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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF DANTROLENE SODIUM IN BULK AND PHARMACEUTICAL DOSAGE FORM

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***Abstract*:** The goal of this research is to develop and validate a stability indicator method for Dantrolene sodium Capsules using the RP-HPLC technology. Dantrolene sodium is a medication used to relax the skeletal muscles and treat muscular spasms. In this study, a new validated stability-indicating RP-HPLC technique for quantitative detection of Dantrolene sodium (DAN) in capsule formulation was established. The column was a Phenomenex Luna C18 column (250 mm × 4.6 mm id; 5µm particle size) with a mobile phase of Acetonitrile: Water in 0.1% Triethylamine (30:70v/v) at a flow rate of 1 ml/min. Eluents were monitored using a UV detector at 380 nm. The calibration curve for DAN was linear across concentration ranges of 2.5-12.5µg/ml (R2 = 0.9998). DAN was exposed to several stress conditions, including acidic, alkaline, oxidative, photolytic, and thermal degradation. The suggested approach was found to provide effective separation of pure medication and degraded products. The proposed method was successfully used to test the stability of DAN in capsule formulations and validated in accordance with ICH recommendations.

KEY WORDS: **Dantrolene sodium, Forced degradation, RP-HPLC, method validation.**

I. INTRODUCTION

Dantrolene Sodium (Fig.1) is Chemically Dantrolene sodium is a 1-{[5-(4-nitrophenyl)-2-furyl] methylidene amino} imidazolidine-2,4-dione, that acts by interfering with excitation-contraction coupling in the muscle fiber. It is used in spasticity and other neuromuscular abnormalities. Although the mechanism of action is probably not central, dantrolene is usually grouped with the central muscle relaxants[1]. On literature survey, several methods were reported for the estimation of DAN individually and in combination with other drugs[2-7] So we have developed a novel, simple, rapid, accurate, precise and highly sensitive RP-HPLCmethod for estimation of DAN in bulk and Capsules dosage form and validated according to ICH guidelines.

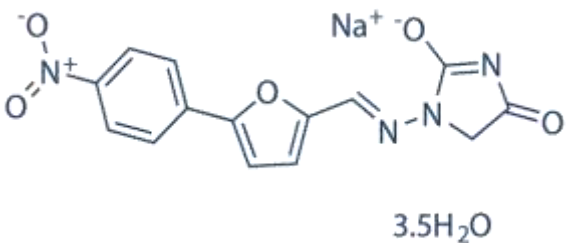

Figure 1Structure of Dantrolene Sodium[1]

II. MATERIALS AND METHODS

Reagents and Chemicals

Analytically pure sample of DAN was procured from Par Formulations, Pudhupakkam, Chennai, Pvt. Ltd. (India). The Pharmaceutical dosage form used in the study labelled to contains 25 mg of DAN was purchased from Local Pharmacy. All chemicals and reagents were of HPLC grade (Loba Chemie Pvt. Ltd.) and were purchased from Sudhagar Biological and Chemicals Pvt. Ltd., Chennai, India.

The instrument and chromatographic conditions

Shimadzu HPLC system, Prominence-i LC-2030 Plus (Shimadzu corporation Kyoto, Japan) consisted of a pump (LC - 2030 Plus Parallel type double Plunger, SPD-20A UV- Visible detector) run under Lab solutions software, with automating injecting facility programmed at 20 µL capacity per injection was used. The column used was Phenomenex Luna C_{18} (250 mm × 4.6 mm, 5.0 µm particle size). Different mobile phases were tested in order to find the best condition for separation of DAN. The mobile phase contained Acetonitrile: Water in 0.1% Triethylamine (30:70, v/v) and the flow rate was maintained at 1.0 ml/min. UV detection was carried out at 380 nm. The mobile phase and samples were filtered through a 0.45 µm membrane filter. Mobile phase was degassed by Sonica ultrasonic cleaner (model 2200 MH) prior to use. The other instrument used are hot air oven.

