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Original Article

# STABILITY-INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ANALYSIS OF DOXEPIN HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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**ABSTRACT**

**Objective:** A simple, reliable, and rapid RP-HPLC method showing stability has been established to detect Doxepin Hydrochloride (DOX) with its degraded products. The proposed method has been validated for specificity, linearity, system suitability, accuracy, precision, robustness, LOD, and LOQ as per ICH guidelines. All parameters were found to be within the accepted limits, affirming the method's reliability.

**Methods:** Analysis was conducted using RP-HPLC on a Phenomenex C18 Luna column (250 mm × 4.6 mm id, 5 µm) with a mobile phase comprising methanol, acetonitrile, and buffer (40:30:30, v/v/v) and a flow rate of 0.5 ml/min. The detection was performed with a UV detector set at 254 nm. Diverse methods have been employed to investigate forced degradation studies, including acid-base hydrolysis, photolysis, thermal degradation, and oxidation. These studies were conducted both in bulk and in capsule formulations of DOX.

**Results:** The retention time (tR) of DOX was 2.92 min, and all parameters met acceptable limit values. The response exhibited linearity over a concentration range of 10 to 50 µg/ml ($R^2$= 0.9974). The percentage of DOX recovered from the pharmaceutical cream dosage form ranged from 97.67% to 101%. Sensitivity levels for the developed method were indicated by limit of detection (LOD) and limit of quantification (LOQ) values of 0.40–0.50 µg/ml. The proposed method was validated according to ICH guidelines.

**Conclusion:** Hence, a simple, reliable, accurate, and precise HPLC method was developed, proving suitable for the analysis of DOX in both bulk and commercial formulations.

**Keywords**: Doxepine hydrochloride, HPLC, Validation, Stability studies


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## INTRODUCTION

Tricyclic Antidepressants (TCAs) constitute a group of drugs primarily employed in treating patients with major depression. They are also utilized for various psychiatric disorders, such as panic disorder, obsessive-compulsive disorder, sleep disorders, eating disorders, and attention deficit hyperactivity disorder [1].

Doxepin Hydrochloride (DOX) is chemically known as Benzoxepin-11-ylidene (3E)-3-(6H-benzo[C][1])-N, dimethylpropan-1-amine. Functioning as a tricyclic antidepressant and anxiolytic psychotropic agent, DOX serves as a second-line treatment for depression, anxiety disorders, and chronic idiopathic urticaria. It is marketed under various brand names, such as Aponal, Adapine, Doxal (Orion), Deptran, Sinequan, Spectra, Doxin, Doxetar, and Sinequan. Additionally, DOX is utilized for sleep maintenance treatment under the trade name Silenor. The IUPAC name and molecular formula are 3-(dibenzo)[b,e]oxepin-11(6H)-ylidene)-N, N-dimethylpropan-1-amine, and C19H21ClNO, respectively [2, 3].

The fundamental objective of process validation is to establish the foundation for documented data and controlled procedures, ensuring that drug products adhere to specified criteria for identity, strength, quality, and purity as outlined by the relevant method. Products must be designed with a focus on incorporating quality, safety, and efficacy [4]. Stability studies play a crucial role in drug development, verifying the identity, potency, and purity of ingredients and formulated products. A key aim of stability studies is to determine the shelf life of a drug, contributing significantly to the overall assessment of its quality and durability [5].

Forced degradation experiments play a pivotal role in the development of analytical methods, enhancing our comprehension of active pharmaceutical ingredients (API), and assessing the stability of drug products. Additionally, these experiments offer valuable insights into degradation pathways and the resulting products [6].

The literature describes several analytical methods for the determination of DOX, either alone or in combination with other drugs, employing diverse detection techniques such as the spectrofluorimetric method [7, 8] and LCMS [9]. In a study focused on serum analysis, the quantification of four compounds—E-doxepin, Z-doxepin, E-desmethyl doxepin, and Z-desmethyl doxepin—was achieved simultaneously using High-Performance Liquid Chromatography (HPLC) [10].

An extensive literature survey reveals several analytical methods, both individual and combined, for estimating DOX in tablet dosage forms [11–13]. However, according to the literature review, only a few methods have been published for a stability-indicating HPLC analysis of DOX in combination. The primary objective of this study was to develop a simple, accurate, precise, and economical stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for determining DOX in the presence of its degraded products. The developed method has undergone validation in accordance with ICH guidelines [14, 15].

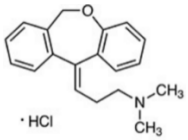



**Fig. 1: Chemical structure of DOX**

## MATERIALS AND METHODS

### Chemicals and reagents

The pure DOX drug was acquired as a gift sample from Aurobindo Pharma Limited, Hyderabad, India. The pharmaceutical formulation

DOX, manufactured by Sun Pharma Limited, Assam, India, was purchased from a local pharmacy in Bengaluru. HPLC-grade acetonitrile and water were sourced from Merck Specialties, Private Ltd.

