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# SIMULTANEOUS ESTIMATION OF ACECLOFENAC AND THIOCOLCHICOSIDE IN PHARMACEUTICAL DOSAGE FORM BY SPECTROPHOTOMETRIC METHOD

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## ABSTRACT

Three simple, rapid, precise and accurate spectrophotometric methods have been developed for simultaneous analysis of Aceclofenac (ACE) and Thiocolchicoside (THI) in their combined dosage form. Method A,Simultaneous equations method involves measurement of absorbance at 275 nm for ACE and 254 nm for THI, Method B,Absorption Ratio method involves measurement of absorbance at 275 nm for ACE and 264 nm for THI & Method C,Ratio first order derivative method involves measurement of amplitude at 265 nm for ACE and 240.60 nm for THI. Methanol used as a solvent. In these three UV spectrophotometric methods, first method linearity lies between 5-30 µg/mL for both ACE & THI, while for second and third method linearity lies between 10-35 µg/mL for both ACE & THI respectively with correlation coefficient > 0.998. The methods were validated according to ICH Guidelines. All validation parameters were within the acceptable range. These developed methods can be applied for routine analysis.

**KEYWORDS:** **Aceclofenac,Thiocolchicoside,Simultaneous Equations Method, Q-Absorbance ratio method, Ratio First Derivative Method.**

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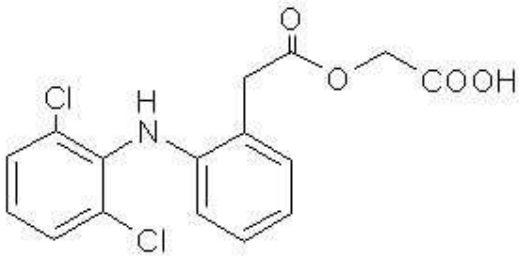
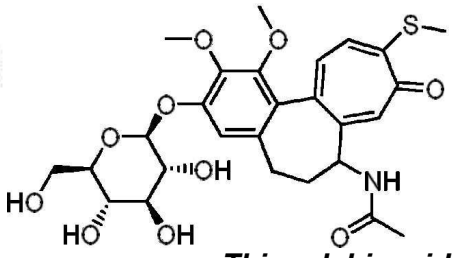
## INTRODUCTION

Chemically, thiocolchicoside (THC) is N-[3-(B-D-glucopyranoxyloxy)-5,6,7,9-tetrahydro-1,2 dimethoxy 10-(methylthio)-9-oxobenzo[a]heptalen-7yl] acetamide. It has selective affinity for γ-amino-butyric acid (GABA) receptors and acts on the muscular contracture by activating the GABA- inhibitory pathways thereby acting as a potent muscle relaxant [1]. Literature survey reveals that Thiocolchicoside can be estimated by spectrophotometry [2], HPLC [3, 4] and by HPTLC [5] methods individually or in combination with other drugs. Chemically, aceclofenac (ACE) is 2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyacetic acid. Aceclofenac is used as anti-inflammatory drug [6]. Aceclofenac alone or in combination with the other drugs is reported to be estimated by TLC-densitometry, differential spectrophotometry [7, 8, 9] HPLC[10, 11] and fluorimetry [12]. Since no spectrophotometric methods are reported for the simultaneous estimation of Thiocolchicoside and aceclofenac in combination therefore in the present work, a successful attempt has been made to estimate both these drugs simultaneously by Various UV spectrophotometric methods. The proposed methods were optimized & validated as per ICH guidelines [13].




***Aceclofenac***




***Thiocolchicoside***

## MATERIALS AND METHODS

### *Apparatus and Software*

Shimadzu UV-1800 double beam spectrophotometer connected to a computer loaded with Shimadzu UV Probe 2.32 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-400 nm. Contech analytical balance with minimum weighing capacity of 1mg was used in the study.

