

# Comparison of RP-HPLC and UV Spectrophotometric Methods for the Determination of Orphenadrine Citrate and Diclofenac Sodium in Pharmaceutical Drugs

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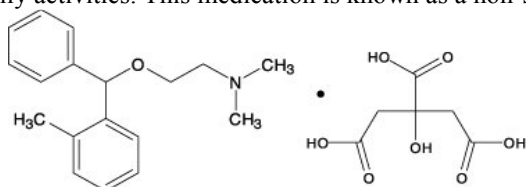
## Abstract

This paper describes the development and evaluation of a RP-HPLC and UV spectrophotometric methods to quantify Orphenadrine Citrate and Diclofenac Sodium in Tablets and Injections. RP-HPLC analysis were carried out using a C<sub>18</sub> Knauer column and a mobile phase composed of (1% Triethylamine aqueous buffer adjust pH=2 by H<sub>3</sub>PO<sub>4</sub> 85%: Methanol: Acetonitrile) ; (35: 20: 45) v/v%, with a flow rate of 2.0 mL/min and UV detection at 195 nm. For the spectrophotometric analysis, (Ultra pure water: Methanol) ; (50: 50) v/v% was used as solvent and the wavelength of 264, 277 nm was selected for Orphenadrine Citrate and Diclofenac Sodium, respectively the detection. Both methods were found to quantify Orphenadrine Citrate and Diclofenac Sodium in Tablets and Injection accurately. Therefore RP-HPLC and UV methods presented the most reliable results for the analyses of Tablets and Injection.

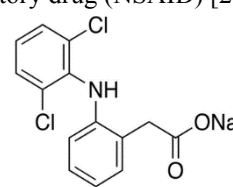
**Keywords:** Orphenadrine Citrate; Diclofenac Sodium; UV spectrophotometry; RP-HPLC; pharmaceutical formulations.

## 1. INTRODUCTION

Orphenadrine Citrate (OC), "IUPAC" chemically known as N,N-dimethyl-2-[(2-methylphenyl)-phenylmethoxy]ethanamine;2-hydroxypropane-1,2,3tricarboxylic acid (**Figure 1**). Diclofenac Sodium (DS),"IUPAC" chemically known as Sodium; 2-[2-(2,6-dichloroanilino) phenyl]acetate (**Figure 2**). OC was one of the sparingly soluble in water-soluble that is a tasteless and odorless whiteness crystal Powder [1]. OC has been identified as one of the most important Anticholinergic. Orphenadrine was used to treat acute muscle aches or spasms/pains. It was usually combined with rest, physical therapy, and other treatment [2]. DS was one of the water-soluble that is a tasteless and odorless whiteness crystal Powder [1]. Ds was used to relieve pain, swelling (inflammation), and joint stiffness caused by arthritis. Reducing these symptoms helps you do more of your normal daily activities. This medication is known as a non-steroidal anti-inflammatory drug (NSAID) [2].



**Figure 1.** Chemical structure of Orphenadrine Citrate.



**Figure 2.** Chemical structure of Diclofenac Sodium.

Different methods have been developed for the determination of OC in pharmaceutical formulation including colorimetric [3], spectrophotometry [4], capillary electrophoresis [5], Gas Chromatography [6]. The determination of DS in pharmaceutical formulation including colorimetric [7], spectrophotometry [8], capillary electrophoresis [9], Flow injection [10] Spectrofluorescence [11-12] Voltametry [13], RP-HPLC [14].

## 2. EXPERIMENTAL

### 2.1. Instruments and analytical conditions

All HPLC measurements were made on a Waters 1525 Binary HPLC Pump, consisting of a 7725i manual injector with a 20  $\mu$ L loop, integrated UV-Vis detector (Milford, MA). The system employed a 250 mm  $\times$  4.6 mm C<sub>18</sub> column Wat 054275 (Milford, MA) and particle size of 5  $\mu$ m guard column. The detector was utilized at 195 nm and UV spectra from 200 to 400 nm were recorded on line for peak identification. The mobile phase consisted of (Triethylamine 1% buffer :Methanol: Acetonitrile) ; (35: 20: 45) v/v%, at flow rate of 2.0 mL/min. The injection volume was 20 $\mu$ L. A Jasco V-630 UV-Vis spectrophotometer with 1 cm quartz cells was used for all absorbance measurements under the following operating conditions: Scan speed medium (400 nm/min), scan range 200–1100 nm and slit width 2 nm. Spectra were automatically obtained by Jasco system software. pH measurements were made with ORION 250A (USA) with combined glass pH electrode.