**Instrumentation and chromatographic conditions**

Chromatographic analysis was conducted using a Shimadzu HPLC system equipped with a UV detector and an automatic injector with a 100 µl sample loop. A Phenomenex Luna C18 column (250 mm × 4.6 mm id, 5 µm particle size) was employed, and Shimadzu LC solution software facilitated data processing. The flow rate was maintained at 1.0 ml/min, and UV detection was performed at a wavelength of 220 nm (fig. 2). Additional equipment included an Ultra Sonicator (Dakshin, Sakinaka, Mumbai), a digital analytical balance (AUX 220, Shimadzu, Japan), and a 0.45 µm membrane filter. Optimized chromatographic conditions are summarized in table 1.

**Table 1: Chromatographic conditions**

| Parameters | Optimum chromatographic conditions |
|---|---|
| Instrument | RP–HPLC Shimadzu (Japan) equipped with a SPD-20A UV-Visible detector and LC-20AT |
| Injector | Rheodyne 20 µ loop |
| Column | Phenomenex Luna (ODS) C18 250 mm x 4.6 mm,5 µm |
| Detector | UV detector |
| Wavelength | 254 nm |
| Flow rate | 0.5 ml/nm |
| Injection volume | 20 µl |
| Mobile phase | Methanol: acetonitrile: buffer (40: 30: 30) v/v/v |
| pH | 2.5 with Orthophosphoric acid (OPA) |

**Preparation of buffer**

100 ml of HPLC-grade water was used to dissolve 0.136 mg of dihydrogen phosphate (H2PO4). The pH of the solution was adjusted to 2.5±0.5 using Orthophosphoric acid (OPA).

**Preparation of mobile phase**

The mobile phase, consisting of methanol: acetonitrile: buffer (40:30:30, v/v/v), was prepared, filtered, and degassed. This composition was chosen as the optimal mobile phase for DOX, as it exhibited excellent resolution and accurate peak characteristics.

**Standard stock solution (10 µg/ml)**

An accurately weighed quantity of 10 mg of DOX was dissolved in the diluent (mobile phase) and sonicated until completely dissolved. The solution was then transferred to a 10 ml volumetric flask, and the volume was adjusted with the diluent to obtain a solution with a concentration of 1000 µg/ml. Subsequently, 1 ml of this solution was pipetted out and filled up to the mark with the mobile phase in another 10 ml volumetric flask to achieve a concentration of 100 µg/ml. Further dilution was performed by transferring 1 ml of this solution to a 10 ml volumetric flask and adjusting the volume to 10 ml with the mobile phase solution, resulting in a final concentration of 10 µg/ml.

**Sample stock solution**

Five 10 mg DOX capsules were weighed, and a powder equivalent to 10 mg of DO was transferred to a clean, dry 10 ml volumetric flask. The powder was then mixed with the appropriate amount of mobile phase (methanol: buffer: acetonitrile, 60:30:30 v/v/v) and sonicated until fully dissolved. The volume was adjusted to the mark with the mobile phase, resulting in a standard solution concentration of 1000 µg/ml.

Subsequently, 1 ml of the above-mentioned solution was transferred to a 10 ml volumetric flask, and the volume was adjusted to 10 ml with the mobile phase, yielding a concentration of 100 µg/ml. Further dilution was performed by transferring 1 ml of this solution to another 10 ml volumetric flask, and the volume was raised to 10 ml with the mobile phase to achieve a concentration of 10 µg/ml.

To clarify the filtration step, filter the final solution through a 0.45 µm Millipore PVDF filter and collect the filtrate after discarding 5 ml of the initial filtrate.

**Selection of wavelength for detection**

The HPLC method is sensitive to the choice of the detection wavelength. An ideal wavelength provides a robust response for drugs while facilitating the detection of impurities. In this case, the wavelength was chosen from the spectrum at 254 nm.

**Determination of retention time of DOX**

The standard stock solution of DOX with a concentration of 10 µg/ml was injected into the HPLC system at a flow rate of 0.5 ml/min, and the wavelength used was 254 nm. The retention time (tR) of the drug was then recorded.

**Validation of developed RP-HPLC method for DOX analysis**

The developed HPLC method underwent validation following the ICH Q2 (R1) criteria. This validation encompassed assessments for linearity, accuracy, precision, LOD, LOQ, system suitability, specificity, and robustness. These evaluations were conducted to ensure that the method's performance meets the necessary criteria for its intended analytical application [16-18].

**System suitability**

This test serves to verify the operational capability of the analytical system and its ability to generate precise and accurate results. In a 10 ml volumetric flask, a 1.0 ml portion of the 100 µg/ml DOX standard solution was added, and the volume was adjusted to 10 ml with HPLC-grade Methanol: Acetonitrile: Buffer (40:30:30 v/v/v) mobile phase to achieve a concentration of 10 µg/ml. The solution was then sonicated for 15 min.

Subsequently, 20 µl of this standard solution was injected into the HPLC system, and the chromatogram was analyzed for the drug retention time, peak area, and peak resolution. These observations contribute to the assessment of the system's performance and its ability to generate reliable analytical data.

**Specificity**

In a 10 ml volumetric flask, 1 ml of the 100 µg/ml standard solution of DOX was added, and the volume was adjusted to 10 ml using HPLC-grade Methanol: Buffer and Acetonitrile (mobile phase) to obtain a concentration of 10 µg/ml. The resulting solution was sonicated for 15 min.