### *Materials*

Active pharmaceutical ingredient (API) working standards of Aceclofenac, purchased from Yarrow Chem Products, Mumbai and Thiocolchicoside Gift sample provided from Hindustan Herbal Pvt. Ltd, Rohtak. Test samples (Tablet with composition 100 mg Aceclofenac and 8 mg Thiocolchicoside) were purchased from local Pharmacy.(ZERODOL TH 8, Marketed by IPCA Laboratories), were purchased from local market.

***Solvent used:*** Methanol- AR grade

### *Preparation of Stock Solution:*

Accurately weighed ACE and THI (in quantities of 25.0 mg) were transferred to two separate 25 ml volumetric flasks, dissolved with the use of Methanol and volume was made up to the mark with same Methanol to obtain stock solution of ACE (1000 µg/mL) and THI (1000 µg/mL).

### *Method A:Simultaneous Equations Method (SEM)*

From the stock solution of 1000 µg/mL, working standard solutions of drugs were prepared by appropriate dilutionand were scanned in entire UV range to determine the λ max. ACE has λ max at 275 nm while THI has λ max at 254 nm respectively (Fig.1).

Standard solutions were prepared having concentration 5-30 µg/mL for both drugs. The absorbances of these standard solutions were measured at 254 nm and 275 nm and calibration curves were plotted at these wavelengths. By applying Vierodt's equation,

$$CX = A2*aY1 – A1*aY2/ aX2*aY1 – aX1*aY2 \quad \cdots\cdots (1)$$
$$CY = A1*aX2 – A2*aX1/ aX2*aY1 – aX1*aY2 \quad \cdots\cdots (2)$$

***Method B: Q-Absorbance ratio method (ARM)***

Q-Absorbance method uses the ratio of absorbances at two selected wavelengths, one at isoabsorptive point and other being the λmax of one of the two compounds. Fromthe stock solutions, working standard solutions of ACE (20 µg/mL) and THI (20 µg/mL) were prepared by appropriate dilution and were scanned in the entire UV range to determine the maximum absorbance(λmax) and isoabsorptive point. ACE and THI have λmax at 275 nm and at 254 nm,respectively. Both the drugs were found to have same absorbance at 264 nm (iso-absorptive point). The wavelengths selected for analysis were 264 nm and 275 nm respectively (Fig.2). A series of standard solutions ranging from 10-35 µg/mL for ACE and THI both were prepared and the absorbance of solutions were recorded at 275 and 264 nm to plot a calibration curveof absorbance versus concentration. The calibration curves were found to be linear in the concentration rangeunder study. Absorptivity values of ACE andTHI were determined at selected wavelengths and are presented in Table-1.The concentration of two drugs in mixture was calculated by using following equations:

$$Cx = \frac{Q_M - Q_Y}{Qx - Q_Y} X \frac{A_1}{ax_1} \quad \cdots\cdots (3)$$

$$C_Y = \frac{Q_M - Q_X}{Q_Y - Q_x} X \frac{A_1}{aY_1} \quad \cdots\cdots (4)$$

Where, $C_X$ and $C_Y$ are the concentration of THI and ACE respectively, $Q_M$ is the ratio of absorbance of sample at 275 and 264 nm, $Q_X$ is the ratio of absorptivity of THI at275 and 364 nm, $Q_Y$ is the ratio of absorptivity of ACE at 275 and 264 nm, A is the absorbance of sample solution at 264 nm, $ax_1$ and $ay_1$ are the absorptivities of THI and ACE at 264 nm, respectively.

***Method C: Ratio First Derivative Method (RFD)***

The solutions of standard ACE and THI were prepared in the range of 10-35 µg/mL for both. The absorption spectra of the solutions of ACE and THI were recorded in the range of 200 nm to 400 nm and were stored in the memory of the instrument and Absorption spectra converted to ratio spectra by dividing ACE 10-35 µg/mL each with 35 µg/mLof THI and THI 10-35 µg/mL, each spectra is divided with 35 µg/mL ACE. Now transformed to first derivative with Δλ = 8 nm and scaling factor 10(Fig. 3, 4). Measure the amplitude at 265 nm and 240.60 against reagent blank and content calculated by substituting corrected absorbance and absorptivities of ACE and THI at regression equation of RFD.

