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Method to Develop and Stress Degradation Profile of *N-(2,6-dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl}Acetamide* Studied by UV Spectroscopy

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MSKA and AL designed the study, performed the statistical analysis, wrote the protocols, and wrote the first draft of the manuscript. Authors HHF and KR managed the analyses of the study. Authors IA and SI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Synthetic chemistry has always served as a back bone to the medicinal and pharmaceutical chemistry in terms of drug development and drug optimization. It helped in a great deal in finding new lead compounds and synthesizing new drugs. A new molecule N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)-1,3,4-oxadiazol-2-yl] sulfanyl} acetamide, was synthesized from the fusion of Indole acetic acid with 1,3,4-oxadiazole. This pharmacologically active entity lacks a suitable method for its analysis.
Aim: The present research aimed to develop a UV visible spectroscopic method for the purpose

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followed by its validation according to ICH guidelines.
Methodology: The method was developed at 225 nm (λ_{max}). Then the accuracy, precision, sensitivity (Limit of detection & Limit of quantification), specificity, robustness and ruggedness were calculated. The analyte was exposed to multiple stress conditions to figure out method's specificity.
Results: The developed method showed the linearity within a range (0.5 – 50 µg/mL) with correlation coefficient (R^2) = 0.9997. The accuracy of the developed method was figured out by recovery analysis and it was within 95.556 – 104.321 %. The precision analysis *i.e.* interday (0.505591 %), intraday (0.231661 %) and repeatability (0.06478 %), were within the acceptance criteria *viz.* % RSD less than 2 % and LOD & LOQ were found to be 0.523356 and 1.58598 µg/mL. All the validation parameters were within the acceptance limits making the method unique and acceptable. In addition to that it was found to be easy, reliable and analyst friendly (ruggedness, 0.520889 %). The analyte when exposed to stress conditions *viz.* acidic (0.1N H_2SO_4) and basic (0.1N NaOH) environment, oxidative stress (3 % H_2O_2), UV light and altered temperature and humidity (80 ºC+75% RH) for 24 hr, it was found deteriorated. The analyte was 65.56 % degraded in acidic, 39.63 % in basic, 45.18 % under oxidative stress and 61.85 % under altered conditions of temperature and humidity. There was a complete loss of analyte (87.78 %) when exposed to UV light.
Conclusion: The results clearly states that the method is simple, sensitive, specific, precise and accurate, thus can be employed for the quantitative estimations of the analyte.

Keywords: Method development; validation; synthetic; stability; forced degradation study.

1. INTRODUCTION

When there are no authoritative procedures available, new techniques *viz.* analytical methods, are being devised for the assessment of the novel product. These procedures are upgraded and substantial through fundamental runs [1]. Analytical method refers to a procedure or a way to perform analysis for the analyte. It must be detailed enough to explain explicitly all the steps for the required to perform each analytical test [2]. Its purpose must be understood as it will direct the validation parameters, which are obligatory to be evaluated, as validated analytical systems assume a critical job in accomplishing this objective [3,4].

From the phases of medication advancement to showcasing and post promoting, scientific procedures assume an extraordinary part, be it understanding the physical and substance soundness of the medication, sway on the choice and plan of the measurement structure, surveying the strength of the medication particles, quantitation of the pollutants and ID of those contaminations which are over the set up edge fundamental to assess the poisonousness profiles of these debasements to recognize these from that of the API, when relevant and evaluating the substance of medication in the advertised items. The examination of medication and its metabolite which might be either quantitative or subjective is widely applied in the pharmacokinetic studies [5].

In the current project, a stability indicating method has been developed and validated for a new molecule named *N-(2,6-dimethylphenyl)-2-{[5-(1*H*-indol-3-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide* Fig.1.

Literature states that the compound showed antibacterial action against two gram-positive (*Bacillus subtilis,* and *Streptococcus aureus*) and three gram-negative (*Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella typhi*) bacteria when compared with ciprofloxacin. The molecule has also proven its efficacy as hemolytic agent and in enzyme inhibition analysis by showing inhibition of butyrylcholinesterase, lipoxygenase and *α*-glucosidase. Molecular docking analysis of compound was also reported to quantify the biological potential of the drug and it showed the interaction of the lead with the three major active sites of the amino acid moiety via its indole ring [6].

For a drug to become a potential drug candidate, it requires an analytical method for its identification and analysis. The method keeps a check and is helpful in lying down parameters [5]. It was selected as it was found the most effective in its pharmacological performance among the various activities that were carried out among its series. Yet literature fails to disclose any

evidence related to analytical procedure or methods for the analyte or any member of its series till date. Being most dynamic, this pharmacologically active entity was opted for the method development followed by its validation.

2. EXPERIMENTAL

2.1 Instruments

Spectroscopic analysis was conceded out using double beam Shimadzu recording Ultraviolet Visible Spectrophotometer (UV-2550, made in Japan) connected to a computer installed with *Shimadzu UVPC version 3.9 software* with 1 nm spectral band width and 0.3 nm of wavelength accuracy, with path length of 10 mm and matched quartz cells of 1 cm, was utilized for analytical purpose.

2.2 Solubility Test

The solubility of the molecule was checked in water (H_2O), methanol (MeOH), ethanol (EtOH), acetonitrile, hydrochloric acid (1N HCl), sodium hydroxide (5% NaOH), nitric acid (HNO_3), sulphuric acid (1N H_2SO_4) and chloroform ($CHCl_3$).

