International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 9, Issue 4, 2017

Original Article

# STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DOSULEPIN HYDROCHLORIDE AND METHYLCOBALAMIN IN TABLET DOSAGE FORM

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*Received: 17 Apr 2017, Revised and Accepted: 14 Jun 2017*

**ABSTRACT**

**Objective:** To develop an accurate, simple, rapid, precise, economic and stability indicating RP-HPLC method for the simultaneous estimation of dosulepin hydrochloride and methylcobalamin in tablet dosage form and validate as per ICH guidelines.

**Methods:** The column used was Kromasil $C_{18}$ (250 X 4.6 mm, 5 µm), with a mobile phase containing acetonitrile: phosphate buffer pH 3 adjusted with o-phosphoric acid (60:40) with a flow rate of 1 ml/min. The effluents obtained were monitored at 285 nm with photodiode array detector. Dosulepin hydrochloride and methylcobalamin were subjected to stress degradation conditions like hydrolysis (acid and base), oxidation, thermal and photolysis degradation. The samples subjected to stress degradation were analysed by the developed method.

**Results:** The retention time for dosulepin hydrochloride and methylcobalamin was found to be 7.99 min and 1.97 min, respectively. The linearity of developed method was achieved in the range of 165-495 µg/ml for dosulepin hydrochloride and 5-15 µg/ml for methylcobalamin. The detection (LOD) and quantitation (LOQ) limits were found to be 0.75 µg/ml and 2.28 µg/ml for dosulepin hydrochloride and 0.040 µg/ml and 0.121 µg/ml for methylcobalamin respectively. In the stability studies, it was observed that there is no interference of the degradation products with drug samples.

**Conclusion:** An accurate simple, rapid, precise, linear and stability indicating RP-HPLC method was developed for simultaneous quantitative estimation of dosulepin hydrochloride and methylcobalamin both in bulk and pharmaceutical formulation. The method was validated as per ICH guidelines. This method holds good for the routine analysis of dosulepin hydrochloride and methylcobalamin in various pharmaceutical industries as well as in academics.

**Keywords:** Stability, RP-HPLC, Validation, Dosulepin hydrochloride, Methylcobalamin


DOI: http://dx.doi.org/10.22159/ijap.2017v9i4.19204

## INTRODUCTION

Dosulepin hydrochloride (fig. 1) chemically is 1-Propanamine, 3-dibenzo [b, e] thiepin-11(6H)-ylidene-N,N-dimethyl-, hydrochloride. Dosulepin belongs to the category of drugs referred to as tricyclic antidepressants and having anxiolytic properties. Dosulepin works by preventing serotonin and noradrenaline from being reabsorbed back into the nerve cells in the brain. This helps prolong the mood lightening effect of any released noradrenaline and serotonin. In this way, dosulepin helps relieve depression [1].

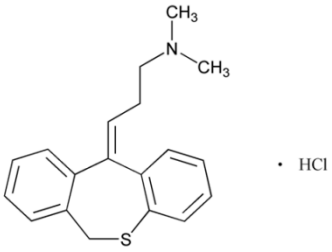



**Fig. 1: Chemical structure of dosulepin hydrochloride [2]**

Methylcobalamin chemically is alpha-(5,6-dimethylbenzimidazolyl) methylcobalamin (fig. 2). It is a form of vitamin $B_{12}$(cyanocobalamin) and differs from that the cyanide is replaced by a methyl group. Vitamin $B_{12}$ is an essential material for the growth, cell reproduction, hematopoiesis and synthesis of genetic material like nucleoprotein and myelin. Cobalamin is generally used in the treatment of peripheral neuropathy, diabetic neuropathy and as a preliminary treatment for amyotrophic lateral sclerosis. Cobalamin is necessary for DNA synthesis, the formation of red blood cells and maintenance of the nervous system, growth and development of children [3-5].