## 2.2. Solvents and materials

All chemicals and reagents were of analytical or pharmaceutical grade and solutions were prepared with (Methanol : Ultra pure Water) ; (50: 50)v/v% and Ultra pure water for more dilutions. Orphenadrine Citrate and Diclofenac Sodium were obtained from Aurobindo (India). The purity of OC and DS were 98.0% and 99.9% according to BP [1]. Methanol; Acetonitrile (HPLC grade) were obtained from Merck. Triethylamine was obtained from Sigma Aldrich. Pharmaceutical preparations containing OC and DS were purchased from commercial sources in the local market.

## 2.3. Preparation of Standard and Sample Solutions

The standard stock solutions were prepared by dissolving 10 mg of Orphenadrine Citrate and Diclofenac Sodium reference standard, respectively in 10 mL of Methanol: Ultra pure water (50: 50 v/v%) to get a concentration of 1 mg/mL. An aliquot of 100  $\mu$ L of the obtained solution was transferred to a 10 mL volumetric flask. The final volume was adjusted with Ultra pure water for spectrophotometric and chromatographic analysis, resulting in solutions of 10  $\mu$ g/mL.

The sample solutions were prepared by dissolving 10 mg of Orphenadrine Citrate and Diclofenac Sodium powder for Tablets or Injections in 10 mL of water to get a concentration of 1 mg/mL. An aliquot of 100  $\mu$ L of this solution was transferred to a 10 mL volumetric flask. The final volume was adjusted with water for spectrophotometric analysis and chromatographic analysis, to obtain a solution at 10 $\mu$ g/mL of Orphenadrine Citrate and Diclofenac Sodium.

## 2.4. Procedure for pharmaceutical formulations

Twenty Tablets and Injections contents were powdered and mixed thoroughly for DC Formulation Pharmaceuticals. An amount corresponding to 10 mg of DC was weighted, and OC was weighted Powder and mixed thoroughly for OC Formulation Pharmaceuticals, dissolved with 0.2 mL of (Methanol: Ultra pure Water) (50: 50)v/v% solution and diluting to 100 mL with the Ultra pure water. The volumetric flask was sonicated for 10 min. The solution was filtered through a Whatman filter paper (No. 1) and the resulting solution was used for analysis by the recommended procedures in the concentration range.

## 3. RESULTS AND DISCUSSION

During the chromatographic method development, Ultra pure Water showed to be a more adequate Organic solvent than Methanol, regarding the Orphenadrine citrate and Diclofenac sodium retention.

After the evaluation of the Orphenadrine citrate and Diclofenac sodium UV spectrum in various solvents: Methanol (100%), (Ultra pure Water: Methanol) (50:50)v/v%, (**Figure 3, 4**), the wavelength of 264, 276 nm was chosen due to the adequate molar absorptivity of Orphenadrine citrate and Diclofenac sodium, respectively in this region and to minimize possible interference from other compounds and solvents in the samples.

