from pH 5 to 8. Beyond this range they indigo rapid degradation.

Penicillins forms penicilloic acid (1), which in turn loses carbon dioxide to form penilloic acid (2). Most penicillins are also readily degradated by acids. Madly acidic conditions produce penicillenic acid (3), which forms penicilloic acid (1) which base, or penillic acid (4) which further acid treatment. The complete hydrolysis of penicillin produces penicillamine (5) and penicilloaldehyde (6). All the degradation path-ways are summarized in Scheme 1.

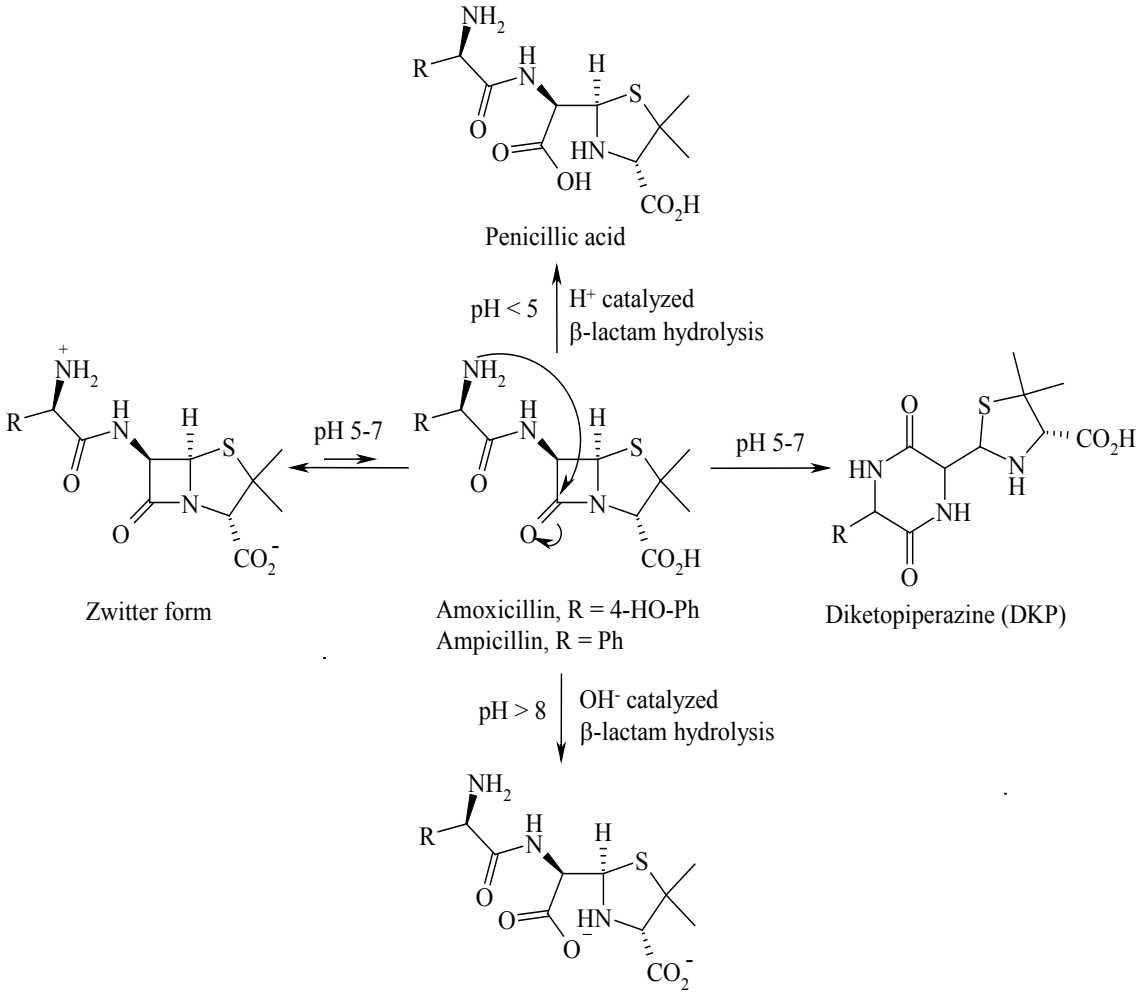
Both ampicillin and amoxicillin are among the most prescribed antibiotic drugs today. They are amino derivatives of benzylpenicillin. The difference between the two aminopenicillins is that amoxicillin has an additional hydroxyl group at the 4-position of the benzyl group. On the other hand the presence of the amino group has an impact on the stability and degradation pathways. For example aminopenicillins have increased stability under acidic conditions; the enhanced stabilities are attributed to the presence of the predominant zwitterion forms of both drugs at pH 5. At below 5, amoxicillin degrades mainly via hydrolysis of its *β*-lactam ring. At pH 5 to 7, the formation of diketopiperazine becomes its major degradation pathway, resulting from the intramolecular attack of the *β*-lactam ring by the benzylic amino group. Above pH 8, hydroxide ion-catalyzed hydrolysis predominates. The degradation pathways of aminopenicillins at the axample of amoxicillin are summarized in Scheme 2.

The *cephalosporins* are a group of antibiotics closely related to penicillin. In cephalosporins, the β-lactam rings is fused to a dihydrothiazine ring instead of the thiazoline ring of penicillins (Tabl 1). These antibiotics are not destroyed by the enzyme penicillinase, but are degraded by cephalosporinase, and acetylesterase.

In general, the degradation path ways of cephalosporins are similar to these of penicillins: at both low and high pH ranges, the hydrolysis of the *β*-lactam ring is usually the major pathway. In the mid-range pH, formation of the diketopiperazine degradant becomes a significant path-way. An example can be found in the case of cefaclor, a second generation cephalosporin antibiotic that is still widely today.



Scheme 1. Known degradation behavior of penicillins under different hydrolytic conditions.



Scheme 2. Major degradation pathways of aminopenicillins under of different pH ranges.

Reaction of S-oxidation and perhydrolisis. Penicillins Spectrophotometric assay methods

The iodometric method is unselective productions as well as their degradation products, are oxidised by iodine. It is therefore not suitable for control of the purity of the antibiotics of this group. The ultraviolet spectrophotometric method, is suitable for low concentrations of *β*-lactams. The direct spectrophotometric methods suffer a lack of specificity because all compounds containing the *β*-lactam ring absorb in the range 250-270 nm.

Before the development of a chemical assay for a penicillins, commonly occurring impurities must be considered for their potential interference. The most important of these impurities are, in order of relative abundance, penicilloic acids, penicillenic acids, penillic acids, and impurity penicillins. Penicilloic acids cans paralled the assay response of the parent penicillin in detection techniques, which rely upon a chemical or spectrophotometric property of the side chain moiety. Penicillenic and penillic acids contain as side similar to that of the parent penicillin. In addition, penicillenic acids have strong ultraviolet absorption due to the oxazone structure. For example, the molar absorptivity for benzylpenicillenic acid is 26.600 at the absorption maxima at 322 ±1 nm in an ethanol solution. benzylpenicillic acid has a ε of 7100 at an absorption shoulder at 325 ± 1 nm. These acid degradation produces would not be expected to interfere with methods based upon the β-lactam structure [1].

Some penicillins can be contaminated with small amounts of other penicillins. These impurity penicillins interfere in assay methodology based upon *β*-lactam chemistry. If the parent penicillin side chain and the impurity penicillin side chain differ sufficiently, a method based upon side chain chemistry may be appropriate.

For degraded penicillin samples or crud samples the number and quantity of impurities present increase to include penilloic acid, penamaldates, the side chain precursor (eq. phenylacetic acid from benzylpenicillin), and 6-aminopenicillanic acid.

The ultraviolet spectrophotometric method, based upon acid degradation to penicillenic acid has been applied to penicillins. This assay method is selective for the intact penicillin molecule. The ultraviolet absorption at 322 nm is due to the formation of the oxalone structure of the acid rearrangement product, penicillenic acid. Penilloic acid, 6-aminopenicillanic, cephalosporins, and other antibiotics do not interfere.

Experimental conditions vary widely because of the differences in stability characteristics of penicillins in acidic solutions. In addition, both cooper and mercury catalysts have been used at various concentrations. This ultraviolet spectrophotometric procedure for penicillins is rapid and sample.

The most common degradation product of penicillins, penilloic acid, is present in all penicillin samples. This impurity is easily determinated by the iodometric titration method. The absorption of iodine of an intact sample is measured under neutral or weakly acidic conditions.

Excessive acid inactivation of penicillin produces penicillevic and penillic acids, and the characteristic yellow colour of crud penicillin products. These acid degradation product may be detected by measuring in neutral solution.

The applied to a number of penicillins as summarized in Table 3.

Although assay precision varies standard deviation is approximately 2 %. The major advantage of this procedure is sensitivity. Approximate measuring concentration are summarized in Table 3.

Table 3. Assay conditions for spectrophotometric method determination of penicillins based upon acid and catalytic degradation.

Name of penicillin	Catalyst	pH	t, °C	Time, min	λ_{max}, nm	Approx. conc. for assay, μg mL^{-1}
Benzylpenicillin	Cu(II)	4.6	100	15	322	12
		1.8	20	30	322	8
Penicillin O	Hg(II)	1.8	20	30	322	6
Phenoxymethyl-penicillin	Hg(II)	1.8	20	30	322	40
Ampicillin	Cu(II)	5.2	75	30	320	10
Oxacillin	Hg(II)	1.8	20	30	338	5

The ultraviolet spectrophotometric method has applied to phenoxymethylpenicillin; however, sensitivity is poor due to the acid stability of phenoxymethylpenicillin. The intact ultraviolet absorption of phenoxymethylpenicillin at 268 and 275 nm is the basis of a non-selective direct spectrophotometric method. The British Pharmacopeia spectrophotometric method is made more selective by initial precipitation of phenoxymethylpenicillanic acid from acidic solution. The direct ultraviolet assay of ampicillin in the aqueous solution at the 258 nm has been described.

As already noted the major product resulting from mild alkaline hydrolysis of penicillins is the dibasic penicilloic acid. Alkaline hydrolysis of penicillin V is usually first order with respect to penicillin but at pH > 10. It was found that the hydrolysis was catalazed by OH^- and was second order. The rate of attack on the anion was seven times greater than that on the undissociated acid [2]. Alkaline hydrolysis of Penicillin G shows that incorporation of the amide side chain and acid group increases the reaction barrier, whereas, the thiazolidine ring lowers the barrier [3].

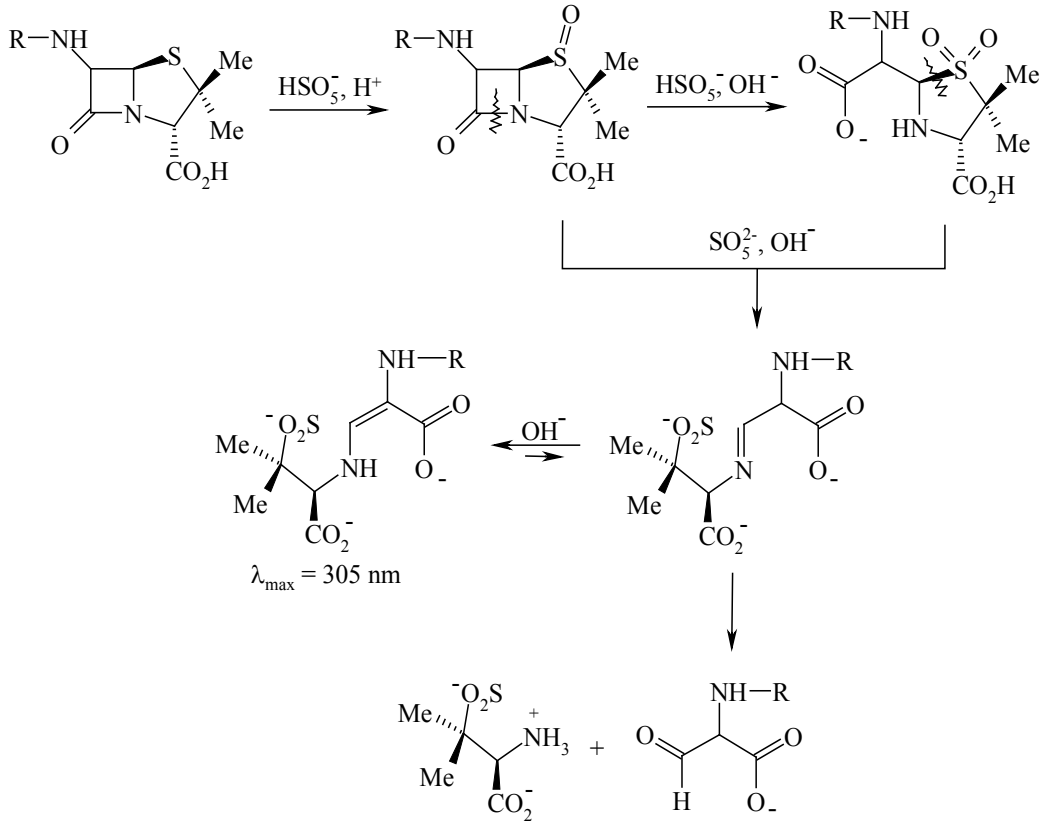
Method is based on the oxidation reaction of Ampicillin with alkaline hydrogen peroxide; the absorbance is measured at 302 nm for a fixed time of 70 min, procedure was achieved at room temperature [4].

