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# DEVELOPMENT AND VALIDATION FOR ESTIMATION OF METHOCARBAMOL AND MELOXICAM IN PHARMACEUTICAL DOSAGE FORM BY UV METHOD

**Nehal R. Vadaviya, Mr.DarshilShah*and Dr. Dilip G. Maheswari**
L. J. Institute of Pharmacy, Between Sarkhej Circle &Katariya Motors, S.G. Road, Ahmedabad, Gujarat-382210, India.
Email: darshilshah89@yahoo.com

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**Abstract**

A simple, accurate, rapid and reproducible UV-spectroscopy method have been developed and validated for the estimation of Methocarbamol and Meloxicam in Pharmaceutical dosage form. The absorbance of drug measured at 224 and 365 nm wavelength for Methocarbamol and Meloxicam respectively. The range of linearity was found to be 4, 8, 12, 16, 20µg/ml for both the drugs with the linearity equation y = 0.0768x + 0.0033 and y= 0.0354x + 0.0467 for Meloxicam and Methocarbamol respectively. Correlation coefficient is 0.999 and 0.998 for Meloxicam and Methocarbamolrespectively.The % RSD values for interday and intraday were found to be less than 2%. And % Recovery were in between 98.05-100.28%. LOD and LOQ were found to be 0.03 and 0.7 respectively for Meloxicam and 0.002 and 0.2 respectively for Methocarbamol. The result concluded that the developed method is accurate, precise and reproducible as per ICH guideline (Q2R1).

**Keywords:** Method development, Validation, Absorption Correction Method, Meloxicam, Methocarbamol

## Introduction [2-6]

Methocarbamol (2-hydroxy-3-(2-methoxyphenoxy)propyl carbamate) is a muscle relaxant with molecular formula$C_{11}H_{15}NO_5$and molecular weight 241.244 g/mol. Methocarbamol is carbamate derivative of guaifenesin, is a central nervous system depressant with sedative and musculoskeletal relaxant properties. It works by blocking nerve impulses or pain sensation that is sent to brain. Methocarbamol is indicated as an adjunct to rest, physical therapy, and other therapies for the relief of discomfort associated with acute, painful muscloskeletal conditions.

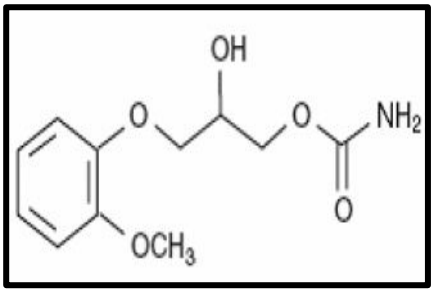


**Figure1: Chemical structure of Methocarbamol**

Meloxicam (4-hydroxy-2-methyl-N-(5-methyl-2 thiazoyl) 2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide) is a NSAID with molecular formula $C_{14}H_{13}N_3O_4S_2$ and molecular weight 351.401 g/mol. Meloxicam is an oxicam derivative drug. It contain enolic acid group of non-steroidal anti-inflammatory drugs (NSAIDs). Meloxicam is used to relieve pain, tenderness, swelling, and stiffness caused by osteoarthritis and rheumatoid arthritis. It works by stopping the body's production of a substance that causes a pain, fever, and inflammation.

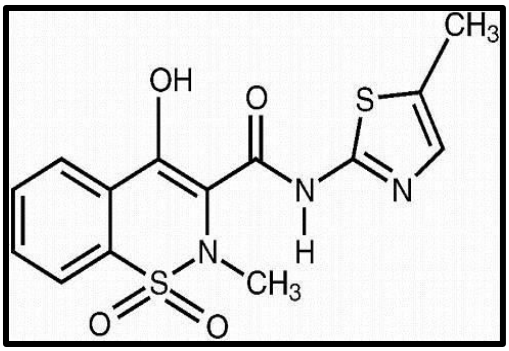


**Figure 2: Chemical structure of Meloxicam**

Meloxicam is NSAID (non-steroidal anti-inflammatory drug), it is used for pain and pain related disorders while Methocarbamol is a muscle relaxant, it is used in pain. Combination of these two drugs provides an improved therapeutic response as compare to either drug alone, this combination is used to control and treat inflammatory musculoskeletal diseases.

