Current Topics in Analytical Chemistry

Review

Spectrophotometric and spectrofluorimetric determination of the 2-and 10-disubstituted phenothiazines using peroxy acid oxidation

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ABSTRACT

This article provides an overview of the state of the art in pharmaceutical applications of absorption spectroscopy and spectrofluorimetry in the analysis of phenothiazines by indirect method using peroxides. This review presents the fundamentals for the beginner and, for the expert, discusses both qualitative and quantitative analysis problems. Several sections focus on the determination of phenothiazines, in various matrices, the coupling of spectrophotometric methods, and the problems associated with the use of chemical reactions prior to spectrophotometric and spectrofluorimetric measurements. The final section provides a survey of the spectrophotometric and spectrofluorimetric determination of the main families of phenothiazines in the form of the corresponding sulfoxides obtained with peroxyacids as new analytical reagents, emphasizing the achievements of the last decade.

KEYWORDS: derivatization, phenothiazine derivatives, Oxone, diperoxydicarboxylic acids, spectrophotometry, spectrofluorimetry.

1. Introduction

Phenothiazine drugs are currently formulated in a variety of dosage forms either as an individual medicament or in combination with one or more other drugs [1].

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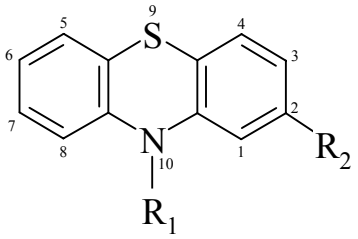
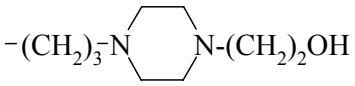
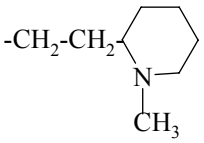
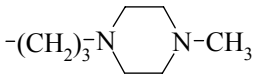
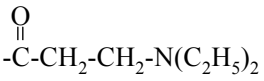
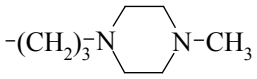
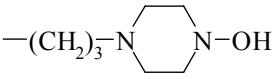
The chemical structures of the most common 2- and/or 10-disubstituted phenothiazine derivatives are described in Table 1.

Among the methods used to assay the phenothiazines in pharmaceutical preparations are ultraviolet and visible spectrophotometric procedures [2]. The advantages of these methods are low time and labor consumption. The precision of these methods is also excellent. The use of UV–Vis spectrophotometry especially applied in the analysis of pharmaceutical dosage forms has increased rapidly over the last few years [2].

The ring system (1,4-Thiazine condensed with two benzene rings) of the phenothiazines possesses an intense and characteristic spectrum. The spectra of the phenothiazines are very similar and are characterized by an intense band at 250-255 nm and a group of less-intense bands around 300-310 nm [2]. The intensity ratio of the two bands is a characteristic value which allows to identify and estimate the purity of phenothiazines [3]. The intense spectra of phenothiazines present wide possibilities for their quantitative determination in various formulations. The simple spectrophotometric methods usually involve the dilution (or extraction) of the preparation followed by a measurement of absorbance in the ultraviolet region. These procedures based on natural absorption lack specificity and are subject to interference from other ultraviolet absorbing drugs, coloring and flavouring agents or the oxidation products of the phenothiazine drugs [4].

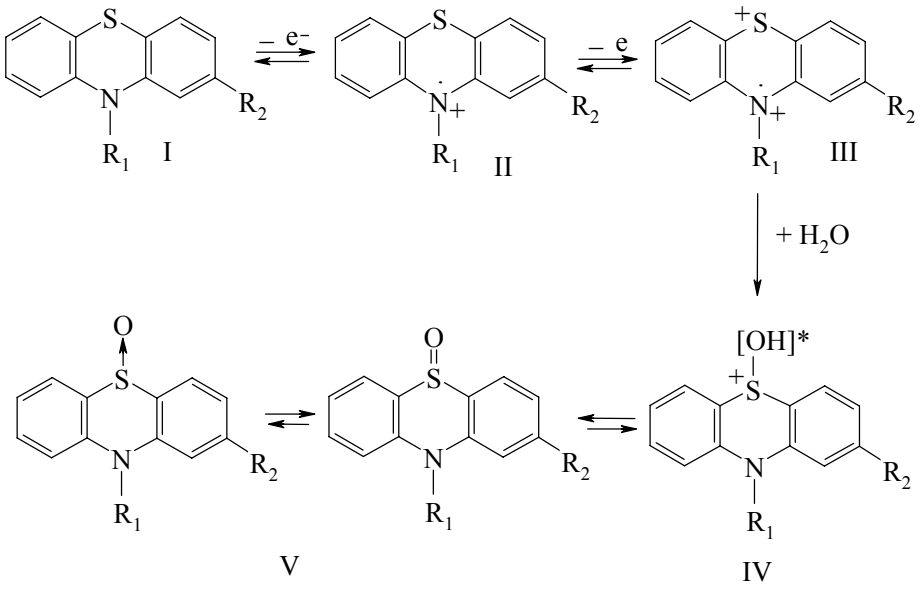
Table 1. Chemical structures of some 2- and/or 10-disubstituted phenothiazine derivatives.



Preparation	Substituents		Symbol
	R_1	R_2	
Chlorpromazine	$-CH_2-CH_2-CH_2-N(CH_3)_2$	-Cl	CPH
Levomepromazine	$-CH_2-CH(CH_3)-CH_2-N(CH_3)_2$	$-OCH_3$	LMPH
Promethazine	$-CH_2-CH(CH_3)-N(CH_3)_2$	-	PMH
Perphenazine		-Cl	EPH
Thioridazine		$-SCH_3$	TRDH
Trifluperazine		$-CF_3$	TFP
Ethacyzin		$-NHC(O)OC_2H_5$	ET
Prochlorperazine		-Cl	PCP
Pericyazine		-CN	PRC

The official compendia [5] recommended for the determination of phenothiazines in bulk, or in pharmaceutical forms, involve measurements of the absorbance at selected wavelengths. The proposed pharmacopoeial procedures require intensive isolation and purification steps in the case of the assay of phenothiazines in their pharmaceuticals forms. The main disadvantage of direct UV-spectrophotometry is the sensitivity to excipients usually presented in pharmaceutical preparations.

Phenothiazines are easily oxidized by different oxidizers [6]. The reaction scheme for the oxidation process as follows:



Scheme 1. The reaction scheme for the oxidation process of 2,10-disubstituted phenothiazines.