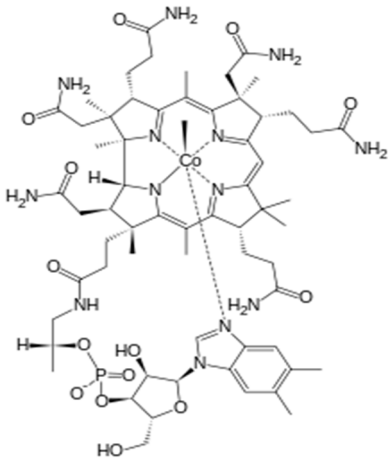



**Fig. 2: Chemical structure of methylcobalamin [6]**

Combination therapy of dosulepin hydrochloride and methylcobalamin is used for the treatment of neuropathic pain.

In literature, various spectrophotometric methods and RP-HPLC methods have been reported for quantitation of dosulepin hydrochloride and methylcobalamin in individual and combined available market formulation [1, 7-16]. But only one assay has been reported for the stability of the same [17]. The present study focuses on developing a new stability assay method for dosulepin hydrochloride and methylcobalamin in combination. This method will provide new insight into the stability studies of this combination. Moreover, this novel validated method (as per ICH guidelines [18-19]) has applicability in industry as well as academia.

## MATERIALS AND METHODS

Pharmaceutical grade dosulepin hydrochloride was procured from Elite Pharmaceutical Private Limited, Ahmedabad and methylcobalamin was obtained from Aum Laboratories, Ahmedabad. The marketed formulation Prothiaden M containing dosulepin hydrochloride 50 mg and methylcobalamin 1500 mcg was purchased from the local market. Methanol, orthophosphoric acid, acetonitrile and HPLC grade water were obtained from Merck. All solvents used were HPLC grade. RP-HPLC Shimadzu (LC 20ATVP) model with Spin chrome (LC Solutions) software was employed in this method. Analytical column used for the separation of analytes was Kromasil $C_{18}$ (250 X 4.6 mm, 5 µm).

### Methods

### Selection of wavelength

Standard solutions of dosulepin hydrochloride and methylcobalamin were prepared at the concentration of 10 µg/ml and scanned using UV-Visible spectrophotometer at the range of 200-400 nm. The isosbestic point selected for simultaneous estimation was 285 nm based on combined UV spectrums of dosulepin hydrochloride and methylcobalamin (fig. 3).

### Chromatographic conditions

The developed method used a reverse phase, Kromasil $C_{18}$ column (250 X 4.6 mm, 5 µm). The mobile phase used was acetonitrile: phosphate buffer pH 3 adjusted with o-phosphoric acid (60:40) at a flow rate of 1.0 ml/min and a detection wavelength of 285 nm using a photodiode array detector.

### Preparation of phosphate buffer

Accurately weighed 2.7 g of potassium dihydrogen phosphate ($KH_2PO_4$) and 6 ml of triethylamine were dissolved in 800 ml of water; the pH was adjusted to 3.0 with o-phosphoric acid and final volume adjusted to 1000 ml with water. The buffer was filtered to remove all fine undissolved particles.

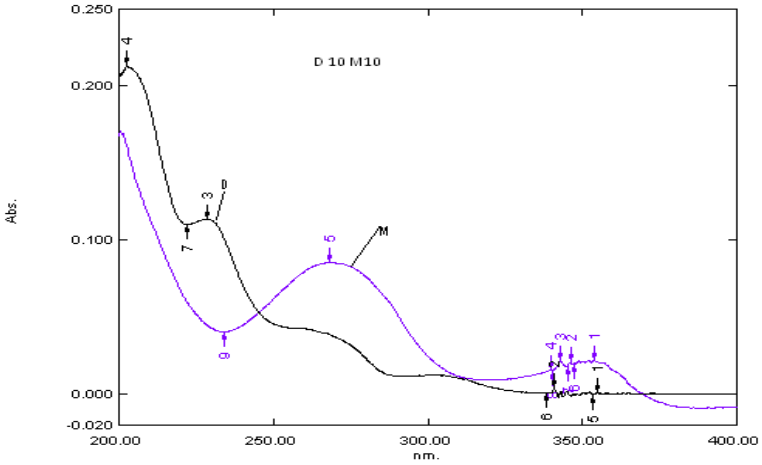



**Fig. 3: UV overlap spectrum of dosulepin hydrochloride and methylcobalamin**

### Preparation of mobile phase

A mixture of 60 volumes of acetonitrile and 40 volumes of phosphate buffer was prepared. The mobile phase was sonicated for 10 min to remove gases.

