

Environmentally Benign UV Spectrophotometric and Stability-Indicating RP-HPLC Methods for Tedizolid Determination: Method Development, Validation, Forced Degradation and Greenness Assessment

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ABSTRACT

Objective:

This study aims at developing and validating of three safe UV spectroscopic techniques (zero order, first derivative and area under the curve) as well as a simple and stability-indicating RP-HPLC assay for the quantitation of Tedizolid in its bulk as well as dosage form.

Significance:

Development of green, rapid, reliable and cost-effective methods is in great demand in pharmaceutical quality control and stability studies. The application of principles of green analytical chemistry with the optimized and validated method has yielded the facile, accurate and eco-friendly method for regular pharmaceutical analysis.

Methods:

Validation was successfully carried out for three UV spectrophotometric techniques using green distilled water and a fast, stable and specific RP-HPLC. Forced degradation of the target drug was performed by RP-HPLC and green analysis of all methods were performed using standard methods Analytical Eco-Scale, BAGI, MoGAPI and ACS Green Chemistry Institute Analytical Procedure Calculator (ACS-GCI).

Results:

The UV techniques resulted linear over 3-15 µg/mL ($r=0.999$), whilst the RP-HPLC technique was linear over the interval 5-25 µg/mL ($R=0.997$), providing RT 2.7 min. All presented acceptable levels of specificity, accuracy (99.91-100.80%), precision (%RSD <2), robustness and sensitivity. RP-HPLC clearly allowed the separation of Tedizolid from the respective impurities (in acidic, alkaline, oxidative, thermal and photolytic stress). Green evaluation presented excellent properties and values of Analytical Eco-Scale (100% for the UV and 86% for the RP-HPLC techniques).

Conclusions:

The proposed UV spectrophotometric and RP-HPLC methods were simple, fast, precise, validated, stability-indicating and eco-friendly and are useful in the quality control and stability studies of Tedizolid both in bulk drug and in tablet dosage forms.

KEYWORDS: Tedizolid; Green Analytical Chemistry; RP-HPLC; UV Spectrophotometry; Stability-Indicating Method; Method Validation

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1. INTRODUCTION

Antimicrobial resistance (AMR) has come up as one of the biggest global public health issues, because it really weakens how well existing antibiotics work, and it also makes infectious diseases heavier to manage. At the same time, the rapid rise of multi drug resistant (MDR) Gram-positive pathogens, especially methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE) and penicillin-resistant *Streptococcus pneumoniae*, has seriously cut down the treatment value of usual antimicrobial agents. These hard-to-treat organisms often go with longer hospital stays, higher financial burdens for care, more complications, and even greater death rates, so there's a clear and urgent need for fresh antibacterial options, ones that show better performance and safer profiles.^[1,2]

Tedizolid phosphate is a second generation oxazolidinone prodrug that is approved for acute bacterial skin and skin structure infections, ABSSSI, caused by susceptible Gram-positive organisms. After oral or intravenous administration, tedizolid phosphate is quickly converted by endogenous phosphatases into its active component, tedizolid. This active form blocks bacterial protein synthesis via selective attachment to the 50S ribosomal subunit, not just in a general way, but in a fairly targeted fashion. When it is compared with linezolid, tedizolid shows stronger antibacterial potency, better performance against resistant bacterial strains, a once daily dosing schedule and a shorter overall course. It also tends to have a lower rate of haematological adverse events, which matters a lot clinically. Because of these pharmacological benefits tedizolid has turned into a useful therapeutic choice for dealing with complicated Gram-positive bacterial infections.^[3,4]

Reliable analytical methods are sort of essential during the whole pharmaceutical product lifecycle, so that drug identity stays consistent, plus potency, purity, stability, and overall quality. Regulatory bodies insist on validated analytical procedures not only for raw materials, but also for finished dosage forms, stability experiments, and the daily routine