***Assay Preparation***

For the estimation of drugs in the commercial formulations, twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Take Tablet powder equivalent to 100 mg of aceclofenac or 8 mg Thiocolchicoside and transferred to 100 mL volumetric flask. Then 92 mg of standard Thiocolchicoside was weighed & added in the same volumetric flask; 35 mL methanol was added the flask was shaken and volume was made up to the mark with Methanol and sonicated for 20 min. The

above solution was filtered through whatman filter paper (#42) discarding first few mL of Filtrate. By making appropriate dilution final solution should contain 20 µg/mL Aceclofenac & Thiocolchicoside. Absorption of the assay preparation measured at 254 nm and 275 nm against reagent blank and content calculated by substituting corrected absorbance and absorptivities of ACE and THI at two wavelengths into Vierodt's equation (Method A).Absorbance of the resulting solution was measured at 264.0 nm and 275.0 nm against methanol. The concentration of ACE and THI can be obtained by equation (Method B). Absorption spectra converted to ratio spectra as Δλ = 8 nm and scaling factor 10 and measure the amplitude at 265 nm and 240.60 against reagent blank and content calculated by substituting corrected absorbance and absorptivities of ACE and THI at regression equation of RFD (Method C).

***Validation***

The methods were validated with respect to linearity, accuracy, precision,LOD and LOQ.

***Linearity***

In method-A linearity was observe between 5-30 µg/mL for both drug while in method B and C linearity follow in the range of 10-35 µg/mL.

***Precision***

The intraday and interday precisions of the proposed spectrophotometric methods were determined by estimating the corresponding responses 3times on the same day and on 3 different days over a period of 1 week for3 different concentrations and 3 replicates of ACE and THI and the results are reported in terms of relative standard deviation(RSD); Table 1

***Accuracy***

It was determined by calculating the recovery of ACE and THI by standard addition method. Accuracy study was performed by addition of known amounts of studied drugs to a known concentration of commercial product (standard addition method). Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Aceclofenac and for Thiocolchicoside is found out. Recovery between 98 % - 102 % justifies the accuracy of the method. The preparation of solution for the recovery study is given in the Table-2.

***Limit of Detection and Limit of Quantitation***

The LOD and LOQ were determined separately base on the standard deviation of the y-intercept and slope of standard calibration curve. LOD= 3.3*σ/S and LOQ= 10*σ/S where, σ is the standard deviation ofthe intercept of regression line and S is the slope of calibration curve.

## RESULTS AND DISCUSSION

For all the three methods linearity was observed in the concentration range of 5-35 µg/ml for both ACE and THI. Marketed brand of tablet was analyzed and amount of drug determined by proposed methods ranges from 99.30 to 100.82 as shown in Table3.