Following sonication, 20 µl of this standard DOX solution was injected into the HPLC system, and a chromatogram was recorded. This process allows for the analysis of the drug's behavior in the system and the recording of relevant chromatographic data for further evaluation.

**Linearity**

Portions of 1, 2, 3, 4, and 5 ml were taken separately from the 100 µg/ml reference solution of DOX. Each portion was placed in a set of 10 ml volumetric flasks, and the volume was adjusted to 10 ml using HPLC-grade Methanol: Buffer: Acetonitrile (mobile phase) to achieve concentrations ranging from 10 µg/ml to 50 µg/ml. Subsequently, 20 µl of each of these standard solutions was introduced into the HPLC system.

To establish a calibration curve for DOX, the peak areas were plotted on the Y-axis, and the corresponding concentrations were plotted on the X-axis. This calibration curve provides a quantitative relationship between the concentration of DOX and the corresponding peak areas, facilitating the accurate determination of concentrations in subsequent analyses.

**Accuracy**

Accuracy was assessed through percentage recovery studies involving the addition of known quantities of standard drugs. By calculating the percentage recovery of the added analyte, the accuracy of the method was determined. Three distinct DOX

concentration levels, namely 80%, 100% and 120% were selected for this evaluation.

In the suggested method, samples were analyzed in six replicates at each concentration level. DOX recovery studies were conducted by adding standard DOX at concentrations of 8, 10, and 12 µg/ml to samples with a known concentration of 10 µg/ml, utilizing the standard addition method. The percentage recovery of DOX was then calculated, providing insights into the accuracy of the analytical method across different concentration levels.

**Precision**

Precision was assessed by evaluating the performance under intraday and interday variations of DOX at concentrations of 10, 20, and 30 µg/ml. For intraday precision, six replicates of each concentration of both standard and sample solutions were consecutively administered on the same day. To ensure interday precision, the same standard and sample solutions were injected on three different days.

The precision results are reported as the percentage relative standard deviation (% RSD). This statistical measure provides insights into the variability of the results, helping to gauge the precision of the analytical method across different concentrations and time intervals.

**Linearity and range**

To assess linearity, six standard solutions were prepared at concentrations of 10, 20, 30, 40, and 50 µg/ml. These solutions were chosen to cover a range of concentrations with an accuracy target of 98% to 102% and a precision of less than 2% RSD. The optimized method conditions were employed to administer these solutions, and the response, measured in terms of peak area, was recorded. The obtained data was then used to plot a graph of peak area against the respective concentrations.

The linearity was evaluated by constructing a linear relationship between the peak area and concentration. Key parameters such as the correlation coefficient, intercept, and slope were calculated to characterize this relationship. Additionally, a separate linearity curve was generated for DOX, with concentration plotted on the X-axis and its response factor on the Y-axis. This comprehensive analysis helps confirm the linear behavior of the method over the specified concentration range.

**LOD and LOQ**

LOD and LOQ were determined following the equations recommended by the International Conference on Harmonization (ICH) guidelines.

$$LOD=3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ = Standard deviation of Y-intercept. S = Slope of the calibration curve.

**Robustness**

The robustness of the analytical method was assessed by introducing slight variations in HPLC conditions. These variations included changes in flow rate (0.48 ml/min and 0.52 ml/min), mobile phase ratios (38:32:28 and 42:28:32), and wavelengths (252 nm and 256 nm). A 20 µl portion of the prepared solution was injected into the HPLC system under each set of conditions, and the results were recorded.

This robustness study is crucial for evaluating the method's ability to remain reliable and consistent in the face of small changes in experimental parameters. The recorded data helps ensure that the method is robust and can produce consistent results even when subjected to minor variations in conditions.

**Force degradation studies**

Forced degradation studies were conducted to obtain a representative sample for the development of stability-indicating methods for the drug substance. The choice of exposure conditions aimed to simulate the product's decomposition under normal conditions of manufacturing, storage, and usage, recognizing that each condition is distinct [19].

Standard samples of DOX underwent various stress conditions for forced degradation studies, including acidic, alkaline, oxidative, thermal, photostability, and neutral conditions. In acidic and alkaline degradation, samples were treated with 0.1 N HCl for 30 min at 60 °C and 0.1 N NaOH for 30 min at 60 °C, respectively. Oxidative degradation was induced using 5% v/v H2O2 at 60 °C for 30 min. Thermal degradation was assessed by exposing the powder sample to a hot air oven at 105 °C for 6 h. Photostability testing involved placing the sample in a UV chamber and exposing it to UV light for 24 h.

Following the specified degradation period, all samples were returned to room temperature and analyzed under optimized chromatographic conditions to assess the stability of the drug. This comprehensive degradation study provides insights into potential degradation pathways and allows for the identification of degradation products, enabling the development of stability-indicating methods.

**Statistical analysis**

All samples underwent analysis in six replicates, and Relative Standard Deviation (RSD) values were calculated for each set of samples. These RSD values serve as a measure of the precision and consistency of the analytical method across the replicates.

It's worth noting that the proposed method does not require approval from an ethics committee or the informed consent of the patient. This suggests that the analytical procedure is likely non-invasive or does not involve human or animal subjects directly, eliminating the need for ethical approvals typically required in clinical or biological research.