quality control work. So, analytical methods really need to deliver enough selectivity, accuracy, precision, sensitivity, and robustness, all while they are in line with current regulatory expectations, this includes the International Council for Harmonisation (ICH) Q2(R2) guideline. More and more people are talking about lifecycle management of analytical procedures, and that makes it even more important to design methods that are scientifically dependable, but also economical and practical for routine work on the plant floor. Several analytical techniques have been described for finding tedizolid in biological matrices and in pharmaceutical products. For instance, high performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection; ultra-high-performance liquid chromatography together with tandem mass spectrometry (UHPLC-MS/MS); liquid chromatography-mass spectrometry (LC-MS/MS), and also fluorescence based spectroscopic methods. Most of these approaches were originally made for pharmacokinetic investigations and therapeutic drug monitoring, using plasma or serum as the sample type. Even though these options can give strong sensitivity and good selectivity, they often come with sophisticated instrumentation and higher operational costs, plus they need lengthy sample preparation, very trained staff, and they consume a fair number of organic solvents. Because of that, their routine use in many quality control labs can be rather limited.

Ultraviolet-visible (UV-Visible) spectrophotometry, still, is one of the most used analytical approaches for routine pharmaceutical analysis since it is simple affordable, quick, and needs only minor sample preparation. More advanced UV ways like derivative spectrophotometry and area under the curve (AUC) approaches also help in boosting analytical selectivity plus sensitivity, and they do not really make the work any harder operationally. Even so, only a limited number of UV spectrophotometric methods have been published for tedizolid determination in pharmaceutical dosage forms, which points to a need for extra validated procedures that can actually fit routine quality control.^[5,6]

Reverse-phase high-performance liquid chromatography, often just called RP-HPLC, still stays the reference analytical method for pharmaceutical assays, mainly because it provides excellent resolution, precision, and reproducibility plus it can cleanly separate degradation products from the parent drug. Stability-indicating RP-HPLC methods are especially useful because they can quantify the active pharmaceutical ingredient while impurities, breakdown products, and even formulation excipients are sitting there in the mix. These kinds of approaches matter a lot through pharmaceutical development, stability testing, regulatory filings, and later on post-marketing quality surveillance. Even if chromatographic methods for tedizolid have been reported, only a limited number of works really concentrate on creating fast, stability-indicating methods that are meant for everyday pharmaceutical quality control, using simplified chromatographic conditions.^[7]

In recent years, Green Analytical Chemistry (GAC) has become kind of a central part in pharmaceutical analytical research. It's basically about making sure analytical procedures have a smaller environmental footprint, like by cutting down on hazardous solvent use, lowering the energy demand, reducing waste output, and also improving operator safety, all while not letting the analytical performance drop. A bunch of assessment tools, for example the Analytical Eco-Scale, Blue Applicability Grade Index (BAGI), the Modified Green Analytical Procedure Index (MoGAPI), and the ACS Green Chemistry metrics, have been brought in so the environmental sustainability of methods can be measured in a more quantitative way. Using these greenness appraisal tools during method development fits the more modern regulatory mindset, and it also helps support sustainable lab routines, really.^[8]

After looking over the published literature in a broad way it becomes clear there are a few big research gaps. In most cases, the analytical methods reported for tedizolid are aimed at bioanalytical work rather than actual pharmaceutical quality control. There are only very limited studies that mention simple UV spectrophotometry using solvents that are considered more environmentally friendly, and just a small number of papers actually put together stability-indicating RP-HPLC with well-planned forced degradation experiments. On top of that, many of the published approaches do not really do a full greenness check using more than one widely used, internationally recognized evaluation tool. So

overall, there is still a clear need for analytical procedures that are simple, quick, not expensive, environmentally sustainable, fully validated following the current ICH Q2(R2) requirements, and appropriate for routine quality control of tedizolid drug products. So, in the present work we tried to develop and validate three green UV spectrophotometric methods, meaning the zero-order technique, the first-derivative approach, and the area-under-curve method. Alongside that, we also developed a rapid stability-indicating RP-HPLC method for measuring tedizolid in both bulk drug and tablet dosage forms. The proposed methods were validated following the ICH Q2(R2) guidelines, tested on commercial formulations, and backed up by forced degradation studies. Finally, we also ran a more comprehensive environmental assessment using Analytical Eco-Scale, BAGI, MoGAPI, and ACS Green Chemistry metrics, so we could clearly show both analytical performance and environmental sustainability at the same time.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

The tedizolid phosphate reference standard (purity >99%) was got as a gift sample from a well-known pharmaceutical manufacturer. The commercially available Starizo® tablets, which contained Tedizolid phosphate, corresponding to 200 mg Tedizolid were purchased from the local market and then used during assay work. For the chromatographic analysis, HPLC grade acetonitrile, along with dipotassium hydrogen phosphate as an analytical reagent grade, and formic acid were used, no other changes. For the UV spectrophotometric studies distilled water was used throughout, as the analytical solvent. Overall, all chemicals, reagents that were part of the experiment, were either analytical grade or HPLC grade.

