REPORT

Development and implication of methodology for aceclofenac in pure and pharmaceutical formulations by microwave assisted spectrophotometry

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Abstract: A simple, fast and cost effective method for determination of Aceclofenac in pure and tablet formulations has been developed and validated. The method is based on formation of stable blue coloured complex (λ_{max} = 740nm) of Aceclofenac with ammonium molybdate in presence of sulfuric acid under microwave irradiation for 5min. Peak shift in FT-IR spectra also indicated the formation of complex. Measurement of absorbance was carried out at λ_{max} = 740nm. The reaction obeys Beer's law over the concentration range of 50-250µg/ml of Aceclofenac. The RSD (intra-day and inter-day precision) for the drug is not greater than 0.95% and recoveries were found to be in range of 99.01-99.99%. The proposed method can be used successfully for routine analysis of Aceclofenac in pure and tablet formulations.

Keywords: Aceclofenac, ammonium molybdate, microwave, FT-IR, spectrophotometry.

INTRODUCTION

Aceclofenac (ACF), a second generation agent and phenyl acetic acid derivative (fig. 1) is a long acting non-sedative antihistamine which is used to prevent the symptoms of allergic reactions mainly for rhinitis and chronic urticaria. It is also used in combination with a decongestant such as pseudoephedrine hydrochloride. The drug is famous for its improved pain relieving therapy and reduced side effects some of which are arrhythmias, hepatitis and hypersensitivity. Its good absorption ability makes it almost 100% bioavailable (Seth 2007).

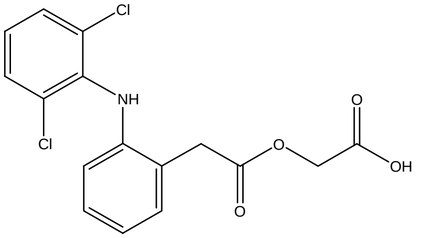

Fig. 1: Structure of Aceclofenac

Considering the importance of this drug various efforts had been put in to define methods for its analysis which range from simplest to complex e.g. titrimetric (British Pharmacopeia, 2002), spectrophotometric (Zawilla *et al*., 2002, El Kousy *et al*., 1999, Modak *et al*., 2010, Zameeruddin *et al*., 2011, Rath *et al*., 2011, Rajmane *et al*., 2009), spectrofluorimetric (El Kousy *et al*., 1999), HPLC (Shaik *et al*., 2008, Satyanarayana *et al*., 2011, Kumar *et al*., 2010, Godse *et al*., 2009), solid-liquid extraction (Bhalerao *et al*., 2010) stripping voltamerty (Posac *et al*., 1995). Most of the spectrophotometric methods were studied below 350 nm i.e. UV-region (Modak *et al*., 2010, Zameeruddin *et al*., 2011, Rath *et al*., 2011, Rajmane *et al*., 2009) while only few colorimetric methods are reported using p-dimethylaminocinnamaldehyde and p-dimethylaminobenzaldehyde along with addition of other reagents which require long heating time for compete development of the color(Zawilla *et al*., 2002, El Kousy *et al*., 1999). In these methods the mean recovery was 98.81% which improves considerably to 98.6 - 102.1% when spectroflourometric method is used but with trade off of %RSD which increases from 0.64% to ≤ 1.1 (Modak *et al*., 2010) and 2% (Satyanarayana *et al*., 2011). When compared to HPLC (with RSD >2.0 %) (Shaik *et al*., 2008), spectrophotometric methods demonstrated better besides being simple and cost-effective (with RSD 0.84% (Zawilla *et al*., 2002).

Hence, spectrophotometric methods, in general, are far more convenient and economical. Considering the importance of ACF and reliability of spectrophotometric methods, a study was initiated in order to overcome the existing disadvantages of the already developed spectrophotometric methods like more time consuming, reduced sensitivity etc. The losses of analyte, time reduction and atmospheric contamination are questionable when conventional procedures are adopted. For this purpose focus has been driven on microwave (MW) in order to overcome these problems and its application in various areas with different samples.

