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ARTICLE

Infrared and Electronic Spectroscopy for Assay of Dosulepin in Pharmaceuticals: *Stability Indicating Study and Quantification Approach*

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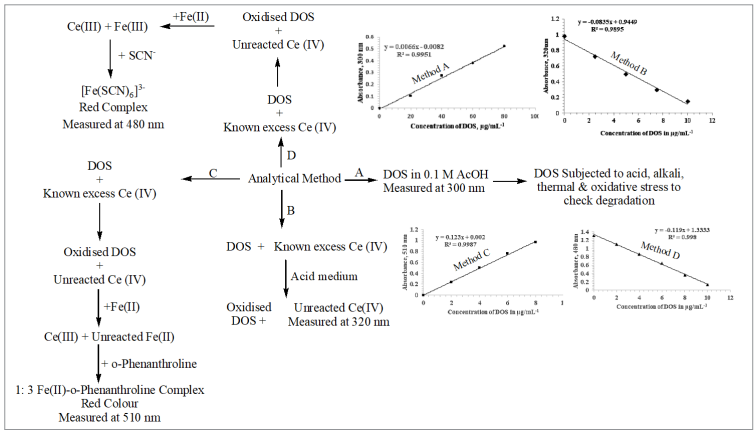

Four simple, precise, and cost-effective spectrophotometric methods were designed and validated to assess Dosulepin hydrochloride (DOS) in pure and dosage form. Two of them are direct UV (Methods A and B), and the other two are indirect visible spectrophotometric methods (Methods C and D). Method A is based on the measurement of the chromophoric activity of DOS in 0.1 M acetic acid (AcOH) at 300 nm. Method B involves the measurement of absorbance due to cerium (IV) left in excess after oxidizing DOS at 320 nm. The unreacted cerium (IV) was treated with a large excess of iron (II), which results in iron (III) and cerium (III). The surplus iron (II) forms a red colored complex with o-phenanthroline at a slightly higher pH was measured at 510 nm in Method C. In Method D the iron (III) formed in the redox reaction between unreacted cerium (IV) and iron (II) was made to form a red colour complex with thiocyanate and measured at 480 nm. The methods are applicable over good linear ranges of 1.0-80.0, 0.25-10.0, 0.5-8.0 and 0.50-10.0 µg mL^{-1} with actual molar absorptivity values of 2.07×10^3, 3.11×10^4, 4.08×10^4 and 3.7×10^4 L $mol^{-1}cm^{-1}$ for Method A, B, C and D, respectively. The validating parameters like limit of detection (LOD), quantification (LOQ), Sandell sensitivity and others have been reported. The methods proposed were successfully applied to quantify DOS in pharmaceuticals. The Fourier Transform Infrared (FT-IR) spectra of the post degradation DOS were studied, compared with that of pure drug and reached to the possible effect of degradation to stress by stability indicating property of Method A.

Keywords: Dosulepin hydrochloride, cerium (IV), spectrophotometry, chromophore, pharmaceuticals

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INTRODUCTION

Dosulepin hydrochloride (DOS), also referred as dothiepin hydrochloride (Figure 1),[1] is (E)-3-(dibenzo[b,e] thiepin-11(6H)-ylidene)-N,N dimethyl propan-1-amine, hydrochloride. It is a tricyclic antidepressant that is used to treat endogenous depression and has anxiolytic qualities as well. DOS helps in relieving depression by preventing the reabsorption of serotonin and noradrenaline into the nerve cells of the brain.[2-4]

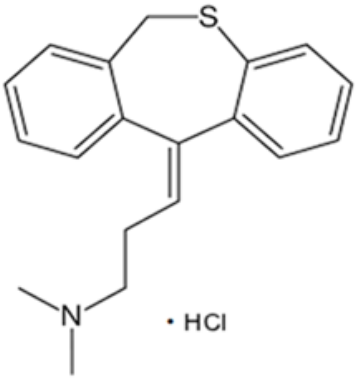


Figure 1. Chemical structure of DOS.

European Pharmacopoeia (EP) recognizes DOS as official one.[5] The EP describes a procedure of non-aqueous potentiometric titration of DOS in acetic acid (AcOH) and acetic anhydride medium against 0.1 M perchloric acid for quantification.

The results of the comprehensive literature review demonstrated that different methods were used to ascertain DOS quantification in pharmaceutical and biological substances. The techniques covered here include liquid chromatography,[6–10] gas chromatography,[11–13] high performance liquid chromatography,[1,2,14–20] conductometry,[21,22] potentiometry,[23,24] and capillary electrophoresis.[25]

Different authors also reported a number of spectrophotometric techniques in addition to these. Sameer et al.[3] developed two techniques for spectrophotometric estimation of DOS by measuring the drug in 0.1 M HCl at 229 nm and in methanol at 231 nm. The evaluation of the limit of detection (LOD), limit of quantification (LOQ), and other regression parameters, successful application of the method to dosage forms and recovery studies were also presented. Heba et al.[4] developed a spectrophotometric method for DOS based on the formation of a binary complex with mercurochrome that was measured at 557 nm with LOD and LOQ of 0.41 and 1.26 μg mL^{-1}. The method was validated statistically. Three techniques for determining DOS were developed by Wafaa[26] and quantified at 423, 498, and 625 nm with respectable sensitivity and regression parameters. Two approaches for quantifying DOS and other two species were published by Hisham et al.[27] based on the generation of mixed anhydrides with malonic and acetic acids, which may be detected in the 329-333 nm range. A method for spectrophotometric detection of DOS was established by Walash et al.[28] in which DOS was measured at 540 nm in an acetate buffer at pH 3.7. The LOD and LOQ values were found as 0.18 and 0.54 μg mL^{-1}, respectively. The technique was used with effectiveness in dosage formulations. Two spectrophotometric approaches have been published by Basavaiah et al.[29] based on the production of an ion pair complex of DOS with bromophenol blue (Method 1) and bromocresol green (Method 2) which were measured at 425 and 430 nm, respectively. For Methods 1 and 2, the LOD and LOQ were reported to be 0.18 and 0.53, 0.17 and 0.50 μg mL^{-1}, respectively. Two spectrophotometric techniques based on the production of an ion pair complex of DOS with bromocresol blue and eriochrome black-T were also reported by Umamaheswar et al.[30] The complexes formed were extracted and measured at 418 and 508 nm, respectively. The procedures were statistically evaluated. Two methods for determining DOS were developed by Sameer et al.[31] Method A was based on the formation of an ion pair complex with Alizarin red-S, its extraction into dichloromethane, and quantification at 445 nm. Method B was based

on the breaking of the formed ion-pair complex and the measurement of free alizarin red-S at 570 nm. The techniques were put to use with dosage formulations and the results were statistically validated. Two techniques for quantifying DOS were reported by Elham.[32] The oxidation of DOS with alkaline $KMnO_4$ was kinetically examined in Method A for a fixed 25 minutes. The coloured manganate's wavelength was fixed at 610 nm for measurements. The absorbance at 470 nm was determined at a set time of 60 minutes for Method B, which was based on the reaction of DOS with 4-chloro-7-nitrobenzofurazan. By creating binary compounds with bromophenol blue, bromothymol blue, bromocresol purple, and bromophenol red, Sane et al.[33] devised a spectrophotometric method for quantifying DOS. In order to measure DOS, Sameer et al.[34] reported two spectrophotometric procedures. The method was based on the addition of a known excess of a bromate-bromide mixture in an acidic medium, which caused the bromination of DOS. The excess of bromine was then estimated by measuring the absorbance at 540 nm with a fixed amount of meta-cresol purple. The sensitivity and regression parameters were reported. Using the hydrochloride of dothiepin as the n-donor and 2,3-dichloro-5,6 dicyano-p-benzoquinone (DDQ) or p-chloranilic acid (p-CA) as the -acceptors, Elham et al.[35] established spectrophotometric charge-transfer complex production, which formed brightly colored complex. The colored products were spectrophotometrically detected using DDQ and p-CA at 460 and 525 nm respectively.

