# A Difference Spectrophotometric Method for Determination of Alimemazine Based on the Absorbance of its Sulphoxide

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*A new difference spectrophotometric method for the analysis of alimemazine in commercial formulations has been proposed. The method is based on oxidizing the drug with potassium caroate for the formation of corresponding sulfoxide ($\varepsilon = (4.7 \pm 0.06) \cdot 10^3$ L mol$^{-1}$ cm$^{-1}$) and measuring the absorbance of the solution at 342 nm using an unoxidized drug of the same concentration as blank. The graph of Beer's law for alimemazine tartrate showed that the ΔA values measured at the corresponding wavelength of maximum absorbance difference are directly proportional to the drug concentration in oxidized solutions in the range 2-30 μg/mL. The characteristics of the calibration curve were $A = (b \pm \Delta b) \cdot C - (a \pm \Delta a) = (0.0539 \pm 0.0014) \cdot C - 0.017 \pm 0.026$; LOD = 0.68 μg mL$^{-1}$; LOQ = 2.07 μg mL$^{-1}$. The proposed method was successfully applied for the analysis of alimemazine-containing commercial pharmaceutical formulations: Teraligen Valenta tablets 5.0 mg and Theralene 4%, oral solution. RSD ≤ 1.8% (δ= –0.82% and –1.30% respectively).*

**Keywords:** alimemazine, oxone, phenothiazine sulphoxide, spectrophotometry, pharmaceutical analysis, quantitative determination, tablets, oral solution

In recent decades, there has been a tendency to increase the prevalence of psychogenic diseases, such as neurosis and other diseases with mild mental disorders, the occurrence, course, compensation and decompensation of which are determined mainly by psychogenic factors. Among the drugs used to treat such diseases, alimemazine tartrate (Alz) has shown its effectiveness. The drug was first synthesized in 1958 in France in the company's laboratory Theraplix [1]. It refers to aliphatic derivatives of phenothiazine with a simple aliphatic bond [2]. The chemical structure is similar to promethazine and levomepromazine. It differs from promethazine by the presence of an additional methylene group ($CH_2$) in the side chain, and from levomepromazine by the absence of an $OCH_3$ group in position 2 of the phenothiazine core (Fig. 1).

Alimemazine (Trimeprazine, trade names Theralene, Teraligen) is an antipsychotic drug with antihistamine, antispasmodic, dopamine-blocking and moderate α-adrenergic blocking action. It has antiemetic, hypnotic, sedative and antitussive effects [3].

According to pharmacological properties, it occupies an intermediate position between promethazine, which is an antihistamine drug with sedative activity, and the antipsychotic drug chlorpromazine. More active in antihistamine and sedative action than promethazine, and has properties characteristic of chlorpromazine and other phenothiazine antipsychotics. Compared with chlorpromazine, it has a less pronounced adrenoceptor blocking effect; has weak anticholinergic activity.

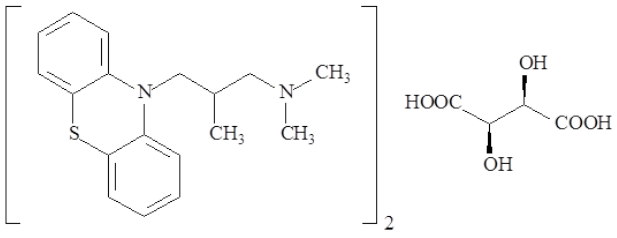


**Fig. 1.** Structural formula of alimemazine tartrate, (2RS)-N,N,2-Trimethyl-3-(10H-phenothiazin-10-yl) propan-1-amine (2R,3R)-2,3-dihydroxybutanedioate (2:1).

The British Pharmacopoeia for the determination of Alz in its pure form proposed acidimetric titration in non-aqueous medium with the end point determination using crystal violet as indicator [4] or potentiometrically [5]. On the other hand, it is recommended using the derivatization spectrophotometry with peroxyacetic acid as oxidizing agent for both oral solution after preliminary extraction and alimemazine tartrate tablets [6].

Known simple direct spectrophotometric methods usually include extraction or strong dilution of the drug followed by absorbance measurement in the ultraviolet region [7]. A direct spectrophotometric procedures lack specificity, determination may be interfered with by other UV-absorbing drugs, dyes, and flavorings, or oxidation products of phenothiazine drugs, which have been shown to be the corresponding phenothiazine sulfoxide and sulfone [8].