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MATERIALS AND METHODS

Chemicals and reagents

All the analytical grade reagents are used in this study. ACF was collected from AGP Pvt. Ltd., B-23 S.I.T.E., Karachi, Pakistan and Ammonium molybdate was purchased from BDH. Analytical grade concentrated sulfuric acid used in this study was supplied by E. Merck. Doubly distilled water and ethanol (BDH) was used throughout the study where applicable.

Instruments

Orient Microwave, Cecil CE-2041 spectrophotometer with 1cm quartz cell for the measurements of absorbance.

Preparation of stock, standard and working solutions

Stock solution of ACF (w/v) was prepared in ethanol to the concentration of 1mg/ml and stored in refrigerator. Standards were prepared by using stock solution as per requirement by further dilution. 10% (w/v) solution of ammonium molybdate was prepared in distilled water.

Preparation of pharmaceutical preparations

For evaluation tablets containing 100mg ACF were powdered, weighed. The powdered tablet was dissolved in ethanol and filtered through Whatman paper. The filtrate was further diluted with ethanol to get 1mg/ml solution of ACF.

General Procedure

To an aliquot of ACF containing 10-250µg/10ml, 0.5ml of concentrated sulfuric acid was added followed by 4ml of 10% (w/v) ammonium molybdate solution. After that the contents were given pulse for 5min in MW at 200W, allowed to cool to room temperature prior to making volume up to the mark (10ml). The absorbance of the resulting blue colour showed λ_{max} at 740nm. To confirm the development of color is due to ACF, a blank was also analyzed in which all the reagents are added except ACF.

The same experiment was repeated by doing conventional heating in water bath in order to determine the effectiveness of MW. In this case, the color developed after heating for 25min at 90°C which show low absorbance as compared to the one developed using MW. Thus, MW heating considerably improved the reaction rate and hence, colour development.

The evaluation was repeated with different concentration of ACF and after plotting absorbance against concentration a graph was obtained i.e. Standard calibration curve (fig. 2). The color reaction obeys Beer's law in concentration range of 50-250µg/ml of ACF.

Procedure for determination of Acelofenac in tablets

An aliquot of tablet solution containing 50-250µg/ml was taken in test tube and colour was developed by following the general procedure. The absorbance of developed colour has absorption maxima at 740nm. Standard calibration curve was used to calculate tablet quantity. The values found were in agreement with the reported ones.

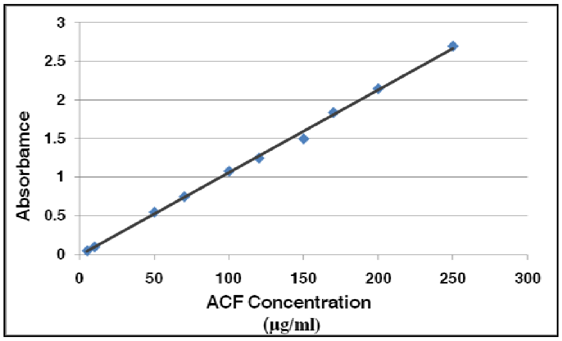

Fig. 2: Standard calibration curve of ACF

RESULTS

Absorption spectrum of coloured complex

The reaction of ACF with ammonium molybdate in presence of sulfuric acid completed after giving pulse at 200W for 5min. The blue colored complex have the absorption maxima at 740nm under optimum conditions (fig. 3).