### Preparation of standard solutions

A standard stock solution of dosulepin hydrochloride was prepared by dissolving 330 mg of dosulepin hydrochloride in 10 ml of mobile phase. After that, the solution was filtered and sonicated for 5 min and volume adjusted to 100 ml with mobile phase giving a final concentration of 3300 µg/ml. Similarly, a standard stock solution of methylcobalamin was prepared by dissolving 10 mg of methylcobalamin in 10 ml of mobile phase and the solution was filtered, sonicated for 5 min. The final concentration of 100 µg/ml was achieved by adjusting the volume to 100 ml.

### Sample preparation

Twenty tablets were weighed and finely powdered. The powder equivalent to 330 mg dosulepin hydrochloride and 10 mg methylcobalamin was accurately weighed. This powder was transferred to the volumetric flask of 100 ml capacity and dissolved in 50 ml of mobile phase. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with the mobile phase. The above solution was filtered through Whatman filter paper (0.45µ). This solution is expected to contain 3300 µg/ml of dosulepin hydrochloride and 100 µg/ml methylcobalamin. The same solution was used for the estimation of dosulepin hydrochloride and methylcobalamin.

### Stress degradation studies

Forced degradation studies were performed to know the degradation products and to establish degradation pathway for dosulepin hydrochloride and methylcobalamin. The study involves acid hydrolysis wherein sample solution was treated with 0.1 M HCl at room temperature for 24 h. The sample solution was treated with 0.1 M NaOH at room temperature for 24 h to study alkali hydrolysis. Oxidative degradation studies involved 3% $H_2O_2$ treatment of sample solution at room temperature for 24 h. The samples were placed in hot air oven at 105 °C for 6 h to study thermal degradation. For photolytic stress studies, samples were exposed to sunlight for 7 d [19].

## RESULTS AND DISCUSSION

### Method development

Different chromatographic conditions were tried for better separation and resolution. Kromasil $C_{18}$ (250 X 4.6 mm, 5 µm) column was found satisfactory. Peak purity of dosulepin hydrochloride and methylcobalamin was checked using photodiode array detector and wavelength of 285 nm was considered satisfactory for detecting both the drugs with adequate sensitivity. A variety of solvents in different ratios over a wide range of pH were tried, but either peak shape was broad or resolution was not good. Repeated trials were performed in order to obtain good, sharp peak with an efficient resolution between two peaks of dosulepin hydrochloride and methylcobalamin. This was achieved by performing isocratic HPLC on a $C_{18}$ column. The run time was 12 min in an isocratic trial with the use of mobile phase consisting of acetonitrile: phosphate buffer pH 3.0 (60:40) on a Kromasil $C_{18}$ (250×4.6 mm, 5 µm) column with flow rate 1.0 ml/min and detection wavelength 285 nm. This developed method gave satisfactory results in terms of retention time, resolution, symmetry and sensitivity. A typical RP-HPLC chromatogram for simultaneous determination of dosulepin hydrochloride and methylcobalamin from the standard can be seen in fig. 4.

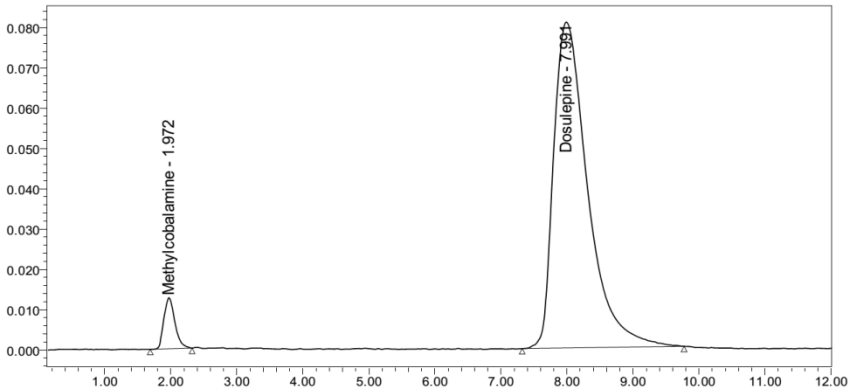



**Fig. 4: Typical chromatogram of standard solution**

### Method validation

The developed RP-HPLC method was validated for parameters like system suitability, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness according to ICH guidelines [18-19].

