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# Simultaneous Estimation of Dosulepin and Methylcobalamin in Bulk and Pharmaceutical Formulation by Reverse Phase High Performance Liquid Chromatography (RP-HPLC)

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***Abstract:*** *A simple, specific and cost effective RP-HPLC method was developed for simultaneous estimation of Dosulepin and Methylcobalamin in bulk. In addition the developed method was applied to the suitable combined tablet dosage form ie., Prothiaden. As there is no UV or HPLC method for the simultaneous estimation of Dosulepin and Methylcobalamin, a need was felt to develop the method for the analysis of both drugs simultaneously. Chromatography was carried on Zorbax C8 column (150x4.5, 5) with mobile phase comprising of Ortho Phosphoric Acid buffer and Methanol in the ratio of 50:50 v/v. The flow rate was adjusted to 0.8 ml/min with PDA detection at 219.8 nm. The retention times of Dosulepin and Methylcobalamin were found to be 3.8 min, 1.9 min respectively and other replicate standard system suitability parameters are within the limit and uniform. The different analytical parameters such as accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ) were determined according to the International Conference on Harmonization (ICH) Q2B guidelines. The detector response was linear in the range of 0.9-2.7 mg/ml., 0.018-0.054 mg/ml for Dosulepin and Methylcobalamin respectively. The proposed method was successfully applied for the reliable quantification of active pharmaceuticals present in the commercial formulations.*

***Keywords:*** *Dosulepin, Methylcobalamin, HPLC, Methanol, Ortho Phosphoric Acid.*

## I. Introduction

Dosulepin (Fig. 1) is chemically 3-(6H-dibenzo[b,e]thiepin-11-ylidene)propyldimethylamine hydro-chloride[1,2]. DOS is a Tricyclic antidepressant (TCA), which inhibit the active reuptake of biogenic amines NA and 5-HT in to their respective neurons [3]. Methylcobalamin (Fig. 2) is a dark red crystalline powder and it has been referred for neurological illness, diabetic neuropathy, hearing loss and Alzheimer's disease. Methylcobalamin is chemically Coα-[α- (5, 6-dimethylbenz-1H-imidazolyl)]- Coβmethylcobamide [1-5]. Literature survey revealed there is no published chromatographic method for this combination of drug.

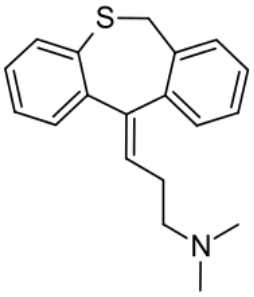

Fig. 1

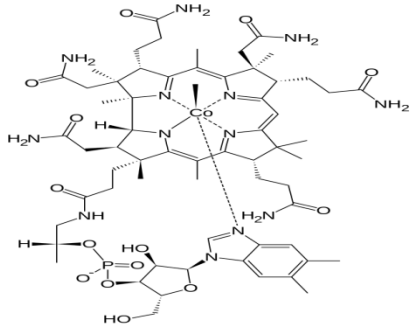

Fig.2

The present paper describes a simple, accurate and precise method for reverse phase liquid chromatographic estimation of DOS and MCA in combined tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [6,7]. In the present work, a successful attempt has been made to estimate both these drugs simultaneously by RP-HPLC method [8,9,10].

## II. Materials And Methods:

### 2.1. Materials:

#### 2.1.1. Chemicals and Reagents:

Working standards of pharmaceutical grade Dosulepin and Methylcobalamin were obtained as generous gifts from Dr.Reddy's laboratories (Hyderabad, AP, India) used as such without further purification. The pharmaceutical dosage form used in the study was Prothiaden.

Methanol (HPLC grade), Orthophosphoric acid (AR grade) purchased from Merck specialities Pvt.ltd (Mumbai, India) and double distilled water used for analysis.

#### 2.1.2. Instrumentation and chromatographic condition:

Chromatography was carried out on Zorbax C8 column (150x4.5, 5) with mobile phase comprising of orthophosphoric acid buffer and Methanol in the ratio of 50:50 v/v. The flow rate was adjusted to 0.8 ml / min with PDA detection at 219.8 nm.

#### 2.1.3. Preparation of standard solution:

Standard stock solutions of pure drugs were prepared separately by dissolving 18 mg of Dosulepin in 10 ml water and 3.6 mg of Methylcobalamin in 100 ml water to get concentrations 1.8 mg/ml and 0.036 mg /ml respectively.