2.2. Instrumentation

The UV spectrophotometric assessment was done with a Shimadzu UV-1800 double-beam UV-Visible spectrophotometer, using UV Probe software, and set with matched 1 cm quartz cells. For the chromatographic part, a Shimadzu HPLC setup was used, fitted with a UV detector. The separation step used an Enable C18 column, dimensions were 250 × 4.6 mm and 5 µm particle size, with the usual setup. Analytical weighing was made using a calibrated electronic balance and sonication was carried out in an ultrasonic bath.

2.3. Drug and Pharmaceutical Formulation

The tedizolid phosphate reference standard, was used for method development along with validation, of course. Marketed Tedizolid tablets (Starizo®) were analysed in order to see how far the developed methods can actually fit into routine pharmaceutical quality control. The tablet powder, equivalent to the labelled amount of Tedizolid was weighed accurately, then it was dissolved in the appropriate analytical solvent, sonicated, filtered, and afterwards suitably diluted before going into UV spectrophotometric and RP-HPLC analysis.

2.4. UV Spectrophotometric Method Development

2.4.1. Solvent Selection

A few solvents got evaluated, sort of based on how well Tedizolid dissolves, along with spectral behaviour, overall analytical sensitivity, environmental safety, and how cost-effective things turned out. Distilled water ended up giving complete drug solubility, pretty steady absorbance profiles, nearly no background interference, and strong environmental compatibility. So in the end, distilled water was picked as the analytical solvent, for all the UV spectrophotometric methods.^[9]

2.4.2. Selection of Analytical Wavelength (λ_{max})

A standard solution of Tedizolid got prepared and scanned across the 200–400 nm window against distilled water, taken as the blank. The absorbance scan showed a prominent peak at around 298 nm, and that particular wavelength was chosen as the analytical point for the zero-order UV method.^[10]

2.4.3. Zero-Order UV Spectrophotometric Method

A standard stock solution of Tedizolid (1000 µg/mL) was made using distilled water, then it was diluted again to get what we can call a working stock solution 100 µg/mL. Later on, suitable aliquots were taken and diluted further, in order to form calibration standards across the concentration area of 3–15 µg/mL. For every solution, the absorbance was read at 298 nm, and after that a calibration curve was formed by putting absorbance on the y-axis and concentration on the x-axis, sort of plot wise. Then the method was applied for numerical determination of tedizolid in the bulk drug material as well as in the commercial tablet formulations.^[11]

2.4.4. First-Derivative Spectrophotometric Method

The zero-order absorption spectra were mathematically transformed into first-derivative spectra, using UV Probe software, with optimised

derivative parameters in place. The derivative signal amplitude was then taken at 317 nm, where the best analytical sensitivity and linearity were seen. Calibration standards, prepared across the concentration interval of 3–15 µg/mL were checked, and the measured derivative amplitude got plotted against concentration for the final quantitative determination of Tedizolid.^[12]

2.4.5. Area Under Curve (AUC) Method

The AUC spectrophotometric method was put together by kind of integrating the spectral area across the wavelength range 288–308 nm, sort of the usual. Then calibration solutions with 3–15 µg/mL tedizolid were analysed and the summed absorbance area became the basis for quantitative estimation. After that, the method was validated as well, and later used on pharmaceutical dosage forms, basically to see how it performs in real units.^[13]

2.5. RP-HPLC Method Development^[14–17]

2.5.1. Column Selection

A number of different reversed-phase chromatographic columns were assessed until adequate peak symmetry, theoretical plate count, and retention behaviour was achieved. The column that gave the most promising chromatographic profile was the Enable C18 (250 4.6 mm, 5 µm) analytical column.

