

The Alternative Spectrofluorometric Method for the Determination of Alimemazine in Pharmaceutical Preparations

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Abstract: This study introduces an oxidative derivatization procedure enabling the indirect spectrofluorometric assessment of alimemazine tartrate (ALZ). ALZ was derivatized using potassium hydrogen peroxomonosulphate (Oxone®) to yield a strongly fluorescent sulfoxide. A fast, simple, and highly sensitive fluorescence method for ALZ tartrate determination was developed based on the emission from its oxone-oxidized product in 0.05 M sulfuric acid ($\lambda_{\text{ex}} = 340 \text{ nm}$; $\lambda_{\text{em}} = 380 \text{ nm}$). The calibration curve displayed linearity across the concentration range of 0.1–13.5 $\mu\text{g/mL}$, and the LOQ (10S) was 0.42 $\mu\text{g/mL}$, demonstrating the potential for a quantitative assay in pharmaceutical formulations, such as the film-coated tablets Theralen® 5 mg and Teraligen® 5 mg, Theralen® 4% oral drops ($|\frac{(\bar{x} - \mu)}{100/\mu}| < \text{RSD } t_{\alpha/\sqrt{n}}$), supporting its suitability for routine quality control and pharmaceutical analysis.

Keywords: spectrofluorimetry; potassium caroate; oxone; determination; alimemazine tartrate; alimemazine sulphoxide; pharmaceuticals.

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1. Introduction

Alimemazine (ALZ, trimeprazine), which is marketed under the names Theralen and Teraligen, is an antipsychotic medication that has antihistaminic, antispasmodic, dopamine-blocking, and moderate α -adrenergic blocking properties, in addition to antiemetic, hypnotic, sedative, and antitussive effects [1].

The chemical structure of the compound is comparable to that of promethazine and levomepromazine. It differs from promethazine by incorporating an additional methylene (CH_2) group in the side chain and from levomepromazine by the absence of a methoxy (OCH_3) substituent at the C-2 position of the phenothiazine ring system (Figure 1).

The British Pharmacopoeia suggests the use of acidimetric titration in a non-aqueous medium for the determination of ALZ in its pure form [2]. It also recommends derivatization spectrophotometry utilizing peroxyacetic acid as an oxidizing agent for ALZ tartrate tablets [3] and oral solutions [4]. This method minimizes interference from excipients or oxidation products during the assay.

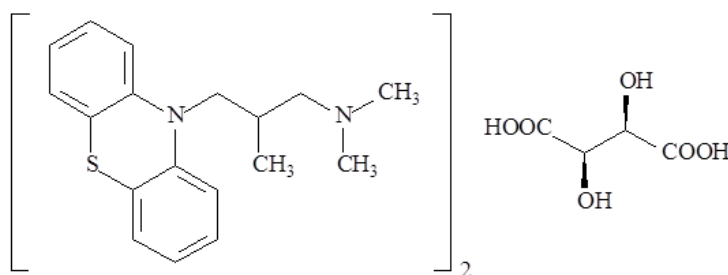


Figure 1. Structural formula of ALZ tartrate, (2RS)-N,N,2-trimethyl-3-(10H-phenothiazin-10-yl)propan-1-amine (2R,3R)-2,3-dihydroxybutanedioate (2:1).

The liquid chromatographic analysis of ALZ was accomplished by developing a post-column chemical derivatization technique. Peroxyacetic acid served as the derivatization agent, producing either a chromophoric radical cation or a fluorescent sulfoxide, depending on the experimental parameters. Both products were effectively employed to detect ALZ following chromatographic separation. Post-column derivatization results in a small peak area in the chromatogram due to the radical cation's low molar absorption and instability. As expected, the instability of phenothiazine radicals makes determination on the basis of sulfoxide fluorescence the most reliable and sensitive method [5].

Owing to its instability, peroxyacetic acid in water remains in equilibrium with hydrogen peroxide and acetic acid, along with a portion of the intact acid. Aqueous solutions of this oxidizing agent are also associated with a strong, pungent, and irritating smell.