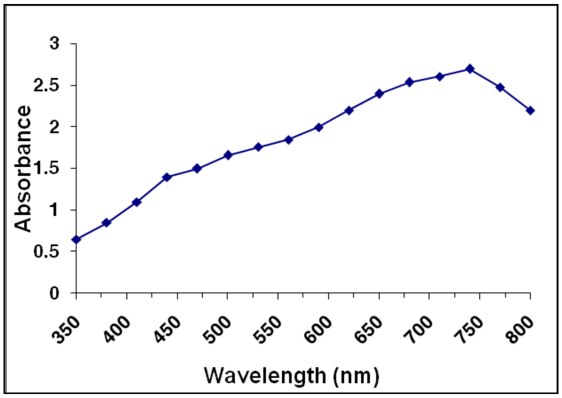

Fig. 3: λ_{max} of ACF with ammonium molybdate

Optimization of parameters

The optimum conditions for the development of maximum colour at λ_{max} 740nm were established by varying the parameters one at a time and observing the effect produced. In each of these parameters room temperature should be maintained prior to dilution and absorbance measurement.

Effect of Microwave power and conventional heating

To study the effect of MW power, 1.0ml of the ACF standard solution was taken in 12 test tubes to each of which 0.5ml of sulfuric acid and 1.0ml of 10% ammonium molybdate solution were added. The contents of 6 test tubes were pulsed for 5min by varying the MW power over the range of 80-500W and maximum absorbance was recorded at 200W. While rest of the 6 test tubes were kept in water bath at 10, 30, 50, 70, 90 and 110°C for 25 min and absorbance was taken at 740nm. The maximum absorbance was recorded at 90°C but the absorbance shown is comparatively less than the one shown by MW heating (fig. 4).

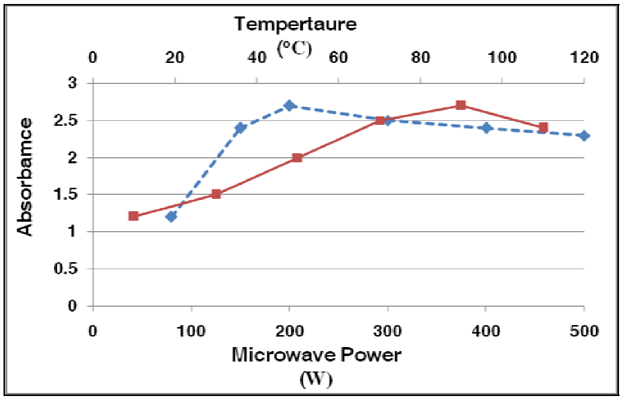

Fig. 4: Comparison between of microwave (…) and conventional (—) heating temperature on the colour development.

Heating time

Heating time evaluation for both MW and conventional was also studied between 1 to 10min by keeping the microwave power at 200W and 5-30min by maintaining temperature at 90°C, respectively. It was found that in case of MW, maximum absorbance was observed after 5min of heating while for conventional system, heating for 25min is essential for complete reaction between the drug and reagent which results in maximum absorbance under the conditions studied (fig. 5).

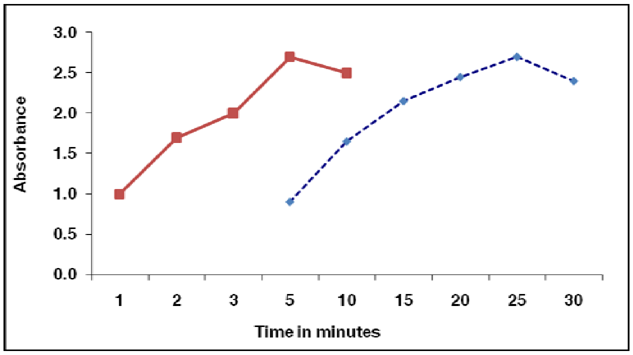

Fig. 5: Time comparision between conventional heating (…) and microwave heating (—)

Influence of reagent and acid on color development

The concentration of ammonium molybdate was studied using different amounts of w/v solutions in range of 0.1-5.0 ml. The results showed that 400 mg/ml of ammonium molybdate is optimum for ACF (fig. 6) and color stability above and below this concentration reduces. Effect of different acids, like hydrochloric acid and nitric acid was also studied and it was found that the blue colour developed in presence of these acids have low absorbance.