III. PREPARATION OF STANDARD AND SAMPLE SOLUTIONS

Diluent: Mobile phase was used as the diluent in the ratio of 1:1

Mobile phase

Acetonitrile: Water in 0.1% Triethylamine (30:70, v/v) is programmed as RP HPLC method.

Preparation of stock standard solutions

Stock standard solution of DAN (2500 μg/ml) was prepared by dissolving 25 mg of DAN in 10 ml of diluent in 10 ml volumetric flask with vigorous shaking (Stock-I), From this stock solution 1 ml was pipetted and diluted to 10 ml with methanol (Stock-II).

Preparation of working standard solution

From above standard stock solution of (Stock-II) 0.2 ml of DAN solution was taken into 10 ml volumetric flask, separately and was made to the mark with the mobile phase to get 5 μg/ml of DAN.

Preparation of sample stock solution

The average weight of 20 Capsules was determined. Sample stock solution was prepared by dissolving tablet powder equivalent to 25 mg of DAN and was transferred to a 10 ml volumetric flask. Then 5 ml diluent was added and sonicated for 10 mins to ensure complete solubilization of drug. After sonication, volume was made up to the mark with diluent. Filter the sample stock solution with Whatman filter paper and 1ml of the solution was further diluted to 10 ml to get final concentration.

Preparation of sample solution

From above sample stock solution of DAN, 0.2 ml was withdrawn and diluted to 10 ml using diluent to get concentration of 5 μg/ml of DAN.

IV.Validation[8]

The proposed method was validated as per ICH guidelines.

Linearity

Different aliquots of 0.1 - 0.5 ml of DAN was transferred into series of 10 ml volumetric flasks separately, and the volume was made up to the mark with diluent to get concentrations such as 2.5, 5, 7.5, 10, 12.5 µg/ml.

Accuracy

To the pre-analyzed sample solution, a known amount of standard stock solution was added at different levels i.e. 50, 100 and 150%. The solutions were reanalyzed by proposed method.

Precision

The reproducibility of this method was determined by analyzing tablet at different time intervals on same day in triplicates (Intra-day assay precision) and on three different days (Inter-day assay precision).

V. FORCED DEGRADATION STUDIES [9]

Hydrolytic degradation under acidic condition

Pipetted out 0.2 ml of sample stock solution into a 10 ml volumetric flask and added 0.2 ml of 0.1 N HCl. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralizedwith 0.1 N NaOH and made up to 10 ml with diluent.

Hydrolytic degradation under alkaline condition

Pipetted out 0.2 ml of sample stock solution into a 10 ml volumetric flask and added 0.2 ml of 0.1 N NaOH. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralizedwith 0.1 N HCl and made up to 10 ml with diluent.

Oxidative degradation

Pipetted out 0.2 ml of sample stock solution into a 10 ml volumetric flask and added 0.2ml of 0.1% w/v of hydrogen peroxide. Then, the volumetric flask was kept at 60ºC for 24 hours andthen the volume was made up to the mark with diluent.

Photo degradation

Pipetted out 0.2 ml of sample stock solution into a 10 ml volumetric flask and expose tosunlight for 24 hrs and the volume was made up to the mark with diluent.

Thermal induced degradation

DAN sample was taken in petri dish and kept in Hot air oven at 60 °C for 24 hours. Then the sample was taken and diluted with diluent and injected into HPLC and analyzed.

VI. RESULTS AND DISSCUSION

Method development and optimization

The HPLC procedure was optimized with a view to develop a suitable LC method for the determination of DAN in Capsule dosage form. Initially, acetonitrile, buffer and water in different ratios were attempted. But DAN gave tailed peak. So, 0.1% of Triethylamine was added in water and mixtures of acetonitrile and the different ratios of acetonitrile and water were attempted. It was found that acetonitrile: water in 0.1% of Triethylamine in the ratio of 30:70 (v/v) gave acceptable retention times (2.265 min of DAN) with flow rate of 1.0 ml/min as shown in figure 3 and also performed mobile phase blank as shown in figure 2.