The vital importance of this drug has prompted the development of many analytical methods for its

determination. In addition to official various spectrophotometric procedures based on oxidation reactions with the formation of intensely colored radical cations were also developed [9]. However, it is known that the color stability of the radical cation depends mainly on the used oxidizing agent and the acidity of the medium. In the case of a strong oxidizing agent, the color of the radical quickly disappears due to the second step of the reaction leading to the formation of a colorless sulfoxide. This effect can lead to decreased assay sensitivity and reproducibility. Also, some of these methods have some drawbacks, such as a high acidic environment, while others do not have a sufficiently high sensitivity and require a very long heating time [9].

The difference spectrophotometric method of analysis of phenothiazine preparations in various commercial dosage forms deserves attention. The determination can be made as rapidly as by direct spectrophotometry and is specific for the intact phenothiazine preparation. The method includes oxidizing an aliquot of the drug solution with peroxyacetic acid (previously obtained by the reaction of hydrogen peroxide and acetic acid) with the formation of phenothiazine sulfoxide and measuring the absorbance of the solution at 342 nm using an unoxidized drug of the same concentration as blank. The resulting absorbance difference is proportional to the intact phenothiazine drug and is independent of the presence of excipients, degradation products, or other drugs in the formulation. Provided that these substances have not changed under the action of an oxidizing agent, their concentrations in the test and reference solutions are the same, and they have zero difference in absorbance [10].

A post-column chemical derivatization method for the liquid chromatographic determination of phenothiazines has been developed. Peroxyacetic acid is introduced as a derivatizing agent for phenothiazines, giving colored radical cations or fluorescent sulfoxides, depending on the reaction conditions. Both reaction products were successfully used to detect phenothiazines after their liquid chromatographic separation. Fluorescent spectroscopic detection of sulfoxides has proven to be a more reliable and sensitive method. Limits of detection ranged from 4 nM for triflupromazine and trimeprazine to 300 nM for phenothiazine for fluorescent spectroscopic detection of sulfoxide, and from 0.3 μM for phenothiazine and triflupromazine to 2 μM for trifluperazine for spectroscopic detection of the radical cation in the UV-visible region. Calibration functions for the fluorimetric determination of sulfoxide ranged from two to more than three decades, starting from the limit of quantitation [11]. However, peroxyacetic acid is an unstable compound, and its solution is an equilibrium mixture of hydrogen peroxide, acetic acid and, in fact, peroxyacetic acid in water. In addition, the peroxyacetic acid solution has a strong irritating malodor.

Known chromatographic methods have selectivity and sufficient sensitivity to determine the usually low therapeutic concentrations of phenothiazine derivatives in biological fluids. These methods are useful when the sample matrix is complex and the drug concentration is low. In pharmaceutical analysis, where API concentration levels are quite high, the main goal is to develop fast, simple, reproducible and inexpensive methods that can easily be used in routine assays and in quality control laboratories. Spectrophotometry, due to its simplicity, is very useful for the qualitative and quantitative analysis of drugs in pharmaceuticals.

In general, analytical methods for the quantitative determination of alimemazine tartrate are not quite perfect: they require a lot of time, the use of relatively expensive instruments, unstable reagents and toxic solvents, which violates the basic principles of “green chemistry”, which violates the basic principles of “green chemistry”.

It seems promising to carry out the analysis of Alz in the form of the corresponding stable S-oxide, which is easily obtained in a slightly acidic medium using aliphatic diperoxy acids or inorganic Caro peroxoacid [12-13].

The present work describes a new difference spectrophotometric method for determination of Alz in the form of its corresponding sulfoxide obtained with potassium caroate.

## Materials and Methods

*Reagents and instruments*

Thin-layer chromatographic properties of tested compounds were investigated on TLC aluminium plates (10×15 cm) coated with 8-12 μm silica gel, 254 nm PTSH-AF-V-UF (Sorbfil).

NMR spectra were taken on a 300 MHz NMR Spectrometer (Bruker, Germany), solvent $CD_3OD$, internal standard tetramethylsilane.

IR spectra were recorded on a SPECORD M-80 spectrometer, (Zeiss, Germany). Disks were prepared by mixing 200 mg of potassium bromide and 2 mg of the examined compound (1% concentration) followed by compression in the standard manner.

UV measurements were performed using double–beam UV-Visible spectrophotometer, model – UV 1800 (Shimadzu, Japan), UV-Probe 2.62 software and a pair of 1 cm matched quartz cells. Specol 11 spectrophotometer, (Zeiss, Germany) with an EK 5 attachment and 5 cm cells were used also.