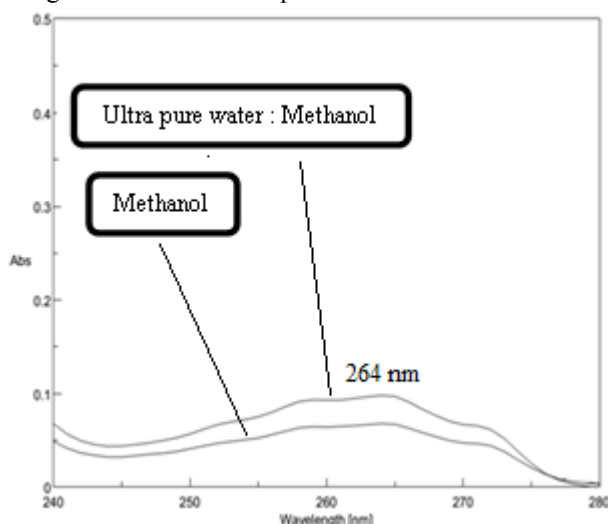


Figure 3. Chemical structure of Orphenadrine Citrate

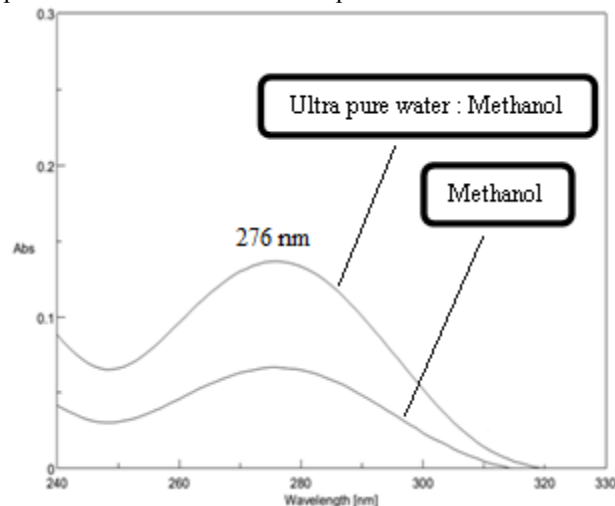


Figure 4. Chemical structure of Diclofenac sodium

### 3.1. Validation

A linear relationship was found between the Orphenadrine citrate and Diclofenac sodium concentrations and the response of both UV and HPLC methods. The regression analysis data are presented in (**Table 1, 2**). High regression coefficient ( $r^2$ ) values were obtained (0.9983 and 0.9997) for OC (0.9994 and 0.9999) for DS, respectively. A random pattern of the regression residues was found and no significant deviation of linearity was

detected in the assayed range.

### 3.2. Linearity

Standard solutions containing 1000 µg/mL of Orphenadrine citrate and Diclofenac sodium in (Methanol: Ultra pure water); (50:50)v/v% were prepared, in triplicate. Aliquots of these solutions were diluted in water. Seven and Six different concentrations, corresponding to 0.5, 1.0, 5.0, 10.0, 15.0, 20.0 and 25.0 µg/mL of Diclofenac Sodium and to 10.0, 25.0, 50.0, 100.0, 150.0 and 200.0 µg/mL (Figure 5) Orphenadrine Citrate (for UV analysis) (Figure 6) and eight different concentrations, corresponding to 0.10, 0.50, 1.0, 5.0, 10.0, 20.0, 40.0, and 80.0 µg/mL of Orphenadrine citrate and Diclofenac sodium (for HPLC analysis) (Figure 7). Calibration curves with concentration versus peak area (Figure 7A, 7B) for OC, Ds, respectively. Or absorbance were plotted for each method (Figure 5A, 6A) for DS, OC, respectively. And the obtained data were subjected to regression analysis using the least squares method (Table 1, 2).