2.3 Melting Point

The melting point of the sample was determined with the aid of Griffin and George melting point apparatus by the following procedure [7].

Take a capillary melting point tube. A small amount of the compound was taken with microspatula, on a clean weighed dish. The open end of the tube was pushed into the compound and about 2 mm of sample was filled in the capillary tube. The tube is then tapped so that the sample in the tube is uniformly packed. Place the capillary melting point tube in the Griffin and George melting point apparatus chamber. Start with a setting of "2" to "2 ½"; the temperature should slowly rise. The sample should be observed continuously, so that the melting point of the sample is not missed. The melting range was recorded, which begins when the sample first starts to melt T_1 ºC and ends when the sample is completely melted T_2 ºC. The test was performed in triplicates. The melting point can be calculated with the following expression;

$$Metling\ point = \frac{T_1℃ + T_2℃}{2}$$

2.4 Fourier Transform Infrared Spectrophotometer (FTIR)

FTIR is a supportive and latest technique for the identification of a substance. The FTIR analysis was carried out according to the procedure reported in literature [8].

The sample powder was used for FTIR analysis. About 10 mg of the standard and sample was placed in 100 mg of potassium bromide (KBr) pellet, in order to prepare the translucent sample discs. The sample was then stacked in FTIR spectroscope with an output run from 400 to 4000 cm^{-1} with goals of 4 cm. Furthermore, the FTIR spectrum was recorded and interpreted.

2.5 Determination of λmax

The first step in the determination of λmax of the analyte i.e. the wavelength at which is absorbs maximum radiation.

2.5.1 Preparation of stock solution

The standard stock solution was prepared by dissolving the drug sample in methanol and made the volume up to the mark (100 mL). The resultant concentration of the standard stock solution is 1 mg/mL or 1000 µg/mL.

2.5.2 Preparation of working solution

The working solution was prepared by taking 10 mL of standard stock in a 100 mL volumetric flask. The volume was made up to the mark with methanol (100 mL). The working solution (100 µg/mL) of sample was then subjected to scanning by UV-Visible spectrophotometer in the range of 200 – 800 nm against methanol as blank. The wavelength corresponding to the maximum absorbance was recorded which was λ_{max}.

2.6 Method Validation

2.6.1 Linearity

Linearity of the developed method was assessed by the following procedure [9];

0.05 mL from the working solution (100 µg/mL) was taken in a test tube. It was further diluted

with methanol and the volume was made up to 10 mL by using methanol to produce 1 µg/mL solution. Similarly, series of dilutions were prepared by taking 0.1 mL, 0.2 mL, 0.3 mL, 0.5 mL, 0.7 mL, 1 mL, 2 mL, 3 mL, 4mL and 5mL was diluted. The volume was made up to 10 mL using methanol to produce 1 µg/mL, 2 µg/mL, 3 µg/mL, 5 µg/mL, 7 µg/mL, 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL, respectively. The aliquots were then scanned at 225 nm against methanol as blank in UV-Visible spectrophotometer and absorbance was calculated individually. The calibration curve was constructed by plotting absorbance at y-axis and concentration at x-axis. A linear plot was drawn and linear regression equation was applied.

2.6.2 Accuracy

The accuracy of the developed method was evaluated by the recovery studies at three levels *viz.* 80 %, 100 %, 120 % by standard addition methodology. The average percentage recovery and percentage RSD was calculated to evaluate the method's accuracy [10].

2.6.3 Precision

The precision studies were demonstrated by the interday (repeatability), intraday and change of laboratory (reproducibility) variation studies. It was performed by three replicate analysis of the same working solution. The mean, standard deviation and percentage RSD was calculated to evaluate the method's precision [11].

2.6.3.1Intraday study

For intraday variation study three concentrations 10, 30, and 50 µg/mL were prepared and the absorbance of the aliquots were analyzed at 0, 5 and 10 h with the aid of UV-visible spectrophotometer at 225 nm wavelength.

2.6.3.2 Interday study

For intraday variation study three concentrations 10, 30, and 50 µg/mL were prepared and the absorbance of the aliquots were analyzed for three consecutive days with the aid of UV-visible spectrophotometer at 225 nm wavelength.

2.6.3.3 *Repeatability study*

For repeatability studies three concentrations 10, 30, and 50 µg/mL were prepared and the absorbance of the aliquots were analyzed in LAB I and LAB II respectively with the aid of UV-Visible spectrophotometer at 225 nm wavelength.

2.6.4 Limit of detection and limit of quantification (LOD & LOQ)

Both LOD and LOQ was calculated with the linearity studies [12]. The steps involved are as under;

The sample concentration (1 µg/mL) was prepared by taking 0.1 mL from the working solution diluted with 10 mL of the methanol in test tube. Similarly, series of dilutions were prepared by taking 0.5 mL, 1 mL, 2 mL, 3 mL and 5mL was diluted. The volume was made up to 10 mL using methanol to produce 1 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL. The sample concentrations were prepared in five replicates. Subsequently, five calibration curves were constructed by analyzing the prepared concentrations. The standard deviations of Y-intercepts and mean slopes of the calibration plots were determined from the linearity data by applying linear regression equations. The absorbance of the aliquots were analyzed with the aid of UV-Visible spectrophotometer at 225 nm wavelength. The LOD was calculated by the following expressions;

$$LOD = 3.3 \times \frac{\sigma}{b}$$

The LOQ was calculated by the following expression;

$$LOQ = 10 \times \frac{\sigma}{b}$$

Where,

σ = Standard deviation of the peak areas of the drug (n = 5)
b = Slope from linearity curve

2.6.5 Specificity

For specificity of the developed method, four samples were taken for the study *i.e.* pure drug solution, blank, placebo and drug + placebo solution at concentration 10 µg/mL. The scans of the aliquots were obtained with the aid of UV-Visible spectrophotometer at 200 – 800 nm wavelength [13].