Other used instruments: analytical balance RADWAG AS 200/C, pH–meter I-160MI with universal glass electrode, IKA orbital shaker KS4000i.

*Pure standard*

Alimemazine tartrate (alimemazine hemitartrate, $C_{18}H_{22}N_2S \cdot 0.5\ C_4H_6O_6$), Merck (≥98.0% (HPLC, TLC)). White-yellow crystalline powder, odorless, and has a bitter taste. It is freely soluble in water and in acetic acid, sparingly soluble in ethanol, and practically

insoluble in diethyl ether. The pH of a solution of Alz is between 5.0 and 6.5. CAS number: 4330-99-8. Melting point 161°C.

*Pharmaceutical formulations*

Teraligen, film-coated tablets 5 mg 100 pcs. Valenta Pharmaceutical JSC (rf), batch number 1700920. The average weight of the tablet - 0.1675 g. Round biconvex tablets, dark pink with a score; two layers are visible on the transverse section: dark pink and white core.

One tablet contains: *Active substance*: Alimemazine tartrate - 5.0 mg; *Excipients*: lactose monohydrate - 73.4 mg, microcrystalline cellulose - 60.8 mg, pregelatinized starch - 16.0 mg, colloidal silicon dioxide (aerosil) - 1.6 mg, croscarmellose sodium - 1.6 mg, magnesium stearate - 1.6 mg. *Shell composition*: Opadry II 85F34655 - 5.0 mg: partially hydrolyzed polyvinyl alcohol - 40.00 %, macrogol-3350 - 20.20 %, talc - 14.80 %, titanium dioxide E 171 - 19.44 %, carmine dye red E 120 - 4.50 %, aluminum lacquer based on sunset dye yellow E 110 - 1.05%, aluminum lacquer based on indigo carmine E 132 - 0.01 %.

Theralene 4 %, oral solution, 30 ml. LABORATOIRE X.O (France), SN 12339257564780. The active substance is Alz tartrate 5 g, corresponding quantity of Alz base 4 g for 100 mL of oral solution, 1 mL of solution contains 40 mg of Alz base. The other components are: propyl parahydroxybenzoate (E216), methyl parahydroxybenzoate (E218), levomenthol, cochineal red A (E124), raspberry flavor, sodium cyclamate, sucrose solution, ethyl alcohol 96.5°, glycerol, citric acid monohydrate, purified water.

*Chemicals*

Potassium salt of peroxymonosulfuric acid in the form of "Oxone" (Sigma-Aldrich), which is a ternary compound $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$. "Oxone" is a registered trademark of DuPont. Oxone has a longer shelf life than potassium peroxymonosulfate [14]. A white, water-soluble solid, Oxone loses < 1% of its oxidizing power within a month. The standard electrode potential for potassium peroxymonosulfate is +1.81 V with a half-reaction to form hydrosulfate (pH = 0) [15]. All other chemicals used throughout this study were of analytical grade.

*Preparation of S-oxidation product of Alz*

0.05 g of pure Alz was shaken with 25 mL of 0.1 M $H_2SO_4$ and mixed with 5 mL of $2 \cdot 10^{-2}$ mol $L^{-1}$ $KHSO_5$, kept for 2-3 min, and 1 M $NH_4OH$ was added dropwise to pH > 8.5. Then oxidative degradation product was extracted with chloroform (3×30 mL). The mixture was shaken for 10 min on an orbital shaker, left to settle for 10 min until the layers separated, and the organic layer was filtered. The obtained chloroform extractions were combined and further research was carried out.

*Standard solutions*

*Working standard solution (WSS) of Alz*

Exact weight of Alz tartrate CRS, containing 12.50 mg of Alz tartrate ($C_{36}H_{44}N_4S_2$, $C_4H_6O$) were transferred into a 25 ml volumetric flask, 15 ml of 0.1 M $H_2SO_4$ was added, shaken until complete dissolution and adjusted to the mark with the same solvent.