A kinetic spectrophotometric method has been developed for the determination of ampicillin (I) and amoxicillin (II). The method involves hydrolysis of the antibiotics with 1.0 M HCl, neutralization with 1.0 M NaOH followed by addition of palladium (II) chloride in the presence of 2 M KCl. The produced yellow colour is measured at 335 nm. The proposed method is valid over the concentration range 8 – 40 μg mL^{-1} and 10 – 40 μg mL^{-1} for I and II respectively with minimum detectability of 0.73 μg mL^{-1} and 0.76 μg mL^{-1} for I and II respectively. The determination of the studied compounds adopting the fixed concentration method is feasible with the calibration equations obtained, but the fixed time method has been found to be more applicable. The proposed method was applied to commercial dosage forms and the results obtained were in good agreement with those given by USP method [5].

An original common kinetic-spectrophotometrical method for the determination of penicillins, such as, benzylpeniciillin sodium [6] ampicillin [7–8], oxacillin [9], amoxicillin [10] and phenoxymethylpenicillin potassium, Penicillin V [11] in pure form and pharmaceutical formulations are described. The method is based on the formation of a typical hydrolytic products by couple with peroxomonosulphate S-oxidation and perhydrolysis reactions in alkali medium, monitored in situ at λ = 275-305 nm. The analytical parameters have been evaluated and the results obtained using standard additions are in agreement with the reference methods.

For example, oxidative-hydrolytic transformations of Ampicillin by means of potassium peroxomonosulfate in alkali medium is discribed on the Scheme 3.

Without peroxomonosulphate in the given conditions diring the first 30-40 min the products formation was not observed. The need for eccess of peroxomonosulphate was explaned by the fact that part of it is involved in the process of hydrolytic cleavage of the formed at the firs stage reaction of corresponding penicillin S-oxide (nucleophilic catalysis of *β*-lactam and thiazolidin cycles hydrolysis) [12].



Scheme 3. Mechanism of Ampicillin chemical transformations by means of potassium peroxomonosulfate in alkali medium.

Recently, this method was devoted to a review of literature [13].

On the Fig. 1, for example , is described electronic spectra of the products of alkaline hydrolysis (1) and perhydrolysis of sodium oxacillin (2-6) in the time.

The formation of characteristic wave with maximum under certain wavelength cogently show of one of several similar by the structure reaction products, that possibly belong to substituted derivates of *N*-acryl-*β*-penicillamin sulfinate (IV) [14].

Alternatively, a perhydrolysis method has been developed which is specific for ampicillin and oxacillin [12, 15]. These approaches can be used to evaluate the target analyte in the presence of its degradation products.

For the first time possibility and advantages of derivatization application by means of peroxide for quantitative determination of *β*-lactam antibiotics were shown on the example of catalyzed by hydrogen peroxide or peroxy acid reactions of alkali hydrolysis of penicillins – so called reaction of perhydrolysis using anions of hydrogen peroxide or peroxy acid ions in alkali medium [16-17]. According to the research results a simple and selective enough kinetics spectrophotometric procedure of individual (ampicillin in the presence of oxacillin) determination of ampicillin and oxacillin in the combined two-component formulation "Ampiox" (ampicilin trihydrate, 0.125 g; oxacillin potassium salt, 0.125 g) was developed. It was based on the oxidation of both antibiotics by diperoxiadypine acid during 1 min to the corresponding S-oxides followed by alkaline hydrolysis of their *β*-lactams groups of corresponding S-oxides at room temperature by means of deperoxiacidic anions. The reaction rate was determined by absorption of yellow products at 305 nm (ampicillin perhydrolysis product) and characterized by tangent value of a straight linear dependence of the kinetic curve. Calibration graphs shows linear dependence within the concentration 1-50 μg mL^{-1} for both antibiotics under study. Combination of the results of the iodometric determination (by reverse titration of diperoxiacid excess in S-oxidation reactions in an independent experiment) of the total content of components in a mixture with kinetic results of a quick reacting component (ampicillin) allows to determine the content of penicillin in binary mixtures. The limits of determination (LOQ) for ampicillin and oxacillin were 1.0 μg mL^{-1} and 2.0 μg mL^{-1}, respectively. The precision of the analysis is confirmed by "introduced-found" method by analyzing pre-made mix ("Ampiox" preparation model) with exactly known content of components. During the preparation analysis RSD ≤ 3.3 %. The results of the average content of components in the product determination correspond to the results obtained in the suggested spectrophotometric method [18].

A very simple and fast kinetic fluorimetric method for the determination of ampicillin is described. It is based on the stopped-flow technique and addition of hydrogen peroxide to the reaction medium, and requires no prior heating to hydrolyse the *β*-lactam ring unlike most hither to known determination [19]. Kinetic data can be obtained only a few seconds after the reactants have been mixed, which allows ready application of the method to routine analyses. The calibration graph is linear over the range 0.025 – 0.7 μg mL^{-1} ampicillin, the detection limit is 20 ng mL^{-1} and within- and between-assay precision is 2.6 % resp. 2.7 %. The method was satisfactory applied to the analysis serum samples by including a preliminary deproteination step using trichloroacetic acid. Mean recovery is 102.1 %.

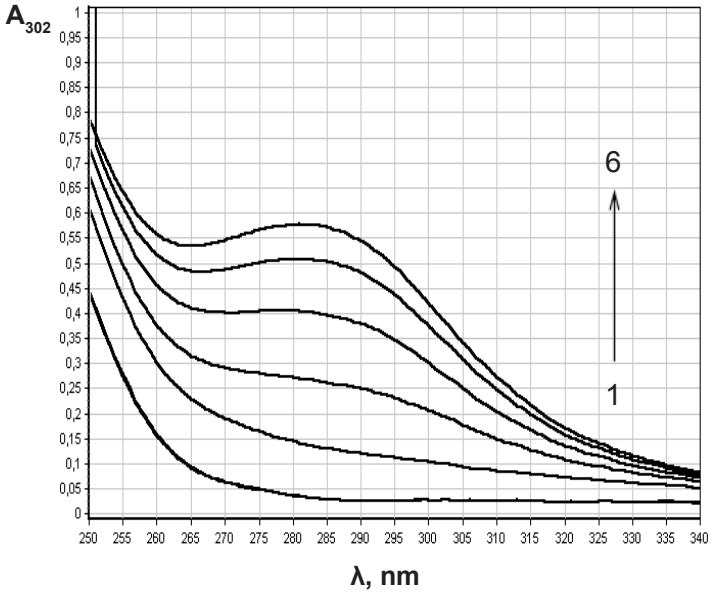


Fig. 1. Electronic spectra of light absorbance of the products of alkaline hydrolysis (1) and perhydrolysis of sodium oxacillin (2-6) in the time, min: 1) 0-20, 2) 5, 3) 10, 4) 15, 5) 20, 6) 25; c(NaOH) = $6.1 \cdot 10^{-3}$ mol L^{-1}; c($KHSO_5$) = $1.2 \cdot 10^{-3}$ mol L^{-1}; c(Na-oxac.) = 30 μmol mL^{-1}.

Spectrophotometric assay methods. Cephalosporins

Cephalosporins are another very important class of *β*-lactam-based antibiotics, which emerged after the penicillin family was introduced. A direct ultraviolet spectrophotometric method also has been described for the determination of cephalosporins. UV-spectrophotometric method for determination of cefazoline sodium using purified water and phosphate buffer was proposed by Pedroso and Salgado [20]. The absorbance was measured at 270 nm and the linear range was between 8-28 μg mL^{-1}.

Game et al. [21] developed two spectrophotometric methods for the determination of cefuroxime axetil in bulk drug and tablets. In the first method, the UV spectrum of Cefuroxime axetil in 0.1 mol L^{-1} HCl was obtained which exhibits absorption maxima (λ_{max}) at 281 nm. Beer's law was obeyed over the range 2 to 30 μg mL^{-1}. Method II is the 1st derivative spectrophotometric method. In this method the simple UV spectrum of Cefuroxime Axetil in 0.1 mol L^{-1} NaOH was obtained and derivatised to 1st order. Maxima occur at 266 nm and minima at 300 nm. A calibration curve was prepared by plotting the absorbance difference between maxima and minima vs. concentration. The linearity range was found to be 4-30 μg mL^{-1}.

A simple, precise, accurate, sensitive and rapid Simultaneous Equation method also was developed for simultaneous estimation of Cefuroxime Axetil and Linezolid in tablet dosage form. The proposed method was applied for the determination of Cefuroxime Axetil and Linezolid in tablet formulation, for determination of sampling using two wavelengths, Axetil and Linezolid were scanned in 200-400 nm range and sampling wavelengths were 276.60 nm for Axetil and 257.40 nm for Linezolid are selected for development and validation of simultaneous equation method. For this method linearity observed in the range of 2-6 μg mL^{-1} for Axetil and 2.4-7.2 μg mL^{-1} for Linezolid and in their pharmaceutical formulation with mean percentage recoveries 99.90±0.005 and 100.02±0.009, respectively. The method was validated according to ICH guidelines and can be applied for routine quality control testing. LOQ (μg mL^{-1}) (*n*=6) Axetil at 276.60 nm [22].

Different reagents have been reported in order to increase the selectivity of the methods based on the own cephalosporins absorption of their products of acid or basic degradation. The hydrolytic degradation of antibiotics is very often used as a preliminary step in the analytical procedures for their determination.

Pritamand and co-workers [23] reported a UV spectrophotometric method for the determination of cefuroxime axetil in bulk and in formulation. The λ_{max} of cefuroxime axetil in 0.1 M HCl was found to be 281 nm. The drug was found to be linear in the concentration range 0.4-2 μg mL^{-1} with correlation coefficient value of 0.998.

Arun et al. [24] reported a spectrophotometric method in UV region for the determination of ceftazidime in bulk dosage form. The solution of ceftazidime in 0.1 mol L^{-1} HCl shows maximum absorbance at 261 nm, Beer's law was obeyed in the concentration range of 2-10 μg mL^{-1}.

As soon these methods have a low sensitivity and are not sefficient selective.

Satry et al. reported a method for the assay of cefadroxil and ceftezoxime in pharmaceuticals. The method was based on the addition of sodium hydroxide followed by iodine and wool fast blue and the absorbance was measured at 540 nm. The linear range was between 0.8-9.9 μg mL^{-1} [25].

Rageh et al., [26] have proposed spectrophotometric method for the determination of cefaclor monohydrate, cefadroxil monohydrate, cephalexin anhydrous, cefradine anhydrous, cefotaxime sodium, cefoperazone sodium, ceftriaxone sodium, ceftazidime penthydrate, cefazolin sodium, cefixime and cefpodoximeproxetil, in bulk drug and in pharmaceutical formulations. The method was based on hydrolysis of the studied drugs using 0.5 mol L^{-1} NaOH at 100°C and subsequent reaction of the formed sulfide ion with NBD-Cl (4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole) to form a yellow-colored chromogen measured at 390 nm.

A kinetic method for the accurate determination of cephalexin has been described. A solution of cephalexin is reacted with $5 \cdot 10^{-3}$ mol L^{-1} cobalt(II) nitrate in $1 \cdot 10^{-3}$ mol L^{-1} sodium hydroxide at 60°C for a fixed time of 6 min, after which the absorbance of the reaction product is measured at 310 nm. The concentration of cephalexin is calculated by using the corresponding calibration equation for the fixed-time method. The method has been applied to proprietary drugs and the results were compared statistically with those given by the BP method. The determination of cephalexin by the fixed-concentration and rate-constant methods is feasible with the calibration equations obtained but the fixed-time method has been found to be more applicable [27].

The analysis of cefixime in pure form and in pharmaceuticals through complexation with Cu^{2+} using acetate-NaOH buffer in mixture water: methanol was also conducted. The complex has maximum absorbance at λ=410 nm. The reaction between cefixime and Cu^{2+} occurred at a stoichiometric ratio of 1:1. All reaction conditions were optimized to obtain the complex. Under optimum conditions, Beer's law was obeyed at concentrations ranging from 0.2267-22.671 μg mL^{-1} as prescribed by Ramadan et al. [28].

Spectrophotometric method for the determination of some cephalosporins based on the hydrolysis of the cephalosporin in sodium hydroxide solution to produce the sulphide ion and the conversion of the sulphide with the p-phenylenediamine to form a violet color was reported by Alothman and Abdalla [29]. Acetaminophen was hydrolysed in sulphuric solution and the resulting p-aminophenol was oxidized with

sulphide ion in the presence of Fe^{3+} to form a red product. The method was successfully applied to the assay of some cephalosporins in drug formulations.

Zhao et al. [30] proposed indirect spectrophotometric determination of sodium ceftriaxone. Sodium ceftriaxone was degraded completely in the presence of 0.20 mol L^{-1} sodium hydroxide in boiling water bath for 20 min. The method was linear in the range of 0.70 - 32 μg mL^{-1} and the LOD was found to be 0.60 μg mL^{-1}.

Buhl and Szpikowska-Sroka [31] reported spectrophotometric method for the determination of cefotaxime, ceftriaxone and cefradine with leuco crystal violet. The determination was based on the reduction of potassium iodate in acidic medium, followed by hydrolysis of β-lactam ring of cephalosporins with sodium hydroxide. The formed iodine reacts with leuco crystal violet. The crystal violet dye of maximum absorption at 588 nm is formed. Beer's law was obeyed in the concentration range 0.8-4.8, 0.4-1.6 and 0.2-2.0 μg mL^{-1} for cefotaxime, ceftriaxone and cefradine, respectively. The molar absorptivity of the colored compound is $8.4 \cdot 10^4$ L mol^{-1} cm^{-1} for cefo-taxime, $2.4 \cdot 10^5$ L mol^{-1} cm^{-1} for ceftriaxone and $1.6 \cdot 10^5$ L mol^{-1} cm^{-1} for cefradine. The analytical parameters were optimized and the method was successfully applied to the determination of cefotaxime, ceftriaxone and cefradine in pharmaceuticals.