### System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and tailing factor were evaluated. The system suitability parameters were tabulated in table 1. All the parameters were found to be within the limits [20-21].

### Linearity

The linearity of the method was determined by assaying dosulepin hydrochloride and methylcobalamin standard stock solution at five different concentrations. Concentrations of dosulepin hydrochloride taken were 165, 247.5, 330, 412.5 and 495 µg/ml and for methylcobalamin 5, 7.5, 10, 12.5 and 15 µg/ml. The calibration curves were plotted between the responses of peak area versus concentration of the analyte. Least-squares linear regression analysis was performed for the calibration curves (fig. 5 and 6). The results have shown an excellent correlation between peak areas and concentration within the concentration range of 165–495 µg/ml for dosulepin hydrochloride, 5–15 µg/ml for methylcobalamin (table 2). The correlation coefficients were found to be 0.999 for dosulepin hydrochloride and 0.994 for methylcobalamin, which meet the method validation acceptance criteria [20-21] and hence the method was said to be linear for both the drugs.

### Precision

The precision of the method was verified by two various methods-repeatability and reproducibility (intraday and interday precision)

**Table 1: Results of system suitability studies**

| Parameters | Acceptance limits | Dosulepin hydrochloride | Methylcobalamin |
|---|---|---|---|
| Retention time | - | 7.991 | 1.972 |
| Theoretical plates | NLT 2000 | 2770 | 2344 |
| Tailing factor (T) | NMT 2 | 1.736 | 1.298 |
| Resolution | NLT 2 | 9.55 | 9.55 |

#NLT: Not less than. # NMT: Not more than

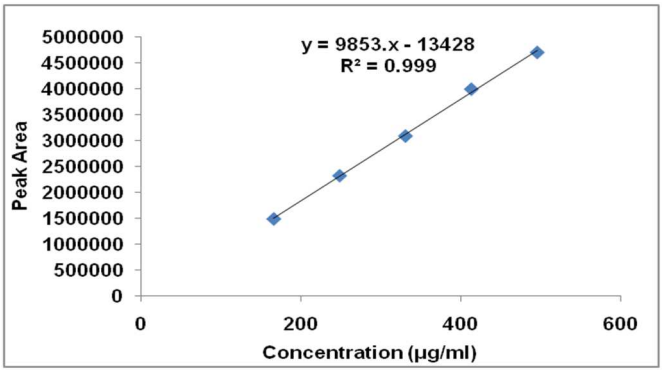



**Fig. 5: Linearity chart for dosulepin hydrochloride**

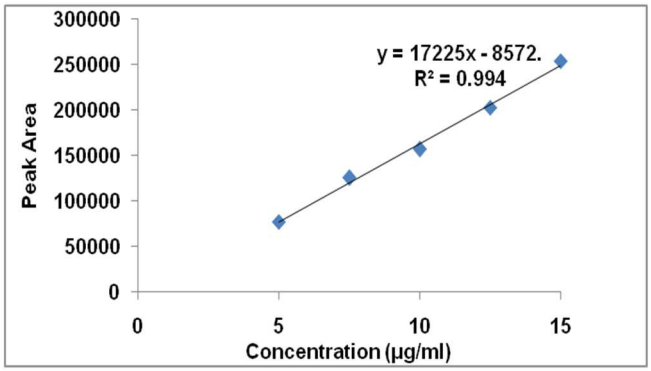



**Fig. 6: Linearity chart for methylcobalamin**

**Table 2: Linearity data for dosulepin hydrochloride and methylcobalamin**

| Parameters | Dosulepin hydrochloride | Methylcobalamin |
|---|---|---|
| Linearity range (µg/ml) | 165-495 | 5-15 |
| Regression equation | y = 9853. x-13428 | y = 17225x-8572 |
| Correlation coefficient | 0.999 | 0.994 |
| Average %RSD (Peak area, n=5) (Linearity) | 0.16 | 0.39 |

# n: Number of injections for liniarity= 5, # %RSD: Relative standard deviation

**Repeatability**

The sample solution was prepared at working concentration of 330 µg/ml for dosulepin hydrochloride and 10 µg/ml for methylcobalamin as described earlier. Repeatability analysis was carried out by injecting the sample 6 times for the same concentration of both the drugs. The results of repeatability study are provided in table 3.