*Stock standard solution of 0.02 M $KHSO_5$*

About 0.7 g of Oxone was dissolved in 70 mL of double-distilled water in a 100 mL volumetric flask, made up to the mark with the same solvent at +20 °C and mixed thoroughly. The exact concentration was determined by iodometric titration. To do this, 10 mL of the solution was taken with a pipette and transferred into a 100 mL volumetric flask. The volume was adjusted to the mark with double-distilled water at +20 °C. Then 10.00 mL of the resulting solution was taken and transferred to a 100 mL conical Erlenmeyer flask, 1 mL of a 0.01 mol $L^{-1}$ $H_2SO_4$ solution was added and, with vigorous stirring, 2 mL of a 5% KI solution. The released free iodine was immediately titrated with 0.01 mol $L^{-1}$ sodium thiosulfate solution. Based on the results of three repeated experiments, the molar concentration of $KHSO_5$ was calculated using the formula:

$$c(KHSO_5) = V(Na_2S_2O_3) \times 0.0100 \times 100.00 / 10.00 \times 10.00 \times 2.$$

*Working standard solution of 0.002 M $KHSO_5$*

10 mL of stock standard solution of 0.02 M $KHSO_5$ was accurately transferred into 100 mL volumetric flasks and completed to volume with double-distilled water.

*Procedure of color reagent selection for Alz and its sulfoxide TLC detection*

Tested solutions of Alz and chloroform extraction of various concentrations were prepared and the corresponding solutions were quantitatively applied by a capillary to the center of a 2×2 cm TLC plate. After solvent evaporation, the appropriate reagents listed in the literature [8, 16] were applied to the same point by a capillary. After a certain time, the formation of color was observed. In parallel, a control experiment was conducted.

*Procedure for calibration curve plotting*

Solution A. Aliquots of 0.10, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 mL of the WSS of Alz were accurately transferred into a set of 25 mL volumetric flasks and 12.5 mL of 0.2 M $H_2SO_4$ was added, 1.5 mL 1.8 mmol $L^{-1}$ $KHSO_5$, adjusted to the mark with distilled water and mixed.

Solution B. 12.5 mL of 0.2 M $H_2SO_4$, 1.5 mL of 1.8 mmol $L^{-1}$ $KHSO_5$ were added to a 25 mL volumetric flask, adjusted to the mark with distilled water and mixed. The absorbance of solution A was registered in a 1 cm cell at 342 nm, using solution B as blank.

*Procedure of calibration curve plotting using differential method*

Solution C. Aliquots of 0.10, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 mL of the WSS of Alz were accurately transferred into a set of 25 mL volumetric flasks, adjusted to the mark with 0.1 M $H_2SO_4$ and mixed. The absorbance of solution A was recorded in

a 5 cm cell at 342 nm, using solution C as blank.

*Application of proposed method for the analysis of tablets Teraligen Valenta JSC "Valenta Pharm" 5.0 mg*

An exact weight of the powder of crushed tablets, 0.4169 g (average weight of the tablet is 0.1666 g) was placed in a 25 mL volumetric flask, 15 mL of 0.1 M $H_2SO_4$ was added, shaken for 10 min, adjusted to the mark with the same solvent and filtered two times. An aliquot of 1.00 mL of obtained solution was transferred into a 25 mL volumetric flask and the same procedure was applied as described under the procedure for calibration curve plotting (Solution A). The absorbance of Solution A was recorded in a 5 cm cuvette at 342 nm, using solution C as blank.

The content of Alz tartrate $(C_{18}H_{22}N_2S)_2 \cdot C_4H_6O_6$ in dosage form as a percentage of the declared amount ($X$) is calculated by the formula:

$$X = (A_1 \cdot a_0 \cdot P \cdot G)/(a_0 \cdot a_1 \cdot L),$$

where $A_1$ is absorbance of the tested solution
$A_0$ is absorbance of Alz tartrate CRS solution
$a_1$ is weight of crushed tablets powder, mg
$a_0$ is weight of Alz tartrate CRS, mg
P is content of Alz tartrate in Alz tartrate CRS, %
G is average weight of tablet, mg
L is declared amount of Alz tartrate per one tablet, mg

*Application of proposed method for the analysis of solution (drops) of Alz 30 ml 40 mg mL$^{-1}$ (calculated on Alz base)*

An accurately measured volume of 0.25 mL of the analyzed solution is transferred into a 25 mL volumetric flask and diluted to the mark with 10 % ethanol solution. Stopper the flask and mix thoroughly. An aliquot of 1.00 mL of obtained solution was transferred into a 25 mL volumetric flask and the same procedure was applied as described under the procedure for calibration curve plotting (Solution A). The absorbance of Solution A was recorded in a 5 cm cuvette at 342 nm, using solution C as blank.