#### 2.1.4. Preparation of sample solution:

20 tablets were weighed accurately, powdered and equivalent weight was calculated. The equivalent weight of twelve tablets were taken and dissolved in 30 ml of water, sonicated for 25 minutes, made up to 50 ml volume and filtered to get the concentration 1.8 mg/ml of Dosulepin and 0.036 mg /ml of Methylcobalamin.

### 2.2. Validation:

The developed method was validated with different analytical parameters such as accuracy, precision, linearity, limit of detection, limit of quantification and robustness according to the international conference on harmonization (ICH) Q2B guidelines.

#### 2.2.1. Precision:

Precision of these methods was checked by analyzing the samples at three different time intervals of the same day (intraday precision (table-2)) as well as on different days (interday precision). Robustness for HPLC method was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 0.8 mL/min to 0.6 mL/min and 1.0 mL/min while ratio of the mobile phase was changed by ± 1%.

Table-2: Validation parameters:

| Validation Parameter | Dosulepin | Methylcobalamin |
|---|---|---|
| ystem precision | 0.1% | 0.1% |
| Tailing factor | 1.1 | 1.4 |
| Theoretical plate count | 4670 | 3130 |
| Linearity | 900-2700 µg/ml, | 18-54 µg/ml |
| Regression equation | y=21048x+11130 | y = 22600x + 1828 |
| Regression coefficient | 1 | 1 |
| Detection limit (µg/mL) | 5.86µg/ml | 1.69 µg/ml |
| Quantitation limit (µg/mL) | 19.55µg/ml | 5.63 µg/ml |
| Accuracy (% recovery) | 101% | 97% |
| Precision | | |
| Intra-day Precision | | |
| Assay value | 100% | 100% |
| %RSD | 0.97 | 0.57 |
| Inter-day Precision | | |
| Assay value | 100% | 99% |
| %RSD | 0.1 | 0.2 |

#### 2.2.2. Recovery studies:

To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 50, 100, 150% levels (table 2 and 3). From the total amount of drug found percentage recovery was calculated.

Table-2 : Recovery studies of Dosulepin

| %Concentration | Area | Amount Added(µg) | Amount Found(µg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 10588540 | 900 | 904 | 101% | |
| 100% | 21044263 | 1800 | 1815.5 | 101 % | 101% |
| 150% | 31548507 | 2700 | 2695.4 | 100% | |

**Table-3:** Recovery studies of Methylcobalamin

| %Concentration | Area | Amount Added(µg) | Amount Found(µg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 1124612 | 18 | 17.98 | 100% | |
| 100% | 2255945 | 36 | 34.98 | 97 % | 100% |
| 150% | 3372120 | 54 | 54.22 | 100% | |

### 2.2.3. Linearity:

**LOD and LOQ:**

Limit of Detection (LOD) and Limit of quantification (LOQ) were calculated by using the values of slopes and intercepts of the calibration curves for both the drugs. LOD and LOQ values for Dosulepin were found to be 5.86 µg/ml and 19.55 µg/ml and for Paracetamol 1.69 µg/ml and 5.63 µg/ml respectively.

### 2.2.4. Robustness:

Method robustness was determined by the small changes in chromatographic conditions like as 0.2ml flow rate and ±5°c temperature and inject the sample observe the result there were no marked changes compare to other analysis. Results of the Robustness were shown in table-3.

Table-4: Robustness study

| Parameters | Changes | Retention Time |
|---|---|---|
| Dosulepin | | |
| Flow rate (ml/min) | 0.6 | 4.36 |
| | 1.0 | 3.42 |
| Temperature | 45°C | 4.87 |
| | 55 °C | 5.31 |
| Methylcobalamin | | |
| Flow rate (ml/min) | 0.6 | 2.20 |
| | 1.0 | 1.72 |
| Temperature | 45°C | 2.35 |
| | 55°C | 2.65 |