Flow-injection spectrophotometric method for the determination of cefadroxil (I) and cefotaxime (II) was described by Metwally et al., [32]. The method was based on the hydrolysis of the cephalosporin with sodium hydroxide whereby the sulfide ion was produced. Linear calibration curves were obtained in the range 36.34-109.2 and 95.48-477.4 μg mL^{-1} for I and II, respectively. The experimental LODs were 0.036 and 0.048 μg mL^{-1} for I and II, respectively. The total flow-rate is 5.3 mL min^{-1} for both drugs. Alternately, the sulfide ion produced is allowed to react with p-phenylenediaminedihydrochloride (PPDD) and Fe^{3+}, and the violet color produced is measured at 597 nm (method B). Linear calibration graphs are obtained in the range 0.5-400 and 0.5-450 μg mL^{-1} for I and II, respectively. The LODs were 0.4 and 0.2 μg mL^{-1} for I and II, respectively. The methods were successfully applied to the analysis of some pharmaceutical formulations, particularly of the injection and capsule types.

Tabl. 4 summarises the main parameters of different proposed methods that use the spectroscopy UV-vis. The determination of cephalosporins by alkaline degradation to sulfide hydrogen has been proposed [33].

Cephazolin [34] suffered acid degradation when is heated in the presence of sulphuric acid. This process can be observed by means of the disapparition of the band of 270 nm and the apparition of a new band at 302 nm due to the hydrolysis product, 2-mercapto-5-methylthiadiazole and there is also absorption at about 250 nm by unidentified degradation products.

A manual visible spectrophotometric method for the determination of cephalosporins by alkaline degradation to sulphide and formation of methylene blue has been adapted for use with an air-segmented AutoAnalyzer I system. The system has been tested for the determination of twelve cephalosporins; rectilinear calibration graphs were obtained with good precision in the general range 8 - 80 μg ml^{-1} of cephalosporin. The automated procedure was tested as a method of determining trace amounts of cephalosporins and other sulphide-producing impurities in penicillin G and V samples. The detection limit was calculated to be 1–2 μg g^{-1} of cephalosporin in penicillin samples [35].

A simple, selective and accurate spectrophotometric method has been developed for the determination of the cephalosporin-type antibiotic cephazolin. The method is based on the fast preliminary acid hydrolysis of cephazolin and the spectrophotometric determination of one of its degradation products – 2-mercapto-5-methylthiadiazole [34].

Methods for determination of individual cephalosporins and their corresponding acid-induced degradation products are described, based upon second derivative spectrophotometry. Linear plots of D_2[dbnd]($d^2A/d\lambda^2$) values with negligible intercept were obtained versus concentration in the range of 0.4–2.4 mg% for both intact and degraded cephalosporins. Kinetic investigation of the cephalosporins degradation proved that the proposed method is applicable to stability determinations Compared with the official method the assay results of the different cephaiosporins pharmaceutical preparations were of equal accuracy (t-test) and reproducibility (F-test) [37].

First-and second-derivative Spectrophotometry, with a zero-crossing technique of measurement, has been used for the quantitation of two-component mixtures of cepnaloridine and cephalothin Na, which are cephalosporins with closely overlapping spectral bands. Beer's Law is followed for up to 28 and 36 μg mL^{-1} of cephaloridine in the first- and second-derivative modes, respectively, and up to 36 μg mL^{-1} of cephalothin Na in both modes. Detection limits at the 0.05 level of significance were calculated to be 0.13 and 0.37 μg mL^{-1} of cephaloridine and cephalothin Na, respectively, in the first-derivative mode, and 0.25 and 0.29 μg mL^{-1}, respectively, in the second-derivative mode. The recovery of these antibiotics in mixtures of injectable dosage forms are also reported [38].

Table 4. Analytical properties of proposed methods for the analysis of Cephalosporins by spectrophotometry UV-vis.

Reagent	Drug	λ , nm	Reaction time; *t*,	ε, L mol^{-1} cm or slope of calibr.	Interval dinamic	*LD*, μg mL^{-1}	Considerations	Ref.
NaOH (hydr.)	Cehalexin Cefuroxime 7-ACA	667	30 min; 100 ºC 50 min; 100 ºC 40 min; 100 ºC	$2.39\cdot10^4$ $0.52\cdot10^4$ $1.76\cdot10^4$			Formation of methylene blue	[33]
NaOH (hydr.)	Cephalexin	667	30-60 min; 100 ºC	$7.0\cdot10^{-3}$ mL μg^{-1}	8-80 μg mL^{-1}	0.3	Flow continuous, automate, formation of methylene blue	[35]
	7-ACA			$2.1\cdot10^{-3}$ mL μg^{-1}		0.5		
	Cefuroxime			$2.0\cdot10^{-3}$ mL μg^{-1}		0.8		
	Cefazolin			$7.0\cdot10^{-3}$ mL μg^{-1}		0.6		
H_2SO_4 (hydr.)	Cefazolin	302	20 min; boiling water bath	$1.3\cdot10^4$	1-10 μg mL^{-1}		Medium Sulfuric acid	[34]
NaOH (hydr.)	Cephalexin	226 first derivative	20 min, pH 7.0		0.2-0.4 mg %		Direct determ. Products basic degr. and first derivat. zero cross.	[36]
H_2SO_4 (hydr.)	Cefazolin, caps., vial, suspens.	263	20 min; boiling water bath		0.4-2.0 mg %		Direct determ. products acid degr. and second derivat.	[37]
	Cephalexin Cefotaxime	250/275 292	20 min 20 min					
$Co(NO_3)_2$, NaOH (hydr.)	Cephalexin, tablets	310	6 min; 60 ºC					[38]

Assay of degraded cephalosporins

In paper [39] different methods proposed for the analysis of cefoxitin, cefotaxime, cephalexin and cephazolin were compared. These cephalosporins have also been assayed spectrophotometrically after preliminary acid hydrolysis [34]. Acid degradation proceeds through cleavage of the side chain amide linkage. The determination of cephalexin in pharmaceutical preparations was also studied.

These products are prepared as in ref. [37]: 20 mg cephazolin, cefoxitin, cefotaxime and cephalexin, 25 mg of 7-ACA or 10 ml of water for the preparation of the blank, were transferred into 100 ml volumetric flasks using 10 ml of 4.5 mol L^{-1} sulphuric acid. The solution was heated in a boiling water bath for 20 min, then it was cooled and neutralized with 6.0 mol L^{-1} sodium hydroxide solution and made up the volume to the mark with water (or sulphuric acid 0.05 mol L^{-1} for cefotaxime). Different volumes between 0.4 and 1.8 ml of stock solutions of cephazolin, cefoxitin, cefotaxime were diluted appropriately to 50 ml. Different volumes between 0.2 and 1.6 ml of the stock solutions of cephalexin and 7-ACA, respectively, or 5 ml of the blank solution were diluted to 25 ml. The absorbance was recorded between 210 and 400 nm against distilled water.

For cephazolin, cefotaxime and cephalexin the precision achieved by intact or acid degraded UV-vis spectrophotometry and HPLC was good and similar. Worse precision was obtained by iodometric method. The USP iodometric method provided the following contents for three replicates 106 ± 7 % for cephazolin, 105 ± 10 % for cefoxitin and 107 ± 10 % for cefotaxime. The precision obtained when these methods are applied to the determination of cephalexin in different pharmaceutical sample was similar to that obtained for cephalexin standard. The best accuracy was obtained then measures made with spectrophotometer of diodes using H-point Standard Additions Method (HPSAM). HPSAM permit the determination of cephalosporins in

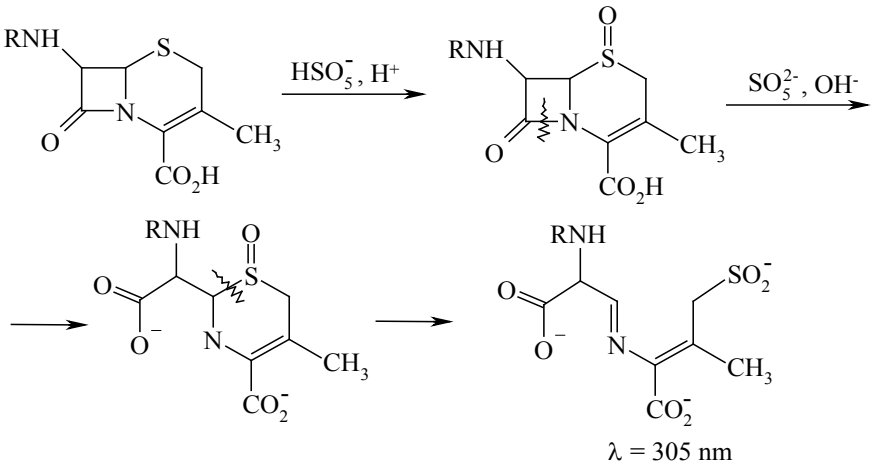
the presence of degraded products. Also, degraded cephalosporins spectra present more significance difference than intact drugs spectra.

The kinetic spectrophotometric method

A new, simple, and sensitive kinetic spectrophotometric method is described for analysis of cefadroxil by measurement of its absorbance at 470 nm after hydrolysis with NaOH at 80 °C. Studies of the method's precision and accuracy gave a standard deviation of 0.44 μg mL^{-1} and a relative standard deviation of 1.93 %. The method determines cefadroxil over the concentration range 10-100 μg mL^{-1}. Calculated activation parameters were 12.42, 25.54, 26.25, and 69.00 Kcal/mole for $\Delta E^{\#}$, $\Delta G^{\#}$, $\Delta H^{\#}$, and $\Delta S^{\#}$, respectively. Cefadroxil concentrations can be determined from the regression line equation y = 0.627x - 8.14 x 10^{-5}, with a correlation coefficient of < 1.0000. The method was validated by assay of cefadroxil in commercial capsules and tablets. Recoveries of common excipients were 99.6, 100.00, 99.2, 99.6, and 100.4 % for lactose, glucose, starch, sucrose, and fructose, respectively. Results were compared with those of a reference method. Calculated *t* and *F* values indicate no significant difference between the 2 methods. The degradation products of cefadroxil do not affect determination of cefadroxil [40].

The kinetic spectrophotometric method, based on the on the couple reactions of drug S-oxidation and perhydrolysis also has applied to the cephalosporins.

A simple, reliable, and sensitive kinetic spectrophotometric method was developed for determination of Cephalexin monohydrate. The method depends on couple reactions: Cephalexin S-oxidation with potassium peroxomonosulfate and alkali hydrolysis by peroxomonosulfate (Scheme 4). The reaction is followed spectrophotometrically by measuring the rate on the change of absorbance at 305 nm (Fig. 2).



Scheme 4. Mechanism of Cephalexin chemical transformations by means of potassium peroxomonosulfate and alkali.

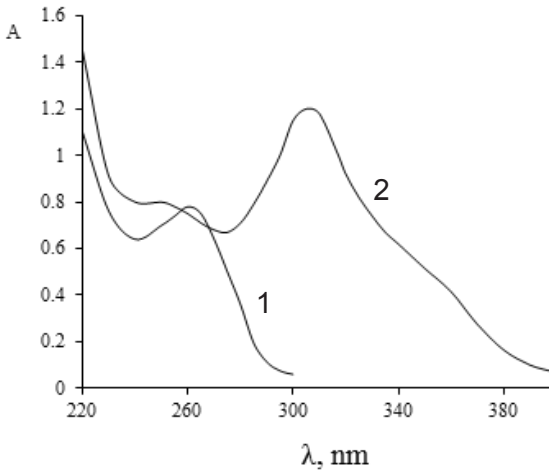


Fig. 2. Electronic spectra of Cephalexin absorption (1) and product of conjugated reactions of S-oxidation and perhydrolysis with potassium peroxomonosulfate (2). c(Cph) = 1·10^{-4} mol L^{-1}; c($KHSO_5$) = 2·10^{-4} mol L^{-1}; c(NaOH) = 0.01 mol L^{-1}.

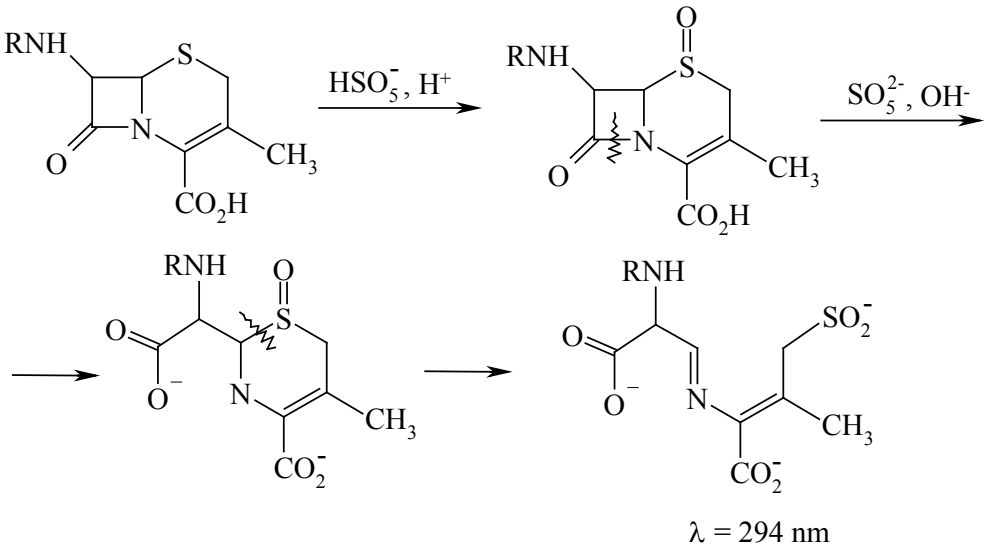
The optimum operating conditions regarding reagent concentrations and time were established. The tangent method is adopted for constructing the calibration curve for determination of Cephalexine concentration in pure powder, which was found to be linear over the concentration range 1–16 μg mL^{-1}. The optimized conditions yielded a calculated limit of quantitation (*LOQ*) 1.0 μg mL^{-1}. The standard method has been applied for the determination Cephalexine concentration in pharmaceutical preparation "Cefalexin" 250 mg to 5 mL. The mean recovery ranges from 100.00 to 104.48 % with RSD = 2.00 % (pure power); from 98.49 to 103.77 % with RSD = 2.17 % (preparation) [41].