2.6.6 Robustness

For robustness of the developed method the sample concentration (10 µg/mL) was prepared.

The absorbance of the aliquots were analyzed with the aid of UV-Visible spectrophotometer at (225 ± 2) 223 and 227 nm wavelengths. The mean, standard deviation and percentage RSD was calculated to evaluate the method's robustness [14].

2.6.7 Ruggedness

For ruggedness of the developed method nine samples of three concentrations 10, 30, and 50 µg/mL were prepared. The absorbance of the aliquots were analyzed by two different analyst with the aid of UV-Visible spectrophotometer at 225 nm wavelength. The mean, standard deviation and percentage RSD was calculated to evaluate the method's ruggedness [15].

2.7 Forced Degradation Study

Specificity of the method was established by forced degradation stability studies. The drug was exposed to standard acid, base hydrolytic, oxidative, radiation exposure and thermolytic stress conditions **[**16].

The samples for this analysis was made in duplicates, one of which served as control and other as test sample. For acid hydrolytic, the desired concentration of the drug (10 µg/mL) of control sample was prepared in methanol while test sample was prepared in 0.1N H_2SO_4. For base hydrolytic study, the desired concentration of the drug (10 µg/mL) of control sample was prepared in methanol while test sample was prepared in 0.1N NaOH. For oxidative stress study, concentration of the drug (10 µg/mL) of control sample was prepared in methanol while test sample was prepared in 3 % H_2O_2. For ultraviolet radiation exposure test, the drug as control sample was packed in aluminium foil and the test sample was directly exposed to ultraviolet light under ultraviolet lamp. For temperature and humidity stress, the control sample and test sample was kept under 25 ºC + 75 % RH and 80 ºC + 75 % RH, respectively. All the stress conditions remained for 24 hours. Then the absorbance of the aliquots were analyzed with the aid of ultraviolet visible spectrophotometer at 225 nm wavelength. The drug content was estimated by linearity equation and percentage drug found was calculated.

2.7.1 Study design

The study design for forced degradation study is shown in Table 1.

2.8 Statistical Analysis

For statistical analysis, *i.e.* calculation of mean, standard deviation (σ), percentage relative standard deviation (% RSD), standard error of mean (SEM), regression analysis, generation of scatter plot, Microsoft Excel *version* 2016. Data is represented by mean ± SEM.

3. RESULTS AND DISCUSSION

The solubility of the drug was checked in nine different solvents at 1 mg/mL concentration. However the drug was found to be stable and soluble in polar solvents *i.e.* methanol, chloroform, ethanol, acetonitrile except water, from very soluble to sparingly soluble. It was found insoluble in acids hydrochloric acid, nitric acid, sulphuric acid. It was also slightly soluble in 5% sodium hydroxide Table 2.

3.1 Melting Point

Melting point also called liquefaction point, is an old, primary and easy method for the identification of a compound. It is referred to as the temperature at which solid and liquid phase co-exist in equilibrium with each other. Normally at this temperature the solid begins to change in liquid phase. The method is now obsolete is most cases as most of the compounds share almost similar or nearby melting points due to which it is sometimes tough to identify the compounds [17]. The melting point of the sample was calculated as an average of its two temperatures (when the solid start to melt and when it's totally melted) and it was found to be 146 ºC approximate Table 3.

3.2 Fourier Transform Infrared Spectroscopy

The FTIR spectroscopy is a modern technique which measures the absorption or emission IR spectrum of a compound. Generally in characterization analysis this techniques helps in the identification of functional groups which in turn helps to draw the possible structure of the molecule. Currently it is widely employed to identify the compounds based on their spectrum similarity with the reference standard. Similar compounds give rise to similar IR spectra and confirms the identity [18]. Presently, the FTIR spectroscopy was carried out for the sample Fig. 2. The sample IR graph showed sharp drops at

3263.16 cm^{-1}, 1654.42 cm^{-1}, 1458.23 cm^{-1}, 1364 cm^{-1} and 741.64 cm^{-1} confirming the structural functional groups present.

3.3 Determination of λmax

λ_{max} refers to the specific wavelength at which maximum amount of ultraviolet radiation is absorbed. Every compound almost shares a different λ_{max}. It goes about as a solitary quantitative parameter to look at the absorption range of various molecules. It is helpful as in method development as it eases the process for us to make the analyte identifiable or analyte in question more prominent. It also helps to ensure in case if UV spectrometer is used as a detector in any other technique such as HPLC system [19].