**Material and Methods**

**Instrumentation**

Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model-1800). All weighing were done on electronic analytical balance (Wensar Dab220).

**Chemicals and Reagents**

The bulk drug Meloxicam and Methocarbamolobtained from ZydusCadila, Ahmedabad.Analytical grade methanol was procured from Merck Fine chemicals (Mumbai).

**Preparation of Standard Solution**

**(A) Preparation of Standard Solution of Meloxicam**

**Preparation of Standard Stock Solution of Meloxicam (100µg/ml)**

Accurately weighed quantities of MEL 10 mg was transferred to 100 ml volumetric flask, dissolved in 10 ml of Methanol and sonicate it and diluted up to mark with Methanol to give a stock solution having strength of 100µg/ml.

**Preparation of Working Standard Solution of Meloxicam**

From the above stock solution pipette out 0.4mL, 0.8mL, 1.2mL, 1.6mL, and 2.0mL of solution and transferred to 10 mL volumetric flask and make up the volume up to 10 mL with methanol to Produce concentration 4, 8, 12, 16 and 20µg/mL respectively.

**B) Preparation of Standard Solution of Methocarbamol**

**Preparation of Standard Stock Solution of Methocarbamol (100µg/ml)**

Accurately weighed quantities of MET 10 mg was transferred to 100 ml volumetric flask, dissolved in 10 ml of Methanol and sonicate it and dilutedup to mark with Methanol to give a stock solution having strength of 100µg/ml.

**Preparation of Working Standard Solution of Methocarbamol**

From the above stock solution pipette out 0.4 mL, 0.8 mL, 1.2 mL, 1.6 mL, and 2mL of solution and transferred to 10 mL volumetric flask and make up the volume up to 10 mL with methanol to Produce concentration 4, 8, 12, 16 and 20 µg/mL respectively.

**Preparation of Calibration Curve**

**(A)Calibration Curve for Meloxicam**

Calibration curve for MEL consists of different concentrations of stock MEL solution ranging from 4-20µg/ml. The solutions were prepared by pipetting out 0.4, 0.8, 1.2, 1.6 and 2.0 ml of the working standard solution of MEL (100µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol.

**(B) Calibration Curve for Methocarbamol**

Calibration curve for MET consists of different concentrations of stock MET solution ranging from 4 – 20 µg/ml. The solutions were prepared by pipetting out 0.4, 0.8, 1.2, 1.6 and 2.0 ml of the working stock solution of MET (100µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol.

**Validation**

**Linearity and Range**

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 4-20µg/ml and 4-20 µg/ml for MEL and MET respectively (n = 5).

The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient and regression line equations for MEL and MET were calculated.

**Precision**

**(A) Repeatability**

Aliquots of 1.2 ml of working standard solution of MEL (100 µg/ml) were transferred to a 10 ml volumetric flask. Aliquots of 1.2ml of working standard solution of MET (100 µg/ml) were respectively transferred to a 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 12µg/ml solution of MEL and 12µg/ml solution of MET. The absorbance of solution was measured six times and % RSD was calculated.

**(B) Intraday precision**

Aliquots of 0.4, 1.2, and 2.0 ml of working standard solution of MEL (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.4, 1.2 and 2.0 ml of working standard solution of MET (100 µg/ml) were respectively transferred to the same series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 4, 12 and 20µg/ml solution of MEL and 4, 12 and 20µg/ml solution of MET. Solution was analyzed 3 times on the same day and % RSD was calculated.

