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# METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DOSULEPIN IN PURE AND DOSAGE FORM BY USING HPLC

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**Abstract:**

*A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Dosulepin in its pure form as well as in tablet dosage form. Chromatography was carried out on Xterra C18 (4.6×150mm, 5µ) column using a water (100% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 230nm. The retention time of the Dosulepin was 2.1 ±0.02min. The method produce linear responses in the concentration range of 5-25µg/ml of Dosulepin . The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.*

**Keywords:** *Dosulepin , RP-HPLC, validation.*

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## INTRODUCTION:

**Instrumentation of HPLC**

The basic liquid chromatograph consists of six basic units. The mobile phase supply system, the pump and programmer, the sample valve, the column, the detector and finally a means of presenting and processing the results [1-6].

**Mobile phase (solvent) reservoirs and solvent degassing**

The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase**.**

**Mobile phase**

Mobile phases in HPLC are usually mixtures of two or more individual solvents. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. The two most critical parameters for nonionic mobile phases are strength and selectivity [7-9].

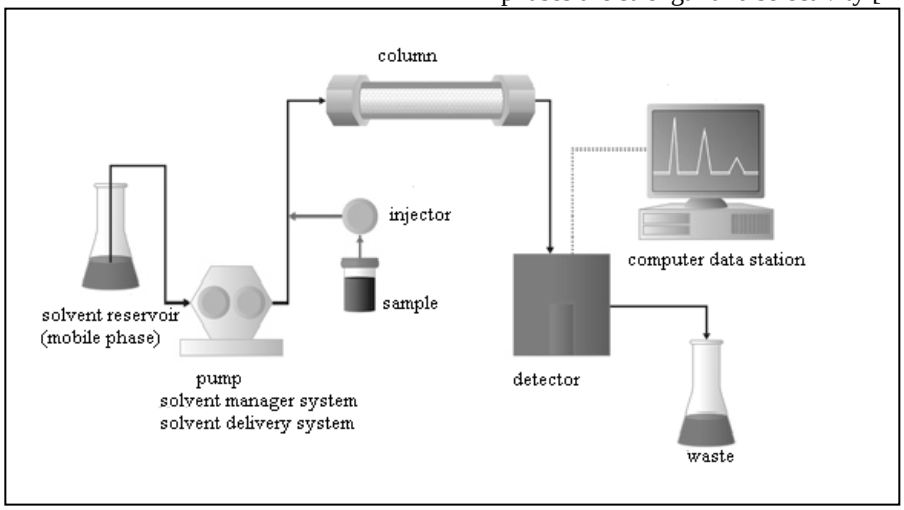


**Fig.1: Components of HPLC instrument block diagram**

**Mobile phase preparation**

Mobile phases must be prepared from high purity solvents, including water that must be highly purified. Mobile phases must be filtered through ≤1 µm pore size filters and be degassed before use.

**Degassing of solvents**

Many solvents and solvent mixtures (particularly aqueous mixtures) contain significant amounts of dissolved nitrogen and oxygen from the air. These gasses can form bubbles in the chromatographic system that cause both serious detector noise and loss of column efficiency. These dissolved gases in solvent can be removed by the process of degassing. Every solvent must be degassed before introduction into pump as it alter the resolution of column and interfere with monitoring of the column effluent.

Degassing is done in many ways:

1. By warming the solvents
2. By stirring vigorously with a magnetic stirrer
3. By subjecting to vaccum filtration
4. By ultra sonication (using ultrasonicator)

**ANALYTICAL METHOD VALIDATION**

Method validation as per ICH can be defined as "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".[22,20,26]

**Objective of validation**

There are two important reasons for validating assays in the pharmaceutical industry.The first, and by for most important is that assay validation is an integral part of the quality control system.The second is that current good manufacturing practice regulation requires assay validation. In industry it would be difficult to confirm that the product being manufactured is uniform and that meet the standards set to assure fitness for use. The varying nature of the differences between the analytical development laboratory and quality control laboratory is a good reason for validation program.

Method validation study includes Specificity / Selectivity, Linearity, Accuracy, Precision, Limit of

detection, Limit of Quantitation, Robustness, System suitability and Stability criteria.