Despite the large number of spectrophotometric methods that have been published, no reports were found for the DOS using cerium (IV) following the direct and indirect method of estimations. The bulk of them involve the use of expensive chemicals and organic solvents for extraction, making them not environmentally friendly. Further, these techniques fell short in terms of accuracy and precision, and most of them require specialized workers, well-equipped laboratories, and strict working conditions in order to conduct analysis. Therefore, there is room for method development in terms of sensitivity and simplicity.

In light of this, an effort was made to create straightforward, precise, affordable, and cost-effective methods for measuring the DOS, and the authors believe they have achieved their goal.

MATERIALS AND METHODS

Instruments

A flexible benchtop Agilent Cary 630 Fourier Transform Infrared (FTIR) spectrometer (Agilent Technologies Ltd, Mumbai, India) have been utilized for measurements. It operates with the MicroLab Pharma Software in the spectral range 4,000 to 400 cm^{-1} with ≤ 2 cm^{-1} resolution. The thermal detector containing 1.3 mm diameter, thermoelectrically-cooled deuterated triglycine sulfate (DTGS) and potassium bromide pellet method were employed.

Shimadzu UV-Visible 1800 spectrophotometer capable of measuring the absorption in the wavelength range of 190-1100 nm with a band width of 1.0 nm was used for UV measurements in the fast scan mode. The quartz cuvette with 1 cm path length was utilized for the measurements.

Reagents

The chemicals and reagents utilized were all of the analytical variety. Except as otherwise noted, all works were performed using distilled water (DW) of conductivity 1.34 µS cm^{-1}. The gift sample of pure DOS was obtained from Taj Pharma India Ltd, Hyderabad.

Acme Generics LLP, Himachal Pradesh, India's Prothiaden tablets (50 mg DOS/tablet) were bought from commercial sources. The standard glacial acetic acid (AcOH, Merck, Mumbai, India, 98% Pure) was diluted to resulting 0.1 M solvent. By diluting the necessary volume of concentrated H_2SO_4, a 2 M H_2SO_4 was created. The needed quantity of cerium (IV) sulphate (85-115% pure) (Loba Chemie Ltd, Mumbai, India) was dissolved in 0.5 M H_2SO_4 and heated. After cooling, the solution was standardised with sodium oxalate solution.[36] The requisite aliquots were pipetted and diluted to obtain 128 and 300 µg mL^{-1} cerium (IV). In order to make 1000 µg $mL^{-1}$of ferrous sulphate solution, 1.0 g of $FeSO_4.7H_2O$ (98% pure; from Thermo Fisher Scientific India Pvt. Ltd. Mumbai) was dissolved in 5 mL of 2 M H_2SO_4 and diluted to a volume of 1 L using distilled water. The required volume of this was diluted to obtain 500 µg mL^{-1} with

respect to iron (II). A 20% (w/v) ammonium thiocyanate (NH_4SCN) solution was prepared by dissolving 20 g of the chemical (S. D fine chem Ltd, Mumbai, India, 98% pure) in about 70 mL of water and made up to mark with water in a 100 mL volumetric flask. A 1000 μg mL^{-1} of o-phenanthroline was prepared by dissolving 0.5 g of chemical (99.5% pure from Himedia Laboratories, Mumbai, India) in approximately 300 mL of water followed by heating and then adding water to the mark in a 500 mL volumetric flask. A 100 mL volumetric flask was filled to the proper level with water after 20.50 g of sodium acetate (NaOAc) (S.D. Fine Chem Ltd., Mumbai, 98%pure) was dissolved in approximately 80 mL of water to prepare 2.5 M NaOAc.

Experimental procedures

Method A

Different aliquots of the 100 μg mL^{-1} DOS solution in 0.1 M AcOH, equivalent to 1.0–80 μg mL^{-1}, were correctly measured and carefully placed into a series of 10 mL standard flasks. The flasks were added with 0.1 M AcOH to reach 10 mL. At 300 nm, the absorbance of each solution was recorded in comparison with a reagent blank (10 mL aqueous solution contains 0.1 M AcOH).

Method B

Into a series of 10.0 mL volumetric flasks different aliquots of 25 μg mL^{-1} of DOS to get 0.25 – 10 μg mL^{-1} solutions were measured and transferred carefully. One mL of 128 μg mL^{-1} cerium (IV) has been added to each flask and the contents were mixed well and allowed to stand for 10 min. DW was used to further dilute the contents to the proper concentration. The absorbance of each solution was then measured at 320 nm in comparison to water.

Method C

Various aliquots of 40 μg mL^{-1} of DOS solution were introduced to a series of 10.0 mL volumetric flasks to get 0.5 – 8.0 μg mL^{-1} of DOS solution followed by the addition of 1 mL of 300 μg mL^{-1} of cerium (IV). Ten minutes were given for the contents to stand. To each of the flasks 1 mL of 500 μg mL^{-1} of $FeSO_4$ solution was added, mixed, and allowed to stand for 5 min, followed by the addition of 1 mL of 1000 μg mL^{-1} o-phenanthroline and 1 mL of 2.5 M NaOAc. Each solution was brought to the mark with DW, mixed and absorbance was measured at 510 nm against a reagent blank after the solutions had been well mixed.

Method D

Accurately measured aliquots of the 40 μg mL^{-1} DOS solution were transferred into a series of 10 mL calibrated flasks to obtain 0.5 – 10 μg mL^{-1} standards. Each flask received 1 mL of the 300 μg mL^{-1} cerium (IV) solution. Ten minutes were given for the contents to stand. Each flask received 1 mL of a 500 μg mL^{-1} $FeSO_4$ solution, which was mixed and left to stand for 5 minutes before adding 1 mL of 20% (w/v) NH_4SCN solution. The flask's contents were thoroughly mixed and made up to the mark with DW. At 480 nm, the absorbance of each solution was noted after against a water blank.

The calibration curves were constructed by plotting the absorbance measured versus the concentration (μg mL^{-1}) of DOS. The unknown concentrations were found by calibration graph or by regression equation derived from concentration-absorbance data.