The first step is a reversible electron abstraction from the phenothiazine (I) to the colored semiquinone free radical (II) (see Scheme 1). The distribution of π-electrons in the 2,10-disubstituted phenothiazines, according to theoretical considerations, may lead to the formation of some resonance forms of free cation radical [7]. The radical is stable for a certain period of time depending on phenothiazine substitution, pH and buffer used. [8] The free radical can lose another electron, giving the colorless Phenothiazonium ion (III) (see Scheme 1). Next, compound (III) hydrolazes to the phenothiazine derivative S-oxide (V) [6].

Oxidation is interesting for the determination of phenothiazines because it enhances the spectroscopic properties by allowing detection in the visible spectral region.

Many spectrophotometric methods for 2,10-substituted phenothiazine determination based on their oxidation to colored radical cations and the subsequent measurement of absorbance in the visible region have been proposed [9].

The descriptions given in the presented review methods, based on their oxidation reaction, can be alternatives. The absorbance of colored phenothiazine radicals is less liable to spectral interferences from others ingredients of pharmaceuticals. The described methods offer advantages in their simplicity, rapidity and common access to instrumentation.

However, it is known that stability of color cation radical depends mainly on the oxidation agent used. In the case of a strong oxidant, the color of radical disappears quickly due to the second step of reaction which leads to the formation of a colorless sulphoxide. This effect can result in the decrease of sensitivity of assay and reproducibility.

Also, some of these methods have some disadvantages, such as a high acidic medium [10] and others don't have sufficiently high sensitivity and require a very long heating time [11].

Usually, in order to resolve such problems, the use of kinetic methods is recommended. In combination with the stopped-flow mixing technique, kinetic methodology is highly suitable for this purpose as it allows the mixing of the sample and reagent solutions automatically, as well as making measurements immediately after mixing.

A high-sensitive stopped-flow kinetic spectrophotometric method for the determination of perphenazine by its oxidation with potassium persulphate in sulphuric acid media, which was applied to monitor the change of the absorbance of the intermediate at 526 nm within the original few seconds, has been developed. It was found

that the initial reaction rate increased linearly with an increase in the perphenazine concentration in the range from (1-16)·10^{-5} mol L^{-1}. LOD was calculated to be 5.3·10^{-6} mol L^{-1} [12].

During the development of a method for the analysis of Promethazine with persulphate, conditions were found under which a pinkish red-colored product exhibiting maximum absorbance at 515 nm was stable for 12 hours [13].

The red color was found stable in different solvents like water, methanol or ethanol. There was no significant effect of pH on the intensity of the color. The reaction was selective for promethazine–HCl, with 1 μg mL^{-1} as the visual limit of identification and provides a basis for a new spectrophotometric determination method. The calibration graph was linear in the range of 0.001-0.125 mg mL^{-1} of promethazine–HCl (r=0.987). The apparent molar absorptivity was 2.22·10^3 mol^{-1} cm^{-1}. The method was successfully applied to pure and pharmaceutical formulations of promethazine–HCl. The quantitative assessment of tolerable amounts of possible interferants was also studied. The results are reproducible within ±1% and in good agreement with those obtained by the standard procedure. The average recoveries obtained by the proposed method ranged from 97% to 100.4%.

The oxidation reaction with hydrogen peroxide also was used for the determination of phenothiazines [14].

Aqueous and micellar catalysis of horseradish peroxidase has been studied in a sequential injection system (SIA) by using hydrogen peroxide oxidation of two phenothiazines: water-soluble chlorpromazine and less water-soluble perphenazine micellised in a Tween 80 medium. The coloured free-radical intermediate formed was monitored spectrophotometrically at 527 nm. The system enables the determination of chlorpromazine in water and perphenazine in micellar medium up to 1.25·10^{-4} mol L^{-1}, with quantification limits of 2·10^{-5} and 1.25·10^{-5} mol L^{-1}, respectively. Relative standard deviation (RSD) values were in both cases less than 1.6%. The optimized SIA system consumed 125 μL of a sample, 1.5 μg of peroxidase and 5 × 10^{-8} mol of hydrogen peroxide per determination, which guarantees economy in the use of sample and reagents with reduced residue production, in good agreement with the current recommendations of green chemistry.

The potential effect of several compounds commonly used as excipients on analytical signals has been studied and no interfering effect was noticeable. Statistical comparison of the results obtained with the proposed methodology and with the reference methods showed good agreement and indicate no significant difference at the 95% confidence level [15].

An original, simple and sensitive flow-injection spectroelectroanalytical method for the determination of chlorpromazine in pure form or in pharmaceutical formulations has been described. The method is based on the formation of a stable cationic radical by electro-oxidation in sulfuric acid medium (0.1 mol L^{-1}), monitored *in situ* at λ = 524 nm. The determination of chlorpromazine hydrochloride in pure form or in pharmaceutical formulations was explored, considering the amperometric and the absorptiometric signals. The association of these two signals enhanced the selectivity of the analysis and proved decisive when other electroactive compounds or excipients like ascorbic acid were present in the formulation. The analytical parameters have been evaluated and the results obtained using standard additions are in agreement with the reference methods [16]. The descriptions given in the methods below, which are based on a peroxyacid oxidation reaction, could be alternatives.

2. Indirect spectrophotometric determination of 2- and/or 10-disubstituted phenothiazine derivatives in the form of sulfoxides obtained using peroxyacid

In order to improve the analytical properties of phenothiazine determination by indirect methods peroxy acids have been proposed as analytical reagents [17]. An original method for the rapid determination of phenothiazine drugs in a wide variety of pharmaceutical preparations has been described. The drugs are determined by a difference spectrophotometric technique based upon the absorbance of the sulphoxide derivative of the drug relative to the absorbance of the solution of the underivatized drug. The sulphoxide

derivatives are formed rapidly and quantitatively at room temperature by the addition of a solution of peroxyacetic acid, prepared by the slow reaction of hydrogen peroxide and glacial acetic acid on standing. The difference in the absorbance of the solutions is proportional to the concentration of the phenothiazine drug in the preparations and is specific for the intact drug in the presence of oxidative and photochemical decomposition products, excipients and co-formulated drugs.