**Accuracy**

The accuracy of measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose "true value" is known) is analyzed and the measured value should ideally be identical to the true value.Typically, accuracy is represented and determined by recovery studies but there are three ways to determine accuracy:

1. Comparison to a reference standard
2. Recovery of the analyte spiked into blank matrix or
3. Standard addition of the analyte.

The ICH documents recommended that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentrations levels the specified range (i.e., three concentrations and three replicates of each concentration). Accuracy was tested (%Recovery and %RSD of individual measurements) by analyzing samples at least in triplicate, at each level (80%, 100% and 120% of label claim) is recommended. For each determination fresh samples were prepared and assay value is calculated [4-8].

**Acceptance criteria:** The accuracy should be within 98-102%.

**Precision**

Precision can be defined as "the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample". A more comprehensive definition proposed by the International Conference on Harmonization (ICH) divides precision into three types:

1. Repeatability
2. Intermediate precision
3. Reproducibility

**Repeatability:**

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision and involves multiple measurements of the same sample (different preparations) by the same analyst under the same conditions.At least

1. 5 or 6 determinations
2. At two or three different concentrations

Should be done and the relative standard deviations were calculated. The % RSD can be calculated by,

$$\%\ \mathbf{RSD} = \frac{\mathbf{SD} \times \mathbf{100}\ \%}{\mathbf{MEAN}}$$

Where, RSD = relative standard deviation

SD = standard deviation

The standard deviation SD is given by,

$$\sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - x)^2}$$

**Intermediate precision:**

It is the agreement of complete measurements (including standards) when the same method is applied many times within the same laboratory. Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment etc.

**Reproducibility:**

Reproducibility expresses the precision between laboratories and is often determined in collaborative studies or method transfer experiments.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.[22]

**Acceptance criteria**: The % RSD should be less than 2.

**Linearity:**

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. It is a measure of how well a calibration plot of response vs. concentration approximates a straight line. Linearity can be assessed by performing single measurements at several analyte concentrations. The data are then processed using a linear least squares regression. The Resulting plot slope, intercept and correlation coefficient provide the desired information on linearity [9-15].

**Acceptance criteria**: Correlation coefficient ($R^2$)>0.998.

**Specificity**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present; these include impurities, degradants and matrix etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures. This definition has the following implications:

Identification: To ensure the identity of an analyte.

Purity Tests: To ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content etc.

Assay (content or potency): To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample [16-20].

**Acceptance criteria:** No interference should be present.

**Limit of Detection**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD can be defined as the smallest level of analyte that gives a measurable response. The detection limit is usually expressed as the concentration of the analyte (percentage parts per million) in the sample. It is usually determined by 3 ways:[21]

1. Based on Visual Evaluation
2. Based on Signal-to-Noise
3. Based on the Standard Deviation of the Response and the Slope

The limit of detection may be expressed as

$$\mathbf{LOD} = \frac{\mathbf{3.3\sigma}}{\mathbf{S}}$$

Where, σ = the standard deviation of the response
S = the slope of the calibration curve.

**Acceptance criteria:** S/N should be 3:1

**Limit of Quantitation**

The quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ is usually expressed as the concentration of the analyte (percentage parts per million) in the sample. . It is usually determined by 3 ways:[26]

1. Based on Visual Evaluation
2. Based on Signal-to-Noise
3. Based on the Standard Deviation of the Response and the Slope

The limit of Quantitationmay be expressed as:

$$\mathbf{LOQ} = \frac{\mathbf{10\sigma}}{\mathbf{S}}$$

Where, σ = the standard deviation of the response
S = the slope of the calibration curve.

**Acceptance criteria:**S/N should be 10:1

**Robustness**

It is defined as a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.It involves a number of method parameters that are varied within a realistic range and the quantitative influence of the variables is determined. [22]

The typical variations are:

- Influence of variations in mobile phase composition.
- Temperature.
- Flow rate.

**System Suitability Testing [22-24]**

Prior to the analysis of samples each day, the operator must establish that the HPLC system and procedure are capable of providing data of acceptable quality. This is accomplished with system suitability experiments, which can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The parameters that can be used to determine system suitability prior to analysis, includes Plate number (N), Tailing factor, k and/or α, Resolution (Rs)and Relative standard deviation (RSD) of peak height or peak area for repetitive injections. Typically at least two of these criteria are required to demonstrate system suitability for any method.