Procedure for tablets

Weighing and lyophilizing were done on ten Prothiaden tablets. The amount of tablet powder needed for each method was 10 mg for Method A, 25 mg for Method B, and 40 mg for Methods C and D were weighed and put into individual 100 mL volumetric flasks. Each was thoroughly shaken for 20 minutes with about 70 mL of 0.1 M AcOH before being filtered using Whatman No. 1 filter paper into additional 100 mL volumetric flasks. The filtrate was diluted to the mark with DW to get 100, 250, and 400 μg mL^{-1} of tablet extract respectively. A 5.0 mL of each tablet extract of 250 and 400 μg mL^{-1} extracts were diluted 10 times further with the working solvent and the solute of 100, 25 and 40 μg mL^{-1} DOS were put to use for the analysis using the general process already mentioned in Methods A, B, C and D above.

RESULTS AND DISCUSSION

Methodology

The chromophoric activity of DOS in 0.1 M AcOH is measured in method A. The wavelength maximum for DOS in 0.1 M AcOH medium was found at 300 nm. The absorbance at 300 nm grows linearly along with DOS concentration. As cerium (IV) is reduced to cerium (III) by the DOS in Method B, the absorbance of cerium (IV) left was measured at its maximum wavelength of 320 nm. As the DOS concentration rises, the absorbance falls. The absorption spectra of DOS in 0.1 M AcOH and cerium (IV) in 2 M H_2SO_4 medium are given in Figure 2.

Methods C is based on the measurement of absorbance of the red-coloured complex formed between left over iron (II) and o-phenanthroline complex at 510 nm. Method D involves the complexation of iron (III) produced with thiocyanate and the resultant iron hexathiocyanate was measured at 480 nm. Method C and D follow the indirect estimation of DOS, as the generated iron (II)-o-phenanthroline complex concentration increases linearly with the concentration of DOS, and the concentration of iron (III)-thiocyanate complex decreases linearly with the concentration of DOS.

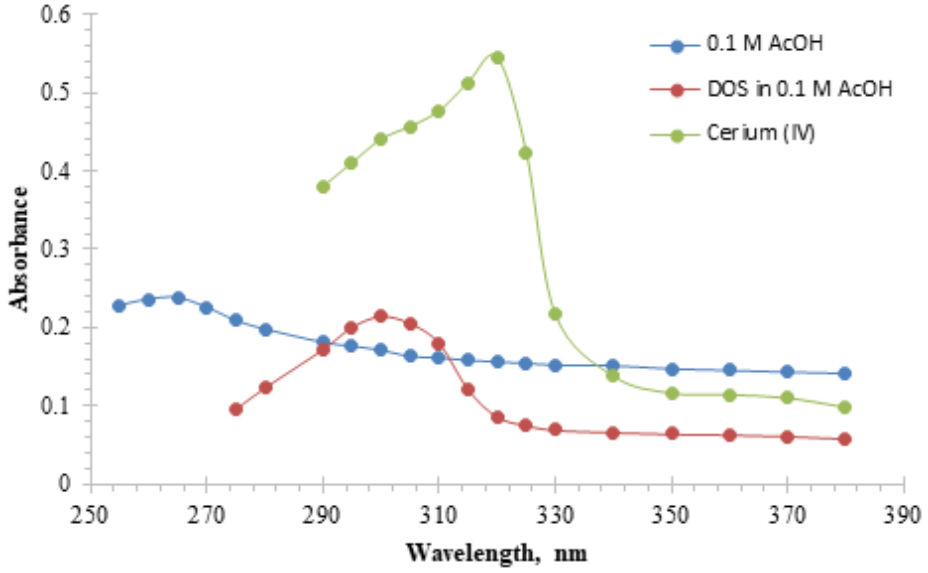
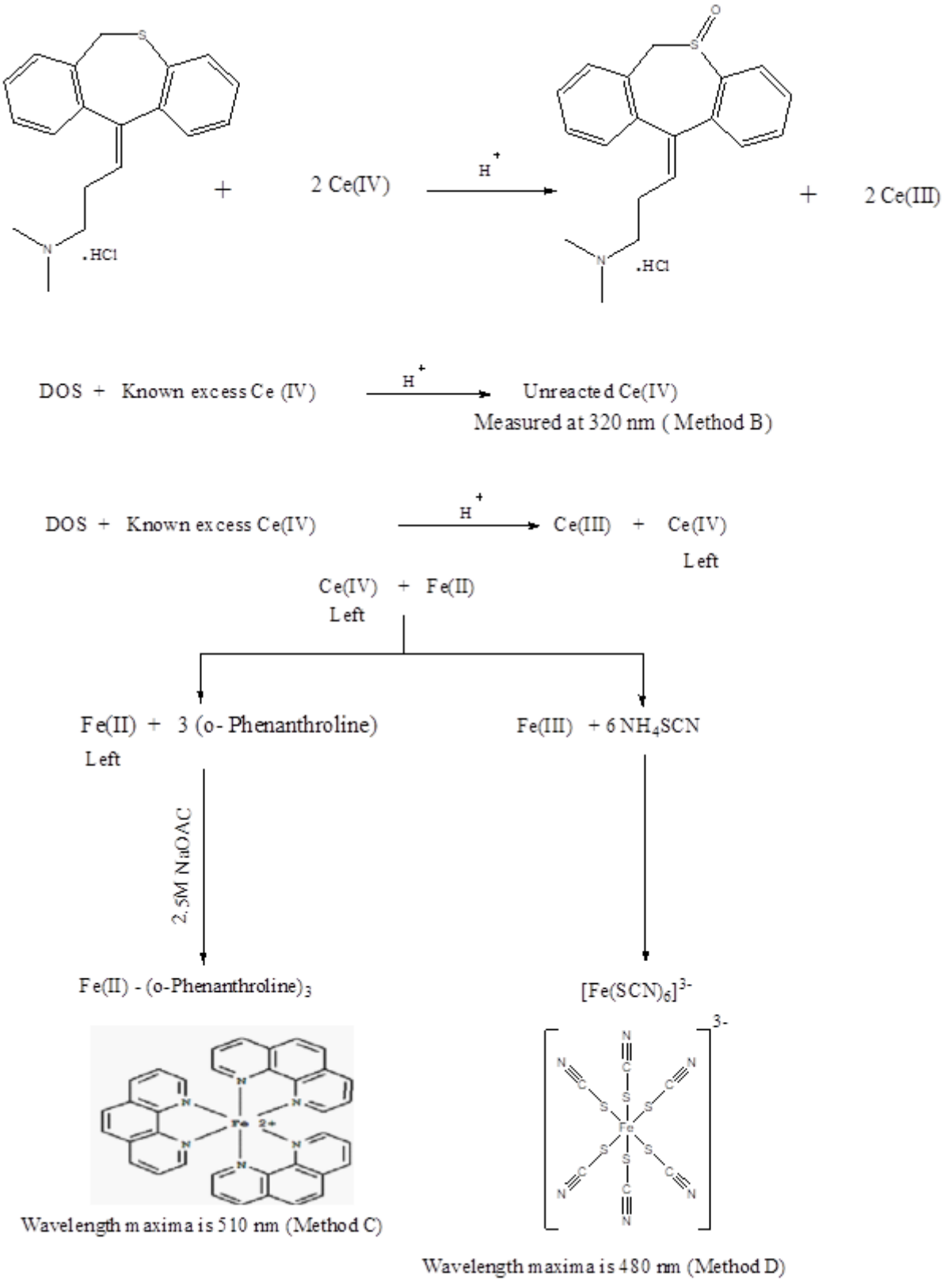


Figure 2. Absorption spectra of 0.1 M AcOH, DOS in 0.1 M AcOH and cerium (IV) solution.