For determining the weight per ml of the oral solution of promethazine hydrochloride, to a weighed quantity containing 10 mg of PMT Hydrochloride add 25 ml of water and 5 ml of a 5% w/v solution of sodium hydroxide. Extract the mixture with two 50 ml quantities of chloroform, shaking vigorously for 1 minute each, evaporate the combined extracts to dryness at about 30 °C at a pressure of 2 kPa and dissolve the residue in sufficient 0.1 mol L^{-1} hydrochloric acid to produce 50 ml (solution A). Dilute 10 ml of solution A to 50 ml with water (solution B). To a further 10 ml of solution A add 5 ml of peroxyacetic acid solution, allow to stand for 10 minutes and add sufficient water to produce 50 ml (solution C). Measure the absorbance of solution C at the maximum at 336 nm, using solution B in the reference cell and measure the absorbance of solution B at the same wavelength using water in the reference cell. Repeat the procedure using a 0.02% w/v solution of promethazine hydrochloride BP CRS in 0.1mol L^{-1} hydrochloric acid in place of solution A and dilute 10 ml of solution A to 50 ml with water (solution B). To a further 10 ml of solution A add 5 ml of peroxyacetic acid solution, allow to stand for 10 minutes and add sufficient water to produce 50 ml (solution C). Measure the absorbance of solution C at the maximum at 336 nm, using solution B in the reference cell and measure the absorbance of solution B at the same wavelength using water in the reference cell. The result is not valid if the absorbance of solution B is more than 0.10 [18]. It is clearly shown that sample frequency of this method is very low, which implies lengthy extraction process.

In order to improve the analytical properties of this spectrophotometric method long-chain aliphatic diperoxy acids (diperoxyazelaic acid, diperoxyadipic acid) and potassium peroxymonosulfate (Oxone) were proposed as oxidative reagents. The concentration of aliphatic diperoxy acid or Oxone in the oxidizing reagent determined by the iodometric method is sufficiently high under these conditions to oxidize the phenothiazine drugs to the sulphoxides rapidly and quantitatively without heating the solution. The products formed by the reaction of phenothiazine derivatives with oxidizing reagent under the conditions of the assay was confirmed as sulphoxides of phenothiazine derivatives by comparison of its ultraviolet absorption spectrum with those of authentic sulphoxides.

The proposed methods are characterized by simplicity, sensitivity, and good precision. The determination of phenothiazines by spectrophotometric method is preferable to other conventional methods because they are fast and precise (RSD values ranging from 0.6 to 2.5%) [19]. A new method has also been described for the rapid determination of phenothiazines drugs in pharmaceutical preparations. The drugs were determined by a difference spectrophotometic technique based upon the absorbance of the sulphoxide derivative of the drug relative to the absorbance of the solution of the underivatized drug. The sulphoxide derivatives are formed rapidly and quantitatively at room temperature by the addition of the solution of potassium peroxomonosulphate (Oxone). The difference absorbance of the solution is proportional to the concentration of the phenothiazine drug in the preparation and is specific for the drug in the presence of colouring and flavouring agents, excipients and most co-formulated drugs [20-23]. The precision (%RSD) is close to 3%.

Methods for the determination of phenothiazine preparations such as Prochlorperazine (syn. Vertinex) (using Oxone as an oxidant) [24] and Pericyazine [25] (syn. Neuleptil), as well as Tizercin (using diperoxyazelaic acid) in various commercial preparations by indirect spectrophotometry, have been described [26]. The analysis can be performed quickly, like direct spectrophotometric determination, and it is sufficiently specific. The method involves the oxidation of the drug in an aliquot of a solution of diperoxy acid or Oxone with the formation of a corresponding sulfoxide

derivative of phenothiazine and measuring the absorbance of the solution in the range 320-350 nm using a reagent solution of the same concentration in the compensation solution. The absorption difference obtained is proportional to the concentration of the native phenothiazine derivative in the drug, and its magnitude is not affected by the presence of excipients, degradation products, or the presence of other drugs.

Recently, the oxidative derivatization method by means of diperoxyazelaic acid for the indirect spectrophotometric determination of perphenazine dihydrochloride was presented [27]. Diperoxyazelaic acid is introduced as a derivatizing agent for perphenazine, yielding the sulfoxides. This reaction product was successfully employed for the spectrophotometric determination of the perphenazine dihydrochloride. The UV spectroscopic detection of the sulfoxide proved to be the more robust and sensitive method. At 342 nm, the molar absorptivity is $5.45 \cdot 10^3$ $L \cdot mol^{-1} \cdot cm^{-1}$. Figure 1 demonstrates dependence of the UV-spectra of the S-oxidation product of perphenazine on its concentration.

The elaborated method allowed determination of perphenazine dihydrochloride in the concentration range 1-40 $\mu g \cdot ml^{-1}$. Limits of quantification (LOQ=10S) is 3.3 $\mu g \cdot ml^{-1}$. A new spectrophotometric technique was developed and the possibility of quantitative determination of Perphenazine dihydrochloride in Perphenazine Tablets was demonstrated. The present method is precise, accurate and other inactive excipients of drug did not interfere. RSD = 2.00%; $\delta = (\bar{x}-\mu)\ 100\%/\mu = -0.85$ %.

The optical characteristics and analytical parameters for the determination of phenothiazine drugs are summarized in Tables 2 and 3.

The use of derivative UV-spectrophotometry was proposed for the simultaneous quantification of promazine hydrochloride and promazine sulphoxide [28]. In this method, mathematical parameters were established for generating derivative spectra of the analytes. The determination of promazine hydrochloride was achieved by the first-order derivative at wavelength 268 nm, while the determination of promazine sulfoxide was achieved by applying the third-derivative spectrum based on measurements at wavelength in the range of 342-344 nm. The recovery of promazine hydrochloride in the presence of promazine sulphoxide ranged from 95.0 to 106.5%. Usually, derivatization spectrophotometric methods suffer from spectrum interferences.

Georg Diehl and Uwe Karst [29] have presented a first post-column chemical derivatization method for the liquid chromatographic determination of phenothiazines. Peroxyacetic acid is introduced as a derivatizing agent for phenothiazines, yielding the colored radical cations or fluorescent sulfoxides, depending on reaction conditions. Both reaction products were successfully employed for the detection of the phenothiazines after their liquid

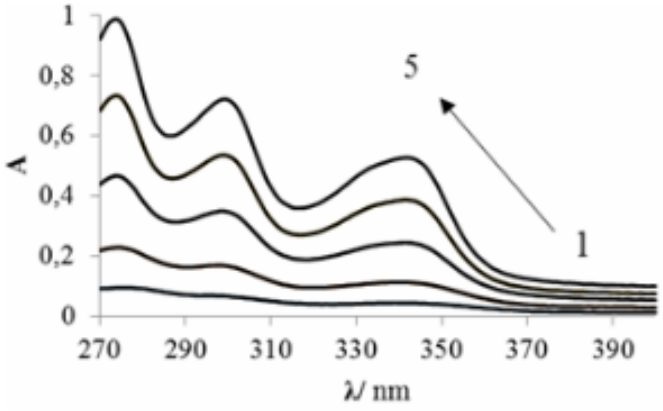


Figure 1. Dependence of the UV-spectra of the S-oxidation product of perphenazine on its concentration, C, $\mu g\ mL^{-1}$: *1* – 4; *2* – 10; *3* – 20; *4* – 30; *5* – 40. 0.4 mol L^{-1} HCl.