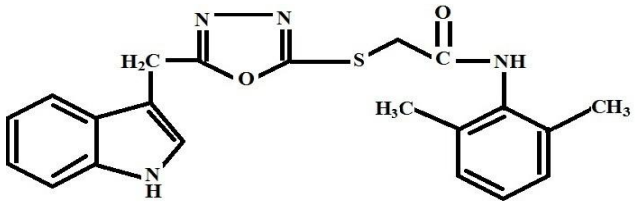

Fig. 1. Structure of *N-(2,6-dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamid*

Table 1. Study deign of forced degradation study for *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl} acetamide*

Parameters	Sample with treatment		Duration of exposure (hr)
	Control	Test	
Acid hydrolysis	Methanol	0.1N H_2SO_4	24
Base hydrolysis	Methanol	0.1N NaOH	24
Oxidative stress	Methanol	3% H_2O_2	24
Temperature and humidity	25 ºC+75 % RH	80 ºC+75% RH	24
UV light exposure	Wrapped in aluminium foil and exposure	Direct exposure	24

**RH – Relative humidity*

Table 2. Solubility study in different solvent systems for *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl} acetamide*

Sr. No.	Solvents	Solubility status
1	Water (H_2O)	Insoluble
2	Methanol (CH_3OH)	Very Soluble
3	Ethanol (C_2H_5OH)	Freely soluble
4	Acetonitrile	Sparingly soluble
5	1N Hydrochloric acid (HCl)	Insoluble
6	5% Sodium hydroxide (NaOH)	Slightly soluble
7	Nitric acid (HNO_3)	Insoluble
8	Sulphuric acid (H_2SO_4)	Insoluble
9	Chloroform ($CHCl_3$)	Soluble

Table 3. Melting point by Griffin and George melting point apparatus of *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl}acetamide*

Sr. No.	T_1°C	T_2°C	Melting Point °C
1	144	148	146
2	143	148	145.5
3	143	149	146
			Mean = 145.83 ≈146

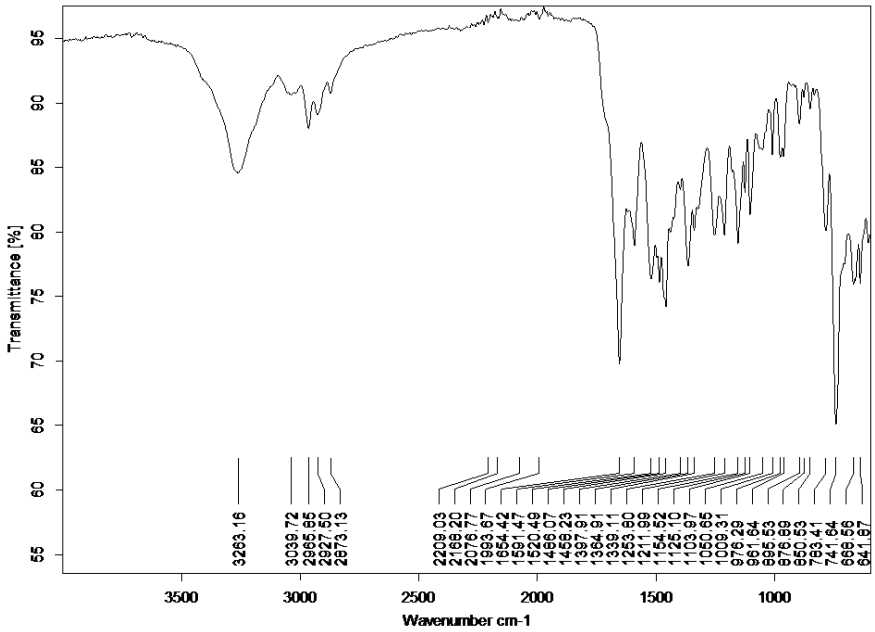

Fig. 2. FTIR spectrum of sample *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl} acetamide*

The maximum wavelength was recorded by running the scan in UV-Visible region from 200 to 800 nm. The maximum absorption of the electromagnetic radiation was found at 225 nm wavelength. It was denoted as λ_{max} Fig. 3.

3.4 Linearity

Linearity is the property of a scientific relationship or function which implies that it very well may be graphically spoken to as a straight line [20].

In the first step of validation linearity was figured out. Incremental or replicate analysis was employed for the purpose *viz.* a number of solutions were prepared in gradually increasing concentration. Presently, five concentrations (10 – 50 µg/mL) were made and absorbance was recorded at 225 nm wavelength against them Table 4.

The linear relationship between concentration and absorbance data of the samples was noticed and a calibration curve was obtained by plotting a graph. The trend line was drawn and slope was calculated by linear regression equation (Fig 4).

3.5 Accuracy

The accuracy of an analytical procedure communicates the closeness of understanding between the worth which is acknowledged either as a traditional genuine value or an acknowledged reference value and the value found. This is often termed trueness [21].

The accuracy analysis was carried out at three levels 80, 100, and 120 %. The results were noted and tabulated in terms of recovery studies or standard addition method Table 5. The accuracy range was found to be 98.621 – 100.674.

3.6 Precision

The precision of an analytical method communicates the closeness of agreement (level of disperse) between a progression of estimations acquired from a number of experiments of the homogeneous sample under the recommended conditions [22]. The precision study was carried out in interday and intraday variation study.

The results are as under;

3.6.1 Intraday precision

The intraday study was carried out by performing the analysis at different times on a same day. 5 hours gap was decided and statistical parameters were noticed. The outcomes fall