The probable reaction schemes proposed for Methods B, C and D are presented in Scheme 1.[37,38]



Scheme 1. Reaction schemes for Method B, C and D.

Optimization of variables

By changing one variable at a time while holding the others constant, many factors, including the amount of oxidant, the choice of acid, the acid concentration, the solvent, the concentration of metal ions, and the concentration of ligands, were optimized one after the other. The optimization was based on the maximum absorbance obtained at the respective λ_{max} in each method. The effective chromophoric behaviour of DOS was not seen when it was dissolved in solvents like H_2O, alcohol and methylene chloride[5] but, the chromophoric behaviour of DOS in AcOH was found to be satisfactory. So, 0.1 M AcOH was selected as a solvent in Method A, DOS showed maximum absorbance at 300 nm in 0.1 M AcOH medium. At this wavelength, the linearity was established between the absorbance and concentration of DOS in 0.1 M AcOH, made it possible to find the linear concentration range of method A. It was discovered that 2 M H_2SO_4 was the best acid to utilize for all techniques except Method A after experimenting with various acids of different concentrations. To fix the optimum amount of cerium (IV) to oxidize DOS in Method B, C and D, investigations were carried out using the oxidant, acid, reducing agent, viz, iron (II) and complexing agents namely o-phenanthroline and thiocyanate. Varying volumes of 1000 µg mL^{-1} cerium (IV) were placed in 10 mL standard flasks, acidified with 2 M H_2SO_4 and added 1 mL of 500 µg mL^{-1} iron (II) sulphate as reductant. After the rapid oxidation ensured to be completed, either the unreacted reductant was treated with o-phenanthroline or oxidised reductant treated with thiocyanate in Methods C and D, the contents were diluted to the mark with water and measured the absorbances at respective wavelengths of maximum. The upper limit of absorbance as either ~1 or 1.33 in Method C and D were found when the cerium (IV) present at 128 or 300 µg mL^{-1}. Besides, the linearity was found excellent to satisfy the Beer's law up to these levels of cerium (IV). Hence, the upper Beer's law limit for cerium (IV) was set by preliminary examinations is found as 128 µg mL^{-1} for Method B and 300 µg mL^{-1} for Methods C and D. To evaluate the DOS of detection range, different concentrations were reacted with 1 mL of 128 or 300 µg mL^{-1} of cerium (IV). Based on the absorbance data acquired for Methods C and D, different concentrations of iron (II) were used and optimized at 500 µg mL^{-1}. After experimenting with various concentrations, the sodium acetate (NaOAc) was optimized, and the greatest analytical signal was found at 2.5 M NaOAc. In Method B, the absorbance measured at 320 nm with respect to cerium (IV) decreases as the concentration of DOS increases. Similarly, in Method C, $[Fe(o\text{-phenanthroline})_3]^{+2}$ absorbance increases linearly with DOS concentration at 510 nm, while in method D, $[Fe(SCN)_6]^{3-}$ absorbance declines linearly with DOS at 480 nm.

Study of DOS stability under various stressing media/conditions in Method-A

The stability of the DOS was ascertained by forced degradation studies of DOS solution equivalent to 60 µg mL^{-1} under different stressed conditions. The solution was treated with 0.1N HCl and 0.1N NaOH starting from room temperature up to 70 ºC for seven hours to check the stress degradation by hydrolysis under acidic and basic conditions, respectively. Oxidative degradation was done by treating the solution with 3% H_2O_2 solution at neutral pH for seven hours. The solution was placed in hot air oven for 24 h at 105 °C to check the thermal degradation. For UV stress studies, solution was exposed to UV radiations for 24 hours.[39] The FT-IR (Figure 3) and UV (Figure 4) spectra of these stressed DOS samples were compared with that of pure DOS spectra and the results were summarised in Table I.

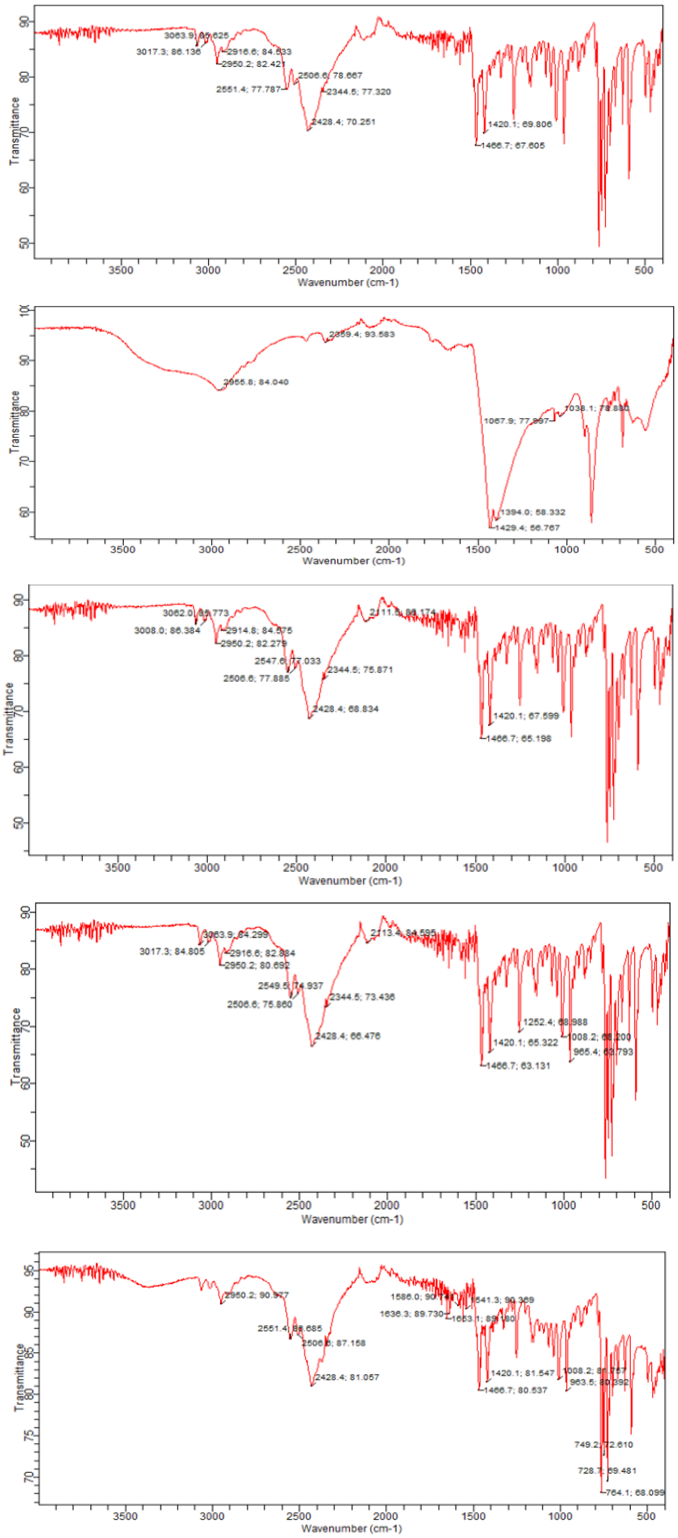


Figure 3. FT-IR spectra of: 3(A) – Pure DOS; 3(B) – Alkaline degraded DOS; 3(C) – Thermal stressed DOS; 3(D) – UV stressed DOS; 3(E) – Acid stressed DOS.