Alz tartrate content calculated on Alz base ($C_{18}H_{22}N_2S$) in dosage form as a percentage of the declared amount ($X$) is calculated by the formula:

$$X = (A \cdot C_{st} \cdot 100\%)/(A_{st} \cdot L \cdot 1.25),$$

where A and $A_{st}$ are absorbance of the test and standard solutions, respectively, at wavelength corresponding to absorbance maximum difference (342 nm)
$C_{st}$ is concentration of the CRS solution in mg per 100 mL
L is declared amount of Alz tartrate calculated on Alz base, mg mL$^{-1}$

## Results and Discussion

It was found that the drug can be determined by a differential spectrophotometric method based on the absorbance of the sulfoxide derivative of the drug in relation to the absorbance of the non-oxidized drug solution. The sulfoxide derivative is formed quickly and quantitatively at room temperature by adding a solution of potassium caroate. The scheme of the oxidation process of Alz with potassium caroate is shown in Fig. 2.

The structure of the formed Alz S-oxidation product (N,N,2-trimethyl-3-(10H-phenothiazin-10-yl sulfoxide) propan-1-amine), which is pale yellow to light yellow solid, was confirmed by melting point (91-94°C), TLC, NMR, IR and UV spectroscopy methods.

$^1$H NMR ($CD_3OD$, 300 MHz): d 7.12–6.9 (m, 8 H, arom), 4.60 (d, J = 7.3 Hz, 2 H, H-15), 2.32 (s, 6 H, H-19, H-20), 2.27 (d, J = 7.4 Hz, 2 H, H-17), 2.13 (m, 1 H, H-16), 1.02 (d, J = 6.0 Hz, 3 H, H-21) ppm.

The resulting sulfoxide was also characterized by IR spectroscopy. The IR spectrum shows a characteristic peak at 1055 cm$^{-1}$ corresponding to the sulfoxide group, which is absent in the spectrum of pure Alz (KBr, cm$^{-1}$): 3416, 2965, 1388, 1301, 1270, 1209, 1055 (S=O), 978, 763.

The UV spectrum of Alz sulfoxide is presented in Fig. 3.

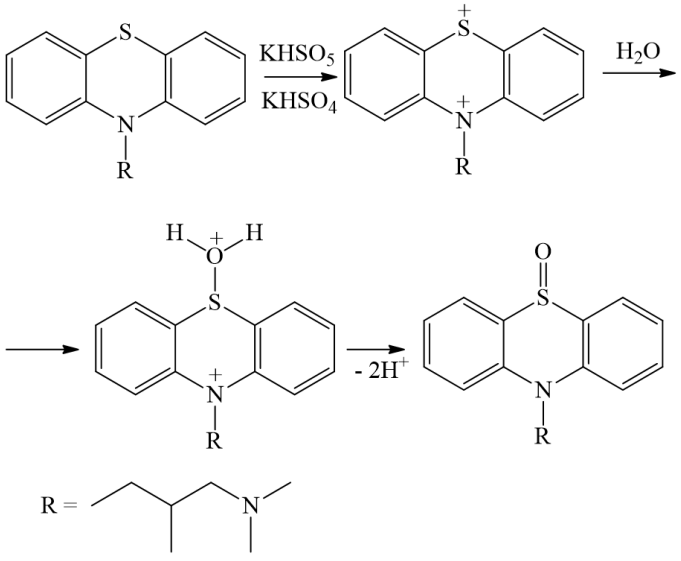


**Fig. 2.** Scheme of the oxidation process of alimemazine with potassium caroate.

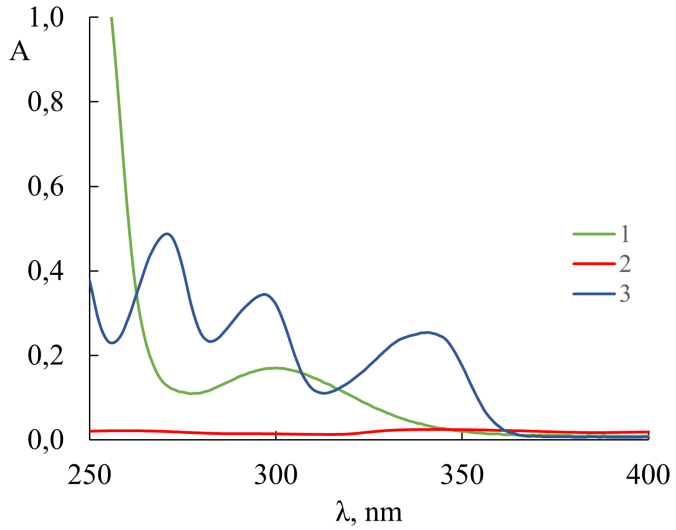


**Fig. 3.** UV spectra of alimemazine hemitartrate ($C_{18}H_{22}N_2S{\cdot}0.5C_4H_6O_6$) (1), $KHSO_5$ (2) and alimemazine S-oxide (3). $c$(Alz) = 53.6 μmol $L^{-1}$; $c(KHSO_5)$ = 108 μmol $L^{-1}$; $c(H_2SO_4)$ = 0.1 mol $L^{-1}$.