2.5.2. Mobile Phase Optimization

Optimization of mobile phase composition different compositions of mobile phase (phosphate buffer and organic modifiers) was screened to find optimum mobile phase. A composition of dipotassium hydrogen phosphate buffer and acetonitrile give good symmetrical peaks with good separation and acceptable system suitability parameters. Thus, it was used as the optimized mobile phase.

2.5.3. Flow Rate Optimization

Flow rates between 0.8–1.2 mL/min were tested whilst optimizing chromatography. A flow rate of 1.0 mL/min produced an optimum mixture between peak symmetry, analysis time and chromatographic efficiency. The 1.0 mL/min flow rate was selected as the optimum.

2.5.4. Selection of Detection Wavelength

Tedizolid appeared sufficiently chromatographically sensitive at 300 nm to produce high detector response with minimal background noise. Thus 300 nm was selected for analytical wavelength detection in RP-HPLC analysis.

2.5.5. Optimized Chromatographic Conditions

Separation of Tedizolid was performed by isocratic elution using an Enable C18 column (250 4.6 mm, 5 m) and mobile phase composed of dipotassium hydrogen phosphate buffer and acetonitrile at a flow rate of 1.0 mL/min. Detection was at 300 nm and sample volume was 20 L. The chromatographic profile of Tedizolid under the established conditions resulted in a sharply and symmetrically peaked product eluting at approximately 2.7 min, which could be routinely used for quantitative measurement.

2.6. Method Validation (ICH Q2(R2))

The devised UV spectrophotometric and RP-HPLC method was validated as per ICH Q2(R2) guideline and parameters that included are system suitability, specificity, linearity & range, accuracy, precision (repeatability and intermediate precision), limit of detection (LOD), limit of quantitation (LOQ), robustness, and ruggedness. Acceptance limits for each parameter were fixed as per current ICH recommendation prior to execution of validation studies.

2.6.1. System Suitability

The system was assessed for parameters such as theoretical plates, tailing factor, reproducibility of the retention times, reproducibility of the peak areas, prior to analysis.

2.6.2. Specificity

The absence of any significant peak or interference at the retention time of Tedizolid was verified by injecting blank, standard, sample and stressed sample.

2.6.3. Linearity

Calibration curve was plotted with the five levels for the UV (3–15 g/ml) and RP-HPLC (5–25 g/ml). Regression analysis was done by the method of least squares.

2.6.4. Accuracy

Recoveries were assessed by spike recovery at 50%, 100% and 150% the level of expected concentration. Percentage recovery was computed using the given formula.

2.6.5. Precision

Repeatability (intra-day) and intermediate precision (inter-day) were assessed using replicate analyses, and results were expressed as percentage relative standard deviation (%RSD).

2.6.6. Limit of Detection (LOD)

LOD values were calculated from the standard deviation of the response and the calibration curve's slope as per ICH guidelines.

2.6.7. Limit of Quantification (LOQ)

Calculation of LOQ values the values for LOQ are calculated as: Standard deviation of the response/Slope of the standard curve.

2.6.8. Robustness

The reproducibility of the system was tested by making slight, intended changes to chromatographic parameters such as detection wavelength and mobile phase flow rate.

2.6.9. Ruggedness

Ruggedness determined by analysis across different operating conditions (different operators on different days)

2.7. Assay of Marketed Formulation

To confirm the utility of the devised procedures, commercially Tedizolid tablets were selected for the assay, conducted by UV spectrophotometry as well as RP-HPLC. From the calculated percentage drug content values were compared with labelled claim.

2.8. Forced Degradation Studies

The ability of the RP-HPLC method to indicate stability of Tedizolid was tested by stress studies where Tedizolid was exposed to the acid, alkali, oxidation, thermal and light degradation conditions recommended by ICH guidelines. After stressing for 24–48 hr, stressed samples were neutralised (where applicable), diluted and filtered through a 0.45m membrane filter before injection onto the optimized RP-HPLC system and the resultant chromatograms were analysed for degradation pattern and peak purity.