The FTIR spectrum of pure DOS shows asymmetric and symmetric stretching bands for methyl group in the region 2916-2950 cm^{-1} and 3017-3063 cm^{-1}. The CH_2 stretching vibrations are also observed in the region 2950-3020 cm^{-1}. The band around 3035 cm^{-1} generally represents the C-H stretching vibrations of aromatic ring and the ring C-C stretching vibrations occurs in the 1420-1625 cm^{-1} region. The asymmetric and symmetric deformations are expected in the range 1420-1466 cm^{-1}. The band in the region 520-530 cm^{-1} represents the C-S stretching vibrations. For DOS the C-N stretching vibration modes are observed in the region 728-1008 cm^{-1}. The nitrogen present in DOS is tertiary in form, the corresponding onium salt is commonly encountered and it displays strong broad N-H stretching vibrations in the region around 2500 cm^{-1}.

The degradation of DOS in the alkaline medium was evident from the comparison of FT-IR spectra of pure DOS with the that of post alkaline degradation drug. The missing of a prominent peak at 2428 cm^{-1} corresponds to the N-H stretching vibrations of 'onium' salt form of tertiary amine group and bands in the region of 794-1033 cm^{-1} due to C-N stretching vibrations indicates the degradation of DOS in alkaline medium. Further, the extension of study of DOS by stressing it to oxidation with peroxide revealed more than 50% degradation. The solid product left post-oxidation was found-insufficient. However, the resulted content was dissolved in 0.1 M AcOH and was recorded the UV spectrum. The absorbance at 300 nm indicated the recovery of 47.26% DOS and confirmed the degradation above 50%. Other than these, DOS was stable under acid, UV and thermal stress, as there was no significant difference between their FT-IR spectra and that of pure DOS. The FT-IR assignments were compared with the band regions calculated by Generalized Gradient Approximation methods developed by Perdew and Wang (GGA-PW91) and Becke-Lee-Yang-Parr (GGA-BLYP) and found to be robust.[40]

The UV spectra of DOS under different stressed conditions indicated that, DOS did not degrade under acidic, thermal and UV stressed conditions and the spectra is similar to that of spectrum of pure DOS in 0.1 M AcOH. The presence of wavelength shifted flat curve in the alkaline stressed DOS and the other with less intensity at 300 nm confirms that, almost complete degradation and partial degradation of DOS in 0.1 M NaOH and 3% H_2O_2, respectively.

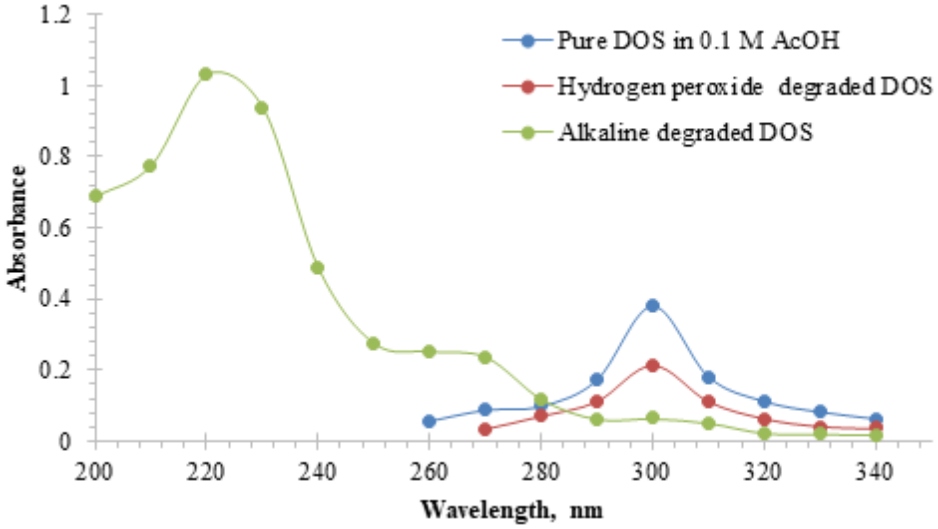


Figure 4. UV- spectra of Pure DOS in 0.1 M AcOH, H_2O_2 and alkaline degraded DOS.

Table I. Results of stability studies of DOS under various stressing conditions in Method A

Stressing media/condition	%Recovery of DOS
HCl	101.73
NaOH	11.42
H_2O_2	47.26
Exposed to UV rays for 24 h	100.94
Exposed to high temperature for 24 h	101.08

Method validation

Linearity and sensitivity parameters

According to International Conference on Harmonisation (ICH) norms,[41] validation checks on the suggested methodologies were performed. The linear correlation between the absorbance at respective λ_{max} and concentration of DOS were established in the range given in Table II. The regression Equation 1 was derived for each calibration line.

$$Y = a + bX \qquad \text{Equation 1}$$

where Y is the absorbance of a 1 cm layer of solution, *a* is the intercept, *b* is the slope and X is the concentration in µg mL^{-1}.

The obtained calibration curves (Figure 5) are experimental and calibration equations were derived using the Least Squares method. The non zero intercept values reported are for the best curve fitting line. For all the approaches, a, b, regression coefficient (R^2), Beer's law range, molar absorptivity, and Sandell sensitivity values were computed and provided in Table II. The limits of detection (LOD) and quantification (LOQ) were calculated following to ICH guidelines[41] using the formula LOD=3.3S/b and LOQ= 10S/b, where S is the standard deviation (SD) of blank absorbance values and b is the slope of the calibration plot.