**(C) Interday Precision**

Aliquots of 0.4, 1.2, and 2.0 ml of working standard solution of MEL (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.4, 1.2 and 2.0ml of working standard solution of MET (100 µg/ml) were respectively

transferred to the same series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 4, 12 and 20µg/ml solution of MEL and 4, 12 and 20µg/ml solution of MET. Solution was analyzed 3 times on the 3 different days and % RSD was calculated.

**Limit of Detection (LOD)**

The LOD is estimated from the set of 5 calibration curves used to determine method linearity.

The LOD may be calculated as,

LOD = 3.3 $*SD/Slop$e

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

**Limit of Quantification (LOQ)**

The LOQ is estimated from the set of 5 calibration curves used to determine method linearity.

The LOQ may be calculated as,

$LO$Q = 10 $*SD/Slop$e

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

**Accuracy**

The accuracy of the method was determined by calculating recovery of MEL and MET by the standard addition method. Aliquots of 0.64, 0.8, and 0.96 ml of working standard solution of MEL(100 µg/ml) were added at 80, 100 and 120 % level to pre-analyzed 0.8 ml sample solutions of MEL and METtransferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 14.4, 16 and 17.6µg/ml solution of MEL.

Aliquots of 0.64, 0.8, and 0.96 ml of working standard solution of MET (100 µg/ml) were added at 80, 100 and 120 % level to pre-analyzed 0.8 ml sample solutions of MEL and MET transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 14.4, 16 and 17.6 µg/ml solution of MET. Absorbance of solution was measured at selected wavelengths for MEL and MET. The amount of MEL and MET were calculated at each level and % recoveries were calculated by measuring the absorbance and fitting the values in equation. Accuracy was assessed using three concentrations and three replicates of each.

**Absorption Correction Method**

This method was based on UV spectrophotometric determination of two drugs, using absorbance correction method. It involves measurement of absorbances at two wavelengths 224nm(λmax of Methocarbamol(MET)) and 365nm (λmax of Meloxicam (MEL)) in methanol

From the overlay spectra of two drugs, it is evident that at the wavelength of MEL (365 nm) MET having absorbance 0 and at the wavelength of MET (224 nm) MEL having some absorbance.

Equation for absorption correction method as following:

$$A = abc$$
$$Cx = A1 / ab$$
$$Cx = A1 / ax1 * b \quad (1)$$

$$A2 = A\,Telm + A\,meto$$
$$A2 = (ay2 * cy * b) + (ax2 * cx * b)$$
$$A2 = (ay2 * cy) + (ax2 * cx)$$
$$Cy = [A2 - (ax2 * cx)] / ay2 \quad (2)$$

Where,

A1 = absorbance of mixture at 224 nm ($\lambda_1$)

A2 = absorbance of mixture at 365 nm ($\lambda_1$)

ax1 = absorptivity of METHO at 224nm

ax2 = absorptivity of MELO at 365nm

$C_X$ = Concentration of Methocarbamol

$C_Y$ = Concentration of Meloxicam

**Results and Discussion**

A reliable absorption correction method was developed for simultaneous estimation of Meloxicam and Methocarbamol in synthetic mixture by UV Spectrophotometry. Beers law was obeyed in concentration range of 4-20 µg/ml for Meloxicam and 4-20 µg/ml for Methocarbamol at 365 nm and 224 nm wavelengths. The correlation coefficients for Meloxicam and Methocarbamol were found to be $R^2$ = 0.999 and 0.998. The mean % recoveries were found to be in the range of 99.15- 99.56% and 99.08 -100.28%. The proposed method was precise, accurate and reproducible and acceptable recovery of the analyte, which can be applied for the analysis of Meloxicam and Methocarbamol in pharmaceutical dosage form.