The method was found to be precise as the % RSD values calculated were within the limits for repeatability [20-21].

**Reproducibility (intraday and interday precision)**

Intraday and interday precision were carried out using three replicates of different concentrations (165, 330 and 495 µg/ml for dosulepin hydrochloride; 5, 10 and 15 µg/ml for methylcobalamin). The results of intraday and interday precision are tabulated in table 4.

The method was found to be precise as the % RSD values calculated were within the limits for reproducibility [20-21].

**Table 3: Repeatability data for dosulepin hydrochloride and methylcobalamin**

| Concentration of dosulepin hydrochloride | Peak area of dosulepin hydrochloride (n=6) | Concentration of methylcobalamin | Peak area of methylcobalamin (n=6) |
|---|---|---|---|
| 330 µg/ml | 2883398 | 10 µg/ml | 248458 |
| | 2844178 | | 245485 |
| | 2861434 | | 244339 |
| | 2888642 | | 243111 |
| | 2900001 | | 247727 |
| | 2833170 | | 248069 |
| Mean | 2868471 | Mean | 246198 |
| SD | 26493.98 | SD | 2210.877 |
| %RSD | 0.92 | %RSD | 0.89 |

# n: Number of injections= 6, # SD: Standard deviation, # %RSD: Relative standard deviation

**Table 4: Intraday and interday precision data for dosulepin hydrochloride and methylcobalamin**

| Drug | Concentration (µg/ml) | Intra-day precision | | Intra-day precision | |
|---|---|---|---|---|---|
| | | mean±SD (n=3) | % RSD | mean±SD (n=3) | % RSD |
| Dosulepin hydrochloride | 165 | 1475903±3817 | 0.25 | 1499656±20976 | 1.39 |
| | 330 | 3075762±14929 | 0.48 | 3125024±42235 | 1.35 |
| | 495 | 4714408±19769 | 0.41 | 4731060±57485 | 1.21 |
| Methylcobalamin | 5 | 77230±645 | 0.84 | 77273±1183 | 1.53 |
| | 10 | 157826±978 | 0.62 | 156792±2612 | 1.66 |
| | 15 | 253929±1337 | 0.52 | 254524±4647 | 1.82 |

# n: Number of injections= 3, # SD: Standard deviation, # %RSD: Relative standard deviation

**Accuracy**

The accuracy of the method was determined by recovery studies. The percent mean recovery of both the drugs at three different levels (50 %, 100 % and 150%) was determined. The recovery studies were performed in triplicates for each level. The percentage recovery and mean percentage recovery calculated for the drug is shown in table 5. The observed data were within the required range [20-21], which indicates good recovery values, affirming the accuracy of the method developed.

**Table 5: Results of accuracy**

| Drug | Level (%) (n=3) | Amount taken (µg/ml) | Amount recovered (mean±SD) | % Recovery |
|---|---|---|---|---|
| Dosulepin | 50 | 165 | 164.64±1.196 | 99.78 |
| hydrochloride | 100 | 330 | 327.56±1.659 | 99.26 |
| | 150 | 495 | 495.03±3.673 | 99.98 |
| Methylcobalamin | 50 | 5 | 4.995±0.056 | 99.96 |
| | 100 | 10 | 9.92±0.027 | 99.21 |
| | 150 | 15 | 15.081±0.149 | 100.56 |

#n: Number of injections= 3, #SD: Standard deviation

**Robustness**

To determine the robustness of the developed method, experimental conditions were deliberately altered, and the system suitability parameter retention time and peak area were evaluated. The solutions prepared as per the test method described earlier and injected at different variable conditions like flow rate (0.8, 1, 1.2 ml/min.), mobile phase ratio of acetonitrile and phosphate buffer (59:41, 60:40, 61:39) and pH (2.8, 3, 3.2). The assessments of system suitability parameter like retention time and peak area were compared with that of method precision. The method was found to be precise as the % RSD values calculated were within the limits for robustness [20-21] indicating method was robust. Robustness data are given in table 6.