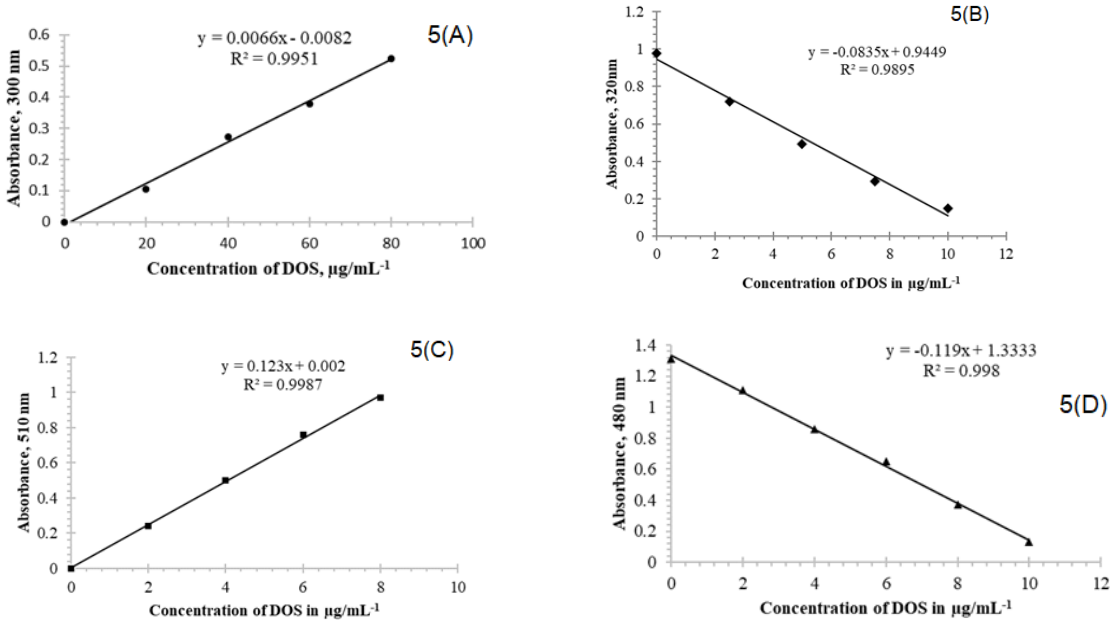


Figure 5. Calibration plots for: 5(A) – Method A; 5(B) – Method B; 5(C) – Method C; 5(D) – Method D.

Table II. Sensitivity and regression parameters for proposed methods

Parameter	Method A	Method B	Method C	Method D
λ_{max}, nm	300	320	510	480
Linear range, μg mL^{-1}	1.0-80.0	0.25-10.0	0.5-8.0	0.5-10.0
Molar absorptivity, L mol^{-1} cm^{-1}	2.067 x 10^3	3.1086 x 10^4	4.08 x 10^4	3.70x 10^4
Sandell sensitivity, μg cm^{-2}	0.1605	0.0106	0.0081	0.0089
LOD, μg mL^{-1}	0.2347	0.0883	0.1204	0.7070
LOQ, μg mL^{-1}	0.7113	0.2676	0.3650	2.1424
Regression Data, Y=*a* + *b*X				
Intercept (a)	-0.0082	0.9449	0.0020	1.3333
Slope (m)	0.0066	-0.0835	0.1230	-0.1190
Regression coefficient (R^2)	0.9951	0.9895	0.9987	0.9980

Accuracy and precision

Triplicates of three different amounts of DOS were used to study the intra-day and inter-day variations to test the accuracy and precision of the suggested approaches. Intra-day measurements include the analysis of drug three times (forenoon, afternoon and at evening) within a day. Whereas, the inter-day analysis was performed on three consecutive days under optimal experimental conditions using developed procedures. The percentage relative error (%RE) and percentage relative standard deviation (%RSD) between the amounts obtained by measurement and the amounts subjected were evaluated, and used to assess the correctness and closeness of the proposed methods. The study findings presented in Table III imply a high degree of precision and accuracy between the computed and each individual values.

Table III. Intra-day and Inter-day Accuracy Precision

Method	DOS taken μg mL^{-1}	Inter-day accuracy and precision			Intra-day accuracy and precision		
		DOS found* μg mL^{-1}	%RE	%RSD	DOS found* μg mL^{-1}	%RE	%RSD
A	10.00	10.05	0.51	1.45	10.10	1.00	2.21
	20.00	20.12	0.63	1.83	19.93	0.34	1.12
	30.00	28.93	3.56	3.05	29.07	3.07	0.77
B	2.50	2.53	0.01	1.54	2.53	1.46	1.24
	5.00	4.96	0.36	3.71	5.17	3.48	2.48
	7.50	7.63	0.16	1.67	7.56	0.86	0.48
C	2.00	1.94	2.72	2.42	1.91	4.08	4.25
	4.00	3.98	0.34	3.12	4.01	0.34	3.10
	6.00	5.91	1.36	1.37	6.05	0.90	2.05
D	2.00	2.04	2.20	4.10	1.96	1.96	4.28
	4.00	4.11	2.90	3.11	4.03	0.84	3.18
	6.00	5.93	1.02	2.16	5.99	0.09	2.80

*Mean values of three determinations

Robustness and ruggedness

The proposed procedures were shown to be reliable, despite purposefully varying the acid content, solvent, and other parameters, there was no appreciable change in RSD values above 5%.

Three analysts were performed the assays independently utilizing various lab instruments and spectrophotometers to test the robustness of the methods. It was found that the % RSD values were less than 5%, which amplifies the fact that the suggested approaches were reliable.

Applications to tablet analysis and statistical evaluation of results

Commercially available DOS tablets were evaluated using the suggested methodologies and the standard procedure of a reference method. As mentioned in the reference method, 0.1 M perchloric acid was the titrant for the potentiometric estimation of DOS in an anhydrous acetic acid and acetic anhydride medium.[5] The true titrating agent, however, was the acetyl perchlorate that generated during the course of titration.[42] The study findings were presented in Table IV. The results of proposed methods are not deviated with respect to accuracy as well as precision as it can be realised from calculated F and *t*- values in the Table IV. Thus, the proposed methods are confirmed and as evolved at acceptable accuracy and precision.

Table IV. Results of analysis of prothiaden tablets by proposed methods and reference Method

Nominal DOS amount (mg/tablet)	Found* (Percent label claim ± SD)				
	Reference method	Method A	Method B	Method C	Method D
50.00	100±0.57	99.92±0.64 *F* = 1.26 *t* = 0.21	100.06±0.59 *F* = 1.07 *t* = 0.16	99.93±0.87 *F* = 2.33 *t* = 0.15	100.17±0.81 *F* = 2.02 *t* = 0.39

*Mean value from three determinations

The tabulated *F* and *t* values at 95% confidence level for four degrees of freedom are 6.39 and 2.77, respectively.

Recovery study

To further evaluate and establish the reliability of the suggested procedures, the recovery tests were carried out using a standard addition procedure. Pre-analyzed tablet powder was spiked with pure DOS at three distinct concentration levels before being analyzed in triplicate. It was determined the total DOS present. The percentage recovery of DOS ranged from 97.77 to 103.34% in all cases (Table V), indicating that the co-formulated substances showed no effect on the test. This demonstrates that the assay methods were precise and the percentage recovery values were acceptable.