In general, analytical methods for the quantitative determination of ALZ are not ideal; they require the use of an unstable oxidizing agent, a peracetic acid solution prepared in situ, and toxic solvents, which violate the basic principles of "green chemistry".

We previously developed a differential spectrophotometric method for the analysis of ALZ in the form of its sulfoxide obtained by using stable, commercially available Oxone® [6].

Oxone® is a stable ternary compound $2\text{KHSO}_5 \cdot \text{KHSO}_4 \cdot \text{K}_2\text{SO}_4$ [7]. An acidic medium releases free KHSO_5 , a strong oxidizing agent with an oxidation potential of +1.81 V, and undergoes a half-reaction to form hydrogen sulfate (pH = 0) [8]. Oxone has already proven to be a very promising analytical reagent for the analysis of drugs [9,10], particularly phenothiazine derivatives [6,11–16]. Our research introduces, for the first time, the use of KHSO_5 as a selective derivatizing agent to generate a stable, highly fluorescent sulfoxide derivative of ALZ for conducting fluorescence-based analysis of the compound.

This work aimed to develop a new method for the quantitative determination of ALZ in drugs using fluorescence spectroscopy with KHSO_5 as the analytical reagent.

2. Materials and Methods

2.1. Reagents.

Alimemazine tartrate, Syn. alimemazine hemitartrate ($\text{C}_{18}\text{H}_{22}\text{N}_2\text{S}_{0.5}\text{C}_4\text{H}_6\text{O}_6$) (Merck), $\geq 98.0\%$ (HPLC, TLC) was used as the working standard.

Dosage forms were collected from commercial sources.

Alimemazine tartrate (6.25 mg, equivalent to 5.0 mg ALZ base), LOT: LC309; Expiry: 31-10-2027; SN: 36114290886463.

Theralen tablets were film-coated with 5 mg No. 50 for oral use. Composition: One tablet contained 5 mg ALZ. Manufacturer: LABORATOIRE X.O. (France).

Teraligen film-coated tablets (5 mg, 100 pcs) manufactured by Valenta Pharmaceutical JSC, series 1700920, were examined. The tablets are round, biconvex, dark pink, and scored, with a visible two-layer structure on the cross-section: a dark pink shell and a white core. The average weight of a tablet is 0.1675 g. Each tablet contains 5.0 mg of the active ingredient ALZ tartrate and the following excipients: lactose monohydrate (73.4 mg), microcrystalline cellulose (60.8 mg), pregelatinized starch (16.0 mg), colloidal silicon dioxide (1.6 mg), croscarmellose sodium (1.6 mg), and magnesium stearate (1.6 mg). The coating (Opadry II 85F34655, 5.0 mg) comprises partially hydrolyzed polyvinyl alcohol (40.00%), macrogol-3350 (20.20%), talc (14.80%), titanium dioxide E171 (19.44%), carmine red E120 (4.50%), and aluminum lacquers based on sunset yellow E110 (1.05%) and indigo carmine E132 (0.01%).

Theralen[®] 4% ALZ (tartrate) 30 mL 4 % oral (solution) drops. Composition: ALZ tartrate - 5 g (quantitatively corresponding to 4 g of ALZ base), 100 mL; *Excipients*: propyl p-hydroxybenzoate (E 216), methyl p-hydroxybenzoate (E 218), cochineal red A (E 124), sucrose solution, and ethanol (alcohol). The strength of alcohol by volume is 10.7% (vol/vol). Manufacturer: Laboratoire X.O. (France). LOT: LA071; PC: 03400931053088; SN: 12330257564780.

According to the Certificate of Analysis, the average content of the drug (ALZ active substance) was 3.96 % of the ALZ base (limits of not less than 3.8 % and not more than 4.2 %, that is 95-105 %).

Reagent. In the experiments, oxone was used to oxidize ALZ to its S-oxide. The active ingredient of oxone is KHSO (CAS 10058-23-8). It is highly soluble in water, with a solubility exceeding 250 g/L at 20°C. This oxidation process is also regarded as "green", as it does not produce toxic byproducts. A 0.005 mol/L solution of potassium caroate was made by stirring a portion of approximately 0.15-0.2 g of oxone with 100 mL of double-distilled water and standardizing it iodometrically [17].

