# Development, Validation and Forced Degradation Study of Beclomethason Dipropionate in Bulk and Pharmaceutical Dosage Form by HPTLC Method

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***ABSTRACT:-***

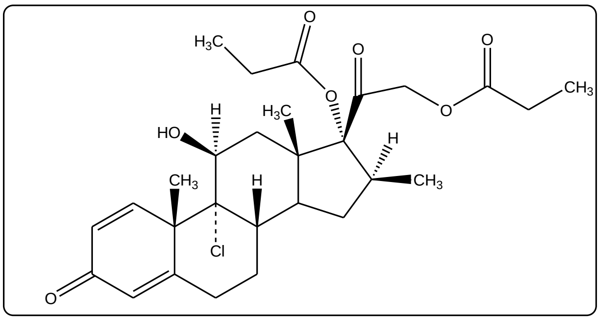
***Objective:-****The Present work describes the development and validation of a new simple, accurate, precise and stability-indicating HPTLC method for the determination of Beclomethason Dipropionate in the capsule dosage form.* ***Methods:-****The chromatographic separation was achieved by using Ethyl acetate: Toluene: Formic acid (4:6:0.2 v/v/v) as mobile phase and UV detection at 252nm with Rf value 0.64. This developed method was validated based on linearity, accuracy, precision, the limit of detection, the limit of quantitation and robustness as per ICH guidelines.* ***Result:-****The described method was linear over a concentration range of 200-1000 ng/band with correlation coefficient ($r^2$)> 0.9995. The recovery was found to be 98.14%. The limit of detection (LOD) and the limit of quantification (LOQ) for Beclomethason was found to be 28.30 ng/band and 85.76 ng/band respectively. The drug was shows a stress conditions of acid hydrolysis, alkali hydrolysis, photolysis, thermal degradation.****Conclusion:-****The proposed stabilityindicating method can be used for the determination of Beclomethason Dipropionate in bulk samples and in the pharmaceutical dosage form.*

***KEYWORDS:-*****Hydrolysis, ICH guideline, Mobile phase, TLC Plate.**

**DOI Number: 10.14704/nq.2022.20.13.NQ88173** **Neuro Quantology 2022; 20(13):1392-1400**

## Introduction:-

Beclomethason Dipropionate chemically known as 9a-chloro-11b-hydroxy-16b-methyl-3, 20-dioxopregna 1,4-diene-17,21-diyldipropionate. It is a corticosteroid hormone receptor agonist with anti-inflamatory, antiallergic, antiasthmatic and immunomodulating properties [1].These has multiple anti-inflammatory effect, inhibiting both Inflammatory cells and release of inflammatory mediators. Beclomethasone enters the nucleus where it binds and activates specific nuclear receptors, resulting in an altered gene expression and inhibit proinflammatory cytokine production [2]. These anti-inflammatory actions play an important role in controlling symptoms and improving lung Function in asthma.



It is white or creamy white, odorless powder, soluble in chloroform, acetone and alcohol and slightly soluble in water[3]. These molecules have been officially reported in IP, EP and USP. Formulation containing this component is available in the form of inhaler, Rotacaps, cream, pills, nasal spray[4].

Literature Survey reveals that a few analytical methods are developed for the determination of Beclomethason Diproponate using UV-visible spectroscopy, HPLC, Capillary electrophoresis, liquid chromatography and mass spectrometry in bulk and capsules. But there were no method developed for stability indicating hptlc method for estimation of beclomethason dipropionate[5]. The main objective of study is to provide simple, rapid, reliable, economic, specific and sensitive method has been developed for estimation of beclomethason dipropionate in capsule formulation and in bulk drug. As compared to the other analytical technique HPTLC is the most simple, less time consuming technique, minimal use of solvent and low cost method and its validation according to ICH guideline[6].

**MaterialsAnd Methods:-**

Beclomethason Dipropionate was provided as gift samples from industry. Marketed formulation Capsule (Mfg. by CIPLA Ltd.) with dose of 400 mcg was procured from local market, Pune. Ethyl Acetate, Toluene, Formic acid was used as solvent for mobile phase. Analytical grade solvent and TLC aluminum plates precoated with silica gel F254 used for this study were purchased from Merck Pvt. Ltd, Mumbai.