2.9. Greenness Evaluation

The environmental friendliness of the designed UV spectrophotometric and RP-HPLC methods was comprehensively assessed based on the data collected during validation using four complementary GAC-tools such as Analytical Eco-Scale, Blue Applicability Grade Index (BAGI), Modified Green Analytical Procedure Index (MoGAPI), and ACS-GCI APC. Their usage gave the chance to cover all stages of sample workflow and to estimate reagent's risks, energy use, waste produced, occupational safety, analytical usability, and to give an overall indication of eco-sustainability. The overall evaluation using greenness is different to conventional method validation since it includes all analytical procedure stages, from the sample treatment and the choice of solvent up to instrumental operations, and related

expenses, waste and safety of analyst, therefore various indicators should be used for the overall evaluation of the analytical procedure's ecological safety.^[18–20]

2.9.1. Analytical Eco-Scale^[21,22]

Analytical Eco-Scale was used as a semi quantitative tool in the assessment of the greenness of the developed analytical procedures. A green analytical procedure should be given a score of 100 with points deducted from the score for all items adversely affecting environmental conditions. Penalty points were awarded as per the guidelines suggested by Gauszka and co-workers for the following points;

- Chemicals/Reagents used.
- Nature & hazard classification.
- Amounts of Chemicals/Reagents used.
- Energy used in analysis.
- Occupational hazard of chemical used.
- Sample preparations.
- Amount & method of waste disposal.

The final Eco-Scale score was calculated using the following equation:

$$\text{Analytical Eco-Scale Score} = 100 - \sum \text{Penalty Points}$$

The greenness of each analytical procedure was interpreted according to the following criteria:

Eco-Scale Score	Interpretation
>75	Excellent green analytical method
50–75	Acceptable green analytical method
<50	Inadequate green analytical method

Points in RP-HPLC The factors awarded points for the RP-HPLC were; use of acetonitrile; the phosphate buffer; the use of formic acid; the quantity of solvent consumed; the energy requirements of the instrumental set; the requirement for membrane filtration; and employee exposures. The UV spectrophotometric method utilised purified water for the purpose of the determination and the simplicity of the procedure, together with the very small sample requirements of the method; lead to a much lower total number of points.

2.9.2. Blue Applicability Grade Index (BAGI)^[23]

The overall practical applicability and greenness of developed analytical methods were evaluated simultaneously by using The Blue Applicability Grade Index (BAGI). This metric accounts for not only analytical performance but also operational practicability, as is not typical of green metrics. The following analytical parameters were incorporated into BAGI scoring:

- Type of analytical method
- Analytical technique used
- Requirements for sample preparation
- Number of samples analysed per hour
- Reagents and materials used
- Necessity for pre-concentration
- Level of automation
- Sample volume required
- Applicability for routine analysis.

All parameters were individually evaluated by the BAGI scoring algorithm and then the total BAGI was automatically calculated by means of the BAGI assessment platform. The higher BAGI values mean better combined practical applicability and greenness of the analytical methods. The BAGI star diagram for UV spectrophotometric and RP-HPLC methods was visually plotted, for enabling an objective comparison of the greenness and analytical feasibility.

2.9.3. Modified Green Analytical Procedure Index (MoGAPI)^[24]

The Modified Green Analytical Procedure Index (MoGAPI) is an index used to estimate the total environmental burden of a process for an entire analytical procedure ranging from sample sampling to waste. The MoGAPI method is a pictograph with different colours and it estimates the environmental impact for each stage of the analytical workflow:

- Sample sampling
- Sample preservation
- Transportation
- Sample storage
- Type of analytical method
- Extraction procedure
- Solvents used (including quantity)
- Further sample manipulation
- Health impacts
- Physical risks
- Instrumental power
- Occupational impact
- Waste generation
- Waste disposal

Each element of the analytical procedure was estimated using predefined criteria of the MoGAPI and resulted in pictograms showing greener and redder stages of the analysis. Greener parts correspond to the procedures of low impact while greener and redder part correspond to procedures of high impact.