Table 2. Optical characteristics and analytical parameters of phenothiazine derivative sulfoxides.

Characteristics	PCP (with diperoxyazelaic acid)	PRC (using Oxone)	LMPH (with diperoxy-azelaic acid)	EPH (with diperoxy-azelaic acid)
λ_{max} /nm	336-338	360-362	332-333	342-343
Molar absoprtivity, $\varepsilon \pm \Delta\varepsilon$ ($L \cdot mol^{-1} \cdot cm^{-1}$)	$(2.7 \pm 0.1) - 3.9) \cdot 10^3$	$(5.5 \pm 0.15) \cdot 10^3$	$(3.5 - 4.0) \cdot 10^3$	$(5.6 - 7.0) \cdot 10^3$
The Beer's law limits, 10^{-5} mol L^{-1}	0.2-11	0.2-11	0.4-14	0.4-10
Limit of quantification, *LOQ*, mol L^{-1}	$4.5 \cdot 10^{-6}$	$5.8 \cdot 10^{-6}$	$5.7 \cdot 10^{-6}$	$4.2 \cdot 10^{-6}$

Table 3. Optical characteristics and analytical parameters for the determination of phenothiazine drugs.

Characteristics, Parameters	CPH (using Oxone)	PMH (using Oxone)	LMPH (using Oxone)	EPH (using Oxone)	TRDH (using diperoxy-adipic acid
λ_{max} /nm	341-342	335-337	332-333	341-342	349-350
Molar absoprtivity, $\varepsilon \pm \Delta\varepsilon$ ($L \cdot mol^{-1} \cdot cm^{-1}$)	5350 ± 300	5300 ± 100	6090 ± 300	5300 ± 300	4950 ± 400
The Beer's law limits, 10^{-5} mol L^{-1}	0.35-11	0.2-11	0.6-14	0.4-10	0.9-15
Limit of quantification, *LOQ*, mol L^{-1}	$3.5 \cdot 10^{-6}$	$1.6 \cdot 10^{-6}$	$5.7 \cdot 10^{-6}$	$4.2 \cdot 10^{-6}$	$8.6 \cdot 10^{-6}$
Regression equation* A = *bc* + *a*					
Regression coefficient (*r*)	0.999	0.999	0.999	0.999	0.999
Slope ($b \pm \Delta b$) /L mol^{-1}	5350 ± 291	5293 ± 111	6088 ± 297	5304 ± 290	4952 ± 423
Intercept ($a \pm \Delta a$)	-0.00005 ± 0.02	-0.001 ± 0.0075	-0.02 ± 0.03	0.01 ± 0.02	0.02 ± 0.04
Dispersion (S^2) (n = 5-7; P = 0.95)	$1.9 \cdot 10^3$	$2.2 \cdot 10^3$	$3.25 \cdot 10^3$	$2.0 \cdot 10^3$	$1.1 \cdot 10^3$

* A = *bc* + *a*, where «*A*» is the absorbance, «*c*» - concentration in mol L^{-1}

chromatographic separation. The fluorescence spectroscopic detection of the sulfoxides proved to be the more robust and sensitive method. Limits of detection ranged from 4 nM for triflupromazine and trimeprazine to 300 nM for phenothiazine for the fluorescence Phenothiazine spectroscopic detection of the sulfoxide and from 0.3 μM for phenothiazine and triflupromazine to 2 μM for trifluperazine for the UV-Vis spectroscopic detection of the radical cation.

3. Assay of the phenothiazines in the presence of their sulphoxides

The simultaneous determination of the phenothiazines in the presence of their sulphoxides is an important task. The structural similarity of the sulphoxides

and the parent compounds leads to difficulty in the conventional UV spectrometric methods of analysis for this group of compounds, as the UV spectra of these compounds overlap extensively. This invalidates the usual compendial procedure of using absorbance measurement at a single wavelength.

Several methods have therefore been proposed for addressing this problem. Usually in order to resolve such a problem, chromatographic methods are recommended.

Some workers [30] have used high-performance liquid chromatography (HPLC) for various members of this class of compound.

A rapid, simple, and accurate high-performance liquid chromatographic procedure using an amino-bonded microparticulate column for the determination of chlorpromazine hydrochloride and its two oxidation products, such as chlorpromazine sulfoxide and chlorpromazine sulfone, in commercially available pharmaceutical dosage preparations has been reported. Simultaneous identification and quantitation of chlorpromazine sulfoxide and chlorpromazine sulfone in the presence of at least 100 times the amount of the parent compound were achieved with selective fluorometric detection. Identification and quantitation of chlorpromazine hydrochloride were achieved by changing the fluorescence detection mode. A typical chromatographic run was completed within 15 min. The described procedure is simpler, more sensitive, and more accurate than the USP XIX methods, particularly for the analysis of chlorpromazine sulfoxide in injectables. Quantitation of chlorpromazine sulfoxide and chlorpromazine sulfone at levels as low as 0.1 μg, representing 0.1% contamination, was demonstrated [31].

This method requires specialized equipment and expertise and can be laborious for routine analysis. The HPLC technique, though rapid and sensitive, is relatively expensive involving costly instruments and solvents.

4. The derivative UV-spectrophotometric method

Alternatively, derivative UV-spectrophotometry can be a useful and easily accessible tool that allows the fast and precise resolution of a multicomponent mixture. This approach allows the separation of overlapped spectra and their use for the quantification of compounds.

Derivative spectrophotometry is based on a mathematical transformation of the spectra zero-order curve into derivative spectra. Because the derivatization of spectra allows one to obtain more information by separation of the overlapped signals included in the zero-order spectra, it was employed for the determination of 2,10-substituted phenothiazines and its sulphoxides.