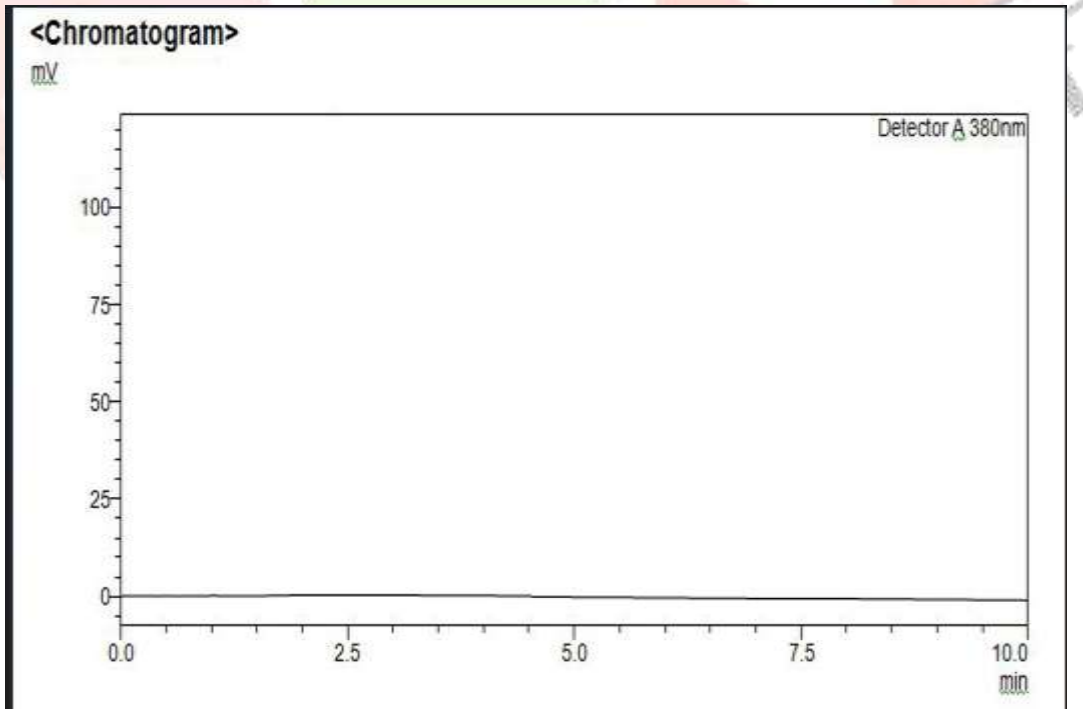

Figure 2. Blank for Optimized Mobile Phase

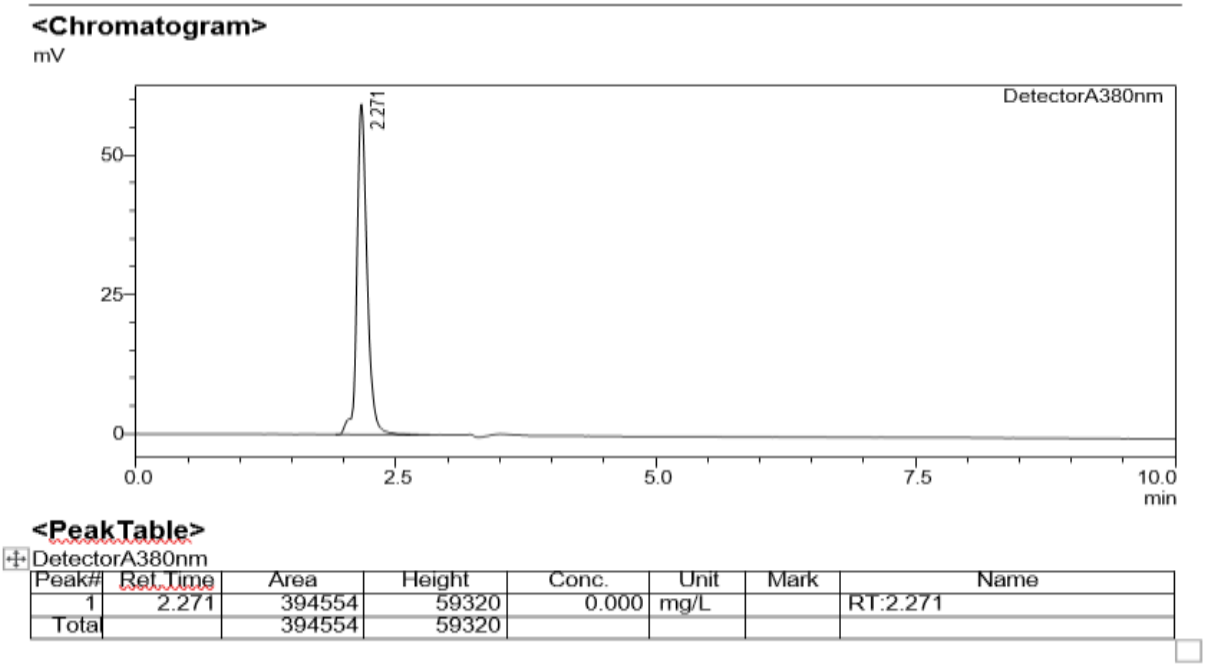

Figure 3. Optimized Chromatogram for Dantrolene sodium

Method validation

The described method has been validated which include parameters like system suitability, linearity, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification).

System suitability

System suitability and chromatographic parameters were validated such as tailing factor and theoretical plates was calculated. The results are given in table 1.

Table 1: System suitability parameters.

Parameters	**DANTROLENE**
Retention time	2.245
Peak area	394897
Tailing factor (T)	1.082
Theoretical plate (N)	6876

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of DAN at different concentrations in the range of 2.5–12.5 µg/ml with correlation coefficient (r^2) of 0.9998. Results are given in table 2.

Table 2: Linearity data for Dantrolene sodium

Drug	**Concentration (µg/ml)**	**Area**
DAN	2.5	195066
	5.0	394554
	7.5	579725
	10.0	770290
	12.5	952536

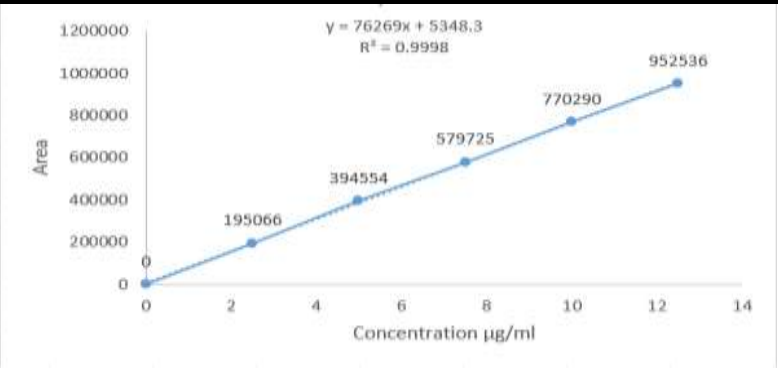

Figure 4. Linearity for Dantrolene sodium

Accuracy

Accuracy of the proposed method was determined by performing the recovery experiment. The recovery experiment was studied by adding known amount of standard DAN and to the Pharmaceutical Product and calculating the recovered standard amount. At 50%, 100% and 150% standard addition level mean recovery of were found to be 99.81%, 100.19% and 100.08% for DAN. The results of recovery experiment are given in table 3.

Table 3: Accuracy for Dantrolene sodium

Drug	percentage	Amount present* µg/ml	Amount Added* µg/ml	Amount Estimated*	Amount recovered	%recovery	SD	%RSD	SE
DAN	**50%**	5	2.5	7.485	2.485	99.813	0.2334	0.2338	0.1347
	100%	5	5	10.019	5.019	100.916	0.2173	0.2169	0.1254
	150%	5	7.5	12.510	7.51	100.08	0.3634	0.3631	0.2091

Precision

Precision was evaluated at the repeatability and intermediate precision levels. For repeatability analysis, six independent portions of a tablet dosage form were processedthrough the full analytical method and results was evaluated obtained by % RSD values of 0.3704 for DAN as shown in table.4.