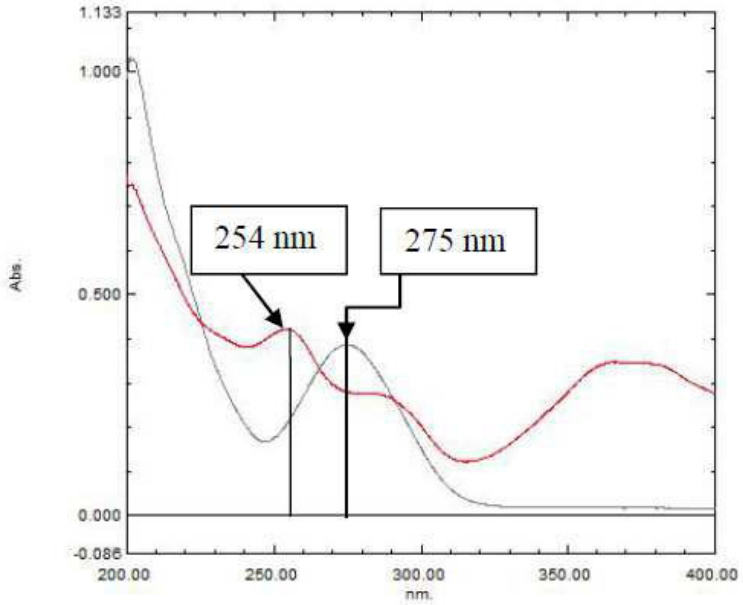



**Figure 1**
***Overlain spectra of 10 µg/mL of ACE and 10 µg/mL of THI***

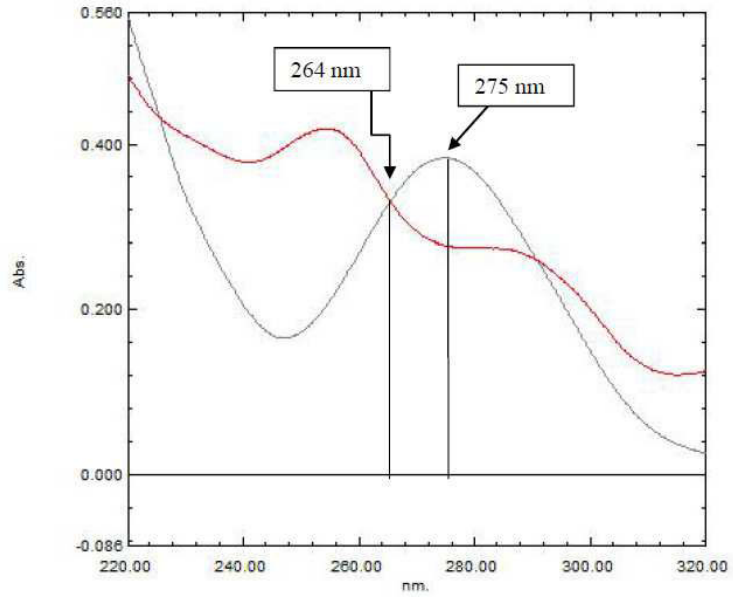



**Figure 2**
***Overlain spectra of 10 µg/mL ACE & THI for selection of Wavelength***

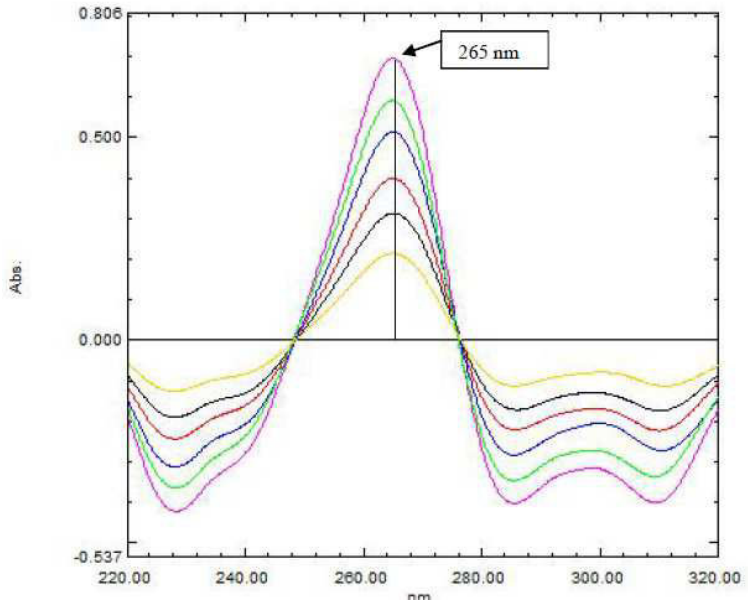



**Figure 3**
***Overlain RFD spectra of standard dilutions of ACE***

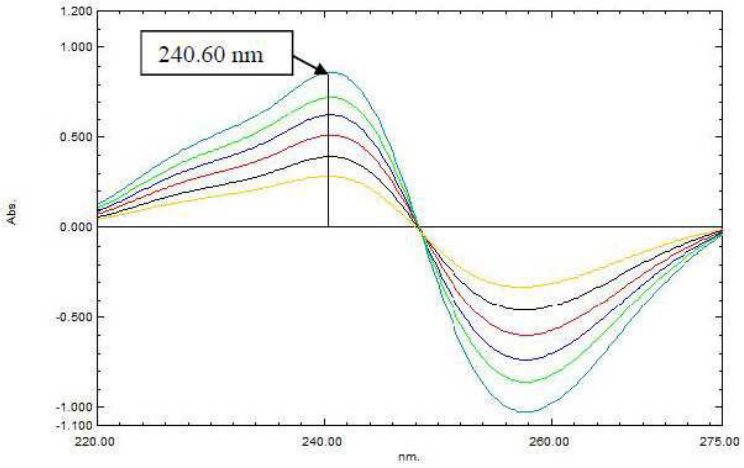



**Figure 4**
***Overlain RFD spectra of standard dilutions of THI***

**TABLE 1**
***SUMMARY OF VALIDATION PARAMETERS BY DEVELOPED METHODS***

| Parameter | Method A | | Method B | | Method C | |
|---|---|---|---|---|---|---|
| | ACE | THI | ACE | THI | ACE | THI |
| Wavelength (nm) | 275 | 254 | 275 | 264 | 265 | 240.60 |
| Beer's Law Limit(µg/ml) | 5-30 | 5-30 | 10-35 | 10-35 | 10-35 | 10-35 |
| Coefficient of correlation | 0.9981 | 0.998 | 0.9981 | 0.998 | 0.9984 | 0.9987 |
| Slope | 0.0341 | 0.0335 | 0.034 | 0.0281 | 0.0192 | 0.023 |
