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Fourth Derivative and Compensated Area under the Curve Spectrophotometric Methods Used for Analysis Meloxicam in the Local Market Tablet

Ruba Fahmi Abbas*, Neda Ibrahim Mahdi, Ali Amer Waheb, Amjad Gali Aliwi, Marwa S. Falih
Department of Chemistry, Collage of Science, Mustansiriyah University, IRAQ.
*Correspondent author email: rubaf1983@uomustansiriyah.edu.iq

Abstract
Two Rapid, direct, ecological friendly and economical spectrophotometric methods were used for estimation of meloxicam in the market Tablet dosage form. The First method is based on the use the fourth order derivative spectrum (D4) and the second method is depended on the ratio of the area under the curve for the two peaks in the drug (Compensated area under the curve). The linear calibration graphs of the two methods were measured in the concentration range (5-35)mg/l and the average of recoveries for local market Tablet (AWA)® were 99.8% for D4 method and 100.2% for CAUC method which indicating a good accuracy and precision for these methods. In this study, the results obtained by these suggested methods have been successfully statistically compared by t-test and Mann-Whitney test showed a good agreement.

Keywords: meloxicam; D4; CAUC; spectrophotometric; Mann-Whitney test

Introduction
Meloxicam Figure 1, is a newer non-steroidal anti-inflammatory drug (NSAID) in the enolic acid group found to inhibit cyclo-oxygenase -2(cox-2)[1], it is used to treatment of ankylosing spondylitis, osteoarthritis and rheumatoid arthritis[2]. Meloxicam drug was used to prepare nano crystal formulation to increase transdermal delivery[3], also made films nano crystals from meloxicam[4] and using meloxicam as an analytical reagent for determination silver and vanadium[5,6]. There are many analytical methods were reported for the estimation meloxicam in biological and pharmaceutical samples they include: flow-injection chemiluminescence [7], liquid-chromatography- tandem mass spectrometry [8], polarographic [9], flow injection spectrophotometric [10, 11], fluorimetric[12] and derivative spectrophotometric methods[13]. In this paper, two precise, simple and nontoxic spectrophotometric methods were used to estimation meloxicam in market Tablet formulation, the first method was based on recording the fourth derivative spectra and the second method was depended on the area of the absorption spectrum for the peaks, the data for these methods were successfully statistically compared using parametric t-test and non-parametric mann-whitney U-test.
Materials and Methods

a. Apparatus: All the spectral measurements analysis were made by using spectrophotometric (VARIAN UV-visible) with 1cm path quartz cells with software program (originlab pro.2016).

b. Material and standard solutions:
   1. 1000 mg/l stock solution of pure meloxicam (M.Wt= 351.403) was prepared by dissolving (0.1gm) from this pure drug in 25 ml of distilled water and complete to 100 ml with the same solvent in 100 ml volumetric flask.
   2. 100 mg/l standard solution was prepared by transferred 25 ml from 1000 mg/l standard solution to 250 ml volumetric flask and then complete the volume to the mark with distilled water.
   3. Preparation of calibration graph was made up by preparation a series of 20 ml volumetric flask, (1,2,3,4,5,6 and7) ml of 100 mg/l meloxicam standard solution were transferred to obtained concentration (5,10,15,20,25,30 and 35) mg/l and then the volume of each flask was completed to the mark with distilled water.
   4. Preparation of local market (AWA)® Tablet was made up by grinded ten Tablets and dissolving 0.1 gm from this powder in 100 ml distilled water to obtained concentration equal to 1000 mg/l , and then repeat the general procedure described under preparation of calibration graph.

Procedure:

1. The fourth derivative spectra were accomplished in the range (300-400 nm), the absolute values for calibration graph were measured at 361 nm.
2. The area under the curve values for the meloxicam were recorded over the wavelength range for the first peak A1(264-277nm) and for the second peak A2(352-378nm), the calibration graph was constructed by the calculated the area under the curve ratios(ratio \( \frac{A_{UC1}}{A_{UC2}} \)) for the meloxicam standard solution (5-35mg/l).