**Table 1: Marketed Formulation Selected For Analysis**

| Trend Name | Content | Mfg. Company |
|---|---|---|
| Beclate | Beclomethason Dipropionate (400 ncg) | CIPLA Pvt. Ltd |

**Instrumentation and Chromatographic Condition**

HPTLC was performed with a Camag (Muttenz, Switzerland) Linomat V applicator, a Camag twin trough TLC chamber, a Camag TLC scanner 3, Camag Wincats software and a Hamilton (Reno, Nevada, USA) Syringe (100 µL).Chromatographic separation of drug was performed by using precoated silica gel TLC plates $F_{254}$, 10x10cm size. Samples were applied on the TLC plates in the form of bands, width 6mm using Hamilton microliter syringe (100µl). Linear ascending development was carried out in 10× 10cm twin trough glass chamber (CAMAG) using mobile phase which consists of Ethyl acetate : Toluene : Formic acid in the ratio of 4:6:0.2 v/v/v. The slit dimension was kept as 5 × 0.45mm. The optimized saturation time for mobile phase was 20 min. Chromatogram was run until the distance of 8 cm. After development the plate was dried and on a Camag TLC densitometric analysis was performed. Scanner equipped with winCATS software at 252 nm. The source of radiation utilized was deuterium lamp. Evaluation was determineusing peak area with linear regression[7]. 

**Preparation of Standard solution, Sample solution and Calibration curve**

**Standard Stock Solution:**

10 mg of Beclomethason Dipropionate was weighed accurately and transferred into 10 ml volumetric flask, sonicate to dissolve and the volume was made up to the mark with methanol (1000 µg/ml), respectively. Calibration standard were spotted on TLC plate using Camag Hamilton 100 µl syringe using automatic sampler applicator Linomate V having a concentration of 200, 400, 600, 800 and 1000 ng/band. The plates were developed using mobile phase containing Ethyl acetate : Toluene : Formic acid (4:6:0.2 v/v/v) and the calibration curve was plotted between peak areas versus concentration.

**Method Validation**

**Precision:-**

Precision was studied as intraday and interday variations. Intraday was assessed by analyzing 6 concentration of BEC for three times within a day (Morning, Afternoon, and Evening). Interday was determined by analyzing sample solution concentration of BEC at different days respectively.

**Accuracy:-**

To check accuracy of the method, recovery studies were carried out by standard addition method at 80%, 100% and 120% sample chosen was 200 ng/band of standard. These solutions were applied on TLC plates in triplicate. This was done to check a recovery of drug at different level in formulation.

**Robustness:-**

Robustness was studied in five replicate at the concentration level of 200 ng/band. In this study, parameters (Mobile phase volume, duration of saturation) were studied and the effects on the results were examined.

**Ruggedness:-**

Ruggedness of the proposed method was studied by two different analysts using the same experimental and environmental condition. The spots of BEC having concentration of 200 ng/band were applied on TLC plates. The development and scanning of spots were performed.

**Sensitivity:-**

Sensitivity of Proposed method was estimated in terms of limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD and LOQ are based on the standard deviation of response of the calibration curve. To calculate the LOD and LOQ the standard deviation of y-intercept and slope of calibration curve were used. Formula for LOD is (LOD= 3.3(SD)/S) and Formula for LOQ is (LOQ= 10(SD)/S) Where, SD =Standard deviation, S = Slope

**Specificity:-**

The specificity of the method was assessed by comparing the chromatogram obtained from the standard drug with the chromatogram obtained from capsule solution[8, 9, 10].

**Forced Degradation studies:-**

**Acidic Degradation:-**

1ml of standard solution was added into 1ml 0.1N methanolic HCl kept at room temperature for 12 hrs. Volume was making up with methanol and spotted on TLC plate. This was run with mobile phase consisting of Ethyl acetate: Toluene: Formic acid in the ratio of 4:6:0.2 v/v/v. The plate was dried and scanned at 252 nm. Densitogram was recorded and % degradation was calculated and densitogram was shown in figure 4

**Basic Degradation:-**

1 ml of standard solution was added into 1 ml 0.1N methanolic NaOH kept at room temperature for 12 hrs.Volume was make up with methanol and spotted on TLC plate. Plate was run with mobile phase consisting of Ethyl acetate: Toluene: Formic acid in the ratio of 4:6:0.2 v/v/v. The plate was dried and scanned at 252 nm. Densitogram was recorded and % degradation was calculated and densitogram was shown in figure 5