Table V. Recovery study of Prothiaden Tablets by the standard addition method

Method	DOS in tablet μg mL^{-1}	Pure DOS added μg mL^{-1}	Total found* μg mL^{-1}	%DOS recovered (Percent ± SD)
A	20.00	10.00	29.33	97.77 ± 1.06
	20.00	20.00	41.33	103.34 ± 1.02
	20.00	30.00	49.33	98.66 ± 0.89
B	2.50	1.25	3.75	100.03 ± 0.78
	2.50	2.50	4.99	99.95 ± 0.66
	2.50	3.75	6.25	100.02 ± 0.71
C	2.00	2.00	3.95	98.83 ± 0.84
	2.00	4.00	6.09	101.56 ± 1.09
	2.00	6.00	7.95	99.41 ± 1.05

(continues on the next page)

Table V. Recovery study of Prothiaden Tablets by the standard addition method (continuation)

Method	DOS in tablet μg mL^{-1}	Pure DOS added μg mL^{-1}	Total found* μg mL^{-1}	%DOS recovered (Percent ± SD)
D	4.00	2.00	5.91	98.62 ± 0.53
	4.00	4.00	8.16	102.06 ± 1.10
	4.00	6.00	9.98	99.83 ± 1.18

*Mean value from three determinations

CONCLUSION

For the purpose of determining DOS, four new spectrophotometric methods were designed and validated using 0.1 M AcOH as solvent in Method A. The oxidative ability of cerium (IV) was used in developing Methods B, C and D. Iron (II), o-phenanthroline and thiocyanate used as reagents in the oxidation and complexation steps post reaction between DOS and cerium (IV). It was possible to determine DOS concentrations as low as 0.71, 0.26 and 2.14 μg mL^{-1} with confidence and a justifiable level of accuracy and precision. The procedures required no harsh experimental conditions and were straightforward, precise, rapid, cost-effective, and free from interference from common diluents and additives in the formulations. The reaction of cerium (IV) with DOS was used to design an assay procedure for the indirect quantification of DOS by reacting excess cerium (IV) with iron (II), followed by complexation of excess iron (II) with o-phenanthroline in Method C and stoichiometrically generated iron (III) with thiocyanate in Method D. The techniques for figuring out DOS in tablets worked well. Due to their straightforward operation and use of inexpensive instruments, these methods offer an advantage over currently used instrumental methods for DOS. Chemicals that were inexpensive and easily accessible were sufficient for the experiment, which increases the cost-effectiveness. It was advised that the techniques be applied in quality control labs.

Conflicts of interest

The authors declare that there is no conflict of interest.

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REFERENCES

(1) Prasanthi, T.; Lakshman, R. A.; Nandini, M.; Hemanth, M.; Bhuvaneswari, M.; Chaitanya, M. Method development and validation for the estimation of dothiepin hydrochloride by using RP-HPLC in pure and tablet dosage form. *Indian J. Pharm. Educ. Res.* **2019***, 53 (2), 310-315.* https://doi.org/10.5530/ijper.53.2.39

(2) Radhika, S.; Ragin, S. Stability indicating RP-HPLC method for simultaneous estimation of dosulepin hydrochloride and methylcobalamin in tablet dosage form. *Int. J. App. Pharm.* **2017**, *9* (4), 69-75. https://doi.org/10.22159/ijap.2017v9i4.19204

(3) Sameer, A. M. A.; Basavaiah, K.; Cijo, M. X.; Vinay, K. B. Validation of UV spectrophotometric methods for the determination of dothiepin hydrochloride in pharmaceutical dosage form and stress degradation studies. *J. Appl. Spectrosc.* **2012,** *79* (5), 780-787. https://doi.org/10.1007/s10812-012-9670-7

(4) Heba, S. E.; Fawzi, A. E.; Fatma, A. A. A.; Amina, M. B. Validated spectrophotometric and spectrofluorimetric methods for determination of dapoxetine hydrochloride and dosulepin hydrochloride in their dosage forms using mercurochrome. *Lumin*. **2018,** *33* (8), 1306-1313. https://doi.org/10.1002/bio.3566

(5) The European Pharmacopoeia, 6th edition, 2007.
(6) Shivakumar Reddy, L.; Prasad Reddy, S. L. N.; Srinivas Reddy, G. Validated stability indicating liquid chromatographic method for simultaneous estimation of dosulepin and methylcobalamin in combined pharmaceutical dosage form. *Orient. J. Chem.* **2014**, *30* (3), 1243-1251. http://dx.doi.org/10.13005/ojc/300340
(7) Dhara, B. D.; Dimal, A. S.; Falgun, A. M.; Usmangani, K. C.; Kashyap, K. B. Liquid chromatographic estimation of dosulepin HCl and methylcobalamin in pharmaceutical formulation. *Res. Rev. J. Pharm. Nanotechnol*. **2014**, *2* (2), 29-35.
(8) Xiang, C.; Ben, M. C.; Shao, G. L.; Fu-Liang, D.; Ping, Z. Determination of dothiepin in human plasma by LC–ESI–MS and its application to bioequivalence studies. *Chromatographia* **2008**, *68* (11), 941-947. http://dx.doi.org/10.1365/s10337-008-0835-8
(9) David, R. B.; Barbara, K. H. Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry. *J. Chromatogr. A* **2011**, *1218* (44), 7901-7913. http://dx.doi.org/10.1016/j.chroma.2011.08.092
(10) Richard, B.; Jason, M. W.; Cobus, G. Qualitative and quantitative temporal analysis of licit and illicit drugs in wastewater in Australia using liquid chromatography coupled to mass spectrometry. *Anal. Bioanal. Chem*. **2018,** *410* (2), 529-542. http://dx.doi.org/ 10.1007/s00216-017-0747-2
(11) Jack, E. W.; Horace, E. H.; Rebecca, O.; Steven, C. H. Determination of doxepin by electron-capture gas chromatography. *J. Anal. Toxicol*. **1978**, *2* (2), 44-49. https://doi.org/10.1093/jat/2.2.44
(12) Nicole, H. J.; Philippe, D. F.; Germain, M. B. Concurrent determination of second-generation antidepressants in plasma by using gas chromatography with nitrogen–phosphorus detection. *Clin. Chem*. **1997,** *43* (11), 2209-2210. https://doi.org/10.1093/clinchem/43.11.2209
(13) Liliana, T.; Andre, L. C.; Sonia, T.; Pedro, C.; Goreti, F. S.; Helena, M. T. Antidepressants detection and quantification in whole blood samples by GC–MS/MS, for forensic purposes. *J. Pharm. Biomed. Anal.* **2016**, *128*, 496-503. https://doi.org/10.1016/j.jpba.2016.06.027
(14) Brodie, R. R.; Chasseaud, L. F.; Crampton, E. L.; Hawkins, D. R.; Risdall, P. C. High performance liquid chromatographic determination of dothiepin and northiaden in human plasma and serum. *J. Int. Med. Res*. **1977**, *5* (6), 387-390. https://doi.org/10.1177/030006057300100201
(15) Shibanoki, S.; Imamura, Y.; Arakawa, Y.; Ishikawa, K. Determination of dosulepin and its metabolite: Application of high-performance liquid chromatography with electrochemical detection. *J. Chromatogr. B Biomed. Appl.* **1987**, *415* (2), 365-371. https://doi.org/10.1016/s0378-4347(00)83228-4
(16) Usharani, G.; Chendrashekar, B.; Devanna, N. Simultaneous estimation of dosulepin and methylcobalamin in bulk and pharmaceutical formulation by reverse phase high performance liquid chromatography (RP-HPLC). *IOSR J. Pharm. Biol. Sci*. **2014**, *9* (3), 55-59. https://doi.org/10.9790/3008-09365559
(17) Aruna, P.; Ajitha, A.; Maheshwar, U. R. Analytical method development and validation of simultaneous estimation of dosulepin and methylcobalamin in tablet dosage form by RP-HPLC. *Int. J. Pharm*. **2014**, *4* (3), 267-274.
(18) Anita, A.; Komal, G.; Mayuri, N.; Priya, M.; Paraag, G. Development and validation of RP-HPLC method for determination of dothiepin hydrochloride in bulk and pharmaceutical dosage form. *Int. J. Chem. Pharm. Anal*. **2013**, *1* (1), 9-13.
(19) Mannfred, K.; Caroline, S. Simultaneous determination of seven tricyclic antidepressant drugs in human plasma by direct-injection HPLC-APCI-MS–MS with an ion trap detector. *Ther. Drug Monit.* **2002,** *24* (4), 537-544. https://doi.org/10.1097/00007691-200208000-00013
(20) Li Wan, P. A.; Irwin, W. J. A high-performance liquid chromatographic assay of cis- and trans-isomers of tricyclic neuroleptic drugs. *J. Pharm. Pharmacol*. **1979**, *31* (8), 512-516. https://doi.org/10.1111/j.2042-7158.1979.tb13574.x

