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Determination of aceclofenac in bulk and pharmaceutical formulations

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Abstract

Three sensitive and reproducible methods for quantitative determination of aceclofenac (AC) in pure form and in pharmaceutical formulation are presented. The first method is based on the reaction between the drug via its secondary aromatic amino group and *p*-dimethylaminocinnamaldehyde (PDAC) in acidified methanol to give a stable coloured complex after heating at 75 °C for 20 min. Absorption measurements were carried out at 665.5 nm. Beer's law is obeyed over concentration range 20–100 ug ml^{-1} with mean recovery 100.33 ± 0.84. The other two methods are high performance liquid chromatography (HPLC) and densitometric methods by which the drug was determined in the presence of its degradation products over concentration range of 20–70 ug ml^{-1} and 1–10 ug per spot and mean recoveries are 99.59 ± 0.90 and 99.45 ± 1.09, respectively. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Aceclofenac; Stability-indicating methods; Spectrophotometric densitometric; HPLC methods

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1. Introduction

Aceclofenac (AC) 2-[[2-[2-[(2,6 dichlorophenyl) amino)phenyl)acetyl)oxy)acetic acid is a non-steroidal analgesic, antipyretic, anti-inflammatory drug [1,2]. Few methods for its determination have been reported such as titrimetric [3], stripping voltammetric [4], spectrophotometric and spectroflourimetric [5], and high performance liquid chromatography (HPLC) [6,7] methods. None of the published methods was capable of determining AC in the presence of its degradation products. The spectrophotometric method is not stability indicating, yet it has the advantage of simplicity availability of equipment, low cost and high sensitivity as it determines AC over a concentration range of 20–100 ug ml^{-1} while the official method determines 50–300 mg. The spectrophotometric method showed also good accuracy and determined AC in dosage form with mean recovery 98.81% and %R.S.D. 0.64% while in the reported spectrophotometric and spectroflourometric methods [5] the recovery was 98.6–102.1% with %R.S.D. ≤ 1.1. The proposed

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chromatographic methods had the advantage of being selective, capable of quantitative determination of AC in the presence of its degradation products, which may result from hydrolysis of AC under inappropriate storage conditions of high temperature and humidity. The recovery of AC in the proposed HPLC method is 99.59 ± 0.90 and that of the densitometric method is 99.45 ± 1.09 with intra- and inter-day assay variations of 0.03–2.72 and 1.05–1.78% for the HPLC method and 0.25–2.42 and 0.98–1.42% for the densitometric method, respectively. The reported HPLC method [6] showed a mean recovery of 93.3–96.8% with intra- and inter-day assay variations of 1.6–7.8% and 2–9.8%, respectively. A simultaneous determination of AC and diclofenac in human plasma by HPLC was reported [7] but this method did not determine AC in the presence of both degradation products. All the proposed methods were applied to pharmaceutical formulation without the interference of excipients. Statistical interpretation of the obtained results was made.

2. Experimental

2.1. Material

1. Aceclofenac standard was kindly supplied by Bristol Myers Squibb (Bristol Myers Squibb company, New York, Cairo).
2. Bristaflam tablets of batch number G01799, labelled to contain 100 mg per tablet.
3. Diclofenac standard was kindly supplied by Aarti Drugs Ltd (Rupal chemical division).