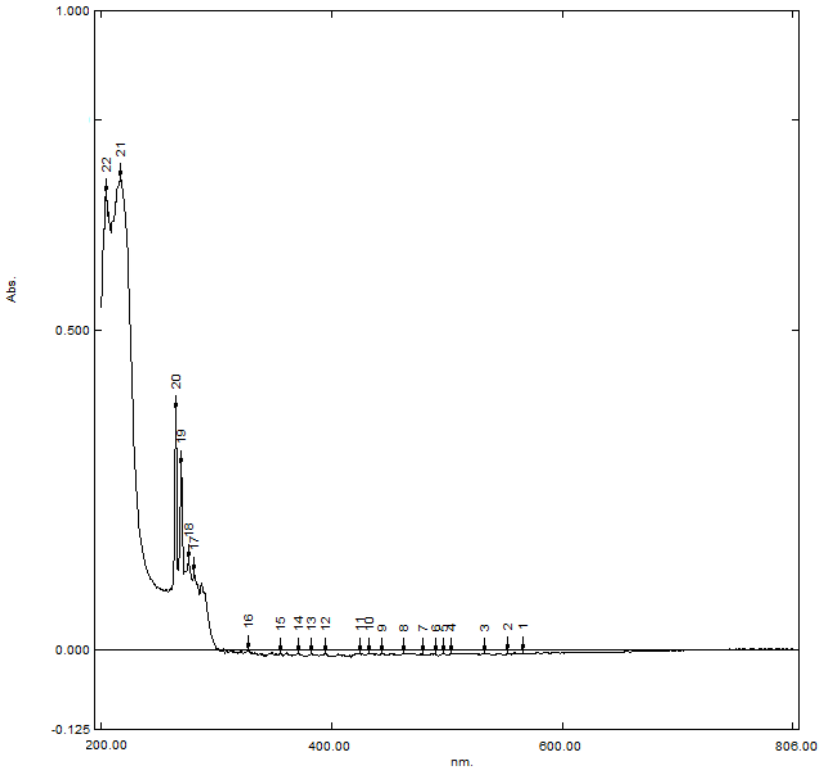

Fig. 3. Ultraviolet visible spectrum of *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide*

inside the approved criteria *i.e.* the % RSD is less than or equal to 2% Table 6.

3.6.2 Interday precision

The interday study was carried out by performing the analysis on three different days and statistical parameters were noticed. The outcomes fall inside the approved criteria *i.e.* the % RSD is less than or equal to 2 % Table 7.

3.6.3 Repeatability analysis

The outcomes fall inside the approved criteria *i.e.* the % RSD is less than 2 % Table 8.

3.7 Limit of Detection and Limit of Quantification

The LOD of an individual explanatory system is the most reduced measure of analyte in a sample, which can be recognized, yet not really quantitated as an accurate worth. As far as possible, LOQ is the most minimal measure of analyte in a sample which can be distinguished however not quantitates [23]. The LOD and LOQ were determined by their respective expressions as mentioned above and utilizing the slope value obtained from linearity equation ($y = 0.0136x + 0.1997$; $R^2 = 0.9983$) Table 9.

Hence Table 10,

3.8 Specificity

Specificity is the capacity to survey the analyte unequivocally within the sight of segments which might be relied upon to be available [24]. The developed method was found to be specific as shown by the UV-visible photometric scans. As there wasn't any interference found. All four samples were ran over 200 to 800 nm. In all scans, neither blank Fig. 5 nor did placebo Fig. 6 show any absorbance at the selected wavelength 225nm. However, the spectrum for reference standard/ pure drug sample Fig. 7 and the drug sample Fig. 8 were comparable and showed peak at 225 nm wavelength. Evidently, the method is selective for the analysis of the said drug.

Table 4. Concentration and absorbance data with Ultraviolet visible spectrophotometer from different concentrations of *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide*

Serial no.	Concentration (µg/mL)	Absorbance
1	0.5	0.090
2	1	0.097
3	2	0.104
4	3	0.108
5	5	0.119
6	7	0.129
7	10	0.147
8	20	0.199
9	30	0.251
10	40	0.307
11	50	0.360

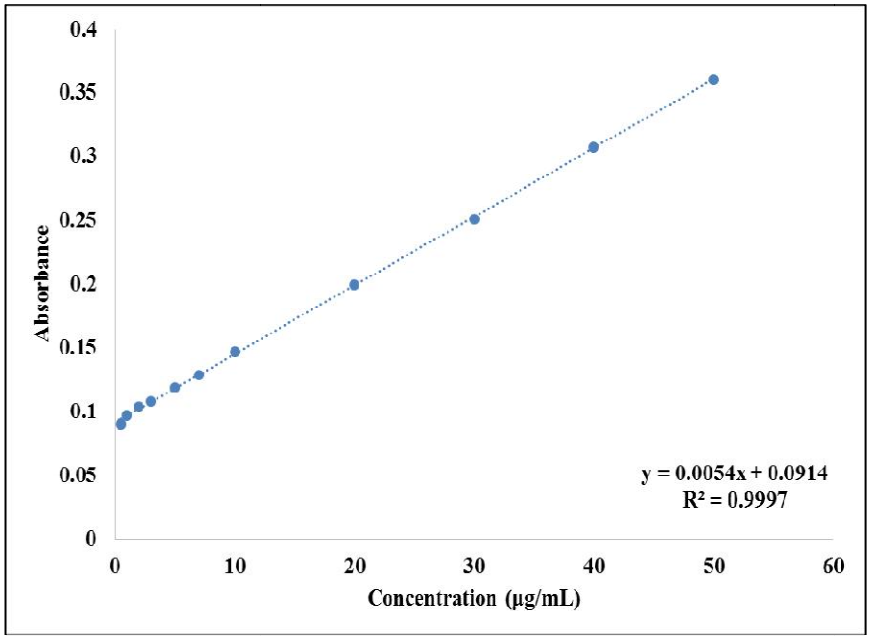

Fig. 4. Linearity curve of *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl} acetamide*