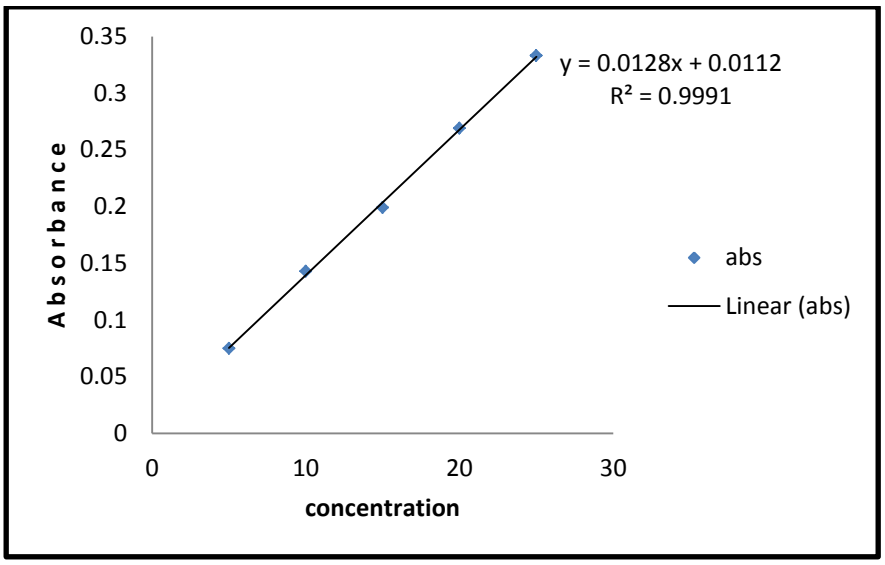


**Figure 3: Calibration curve of Meloxicam at 224nm.**

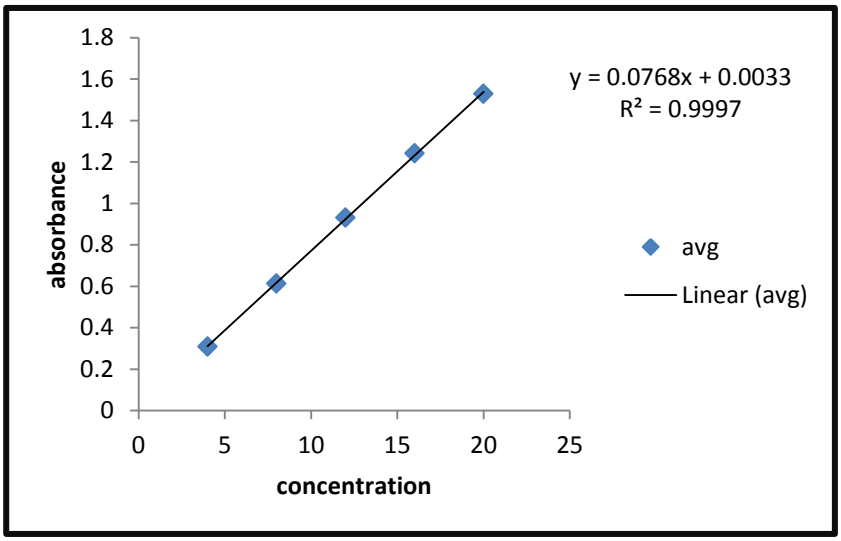


**Figure 4: Calibration curve of Meloxicam at 365nm.**

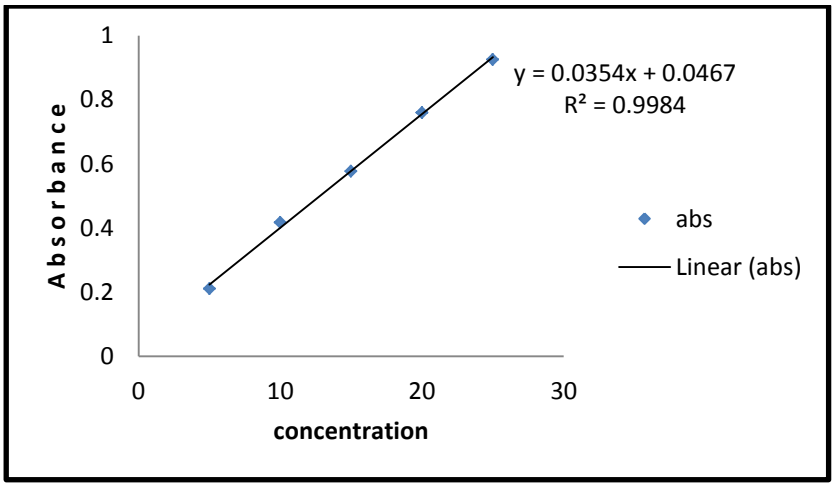


**Figure 5: Calibration curve of Methocarbamol at 224 nm.**

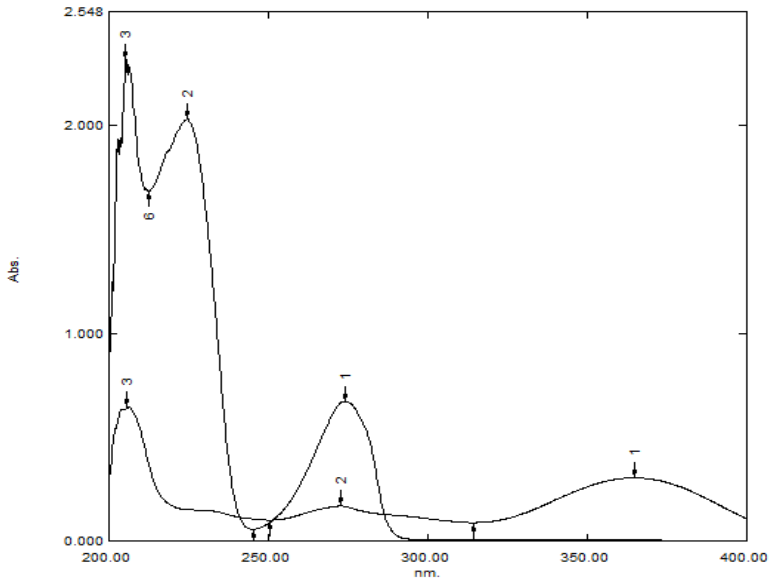


**Figure 6: Overlay spectra of Meloxicam and Methocarbamol**

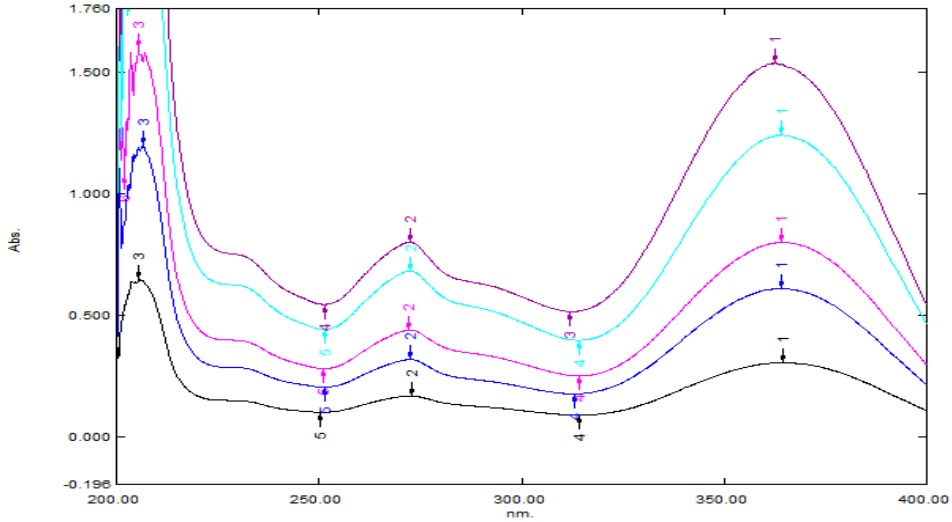


**Figure 7: Overlay spectra of Meloxicam (4-20µg/ml).**

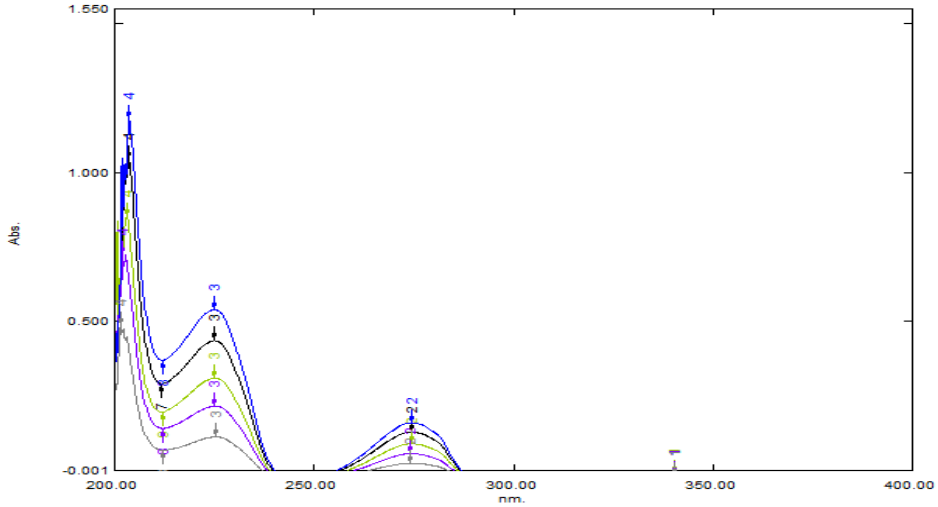


**Figure 8: Overlay spectra of Methocarbamol(4-20µg/ml).**

**Table-1: Linearity data of Meloxicam.**

| AT 365 nm | | | At 224 nm | | |