Two spectrophotometric methods are proposed for the simultaneous quantification of Levomepromazine hydrochloride and its main degradation product Levomepromazine sulfoxide. One of them is based on the first-order derivative spectra generated by the Savitzky-Golay algorithm (third-order polynomial degree, $\Delta\lambda$ = 10 nm). Determination of Levomepromazine hydrochloride and its sulfoxide was realized by measurements of amplitudes of derivative spectra at 332 nm and 278 nm, respectively. The Beer law was obeyed in the concentration range 1.5-50 $\mu g \cdot ml^{-1}$ for Levomepromazine and 2.5-50 $\mu g \cdot ml^{-1}$ for Levomepromazine sulfoxide. The second of the proposed methods utilized the bivariate calibration algorithm. The determination was performed at 302 nm for Levomepromazine and at 334 nm for Levomepromazine sulfoxide. The elaborated methods allowed determination of Levomepromazine in the concentration range 1.0-25 $\mu g \cdot ml^{-1}$ while Levomepromazine sulfoxide was determined in the concentration range 2.0-50 $\mu g \cdot ml^{-1}$ [32].

In pharmaceutical dosage forms, interference in the UV spectra of analytes by other UV-absorbing solutes leads to a requirement for the development of procedures with greater specificity. One method used to address this problem is the technique of derivative spectroscopy, where the composite spectrum is transformed to the second or higher derivative in the wavelength domain. This paper reports the novel application of this technique for the determination of a Chlorpromazine and its sulphoxide impurity in various pharmaceutical dosage forms.

A systematic approach developed for optimisation of the derivative order, graphical measurement and instrumental conditions led to the adoption of

third-order derivative spectroscopy as a method with suitable precision and selectivity for the determination of the phenothiazines. Both the parent compound and the sulphoxide impurity can be assayed in dosage forms by measurement of the amplitudes of the positive peak at 259 nm with respect to the negative peak at 267 nm, and the positive peak at 350 nm with respect to the negative peak at 361 nm, respectively. A new notation for denoting these amplitude measures is proposed, *viz.*, $^3A_{259,267}$ and $^3A_{350,361}$, respectively. By comparison with an independent referee method based on reversed-phase HPLC, the proposed method and the referee method gave statistically similar results for the determination of chlorpromazine and its sulphoxide in injectables, tablets and syrups. The figures of merit for the proposed assays are described in terms of response linearity, 95% confidence limits, relative standard deviation, recovery data and correlation coefficients. It is suggested that similar methodology should be applicable to the analysis of other members of the phenothiazine class of compounds [33].

Modern quantitation of multicomponent mixtures with ultraviolet spectroscopy is tending towards application of computer-aided calculations where mathematical over determination has been favored for reducing random errors in such analysis. Application of this procedure has however been made difficult by the observation of anomalous results. Additional sources of error is reported in the present study. Error patterns were traced to features of the spectra of components of the standards rather than the spectra of the mixtures to be analyzed alone. Calculation efficiency correlated inversely with isoabsorptive points of the standard spectra in the calibration matrix. New computational indices, relative total absorptivity and relative total absorbance (RTA), calculated by the integration of absorption spectra of the components, were shown to be measurements that could be employed to optimize the limit of quantitation of components present at low levels. RTA could be optimized separately for each component and could be a useful tool in automatic weighting factor allocation in future multicomponent analysis routines. Mixtures of Chlorpromazine and its sulfoxide which is of medical, forensic and industrial interest has been chosen as a model in this study. The optimized wavelength range for analysis of Chlorpromazine-Chlorpromazine sulfoxide was determined to be 270-360 nm [34].

5. A spectrofluorimetric method

The increased sensitivity of spectrofluorimetric, compared with absorptiometric, measurements for sulfoxides has been used in several assays for phenothiazines in pharmaceutical formulations, based on the oxidative conversion of the phenothiazines to the corresponding sulfoxide [35].

The properties of ultraviolet radiation were exploited to obtain fluorescent products from three phenothiazine compounds (chlorpromazine, promethazine and perphenazine). Photochemical reactions were studied and compared with chemical oxidation processes. After optimizing the working conditions, these reactions were applied to the determination of the analytes by stopped-flow injection methods (irradiation of the flow cell) and normal flow injection methods (irradiation of the reaction coil). The determination limits thereby achieved were 20 $ng \cdot ml^{-1}$ (chlorpromazine and perphenazine) and 50 $ng \cdot ml^{-1}$ (promethazine) with relative standard deviations of less than 2% in all instances (n = 11). Analyses for these compounds in pharmaceutical preparations were also carried out [36].

The oxidation of promethazine to its corresponding fluorescent sulphoxide has been used to develop a novel kinetic fluorimetric method for the determination of this drug. The use of a stopped-flow mixing technique makes use of an oxidizing reagent unnecessary because the oxidation is rapidly carried out by dissolved oxygen. The method is simple and fast as it only requires a few seconds to obtain kinetic data which allows ready application to routine analyses. The calibration graph is linear over the range 0.5-80 $\mu g\ mL^{-1}$ and the precision (%RSD) is close to 2%. The method was applied to the determination of promethazine hydrochloride in two pharmaceutical preparations [37].

It was found that the oxidation of promethazine to its corresponding sulfoxide derivative is very fast in slightly acid medium and in the presence of an oxidant such as hydrogen peroxide. The oxidation

can also be effected by dissolved oxygen, but the reaction is much slower. In the presence of hydrogen peroxide the reaction rate is roughly twice as fast as in its absence if the batch technique is used. However, in the stopped-flow mixing technique, the presence of hydrogen peroxide does not modify the reaction rate obtained in its absence which is roughly four times higher than that obtained by using the batch technique in the presence of hydrogen peroxide.

Two 10 ml reservoir syringes of the stopped-flow module were filled with the same solution, prepared previously and containing 10 ml of 0.1 mol L^{-1} phthalate buffer (pH 2.8) and a standard or sample solution of promethazine hydrochloride in a final concentration range 0.5-80 μg mL^{-1}, and a final volume of 25 ml. After the two 2 ml drive syringes were filled, equal volumes (0.15 ml) of the solution were mixed in the mixing chamber in each run. The variation of the fluorescence intensity throughout the reaction was monitored at λ_{ex} 330 nm and λ_{em} 370 nm. Fluorescence values were obtained over 15-20 s intervals and processed by linear regression using a microcomputer and software for application of the initial-rate method ('Kinetic Obey'). The reaction rate was determined in about 10 s and each sample was assayed in triplicate. The blank signal was found to be negligible. All measurements were carried out at 20 °C. The concentration of promethazine in the samples was determined by interpolation from the working curves prepared from standard solutions of promethazine hydrochloride (final concentration 0.5-80 μg mL^{-1}) in water.

The detection limit was 0.3 μg mL^{-1}. The relative standard deviation (P = 0.05, n = 10) obtained for 1 and 10 μg mL^{-1} promethazine hydrochloride was 2.6 and 2.2%, respectively. A salient feature of this method is its speed (initial rate measurements are performed in about 10 s).