Molar absorptivity, defined as the slope of absorbance dependence *vs.* concentration $\varepsilon$ (L $mol^{-1}$ $cm^{-1}$) at $\lambda_{max}$ = 342 nm for Alz sulfoxide solution in 0.1 M $H_2SO_4$ is (4.7 ± 0.06) · $10^3$ L $mol^{-1}$ $cm^{-1}$ (Fig. 4).

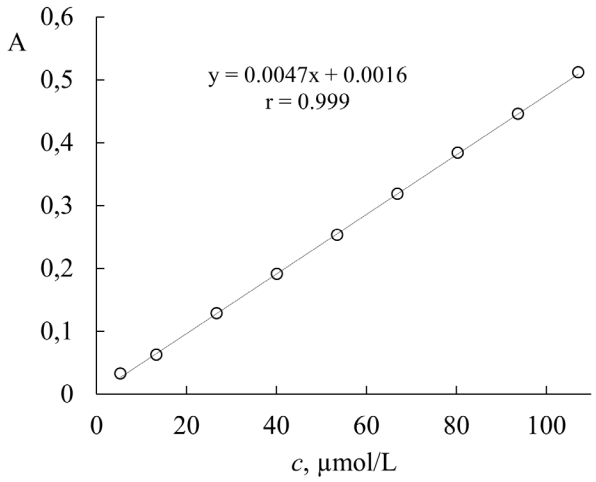


**Fig. 4.** Calibration curve for determination of alimemazine hemitartrate ($C_{18}H_{22}N_2S{\cdot}0.5C_4H_6O_6$), as its sulphoxide *vs* $KHSO_5$ + $H_2SO_4$, $\lambda_{max}$ = 342 nm, $l$ = 1 cm, $c(KHSO_5)$ = 108 μmol $L^{-1}$, $c(H_2SO_4)$ = 0.1 mol $L^{-1}$.

The product formed by the reaction of Alz tartrate with an oxidizing reagent under the conditions of analysis was identified as the sulfoxide by comparison its UV spectrum with the Alz sulfoxide one. Characteristic sulfoxide maxima at 233 nm, 272 nm, 298 nm and 342 nm has been shown. Molar absorptivity ratios of product were as follows ε(233)/ε(342) : (272)/ε(342) : (298)/ε(342) : (342)/ε(342) = 5.3 : 2.0 : 1.4 : 1.0 (see for comparison [17]).

The oxidation product was confirmed as Alz sulfoxide by comparing its Rf values in four TLC systems. The first step in the investigation of chromatographic mobility of Alz and its sulfoxide was the selection of color reagent. Various color producing spraying reagents have been used for the determination of phenothiazines. Results are given in Table 1.

**Table 1.** Identification of alimemazine and its sulfoxide with color reagents.

| № | Reagent | Alime-mazine | Alimemazine sulfoxide |
|---|---|---|---|
| 1. | Dragendorff's reagent | Dark brown | Light brown |
| 2. | Bushard's reagent | Brown | Brown |
| 3. | Wagner's reagent | Brown | Light brown |
| 4. | Froehde reagent | Yellow | Yellow |
| 5. | Schulteze's reagent | Brown | Brown |
| 6. | Mandelin reagent | Blue-violet | Green |
| 7. | Marquis reagent | Violet-red | Yellow-brown |
| 8. | FPN reagent | Red-brown | Brown |

Wagner's reagent was chosen for the treatment and determination of adsorption zones. Considering the basic properties of Alz and its solubility in organic solvents, in the studying of chromatographic mobility, general basic mobile phases and phases, that are given in the literature for phenothiazines, were used. Results are given in Table 2.

It has been shown that Alz and AlzSO have satisfactory chromatographic mobility in all used systems. After processing of corresponding zones, visualization products of Alz and its sulfoxide were stained in brown and light brown. The obtained results indicate that Rf values of the tested sample of Alz in the corresponding mobile phases and color of its spots on the chromatographic plate exactly match the literature

sources [8]. This fact demonstrates the suitability of the applied chromatographic systems. Location of visualization products of Alz and its sulfoxide at the different chromatographic zones confirmed by their Rf values.