A simple, reliable and sensitive kinetic spectrophotometric method was developed for determination of cefadroxil in pure substance and dosage form by the product of two conjugate reactions of peroxoacidic oxidation and perhydrolysis in alkali medium using potassium peroxomonosulfate as analytical reagent ($KHSO_5$) (Scheme 4). The initial rate method was used at 294 nm.

0.39 g (precise weight) of Cefadroxil was placed in a 100 mL volumetric flask, diluted to the mark with double distilled water. Then, 1 mL of this solution was further diluted to 100 mL with double distilled water at 20° C. 10.00 mL of obtained solution was introduced into 100 mL volumetric flask containing 4 mL of 0.02 mol L^{-1} $KHSO_5$ solution and 4 mL of 0.51 mol L^{-1} NaOH solution. The content was shaken and finally diluted to the mark with double distilled water. After the NaOH solution was added the stopwatch was switched on. The obtained solution was transferred to a 1 cm cell to measure the absorbance at the wavelength 294 nm during first 15 min every 2 min against water. A kinetic dependence curve of absorbance A against time, min was obtained. The calculation was performed using the initial rate method (tangent method). Five replicate estimaytions were done in similar way.

Linearity was studied over concentration range 1-7 μg mL^{-1} and correlation coefficient was found to be 0.999 for regression line. The proposed method was validated statistically and checked through recovery studies. Statistical comparisons of the results with the reference methods show excellent agreement and indicate no significant difference in accuracy and precision [42].

The fixed-time kinetic method is used for the determination of cefazolin in pure substance and cefazolin sodium in powder for injection by measurement of the yellow typical products at 305 nm from the hydrolytic degradation of the corresponding cefazolin S,S'-dioxide with potassium peroxomomsuphate in alkali medium. Under optimal conditions ($c(KHSO_5) = 4 \cdot 10^{-4}$ mol L^{-1} and $c(NaOH) = 2.4 \cdot 10^{-2}$ mol L^{-1}, T = 299 K) dependence of conditional reaction rate on the concentration of cefazolin in the range of 1.6–26 mg mL^{-1} is described by the equation: $A_{20} = 0.016 + 0.01$ (R = 0.99). Linear calibration dependence gave reason to the analysis by the standard method. Limit of Quantification (LOQ) for cefazolin sodium was found to be 1.6 μg mL^{-1}. The relative standard deviation as a result of the determination of cefazolin in substance by calibration graph was 2.05 % (accuracy, $\delta = 0.46$ %), in cefazolin sodium powder for injection by method of standard 2.06 % (accuracy, $\delta = 1.42$%) [43].



Scheme 5. Mechanism of Cefadroxil chemical transformations by means of potassium peroxomonosulfate and alkali.

Assay cephalosporins by fluorescent methods

Intensely fluorescent products were formed from cephalexin and cephradine in acidic solution containing hydrogen peroxide by hydrolysis at high temperature. A fluorescent product was formed from cephatrizine in solutions containing mercuric chloride by warming at 60 degree for 50 min after cleavage of the beta-lactam ring with 1 mol L^{-1} NaOH. Each fluorescent product was readily extracted with ethyl acetate or an acetone-chloroform mixture from neutral solutions and could be partitioned into alkaline solution. The urinary excretion of the antibiotics in human volunteers after oral administration was studied using the new methods [44].

Omar et al., [45] developed kinetic spectro-fluorimetric method for the determination of cephalexin, cefotaxime and cefuroxime. The method was based on their degradation under an alkaline condition producing fluorescent products. The reaction was followed spectrofluorimetrically by measuring the rate of change of fluorescence intensity at specified emission wavelength. The initial rate and fixed time methods were used for the construction of calibration graphs to determine the concentration of the studied drugs. The calibration graphs were linear in the concentration ranges 0.2–1.2 and 0.2–2.2 μg mL^{-1} using the initial rate and fixed time methods, respectively.

An accurate, reliable, specific and sensitive kinetic spectrofluorimetric method was developed for the determination of seven cephalosporin antibiotics namely cefotaxime sodium, cephapirin sodium, cephradine dihydrate, cephalexin monohydrate, cefazoline sodium, ceftriaxone sodium and cefuroxime sodium. The method is based on their degradation under an alkaline condition producing fluorescent products. The factors affecting the degradation and the determination were studied and optimized. The reaction is followed spectrofluorimetrically by measuring the rate of change of fluorescence intensity at specified emission wavelength. The initial rate and fixed time methods were used for the construction of calibration graphs to determine the concentration of the studied drugs. The calibration graphs are linear in the concentration ranges 0.2–1.2 μg mL^{-1} and 0.2–2.2 μg mL^{-1} using the initial rate and fixed time methods, respectively. The results were statistically validated and checked through recovery studies.

The method has been successfully applied for the determination of the studied cephalosporins in commercial dosage forms. The high sensitivity of the proposed method allows the determination of investigated cephalosporins in human plasma. The statistical comparisons of the results with the reference methods show an excellent agreement and indicate no significant difference in accuracy and precision [46].

A selective, highly sensitive fluorimetric method is described for the determination of three i aminocephalosporins, namely cephalexin, cefaclor and cephradine. Other cephalosporins free from the amino group did not interfere with the assay. The proposed method involves acid-hydrolysis of the drugs and subsequent alkalinization before measurement. The different experimental parameters affecting the fluorescence intensity were carefully studied and incorporated into the procedure. The method permits the determination of 0.02–0.40, 0.04–0.60 and 0.08–0.80 μg mL^{-1} for cephalexin, cefaclor and cephradine respectively with minimum detectability of 0.2 ng mL^{-1} for all cephalosporins. The proposed method was successfully applied to the determination of these drugs in formulations and biological fluids. The advantages of the described method over other existing methods were discussed.A route for the reaction pathway proposed [47].

A polarographic method assay of cephalosporins

The most frequently used method for the determination of penicillins and cephalosporins, especially in biological fluids, is high-performance liquid chromatography, but polarographic have also been successfully applied [48–50].

Comparison of polarographic with microbiological and iodometric analytical procedures in terms of precision, accurancy and selectivity showed the advantage of the polarographic method in all there aspects.

As was indicated in [51] cephalosporins which do not bear at C-3 a good leaving group, also do not contain a reducible grouping in the side chain at C-7 and have been considered unreducible. Attemps have been made to determine such compounds after an acid or base catalyzed hydrolysis. Thus, cephalexin yields an unidentified reducible product after acid hydrolysis in 5 mol L^{-1} HCl at 80 ºC for 15 min [52]. When the hydrolysis of this compound was carried out in a phosphate buffer pH 7.4 at 80 ºC for 1 hour, the hydrolysis product is reduced at –1.26 V. It is assumed that the reducible degradation product is 3-hydroxy-4-methyl-2(5H)-thiophenone [53]. Carrying out the hydrolysis in 0.1 mol L^{-1} NaOH at 100 ºC for 1 hour resulted in a more extensive degradation. The electroactive species is assumed to contain grouping CH=N–C(COOH)=C, and the reduction at the −0.84 V(SCE) in 0.2 mol L^{-1} NaOH is attributed to the C=N bond, that at −1.34 V to the reduction of the ethylenic bond [54]. Cephalexin, which contains an unsubstituted 3-methyl group, but no reducible group, does not give a reduction peak at a dropping mercury electrode, whereas its degradation product does. The degradation of cephalexin was carried out in 0.1 mol L^{-1} NaOH at 100°C for 20 min. A sensitive reduction peak of cephalexin was obtained by adsorptive stripping voltammetry in 0.1 mol L^{-1} NaOH at an accumulation time of 60 s. The peak potential is −0.80 V (vs. Ag/AgCl). The peak current is directly proportional to the concentration of cephalexin, with a detection limit of $5.0 \cdot 10^{-10}$ mol L^{-1}. The electrochemical

behaviour of cephalexin was studied. The reduction process is quasi-reversible with adsorptive characteristics. The mechanisms of degradation and electrode reaction are discussed [54].

In similar way it is possible to determine cepharadine, either after hydrolysis in 5 mol L^{-1} HCl [55]. A polarographic method has been developed for the quantitative analysis of cephradine and its dosage forms. Direct determinations on capsules are carried out; excipients and coloring matter do not interfere in the determination. The electroactive product is formed by acidic hydrolysis with 5.0 mol L^{-1} HCl and heating at 80 degrees C for 60 min. Two polarographic waves are obtained: I = −0.46 V and II = −0.78 V vs. SCE. Both reduction waves are diffusion controlled. Wave I is preferred for analytical purposes. The precise chemical identity of the electroactive product has not been determined, but UV spectral data and the TLC R_f value are reported. A linear relation is established for levels of cephradine between 10^{-2} and 10^{-5} mol L^{-1} in 5.0 mol L^{-1} HCl [55].

The degration of cephradine was also carried out in NaOH at 100℃ for 20 min. In a supporting electrolyte of 0.3 mol L^{-1} NaOH, a sensitive reduction peak of cephradine was found by single-sweep oscillopolarography.The peak potential is −0.84 V (vs. SCE).The relationship between the peak current and the concentration of cephradine is linear in the range of 2.0×10^{-8}–$5.0\cdot10^{-6}$mol L^{-1} (R = 0.9995) and the detection limit is $5.0\cdot10^{-9}$ mol L^{-1}.The electrochemical behaviour of cephradine at Hg electrode is studied. The parameters of electrode reaction are discussed. The reduction process is quasi-reversible with adsorptive characteristics [56].

Cephalexin gives a reduction wave in 0.03 mol L^{-1} HCl medium at ca. −1.24 V. With cephalexin concentration higher than $2.5\cdot10^{-5}$ mol L^{-1}, another reduction wave is observed at ca. −0.90 V. These reduction waves are attributed to the reduction of ethylenic bond of a six-membered dihydrothiazine ring. When H_2O_2 is present, the reduction wave at ca. −0.90 V is catalyzed by H_2O_2 and its reduction intermediate hydroxyl radical, producing a catalytic wave. However, the reduction wave at ca. −1.24 V remains nearly unchanged. A sensitive polarographic method for the determination of cephalexin is proposed based on the reduction wave of cephalexin. The second-order derivative peak current of the wave at ca. −1.24 V is rectilinear to the cephalexin concentration in the range $1.0\cdot10^{-7}$ to $2.5\cdot10^{-5}$ mol L^{-1}, and the detection limit is $5.0\cdot10^{-8}$ mol L^{-1}. The proposed method is applied to the individual tablet dosage form and human serum [57].

In this paper we present a new electroanalytical method for determination of ceftiofur based on the hydrolysis of this antibiotic in 0.04 mol L^{-1} Britton–Robinson buffer at pH 10 and 60 °C for 60 min (reduction peak at −0.70 V). Conditions were optimized for complete hydrolysis and quantitative determination of ceftiofur in milk. The method can be successfully used for determination of the antibiotic directly from samples of fluid milk and powder milk spiked to concentrations of $6.0\cdot10^{-8}$, $8.0\cdot10^{-8}$, and $10\cdot10^{-8}$ mol L^{-1}, respectively. Recovery test ranged from 98.28 % to 100.83 %. The limits of detection and quantification were $3.73\cdot10^{-10}$ and $1.24\cdot10^{-9}$ mol L^{-1}, respectively. The method has the advantage of eliminating interference from proteins present in the sample, thus obviating the need for exhaustive extraction, which often renders other procedures unfeasible in terms of time and reagent cost. In addition, waste generation was found to be lower than in other methods [58].

The differential pulse polarography (d.p.p.) of several cephalosporins – cephalothin, cephalosporin C, cephaloridine, cephalonium, cefuroxime, cephoxazole, cephalexin, cephradine, desacetyl-cephalosporin C, 7-aminocephalosporanic acid and 7-aminodesacetoxy-cephalosporanic acid – and some degradation products has been studied. Cephalexin, cephradine and 7-aminodesacetoxycephalosporanic acid, which contain an unsubstituted 3-methyl group but no reducible group, do not give d.p.p. peaks at the dropping mercury electrode, whereas certain of their degradation products do. The other cephalosporins, in which the 3-methyl group is substituted, give a d.p.p. peak at about −1 V (pH 2–4). Although these peaks are near the cathodic limit under the conditions used, these cephalosporins can be determined down to about 0.1 μg mL^{-1}. Cephaloridine exhibits two d.p.p. peaks in this region, and can be determined. Cefuroxime, in addition to the peak at −IV, has a larger peak at −0.45 V (pH 2). Cephalonium shows a large well-defined peak at −0.72 V (pH 3) and two overlapping peaks. D.p.p. data for several degradation products including the diketopiperazines formed from cephalexin and cephradine are reported [59].