According to the results obtained, the joint use of kinetic methodology and the stopped-flow mixing technique provides a simple and fast fluorimetric method for the determination of promethazine requiring no reagent addition or sample dilution. The features of the stopped-flow technique ensure that the oxidation of promethazine by dissolved oxygen takes place in a few seconds, so kinetic data can be obtained quickly, a result which provides a useful means of accomplishing automation in routine analyses. On comparing the features of the proposed method with those of the FIA automatic method using cerium(IV) as oxidant [38], the following conclusions can be drawn: the stopped-flow method avoids the high cost of cerium(IV) salts and the environmental problems posed by sulphuric acid waste; also the quantification limit is lower and the linear range of the calibration graph is wider than that of the FIA method (10.3-51.3 μg mL^{-1}).

Kinetic methodology, particularly in association with the stopped-flow mixing technique, currently offers major advantages over equilibrium methodology as it provides a means of accomplishing automation and rapid handling of reagents in routine analyses.

A simple, sensitive and accurate spectrofluorimetric method has been described for the quantitative determination of diethazine and promethazine either in the pure form or in its pharmaceuticals. The method is based on the formation of red fluorescent product of these drugs with Au(III). A linear calibration graph is obtained over the range 0.05-100 ppm for diethazine and promethazine [39].

The properties of ultraviolet radiation were exploited to obtain fluorescent products from three phenothiazine compounds (chlorpromazine, promethazine and perphenazine). Photochemical reactions were studied and compared with chemical oxidation processes. After optimizing the working conditions, these reactions were applied to the determination of the analytes by stopped-flow injection methods (irradiation of the flow cell) and normal flow injection methods (irradiation of the reaction coil). The determination limits thereby achieved were 20 ng ml^{-1} (chlorpromazine and perphenazine) and 50 ng ml^{-1} (promethazine) with relative standard deviations of less than 2% in all instances (n = 11). Analyses for these compounds in pharmaceutical preparations were also carried out [40].

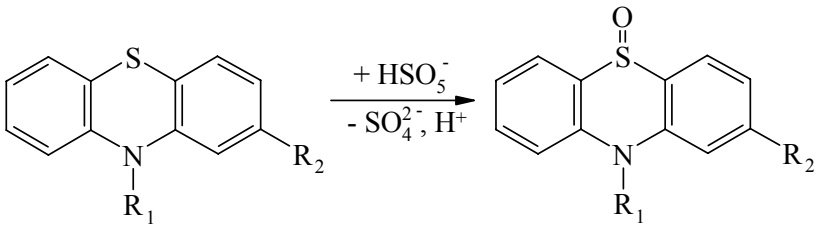
Recently, a new method was elaborated for quantitative determination of ethacyzine hydrochloride (the diethylamino analog of ethmozine) (ET) in the form of corresponding sulfonic derivative obtained with the use of

potassium peroxomonosulphate, through spectrofluorometry (λ_{ex} = 269 nm/λ_{em} = 380 nm). Linear concentration dependence was preserved in the concentration interval (1-8)·10^{-6} mol l^{-1} ET, lg*I* = 97047*c* – 0.003 (r = 0,999). LOQ = 1.1·10^{-6} mol L^{-1}. It was shown that in the determination of ET in ethacyzine, 50 mg tablets (Olainfarm, Latvia), using the developed method, RSD = 1.7% (accuracy, δ = -0.2%) [41].

Other phenothiazines, such as Chlorpromazine, Promethazine, Perphenazine, Trifluoperazine and Thioridazine were also reactive and can be oxidized to the high fluorescent sulfoxides using peroxy acids [42] or Oxone [43-47] (see Scheme 2 for an example of Trifluoperazine). For TFP S-oxide the excitation and emission wave lengths were 274 and 407 nm, respectively. TFP could be determined at the 0.05-4 μg mL^{-1} (I_{fl} = 192,31*c* + 2,8, *r* = 0,999) (Figure 2). The RSD was ≤3.1 % for 0.2-0.5 μg mL^{-1} Trifluoperazine.

Fluorescence spectra of PMH S-oxide, obtained with potassium peroxomonosulfate are shown in Figure 3. The excitation and emission wave lengths for PMH were 317 and 375 nm, respectively. The PMH concentration range in the biological samples was 1-15 μg mL^{-1} (I_{fl} = 65,73c, r = 0,996). RSD < 0.45%; δ = −0.22%…+ 0.36% [45].

Recently, the oxidative derivatization method by means of Oxone for the indirect spectrofluorimetric determination of Prochlorperazine Maleate in pharmaceutical preparations was presented [48]. The excitation and emission wave lengths for PCP S-oxide in 0.01 M sulfuric acid solution were 340



Scheme 2. Mechanism of TFP chemical transformations by means of potassium peroxomonosulfate in acidic medium.

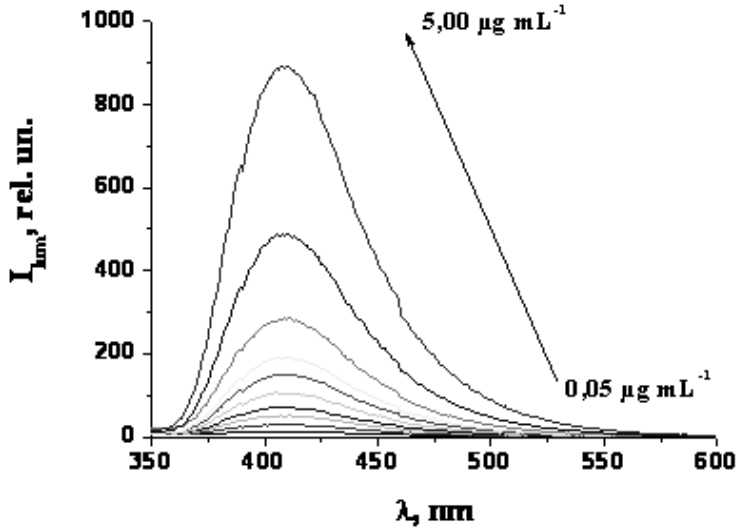


Figure 2. Fluorescence spectra of TFP S-oxide, obtained by means of potassium peroxomonosulfate (λ_{ex} = 274 nm/λ_{em} = 407 nm).