2.9.4. ACS Green Chemistry Institute Analytical Procedure Calculator (ACS-GCI APC)^[25]

The ACS Green Chemistry Institute Analytical Procedure Calculator (ACS-GCI APC) has been employed to evaluate the environmental benefits of the developed RP-HPLC and UV spectrophotometric procedures in accordance with established international principles for green chemistry. The ACS calculator assesses analytical methods based on several key sustainability indicators: -

- Instrumental energy consumption
- Solvent energy consumption
- Solvent environmental impact
- Solvent safety profile
- Waste generation
- Analytical greenness

The information of the analytical procedures, such as instrumental requirements, run time, solvent composition and consumption, sample preparation requirements, and waste generated, was input in the ACS-GCI APC platform and calculated the score of sustainability as well as the overall score. ACS calculator takes into account a broader scope than other greenness evaluation tools by combining together the environmental, workplace, and the operational impacts to a standardized method for a better comparison of different methods.

2.9.5. Overall Greenness Assessment Strategy

For a complete environmental profile, the performance of the developed analytical methods, as to their environmental soundness was evaluated through the simultaneous use of each of the four greenness measures employed separately. The Analytical Eco-Scale determined environmental load through penalty points, BAGI assessed an analytical aptitude together with the sustainability and MoGAPI provided the environmental load of each of the procedural step using a pictorial scale whereas the ACS-GCI APC afforded a summarized environmental footprint from consumptive (solvent, energy) and wasteful criteria of an analytical process. The integrative results obtained from these complementary methods afforded for a reliable and comprehensive assessment of developed UV spectrophotometric and RP-HPLC methods, in compliance with contemporary principles of Green Analytical Chemistry.^[26]

3. RESULTS

3.1. UV Spectrophotometric Method Development

3.1.1. UV Absorption Spectra and Selection of Analytical Wavelength

The UV spectrum of Tedizolid in distilled water has a distinct absorption maximum at 298 nm. The solvent does not exhibit serious interference at baseline and can be scanned without fluctuation. The overlays at each concentration (3-15 g/mL) are similar showing the absence of any peak shift, indicating that this range fits Beer Lambert law. Hence, 298 nm was chosen as the analytical wavelength for developing zero-order UV method (**Figure 1-3**).

The regression equation obtained for the calibration curve was:

$$y = 0.0464x - 0.0060$$

with an excellent correlation coefficient of

$$R^2 = 0.9997$$

demonstrating outstanding linearity between concentration and absorbance.

The analytical performance parameters obtained for the zero-order method were: **Table 1**. Analytical characteristics of the developed zero-order UV spectrophotometric method for Tedizolid determination.

3.1.2. First-Derivative Spectrophotometric Method

First derivative spectra obtained demonstrated a clearly selective analytical band at 317 nm, with a good linear range for the concentration at 3-15 g/mL. Differentiation caused a sharp rise in the analytical specificity reducing background noise. Calibration graph is excellent and presented analytical properties **Table 2**. (**Figure 4,5,6**)

A high level of proportion was observed for derivative amplitude versus concentration in standards calibrated over the range 3-15 g/mL. A linear relationship was generated for the standards and a regression line was established; indicating excellent linearity.

$$y = 0.164x + 0.0306$$

with

$$R^2 = 0.9999$$

The statistical parameters obtained for the first-derivative method were: **Table 2**. Analytical characteristics of the developed first-derivative UV

spectrophotometric method for Tedizolid determination.

3.1.3. Area Under Curve (AUC) Method

An AUC method was prepared by summing the values of absorbance at the wavelength ranges from 288-308 nm. It was found that the AUC methods had excellent linear range of 3-15 g/mL with a good precision and sensitivity. Analytical parameters are summarised in Table 3 and (Figure 7).

3.2. RP-HPLC Method Development and Optimization

3.2.1. Chromatographic Optimization

The optimized condition adopted has a suitable and proper sharp and symmetric peak in terms of Tedizolid with RTs of around 2.7 min on an Enable C18 column using dipotassium hydrogen phosphate buffer in acetonitrile as mobile phase. Representative chromatogram of Blank, standard sample and sample of formulation on optimized mobile and stationary phases given in figures 8-10 whereas optimized chromatographic condition outlined in Table 4.

3.3. Method Validation

The developed UV spectrophotometric and RP-HPLC methods were fully validated in according with the guidelines of validation (ICH Q2(R2)) requirements. Methods were validated for selectivity, linearity, accuracy, linearity, ruggedness, robustness and sensitivity. (Table 5-11).