Differential pulse polarography (d.p.p.) is used to study the degradation of cephalexin. Hydrogen sulphide, evolved during the degradation of cephalexin solutions, was removed continuously in a stream of nitrogen and determined periodically. Other electroactive degradation products were observed by d.p.p. of the degraded sample solutions. The degradation mechanism is highly dependent on pH, the initial concentration of cephalexin, temperature, the particular buffer used, and the presence of dissolved oxygen. The formation and degradation of the diketopiperazine derivative formed by intramolecular aminolysis, particularly at neutral pH, can be followed by means of its polarographic peak at −0.9 V (pH 7.4). Approximately half the total sulphur originally present in cephalexin is liberated as hydrogen sulphide at pH 7.4 at 37 °C. Increasing the degradation temperature to 80 °C and sweeping out the hydrogen sulphide with nitrogen increases the yield of a major product which gives a peak at −1.26 V. At pH 8.5 (80 °C. 100 μg cephalexin mL^{-1}) the percentage of the sulphur evolved as hydrogen sulphide increases with time,

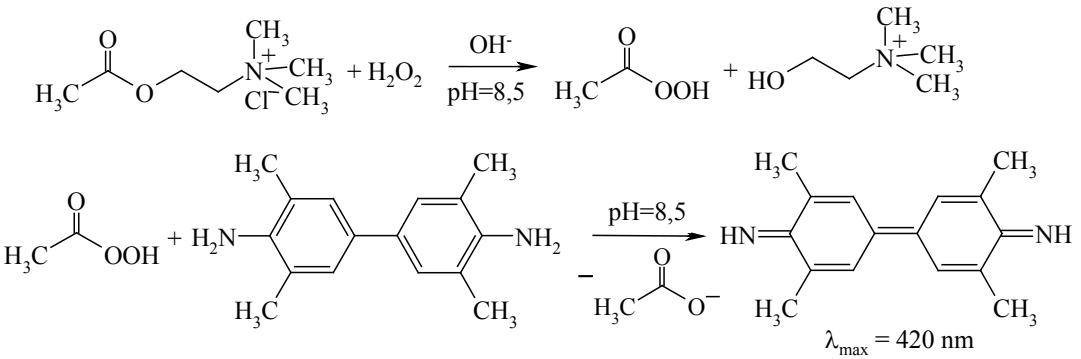
and a peak appears at −0.96 V (probably 2-hydroxy-3-phenyl-6-methylpyrazine) which increases as the peak at −1.26 V becomes smaller. Other products formed under different conditions (concentration, pH, temperature) are reported. At pH 3 (80 °C) only 8 % conversion via intramolecular aminolysis and 5 % evolution of total sulphur is indicated after four hours [60-61].

Cefaclor is not reducible at a mercury electrode, but it can be determined polarographically and by cathodic stripping voltammetry as its initial alkaline degradation product which is obtained in high yield by hydrolysis of cefaclor in Britton-Robinson (B-R) buffer pH 10 at 50 degrees C for 30 min (reduction peak at pH 10, −0.70 V). Differential pulse polarographic calibration graphs are linear up to at least $1 \cdot 10^{-4}$ mol L^{-1}. Recoveries of 93 % of the cefaclor (n = 3) were obtained from urine spiked with 38.6 μg mL^{-1} using this polarographic method with 1 ml urine made up to 10 ml with pH 10 buffer. Using cathodic stripping voltammetry and accumulating at a hanging mercury drop electrode at −0.2 V for 30 s, linear calibration graphs were obtained from 0.35 to 40 μg mL^{-1} cefaclor in B-R buffer pH 10. Arelative standard deviation of 4.2 % (n = 5) was obtained, and the limit of detection was calculated to be 2.9 ng mL^{-1}. Direct determination of cefaclor in human urine (1 ml of urine was made up to 10 ml with pH 10 buffer) spiked to 0.39 μg mL^{-1} was made (recovery 98.6 %) [62].

Electrooxidation of cefadroxil monohydrate was investigated using a glassy carbon electrode depending on pH and supporting electrolyte. It was shown that the determination of the substance from capsules and in oral suspension could be made by differential pulse voltammetry. The results confirm the suitability of the proposed method for the accurate and sensitive analysis of cefadroxil monohydrate. The DPV and spectrophotometric results were compared to those of official high performance liquid chromatographic (HPLC) methods by means of Student's t-test at the 95 % confidence level, and no significant difference was found between them [63].

Assay of esters by kinetic method with use perhydrolysis reactions

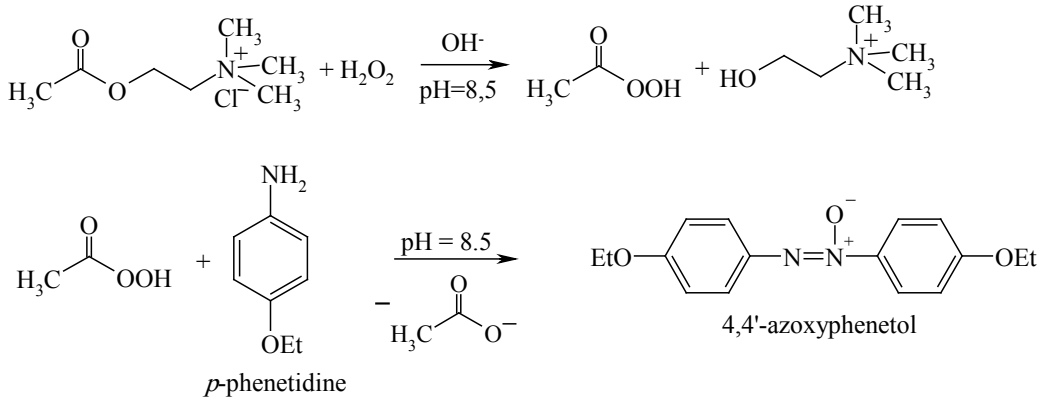
A kinetic spectrophotometric method fixed-time at 10 min has been developed for the determination of Acetycholine Chloride (ACh) using the indicator reaction of catalytic 3,3',5,5'-Tetramethylbenzidine (TMB) oxidation by hydrogen peroxide at pH 8.5 in model solutions and pure substance (Scheme 6). Calibration graph for Acetycholine Chloride has linear dependence in the range 20–100 μmol L^{-1} ($A_{15} = (9.75 \pm 0.66) \cdot 10^2 \cdot c$ ($R = 0.998$), were c, mol L^{-1}) with a limit of quantitation (LOQ) of 2 μmol L^{-1} ACh. For five determinations of 40 μmol L^{-1} and 80 μmol L^{-1} Acetycholine Chloride the reproducibility has RSD of 2.89 % and 2.02 % respectively. The proposed method is more sensitive, simple and expresses in comparance with the well-known one. Acetylcholine Chloride substance contains 98.8 ± 2.5 % of $C_7H_{16}ClNO_2$ [64].



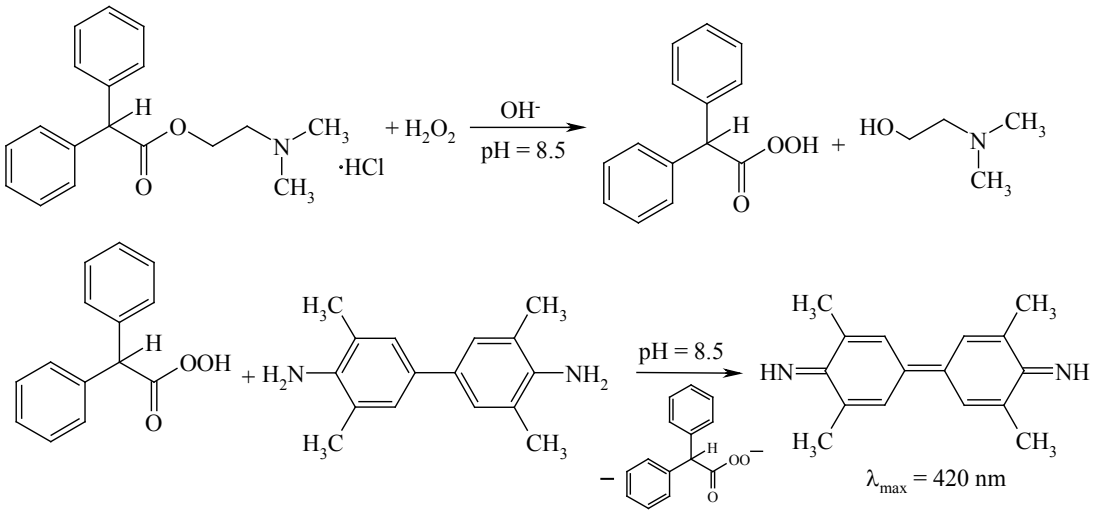
Scheme 6. Chemistry of Acetylcholine perhydrolysis and coupled TMB peroxyacid oxidation.

A new kinetic-spectrophotometric method tangent for the quantitative determination Acetycholine Chloride of the pure substance by indicator reaction of *p*-phenetidine with hydrogen peroxide was proposed (Scheme 7) [65]. Linearity was studied over concentration range $(1\text{-}35) \cdot 10^{-5}$ mol L^{-1} and correlation coefficient was found to be 0.99 for regression line. For series of Ach concentrations ranging from 4 – 40 μg mL^{-1} RSD was below 2.0 %.

A kinetic spectrophotometric method has been developed for determination of alkaloid Spasmolitine using the indicator reaction of catalytic 3,3',5,5'-tetra-methylbenzidine oxidation by hydrogen peroxide at pH 8.5 in pure substance (Scheme 8). Calibration graph for Spasmolitine has linear dependence in the rage 0.01-0.15 mg mL^{-1}, LOQ is $3.9 \cdot 10^{-5}$ mol L^{-1} (13 μg mL^{-1}). At Spasmolitine concentration 0.4 mmol L^{-1} determinations the reproducibility has RSD ≤ 3.33 (δ = -0.68 %). The propose method is more sensitive, simple and expresses in comparance with the well famous one. Recovery - 99.30 ± 4.05 % of $C_{20}H_{25}NO_2$, HCl [66].



Scheme 7. Chemistry of Acetycholine perhydrolysis and *p*-phenetidine coupled peroxyacid oxidation.



Scheme 8. Chemistry of *Spasmolitine* perhydrolysis and coupled TMB peroxyacid oxidation.

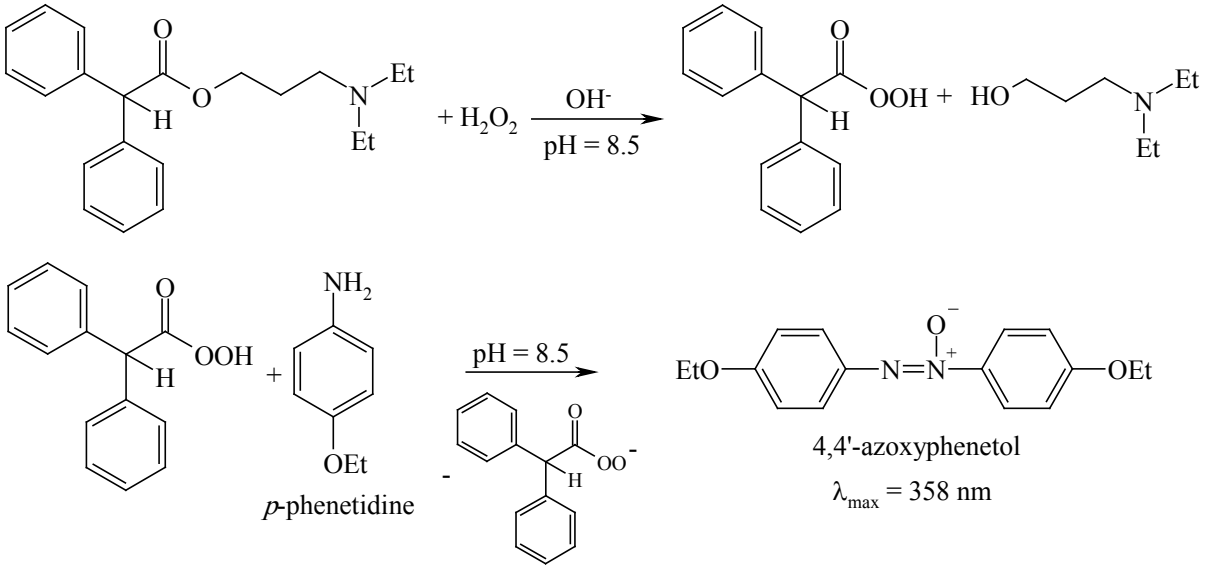
A kinetic spectrophotometric method has been developed for the determination of Arpenal (AP) using the indicator reaction of catalytic *p*-Phenetidine oxidation by hydrogen peroxide at pH 8.5 in model solutions and tablets "Arpenal" 0.05 g. It is based on the system of two coupled reaction: Arpenal perhydrolysis (reaction with excess of H_2O_2 in a weak alkaline medium with diphenyl *peracetic* acid formation) and following *p*-phenetidine (*p*-Ph) oxidation by *in situ* generate diphenyl *peracetic* acid to 4,4'-azoxyphenetole (4,4'-Bis(ethoxy)azoxybenzene) (λ_{max} = 358 nm, lgε = 3.8) (Scheme 9). Its increasing absorbance allows to determine AP. Kinetic spectrophotometric initial rate method was used for computing. Calibration graph for Arpenal has linear dependence in the range $8.6 \cdot 10^{-2} - 6.8 \cdot 10^{-1}$ mg mL^{-1} with LOD and LOD of $2.5 \cdot 10^{-2}$ and $7.6 \cdot 10^{-2}$ mg mL^{-1} Arpenal respectively. For five determinations of 0.342, 0.428 and 0.514 mg mL^{-1} Arpenal the reproducibility has a RSD of 1.91, 1.64 and 1.25 % respectively. AP tablets contains 100.5 ± 2.24 % of $C_{21}H_{27}NO_2 \cdot HCl$. The proposed kinetic spectrophotometric method proved to be selective, simple and rapid (single analysis time does not exceed 10 min) for the quantitative determination of Arpenal in the presence of it hydrolytic cleavage products [67].

In general, Acetylsalicylic acid (ASA) analysis is not realised directly, and a previous quantitative hydrolysis in a basic medium is necessary, converting *acetylsalicylic acid* to salicylate ions for its determination. The hydrolysis is carried out by sodium hydroxide solution.