**RESULTS AND DISCUSSION**

**Method development**

Various combinations of the mobile phase were tested to optimize the RP-HPLC parameters. Satisfactory separation and a well-symmetrical peak of DOX were achieved using the mobile phase Methanol: Buffer: Acetonitrile (40:30:30 v/v/v) with a flow rate of 0.5 ml/min and detection at 254 nm. The mobile phase was sonicated for 10 min and then filtered through a 0.45 µm membrane filter.

The chromatogram in fig. 2 displays a peak response with good resolution. The DOX peak effectively resolved in the solvent system consisting of Methanol: Buffer: Acetonitrile at a ratio of 40:30:30 v/v/v. This optimized chromatographic condition ensures reliable and precise separation of DOX in the analysis.

**Determination of retention time**

A 20 µl aliquot of DOX at a concentration of 10 µg/ml was injected into the system with a flow rate of 0.5 ml/min. The resulting chromatogram of DOX is illustrated in fig. 2, providing a visual representation of the separation and detection under the specified RP-HPLC conditions, including the mobile phase composition and detection wavelength. This chromatogram serves as a valuable tool for assessing the quality of the analysis, confirming the presence, and characterizing the DOX peak in the sample.

**Validation of the optimized method**

**System suitability**

This parameter ensures the proper functioning of the analytical system and verifies that the developed method possesses adequate resolution and reproducibility for the analysis. A 20 µl solution of DOX (10 µg/ml) was injected into the HPLC system, and parameters such as the symmetric factor, theoretical plates, and resolution were recorded and are presented in fig. 3 and table 2.

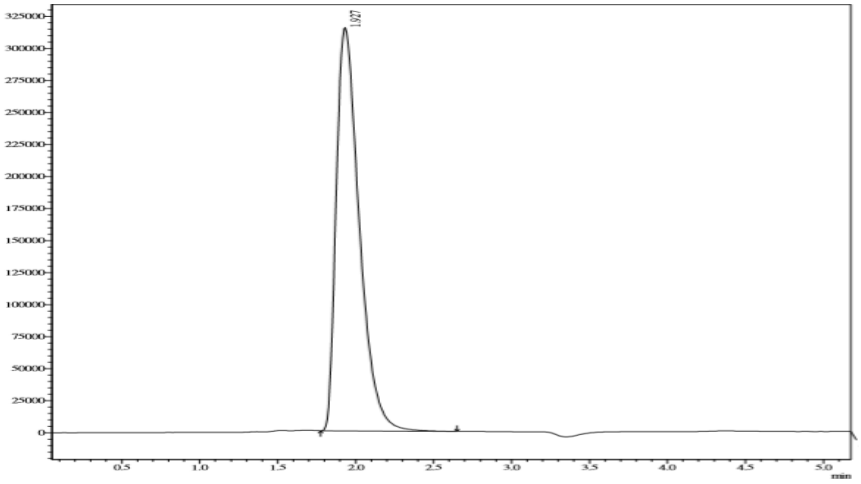


**Fig. 2: Chromatogram of DOX**

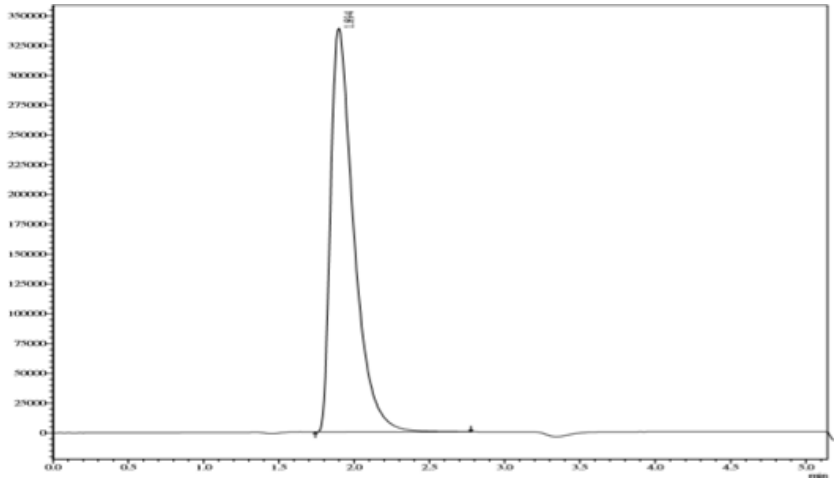


**Fig. 3: Chromatogram of system suitability**

**Table 2: System suitability**

| Parameters | DOX |
|---|---|
| Theoretical plates | 2471 |
| Tailing factors | 1.452 |
| Resolution | 0.0 |

**Specificity**

The specificity test was conducted to assess and confirm that contaminants, degradation products, and diluents do not impact the analyzed samples. The results illustrating the specificity of the established method are presented in fig. 4.

**Linearity**

Linearity studies were conducted to confirm that the test results were directly proportional to the analyte concentration. A 20 µl aliquot of each DOX standard solution (10-50 µg/ml) was injected into the HPLC system. At this concentration range, the calibration curve analysis revealed excellent regression coefficients ($r^2$: 0.9974). The regression equation, expressed as y=22824x-1565, where y is the peak area and x is the concentration, is illustrated in fig. 5.