3.4. Assay of Marketed Tablets

Application of developed method on the assay of commercially marketed Tedizolid tablets the results of assay obtained by the proposed methods were found within pharmacopeial range and well in coincidence with the labelled claim and suitable for routine Pharmaceutical Quality Control (Table 12).

3.5. Forced Degradation Studies

Tedizolid was successfully resolved with its degradation products formed under different stress conditions such as acidic, alkaline, oxidative, thermal and photolytic conditions and the proposed method was found to be stability indicating. None of the degradation products interfere with Tedizolid at retention time as shown in the chromatograms (Figure 11-15) and degradation studies have been summarized in Table 13.

3.6. Greenness Assessment

The environmental impact of the analytical methods developed has been assessed by means of the Analytical Eco-Scale (Eco-Scale for Analytical Laboratories), BAGI, MoGAPI, and ACS Green Chemistry Institute Analytical Procedure Calculator.

UV methods show very good green chemistry evaluation, RP-HPLC methods have a good environmental profile even considering that there has been a low volume usage of the organic solvent. Tables 14 to 17 and Figures 16 to 18 show the results of the eco-evaluation methods for UV and RP-HPLC methods.

4. DISCUSSION

4.1. Performance of the Developed UV Spectrophotometric Methods

The feasibility of applying of distilled water in spectrophotometry was established so that UV spectrophotometric analysis methods that do not hurt analytical properties with an eco-friendly technique without the use of other hazardous substances or organic solvents were developed successfully. Three methods analysed showed outstanding linearity, precision and accuracy, so that they can be applied in pharmaceutical industry quality controls regularly. First-order derivative showed the most analytical sensitivity and zero-order method is simplest; AUC minimized disturbance by narrow wavelength fluctuation.

4.2. Performance of the RP-HPLC Method

A short retention time (approx. 2.7 min) was achieved at rapid chromatographic separation with very good peak symmetry and column efficiency with this optimised RP-HPLC method. Rapid analysis minimizes waste in solvent usage and increases sample throughput, hence resulting in an increased laboratory productivity and efficiency.

4.3. Validation of the Proposed Methods

The validation results showed the accordance with ICH Q2 (R2) requirement and a fine result in linearity, accuracy, precision, robustness, ruggedness has proven UV spectrophotometry and RP-HPLC methods were suitable for quantify the Tedizolid dosage form.

4.4. Stability-Indicating Capability

The method developed also show good specificity by separating Tedizolid from its degradation products in RP-HPLC analyses under stress conditions as per forced degradation. No other interacting peaks were observed thus also indicated suitability of method in determination during stability study.

4.5. Greenness Assessment

The environmental validity of the proposed analytical methods was corroborated by

comprehensive screening performed employing four integrated green tools. The reason for such highest greenness performance of UV methods was that distilled water (DW) as primary solvent and a total single analytical medium was utilized. The other factor which accounted to the superior greenness performance of RP-HPLC method was its short analysis (run) time that has correspondingly resulted in less consumption and generation of waste with a minor contribution of Acetonitrile.

4.6. Comparison with Published Methods

Comparison of the proposed methods with reported methods in order to verify the analytical advantage and novelty of the developed UV spectrophotometric and RP-HPLC methods was made against literature reported analytical methods of Tedizolid regarding, analytical technique, linear range, analytical wavelength, retention time, run time, stability-indicating capacity, greenness and method applicability. To date, Tedizolid was analysed mostly using RP-HPLC, UHPLC, LC-MS/MS, and fluorescence spectroscopic analysis methods on different matrix like, pharmaceutical formulations or bio-fluids. Though, such developed methods achieved adequate analytical sensitivity, their use of expensive instrumentations, rigorous sample preparations, longer chromatographic runs, and highly hazardous organic solvents lead to high cost and environmental load. Additionally, few literature reports discussed comprehensive greenness assessment of analytical methods using the most recognized Green Analytical Chemistry indexes. Three greener UV spectrophotometric methods, and rapid stability-indicating RP-HPLC method, was successfully developed, and validated, in accordance with ICH Q2(R2) recommendations. The present developed UV spectrophotometric methods solely use the usage of distilled water only for the analyses as a medium, thereby eliminating toxic organic solvents while displaying effective analytical behaviour. The optimal method in RP-HPLC provided rapid chromatographic analysis of approximately 2.7 min, which reduced the organic solvent consumption significantly and, in comparison to several existing chromatographic methods, reduced total analysis time to a much lesser extent. The primary superiority of this current work is the comprehensive green assessment performed by using four evaluation tools: Analytical Eco-Scale, BAGI, MoGAPI and the ACS Green Chemistry metrics in the four analyses, thus providing great merit to present analytical