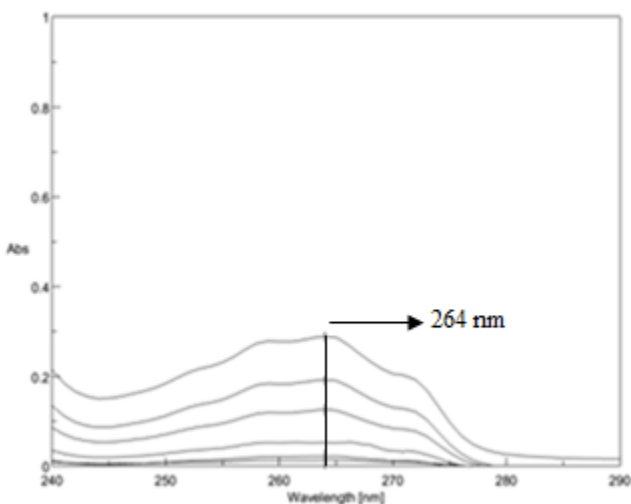


Figure 6. Overlay standards series of Orphenadrine Citrate

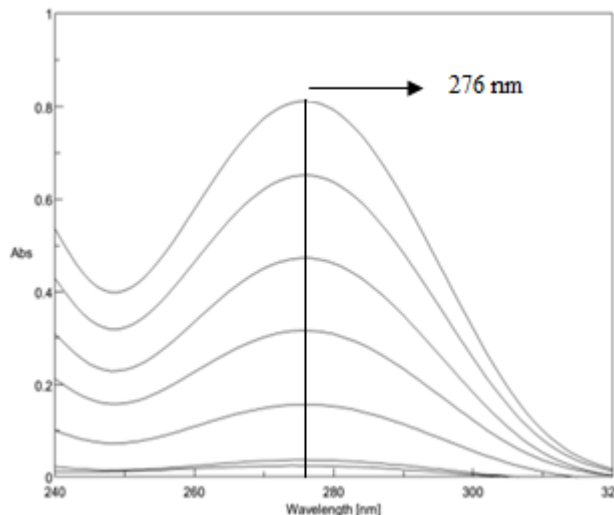


Figure 5. Overlay standards series of Diclofenac sodium

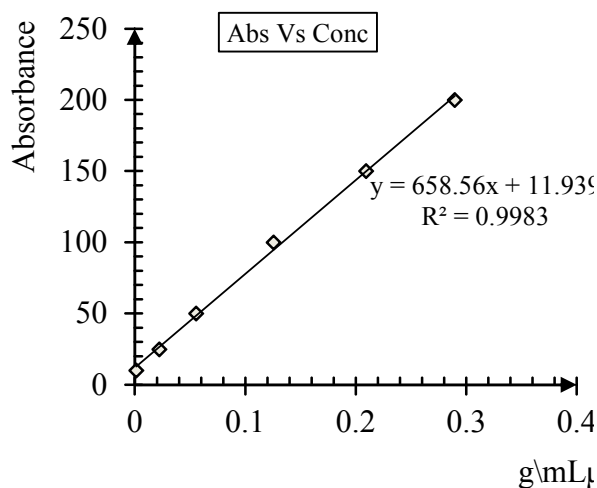


Figure 6A. Overlay standards series of Orphenadrine Citrate

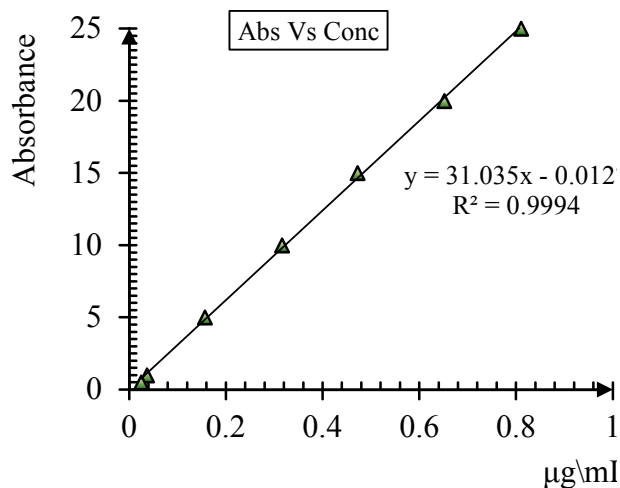


Figure 5A. Overlay standards series of Diclofenac Sodium

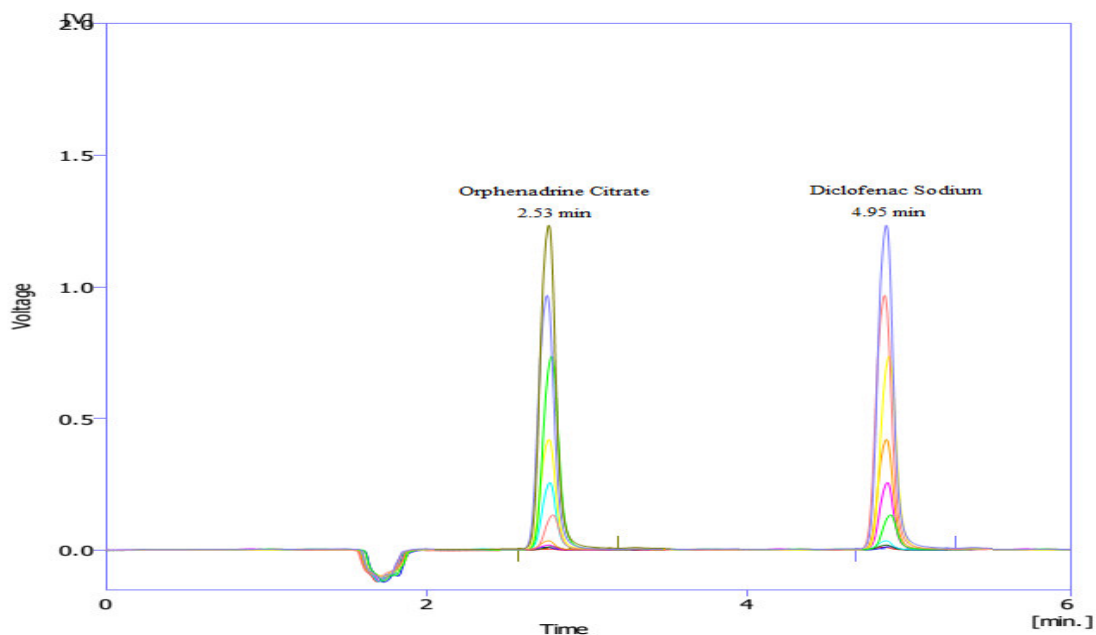


Figure 7. Overlay standards series of Orphenadrine Citrate and diclofenac Sodium

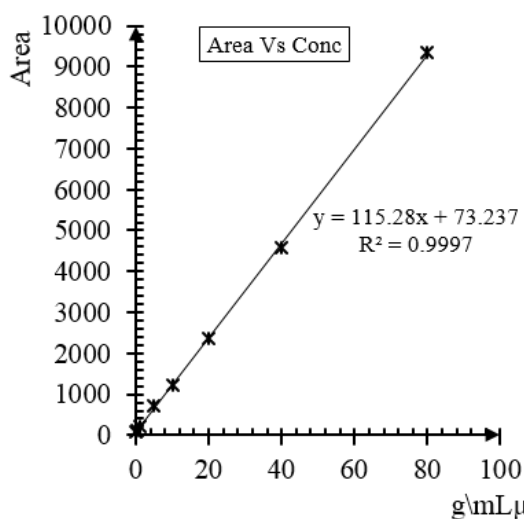


Figure 7A. Overlay standards series of Orphenadrine Citrate

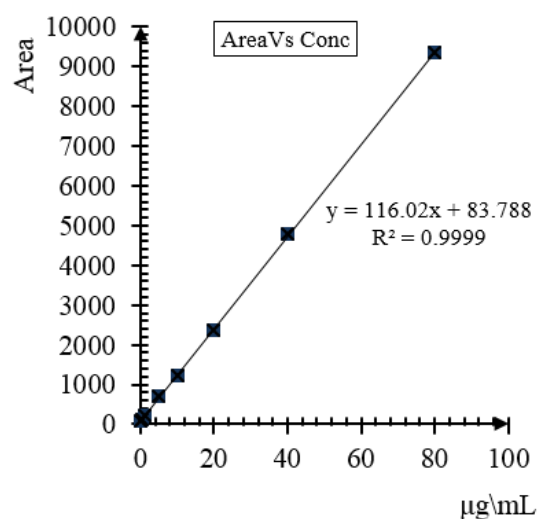


Figure 7B. Overlay standards series of Diclofenac Sodium

Table 1. Overview of the Linearity Data Obtained for of Orphenadrine citrate by the Chromatographic and Spectrophotometric Methods		
Parameter	HPLC	UV
Regression coefficient ( $r^2$ )	0.9997	0.9983
Slope	115.2	658.56
Intercept	73.247	11.939
Concentration range ( $\mu\text{g/mL}$ )	0.1-80.0	10.0–200.0
Number of points	8	6

Parameter	HPLC	UV
Regression coefficient ( $r^2$ )	0.9999	0.9994
Slope	116.02	31.035
Intercept	83.788	0.0127
Concentration range ( $\mu\text{g/mL}$ )	0.1-80.0	0.50–25.0
Number of points	8	7

### 3.3. Precision

The methods were found to be precise as the RSD (%) values for the precision studies were well below 2% (n=3). The results are shown in (Table. 3).