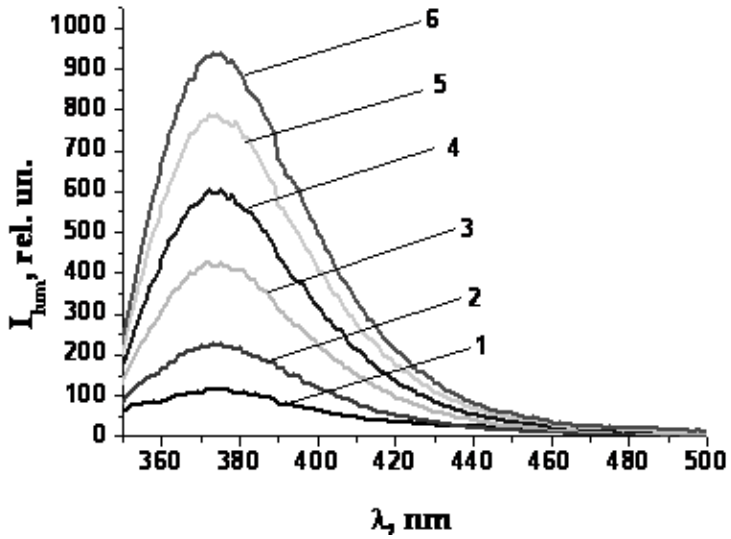


Figure 3. Fluorescence spectra of PMH S-oxide, obtained with potassium peroxomonosulfate. (λ_{ex}= 317 nm/λ_{em} = 375 nm). *c*(PMH S-oxide), μg mL^{-1}: *1*–2,1; *2*–3; *3*–6; *4*–9; *5*–12; *6*–15.

and 380 nm, respectively. The calibration curve is linear in PCP S-oxide concentration range of 0.8-10.0 μg/ml. Limit of quantification (LOQ = 10S) is 0.8 μg·ml^{-1}. The possibility of quantitative determination of Prochlorperazine maleate in Vertinex tablets 5 mg has been shown, RSD <2.3% (δ <RSD).

6. The derivative spectrofluorimetric method

The derivative method has found its applications not only in UV-spectrophotometry but also in fluorimetry. The use of derivative spectrometry is not restricted to special cases, but may be of advantage whenever quantitative study of normal spectra is problematic.

A direct second-derivative spectrofluorimetric procedure for determining sulphoxide impurity in phenothiazines and their formulations has been developed. The method, which has been applied to the analysis of chlorpromazine hydrochloride and prochlorperazine mesylate, as examples of typical phenothiazine substances, and to their formulations, is based on the measurement of the amplitude taken from the minimum at *ca.* 270 nm to the longer wavelength maximum in the second-derivative excitation spectrum of the sulphoxide in pH 8 buffer solution. The method is rapid, accurate and precise, and can be used to measure the concentration of sulphoxide in phenothiazines and their formulations at concentrations down to 0.1% m/m of that of the parent phenothiazine [49].

A thin-layer chromtographic-second derivative spectrofluorimetric procedure has been developed for low levels of sulphoxide (down to 0.01%, w/w) in phenothiazine drug substances and formulations. The method has been applied to the analysis of prochlorperazine maleate, chlorpromazine hydrochloride and promethazine hydrochloride. It has also been applied to the analysis of pharmaceutical formulations of promethazine hydrochloride and comparison of the results with those obtained by a published difference spectrophotometric procedure for promethazine sulphoxide showed that there was good agreement. The method is simple, rapid, accurate and precise and seems to have general application to the determination of low levels of sulphoxide in phenothiazine drug substances or formulations [50].

The development of variable-angle synchronous scanning (v.a.s.s.) in fluorescence spectrometry has been reported, based on a computer-aided spectrofluorimeter. The technique permits a linear path to be scanned at any preselected angle through the emission-excitation matrix defined by (I_{em}, λ_{em}, λ_{ex}), by effectively scanning the emission and the excitation monochromators at different speeds under computer control. When applied to

pharmaceutical dosage forms, v.a.s.s. gave good selectivity for chlorpromazine in the presence of its principal degradation product, chlorpromazine sulphoxide. Good calibration linearity, precision and recovery were observed for both principal drug components. The angle of the scan trajectory can also be varied continuously through the emission-excitation matrix, to describe any desired path under computer control. This novel technique of non-linear v.a.s.s. can provide an improved method for generating diagnostic profiles of drugs and degradation products [51].

Thus, the methods discussed in the above review, based on oxidation reaction of 2- and/or 10-disubstituted phenothiazine derivatives by Oxone and aliphatic diperoxy acids, can be alternatives to the official methods.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. Aronson, J. K. 2015, Elsevier Science, 16th Ed., 7674.
2. Gorog, S. 2018, CRC Press, 405.
3. Pharmacopeia, U. S., XXII, U. and XVII, N. 2009, The United States Pharmacopeia, The National Formulary, United States Pharmacopeial Convention.
4. Georgiynts, V. A., [Bezugly, P. O.], Ukrainets, I. V., Taran, S. C., Blazheyevskiy, M. Ye., Perehoda, L. O., Bevz, N. Yu., Garna, N. V., Gorohova, O. V., Drudko, V. O., Leonova, S. G., Sydorenko, L. V., Hanin, V. A., Burian, G. O., Taran, K. A., Abu Sharkh, A. I., Petrushova, L. O., Dobrova, A. O. 2018, Pharmaceutical analysis : the study guide for students of higher shools. Ed. V. A. Georgiyants – Kharkiv:NUPh : Golden pages, 494 p.
5. The British Pharmacopoeia. 2019, London, United Kingdom, Stationery Office, 6648.
6. Puzanowska-Tarasiewicz, H., Kuzmicka, L., Karpinska, J. and Mielech-Łukasiewicz, K. 2005, Anal. Sci., 21(10), 1149-1153.
7. Udsin, E., Eckert, H. and Forrest, I. S. 1980, Phenothiazines and Structurally Related Drugs: Basic and Clinical Studies, Developments in Neuroscience, Elsevier, Amsterdam, Netherlands, 7, 818.
8. Gupta, R. R. 1988, Phenothiazines and 1,4-Benzothiazines: Chemical and Biomedical Aspects, Bioactive Molecules, Elsevier, Amsterdam, Netherlands, 4, 922.
9. Puzanowska-Tarasiewicz, H., Tarasiewicz, M., Karpinska, J., Kojło, A., Wołyniec, E. and Kleszczewska, E. 1998, Chemia Analityczna, 43(2), 159-178.
10. Srinivasamurthy, K. C. and Seetharamappa, J. 2000, Ind. J. Pharm. Sci., 62(4), 273-276.
11. Basavaiah, K. and Krishnamurthy, G. 1998, Anal. Lett., 31, 1037-1046.
12. Wang, R.-Y. and Lu, Y.-T. 2005, Spectrochim. Acta Part A: Mol. Biomol. Spectrosc., 61(5), 791-1024.
13. Saif, M. J. and Anwar, J. 2005, Talanta, 67(5), 869-872. https://doi.org/10.1016/j.talanta.2005.03.034
14. Basińska, H., Puzanowska-Tarasiewicz, H. and Tarasiewicz, M. 1970, Chem. Anal. [Warsaw, Pol], 15(2), 405-410.
15. Passos, M. L., Saraiva, M. L. M. and Lima, J. L. 2008, Talanta, 77(2), 484-489. https://doi.org/10.1016/j.talanta.2008.03.022
16. Daniel, D. and Gutz, I. G. 2005, J. Pharm. Biomed. Anal., 37(2), 281-286.
17. Davidson, A. G. 1976, J. Pharm. Pharmac., 28(11), 795-800.
18. British Pharmacopeia. 2009, Stationary Office, London, 1-4, 10952.
19. Blazheyevskiy, M. Ye. 2003, Farmats. Zhurn., 1, 64-73.
20. Shlyusar, O. I. and Blazheyevskiy, M. Ye. 2012, News Pharm., 1(69), 51-53.
21. Shlyusar, O. I., Blazheyevskiy, M. Ye. 2012, Farmats. Chasopys, 3(23), 89-92.
22. Shlyusar, O. I. and Blazheyevskiy, M. Ye. 2012, News Pharm., 4(72), 34-38.
23. Shlyusar, O. I., Blazheyevskiy, M. Ye. 2012, Farmats. zhurnal, 4, 71-75.
24. Blazheyevskiy, M. Ye. and Rammal, A. N. 2018, Medychni nauky: istoriya rozvytku, suchasnyy stan ta perspektyvy doslidzhen', L'viv, HO «L'vivs'ka medychna spil'nota», 73-79.
25. Blazheyevskiy, M. Ye. and Moroz, V. P. 2019, French-ukr. J. Chem., 07(02), 52-60. https://doi.org/10.17721/fujcv7i2p52-60