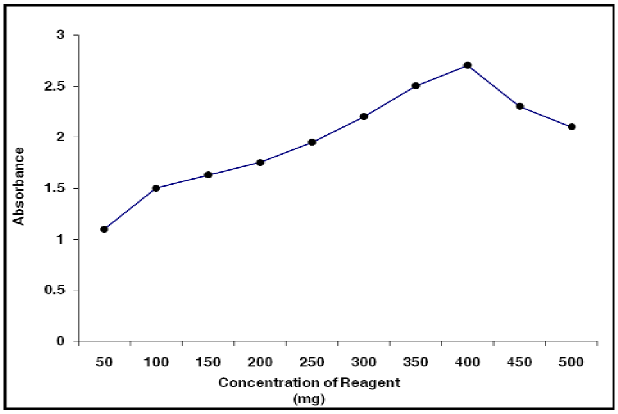

Fig. 6: Concentration of ammonium molybdate regarding the color development.

DISCUSSION

Method validation and optimization

Linearity

The optimization has been done at lower analyte concentration. A linear calibration graph between 50-250µg/ml is obtained. The value of molar absorptivity and correlation coefficient (r) was found to be 0.673 x 10^4 and 0.998. The least squares method was used to calculate the regression from ten points which is average of five determination (Christian *et al*., 2004).

Precision

Table 1 shows the results for the determination of ACF, which confirm that the method has good precision (RSD <0.95), sensitivity, validity and repeatability as indicated by the dose agreement between the actual and found values.

Table 1: Determination of aceclofenac from pure solution

Aceclofenac amount taken (µg/ml)	Aceclofenac amount found* (µg/ml)	Relative standard deviation (%)
10	10.5	0.95
20	19.95	0.50
30	29.6	0.34
60	60.1	0.164
90	90.5	0.110
100	99.0	0.101
140	139.50	0.071
200	210.50	0.047
250	250	0.04

*Each reading is calculated by taking average of five determination.

Applications in pharmaceutical formulations

The proposed method can be used successfully for routine analysis of ACE for the quality control of pure ACF and in dosage formulations. The label claim was found in the range of 99.01-99.99 mg per tablet as shown in table 3. This shows the accuracy of developed the method.

Table 2: FT-IR characteristic band of pure ACF and the complex

ν (cm^{-1})	Absorption band of ACF	Absorption band of ACF-complex
N-H	3572	3440
O-H	3268	3202
Ar C-H	2930	2925
C=O	1717	1651
C-C	1589	1401
COO	1254	1112
O-Mo-O	-	892
C-Cl	747	587

Proposed mechanism

ACF is believed to react with ammonium molybdate in acidic media by formation of some bond between carboxylic group of the ACF resulting in the shifting of C=O peak to higher wave number as shown in table 2. The aromatic peaks in the region of 900-700cm^{-1} are absent indicating the breakage of ACF molecule with release of carboxylic group that further reacts with Mo.

Characterization of colored complex

The colored complex of ACF with ammonium molybdate was isolated from solution by evaporation and characterized using FT-IR. Table 2 shows the comparison between characteristic bands of ACF and the complex.

CONCLUSIONS

The spectrophotometric method for quantitative determination of ACF in range of 50-250μg/ml had been determined with help of ammonium molybdate in acidic media. The colour development took place only in 5min which on other hand requires 25min when conventional heating was used hence, enabling quick and easy determination of the drug. The method provides a quick and economical quality tool for the micro-determination of ACF in both pure and pharmaceutical preparations. This method offers significant advantages in comparison to the other methods which are long, tedious, expensive and required many chemical reagents.

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Table 3: Determination of aceclofenac in pharmaceutical preparations

Drug (Aceclofenac)	Pharmaceutical Preparation	Amount present (Manufacturer's specifications) (mg)	Amount found* (mg)	% RSD	% Recovery
Sample 1	Tablet	100	99.99	0.74	99.99
Sample 2	Tablet	100	99.95	0.73	99.95
Sample 3	Tablet	100	99.01	0.88	99.01

*Each reading is calculated by taking average of five independent measurements.

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