**Table 2.** Parameters of the chromatographic mobility of alimemazine and its sulfoxide.

| Mobile phases | Alimemazine | Alimemazine sulfoxide |
|---|---|---|
| | Rf value | |
| Ethyl acetate-methanol-ammonia (75:10:5) | 0.88±0.02 | 0.59±0.01 |
| Acetonitrile-ammonia (90:10) | 0.85±0.02 | 0.64±0.01 |
| Methanol-ammonia (90:1.5) | 0.73±0.02 | 0.43±0.02 |
| Methanol | 0.46±0.01 | 0.28±0.01 |

*Study of the linearity parameters*

Linearity studies were performed over the range of application of the method using model solutions. Beer's law obedience (Fig. 5) for Alz tartrate shows that ΔA values measured at the corresponding wavelength of the absorbance maximum difference are directly proportional to the concentration of the drug in oxidized and non-oxidized solutions in the concentration range of 2-30 μg/mL. The results of calculations of the linear regression equation are reported in Table 3.

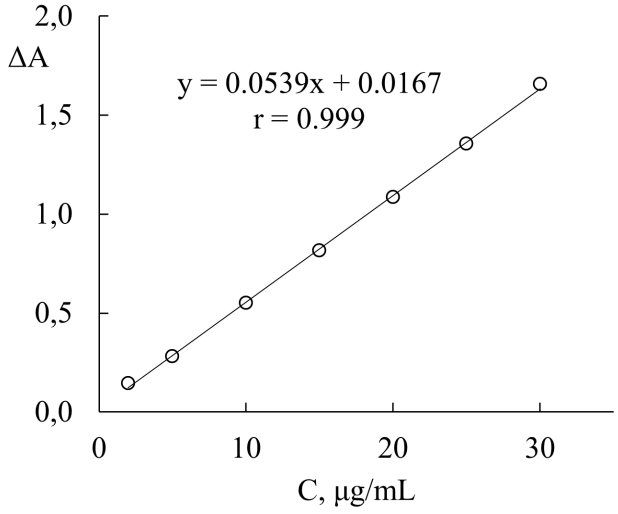


**Fig. 5.** Calibration curve of Alimemazine determination ($C_{36}H_{44}N_4S_2$, $C_4H_6O$), as its sulphoxide *vs* Alimemazine + $H_2SO_4$, $\lambda_{max}$ = 342 nm, *l* = 5 cm, C(Alz) = 2-30 μg mL$^{-1}$, *c*($KHSO_5$) = 108 μmol L$^{-1}$, c($H_2SO_4$) = 0.1 M.

**Table 3.** Regression output.

| Parameter | Data |
|---|---|
| Beer's Law Limit (mg mL$^{-1}$) | 2-30 μg mL$^{-1}$ |
| Regression equation* | A = (0.0539 ± 0.0014) · C - 0.017 ± 0.026 |
| $S_b$ | 0.00062 |
| $S_a$ | 0.011 |
| Correlation coefficient (*r*) | 0.999 |
| LOD (mkg mL$^{-1}$) | 0.68 |
| LOQ (mkg mL$^{-1}$) | 2.07 |

*A = (b ± Δb) · C – (a ± Δa)

*Accuracy and precision*

Accuracy and precision of the developed method were determined at three levels of Alz concentrations. Five replicates of each sample were analyzed. Resulting relative standard deviations did not exceed 1.5% (Table 4), confirming the highly reproducible results and the precision and accuracy of the procedure results. $\delta = (\bar{x} - \mu)\ 100\%/\mu < (t_\alpha \cdot RSD)/\sqrt{n}$.

**Table 4.** Precision and accuracy evaluation (n = 5; P = 0.95).

| Amount taken, μg mL$^{-1}$ | Amount found, μg mL$^{-1}$ | Recovery ± RSD, % | *, % |
|---|---|---|---|
| 5.0 | 5.1±0.1 | 100.9±1.5 | 0.9 |
| 15.0 | 14.9±0.2 | 99.5±0.9 | -0.5 |
| 25.0 | 25.2±0.2 | 100.7±0.8 | 0.7 |

*($\bar{x}$ - μ) 100/μ (%)

This good level of precision was suitable for analysis of Alz in its pharmaceutical dosage forms.

*Specificity*

A number of substances that may be present in dosage forms of phenothiazine formulations, either as degradation products or as a part of combined drug, were examined using absorbance difference assay at analytical wavelength. Alz sulfoxide and excipients of drugs in regulated quantities give zero difference in absorbance and therefore do not interfere with the analysis.