A 0.5 mol/L sulfuric acid solution was prepared from standard titer fixanal.

Throughout the study, all additional chemicals utilized were of analytical grade.

2.2. Apparatus.

UV electronic absorption spectra were measured via a Specord 200 spectrophotometer (Analytik Jena, Germany). Luminescence excitation and luminescence spectra were recorded using a CaryEclipse spectrofluorometer (Agilent Technologies, USA) with excitation and emission wavelengths of 340 nm and 380 nm, respectively. Measurements were performed with 1 cm quartz cells. An ANG50C analytical balance (Axis, Ukraine) was used.

2.3. Procedures.

2.3.1. Sample preparation.

A 0.5 mg/mL stock solution of ALZ in double-distilled water was prepared. An accurate weight of the ALZ pure substance, containing 50.0 mg of ALZ tartrate, was dissolved in 100.0 mL of double-distilled water. This solution was diluted with double-distilled water to yield a 0.1 mg/mL ALZ working solution.

2.3.2. Calibration graph.

Defined portions of the 0.1 mg/mL ALZ working solution were pipetted into 25 mL standard flasks. Then, 2.5 mL of 0.5 mol/L H₂SO₄ and 0.4 mL of 5 mmol/L KHSO₅ were sequentially added to each flask, and the solutions were diluted to the mark with double-distilled water and mixed. After 5 min, the fluorescence was measured. The fluorescence intensity was plotted against the amount of ALZ.

2.3.3. Procedures for dosage forms.

Tablets. 20 tablets were weighed and crushed. A precise quantity of powder, equivalent to around 5 mg of ALZ, was measured and transferred into a small conical flask, 30 mL of double-distilled water was added, the mixture was stirred for 15 min, and filtered into a 50 mL standard flask, the residue was washed with double-distilled water, the combined solution was diluted, and the mixture was washed to a volume of 50.0 mL with the same solvent. A suitable volume was analyzed as described above.

The content of ALZ tartrate (C₁₈H₂₂N₂S)₂·C₄H₆O₆ in the medicinal form as a percentage of the stated amount (*X*) was calculated via formula (1):

$$X = \frac{I_1 \cdot a_0 \cdot P \cdot G}{I_0 \cdot a_1 \cdot L} \quad (1)$$

Where *I*₁ is relative fluorescence intensity of the test solution; *I*₀ is relative fluorescence intensity of the solution of the standard sample of ALZ; *a*₁ is weight of powder of ground tablets, mg; *a*₀ is weight of the standard sample of ALZ, mg; *P* is content of ALZ tartrate in the standard sample of ALZ, %; *G* is average weight of one tablet, mg; *L* is amount of ALZ in one tablet, mg.

Oral solution (drops). An accurately measured 1.00 mL solution was transferred to a 500 mL standard flask and diluted to the mark using a 10% ethanol solution. The flask was sealed and thoroughly mixed. An accurately measured volume equivalent to ≈ 0.1 mg ALZ was transferred to a 25 mL standard flask. Next, 2.5 mL of 0.5 mol/L sulfuric acid was added, followed by 0.4 mL of 5 mmol/L KHSO₅, made up to the mark with double-distilled water, and mixed. After 3 min, the fluorescence was measured.

A similar experiment was carried out with a standard working ALZ solution (*c* = 0.1 mg/mL).

Standard reference solution. The reference preparation of ALZ (approximately 50 mg (exact weight 51.08 mg), which is equivalent to 40 mg of ALZ base) was dissolved in water and brought to 100 mL.