Results and Discussion

Fourth derivative method D4

Derivative spectrophotometric methods were widely used for the analysis of pharmaceutical drugs, this technique is required less time for the analysis, cheaper and easily applied to analysis of pharmaceutical and raw material.

The zero order spectra that have the similar linear relationship between absorbance of the solutions and the corresponding concentration for all orders of derivative [14, 15]:
"A=Є BC" .... For zero order
\[ \frac{d\lambda}{dA} = \frac{d\varepsilon}{d\lambda} bc \]
for all derivative order
So, for the fourth derivative order, the equation became as the following:
\[ \frac{d^4A}{d\lambda^4} = \frac{d^4 \varepsilon}{d\lambda^4} bc \]

Where, \( A= \) is the Absorbance of the solution, \( \lambda= \) is the wavelength, \( \varepsilon= \) is the molar absorptivity, \( l= \) is the width of cell and \( C= \) is the concentration of the solution.

Figure 2 (a,b) show the zero order and fourth derivative spectra of pure meloxicam standard solution. The normal absorbance spectra is called zero order spectra, derivative spectrophotometry technique is based on derivative spectra of the zero-order spectrum, a fourth derivative and all other derivative order are passes through the same wavelength as \( \lambda \) max of the absorbance band for the zero order spectrum. Figure 3 shows that the minimum bands for the fourth derivative at the same wavelength as (361 nm) \( \lambda \) max of the zero order absorbance bands.
Compensated area under the curve method (CAUC)

This method is simple and providing better sensitivity and depended on measuring the area of the drug in the two range of wavelengths [(\(\lambda_2-\lambda_1\)) for peak A1 and (\(\lambda_4-\lambda_3\)) for peak A2] not only depending on the measuring the area at single wavelength, and then calculated the area under the curve ratio \(\frac{AUC_{A1}}{AUC_{A2}}\) for two peaks. Figure 4 is shown The AUC of meloxicam standard solution and the AUC was calculated from the following equations [16, 17, 18]:

\[
\begin{align*}
AUC_{A1} &= \int_{\lambda_1}^{\lambda_2} A_1 d\lambda = \int_{264}^{277} A_1 d\lambda \\
AUC_{A2} &= \int_{\lambda_3}^{\lambda_4} A_2 d\lambda = \int_{352}^{378} A_2 d\lambda
\end{align*}
\]

Where; \(A_1\) and \(A_2\) are the absorbance of meloxicam for the peak A1 and peak A2, respectively. 
\(\int_{\lambda_1}^{\lambda_2} A_1 d\lambda\) and \(\int_{\lambda_3}^{\lambda_4} A_1 d\lambda\) are the area under the curve between (264–277nm) for the peak A1 and (352–378nm) for the peak A2, respectively.
**Calibration graph and validation parameters**

The calibration graph for fourth derivative method was obtained by plotted the absorbance of the fourth derivative spectra vs. the concentration of the meloxicam standard solution. **Figure 5** and the calibration graph for CAUC method **Figure 6** was obtained by plotted the AUC ratio vs. the concentration of the drug.

**Correlation of determination** of the fourth derivative was 0.990 and 0.998 for the CAUC, the other statistical parameters analysis of the data was given from calibration graph are listed in Table 1.

**Table 1: The validation parameters obtain from the calibration graph of meloxicam**

| Method  | Wavelength (nm) | Range (mg/L) | Equation | Y = b x + a |
|---------|----------------|--------------|----------|-------------|
| D4      | 361 nm         | 7-35         | Y = 0.000239x + 0.0001 | 0.000239 - 0.0001 |
| CAUC    | 361 nm         | 7-35         | Y = -0.032x + 3.839 | -0.032 - 3.839 |

