DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR ESTIMATION OF DEXTROMETHORPHAN HYDROBROMIDE

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ABSTRACT
A simple, sensitive and accurate stability indicating HPTLC method has been developed and validated for estimation of Dextromethorphan hydrobromide in bulk and pharmaceutical dosage form. The drug was spotted on precoated silica gel 60 F254 aluminum plates using Toluene: Methanol: Triethylamine (8.5:1:0.5 v/v/v) as mobile phase. The retention factor (Rf) was found to be 0.60±1.92. The detection of band was carried at 225 nm. The drug was subjected to different stress conditions like acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis. The method was successfully validated according to ICH guidelines Q2 (R1). The data of linear regression analysis indicated a good linear relationship over the concentration range of 2000-20000 ng/band with correlation coefficient 0.991. The method found to be simple, sensitive, selective, accurate and repeatable for analysis of and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

INTRODUCTION
Dextromethorphan hydrobromide chemically, it is morphinan, 3-methoxy-17-meth (9, 13, 14)-, hydrobromide. It is antitussive (cough suppressant) drug used for the pain relief and in psychological conditions. It acts on cough centre to elevate the threshold for coughing [1]. Literature survey reveals methods reported viz simple HPLC [2]-[4] and bioanalytical HPLC method [5] for estimation of Dextromethorphan hydrobromide.

To the best of our knowledge no stability indicating HPTLC method has been reported for estimation of Dextromethorphan hydrobromide. The present work describes a simple stability indicating HPTLC method for the determination of
Dextromethorphan hydrobromide in bulk and pharmaceutical dosage form (DMR-20) according to the International conference on harmonization (ICH) guidelines [6][7].

**MATERIALS AND METHODS**

**Reagents and chemicals**

Authentic sample of Dextromethorphan hydrobromide was obtained from Medley Pharmaceuticals Limited, Mumbai. The formulation DMR – 20 labeled to contain Dextromethorphan hydrobromide 20 mg was procured from local market. Methanol (AR grade), Toluene (AR grade) were purchased from S. D. Fine Chemical Laboratories, Mumbai. Hydrochloric acid (HCl), hydrogen peroxide (H₂O₂) and sodium hydroxide (NaOH), Triethylamine were purchased from LOBA CHEMIE PVT. LTD., Mumbai.

**Chromatographic condition:**

Chromatographic separation of drug was performed on aluminum plates precoated with silica gel 60 F₂₅₄, (10 cm × 10 cm with 250 μm layer thickness). Sample was applied on the plate as a band of 4 mm width using Camag 100 μl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of Toluene: Methanol: Triethylamine (8.5:1:0.5 v/v/v). 10 cm × 10 cm CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 15 min saturation conditions and 10 ml of mobile phase was used per run, migration distance was 80 mm. Densitometric scanning was performed using Camag TLC scanner at 225 nm, operated by win CATS software, slit dimensions were 3.00 x 0.45 mm and Deuterium lamp was used as a radiation source.

**Selection of Detection Wavelength**

From the standard stock solution (1000 μg/ml) further dilutions were made using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 225 nm. Representative UV spectrum of Dextromethorphan hydrobromide is shown in Figure 1.

**Preparation of Standard stock solution**

Standard stock solution of drug was prepared by dissolving 10 mg of the drug in 10 ml of methanol to get concentration of 1000 μg/ml. From the standard stock solution, working standard solution was prepared containing 100 μg/ml of Dextromethorphan hydrobromide. 10 μl of the resultant solution was applied on TLC plate to get concentration of 1000 ng/band. Representative densitogram of Dextromethorphan hydrobromide (1000 ng/band) is shown in Figure 2.

**Preparation of sample solution**

A tablet containing 20 mg of Dextromethorphan hydrobromide (DMR-20) was weighed and powdered. Powder equivalent to 10 mg of drug was transferred to 10 ml volumetric flask and volume was made up with methanol to get concentration (1000 μg/ml) and was sonicated for 10 min. Solution was filtered, 4 μl of the resultant solution was applied on TLC plate to get concentration of 4000 ng/band.

![Figure 1: Representative UV spectrum of Dextromethorphan Hydrobromide (10 μg/ml)](image)

**Stress degradation studies of bulk drug**

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 acidic, alkaline, hydrolysis, and oxidation. Dry heat and photolytic degradation were carried out in the solid state. All studies are carried out at concentration level of 16,000 ng/band.

**Alkaline hydrolysis**

To 1 ml of stock solution of Dextromethorphan hydrobromide (10,000 μg/ml), 1 ml of 1 N NaOH was added. The above solution was kept for 4 hours at room temperature. The volume was made up to 10 ml with methanol. 16 μl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 96.01 % of Dextromethorphan hydrobromide was recovered with one peak of degradation. Representative densitogram is shown in Figure 3.