**Oxidative Degradation:-**

1 ml of standard solution was added into 1 ml of 3% $H_2O_2$ kept at room temperature for 6 hrs. Volume was made up with methanol and spotted on TLC plate. Plate was run with mobile phase consisting of Ethyl acetate: Toluene: Formic acid in the ratio of 4:6:0.2 v/v/v. The plate was dried and scanned at 252 nm. Densitogram was recorded and % degradation was calculated and densitogram was shown in figure 6

**Neutral Degradation:-**

1ml of standard solution was added into 1ml Distilled water kept at room temperature for 6 hrs. Volume was made up with methanol and spotted on TLC plate. Plate was run with mobile phase consisting of Ethyl acetate: Toluene: Formic acid in the ratio of 4:6:0.2 v/v/v. The plate was dried and scanned at 252 nm. Densitogram was recorded and % degradation was calculated and densitogram was shown in figure 7.

**Thermal Degradation:-**

Drug sample was kept separately in oven (60°C) for a period of 24 hours. A sample were withdrawn after 2 hr, 4 hr, 6 hr, 12 hr,18 hr, 24 hours, dissolved in methanol to get solution of 1000 µg/ml. Plate was run with mobile phase consisting of Ethyl acetate : Toluene: Formic acid in the ratio of 4:6:0.2 v/v/v. The plate was dried and scanned at 252 nm. Densitogram was recorded and % degradation was calculated and densitogram was shown in figure 8.

**Photolytic Degradation:-**

Drug sample was kept separately under sunlight for a period of 24 hours. A sample were withdrawn after 2 hr, 4 hr, 6 hr, 12 hr,18 hr,24 hours, dissolved in methanol to get solution of 1000 µg/ml. Plate was run with mobile phase consisting of Ethyl acetate : Toluene: Formic acid in the ratio of 4:6:0.2 v/v/v. The plate was dried and scanned at 252 nm. Densitogram was recorded and % degradation was calculated and densitogram was shown in figure [11].

**Result And Discussion**

**Optimization of HPTLC Method**

Optimization of method was done by different mobile phase composition, in varying ratios were tried. Use of mobile phase consisting of Ethyl acetate: Toluene: (4:6 v/v) gave good peak with RF value of 0.64 for Beclomethason Dipropionate but tailing was observed. Therefore to decrease tailing Formic acid was added. The optimized mobile phase was ethyl acetate: toluene: Formic acid (4:6:0.2 v/v/v) which gave sharp and well defined peak at $R_f$ value of 0.64. The densitogram of standard Beclomethason Dipropionate (200 ng/spot) was measured at 252 nm.

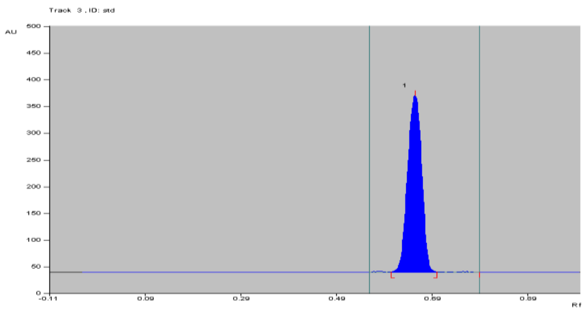


**Figure 1. Typical Densitogram of BDP (Rf= 0.64)**

### Validation of Method

Linearity:-

The calibration curve was plotted between peak areas versus concentration. The linear regression data for the calibration curves (n=5) showed good linear relationship over the concentration range of 200-1000ng/spot for Beclomethason Dipropionate with $R^2$=0.9995. The slope and intercept of the regression equation were 1.4535 and 1038 respectively for the Beclomethason Dipropionate. The linearity was found to be satisfactory and reproducible. The calibration curve for Beclomethason Dipropionate is shown in fig. 2.