The content of ALZ tartrate in terms of ALZ base (C₁₈H₂₂N₂S) in the preparation, as a percentage of the declared amount (*X*), was calculated via formula (2):

$$X = \frac{I \times C_{st} \times 100\%}{I_{st} \times L \times 1.25} \quad (2)$$

Where *I* and *I*_{st} are the relative fluorescence intensities in the experiments with the test and standard solutions, respectively; *C*_{st} is the concentration of the standard reference solution in mg per 100 mL; *L* is the declared amount of ALZ tartrate in terms of ALZ base in 1 mL of solution, mg.

3. Results and Discussion

The product of the ALZ S-oxidation was identified as the sulfoxide, and the S-oxidation pathway of ALZ by KHSO_5 was illustrated in our previous work [6].

3.1. Selection of detection wavelengths.

The absorption (Figure 2) and fluorescence excitation (Figure 3) spectra of the ALZ solution after oxidation with oxone are presented. The absorption spectrum of ALZ in a 0.05 M sulfuric acid solution shows multiple bands in the UV range, peaking at 233, 272, 298, and 342 nanometers.

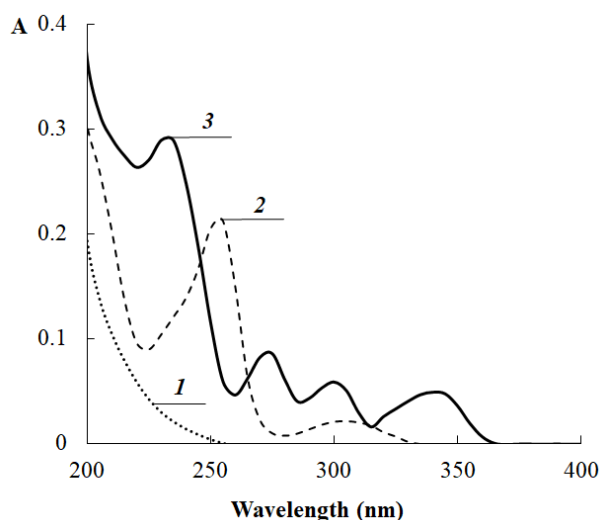


Figure 2. UV absorption spectra of (1) oxone; (2) ALZ tartrate; and the product of its oxidation by (3) KHSO_5 ($c(\text{H}_2\text{SO}_4) = 50 \text{ mmol/L}$, $c(\text{KHSO}_5) = 80 \text{ }\mu\text{mol/L}$, $c(\text{ALZ}) = 5 \text{ }\mu\text{g/mL}$, $l = 1 \text{ cm}$).

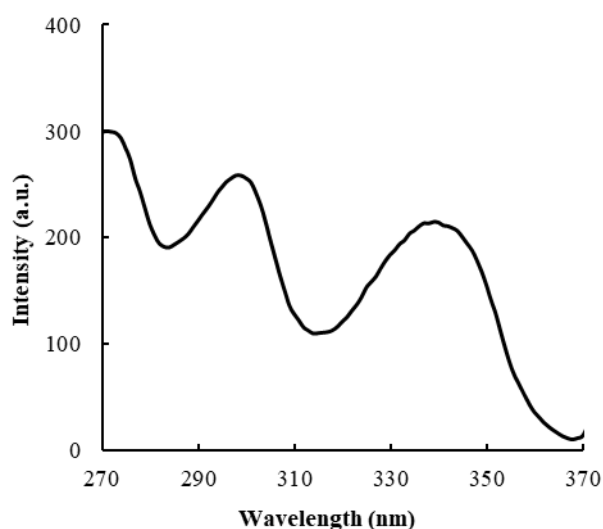


Figure 3. Fluorescence excitation spectra of ALZ solution after oxidation with oxone ($c = 5.0 \text{ }\mu\text{g/mL}$, slits 5-5; $\lambda_{\text{em}} = 380 \text{ nm}$).

As shown in Figure 3, the fluorescence excitation spectrum of a solution of ALZ after oxidation with oxone in the region of 270–400 nm is similar to its absorption spectrum.

Figure 4 shows the fluorescence spectra of the oxidation products of ALZ tartrate with caroate as a function of the concentration of ALZ.

The fluorescence developed immediately, and the maximum intensity remained stable for 1.5 h (no signal decay).