techniques. Based on literature survey, no existing analytical method for the Tedizolid has ever combined these four renowned green metrics along with development of both UV-Vis and stability indicating RP-HPLC method at once. **Table 18.** Comparison of the proposed analytical methods with previously reported methods for Tedizolid determination.

4.7. Novelty and Advantages

New developments have been presented in this paper in terms of analytical and environmental methods for analysis of Tedizolid in its pharmaceutical dosage forms. In contrast with earlier publications dedicated either to chromatographic or to bioanalytical techniques, this paper integrated the multiple approaches in a single validation process. A key advancement presented in this investigation lies in the successful development and validation of three green UV spectrophotometric methods, based on the zero order, first order derivative and area under curve technique, coupled with a rapid stability indicating RP-HPLC method following the guidelines set forth in ICH Q2(R2). Distilled water was employed as solvent for UV methods instead of toxic organic solvents which reduced dramatically the negative environmental impact while retaining the performance of analytical methods. Another innovation was the optimisation of a rapid method RP-HPLC method, with retention times ~2.7min for Tedizolid, offering efficient chromatographic separation with reduction in solvent consumption and increased throughput. Forced degradation studies proved the stability indication of the RP-HPLC method under stress acidic, alkaline, oxidising, thermal and photo conditions. Another important characteristic of this paper is the multidimensional environmental assessment of all four developed methods utilizing four different green analytical chemistry assessment tools, such as Analytical Eco-Scale, BAGI, MoGAPI, and ACS Green Chemistry metrics. The analytical methods developed provide the following benefits: Fast and simple procedure with good accuracy, precision, robustness, reproducibility according to ICH guidelines. Environmentally safe procedures with good green metrics. Stability indicating RP-HPLC method is obtained that separates the Tedizolid from its degradation products. Reduced use of organic solvent, decreased analytical cost and high sample throughput. High applicability in pharmaceutical quality control laboratories. Full validation of analytical method

coupled with thorough green evaluation provides solid basis for sustainable pharmaceutical analysis.

5. Conclusion

Three green UV spectrophotometric methods (zero-order, first-derivative and area-under-the-curve) and a stability-indicating RP-HPLC method were validated according to ICH Q2(R2) guidelines for the determination of Tedizolid in bulk drug and tablet forms. The developed methods were acceptable and may be used for the routine quality control of pharmaceutical samples. Tedizolid is resolved with its degradation products in all stress conditions (acidic, alkaline, oxidative, thermal and photo degradative) in RP-HPLC, proving to be stability-indicating. The proposed methods had superior environmental characteristics, with the three green UV spectrophotometric methods having the greatest greenness, due to the usage of water only as a solvent, and the greenness of the fast stability-indicating RP-HPLC method was considered excellent with a limited consumption of organic solvent and quick running time according to three analytical tools for greenness calculation like ASG and ACS. The main contribution of this work is the introduction of three complementary green UV spectrophotometric methods and a rapid stability-indicating RP-HPLC method that can be used for the routine and stability investigation of Tedizolid containing pharmaceutical products as green and efficient analytical methods.

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❖ AUTHOR CONTRIBUTIONS

Dr. Anil Kumar S Manchi: Conceptualization, Methodology, Supervision, Validation, Resources, Project administration, Writing – review & editing.

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All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

❖ SUPPLEMENTARY MATERIAL

The supplementary material associated with this article includes:

- Representative UV spectra of Tedizolid.
- Additional first-derivative and AUC spectra.
- Individual calibration data for UV and RP-HPLC methods.
- Raw chromatograms obtained during method optimization.
- Complete forced degradation chromatograms.
- Detailed validation datasets.
- Analytical Eco-Scale, BAGI, MoGAPI, and ACS Green Chemistry assessment worksheets.

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