2.2. Reagents and apparatus

1. Ultraviolet/visible spectrophotometer Shimadzu 1601 pc.
2. Shimadzu-Dual wave length lamp flying cs 9301 densitometer.
3. Ultraviolet short wavelength lamp (254 nm).
4. TLC plates: Silica gel/TLC cards with flourescent indicator 254 nm; layer thickness 0.2 mm; 20 × 20 cm aluminum cards (Fluka).
5. HPLC: Hewlett Packard series 1100 equipped with a Quaternary pump, Diode array detector and a manual injector 20 ul loop.
6. Column: spherisorb ODS (200 × 4.6 mm i.d.) particle size of 5 um.
7. Ultrasonic, J.P.Selecta, s-a, CD. 300513, Espain.
8. PDAC: (Merck Dramstadt, Germany) M.p 137–140 °C, methanolic solution 0.25% w/v was stable for at least 2 weeks if refrigerated.
9. Perchloric acid: (Merck) 1% v/v solution in methanol.
10. Methanol: analytical grade (Lab Scan analytical sciences).
11. Mobile phase for densitometric method: chloroform:ethyl acetate:acetic acid (75:25:5, v/v/v) (all are analytical grade. Lab Scan analytical sciences, chloroform is from Acros organics).
12. Mobile phase for HPLC: methanol:acetonitrile:acetic acid 2% (100:150:250, v/v/v) containing 0.3 ml triethylamine (all are HPLCgrade (Lab Scan analytical sciences)). 2% v/v Acetic acid is prepared using deionised water.
13. Filter paper: Whatman number 44.

2.3. Preparation of degradation products

Two degradation products are identified for AC, diclofenac and 1-[2,6-dichlorophenyl]-2-indolinone [8]; the latter is prepared according to the method mentioned by Larsen at al. [9] M.p 128 °C and its puriy was checked through elemental analysis,TLC using chloroform:acetone: formic acid (80:10:3, v/v/v), and the IR (KBr, cm^{-1}) of the product which showed the characteristic CO band at 1715 cm^{-1} and lack the characteristic NH stretching band at 3300 cm^{-1}.

2.4. Stock solutions

Four stock solutions were freshly prepared.

Stock solution A, standard aceclofenac solution 1 mg ml^{-1} in methanol (analytical grade).

Stock solution B, standard aceclofenac solution 1 mg ml^{-1} in methanol (HPLC grade).

Stock solution C, 0.5 mg ml^{-1} of each degradation product in methanol (analytical grade).

Stock solution D, 0.5 mg ml^{-1} of each degradation product in methanol (HPLC grade).

2.5. Preparation of test solution

Weigh and grind 20 Bristaflam tablets. To a quantity equivalent to 100 mg AC in a volumetric flask 100 add 50-ml methanol, sonicate for 45 min and cool. Complete to volume with the same solvent, filter, reject first 10 ml of the filtrate. Proceed as under the procedure of each method.

2.6. Procedures

2.6.1. Spectrophotometric method

2.6.1.1. Construction of calibration curve. Transfer accurately measured aliquots from stock solution A equivalent to 0.2, 0.3, 0.4, 0.6, 0.7, 0.8 and 1 mg ml^{-1} in 10-ml volumetric flask, add 3 ml PDAC (0.25% w/v) and 1 ml 1% v/v perchloric acid. Allow the reaction to proceed at 75 °C for 20 min then cool. Complete to volume with methanol and measure the absorbance at 665.5 nm. Construct the calibration curve.

2.6.1.2. Assay of tablets. Proceed as under calibration curve using different aliquots of test solution equivalent to 0.1–1 mg AC. Calculate concentration from regression equation.

2.6.2. Densitometric method

2.6.2.1. Construction of calibration curve. Transfer accurately measured aliquots equivalent to 0.5–5 mg AC from its stock solution A into a series of 5-ml volumetric flasks, complete to volume with methanol. Apply 10 ul of each solution to HPTLC plate (20 × 20 cm) using 10-ul pipette. Spots are spaced 2-cm apart from each other and 1.5 cm from bottom edge of the plate. The plate is placed in a chromatographic tank previously saturated with developing mobile phase chloroform:ethyl acetate:acetic acid (75:20:5 v/v/v). Develop the plate by ascending chromatography for 16-cm distance, detect the spots under UV lamp (254 nm) and scan at 275 nm. Construct the calibration curve.

2.6.2.2. Assay of prepared mixtures. Transfer accurately measured aliquots of solution A equivalent to 0.5–4 mg of AC solution A into a series of 5-ml volumetric flasks, add from 10 to 90% of the degradation products using stock solution C. Apply 10 ul of each of these solutions. Proceed as under calibration curve starting from 'spots are spaced...' calculate concentration of AC from regression equation.