**Table 2**: Accuracy and precision for this study

| Pure meloxicam | Conc. (taken) | Rec.* | Avg of Rec.% | RSD* |
|----------------|--------------|-------|--------------|------|
| D4             | 15           | 15.091| 100.606      | 2.664|
|                | 25           | 25.158| 100.632      | 3.479|
| CAUC           | 15           | 14.881| 99.206       | 1.442|
|                | 25           | 25.124| 100.488      | 1.852|

**Application and statistical analysis**

The proposed methods are successfully applied to estimation of meloxicam (AWA)® Tablet. Figures (7a, b) and 8 shows the spectra of market meloxicam(AWA)® Tablet was analysis by the two suggested methods, the results in Table 3 are shown the robustness and reliability of this study.

The recoveries results were statistically compared by using parametric t-test and non-parametric mann-whitney U-test as shown in Table 4. The results of the t-test at (95% confidence level) and Mann –Whitney test U did not exceed the theoretical t value 4.303 and theoretical U value 3 for the two suggested methods, which indicate that there is no difference between fourth derivative and CAUC spectrophotometric methods and reported method [19] and refer to a good accuracy and precision in estimation meloxicam in pure and local market Table (AWA)®.
Figure 7: a- the zero order and b- the fourth derivative spectra of market meloxicam (AWA)® Tablet at concentration 10 and 25 mg/l

Figure 8: The CAUC for the market meloxicam(AWA)® Tablet at concentration 10 and 25 mg/l

Table 2: The recovery and relative standard deviations of the local market meloxicam (AWA)® Tablet at 12 and 20 mg.l⁻¹.

| Method  | Meloxicam (AWA) | Rec.% | Average of Rec.% | RSD*% |
|---------|-----------------|-------|------------------|-------|
|         | Awamedica 15mg. Tablet |       |                  |       |
|         | (taken) mg/l | (Found) mg/l |        |                  |        |
| D4      | 10             | 9.965 | 99.650           | 99.785| 1.269            |
|         | 25             | 24.980| 99.920           |       | 0.560            |
| CAUC    | 10             | 9.977 | 99.770           | 100.249| 1.758          |
|         | 25             | 25.182| 100.728          |       | 0.932            |

"*Average of three determination"

Table 4: The comparison of theD4 and CAUC Spectrophotometric methods with reported method

| Statistical Parameters | Tablet Pharmaceutical preparation |
|------------------------|----------------------------------|
| D4 At peak 361 nm      | Meloxicam pure | Meloxicam (AWA)® 15 mg Tablets |
| Present work           | Rec.% | 100.619 | 99.785 |
|                        | S**   | 0.418   |       |
|                        | t*    | 0.530   |       |
|                        | s²    | 0.347   |       |
|                        | Σ Rank| 5       |       |
|                        | U     | 2       |       |
| CAUC                   | Rec.% | 99.847  | 100.249|
|                        | S**   | 0.204   |       |
|                        | t*    | 0.331   |       |
|                        | s²    | 0.080   |       |
|                        | Σ Rank| 5       |       |
|                        | U     | 2       |       |
| Reported method[19]    | Rec.% | 99.940  | 100.020|
|                        | s²    | 0.0032  |       |
|                        | Σ Rank| 5       |       |
"Where, $S^{**} =$ pooled standard deviation

$$= \sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1 + n_2 - 2}}, \quad (n_1-1) \text{ and } (n_2-1)= \text{number of degrees of freedom for this study and standard method, respectively, } t^* = \text{parametric t-test, } T_{\text{theoretical}} = 4.303, \quad T_{\text{calculated}} < T_{\text{theoretical}} \text{ at } 95\% \text{ confidence level, } S^2_1 = \text{variation} = \frac{\sum (x_i - \bar{x})_1^2}{n_1-1}$,$$

$U =$ Mann - Whitney non parametric test($n_1=n_2=2), U=\sum \text{Rank} = \frac{n(n+1)}{2}, \quad U_{\text{theoretical}} = 3, \quad U_{\text{calculated}} < U_{\text{theoretical}}.$

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