**Acid hydrolysis**

To 1 ml of stock solution of Dextromethorphan hydrobromide (10,000 μg/ml), 1 ml of 1 N HCl was added. The above solution was kept for 4 hour at room temperature. The volume was made up to 10 ml with methanol. 16 μl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 96.01 % of Dextromethorphan hydrobromide was recovered with one peak of degradation. Representative densitogram is shown in Figure 3.
made upto 10 ml with methanol. 16 µl of the resultant solution was then applied at TLC plate and densitogram was developed. 66.34% Dextromethorphan hydrobromide was recovered with no peak of degradant. Representative densitogram is shown in Figure 4.

Neutral Hydrolysis
To 1 ml of stock solution of Dextromethorphan hydrobromide (10,000 µg/ml), 1 ml of distilled water was added. The above solution was kept for 4 hours at room temperature. The volume was made upto 10 ml with methanol. 16 µl of the resultant solution was then applied at TLC plate and densitogram was developed. 94.51% of Dextromethorphan hydrobromide was recovered with no peak of degradant. Representative densitogram is shown in Figure 5.

Degradation under oxidative condition
To 1 ml of stock solution of Dextromethorphan hydrobromide (10,000 µg/ml), 1 ml of 6% H₂O₂ was added. The above solution was kept for 4 hour at room temperature. The volume was made upto 10 ml with methanol. 16 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 86.53% of Dextromethorphan hydrobromide was recovered with no peak of degradant. Representative densitogram is shown in Figure 6.

![Figure 2: Representative Densitogram of Dextromethorphan Hydrobromide (1000 ng/band)](image)

![Figure 3: Representative Densitogram of (A) base induced degradation of Dextromethorphan hydrobromide 16,000 ng/band showing degradant (B) Overlain spectra of Dextromethorphan hydrobromide and its alkali degradant](image)
Figure 4: Representative Densitogram of Dextromethorphan hydrobromide after acid degradation

Figure 5: Representative Densitogram of Dextromethorphan hydrobromide after Neutral degradation

Figure 6: Representative Densitogram of Dextromethorphan hydrobromide after Oxidation degradation

Figure 7: Representative Densitogram of Dextromethorphan hydrobromide after Thermal degradation
Degradation under dry heat
Dry heat studies were performed by keeping drug sample in oven (80°C) for a period of 4 hours. Sample was withdrawn, dissolved in methanol and diluted to get 1000 µg/ml. 16 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average of 91.91% dextromethorphan hydrobromide was recovered with no peak of degradant. Representative densitogram is shown in Figure 7.

Photo-degradation studies
The photo degradation study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/m². After exposure, sample was withdrawn, dissolved in methanol and diluted to get 1000 µg/ml. 16 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 47.45% of dextromethorphan hydrobromide was recovered with no peak of degradant. Representative densitogram is shown in Figure 8.

VALIDATION OF ANALYTICAL METHOD
Specificity
The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.998, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity
From the standard stock solution (1000 µg/ml) of Dextromethorphan hydrobromide, Six replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analyzing six concentrations over the concentration range of 2000-20,000 ng/band for Dextromethorphan hydrobromide. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve as shown in figure 8. The results found to be linear with regression equation of y=0.8592x+2599 with R² = 0.991.

Precision
The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies 3 replicates of 3 concentrations were analyzed on the same day and percentage RSD were calculated. For the inter day variation studies, 3 replicates of 3 concentrations were analyzed on 3 consecutive days and percentage RSD were calculated. For intraday precision and inter-day precision results obtained are shown in Table 1.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
From the linearity data the limit of detection and quantitation was calculated, using the formula LOD = 3.3 σ/ S and LOQ = 10 σ/ S where σ is standard deviation of the y intercept of linearity equation and S is slope of the calibration curve of the analyte. The LOD and LOQ were found to be 357.31ng/ band and 1082.76 ng/band, respectively.

Assay
DMR – 20 tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was applied and area was recorded. Basic concentration of sample chosen was 4000 ng/band from tablet solution. Concentration and % recovery was determined from linear equation. Assay results obtained are shown in Table 2.
Table 1: Intraday and interday variation studies data for Dextromethorphan hydrobromide

| Concentration (μg/ml) | Intra-day Precision | Inter-day Precision |
|-----------------------|---------------------|---------------------|
|                       | Average area | % Recovery | % RSD | Average area | % Recovery | % RSD |
| 4000                  | 6065        | 100.85     | 1.15   | 6051.2      | 100.06     | 0.45   |
|                       | 6087.2      | 101.50     |        | 6077.5      | 100.83     |        |
|                       | 6010.3      | 99.26      |        | 6050.2      | 100.03     |        |
| 8000                  | 9490.3      | 100.26     | 0.40   | 9440.8      | 99.309     | 0.40   |
|                       | 9486.7      | 100.21     |        | 9465.6      | 99.670     |        |
|                       | 9440.8      | 99.54      |        | 9411        | 98.876     |        |
| 12,000                | 13076.4     | 101.62     | 0.70   | 13009.9     | 100.80     | 0.71   |