2.6.2.3. Assay of tablets. Proceed as under calibration curve using different aliquots of test solution equivalent to 0.5–5 mg AC. Calculate concentration from regression equation.

2.6.3. HPLC method

2.6.3.1. Construction of calibration curve. Transfer accurately measured aliquots of solution B equivalent to 200–700 ug AC to 10-ml volumet-

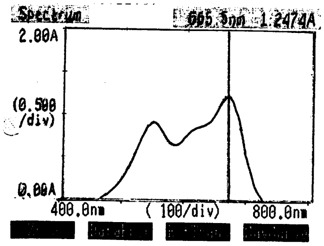


Fig. 1. Spectrophotometric spectrum of the formed complex.

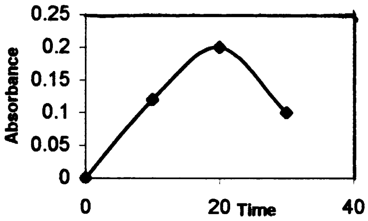


Fig. 2. Absorption intensity of the coloured complex as a function of heating time.

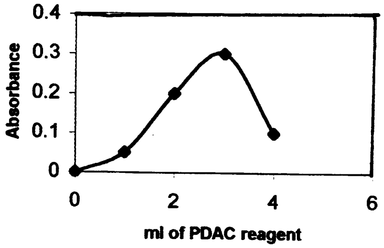


Fig. 3. Effect of PDAC concentration on colour development.

ric flask and complete to volume with methanol (HPLC grade), inject 20 ul of each solution to HPLC (Hewlett Packard 1100) using the mobile phase methanol:acetonitrile:2% acetic acid (100:150:250 v/v/v) containing 0.3 ml triethylamine. Carry out detection at 275 nm, flow rate is 1 ml min^{-1} construct calibration curve.

2.6.3.2. Assay of prepared mixtures. Transfer accurately measured aliquots equivalent to 200–700 ug AC from stock solution B to 10 ml volumetric flask add from 10–90% of degradation products using stock solution D, complete to volume with methanol. Proceed as under calibration curve starting from 'inject 20 ul…'. Calculate concentration from regression equation.

2.6.3.3. Assay of tablets. Proceed as under calibration curve using different aliquots of test solution equivalent to 200–700 ug AC. Calculate concentration from regression equation.

3. Results and discussion

3.1. For spectrophotometric method

The reaction between secondary aromatic amines and *p*-dimethylaminocinnamaldehyde (PDAC) is assumed to take place through condensation of protonated secondary amino group with the carbonyl group of the reagent to produce imminium salt. This reaction is not stability indicating since diclofenac has an aromatic secondary amino group and have been determined by PDAC [10], but the method is valuable for being simple, has low cost and sensitive routine method for the determination of AC in pure and in dosage forms reaction scheme [10].

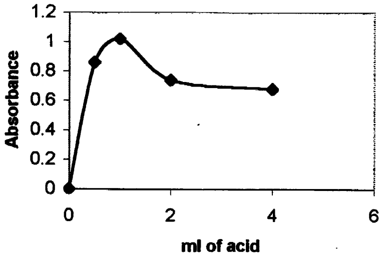
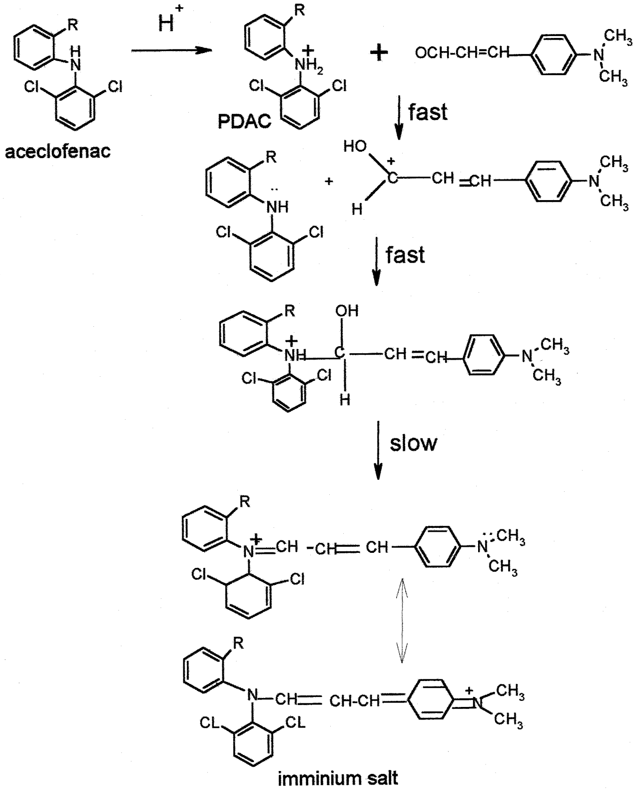