### 3.4. Accuracy

The accuracy of the developed methods was found out by the standard addition method. High recovery values suggest that all three methods are accurate. The results are shown in (Table. 3).

Method	Parameter	Accuracy (Recovery %)	*Precision (RSD %)	LOD, $\mu\text{g/ml}$	LOQ, $\mu\text{g/ml}$
UV	Orphenadrine Citrate (OC)	102.57%	0.92	9.61	10.04
	Diclofenac Sodium (DS)	100.57%	0.86	0.45	0.57
HPLC	Orphenadrine Citrate (OC)	100.17%	0.39	0.09	0.18
	Diclofenac Sodium (DS)	99.27%	0.41	0.08	0.16

\* Average of three determination at each level

### 3.5. Robustness

The HPLC method was found to be robust under deliberate changes in the mobile phase flow rate ( $\pm 0.1$  mL/min), detection wavelength ( $\pm 5$  nm), and organic phase composition ( $\pm 2\%$ ). The results of system suitability for the robustness study are shown in (Figure 7). For the UV spectroscopic methods, changing the slit width shows no significant effect on absorbance, indicating the robustness of the developed methods. No significant changes were obtained in the content of DS and OC during the solution stability studies by the developed methods.

### 3.6. LOD & LOQ

The limit of Determination ( $\text{LOD} = 3.3\sigma/s$ ) and the limit of Quantitation ( $\text{LOQ} = 10\sigma/s$ ),  $\sigma$ - standard deviation of the regression line; s- Slope of calibration curve; was calculated by standard formula as given in ICH.

## 4. Analysis of Tablets & Injections OC and DS

The validated chromatographic and spectrophotometric methods were applied to the analysis of Orphenadrine citrate and Diclofenac sodium in Cross jestic, Medagesic and Voltamed, Diclon (Table 4, 5). Chromatographic analysis showed to be the most sensitive and selective method, and might be applied successfully for Orphenadrine citrate and Diclofenac sodium trace analysis and quantitation in biological matrices. We cannot discharge, However, the analyses time and cost. The spectrophotometric method is clearly less expensive and requires shorter analysis time, besides the ease of handling and lower residues generation.

Since the use of Orphenadrine citrate and Diclofenac sodium as a potent Analgesic and Anti-Inflammatory drug is widespread, the development and validation of simple and reliable methods are essential to assure the quality of the raw materials and pharmaceutical formulations marketed nowadays. A simple method to identify and precisely quantify these drugs may be an important tool to avoid treatment inefficacy and development of resistance due to the exposition to sub therapeutic doses.

<b>Table 4. Diclofenac Sodium Contents in Injectable Samples Obtained by HPLC and UV (n = 6)</b>		
<i>Diclofenac Sodium content (%) ± S.D</i>		
Sample	HPLC	UV
<b>Voltamed 100mg\Tablet</b>	100.84 ± 0.24	104.49 ± 0.62
<b>Diclon 100mg\1mL</b>	101.74 ± 0.36	105.99 ± 0.92
S.D: standard deviation		

<b>Table 5. Orphenadrine Citrate Contents in Injectable Samples Obtained by HPLC and UV (n = 6)</b>		
<i>Orphenadrine Citrate content (%) ± S.D</i>		
Sample	HPLC	UV
<b>Medagesic 25mg\Tablet</b>	101.84 ± 0.14	103.69 ± 0.42
<b>Cross Jesic 35mg\Tablet</b>	101.74 ± 0.23	104.19 ± 0.82
S.D: standard deviation		

### Conclusion

HPLC and UV spectrophotometry were found to be adequate methods to quantify Orphenadrine Citrate & Diclofenac Sodium in Tablets and Injection solutions; the chromatographic and spectrophotometric methods presented the most reliable results. Since these methods are fast and simple, they may be successfully applied to quality control analyses, with the aim of quantifying and identifying Orphenadrine Citrate and Diclofenac Sodium in pharmaceutical products.

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