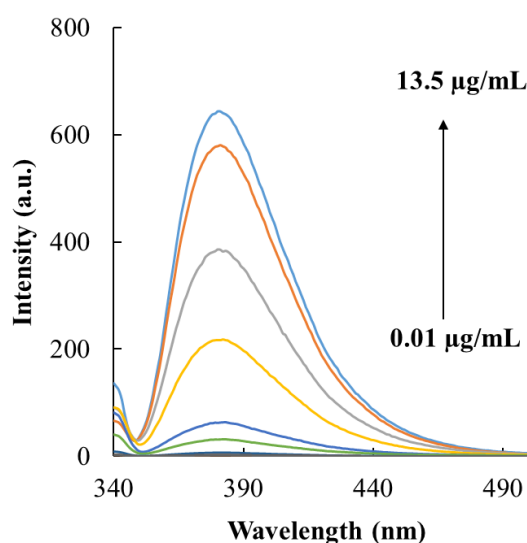


Figure 4. Fluorescence spectra of the product of oxidation of ALZ tartrate by caroate ($c(\text{H}_2\text{SO}_4) = 50 \text{ mmol/L}$, $c(\text{KHSO}_5) = 80 \text{ }\mu\text{mol/L}$, $l = 1 \text{ cm}$, $\lambda_{\text{ex}} = 340 \text{ nm}$).

ALZ S-oxide has a relative fluorescence intensity in aqueous solutions with a maximum excitation wavelength of 340 nm.

The absorption spectrum was obtained to determine the wavelength ranges to be used for the excitation spectrum measurements. The long-wavelength peak detected at 340 nm appears to be concentration-independent and identical to that in the absorption band. The dependence of the fluorescence intensity, I_f , on the molar concentration, c , can be written as:

$$I_f = kQI_0(1 - e^{-\epsilon lc}) \quad (3)$$

Where k is a constant, Q is the quantum efficiency, I_0 is the intensity of incident radiation, l is the cell length, c is the molar concentration, and ϵ is the molar absorptivity of the compound [18].

For very dilute solutions, this equation reduces to the following:

$$I_f = kQI_0\epsilon lc \quad (4)$$

3.2. Validation of the proposed method.

Validation of the developed techniques was carried out in accordance with the ICH guidelines [19]. The validation parameters were limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, linearity, and range.

3.2.1. The linearity parameters study.

The general analytical procedure was applied to 7 standard solutions of ALZ under investigation at varying concentrations. The calibration graph for the spectrofluorometric determination of the ALZ in the form of the corresponding sulfoxide is shown in Figure 5. The calibration graph is linear ($R^2 = 0.999$) in the range of ALZ tartrate concentrations of 0.1–13.5

µg/mL and is described by the regression equation $I = (47.82 \pm 0.69) \cdot c(\text{ALZ})$. The detection limit was 0.14 µg/mL (Table 1).

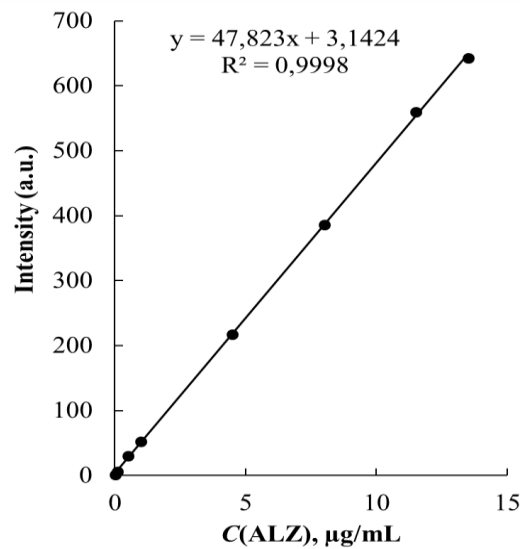


Figure 5. Calibration graph for the spectrofluorometric determination of ALZ tartrate in the form of the corresponding sulfoxide ($c(\text{H}_2\text{SO}_4) = 50 \text{ mmol/L}$, $c(\text{KHSO}_5) = 80 \text{ µmol/L}$, $l = 1 \text{ cm}$, $\lambda_{\text{ex}} = 340 \text{ nm}$).