**Limit of detection (LOD) and Limit of quantitation (LOQ)**

LOD and LOQ were determined using the formulas, LOD = 3.3×SD/S and LOQ = 10×SD/S, where, 'SD' is the standard deviation of the response and 'S' is the slope of the calibration curve. The LOD and LOQ values were found to be 0.75 µg/ml and 2.28/µg/ml for dosulepin hydrochloride and 0.040 µg/ml and 0.121 µg/ml for methylcobalamin.

**Table 6: Robustness study for dosulepin hydrochloride and methylcobalamin**

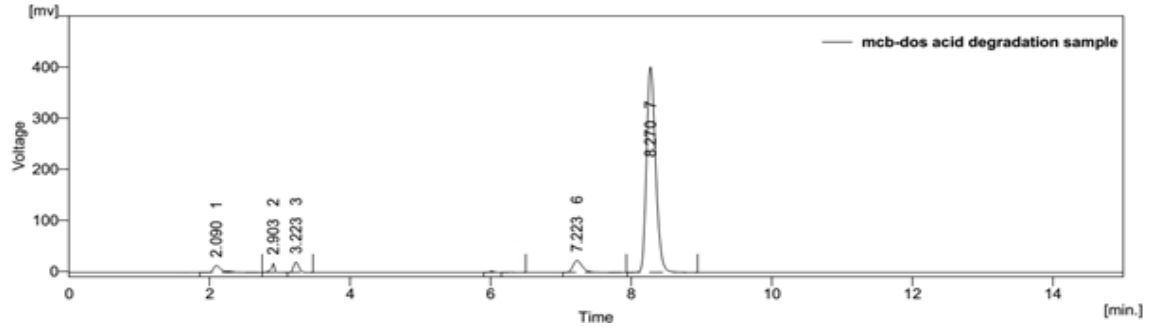
| Parameter | Method condition | Dosulepin hydrochloride | | Methylcobalamin | |
|---|---|---|---|---|---|
| | | Retention time | Peak area | Retention time | Peak area |
| Flow rate (ml/min) | 0.8 | 8.002 | 3030254 | 1.993 | 154856 |
| | 1.0 | 7.991 | 3088441 | 1.972 | 157257 |
| | 1.2 | 7.921 | 3091163 | 1.956 | 159635 |
| | %RSD | 0.55 | 1.12 | 0.9 | 1.51 |
| Mobile phase ratio-acetonitrile: phosphate buffer | 59:41 | 7.958 | 3046672 | 1.966 | 153563 |
| | 60:40 | 7.991 | 3088441 | 1.972 | 157257 |
| | 61:39 | 8.006 | 3093753 | 1.994 | 155209 |
| | %RSD | 0.31 | 0.83 | 0.74 | 1.19 |
| pH | 2.8 | 7.969 | 3092528 | 1.963 | 156843 |
| | 3.0 | 7.991 | 3088441 | 1.972 | 157257 |
| | 3.2 | 7.948 | 3074510 | 1.983 | 154621 |
| | %RSD | 0.26 | 0.30 | 0.51 | 0.91 |

#% RSD: Relative standard deviation

**Forced degradation studies**

Forced degradation studies were performed to demonstrate the stability of the sample in different stressed conditions. The conditions used were acid and base hydrolysis, dry heat, oxidation and sunlight photolysis. The acid hydrolysis showed little degradation of dosulepin hydrochloride and methylcobalamin with degraded products peaks at retention time 7.223 and 2.903. For alkali degradation, degraded product peak was observed at retention time 6.957 and 3.543. Degradation studies under oxidative conditions gave degraded product peaks at retention time 6.943 and 3.867. For the thermal degradation, peaks were observed at retention time 7.497 and 2.790 for degraded products. In photohydrolysis, degraded product peaks were observed at retention time 9.447 and 2.737.