Table 4: Precision result for the proposed method

Sample No.	Dantrolene sodium	
	Peak area response	Assay (%)
1	395464	100.23
2	396892	100.60
3	395938	100.35
4	396706	100.54
5	396794	100.56
Average	399673	101.30
% RSD	0.3704	

Robustness

Robustness study was conducted by deliberate changes in mobile phase composition and flowrate, revealed that there was no significant variation in % assay as shown in table 5.

Table 5: Robustness of the study

Percent assay of the drug	Mobile phase, Acetonitrile: Water in 0.1% Triethylamine		Flow rate, ml/min	
DAN	29:71 (v/v)	31: 69 (v/v)	0.9	1.1
	100.56%	100.25%	100.26%	100.10%

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

The LOD and LOQ was found to be 0.4065 (µg/ml) and 1.2195 (µg/ml) for DAN estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration. The results are given in table 6.

Table 6: LOD and LOQ data for Dantrolene sodium

Drug	LOD	LOQ
DAN	0.4065 (µg/ml)	1.2195(µg/ml)

Forced degradation studies

Results are tabulated in table 7.

Table 7: Summary of forced degradation study.

Stress conditions	Time (hr)		Comment
0.1M HCl	24		Not degraded
0.1M NaOH	24		Not degraded
0.1 % H_2O_2	24		Not degraded
Thermal	24		Not degraded
Photo	24		Degraded (95.76%)

When stress conditions were applied to DAN, the HPLC results showed that there was no degradation occurs in acidic, basic, oxidative and thermal. The drug was degraded in the sunlight which shows that the DAN was sensitive to the Light, the results were shown in figure 5-9.