| Y-intercept | 0.0451 | 0.0679 | 0.047 | 0.0364 | 0.0206 | 0.0459 |
| Intraday (%RSD) | 0.97 | 0.71 | 0.67 | 0.57 | 1.33 | 0.82 |
| Interday (%RSD) | 1.32 | 1.22 | 1.05 | 0.86 | 1.44 | 1.26 |
| LOD (µg/mL) | 0.2425 | 0.2773 | 0.1147 | 0.1097 | 0.0544 | 0.1249 |
| LOQ (µg/mL) | 0.7348 | 0.8404 | 0.3467 | 0.3326 | 0.1649 | 0.3786 |

**TABLE 2**
***RESULTS FOR RECOVERY STUDIES***

| Level of recovery | Amount of drug added | Drug | Method A | | Method B | | Method C | |
|---|---|---|---|---|---|---|---|---|
| | | | Recovery (%)* | ±RSD* | Recovery (%)* | ±RSD* | Recovery (%)* | ±RSD* |
| 80 % | 4 | ACE | 98.10 | 0.5994 | 101.93 | 0.7028 | 100.26 | 1.0603 |
| | 4 | THI | 101.33 | 0.6629 | 100.23 | 0.6941 | 100.72 | 1.3459 |
| 100 % | 8 | ACE | 101.08 | 1.0507 | 100.17 | 0.9791 | 100.12 | 1.0942 |
| | 8 | THI | 101.16 | 1.0405 | 98.52 | 0.8912 | 100.54 | 0.9187 |
| 120 % | 12 | ACE | 101.57 | 0.6948 | 101.93 | 0.7322 | 101.12 | 0.7008 |
| | 12 | THI | 100.65 | 0.7998 | 101.88 | 0.7114 | 100.96 | 0.4475 |