(21) Nour, T. A. G.; Rasha, M. E. N.; Abeer, A. B. Conductimetric determination of the antidepressants, amitriptyline and dothiepin hydrochlorides and tranylcypromine hemisulphate in their pharmaceutical formulations. *J. Pharm. Sci.* **2004**, *29,* 195-201.
(22) Abdel Ghani, N. T.; El-Nashar, R. M.; Bioumy, A. A. Flow injection potentiometric determination of dothiepin hydrochloride. *Anal. Lett.* **2004**, *37* (15), 3237-3254. https://doi.org/10.1081/AL-200040335
(23) Shimaa, A. A.; Ghada, A. S.; Fahima, A. M.; Doha, M. N.; Hala, E. Z. A novel sensor aluminum silicate modified carbon paste electrode for determination of anti-depressant dothiepin HCl in pharmaceutical formulation and biological fluids. *Microchem. J.* **2019**, *148*, 725-734. https://doi.org/10.1016/j.microc.2019.05.007
(24) Fatma, M. A. G.; Ahmed, G. H.; Eman, F. M. Potentiometric method for the determination of lamivudine and dothiepin hydrochloride in pharmaceutical preparations. *Anal. Bioanal. Electrochem*. **2013**, *5* (2), 222-235.
(25) Clark, B. J.; Barker, P.; Large, T. The determination of the geometric isomers and related impurities of dothiepin in a pharmaceutical preparation by capillary electrophoresis. *J. Pharm. Biomed. Anal.* **1992**, *10* (10-12), 723-726. https://doi.org/10.1016/0731-7085(91)80071-g
(26) Wafaa, E. S. H. Extractive colorimetric method for the determination of dothiepin hydrochloride and risperidone in pure and in dosage forms. *Chem. Pharm. Bull*. **2008**, *56,* 1092-1096. https://doi.org/10.1248/cpb.56.1092
(27) Hisham, E. A.; Magda, M. E. H.; Heba, M. E. S.; Magda, M. A. Spectrophotometric and spectrofluorimetric methods for analysis of tramadol, acebutolol and dothiepin in pharmaceutical preparations. *Mol. Biomol. Spectrosc*. **2006**, *65* (5),1087-1092. https://doi.org/10.1016/j.saa.2006.02.008
(28) Walash, M. I.; Belal, F.; El-Enany, N.; Elmansi, H. Spectrophotometric and spectrofluorimetric methods for the determination of dothiepin hydrochloride in its pure and dosage forms using eosin. *Int. J. Biomed. Sci.* **2010**, *6* (4), 327-334. PMID: 23675210.
(29) Sameer, A. M. A.; Basavaiah, K. Spectrophotometric determination of dothiepin hydrochloride in pharmaceuticals through ion-pair complexation reaction. *Chem. Ind. Chem. Eng.* **2012**, *18* (2), 339-347. https://doi.org/10.2298/CICEQ111009010A
(30) Umamaheshwar, K.; Naganjaneyulu, T.; Rambabu, C. Extractive spectrophotometric methods for the determination of dothiepin in pure and pharmaceutical formulations. *Glob. J. Pharmaceu. Sci.* **2017**, *2* (2), 25-29. https://doi.org/10.19080/GJPPS.2017.02.555582
(31) Sameer, A. M. A.; Basavaiah, K. Use of alizarin red S as a chromogenic agent for the colorimetric determination of dothiepin hydrochloride in pharmaceutical formulations. *J. Saudi Chem. Soc.* **2014**, *18*, 107-114. https://doi.org/10.1016/j.jscs.2011.05.018
(32) Elham, A. T. Kinetic spectrophotometric methods for the determination of dothiepin hydrochloride in bulk and in drug formulation. *Anal. Bioanal. Chem*. **2003**, *376* (7), 1131-1136. https://doi.org/10.1007/s00216-003-2040-9
(33) Sane, R. T.; Kothurkar, R. M.; Ladage, K. D.; Tendolkar, R. V.; Gangal, D. P. An extractive econometric method for the determination of dothiepin hydrochloride from pharmaceutical preparations. *Indian J. Pharm. Sci.* **1988**, *50*, 299.
(34) Sameer, A. M. A.; Basavaiah, K. Use of bromate-bromide mixture as a reagent for the determination of dothiepin hydrochloride in pharmaceuticals. *Thai. J. Pharm. Sci*. **2011,** *35,* 147-158.
(35) Elham, A. T.; Suzan, M. S.; Hisham, E. A.; Magda, M. A. Colorimetric methods for the determination of some tricyclic antidepressant drugs in their pure and dosage forms. *Microchim. Acta* **2002**, *140*, 175-182. https://doi.org/10.1007/s00604-002-0904-x
(36) Jeffery, G. H.; Bassett, J.; Mendham, J.; Denney, R. C. *Vogel's textbook of quantitative chemical analysis*, John Mendham, Pearson, **1989**, 5th edition, chapter 10, p. 381.
(37) Raghu, M. S.; Basavaiah, K. Simple, sensitive and eco-friendly methods for the determination of methdilazine in tablets and syrup using cerium (IV). *Chem. Sci. J.* **2012**, *3* (1), 1-14.

(38) Svehla, G. *Vogel's qualitative inorganic analysis*, 7th edition, Pearson education, **2002**, 112-116.
(39) Blessy, M.; Patel, R. D.; Prajapati, P. N.; Agrawal. Y. K. Development of forced degradation and stability indicating studies of drugs – A review. *J. Pharm. Anal*. **2014,** *4* (3), 159–165. https://doi.org/10.1016/j.jpha.2013.09.003
(40) Shojaie, F. Theoretical studies of the vibrational spectra and molecular structures of dosulepin and doxepin. *Phys. Chem. Res.* **2016**, *4* (2), 245-270. https://doi.org/10.22036/pcr.2016.13970
(41) International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonized tripartite guideline, validation of analytical procedures: text and methodology, Q2(R1), complementary guideline on methodology dated 06 November **1996**, ICH, London, UK (2005).
(42) Volgyi, G.; Beni, S.; Takacs-Novak, K.; Gorog, S. Theoretical problems associated with the use of acetic anhydride as a co-solvent for the non-aqueous titration of hydrohalides of organic bases and quaternary ammonium salts. *J. Pharm. Biomed. Anal.* **2010**, *51* (1), 18–23. https://doi.org/10.1016/j.jpba.2009.07.022