**Dosulepin** (3E)-3-(6H-benzo[c][1]benzothiepin-11-ylidene)-N,N-dimethylpropan-1-amine Indicated in the treatment of symptoms of depressive illness, especially where an anti-anxiety effect is required.

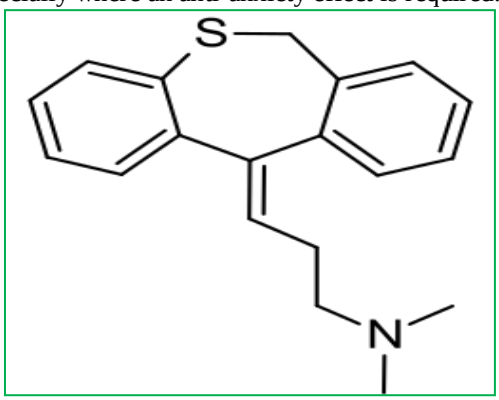


**Fig. 2: chemical structure of Dosulepin**

## MATERIALS AND METHODS:

Accurately measured 1000ml of HPLC Water (100%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

**Instrumentation and Chromatographic conditions**

The analysis was performed by using Xterra C18 column, 4.6×250mm internal diameter with 5 micron particle size column and UV detector set at 230nm nm, in conjunction with a mobile phase of Water in the ratio of 100% v/v (pH 5 adjusted with OPA) at a flow rate of 1ml/min. The retention time of Dosulepin was found to be 2.159 minute. The 10µl of sample solution was injected into the system

**Preparation of standard solution:**

Accurately weigh and transfer 10 mg of Dosulepin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.15ml of the above Dosulepin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

**Mobile Phase Optimization**:

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally,

the mobile phase was optimized to Water in proportion 100%v/v respectively.

**Optimization of Column:**

The method was performed with column like Xterra C18 (4.6×250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow

**Optimized chromatogram):**

Column :Xterra C18 (4.6×250mm) 5µ
Column temperature : Ambient
Wavelength : 230nm
Mobile phase ratio :Water (100% v/v)
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 5minutes

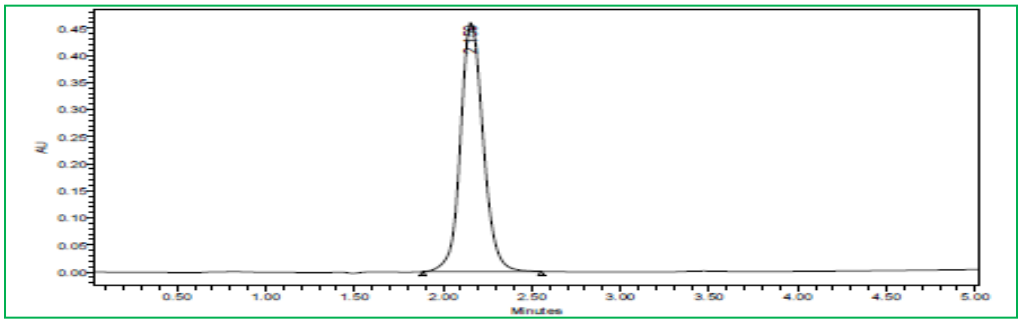


**Fig. 3: Typical chromatogram of mixture of Standard solution.**

**VALIDATION**

**PREPARATION OF MOBILE PHASE:**

**Preparation of mobile phase:**

Accurately measured 1000ml of HPLC Water (100%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

**Diluent Preparation:**

The Mobile phase was used as the diluent.

**Linearity**

The linearity of was obtained in the concentration ranges from 5-25 µg/ml

**Table 1: Linearity data of Dosulepin**

| Concentration Level (%) | Concentration µg/ml |
|---|---|
| 60 | 5 |
| 80 | 10 |
| 100 | 15 |
| 120 | 20 |
| 140 | 25 |