The data for forced degradation are tabulated in table 7. There was no interference of any peak at the retention time of analytes from the blank. RP-HPLC chromatograms for degradation of dosulepin hydrochloride and methylcobalamin can be seen in fig. 7.

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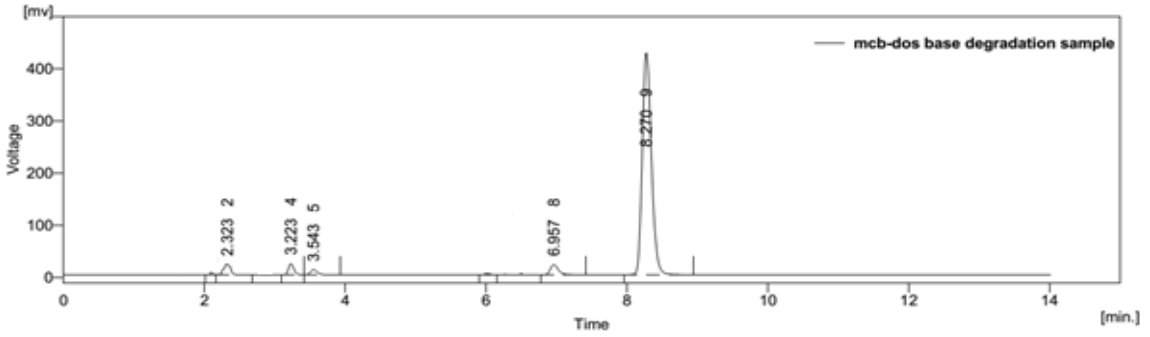


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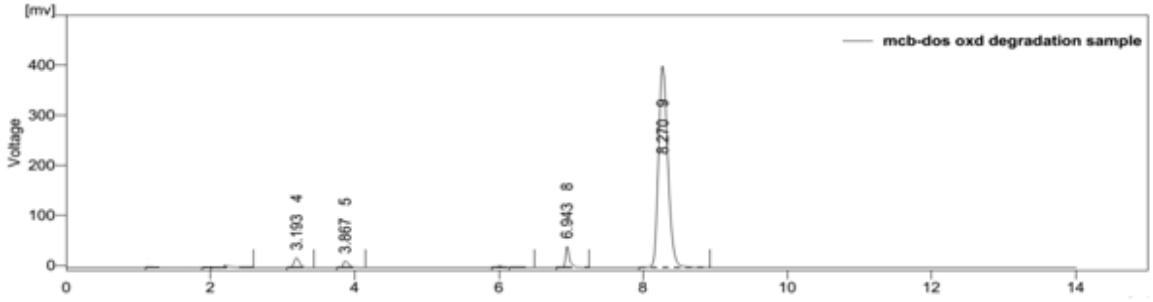


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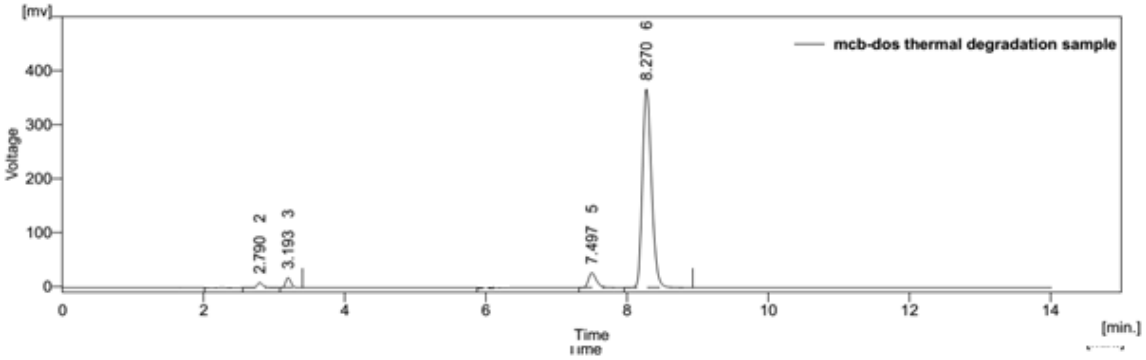