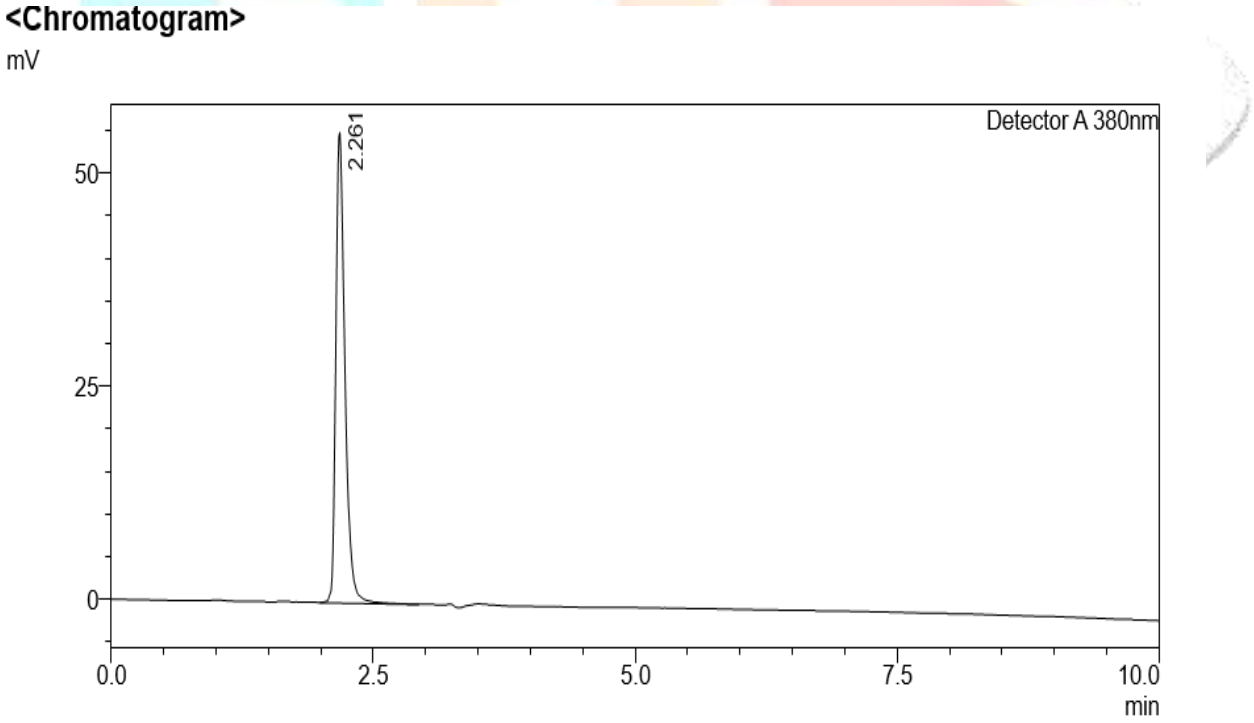

Figure 5 Typical Chromatogram of DAN under forced degradation study-Acid Degradation