26. Blazheyevskiy, M. Ye. and Kuts, A. A. 2018, Medychni nauky: istoriya rozvytku, suchasnyy stan ta perspektyvy doslidzhen', L'viv, HO «L'vivs'ka medychna spil'nota», 59-65.
27. Blazheyevskiy, M., Kucher, M. and Shpychak, O. 2019, French-ukr. J. Chem., 07(02), 96-103. https://doi.org/10.17721/fujcV7I2P96-103
28. Karpinska, J. 2001, Anal. Sci., 17(2), 249-253.
29. Diehl, G. and Karst, U. J. 2000, J. Chromatogr. A, 890(2), 281-287.
30. Akshatha, H. S. and Gurupadayya, B. M. 2018, Asian J. Pharm. Clin. Res., 11(5), 30-37.
31. Takahashi, D. M. 1980, J. Pharm. Sci., 69(2), 184-187. https://doi.org/10.1002/jps.2600690217.
32. Karpińska, J., Wiszowata, A. and Skoczylas, M. 2006, Anal. Lett., 39(6), 1129-1141. doi:10.1080/00032710600620609.
33. Fasanmade, A. A. and Fell, A. F. 1985, The Analyst, 110(9), 1117-1124. doi:10.1039/an9851001117.
34. Fasanmade, A. A. 1994, Anal. Lett., 27(10), 1955-1977.
35. Clark, B. J. and Fell, A. F. 1983, J. Pharm. Pharmacol., 35, 22.
36. Chen, D., Rios, A., Luque de Castro, M. D. and Valcarcel, M. 1991, The Analyst, 116(2), 171-176. doi:10.1039/an9911600171.
37. De La Peña, L., Gómez-Hens, A. and Pérez-Bendito, D. 1993, J. Pharm. Biomed. Anal., 11(10), 893-896. doi:10.1016/0731-7085(93)80046-4.
38. Gomez-Hens, A. and Perez-Bendito, D. 1991, Anal. Chim. Acta, 242, 147-177.
39. Hornyak, I., Kozma, L., Lapat, A. and Tovari, I. 1997, Spectrofluorimetric Determination of Diethazine and Promethazine in Pharmaceutical Preparations. Biomed. Chromatogr., 11(2), 99-101. doi:10.1002/(SICI)1099-0801(199703)11:2<99::AID-BMC658>3.0.CO;2-W.
40. Chen, D., Rios, A., De Castro, M. L., Valcarcel, M. 1991, Analyst, 116(2), 171-176. doi:10.1039/AN9911600171.
41. Blazheyevskiy, M., Yevtukhov, V. and Doroshenko, A. 2017, GISAP: Phys. Math. Chem., 8, 15-17. doi:http://dx.doi.org/10.18007/gisap:pmc.v0i8.1552.
42. Blazheyevskiy, M. Ye. 2006, Zastosuvannya peroksidnih pohIdnih karbonovih kislot u hImIchnomu analIzI. Doctoral Diss. doc. chem. sci: 02.00.02, Dnipropetrovsk, 524.
43. Blazheyevskiy, M. 2017, Application of derivatization by means of peroxy acid oxidation and perhydrolysis reactions in pharmaceutical analysis (monograph), Ivan Franko LNU, Lviv, 106.
44. Blazhetevskiy, M. Ye., Shlyusar, O. I. and Aleksandrova, D. I. 2010, Farmatsiya Ukrayiny. Poglyad u maybutne, NFaU, Harkiv, 133-134.
45. Shlyusar, O. I., Blazheyevskiy, M. Ye. and Aleksandrova, D. I. 2012, Aktualni pitannya meditsiny: suchasniy stan ta shlyahy rozvitku, GO «Lvivska medichna spilnota», Lviv, 76-78.
46. Shlyusar, O. I., Blazheyevskiy, M. Ye. and Aleksandrova, D. I. 2012, Med. Chem., 2(51), 39-43.
47. Blazheyevskiy, M. Ye. 2016, Farmacom, 3, 28-40.
48. Blazheyevskiy, M. Ye., Skrypynets, Yu. V., Yegorova, A. V. and Antonovich, V. P. 2019, MOCA, 14(3), 134-139. doi: 10.17721/moca.2019.
49. Davidson, A. G. and Fadiran, E. O. 1990, Analyst, 115(7), 997-1001. doi:10.1039/AN9901500997.
50. Fadiran, E. O. and Davidson, A. G. 1988, J. Chromatogr. A, 442, 363-370.
51. Clark, B. J., Fell, A. F., Milne, K. T., Pattie, D. M. G., Williams, M. H. 1985, Anal. Chim. Acta, 170, 35-44. https://doi.org/10.1016/S0003-2670(00)81724-9