Table 2: Assay of marketed formulation

| Drug                  | Peak Area | Amount Recovered (μg/ml) | % Recovery | ± %RSD |
|-----------------------|-----------|--------------------------|------------|--------|
| Dextromethorphan hydrobromide | 5988.7    | 3945.18                  | 98.62      | 1.40   |
|                       | 5956.2    | 3907.35                  | 97.68      |        |
|                       | 6050.5    | 4017.10                  | 100.42     |        |
|                       | 6038.3    | 4002.91                  | 100.07     |        |
|                       | 6051.5    | 4018.27                  | 100.45     |        |
|                       | 6089.5    | 4062.52                  | 101.56     |        |

Table 3: Accuracy Studies of Dextromethorphan hydrobromide

| Level | Amount of sample taken (ng/band) | Amount standard spiked (ng/band) | Area   | % Recovery | ±% RSD |
|-------|----------------------------------|----------------------------------|--------|------------|--------|
| 50%   | 4000                             | 2000                             | 7687.3 | 98.70      | 0.60   |
|       |                                  |                                  | 7635.5 | 97.69      |        |
|       |                                  |                                  | 7689.3 | 98.74      |        |
| 100%  | 4000                             | 4000                             | 9427.4 | 99.34      | 1.79   |
|       |                                  |                                  | 9277.6 | 97.16      |        |
|       |                                  |                                  | 9520.3 | 100.69     |        |
| 150%  | 4000                             | 6000                             | 11130.7| 99.29      | 0.92   |
|       |                                  |                                  | 11164.1| 99.68      |        |
|       |                                  |                                  | 11282.6| 101.06     |        |

Accuracy
To check accuracy of the method, recovery studies were carried by spiking the standard drug to the tablet solution, at three different levels 50, 100 and 150 %. Basic concentration of sample chosen was 4000 ng/band. % recovery was determined from linearity equation. The results obtained are shown in Table 3.

Robustness
Robustness of the method was determined by carrying out the analysis under conditions during which Detection wavelength, Time was changed from spotting to development and development to scanning and the effect on the area was noted. It was found that method is robust.

RESULT AND DISCUSSION
Method development: It was observed that the drug showed considerable absorbance at 225 nm. After few trials, Toluene:Methanol:Triethylamine (8.5:1:0.5 v/v/v) was chosen as mobile phase with saturation time 15 min, which gave good resolution and acceptable peak parameters. The densitometric analysis of drug was carried out at 225 nm.

The Rf value was found to be 0.60 ± 1.92. The results indicated the suitability of the method to study the stability of Dextromethorphan hydrobromide under various forced degradation conditions like acidic, basic, hydrolysis, oxidation, dry heat and photolysis. The method was found to be accurate, specific, Precise and robust. Summary of validation parameter and stability study was given in following table.

Table 4: Summary of validation parameters

| S. No. | Parameter           | Dextromethorphan HBr |
|--------|---------------------|-----------------------|
| 1      | Linearity           | Y = 0.8592x + 2599    |
| 2      | Range               | 2,000 – 20,000 ng / band |
| 3      | Precision           | %RSD                  |
| 4      | Assay               | 99.8 %                |
| 5      | Accuracy            | % Recovery            |
| 6      | LOD                 | 357.31 ng / band      |
| 7      | LOQ                 | 1082.76 ng / band     |
| 8      | Specificity         | Specific              |
| 9      | Robustness          | Robust                |
Table 4: Summary of forced degradation study

| Stress condition/Duration | % Assay of active substance | Rf values of degraded products |
|---------------------------|-----------------------------|-------------------------------|
| Acidic/ 1 N HCl/ at room temperature 4 hours | 66.34% | - |
| Alkaline/ 1 N NaOH / at room temperature 4 hours | 96.01% | 0.23 |
| Oxidative/ 6 % H₂O₂ / at room temperature 4 hours | 86.53% | - |
| Neutral / H₂O / at room temperature 4 hrs | 94.51% | - |
| Dry heat / 80ºC/ 4 hours | 91.91% | - |
| UV/200 watt hours/square meter / for 24 hours | 47.45% | - |

CONCLUSION
A simple, precise, accurate, reproducible and stability-indicating HPTLC method without interference from the excipients or from degradation products has been developed and validated for the determination of Dextromethorphan hydrobromide as bulk drug and in tablet dosage form. The developed method can be used for quantitative analysis of Dextromethorphan hydrobromide in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

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Nil

CONFLICT OF INTEREST
The authors declare no conflict of interest

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