Fig. 4. Effect of acid concentration on the intensity of the coloured product of PDAC with AC using 1% $HClO_4$.

reaction scheme[10]



3.1.1. Optimisation of variables

Investigation was carried out to establish the most favourable conditions for the reaction illustrated in the scheme and to achieve maximum colour development at λ_{max} 665.5 nm (Fig. 1) in the quantitative estimation of AC.

The effect of each of the following variables was studied.

3.1.2. Effect of heating temperature, and heating time at assigned temperature

Maximum colour formation was obtained by heating in a water bath at 75 °C for 20 min (Fig. 2).

3.1.3. Effect of PDAC concentration

Different volumes of PDAC reagent (0.25% w/v in methanol) were used. Maximum absorbance was achieved with 3 ml of the reagent (Fig. 3).

3.1.4. Effect of acid type, concentration and volume

The procedure was carried out using methanolic solution of different organic and inorganic acids such as sulphuric, hydrochloric, acetic, perchloric and citric acid. The investigation showed that maximum colour development occurred with 1 ml of 1% v/v perchloric acid (Fig. 4). This may be attributed to specific ion effect.

3.1.5. Choice of suitable solvent

Several solvents were investigated such as water, methanol, ethanol and propanol. Water was reported [9] to cause hydrolysis of the formed imminium salt leading to disappearance of the colour. Methanol was found to be the best solvent giving maximum intensity and stability of the formed colour.

3.1.6. Using the molar ratio method to get the proportion of reaction

By applying this method it was found that AC and PDAC react in the ratio of 1:1 i.e. one molecule was needed from each of the drug and the reagent for completing the reaction.

The linear regression equation for the spectrophotometric method is

$$\text{Absorbance} = 0.01 \times \text{concentration} - 0.01$$

The %R.S.D. values of the slope and intercept for the linearity study were 1.10 and 2.01%, respectively.

3.2. Densitometric method

Several mobile phases were tried to accomplish complete separation of AC from its degradation products. Using the mobile phase chloroform:ethyl acetate:acetic acid (75:20:5, v/v/v) and silica gel/TLC cards with flourescent indicator 254 nm; layer thickness 0.2 mm; 20 × 20 cm aluminum cards (Fluka) complete separation was attained where

R_F of AC = 0.7

R_F of 1-(2,6-dichlorophenyl)-2-indolinone = 0.8

R_F of diclofenac = 0.8

By applying this technique a linear correlation was obtained between the area under the peak and the concentration in the range of (1–10 ug per spot) from which the linear regression equation was calculated.

$$\text{Area} = 2000.70 \times \text{concentration} + 144.20$$

$$r = 0.9980$$

The %R.S.D. of the slope and intercept for the linearity study were 0.16 and 1.58%, respectively.

The proposed method is valid for the determination of AC in the presence of 10–90% of the degradation products, with mean recovery 99.78 ± 0.56.

3.3. HPLC method

3.3.1. Optimisation of chromatographic procedure

Several mobile phases were used, those that were known to quantify diclofenac showed bad resolution of AC from its degradation products. The mobile phase methanol:acetonitrile:acetic acid 2% (100:150:250) containing 0.3 ml triethylamine showed good resolution, good peak symmetry Fig. 5.