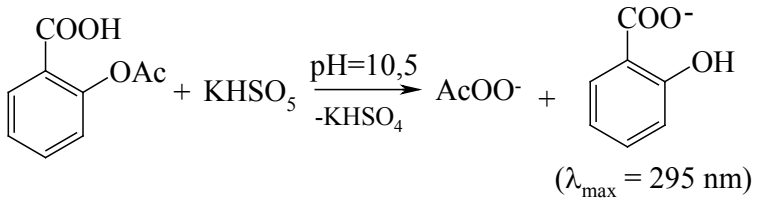
The new spectrophotometric method for the determination of acetylsalicylic acid in pharmaceutical formulations was proposed [68]. It is based on a ligand-exchange reaction. The reaction was followed spectrophotometrically by monitoring the rate of disappearance of the cobalt(II)-1-nitroso-2-naphthol complex in alkaline medium at 410 nm. The optimum operating conditions regarding reagent concentrations and temperature were established. The initial-rate method is adopted for constructing the calibration curve, which was found to be linear over the concentration range 0.72–9.00 μg mL^{-1}. The optimized conditions yielded a theoretical detection limit of 0.35 μg mL^{-1} based on the 3.3S criterion. The interference effects of certain ingredients of powdery drugs, foreign ions and amino acids upon the reaction rate were studied in order to assess the selectivity of

the method. The results are validated statistically and through recovery studies. The point hypothesis test have been performed which indicate that there is no significant difference between the proposed method and the reference method. The developed procedure was successfully applied to the rapid determination of acetylsalicylic acid in commercial pharmaceutical preparations and human control serum. The unique features of this procedure are that determination can be carried out at room temperature and analysis time is short. The newly developed method is simple, inexpensive and efficient for use in the analysis of a large number of samples.

A new kinetic-spectrophotometric method for assay of aspirin, based on the use perhydrolysis reaction by means of potassium peroxymonosulfate as a nucleophilic reagent converting ASA to salicylate ions for its determination (Scheme 10).



Scheme 9. Chemistry of Arpenal perhydrolysis and *p*-phenetidine coupled peroxyacid oxidation.



Scheme 10. Perhydrolysis reaction of aspirin by means of potassium peroxymonosulfate.

The reaction was followed spectrophotometrically by monitoring the rate of appearance salicylate at 295 nm. Tangents method of differential variant of kinetic method analysis was used. The optimum reaction conditions for the perhydrolysis of aspirin with maximum catalytic activity of potassium peroxymonosulfate observed at $3.8 \cdot 10^{-3}$ mol·L^{-1} of potassium peroxymonosulfate and 10.5–11.0 pH range (Fig. 3) In this case the maximum difference in the rate of catalytic (perhydrolysis) and non-catalytic (alkaline hydrolysis) decomposition of ASA was observed and therefore to measure the indicator reaction rate with sufficient accuracy under the first-order reaction kinetics. The hydrolysis product of ASA (salicylate) is monitored at 295 nm during 10 min. This allowed us to develop a new kinetic-spectrophotometric method of aspirin assay in pure substance. It based on an experimentally determined kinetic features of the perhydrolysis reaction.

Calibration graph for aspirin under these conditions was obtained: $tg\alpha = (96.9 \pm 8.3) \cdot c - (0.003 \pm 0.001)$, ($R = 0.999$). It has linear dependence in the range 20–200 μmol L^{-1} of aspirin concentration. From these data a limit of quantitation (LOQ) of 20 μmol L^{-1} was calculated. For five determinations of 8, 12 and 16 μmol·dm^{-3} aspirin the reproducibility has a RSD of 2.36 %, 1.57 % and 0.99 % respectively. Method has satisfactory accuracy ($\delta \leq -0.1$ %). Aspirin substance contains 99.97 ± 0.01 % of acetylsalicylic acid [69] The proposed method does not require the use of toxic solvents or reagent and sophisticated equipment. It is more sensitive, simple and more quickly in comparison with well-known one.

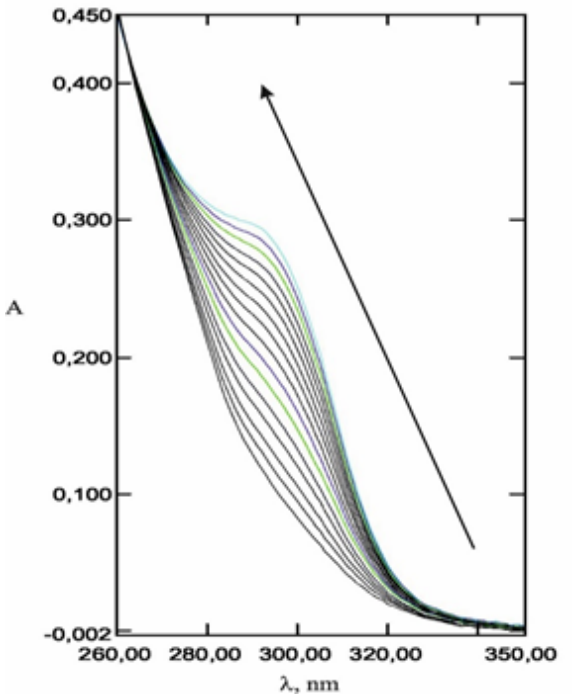
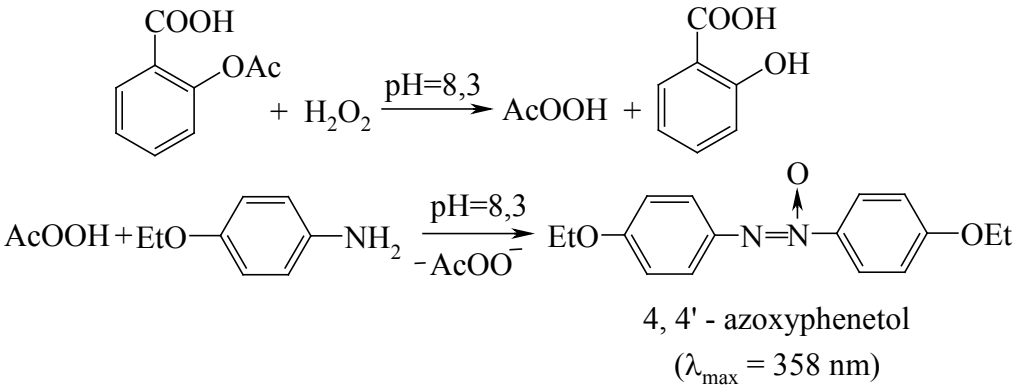


Fig. 3. Electronic spectra of product perhydrolysis of *DL*-lysine acetylsalicylate, obtained by means of potassium peroxomonosulfate (for 30 minutes, every 2 minutes, starting at 30 seconds). c(*DL*-lysine acetylsalicylate) = 28.8 μg mL^{-1}; c($KHSO_5$) = $3.6 \cdot 10^{-3}$ mol L^{-1}; pH = 10.5.

A new also kinetic-spectrophotometric method for assay of acetylsalicylic acid in model solutions and drug "ACELYSINE-KMP" was described. It based on the system of two coupled reaction: acetylsalicylic acid perhydrolysis (reaction with excess of H_2O_2 in weakly alkaline medium to peroxyacetic acid formation) and following *p*-phenetidine oxidation by newly generate peroxyacetic acid to 4,4'-azoxyphenetole (λ_{max} = 358nm), increasing absorbance of it allow to determine acetylsalicylic acid (Scheme 11). Calibration graph for acetylsalicylic acid has linear dependence in the range 22–180μmol L^{-1} with a limit of quantitation (LOQ) of 12μmol L^{-1} acetylsalicylic acid. Calibrating graph equation was find out: tgα=(325.5 ± 21.4) c + (0.0004 ± 0.002) (r = 0.998), where c – molar concentration, mol L^{-1}.

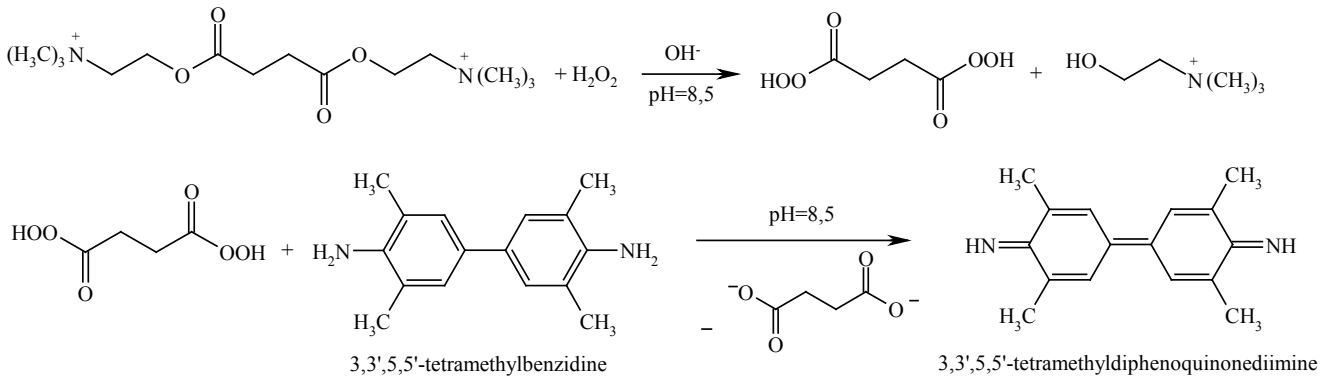
For five determinations of 44 μmol L^{-1}, 88 μmol L^{-1} and 130 μmol L^{-1} ASA the reproducibility has a RSD of 1.18, 1.06 and 0.76 % respectively. The proposed method is more sensitive, simple and more quickly in comparison with the well-known one. Drug "ACELYSINE-KMP" contains 98.95 ± 1.32% of acetylsalicylic acid. Compresent components glycine, lysine and potential hydrolytic cleavage products are not interfering on the acetylsalicylic acid determination [70].

A new kinetic spectrophotometric procedure for the determination of acetylsalicylic acid in micellar medium, either in pure form or in film-coated tablets Cardiomagnil® 75 mg is proposed. The method is based on the indicator reaction of catalytic 3,3',5,5'-tetramethylbenzidine oxidation by generate peracid formed from the acetylsalicylic acid perhydrolysis with excess of hydrogen peroxide in the presence of nonionic surfactant Triton X-100 to give colored 3,3',5,5'-tetramethyldiphenoquinone diimine derivative (λ_{max} = 427nm). The analytical performance of the methods was validated statistically with respect to LOD, LOQ, accuracy, precision and linearity. Proposed method has been successfully applied to the commercial dosage form with the recovery 98.9% and the RSD below 2.0 % [71].



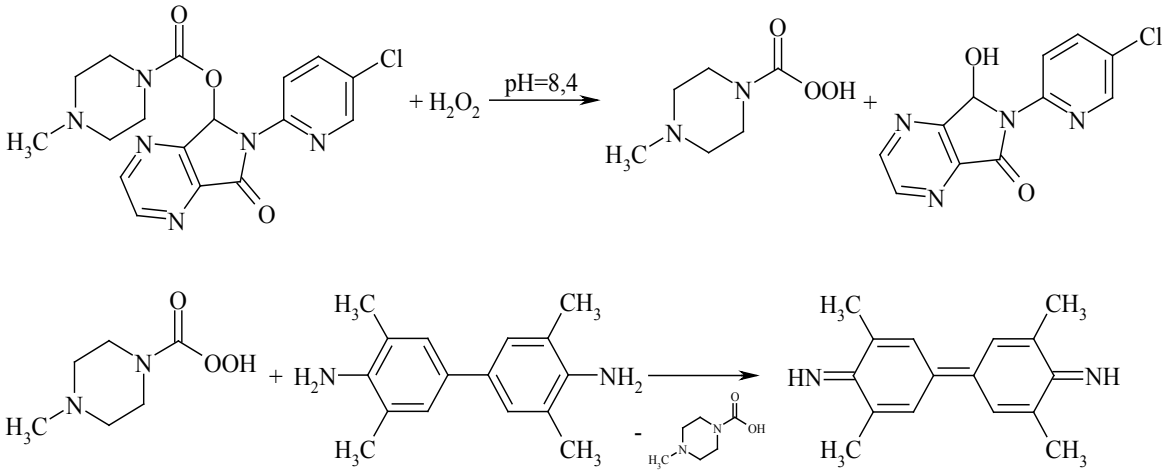
Scheme 11. Chemistry of acetylsalicylic acid perhydrolysis and coupled *p*-phenetidine peroxyacid oxidation.

A novel selective, simple, sensitive, and cost effective kinetic-spectrophotometric method initial rate method for the determination of Suxamethonium (SUX) chloride has been proposed. It is based on the indicator reaction of catalytic 3,3',5,5'-tetramethylbenzidine oxidation (Scheme 12). The analytical performance of the method was validated with respect to LOD, LOQ, accuracy, precision, and linearity for SUX estimation in pure substance and the results were satisfactory. Compared to the reference method, pharmaceutical formulation Lysthenon® contains $0.102 \pm 2.0 \cdot 10^{-3}$ g of $C_{14}H_{30}N_2O_4C_{l2}$, relative to the anhydrous basis (RSD = 1.6 %; δ = -0.3 %) [72].



Scheme 12. Chemistry of SUX perhydrolysis and coupled TMB peroxyacid oxidation.