*Application for the analysis of commercial pharmaceutical formulations*

The proposed method was successfully applied for the analysis of Alz-containing commercial pharmaceutical formulations: Teraligen Valenta tablets 5.0 mg, JSC "Valenta Pharm" and Theralene 4% oral solution, Laboratoire X.O (Table 5). RSD ≤ 1.8%; $\delta = (\bar{x} - \mu)\ 100\%/\mu < (t_\alpha \cdot RSD)/\sqrt{n}$.

**Table 5.** Analysis results of alimemazine in pharmaceutical formulations (n = 7; P = 0.95).

| Parameter | Pharmaceutical formulation | |
|---|---|---|
| | Teraligen tablets, 5 mg | Theralene 4%, oral solution, 40 mg $mL^{-1}$ |
| Labelled amount (μ*) | 4.90 mg | 39.6 mg $mL^{-1}$ |
| Found (± Δ) | 4.86 ± 0.09 mg | 39.1 ± 0.8 mg $mL^{-1}$ |
| Recovery ± RSD, % | 99.18 ± 1.62 | 98.70 ± 1.76 |
| δ, % | - 0.82 | - 1.30 |

*μ was determined according to reference standard pharmacopoeial method [4].

*Greenness profile assessment*

Considering that most of the described analytical methods of Alz determination in its dosage forms were not «green», it was necessary to develop an analytical procedure for the determination of Alz in tablets and oral drops which will meet the principles of «green» chemistry. Assessment of the impact of analytical method on the environment was performed using AGREE (Analytical GREEnness) tool [18] and analytical eco-scale [19]. The result of the evaluation of analytical method using AGREE tool is given in Fig. 6.

According to GAC principles, the environmental impact of the method meets the requirements of excellent «green» analysis with analytical eco-scale total score of 86 (Table 6).

**Table 6.** Analytical eco-scale for assessing the «greenness».

| Parameters | Penalty points |
|---|---|
| Reagents | |
| Oxone | 6 |
| Sulfuric acid | 2 |
| Instruments | |
| Energy | 1 |
| Waste | 5 |
| Total penalty points: 14 | |
| Analytical Eco-Scale total score: 86 | |
| Conclusion | excellent «green» analysis |

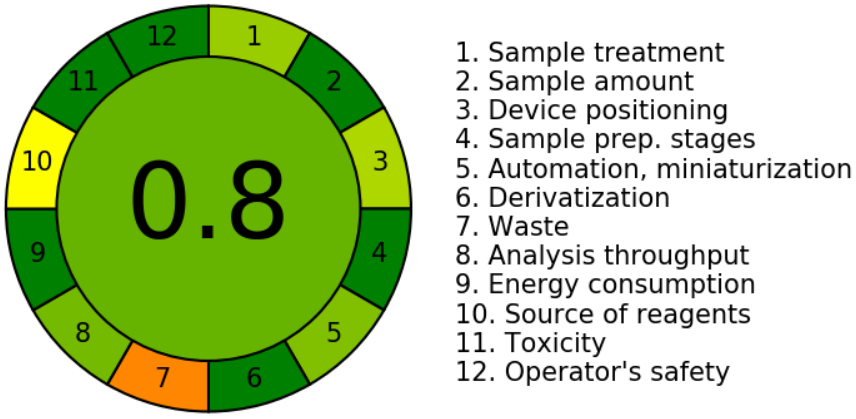


**Fig. 6.** Pictogram of analytical method according AGREE tool.

## Conclusions

A new difference spectrophotometric method for the determination of alimemazine based on the absorbance of its sulphoxide using a new derivatizing reagent, Oxone, was developed. The formation of S-oxidation product - alimemazine sulfoxide was confirmed. The analytical performance of the proposed procedure was validated with respect to accuracy, precision, linearity, LOD and LOQ for alimemazine determination in pure substance with satisfactory results. The possibility of quantitative determination of alimemazine hydrotartrate in Teraligen, tablets 5 mg, Valenta Pharmaceutical JSC (RSD ≤ 1.6 %, δ = -0.82 %) and in Theralene 4%, oral solution, 30 ml Laboratoire X.O (RSD ≤ 1.8 %, δ = -1.30 %) was shown. $\delta = (\bar{x} - \mu)\ 100\%/\mu < (t_{\alpha} \cdot RSD)/\sqrt{n}$. According to GAC principles, the environmental impact of the method responds the requirements of excellent «green» analysis with analytical eco-scale total score of 86.

## Conflicts of Interest

The authors declare no conflict of interest.

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