|---|---|---|---|---|---|
| **Con (µg/ml)** | **Mean absorbance ±SD(n=5)** | **%RSD** | **Con (µg/ml)** | **Mean absorbance ±SD (n=5)** | **%RSD** |
| 4 | 0.308±0.0048 | 1.5 | 4 | 0.1534±0.0023 | 1.5 |
| 8 | 0.6124±0.0038 | 0.62 | 8 | 0.2938±0.0036 | 1.2 |
| 12 | 0.9314±0.0038 | 0.41 | 12 | 0.4574±0.0038 | 0.83 |
| 16 | 1.2418±0.0041 | 0.33 | 16 | 0.6398±0.0027 | 0.43 |
| 20 | 1.5286±0.0040 | 0.26 | 20 | 0.7756±0.0034 | 0.45 |

**Table-2: Linearity data of Methocarbamol (224nm).**

| Con (µg/ml) | Mean absorbance ±SD (n=5) | %RSD |
|---|---|---|
| 4 | 0.115±0.002 | 1.73913 |
| 8 | 0.2168±0.002 | 1.380685 |
| 12 | 0.311±0.003 | 1.150389 |
| 16 | 0.4368±0.004 | 0.931639 |
| 20 | 0.5394±0.003 | 0.659117 |

**Table-3: Repeatability data of Meloxicam.**

| AT 365 nm | | | At 224 nm | | |
|---|---|---|---|---|---|
| **Con (µg/ml)** | **Mean absorbance ±SD (n=6)** | **%RSD** | **Con (µg/ml)** | **Mean absorbance ±SD (n=6)** | **%RSD** |
| 12 | 0.927 ± 0.002 | 0.23 | 12 | 0.451 ± 0.0019 | 0.42 |

**Table-4: Repeatability data of Methocarbamol.**

| AT 286 nm | | |
|---|---|---|
| **Con (µg/ml)** | **Mean absorbance ±SD (n=6)** | **%RSD** |
| 12 | 0.306 ± 0.002 | 0.70 |