Table 1. Regression output.

Parameter	Data
$y = a + bx$	$y = 3.14 + 47.82x$
Correlation coefficient R^2	0.9998
Linear regression equation	$I = 47.82 c$
Slope ($b \pm \Delta b$)	47.82 ± 0.69
Intercept ($a \pm \Delta a$)	3.14 ± 4.89
SD of slope (S_b)	0.28
SD of intercept (S_a)	2.00
LOD	0.14 µg/mL
LOQ	0.42 µg/mL

3.2.2. LOD and LOQ.

The lowest amount of a substance that can be identified in a sample is known as LOD. The smallest amount of analyte that can be measured in a sample with a high level of accuracy and precision is the LOQ. By applying the formulas $\text{LOD} = 3.3(S_a)/b$ and $\text{LOQ} = 10(S_a)/b$, (S_a and b are from Table 1), the LOD and LOQ were determined (Table 1).

3.2.3. Accuracy and precision.

The method's precision was evaluated by analyzing ALZ solutions at three levels (3.0, 5.00, and 10.0 µg/mL) in triplicate to ensure accuracy. The mean recoveries ranged from 101.0 to 102.0 %, with standard deviations ranging from 1.6 to 2.1 %. $|\delta| < \text{RSD } t_{\alpha/\sqrt{n}}$ (Table 2).

Table 2. Evaluation of precision and accuracy ($n = 5$; $P = 0.95$).

Amount taken (μ), µg/mL	Amount found, ($\bar{x} \pm \Delta \bar{x}$), µg/mL	Recovery \pm RSD, %	δ^* , %
3.0	3.05 ± 0.08	101.7 ± 2.1	1.7
5.0	5.1 ± 0.12	102.0 ± 1.9	2.0
10.0	10.1 ± 0.2	101.0 ± 1.6	1.0

* $\delta = (\bar{x} - \mu) 100/\mu$ (%); μ was determined according to the reference standard pharmacopoeial method [2].

3.3. Practical application in pharmaceutical preparations.

The developed analysis method was tested in the determination of ALZ tartrate as an active ingredient in pharmaceutical preparations, namely Theralen and Teraligen tablets, as well as in the oral solution (drops) Theralen 4%. Table 3 provides the obtained analytical data.

According to the Certificates of Analysis, the average content of Alimemazine base in Teralen® oral solution 4% is 3.96% (limits: not less than 3.8 and not more than 4.2 %, i.e., 95-105 %), and in Teralen® Tablets film-coated 5 mg – 5.0 mg/tablet; Teraligen, film-coated tablets 5 mg – 4.86 mg/tablet.

Table 3. Results of quantitative determination of ALZ in test samples.

Pharmaceutical preparation	Taken	Found	Statistical characteristics (P = 0.95)
Theralen® Tablets film-coated 5 mg No. 50 (LABORATOIRE X.O. (France))	0.1557 g (5.0 mg/tablet)*	mg/tabl	$\bar{x} \pm \Delta \bar{x} = 5.05 \pm 0.10$ RSD = ± 2.17 % $\delta^* = +1.0$ %
		4.890	
		5.035	
		5.215	
		5.040	
		5.165	
		5.000	
Theralen® oral solution 4%, 30 mL (SANOFI - AVENTIS FRANCE (France))	1.00 mL (3.96 %)*	mg/mL	$\bar{x} \pm \Delta \bar{x} = 40.00 \pm 0.48$ RSD = ± 1.31 % $\delta^* = +1.01$ %
		40.84	
		40.04	
		40.43	
		39.40	
		39.62	
		39.51	
Teraligen, film-coated tablets 5 mg 100 pcs Valenta Pharmaceutical JSC, series 1700920	0.1675 g (4.86 mg/ tablet)**	mg/tabl	$\bar{x} \pm \Delta \bar{x} = 4.88 \pm 0.09$ RSD = ± 1.95 % $\delta^* = +0.32$ % $\delta^{**} = +0.41$ %
		4.90	
		4.80	
		4.87	
		4.93	
		4.71	
		4.92	
5.00			

$\delta = (\bar{x} - \mu) \cdot 100/\mu$; μ was determined according to * quality certificate data, ** the average content found by the reference method [6].