A simple and express method for the quantitative determination of zopiclone in model solutions of the substance and in "Zopiclone" tablets, 7.5 mg, by the kinetic-spectrophotometric method according to 3,3',5,5'-tetramethylbenzidine oxidation has been developed. It is based on the system of two coupled reaction: 4-methyl-1-piperazineperoxycarboxylic acid generated in zopiclone perhydrolysis reacts with the excess of hydrogen peroxide in the weak alkaline medium with formation of coloured 3,3',5,5'-tetr amethyldiphenylquinone diimine (λ_{max} = 420 nm, pH = 8.4) (Scheme 13). The reaction is performed spectrophotometrically by measuring the rate of change of the absorbance at 420 nm. The method was used for constructing the calibration graph. The initial rate of the reaction was obtained from the linear site of the slope of the initial tangent to the absorbance-time curve. In the pH range of 8.2-8.5 the rate of the coloured product formation becomes maximum. The calibration graph for zopiclone has a linear dependence in the range of 6-36 mg L^{-1} with the limit of detection (LOD) and quantitation (LOQ) of 1.81 and 6.04 mg L^{-1}, respectively. For five determinations of 18, 24 and 30 mg L^{-1} of zopiclone RSD is 1.81, 1.46 and 1.69 %, respectively. The analytical performance of the method was validated statistically with respect to LOD, LOQ, accuracy, precision and linearity for zopiclone estimation in a pure substance and the results were satisfactory. "Zopiclone" tablets compared to the reference method contain 99.83 ± 1.19 % of $C_{17}H_{17}ClN_6O_3$ (RSD = 0.96 %, δ = -0.17 %). The assay of zopiclone in the presence of its hydrolysis products without preliminary separation is an important advantage of the method [73].



Scheme 13. Chemistry of zopiclone perhydrolysis and coupled TMB peroxyacid oxidation.

The method for determination of *DL*-lysine acetylsalicylate in the preparation «Acelysine-KMP» (mixture of *DL*-lysine-acetylsalicylate and glycine) using kinetic-spectrofluorimetric method by the indicator reaction perhydrolysis with potassium peroxomonosulphate at pH 10.5. A fluorescence spectras of product perhydrolysis of *DL*-lysine acetylsalicylate, obtained by means of potassium peroxomonosulfate are shown in Fig. 4. Linear calibration curve in the range (0.5–14.4) mg mL^{-1} of *DL*-lysine acetylsalicylate (λ_{ex} = 298 nm/λ_{em} = 407 nm). The limit of detection (LOD) and quantitation (LOQ) was 0.15 μg mL^{-1} and 0.48 μg mL^{-1}, respectively. The content of *DL*-lysine acetylsalicylate in the preparation

«Acelisyne-KMP» was 1.0 g of (0.8957 ± 0.0205) g (RSD = 1.84 %; δ = -0.73 %). The technique allows to determine the content of the basic substance - prone to hydrolytic decomposition of *DL*-lysine acetylsalicylate - in the presence of the excipients and other drugs or its decomposition products, and is easy to practice and express [74–75].

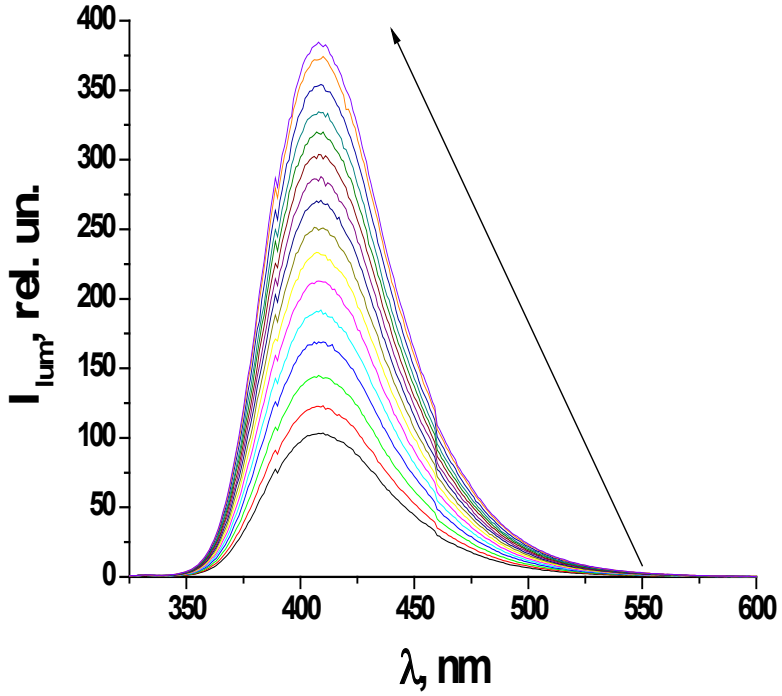
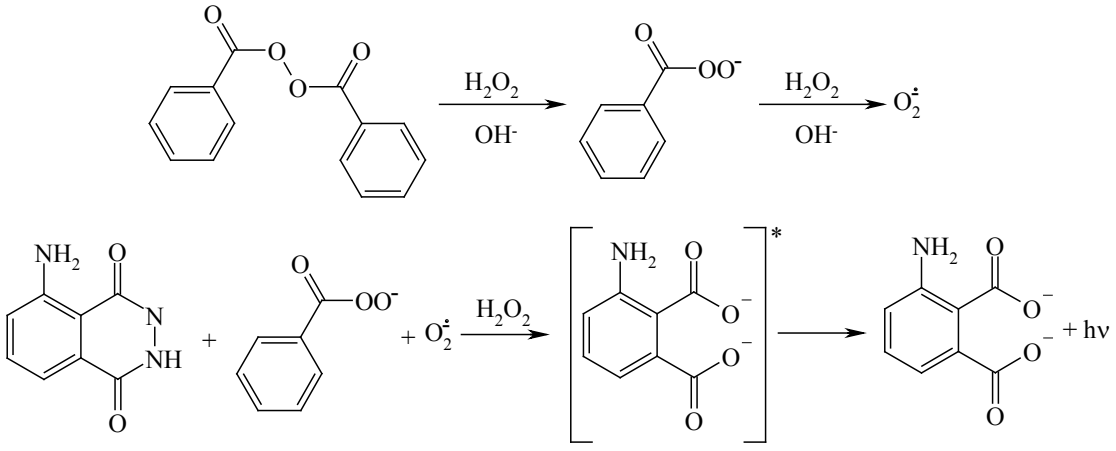


Fig. 4. Fluorescence spectra of product perhydrolysis of *DL*-lysine acetylsalicylate, obtained by means of potassium peroxomonosulfate for 30 minutes, every 2 minutes, starting at 30 seconds (λ_{ex} = 298 nm)/λ_{em} = 407 nm). c(*DL*-lysine acetylsalicylate) = 28.8 μg mL-1; c($KHSO_5$) = $3.6 \cdot 10^{-3}$ mol L^{-1}; pH = 10.5.

Rifampicin is oxidized with hydrogen peroxide to a product that is fluorescent in alkaline solutions. Maximum fluorescence is measured at 480 nm with an excitation wavelength of 370 nm. The lower limit of detection of rifampicin in water is 0.1 μg mL^{-1}. A microbiological assay with *Staphylococcus aureus* 560 as the assay organism verified the reliability of the fluorometric assay. The fluorometric and microbiological assays were applied to the estimation of rifampicin in serum [76].

A new chemiluminescence method for the quantitative determination of the pure substance and preparation content of diacyl peroxides (benzoyl peroxide) by means of the perhydrolysis reaction with use as indicator luminol oxidation reaction (RSD ≤ 2.4 %, δ = -0.39…1.3%) was developed [77] (Scheme 14).



Scheme 14. Perhydrolysis of benzoyl peroxide and coupled Luminol peroxyacid oxidation reaction.

Conclusion

The kinetic spectrophotometric, fluorimetric and voltammetric methods can be easily applied for indirect determination by means of derivatization by peroxy acid oxidation and perhydrolysis (with *peroxy* acid *oxidation* before *alkali hydrolysis)* of the investigated drugs (antibiotics (penicillins and cephalosporins), esters and etc) in pure and dosage forms that do not require elaborate treatment and tedious extraction of chromophore produced. The proposed methods with use peroxy acid and oxone as derivative agents for obtain of derivatives is sensitive enough to enable determination of lower amounts of drug, these advantages encourage the application of proposed method in routine quality control of investigated drugs in industrial laboratories. Finally, method provides advantages of improving selectivity and accuracy, avoiding interference of colored and/or turbidity background of samples because it measures the increase in analytical signal (absorbencies, emission) with time against blank treated similarly and at strong dilution.

References

1. Board NIIR Drugs & Pharmaceutical Technology Handbook. Asia Pacific Business Press Inc.: Delhi, India, 2004. 636 p.
2. Page M.I. The Chemistry of *β*-Lactams. Springler Science and Business Media, 2012. 351 p.
3. Mary G. P. K. S. A theoretical study of biologically important molecules (Doctoral dissertation), 2003.
4. Mohammed O.Y. Optimization of Ampicillin oxidation reaction with hydrogen peroxide and potassium dichromate in different media. *Int. J. Chem. Tech. Res.* 2015, 8(4), 1689-1694.
5. Belal F., El-Kerdawy M. M., El-Ashry S. M. and El-Wasseef D. R. Kinetic spectrophotometric determination of ampicillin and amoxicillin in dosage forms. *Farmaco*. 2000, 55(11-12), 680-686.
6. Blazheyevs'kyy M.Ye., Karpova S.P. Kil'kisne vyznachennya natriyu benzylpenitsylinu u poroshku dlya pryhotuvannya rozchynu dlya in"yektsiy. *Visnyk farmatsiyi*, 2011, no 2, pp. 70-74 (Ukr.).
7. Blazheyevs'kyy M.Ye., Karpova S.P. Kil'kisne vyznachennya natriy ampitsylinu u poroshku dlya pryhotuvannya rozchynu dlya in"yektsiy kinetychnym metodom za reaktsiyeyu perhidrolizu. *Visnyk farmatsiyi.* 2009, no. 3 (59), pp.16-19 (Ukr.).
8. Blazheyevs'kyy M.Ye., Karpova S.P. Kil'kisne vyznachennya ampitsylinu tryhidratu kinetychnym metodom. *Visnyk farmatsiyi*. 2010, no. 1 (61), pp. 23-26 (Ukr.).
9. Blazheyevs'kyy M.Ye., Karpova S.P. Kil'kisne vyznachennya natriyu oksatsylinu v poroshku kinetychnym metodom. *Farmats. Zhurnal*, 2010, no. 3, pp. 64-69 (Ukr.).
10. Blazheyevs'kyy M.Ye. Karpova S.P. Kinetychne vyznachennya amoksytsylinu tryhidratu u likars'kykh formakh kinetychnym metodom. *Farmats. Zhurnal*, 2010, no.5, pp. 55-61 (Ukr.).
11. Blazheyevs'kyy M.Ye. Karpova S.P. Kil'kisne vyznachennya fenoksymetylpenitsylinu kinetychnym metodom. *Farmats. Zhurnal*, 2011, no. 4, S.73-77 (Ukr.).
12. Karpova S. Quantitative determination of ampicillin and oxacillin in the «Ampiox» preparation using potassium peroxomonosulfate. *J. Chem. Pharm. Res.* 2013, 5(10), 57-64.
13. Blazheyevskiy M.Ye. The application of kinetic methods in pharmaceutical analysis. *Methods and objects of chemical analysis*, 2011, 69(1), 4-15.
14. Rogič D. Spectrophotometric determination of penicillanic acid sulfone. *Acta Pharm. Jugosl*. 1985, 35, 47–52.
15. Karpova S.P., Blazheyevs'kyy M.Ye. Kil'kisne vyznachennya ampitsylinu ta oksatsylinu u preparati ampioks za dopomohoyu kaliy hidrohenperoksomonosul'fatu. *Ukr. med. al'manakh*. 2011, 14(6), pp.11-13 (Ukr.).
16. Tymchenko V.O., Blazheyevs'kyy M.Ye. Kil'kisne vyznachennya ampitsylinu v likars'kykh formakh kinetychnym metodom za reaktsiyeyu z peroksykaprynovoyu kyslotoyu. *Tez. dop. nauk. konf. mol. vchenykh ta stud.*, Kharkiv, 2000, S. 57 (Ukr.).
17. Blazheyevs'kyy M.Ye. Kinetychne vyznachennya penitsyliniv u likars'kykh formakh za reaktsiyamy z peroksykarbonovymy kyslotamy. *Farmats. zhurn.*, 2003, no.5, pp. S.66-78 (Ukr.).
18. Blazheyevs'kyy M.Ye. Zastosuvannya peroksydnykh pokhidnykh karbonovykh kyslot u khimichnomu analizi: Doctoral Diss. … dokt. khim. nauk: 02.00.02. DCHTI, Dnipropetrovs'k, 2006, 524 s (Ukr.).
19. Izquierdo P., Gomez-Hens A., Peres-Bendito D. Stopped-flow fluorimetric determination of ampicillin in serum. *Fresenius J. Anal. Chem*. 1992, 342, 606-608.
20. Perdroso T. M., Salgado H. R. N. Validation of cefazoline sodium by UV spectrophotometric method. *Phys. Chem.* 2013, 3, 11-20.
21. Game M.D., Sakarka D.M., Gabhane K.B., Tapar K.K. Validated spectrophotometric methods for the determination of cefuroxime axetil in bulk drug and tablets. *Int. J. Chem Tech Res*. 2010, 2(2), 1259-1262.
22. Chavda R.N., Yadav H. N., Hinge M. A, Shing R. D., Patel E. S., Patel D. R. Development and Validation of Analytical Method for Simultaneous Estimation of Cefuroxime Axetil and Linezolid in Tablet Dosage form. *JPSR*. 2016, 7(4), 172-180.
23. Pritamand J., Manish P., and Sanjay S. Development and validation of UV spectrophotometric method for determination of cefuroxime axetil in bulk and in formulation, International. *J. Drug Dev. & Res.*

2011, 3, 318-322.

24. Arun, K. C., Saravanan, R., Balachandar, M. V., and Kumuthavalli, B. UV-Spectrophotometric determination of ceftazidime in pure and pharmaceutical formulation. *J. Chem. Pharm. Res.* 2010, 2, 424-431.

25. Sastry C. S. P., Rao S. G., and Naidu P. Y. New spectrophotometric method for the determination of some drugs with iodine and wool fast blue BL. *Talanta*. 1998, 45, 1227-1234.