**Accuracy**

Recovery studies were conducted to validate the accuracy of the proposed method, and the results at various levels are presented in table 1. The acceptable range for recovery percentage is 98.0% to 102.0%, and the accuracy study results are detailed in table 3.

**Precision**

Analytical method precision was assessed by analyzing an ample number of samples and calculating their % RSD. The results for intraday and interday precision, using DOX concentrations of 10, 20, and 30 µg/ml, are presented in table 4.

**Table 3: Results of recovery studies**

| Amount of drug taken (µg/ml) | Amount of standard added (µg/ml) | Total concentration found (µg/ml) | % RSD |
|---|---|---|---|
| 10 | 8 | 17.57 | 97.67±0.23 |
| 10 | 10 | 19.75 | 98.75±0.16 |
| 10 | 12 | 22.23 | 101. 04±0.10 |

*Mean ± SD (n=3), SD (Standard deviation), % RSD (Percentage relative standard deviation).

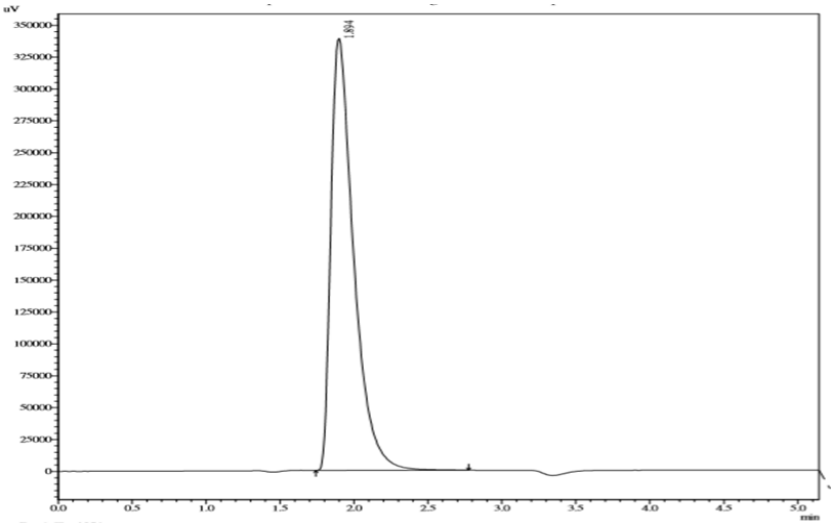


**Fig. 4: Chromatogram of specificity of retention time at 1.92 min**

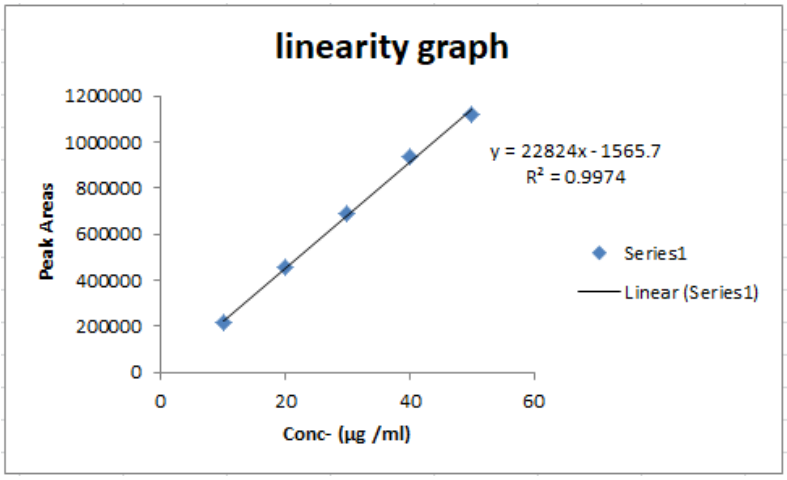



**Fig. 5: Calibration curve of DOX**

**Table 4: Results of intraday and inter-day precision**

| Actual concentration (µg/ml) | Measured mean concentration (µg/ml)* | % RSD |
|---|---|---|
| Intraday | | |
| 10 | 5.18±0.12 | 0.24 |
| 20 | 6.61±0.18 | 0.78 |
| 30 | 13.75±0.02 | 0.86 |
| Interday | | |
| 10 | 5.14±0.18 | 0.36 |
| 20 | 6.63±0.04 | 0.45 |
| 30 | 13.53±0.21 | 1.27 |

*Data are given as mean±SD (n=3), %RSD values lower than 2 % indicate acceptable precision of the developed method.

**LOD and LOQ**

The LOD and LOQ of the proposed method were determined to be 0.495±0.281 µg/ml and 1.503±0.332 µg/ml, respectively.

**Robustness**

Robustness was tested by varying the mobile phase ratio, flow rate and drug wavelength. The results of the robustness of the established method were evaluated by changing the mobile phase ratio, flow rate, wavelength, pH, and molarity of the buffer with the corresponding % RSD values was tabulated in table 5.