The straight line depicted the linearity in the following concentration range 10 – 50 µg/mL. The correlation coefficient (R^2) was found to be 0.9983.
Linearity equation from the curve;
y = 0.0054x + 0.0914
R^2 = 0.9997
Where,
Regression equation = 0.0054x + 0.0914
Slope (b) = 0.0054
Y-intercept = 0.0914
Pearson coefficient of correlation R^2 = 0.9997

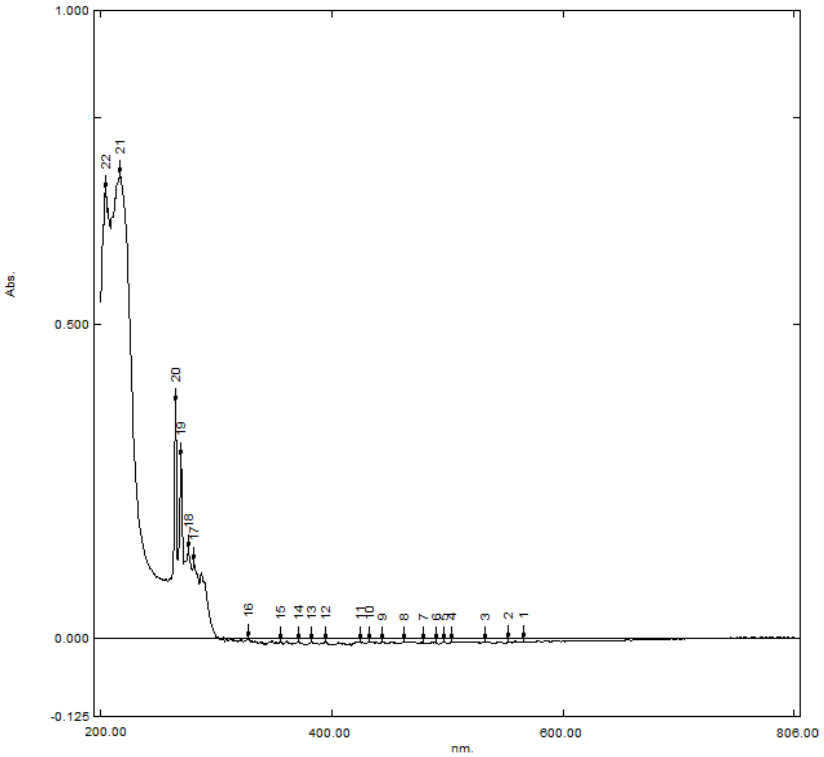

Fig. 5. Ultraviolet visible spectrum of the analyte *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl} acetamide*

3.9 Robustness

The robustness of a systematic methodology is a proportion of its ability stays unaffected by little, however conscious varieties in strategy parameters and gives a sign of its dependability during typical use [25]. In robustness analysis of validation, the impact of little, conscious variations of the scientific parameters on the absorbance of the drug was analyzed. For the said purpose, change in the wavelength was brought about up to 225 ± 2 nm. Acceptable results were obtained as % RSD was less than or equal to 2 %. The results of robustness analysis was calculated and tabulated Table 11. The designated factor *viz.* wavelength persisted unpretentious by minor deviations.

3.10 Ruggedness

The ruggedness is a level of reproducibility of test result under check of condition like an alternate examiner, various instruments and various days [26]. The ruggedness of the developed method was tested in terms of changing the analyst *i.e.* the procedure was performed by two different analysts and results were recorded. The results were within the acceptance criteria *viz.* % RSD less than or equal to 2 % Table 12.

3.11 Forced Degradation Study

The method resulted in stability indicating by showing altered results for the test samples when exposed to deteriorative conditions Table 13. The drug didn't endure harsh conditions and resulted in degradation, yet the method remained selective to the original form.

3.12 Optical Characteristic of Drug

The optical characteristics of drug obtained from the following study are tabulated Table 14.

3.13 Validation Summary

The process was validated in terms all parameters recommended by ICH guidelines Table 15.

Table 5. Accuracy analysis in terms of percentage recovery at 80, 100 and 120 % for *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl}acetamide*

Percentage Recovery analysis	Formulation mg	Analyte added mg	Abs	Analyte found mg	Percentage recovery	Average recovery %	% RSD
80	10	8	0.134	7.889	98.611	96.296	2.40385
80	10	8	0.133	7.704	96.296		
80	10	8	0.132	7.519	93.981		
100	10	10	0.144	9.741	97.407	97.4074	1.90114
100	10	10	0.143	9.556	95.556		
100	10	10	0.145	9.926	99.259		
120	10	12	0.155	11.778	98.148	100.72	3.18948
120	10	12	0.156	11.963	99.691		
120	10	12	0.364	12.081	100.674		

Table 6. Intraday precision analysis by time variation at 0, 5 and 10 h for *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide*

Intraday precision						
Sr. no.	Conc.(µg/mL)	Absorbance*			Standard deviation σ	% RSD
		0 h	5 h	10 h		
1	10	0.1143±0.0017	0.145±0.0008	0.1491±0.0008	0.002646	1.808031
2	30	0.2503±0.012	0.255±0.0017	0.2506±0.0008	0.002603	1.033102
3	50	0.363±0.0015	0.362±0.0021	0.3613±0.0014	0.000839	0.231661

**the results for absorbance are expressed as mean ± SEM*

Table 7. Interday precision analysis by day variation on day 1, day 2 and day 3 from *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide*