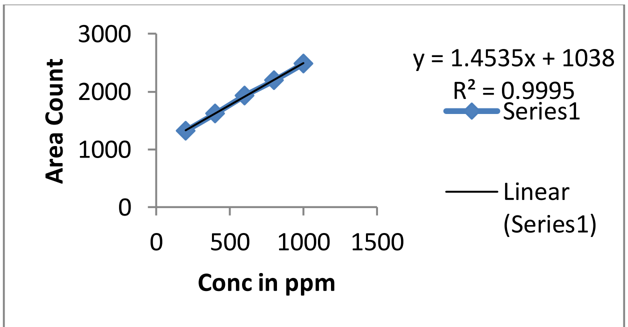



**Fig.2** Calibration curve for Beclomethason Dipropionate

**Table 2. Standard Calibration data for Beclomethason Dipropionate**

| Sr. No | Concentration (ng/band) | Area |
|---|---|---|
| 1 | 200 | 1321.9 |
| 2 | 400 | 1618.55 |
| 3 | 600 | 1928.02 |
| 4 | 800 | 2194.62 |
| 5 | 1000 | 2487.32 |

**Table 3.Linearity regression data for Calibration Curve**

| Sr. No | Parameter | Beclomethason Dipropionate |
|---|---|---|
| 1 | Detection Wavelength (nm) | 252nm |
| 2 | Beers limit (ng/band) | 200-1000 |
| 3 | Regression equation | Y=1.4535x+1038 |
| 4 | Correlation coefficient | 0.9995 |
| 5 | Slope | 1.4535 |
| 6 | Intercept | 1038 |

### Precision:

Intraday and interday precision of the method was assessed by developing the plate after application of 6 replicates of concentration on TLC plate on the same day and the consecutive days respectively. Precision was reported in terms of %RSD. The %RSD values were found to be less than 2% as shown in table 4 and 5.

**Table 4.Result of Intraday precision of Beclomethason Dipropionate**

| Concentration (ng/band) | Peak Area | Mean Area | ± SD | %RSD |
|---|---|---|---|---|
| 200 | 923.89 | 940.78 | 8.890969 | 0.945635% |
| 200 | 940.78 | | | |
| 200 | 941 | | | |
| 200 | 941.95 | | | |
| 200 | 942.5 | | | |
| 200 | 951.15 | | | |

**Table 5. Result of Interday precision of Beclomethason Dipropionate**

| Day | Concentration (ng/band) | Peak Area | Mean Area | ±SD | %RSD |
|---|---|---|---|---|---|
| **Day 1** | 200 | 923.89 | 925.2667 | 10.27464 | 1.110% |
| | 200 | 913.69 | | | |
| | 200 | 941.95 | | | |
| | 200 | 923.89 | | | |
| | 200 | 931.47 | | | |
| | 200 | 916.71 | | | |
| **Day 2** | 200 | 940.78 | 935.438 | 14.37799 | 1.537032% |
| | 200 | 923.89 | | | |
| | 200 | 912.36 | | | |
| | 200 | 941.95 | | | |
| | 200 | 951.15 | | | |
| | 200 | 942.50 | | | |

**Recovery:**

Recovery also known as accuracy, by spiking the band of formulation with 80%, 100%, 120% of pure drug and then finding out the amount of drug recovered. The mean percentage recovery is shown in Table 6.

**Table 6: Result of Recovery Study**

| %Level Discovery | Amount of drug taken (ng/band) | Amount of Std. added (ng/band) | Amount of Drug recovered | % Recovery | % count (w/w) |
|---|---|---|---|---|---|
| **80%** | 200 | 160 | 351.59 | 97.66% | 98.14% |
| **100%** | 200 | 200 | 393.94 | 98.23% | |
| **120%** | 200 | 240 | 433.64 | 98.55% | |

**Robustness:**

Standard deviation of peak area and % RSD was calculated for each parameter. The acceptance criterion for %RSD was found to be NMT 2% which indicates the reliability of method. The robustness of the method is shown in table 7.