**Forced degradation studies**

Stress testing of the drug product was conducted to induce forced degradation, identify degradation pathways, assess drug stability, and confirm the specificity of the analytical methods. Forced degradation studies were performed on both pure and marketed products to validate the stability-indicating conditions employed. These conditions included sunlight exposure, acid hydrolysis, alkali hydrolysis, thermal hydrolysis, and oxidative hydrolysis. The results of these studies, along with a comparison to unstressed conditions, are illustrated in fig. 6 and 7. Analytical results following forced

degradation of the samples are detailed in table 7, accompanied by corresponding fig. depicting the peaks after degradation.

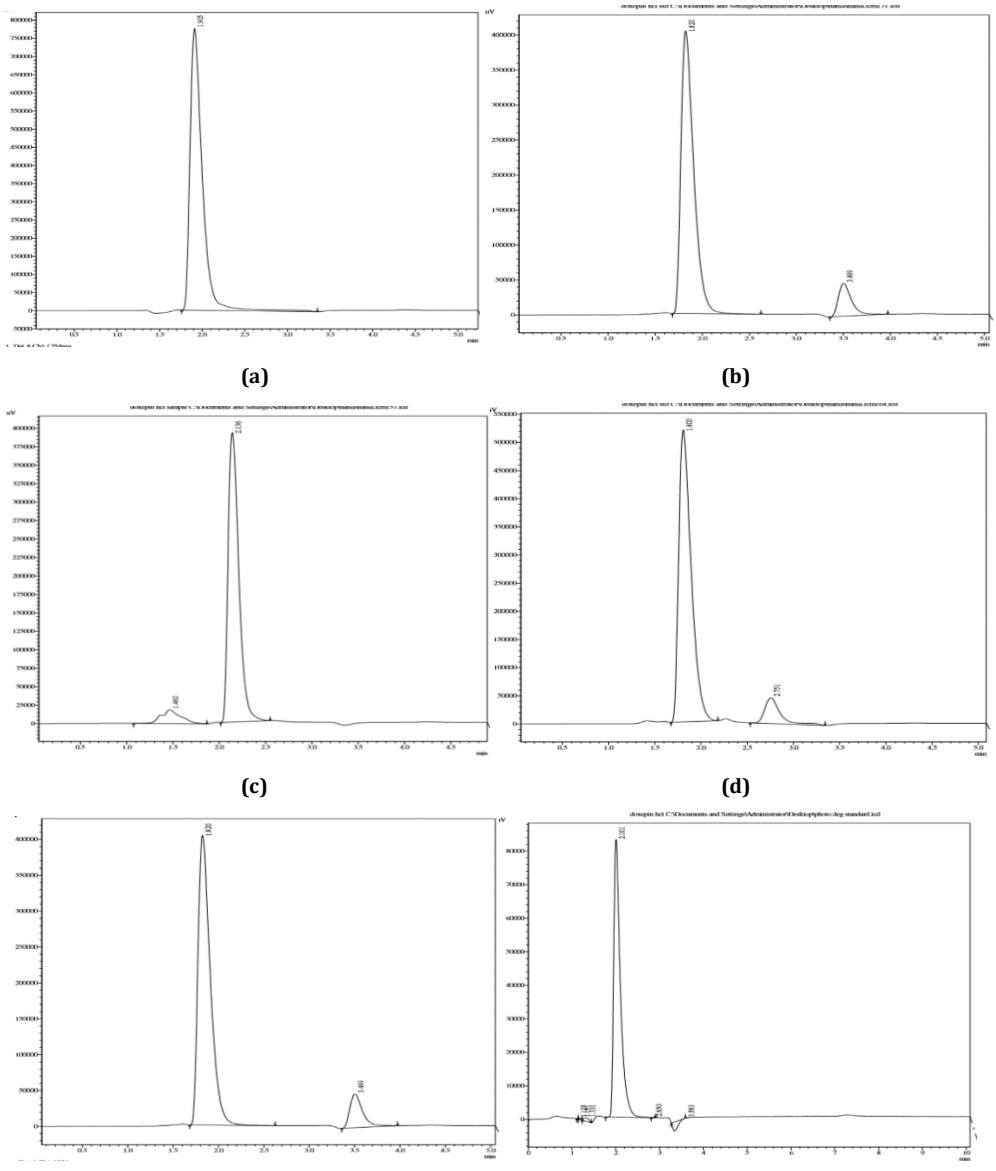
**Table 5: Results of robustness studies**

| Factor | Variation | Retention time (min) | % RSD |
|---|---|---|---|
| Mobile phase (v/v) | 38: 32: 28 | 2.001 | 0.12 |
| | 40: 30: 30 | 1.824 | 0.97 |
| | 42: 28: 32 | 1.711 | 0.05 |
| Flow rate (ml/min) | 0.48 | 2.003 | 0.17 |
| | 0.5 | 1.824 | 0.98 |
| | 0.52 | 1.800 | 0.02 |
| Wavelength (nm) | 252 | 1.906 | 0.08 |
| | 254 | 1.824 | 1.32 |
| | 256 | 1.894 | 0.07 |

The data is expressed as mean ± SD (n = 3), % RSD (Percentage relative standard deviation).