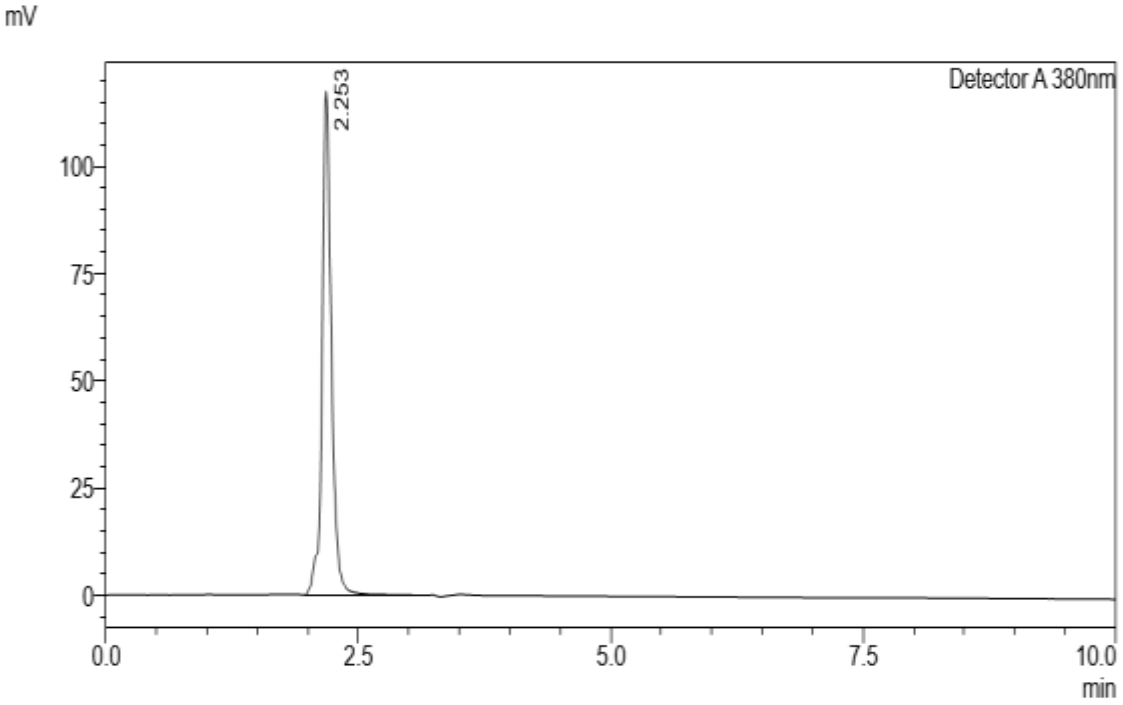

Figure 6 Typical Chromatogram of DAN under forced degradation - Base degradation

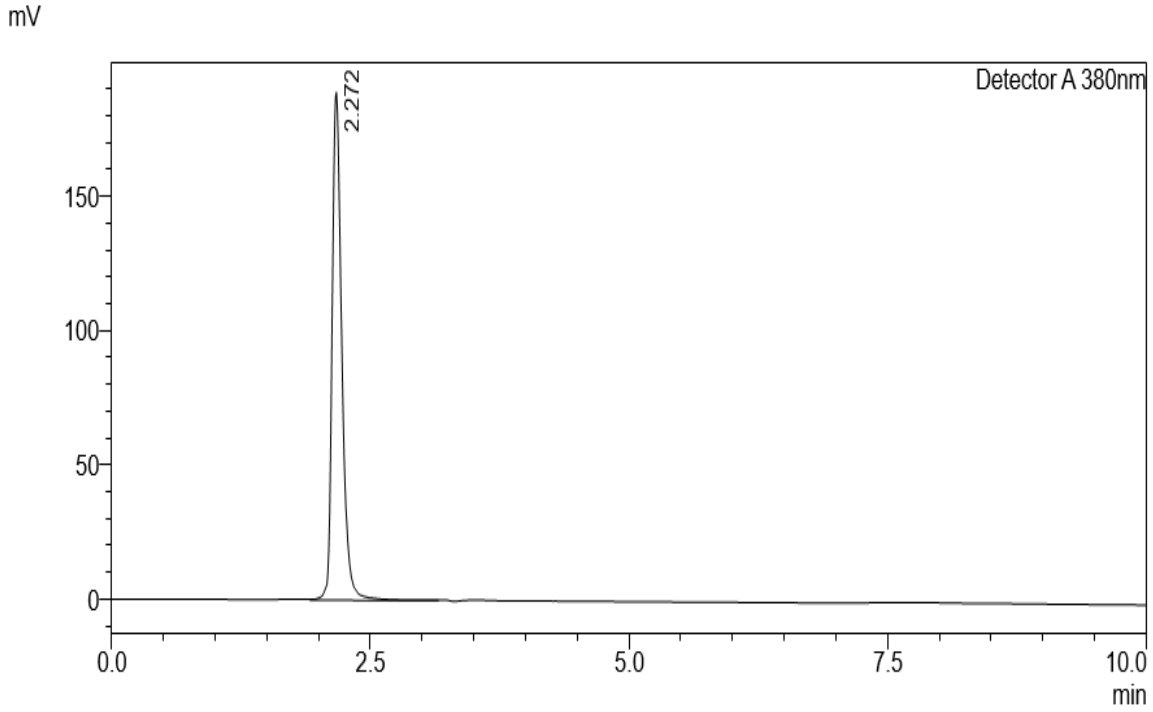

Figure 7 Typical Chromatogram of DAN under forced degradation - Oxidative degradation