## III. Results And Discussion:

Retention times of Dosulepin and Methylcobalamin were found to be 3.8 and 1.9 respectively (as shown in Fig. 3). The detector response was linear in the range of 900-2700 µg/ml, 18-54 µg/ml for Dosulepin and Methylcobalamin respectively. In the linearity study the regression equation and coefficient of correlation for Dosulepin and Methylcobalamin were found to be ($y = 21048x + 11130$, $R^2 = 1$), ($y = 22600x + 1828$ $R^2 = 1$) respectively. Linearity of Dosulepin and Methylcobalamin were shown in Fig. 4 and Fig. 5 respectively. Commercial formulations containing Dosulepin and Methylcobalamin were analyzed by the proposed method. A typical chromatogram of marketed formulation is shown in fig. no.3. Six replicate analysis of formulation were carried out and the mean assay values were found close to 100 %. The tailing factors were <2.0 for both the peaks. The elution order was Dosulepin (RT = 3.8 min) and Methylcobalamin (RT = 1.9 min), at a flow rate of 0.8 mL/min. The chromatogram was recorded at 219.8 nm. System suitability was established by injecting standard solution and results are shown in table no.1.The accuracy of the proposed method was determined by recovery studies. It was confirmed from results that the method is highly accurate (table no.2 and 3). Precision (table no.1) was calculated as interday and intraday variations for both the drugs. Percent relative standard deviations for intraday and interday precision for Dosulepin were 0.97 % and 0.1 % and that for Methylcobalamin were 0.57 % and 0.2 % respectively which are well within the acceptable limit of 2 %. For robustness studies in all deliberately varied conditions, the RSD of contents of Dosulepin and Methylcobalamin were found to be well within the acceptable limit of 2%.

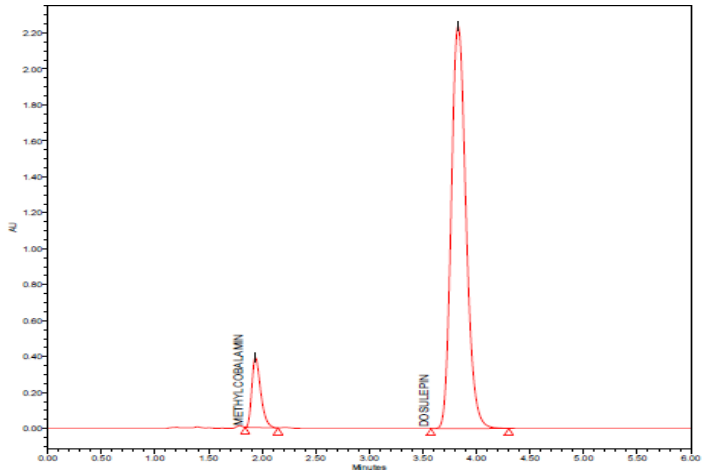


Fig. 3

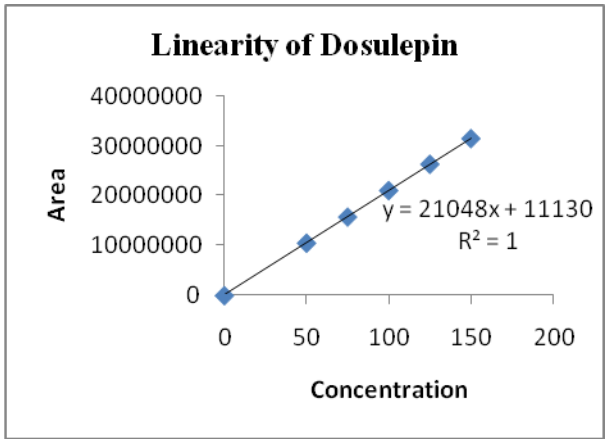


Fig. 4

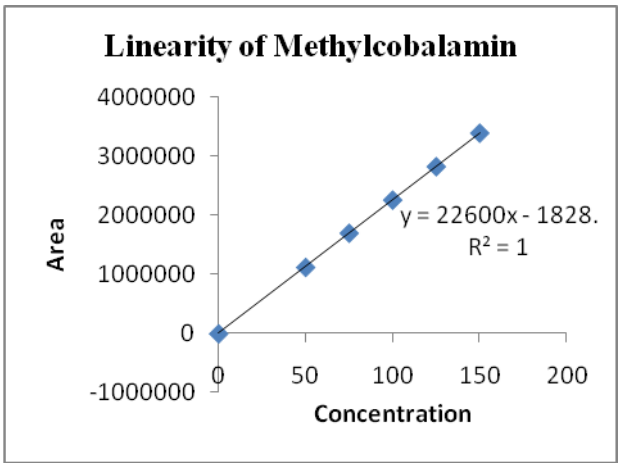


Fig. 5

## IV. Conclusion:

The new HPLC method developed and validated for simultaneous estimation of Dosulepin and Methylcobalamin pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, precise, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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