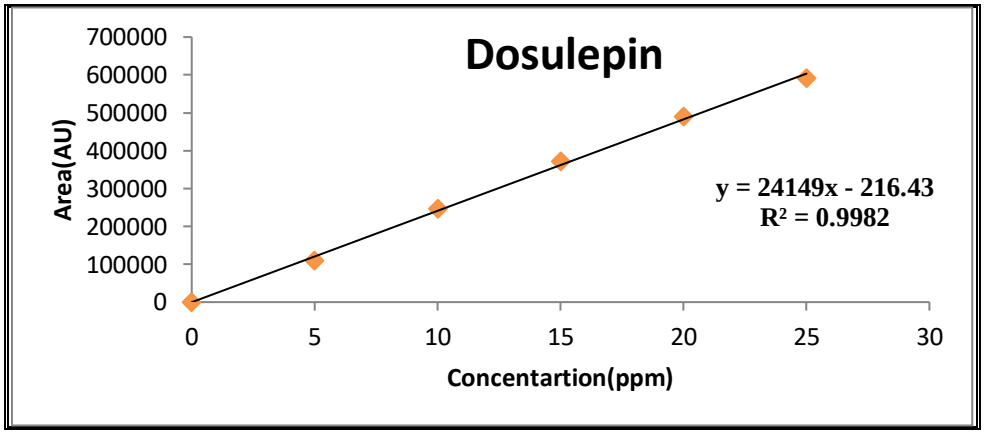


**Fig.4: calibration graph of Dosulepin**

**LINEARITY PLOT**

Linearity of detector response of assay method was found by injecting seven standard solutions with concentration ranging from 5-25µg/mL for Dosulepin. The graph was plotted for concentration versus peak area. The results were shown in Table-1 and fig 4.

**Precision**

**Repeatability**

The precision of test method was determined by preparing six test preparations using the product blend and by mixing the active ingredient with excipients as per manufacturing formula. And the relative standard deviation of assay results was calculated. The results were shown in Table 2

**Table 2: Results of repeatability for Dosulepin**

| S. No | Peak name | Retention time | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Dosulepin | 2.143 | 4110458 | 448125 | 8537 | 1.21 |
| 2 | Dosulepin | 2.147 | 4110200 | 464186 | 9295 | 1.1 |
| 3 | Dosulepin | 2.147 | 4119997 | 456368 | 7758 | 1.1 |
| 4 | Dosulepin | 2.147 | 4112340 | 467995 | 9472 | 1.2 |
| 5 | Dosulepin | 2.149 | 4117162 | 476509 | 9275 | 1.1 |
| **Mean** | | | 4114031 | | | |
| **Std.dev** | | | 4350.25 | | | |
| **%RSD** | | | 0.105742 | | | |

**Accuracy**

Dosulepin tablets content were taken at various concentrations ranging from 50 % to 150 % (50 %, 75 %, 100 %, 125 %, and 150 %) to accurately quantify and to validate the accuracy. The assay was performed in triplicate. The results were shown in Table-3

**Table 3.The accuracy results for Dosulepin**

| %Concentration (at specification Level) | Peak area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 207562 | 7.5 | 7.48 | 99.6 | 99.7% |
| 100% | 412727 | 15 | 14.9 | 99.8 | |
| 150% | 493708 | 22.5 | 22.48 | 99.9 | |

**LIMIT OF DETECTION (LOD)**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.The LOD value for Dosulepin 1.6µg/ml.

**Quantitation limit (LOQ)**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. The LOQ values for Dosulepin 4.8 µg/ml

**ROBUSTNESS**

The robustness was performed for the flow rate 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Dosulepin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard samples of Dosulepin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count. Table 4

**Table 4: Results for Robustness of Dosulepin**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.1ml/min | 409045 | 2.159 | 9622 | 1.1 |
| Less Flow rate of 0.9mL/min | 401847 | 2.629 | 8947 | 1.2 |
| More Flow rate of 1.2mL/min | 458729 | 1.791 | 7855 | 1.15 |

## SUMMARY AND CONCLUSION:

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 230nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Xterra C18 (4.6 x 150mm, 5µm) because it was giving good peak.. In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Dosulepin in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Dosulepin was freely soluble in ethanol, methanol and sparingly soluble in water.

**Table 5.Summary data for Dosulepin**

| Parameters | Dosulepin |
|---|---|
| Retention Time (min.) | 2.159 |
| Linearity (µg/ml) | 5-25 |
| Correlation Coefficient ($r^2$) | 0.998 |
| Slope | 24149 |
| Y – intercept | 216.4 |
| LOD (µg/ml) | 1.6 |
| LOQ (µg/ml) | 4.8 |
| Repeatability (% RSD) n=6 | 0.105742 |
| Intraday Precision (% RSD) | 0.130608 |
| Interday Precision (% RSD) | 0.243947 |
| Accuracy (%) | 99.7 |

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