**Table 6: Assay of DOX in capsule**

| Drug | Labelled | Amount found | % Label claim found |
|---|---|---|---|
| DOX | 10 mg | 1.0002 mg | 99.95 % |



**(a)** **(b)**

**(c)** **(d)**

**(e)** **(f)**

**Fig. 6: (a) Chromatogram of untreated standard solution peak (pure) (b) Chromatogram of acid hydrolysis (pure) (c) Chromatogram of alkali hydrolysis (pure) (d) Chromatogram of oxidation degradation (pure). (e) Chromatogram of thermal degradation (pure), (f) Chromatogram of photodegradation (pure)**

**Table 7: Results of forced degradation studies**

| Parameter (Drug) | Stress conditions | % Degradation (Standard) | % Degradation |
|---|---|---|---|
| Acidic hydrolysis | Refluxed with 0.1 N HCl for 30 m at 60 °C | 6.54 | 7.53 |
| Alkaline hydrolysis | Refluxed with 0.1 N NaOH for 30 m at 60 °C | 3.69 | 7.65 |
| Oxidative | Refluxed with 5 % v/v $H_2O_2$ at 60 °C for 30 m | 5.16 | 6.46 |
| Thermal | Placed in hot air oven 105 °C for 6 h | 6.85 | 7.74 |
| Photodegradation | Placed in UV chamber for 24 h | 1.67 | 3.47 |

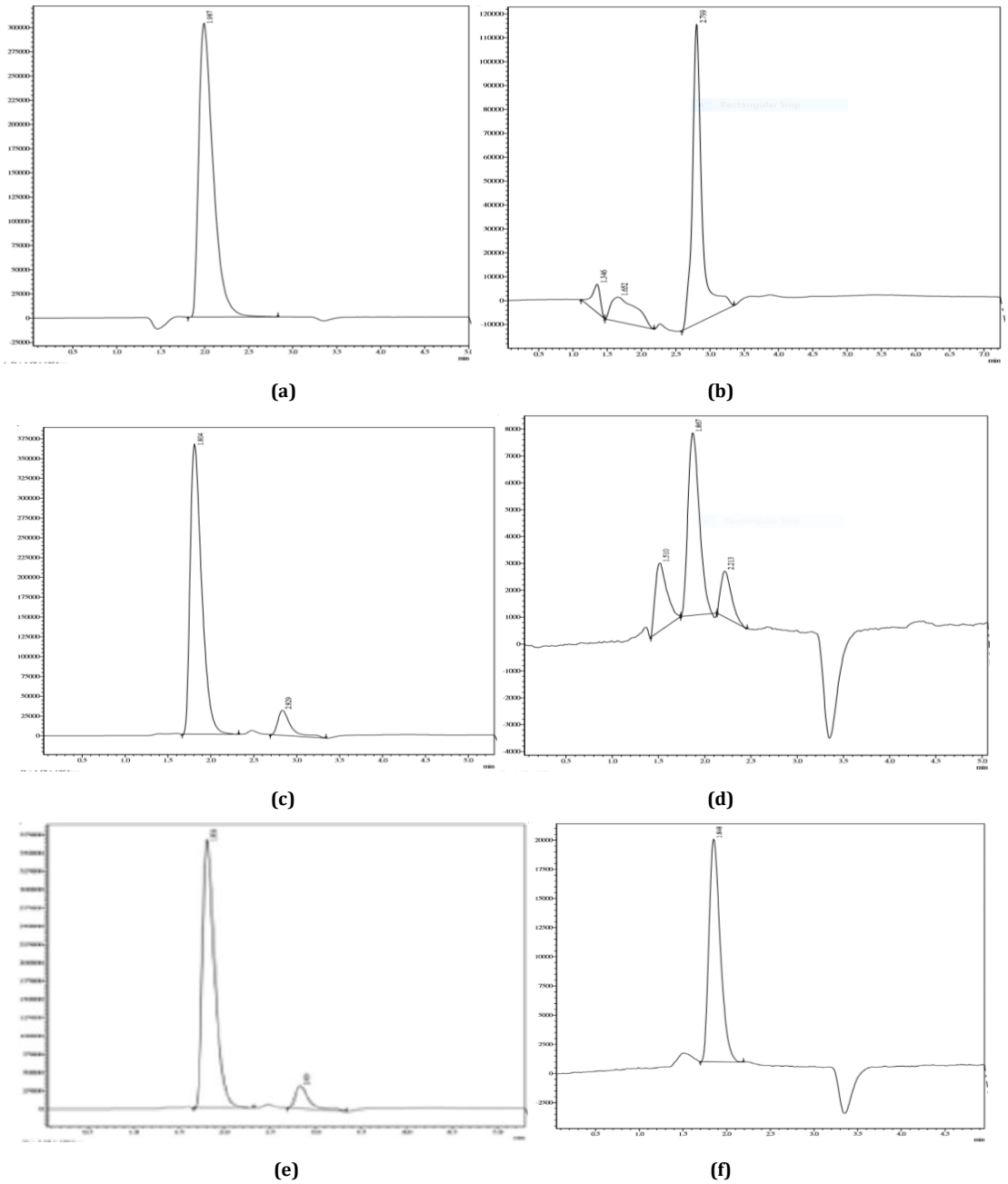


**Fig. 7: (a) Chromatogram of the untreated peak of DOX (marketed), (b) Chromatogram of acid hydrolysis (marketed) (c) Chromatogram of alkali hydrolysis (marketed), (d) Chromatogram of thermal degradation (marketed), (e) Chromatogram of oxidative degradation (marketed), (f) Chromatogram of photodegradation (marketed)**