R_t of AC = 8.87 min

R_t of 1-(2,6-dichlorophenyl)-2-indolinone = 10.27 min

R_t of diclofenac = 12.49 min

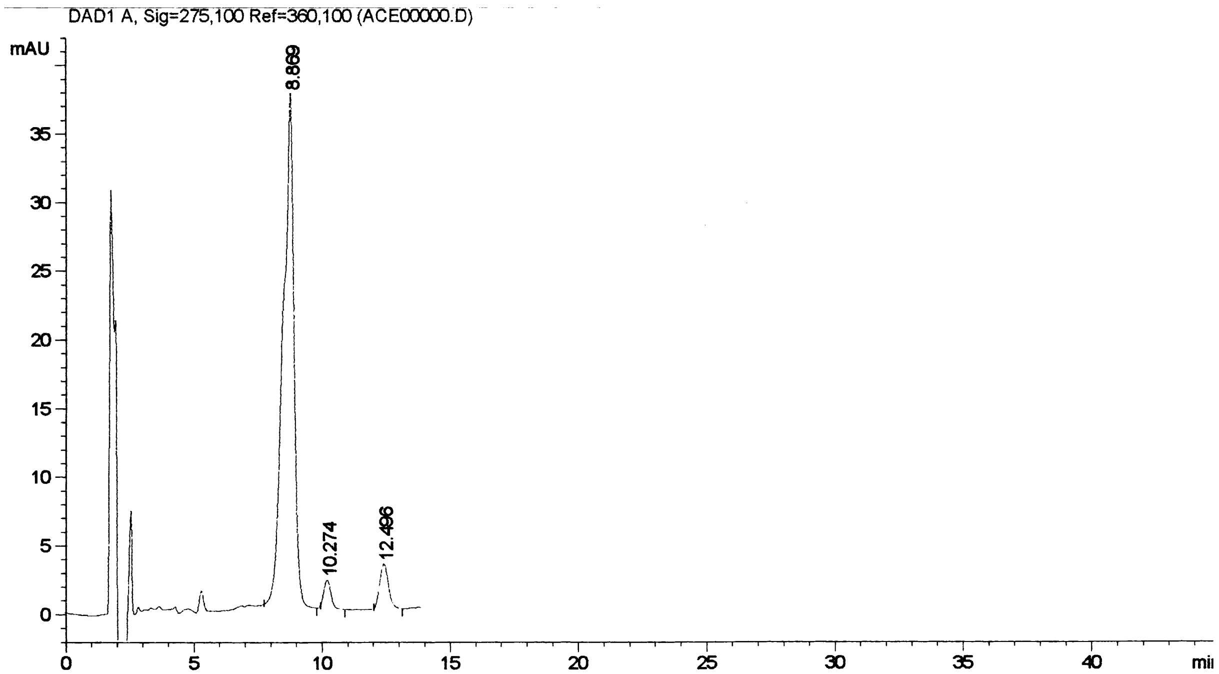


Fig. 5. A typical HPLC chromatogram of AC and its degradation products. R_t of AC is 8.87 min, 1-[2,6-dichlorophenyl]indolinone] is 10.74 min and of diclofenac is 12.49 min.

Table 1
The results of the proposed methods and official method (B.P 2000) in determination of pure drug

Experiment number	Spectrophotometric method[a] (found %)	Densitometric method[a] (found %)	HPLC method[a] (found %)	Official method[a] (found %)
1	101.36	98.30	99.70	99.00
2	100.94	98.80	99.85	100.50
3	99.20	99.00	99.16	100.20
4	100.01	100.27	100.83	99.40
5	100.15	100.90	98.40	100.00
Mean	100.33	99.45	99.59	99.82
%R.S.D.	0.84	1.09	0.90	0.61

[a] Each result is the average of three experiments.