Interday precision						
Sr. no.	Conc. (µg/mL)	Absorbance*			Standard deviation σ	% RSD
		Day 1	Day 2	Day 3		
1	10	0.1396±0.0008	0.1416±0.0018	0.141±0.001	0.001018	0.723374
2	30	0.252±0.0005	0.253±0.0011	0.25±0.0005	0.001528	0.606964
3	50	0.3613±0.0008	0.363±0.015	0.365±0.0015	0.001836	0.505591

**the results for absorbance are expressed as mean ± SEM.*

Table 8. Repeatability precision analysis, at two different labs for *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide*

Change of Lab					
Sr. no.	**Concentration (µg/mL)**	**Absorbance***		**Standard deviation σ**	**% RSD**
		Lab 1**	**Lab 2*****		
1	10	0.1426±0.1429	0.1436±0.0016	0.00071	0.4939
2	30	0.2526±0.2529	0.2533±0.2541	0.00047	0.18633
3	50	0.364±0.3646	0.3636±0.3649	0.00024	0.06478

**the results for absorbance are expressed as mean ± SEM. **Lab 1: Post-graduate Lab for Pharmaceutical Chemistry, ***Lab 2: Post-graduate Lab for Pharmaceutics*

Table 9. Y-Intercepts and slope data from calibration curves for *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl} acetamide*

Sr. no.	Y-intercept	Slope
1	0.0929	0.0053
2	0.092	0.0054
3	0.0943	0.0053
4	0.0935	0.0052
5	0.0935	0.0057
	SD = 0.000853	Mean = 0.00538

Table 10. Summary of Limit of Detection (LOD) and Limit of Quantification (LOQ)

Sr. no.	Parameter	Results
1	LOD	0.523356 µg/mL
2	LOQ	1.585928 µg/mL

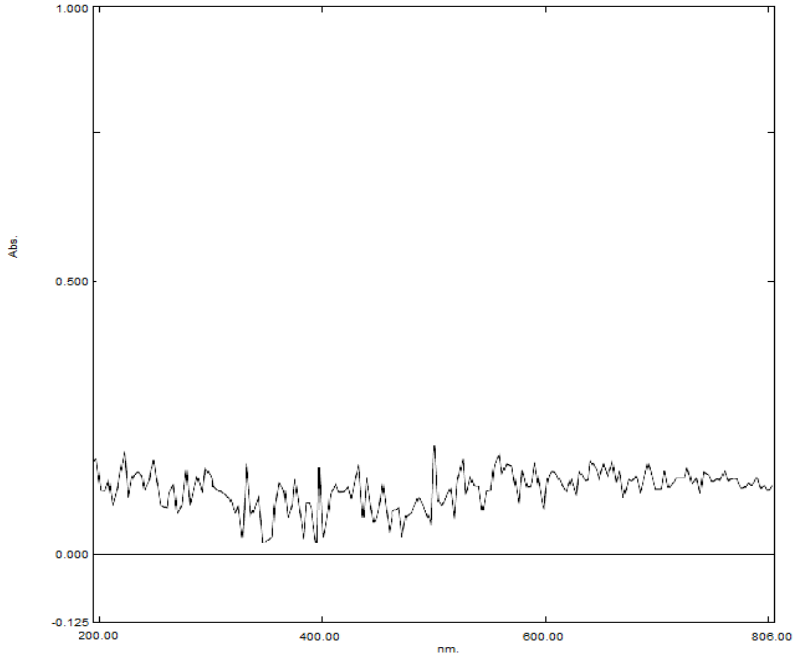

Fig. 6. Ultraviolet visible spectrum of blank (methanol)

Table 11. Robustness analysis of developed method at different wavelengths for *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl} acetamide*

Sr. no.	Concentration µg/mL	Wavelength		
		223 nm	227 nm	
1	10	0.141	0.144	
2	10	0.14	0.145	
3	10	0.139	0.146	
4	10	0.144	0.144	
5	10	0.142	0.143	
		0.1412±0.0008	0.1444±0.0005	**Mean***
		0.00192	0.00114	**Std. deviation**
		1.36228	0.7896	**% RSD**