**Table-5: Intraday Precision data of Meloxicam.**

| AT 365 nm | | | At 224 nm | | |
|---|---|---|---|---|---|
| **Con (µg/ml)** | **Mean absorbance ±SD (n=3)** | **%RSD** | **Con (µg/ml)** | **Mean absorbance ±SD (n=3)** | **%RSD** |
| 4 | 0.303333±0.0015 | 0.50 | 4 | 0.151±0.015 | 1.009 |
| 12 | 0.925±0.002 | 0.21 | 12 | 0.453±0.010 | 0.22 |
| 20 | 1.523±0.001 | 0.06 | 20 | 0.772±0.015 | 0.19 |

**Table-6: Intraday Precision data of Methocarbamol.**

| AT 224 nm | | |
|---|---|---|
| **Con (µg/ml)** | **Mean absorbance ±SD (n=3)** | **%RSD** |
| 4 | 0.112±0.001 | 0.89 |
| 12 | 0.308±0.0014 | 0.45 |
| 20 | 0.536333±0.0015 | 0.28 |

**Table-7: Interday Precision data of Meloxicam.**

| AT 365 nm | | | At 224 nm | | |
|---|---|---|---|---|---|
| **Con (µg/ml)** | **Mean absorbance ±SD** | **%RSD** | **Con (µg/ml)** | **Mean absorbance ±SD** | **%RSD** |
| 4 | 0.299667±0.0024 | 0.83 | 4 | 0.148333±0.0020 | 1.38 |
| 12 | 0.923±0.0028 | 0.30 | 12 | 0.449667±0.0024 | 0.55 |
| 20 | 1.520333±0.0033 | 0.21 | 20 | 0.768±0.0032 | 0.42 |

**Table-8: Interday Precision data of Methocarbamol.**

| AT 286 nm | | |
|---|---|---|
| **Con (µg/ml)** | **Mean absorbance ±SD** | **%RSD** |
| 4 | 0.109333±0.0020 | 1.8 |
| 12 | 0.304667±0.0024 | 0.67 |
| 20 | 0.533±0.0024 | 0.45 |

**Table-9: Accuracy data of Leflunomide and Methotrexate.**

| Name of sample | Level | Amount taken (µg/mL) | Amount added (µg/mL) | Total Amount (µg/mL) | Recovered Concentration (µg/mL) | %Recovery ± SD (n=3) |
|---|---|---|---|---|---|---|
| MEL | 80 | 8 | 6.4 | 14.4 | 14.12 | 98.05±0.03 |
| | 100 | 8 | 8 | 16 | 16.08 | 100.5±0.035 |
| | 120 | 8 | 9.6 | 17.6 | 17.61 | 100.05±0.01 |
| MET | 80 | 8 | 6.4 | 14.4 | 14.25 | 98.95±0.040 |
| | 100 | 8 | 8 | 16 | 15.98 | 99.93±0.026 |
| | 120 | 8 | 9.6 | 17.6 | 17.65 | 100.28±0.025 |

**Table-10: Assay Study Parameter.**

| Meloxicam | | | Methocarbamol | | |
|---|---|---|---|---|---|
| Concentration (µg/mL) | Amount found (µg/mL) | % Assay ± SD (n=3) | Concentration (µg/mL) | Amount found (µg/mL) | % Assay ± SD (n=3) |
| 1 | 1.003 | 99±0.26 | 14 | 14.12 | 100.85±0.20 |

## Conclusion

The proposed Spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of MEL and MET insynthetic Pharmaceutical dosage form. The method utilizes easily available and cheap solvent for analysis of MEL and MET hence, the method is economic for estimation of MEL and MET in pharmaceutical dosage form.

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**Corresponding Author:**

**Mr.DarshilShah*,**

**Email:** *darshilshah89@yahoo.com*