**Table 7: Result of Robustness study**

| Sr. No | Parameter | Robust Condition | Peak area | Mean | SD | % RSD |
|---|---|---|---|---|---|---|
| **1** | Developing Time | 20 min | 972.6<br>971.9<br>974.8 | 973.1 | 1.51 | 0.15% |
| | | 40 min | 986.9<br>987.3<br>989.0 | 987.73 | 1.11 | 0.11% |
| 2 | Mobile phase composition | Ethyl acetate + toluene + Formic acid (4.2:5.8:0.4) | 923.9<br>925.9<br>922.32 | 924.04 | 1.79 | 0.19% |
| | | Ethyl acetate + toluene + Formic acid (3.8: 6.2: 0.4) | 825.13<br>821.33<br>823.8 | 823.42 | 1.92 | 0.23% |

**Specificity:-**

The peak purity of BDP was assessed by comparing their respective spectra at the peak apex, peak start and end positions of the peak. A good correlation was obtained for both drugs. Good correlation values and satisfactory peak purity suggest that there is no interference in the quantification of BD in sample solutions. This verifies that the method is specific. Typical absorption overlain spectra of Beclomethason Dipropionate are shown in Figure3.

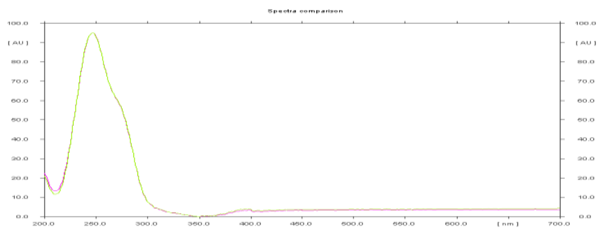



**Figure 3.Overlay Spectra of Beclomethason Dipropionate**

**Sensitivity:-**

The LOD and LOQ of BDP was found to be 28.30 ng/band and 85.76 ng/band

**Table 8. Result of LOD & LOQ**

| Parameters | Result(ng/band) |
|---|---|
| LOD | 28.30 |
| LOQ | 85.76 |

**Stress Degradation Study:-**

Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like hydrolysis, oxidation, temperature, and to establish specific storage conditions, shelf-life and retest period. HPTLC studies under different stress condition are summarized in table no 9.

**Table 9. Result of Stability Study**

| Stress Condition | Time (Hrs) | % Assay of active substance |
|---|---|---|
| Acid hydrolysis (0.1 M HCl) | 6 | 40.98% |
| Base hydrolysis (0.1M NaOH) | 6 | 35.0% |
| Neutral ($H_2O$) | 12 | 59.03% |
| Oxidation(3% $H_2O_2$) | 6 | 46.42% |
| Thermal degradation | 24 | 53.60% |
| Photolytic | 6 | 41.13% |

### Acid degradation:

The drug was degraded at 6 h in acidic conditions. Densitogram was recorded and % degradation was calculated. Densitogram was shown in figure 4

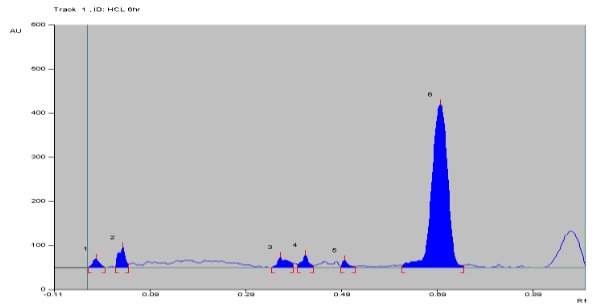


**Figure 4: Acidic Degradation**

### Base degradation:

The drug was degraded at 6 h in Basic conditions. Densitogram was recorded and % degradation was calculated. Densitogram was shown in figure 5

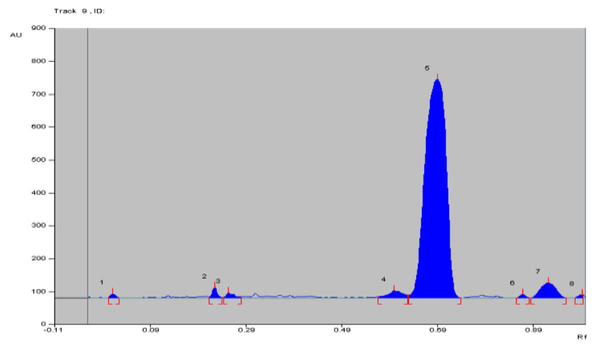


**Figure 5: Basic Degradadtion**

### Oxidative degradation:

The drug was degraded at 6 hrs in oxidative conditions. Densitogram was recorded and % degradation was calculated. Densitogram was shown in figure 6