**the results for absorbance are expressed as mean ± SEM*

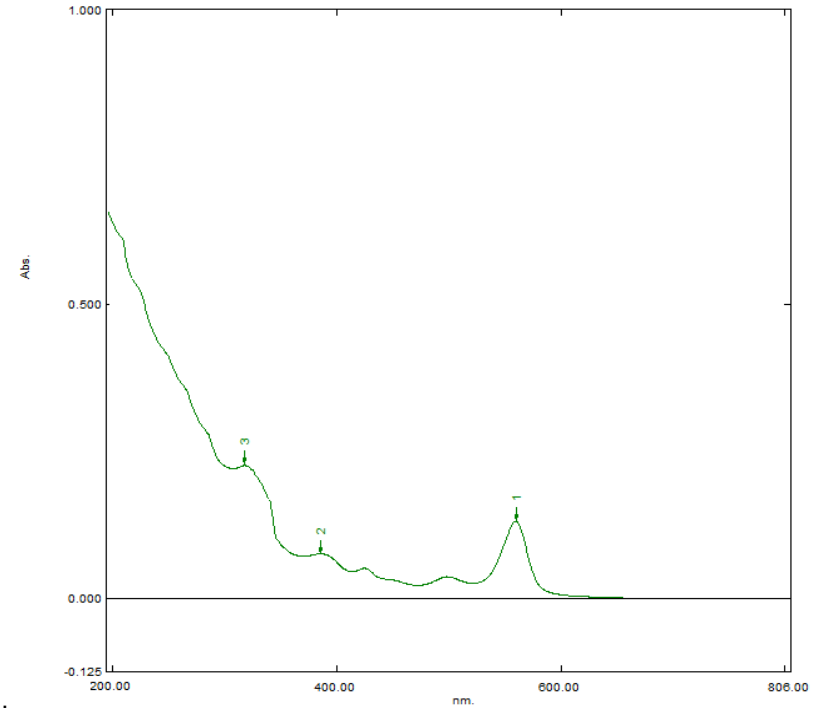

Fig. 7. Ultraviolet visible spectrum of the placebo

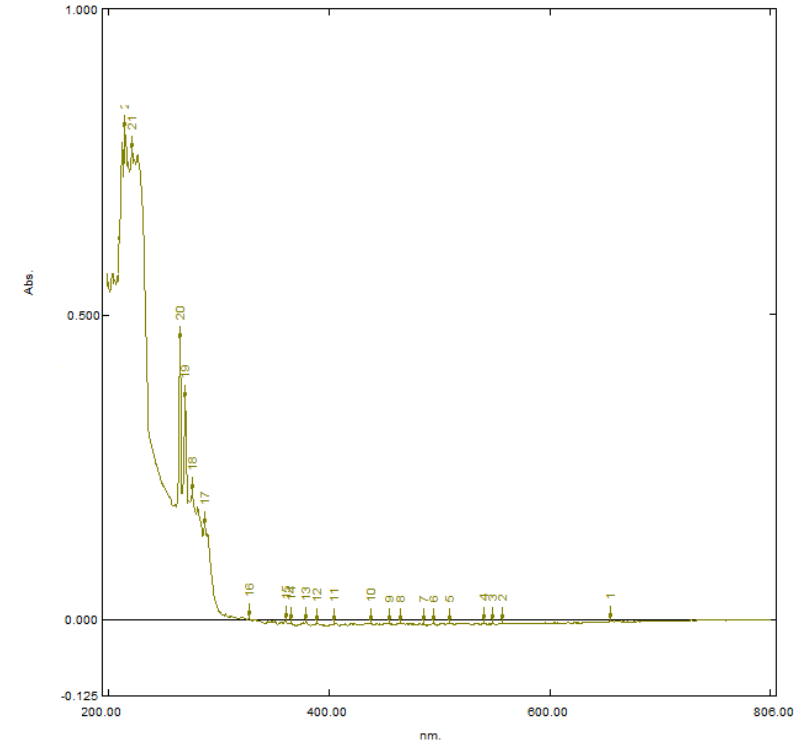

Fig. 8. Ultraviolet visible spectrum of the Placebo + N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl} acetamide

Table 12. Robustness analysis in terms of changing the analyst for *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl} acetamide*

Change of analyst					
Sr. no.	Concentration (µg/mL)	Absorbance*		Standard deviation σ	% RSD
		Analyst 1**	Analyst 2***		
1	10	0.1433±0.0006	0.142±0.0005	0.000943	0.66087
2	30	0.258±0.0017	0.252±0.0015	0.004243	1.663781
3	50	0.3606±0.0014	0.3633±0.0008	0.001886	0.520889

**the results for absorbance are expressed as mean ± SEM. **Analyst 1: Muhammad Shaharyar Khan Afridi*
****Hafiz Hanzalah Fahham*

Table 13. Forced degradation study for *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl} acetamide*

Sr. no.	Degradationtype	Absorbance		Drug found		% Drug found	
		Control	Test	Control	Test	Control	Test
1	Acidic hydrolysis	0.144	0.11	9.74074	3.44444	97.4074	34.4444
2	Basic hydrolysis	0.144	0.124	9.74074	6.03704	97.4074	60.3704
3	Oxidation	0.140	0.121	9	5.48148	90	54.8148
4	UV light exposure	0.142	0.098	9.37037	1.22222	93.7037	12.2222
5	Temperature and humidity	0.143	0.112	9.55556	3.81481	95.5556	38.1481

Table 14. Optical characteristics of *N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)- 1,3,4-oxadiazol-2-yl]sulfanyl} acetamide*

Sr. no.	Parameters	Results
1	λ_{max}	225 nm
2	Beer's Lambert Law limit	0.5 – 50 µg/mL
3	Regression equation	0.0054x + 0.0914
4	Correlation coefficient R^2	0.9997

Table 15. Validation summary of the developed method

Sr. no.	Parameters	Results
1	Precision indicated by % RSD	
	Intraday	0.231661
	Interday	0.505591
	Repeatability	0.06478
2	Accuracy indicated by % recovery	95.556 – 104.321
3	Specificity	No impurities found
4	Robustness indicated by % RSD	0.7896
5	Ruggedness indicated by % RSD	0.520889
6	LOD	0.523356 µg/mL
7	LOQ	1.585928 µg/mL

4. CONCLUSION

New molecules with optimized potential have been discovering day by day and every new drug needs an appreciable method for its identification. A simple, selective, sensitive, reliable and specific method has been developed and validated with good precision and accuracy for the synthetic molecule N-(2,6-Dimethylphenyl)-2-{[5-(1H-indol-3-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl} acetamide, having substantial detection limits. The method maybe used for the accurate assessment of the analyte molecule for both in raw material and in finished product. Therefore, it is concluded that the analyte can be evaluated with the developed method and can be accessed for its stability by the developed method for the future scientific research purposes.

CONSENT

Not Applicable.

ETHICAL APPROVAL

Not Applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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