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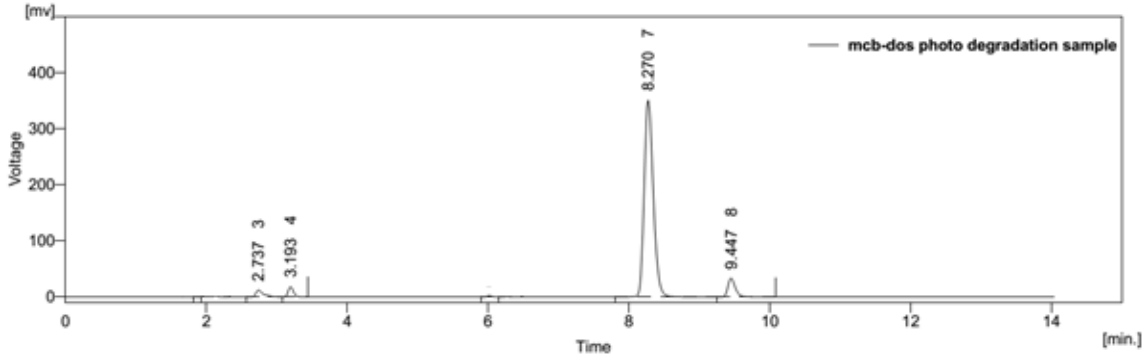


**(E)**

**Fig. 7: Representative chromatograms of acid (A), base (B), oxidative (C), thermal (D), and photolytic (E) degradation of dosulepin hydrochloride and methylcobalamin**

**Table 7: Forced degradation data**

| S. No. | Condition applied | % degradation | |
|---|---|---|---|
| | | Dosulepin hydrochloride | Methylcobalamin |
| 1 | 0.1 M HCl | 26.39% | 22.71% |
| 2 | 0.1 M NaOH | 22.59% | 22.51% |
| 3 | 3 % H2O2 | 28.56% | 34.30% |
| 4 | Heat treatment | 32.48% | 29.72% |
| 5 | Photolysis | 36.40% | 38.20% |

In previous report [17] dosulepin hydrochloride and methylcobalamin were assayed using Inertsil-ODS (250x4.6 mm, 5 µm) column with mobile phase containing 0.02 % orthophosphoric acid and methanol (400:600 v/v), whereas this study uses a novel column, Kromasil $C_{18}$ (250×4.6 mm, 5 µm) column with novel mobile phase consisting of acetonitrile: phosphate buffer pH 3.0 (60:40) wherein retention time of 1.972 for methylcobalamin and 7.991 for dosulepin hydrochloride with better resolution is found. Moreover, various reports of assaying these compounds alone or in other combinations exist but the method developed in this report is first of its kind.

## CONCLUSION

The proposed study, a new stability-indicating RP-HPLC method has been developed for estimation of dosulepin hydrochloride and methylcobalamin in bulk and pharmaceutical dosage form. The developed method was validated and it was found to be simple, sensitive, precise, and robust and it can be used for the routine analysis of dosulepin hydrochloride and methylcobalamin in both bulk and pharmaceutical dosage forms. The forced degradation studies were carried out in accordance with ICH guidelines and the results revealed suitability of the method to study the stability of dosulepin hydrochloride and methylcobalamin under various degradation conditions like acid, base, oxidative, thermal, and photolytic degradations. Finally, it was concluded that the method is simple, sensitive and has the ability to separate the drug from degradation products and excipients found in the dosage form.

## ACKNOWLEDGEMENT

The authors wish to thank Elite Pharma, Ahmedabad and Aum Laboratory, Ahmedabad for providing gift samples.

## CONFLICT OF INTERESTS

Authors have no conflict of interest

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**How to cite this article**

- Radhika Shah, Ragin Shah. Stability indicating RP-HPLC method for simultaneous estimation of dosulepin hydrochloride and methylcobalamin in tablet dosage form. Int J Appl Pharm 2017;9(4):69-75.