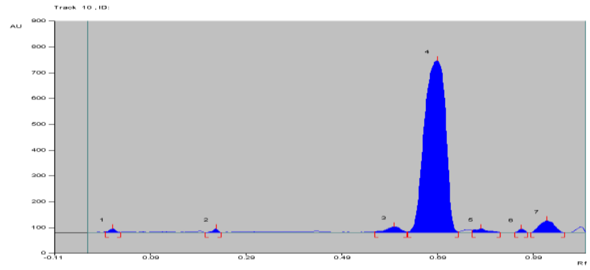


**Figure 6. Densitogram of Oxidative Degradation**

### Degradation Under Neutral Hydrolytic Condition:

The drug was degraded at 12 hrs in neutral conditions. Densitogram was recorded and % degradation was calculated. Densitogram was shown in figure 7

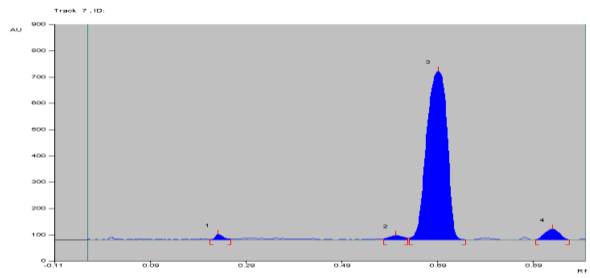


**Figure 7. Degradation under neutral hydrolytic condition**

### Thermal Degradation:

The drug was degraded at 24 h under Thermal conditions. Densitogram was recorded and % degradation was calculated. Densitogram was shown in figure 8

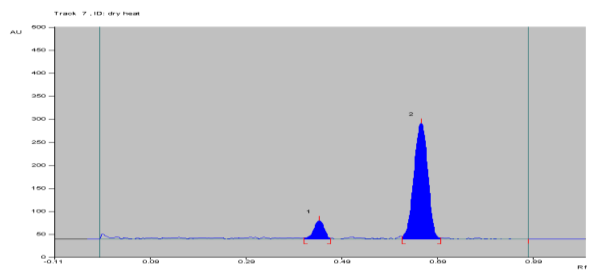


**Figure 8. Thermal Degradation**

**Photo-degradation studies:-**

The drug was degraded at 6 h in photolytic conditions. Densitogram was recorded and % degradation was calculated. Densitogram was shown in figure 9

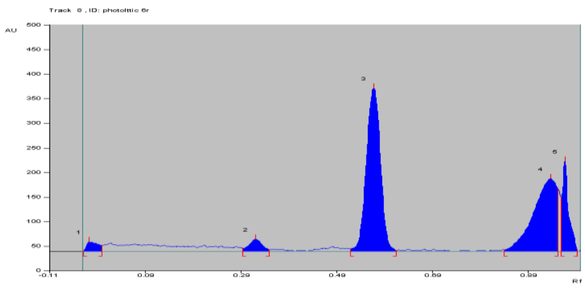


**Figure 9.Photolytic degradation**

**Summary of validation:-**

| Parameters | Observation |
|---|---|
| Linearity range(ng/band) | 200-1000ng/band |
| Regression equation | Y=1.4535X + 1038 |
| Correlation Coefficient | 0.9995 |
| Slope | 1.4535 |
| Limit of Detection | 28.30 |
| Limit of Quantification | 85.76 |
| Recovery | 98.14% |
| Precision[%RSD] | - |
| Interday | Day 1- 1.110%<br>Day 2- 1.5370327% |
| Intraday | 0.945635% |
| Robustness | Robust |

**Conclusion**

The proposed stability – indicating method was simple, precise, accurate, reproducible, and sensitive and can be used for determination of Beclomethason Dipropionate in bulk samples and in pharmaceutical dosage form

**Acknowledgment**

The authors are grateful to Dr. Sonia Singh, Principal and management of the Alard College of Pharmacy, Pune for providing the necessary research facilities also thanks to all who have helped me directly and indirectly in completing this project work.

**Conflict Of Interest**

There is no conflict of interest

**ABBREVATION Used**

**BDP:** -BeclomethasonDipropionate, **RF: -** Retention factor, **LOD: -** Limit of Detection, **LOQ:-** Limit of Quantification, **SD: -** Standard Deviation, **RSD: -** Relative standard deviation, **%:-** Percentage

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