26. Rageh A. H., Elshaboury S. R., Saleh G. A., Mohamed F.A. Spectophotometric method for determination of certain cephalosporins using 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl). Natural Science. 2010, 828-840.

27. Abdulrahman A. Alwarthan, Hamad A. Al-Lohedan Kinetic determination of cephalexin in drug formulations. Talanta, 1994, 41(2), 225-231.

28. Ramadan A., Mandil H., Dahhan M. UV-VIS Spectrophotometric study for determination of cefixime in pure form and in pharmaceuticals through complexation with Cu(II) using acetate-NaOH buffer in water: methanol. *Intern. J. Pharm. Pharm. Sci.* 2013, 5, 428-433.

29. Alothman Z. A., Abdalla M. A. Oxidative coupling for the spectrophotometric determination of certain cephalosporins and acetaminophen in drug formulations. *Arabian J. Chem.* 2011, 4(2), 239-242.

30. Zhao W., Zhang Y., Li Q. Indirect spectrophotometric determination of sodium ceftriaxone with n-propyl alcohol-ammonium sulfate-water system by extraction flotation of copper II. *Clin. Chim. Acta.* 2008, 39, 80-84.

31. Buhl F., Szpikowska-Sroka B. Spectrophotometric determination of cephalosporins with leuco crystal violet. *Chem. Anal*. 2003, 48, 145-149.

32. Metwally F. H., Alwarthan A. A., Al-Tamimi S. A. Flow-injection spectrophotometric determination of certain cephalosporins based on the formation of dyes. *Farmaco*. 2001, 56, 601-607.

33. Abdalla M.A., Fogg A.G., Burgess C. Selective spectrophotometric determination of cephalosporins by alkaline degradation to hydrogen sulphide and formation of methylene blue. *Analyst.* 1982, 107(1271), 213-217.

34. Papazova P., Bontcher M., Kacarova M. New spectrophotometric method for determination of cephazolin. *Talanta.* 1983, 30, 51.

35. Abdalla M.A., Fogg A.G., Barber J.G., Burgess C. Air-segmented continuous-flow visible spectrophotometric determination of cephalosporins by alkaline degradation to hydrogen sulphide and formation of methylene blue and determination of sulphide-producing impurities including cephalosporins in penicillin samples. *Analyst.* 1983, 108, 53-57.

36. El-yazbi F. A, Barary M.N. Determination of Some Cephalosporins Using Derivative Spectrophotometry. *Anal. Lett*. 1985, 18, 629-640.

37. Korany M.A., Elsayed M. Abdel-Hady, Galal S.M. Use of Second Derivative Spectrophotometry For the Determination of Certain Cephalosporins and Their Acid-Induced Degradation Products In Combination. *Anal. Lett*. 1989, 22(1), 159-175.

38. Morelli B. Zero-Crossing Derivative Spectrophotometry for the Determination of Mixtures of Cephaloridine and Cephalothin in Pure and Dosage Forms. *J. Pharm.I Sci*. 1988, 77(7), 615–621. DOI: http://dx.doi.org/10.1002/jps.2600770713

39. Martinez L. G., Falco P.C., Cabeza A.S. Comparicon of several methods used for the determination of cephalosporins. Analysis of cephalexin in pharmaceutical samples. *J. Parm. Biomed. Analysis*. 2002, 29, 405-423.

40. Helaleh M.I.H., Abu-Nameh E.S.M. A kinetic approach for determination of cefadroxil in pharmaceuticals by alkaline hydrolysis, *J. AOAC Inter.* 1998, 81(3), 528-533.

41. Blazheyevs'kyy M.Ye., Labuzova Yu.Yu. Kinetychne spektrofotometrychne vyznachennya tsefaleksynu za produktom reaktsiy peroksokyslotnoho okysnennya ta perhidrolizu. *Visnyk farmatsiyi*, 2010, 2 (62), pp. 30-33 (Ukr.).

42. Blazheyevskiy M.Y. Labuzova Yu.Yu. Kinetic spectrophotometric determination of cefadroxil in pure substance and pharmaceutical dosage form. *J. Chem. Pharm. Research.* 2013. 5(8), 115-121. Access mode: http://jocpr.com/vol5-iss8.html.

43. Blazheyevs'kyy M. Ye., Labuzova Yu.Yu. Kinetychne spektrofotometrychne vyznachennya tsefazolinu za produktom reaktsiy peroksykyslotnoho okysnennya ta perhidrolizu. *Farmats. zhurn*. 2012, no. 4, S.76-80 (Ukrainian).

44. Arita T. Fluorometric determination of cephalexin cephradine and cephatrizine in biological fluids. *Chemical & Pharmaceutical Bulletin (Tokyo)*. 1979, 2273-2280.

45. Omar M. A., Abdelmageed O. H., Attia T. Z. Kinetic spectrofluorimetric determination of certain cephalosporins in human plasma. *Talanta*. 2009, 77, (4), 1394–1404.

46. Ali S.M., Elbashir A., Aboul-Enein H.Y. Spectroscopic Method for Analysis of Cephalosporins in Pharmaceutical Formulations. *World J. Anal. Chem. 2015,* 3(41 A), 21-32.

47. Aly F.A., Hefnawy M.M., Belal F. A selective spectrofluorimetric method for the determination of some α-aminocephalosporins in formulations and biological fluids. *Anal. Lett*. 1996, 29, 117-130.

48. Carlier M., Stove V., Wallis S.C., De Waele J.J., Verstraete A.G., Lipman J., Roberts J.A. Assays for therapeutic drug monitoring of β-lactam antibiotics: A structured review. *Int. J. Antimicrob. Agents*. 2015, 46(4), 367-75. doi: 10.1016/j.ijantimicag.2015.06.016. Epub 2015 Jul 29.

49. El-Shaboury Salwa R., Saleh G. A., Mohamed Fardous A., Rageh Azza H. Analysis of cephalosporin antibiotics. *J. Pharm. Biomed. Analysis.* 2007, 45(1),

1–19.

50. Xu M., Ma H., Song J. Polarographic behavior of cephalexin and its determination in pharmaceuticals and human serum. *J. Pharm. Biomed. Anal*. 2004, 35, 1075-1081.

51. Zuman P. Resent development in elecnroanalytical chemistry of cephalosporins and cefamycins. *Anal. Lett.* 2000, 33(14), 2821-2857.

52. Squella J. A, Nuñez-Vergara L. J., Gonzalez E. M. Polarographic analysis of cephalexin. *J. Pharm. Sci.* 1978, 67(10), 1466-1467. doi: 10.1002/jps.2600671039

53. Fogg A.G., Fayad N.M., Goyal R.N. Differential pulse polarographic determination of cephalexin after hydrolysis in neutral phosphate buffer. *J. Pharm. Pharmacol.* 1980, 32(1), 302-303.

54. Li Q., Chen S. Studies on electrochemical behaviour of cephalexin. *Anal. Chim. Acta*. 1993, 282(1), 145-152.

55. Nuñez-Vergara LJ, Squella JA, Gonzalez EM. DC polarography of cephradine and its application to capsules. *J. Assoc. Off. Anal. Chem*. 1979, 62(3), 556-559.

56. Zeng Yonghuai, Ma Hongyan Electrochemical Behaviour of Cephradine. *Fenxi Huaxue : Chinese J. Anal. Chem.*1997, 25(9), 1006-1009.

57. Xu M., Xu M., Ma H., Song J. Polarographic behavior of cephalexin and its determination in pharmaceuticals and human serum. *J. Pharm. Biomed. Anal.* 2004, 35(4), 1075-1081.

58. Barbosa A. M. J., de Araujo T. A., Trindade M. A. G., Ferreira V. S. A new indirect method based on square-wave voltammetry for ceftiofur determination in bovine milk using an alkaline degradation product. *Microchem. J.* 2011, 297-302.

59. Fogg, A.G., Fayad, N.M., Burgess, C. and Mc Glynn, A. Differential pulse polarographic determination of cephalosporins and their degradation products. *Anal. Chim. Acta*. 1979, 108, 205-211.

60. Fogg, A.G., Fayad, N.M. and Burgess, C. Differential pulse polarographic study of the degradation of cephalexin: Determination of hydrogen sulphide and other degradation products. *Anal. Chim. Acta*. 1979, 110(1), 107-115.

61. Fogg A.G. Martin M.J. Differential pulse polarographic determination of degradation products of cephalosporins: Comparison of the degradation of cephaloglycin in neutral solution with that of cephalexin. *Analyst (London).* 1981, 106(1268), 1213-1217. doi: 10.1039/AN9810601213

62. Rodrigues, L.N.C., Zanoni, M.B.V. and Fogg, A.G. Indirect polarographic and cathodic-stripping voltammetric determination of cefaclor as an alkaline degradation product. *J. Pharm. Biomed. Anal.* 1999, 21(3), 497-505.

63. Özkan S.A., Erk N., Uslu B., Yilmaz N., Biryol I. Study on electrooxidation of cefadroxil monohydrate and its determination by differential pulse voltammetry. *J. Pharm. Biomed. Anal.* 2000, 23(2-3), 263-273.

64. Blazheyevskyy M.Ye., Kryskiw L.S. Acetylcholine kinetic spectrophotometric assay. Ukr. med. al'manakh. 2012, 15, no 3, S.31-33 (Ukrainian)

65. Blazheyevskiy M. Ye. Kinetic method for quantitative determination of acetylcholine. *News of Pharmacy*. 2002, 2(30), 110-113.

66. Blazheyevskiy M.Ye., Kryskiw L.S. Kinetics spectrophotometric determination spazmolitynu. *Farm. Zn. (Kiev),* 2012, no. 6, pp. 67-73 (Ukr.).

67. Blazheyevs'kyy M. Ye., Krys'kiv L. S. Kinetyko-spektrofotometrychne vyznachennya arpenalu za reaktsiyeyu perhidrolizu. *Problemy ekolohichnoyi ta medychnoyi henetyky i klinichnoyi imunolohiyi*. 2014, no. 1, 189-196.

68. Mitić Snežana S., Miletić Gordana Ž., Pavlović Aleksandra N., Toišić Snežana B., Sunarić Slavica M. Quantitative Analysis of Acetylsalicylic Acid in Commercial Pharmaceutical Formulations and Human Control Serum Using Kinetic Spectrophotometry. *Acta Chim. Slov.* 2008, 55, 508–515.

69. Blazheyevskiy M.Ye., Kryskiw L.S. Aspirin kinetic-spectrophotometric assay with peroxymonosulfate. *Actual questions of development of new drugs, Abstracts of XX international scientific and practical conference of young scientists and students*, *Publishing Office, Kharkiv*, 2013, 73.

70. Blazheyevskiy M. Kinetic spectrophotometric determination of acetylsalicylic acid in dosage form "ACELYSIN-KMP" / M. Blazheyevskiy, L. Kryskiw. J. Chem. Pharm. Res. 2013. v. 5, nº 10, 102–107.

71. Blazheyevskiy N., Kryskiw L.S. Kinetic-spectrophotometric determination of acetylsalicylic acid in micellar media. *Peer-reviewed materials digest (collective monograph) published following the results of the LXXXV International Research and Practice Conference and II stage of the Championship in Medicine and Pharmaceutics, Biology, Veterinary Medicine and Agriculture (London, July 24-July 29, 2014).- London: IASHE*, 2014, 46-49. http://files.gisap.eu/sites/files/digest/85%20Internet.pdf (Russ.)

72. Blazheyevskiy Mykola and Kryskiw Lyubomyr Kinetic Spectrophotometric Method for the Determination of Suxamethonium Chloride. *Chemistry & Chemical Technology.* 2015, 9(3), 261-265.

73. Blazheyevskiy M. Ye., Kryskiw L.S. Development of the kinetic-spectrophotometric method for quantitative determination of zopiclone in tablets by the perhydrolysis reaction. *News of Pharmacy*, 2014, *3*, 38-41. http://nbuv.gov.ua/ujrn/vphc_2014_3_11

74. Blazheyevs'kyy M.Ye., Krys'kiv L.S. Kinetyko-spektrofluorymetrychne vyznachennya *D*,*L*-lizynu atsetylsalitsylatu za reaktsiyeyu perhidrolizu z kaliyu hidrohenperoksomonosul'fatom u preparati «Atselizyn-KMP» / M.Ye.Blazheyevs'kyy, *Farmakom*. 2014, 4, 35-41 (Ukr.).

75. Blazheyevs'kyy,M. Ye., Krys'kiv L.S., Yehorova A.V., Skrypynets' Yu.V., Leonenko I.I. Kinetyko-spektrofluorymetrychne vyznachennya

atsetylsalitsylovoyi kysloty u preparati «ATSELIZYN-KMP». *Medychna nauka ta medychna praktyka v Ukrayini: problemy rozvytku ta vzayemodiyi: Mater. mizhn. nauk.-prakt.konf. Odesa, 8-9 lyst. 2013 r. Odesa*: HO «Pivdenna fundatsiya medytsyny», 2013, C. 15-19 (Ukr.).

76. Finkel J.M., Pittillo R.F., Mellett L.B. Fluorometric and Microbiological Assays for Rifampicin and the Determination of Serum Levels in the Dog. *Chemotherapy.* 1971, 16, 380-388.

77. Blazheyevs'kyy M.Ye., Krys'kiv L.S. Khemilyuminestsentnyy metod vyznachennya benzoyilu peroksydu u los'yoni «uhresol» za lyuminolovoyu reaktsiyeyu. *Analitychna khimiya u farmatsiyi : materialy mizhnar. nauk.-prakt. internet-konf.*, *Kharkiv, 19-20 berezn. 2015 Kh. : Vyd-vo NFaU*, 2015, S. 78-79 (Ukr.).