Standard pharmacopoeial procedures for analyzing phenothiazines in bulk or dosage forms recommend absorbance measurements at specific wavelengths or non-aqueous titration. These methods typically involve elaborate purification steps, while direct UV-spectrophotometry is disadvantaged by its sensitivity to excipients usually contained in pharmaceutical products.

A well-known spectrophotometric method [6] for quantitative determination allows for the determination of trimeprazine tartrate in a concentration range of 0.5–40 $\mu\text{g/mL}$ (LOQ = 0.5 $\mu\text{g/mL}$).

3.4. Selectivity.

The following substances produce zero in fluorescence intensity and therefore do not interfere with the assay (placebo experiments): in relation to 5.0 mg of the active substance - ALZ tartrate: 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; 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%.

Spectrofluorimetric determination of ALZ in tablets of 5 mg, as well as 4 % oral solution in the presence of several auxiliary substances, with the corresponding sulfoxide obtained with KHSO₅, is more sensitive, faster, and less time-consuming compared to spectrophotometric methods based on the formation of S-oxidation products, as well as a more straightforward HPLC technique recommended by Ph Eur. The LOQ is 0.42 µg/mL, which is one and a half orders of magnitude lower than in spectrophotometric determination (LOQ = 2.07 µg/mL).

4. Conclusions

In this study, a novel, rapid, and environmentally friendly spectrofluorimetric method was developed for the quantitative determination of ALZ tartrate based on oxidative derivatization with Oxone®. The proposed method offers high sensitivity, selectivity, and simplicity, providing a linear response in the concentration range of 0.1–13.5 µg/mL and an LOQ of 0.42 µg/mL. Compared to traditional spectrophotometric and chromatographic procedures recommended by pharmacopeias, the oxone-based fluorescence approach offers good analytical performance, reduced analysis time, and improved compliance with the principles of green chemistry, owing to the use of a stable, non-toxic oxidizing reagent.

The developed method proved suitable for the ALZ determination in different pharmaceutical forms, including tablets and oral drops, with minimal interference from excipients, RSD < 2.2 %; $|(\bar{x} - \mu) 100/\mu| < \text{RSD } t_{\alpha}/\sqrt{n}$. The high sensitivity of the method additionally allows controlling the admixture of sulfoxide in preparations in a “blank” experiment. The formation of a strongly fluorescent sulfoxide derivative enabled sensitive quantification and provided a viable alternative to methods that employ unstable oxidants, such as peroxyacetic acid.

Future research should focus on further optimizing the reaction conditions to enhance fluorescence intensity and stability, as well as on validating the method across diverse pharmaceutical matrices and biological fluids to assess its applicability in pharmacokinetic and bioavailability studies.

Moreover, the oxidative derivatization mechanism of ALZ with oxone warrants deeper investigation to explore its potential extension to other phenothiazine derivatives. The incorporation of formal green analytical chemistry evaluation tools in future studies would provide quantitative confirmation of the environmental benefits of this method.

Overall, the proposed fluorescence spectroscopic procedure represents a sensitive, reliable, and sustainable analytical method for determining ALZ tartrate in pharmaceutical products. With further validation (including robustness and inter-laboratory testing, as well as intra-day and inter-day testing) and expansion, it holds promise for routine quality control applications and for integration into regulatory and industrial analytical protocols, advancing both analytical performance and environmental responsibility in pharmaceutical analysis.

Author Contributions

Investigation, O.Z. and D.S.; methodology, O.M. and M.B.; formal analysis, O.M. and M.B.; visualization, M.B. and D.S.; writing—original draft preparation, M.B. and D.S.; writing—

review and editing, O.M. and O.Z. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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