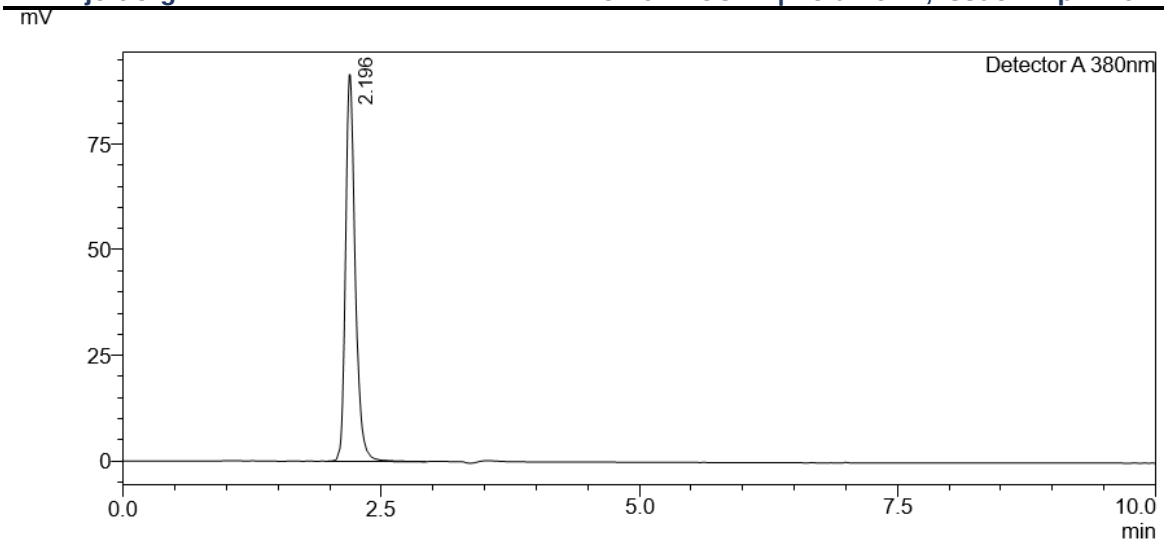

Figure 8 Typical Chromatogram of DAN under forced degradation - Thermal degradation

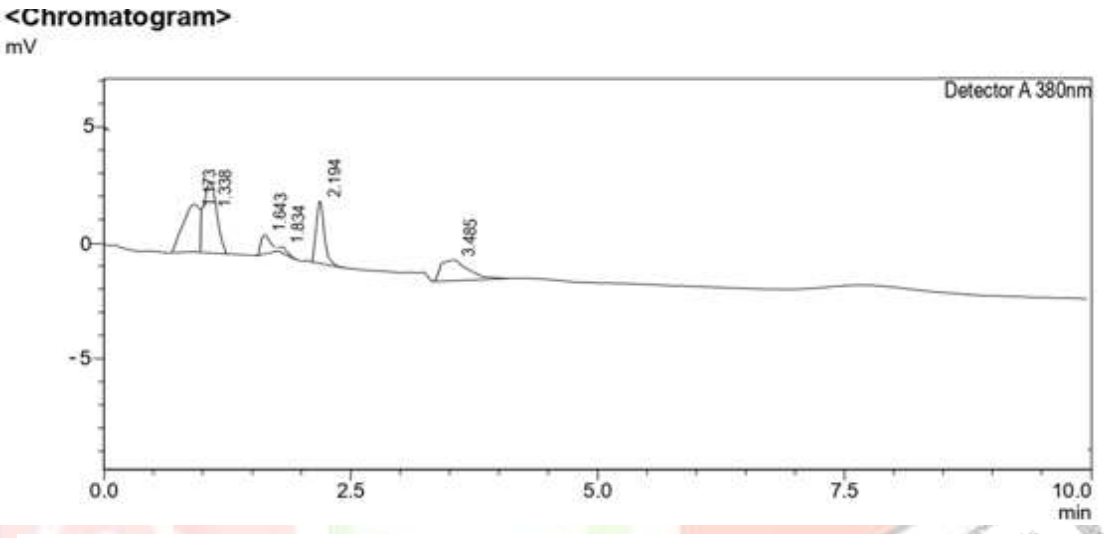

Figure 9 Typical Chromatogram of DAN under forced degradation - Photolytic degradation

VII.CONCLUSIONS

The stability-indicating RP–HPLC method was developed and validated according to ICH guidelines and applied for the determination of DAN in Capsule formulation. The results obtained from validation studies revealed that, the developed method was found to be rapid, simple, accurate, precise, specific, selective and economical. Results obtained from the force degradation, indicates that there was no degradation occurs in acid, base, oxidative, thermal except photolysis. The proposed method has the ability to estimate the drug in capsule dosage form and also used for stability-indicating method to estimate of DAN in bulk powder and Capsule formulation.

VIII. ACKNOWLEDGMENT

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Ethical committee approval: The proposed research does not require permission from an ethics commission. In this research, no matrices including humans or animals were used.

Informed Consent: Not applicable

Peer-review: Externally peer-reviewed

Authorship Contributions

Concept: Data collection or Processing, Writing, and analysis or interpretation.

Conflict of Interest: The authors declared no conflicts of interest.

Financial Disclosure: The authors state that no funding was for this research

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