***Mean of three estimations***

**TABLE 3**
***RESULTS OF SIMULTANEOUS ESTIMATION OF MARKETED FORMULATION (ZERODOL TH 8) FOR METHOD A, B & C***

| Method | Tablet content | Label claim* (%) | RSD (%)* |
|---|---|---|---|
| A | ACE | 100.82 | 0.65 |
| | THI | 99.69 | 0.36 |
| B | ACE | 99.30 | 0.66 |
| | THI | 100.18 | 0.92 |
| C | ACE | 100.53 | 1.04 |
| | THI | 99.94 | 0.47 |

***Mean of six estimations***

## CONCLUSION

The developed all three methods was found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drugs in two component tablet dosage form of ACE and THI. The developed method was validated according to ICH guidelines for linear relation including a coefficient of correlation, accuracy, reproducibility and precision. The RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of ACE and THI in pharmaceutical preparation.

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## REFERENCES

1. J. O. Maryadele; The Merck Index. An Encyclopedia of Chemical, Drug's and BiologicalsNeil.14th Ed: Published by Merck Research Lab, Division of Merck and co. Inc., WhitehosuseStation, NJ, 2006, 1603.
2. Qin Lua, L. C. Christine, E. C. Greg, Ultraviolet absorbance detection of colchicine and related alkaloids on a capillary electrophoresis microchip *Analytica Chimica Acta*, 2006,572, 205–211.
3. Rosso, S. Zuccaro, Determination of alkaloids from the colchicine family by reversed-phase high-performance liquid chromatography *J.Chromatography A.*,1998,825,1, 96–101.

4. W. Sutherland, M. J. Smit, L. Herbst, H. K. L. Hundt, K. J. Swart, A. F. Hundt, Highly specific and sensitive liquid chromatography–tandem mass spectrometry method for the determination of 3-desmethylthiocolchicine in human plasma as analyte for the assessment of bioequivalence after oral administration of Thiocolchicoside, *J. Chromatography A.,* 2002, 949, 1-2, 71–77
5. N. A. El-Ragehy, M. M. Ellaithy, M. A. El-Ghobashy, Determination of thiocolchicoside in its binary mixtures (thiocolchicoside–glafenine and thiocolchicoside–floctafenine) by TLC–densitometry, *Il Farmaco*, 2003, 58, 6, 463-468.
6. Indian Pharmacopoeia, Published by the controller of Publication, Delhi, 2007, 681.
7. N. Y. Hasan, E. M. Abdel, B. E. Elzeany, N. E. Wagieh., Stability indicating methods for the determination of aceclofenac, *Il Farmaco.,* 2003, 58, 2,91-99.
8. S. V. Gandhi, N. S. Barhate, B. R. Patel, D.D. Panchal, K. G. Bothara, A validated densitometric method for analysis of aceclofenac and paracetamol as the bulk drugs and in combined tablet dosage forms, *Act Chromatographi.,* 2008, 20, 2, 175-182.
9. S. S. Chitlange, G. R. Pawbake, A. I. Mulla, S. B. Wankhede, Simultaneous Spectrophotometric Estimation of Diacerein and Aceclofenac in tablet dosage form, *Der pharma Chemica.,* 2010, 2, 1, 335-341.
10. P. Musmade, G. Subramanian, K. K. Srinivasan, High-performance liquid chromatography and pharmacokinetics of aceclofenac in rats, *Anal. Chem. Acta.,* 2007, 585, 1, 103-109.
11. Ojha, R. Rathod, H. Padh, Simultaneous HPLC–UV determination of rhein and aceclofenac in human plasma, *J. of Chromatogr B.,* 2009, 877, 11-12, 1145 - 1148.
12. N. M. El Kousy, Spectrophotometric and spectrofluorimetric determination of etodolac and aceclofenac, *J. Pharm. Biomed. Anal.,* 1999, 20, 1-2, 185-194.
13. ICH, Q2 (R1), Harmonised tripartite guideline, Validation of analytical procedures: text and methodology International Conference on Harmonization ICH, Nov. 2005, Geneva.