We have successfully developed and validated a stability-indicating RP-HPLC method for the analysis of DOX in both bulk and pharmaceutical dosage forms. The development process began with a reverse-phase Phenomenex Luna C18 column (250 mm x 4.6 mm i.d., 5 µm particle size), testing different ratios of methanol, acetonitrile, and buffer (50:30:20 and 40:40:20). This exploration resulted in a DOX peak with high retention time and minimal fronting, achieving a sharp and symmetrical peak with high retention and minimal tailing. The optimized solvent system, Methanol: Acetonitrile: Buffer (40:30:30 v/v/v), exhibited a highly symmetrical and sharp DOX peak with a retention time of 1.92 min at a flow rate of 0.5 ml/min and UV detection at 254 nm. Following

the principles outlined in ICH guidelines, we thoroughly validated the developed chromatographic method.

The system suitability of the developed HPLC technique was assessed, and the number of theoretical plates and tailing factor for DOX was calculated, yielding values of 2471 and 1.452, respectively. Notably, no visible diluent or excipient peaks were observed in the used capsule formulation, demonstrating a high level of specificity for the established method. The proposed HPLC method exhibited a linear calibration graph over a concentration range of 10-50 µg/ml for DOX, with a regression coefficient ($r^2$ = 0.9974). The regression equation was determined to be Y=22824x-1565.7. To further evaluate the accuracy of the method, recovery studies were conducted, resulting in an accuracy range for DOX from 97.67% to 101.04%. These values fell within acceptable limits, indicating that the excipients present in the formulation were not affected by the new analytical method.

Precision studies indicated that the % RSD for DOX was consistently less than or equal to 2.0%, demonstrating a high degree of repeatability and reproducibility for the method. The calculated LOD and LOQ values for DOX affirmed the method's high sensitivity. Robustness studies involving slight variations in chromatographic conditions, such as flow rate, wavelength, and mobile phase, produced satisfactory results within acceptable limits. The developed method was successfully applied to measure DOX in a commercially available capsule form, yielding an assay result of 99.95%. This confirms that the proposed technique is simple, precise, accurate, and highly specific for routine DOX analysis.

Force degradation studies, conducted according to ICH guidelines, subjected the drug to various stress conditions, including acid-base hydrolysis, oxidation degradation, photodegradation, and thermal degradation. These stress conditions proved sufficient to induce drug degradation, and the extent of degradation was quantified by comparing untreated and treated samples.

Under acidic conditions, the degradation % of standard and formulated (capsule) drugs were 6.54 % and 7.53 %, respectively. Under basic conditions, the degradation % of standard and formulated (capsule) drugs were 3.69 % and 7.65 %, respectively. Under oxidative conditions, the degradation % of standard and formulated (capsule) drugs was 5.16 % and 6.46%, respectively. Under photolysis stress, the % degradation of standard and formulated (capsule) drugs were 1.67 % and 3.47 %, respectively. Under thermal stress, the % degradation of standard and formulated (capsule) drugs were 6.85 % and 7.74 %, respectively.

The current method has demonstrated rapidity with a short retention time (1.92 min), enabling the analysis of a large number of samples quickly and cost-effectively compared to that of the previously established method, whose run time was typically varied from 6.121 to 7.936 [11,14]. Consequently, this approach is implemented for routine analysis in the pharmaceutical industry and is well-suited for regular use in quality control laboratories.

A stability-indicating HPLC method was developed for both bulk and marketed dosage forms of DOX. The resulting analytical method proved to be simple, precise, accurate, linear, and robust, meeting the criteria outlined in the ICH guidelines. Notably, the current method offers the advantage of a short retention time and cost-effectiveness compared to the previously established method. This developed method is characterized by simplicity and precision, requiring no complex solvents. Stability studies involved force degradation under various conditions, including acid-base hydrolysis, oxidation, photolysis, and thermal methods in different atmospheres. The stress results indicated that DOX is not stable in acidic, basic, oxidative, photolytic, and thermal stress conditions. Notably, DOX was found to degrade more significantly under acidic and thermal degradation while exhibiting the least degradation under sunlight, alkali, and oxidative stress conditions.

## CONCLUSION

The developed and validated stability-indicating method serves as a valuable tool for studying the storage conditions and shelf-life of DOX. Additionally, it aids in the selection of packaging materials that are less reactive to the drug product. Force degradation studies not only provide insights into the degradation pathway of drugs but also contribute to elucidating the structure of degradants. Advanced analytical instruments can be employed to identify specific degraded products of DOX. The developed method has demonstrated superiority over previously reported methods, offering increased accuracy, sensitivity, speed, and cost-effectiveness. Beyond its immediate applications, this method holds potential for use in clinical research and impurity profiling of drugs.

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## AUTHORS CONTRIBUTIONS

Dr. Rajesh. R conducted the analysis and interpreted the results

## CONFLICT OF INTERESTS

The authors declared no conflict of interest.

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