The composition of the mobile phase was adjusted after varying the organic to aqueous ratio to give the best results. Increasing the proportion of acetonitrile results in complete separation of the three peaks. A further increase in the aqueous proportion results in prolonged retention times. Variable columns were used such as hypersil 5 um BDS C_{18} (250×4.6 mm), u Bondapack 5 um (300×4.6 mm), spherisorb ODS 5 um (200×4.6). All the previous columns showed resolution not less than 10, peaks are sharp and symmetric, eluted within 20 min. Spherisorb was used as it showed minimum elution time with good resolution and it was employed for method validation.

By applying this technique a linear correlation was obtained between the area under the peak and the concentration in the range 20–70 ug ml^{-1} from which the linear regression equation was calculated

$$\text{Area} = 17.77 \times \text{concentration} - 5 \quad r = 0.9990$$

The %R.S.D. of the slope and intercept for the linearity study were 0.4 and 0.85%, respectively.

The proposed method is valid for the determination of AC in the presence of 10–90% of the degradation products with mean recovery of 99.82 ± 0.5.

All solutions are freshly prepared to ensure stability of analyte in solution.

3.4. Validation of the proposed methods

3.4.1. Precision and accuracy

The accuracy and reproducibility of the results in terms of % recovery of pure samples of intact drug analysed by the proposed methods were shown (Table 1). Statistical comparison of the results showed no significant difference (Table 2). Intra-day precision and accuracy of the proposed methods were evaluated by assaying freshly prepared solutions in triplicates at three different

Table 2
Comparison between the results of the official and proposed methods in determination of pure samples

	Official method	Spectrophotometric method	Densitometric method	HPLC method
Range of concentration	50–300 mg	20–100 ug ml^{-1}	1–10 ug per spot	20–70 ug ml^{-1}
Correlation coefficient	–	0.9970	0.9980	0.9990
N	5	5	5	5
$F(6.39)$[a]	–	1.92	3.22	2.19
$t(2.31)$[a]	–	1.11	0.66	0.48

[a] Figures in parentheses represent corresponding tabulated values for F and t at $P = 0.05$.

Table 3
Intra- and inter-day assay variations of AC by the proposed methods

	Spectrophotometric method			Densitometric method[a]			HPLC method		
Intra-day									
0 Day									
Mean of concentration (ug ml^{-1}) $n = 3$	19.78	49.66	78.29	3.985	5.948	7.902	20.24	38.74	61.37
S.D.	0.30	0.56	0.35	0.055	0.066	0.029	0.28	0.59	0.32
%R.S.D.	1.52	1.13	0.45	1.38	1.11	0.37	1.38	1.52	0.52
1 Day									
Mean of concentration (ug ml^{-1}) $n = 3$	20.20	50.24	79.69	3.927	6.005	7.848	19.97	39.36	60.50
S.D.	0.34	0.44	0.81	0.095	0.064	0.047	0.42	0.01	0.36
%R.S.D.	1.68	0.88	1.02	2.42	1.07	0.60	2.10	0.03	0.60
2 Days									
Mean of concentration (ug ml^{-1}) $n = 3$	19.64	49.81	79.70	4.031	6.066	8.065	19.83	40.14	60.77
S.D.	0.56	1.03	0.74	0.010	0.019	0.026	0.54	0.77	0.68
%R.S.D.	2.85	2.07	0.93	0.25	0.31	0.32	2.72	1.91	1.12
Inter-day									
Mean of concentration (ug ml^{-1}) $n = 3$	19.87	49.90	79.23	3.981	6.006	7.938	20.01	39.41	60.88
S.D.	0.29	0.30	0.81	0.052	0.059	0.113	0.21	0.70	0.45
%R.S.D.	1.46	0.60	1.02	1.31	0.98	1.42	1.05	1.78	1.74

[a] Concentration in densitometric method is ug per spot.

Table 4
Comparison between the results of official and proposed chromatographic in determination of AC in the presence of its degradation products

Sample number	% Degradation	Official method[a]	HPLC method[a]	Densitometric method[a]
1	10	107.22	99.80	99.51
2	30	127.60	98.90	98.90
3	50	145.30	100.18	100.30
4	70	175.40	100.20	100.44
5	80	190.15	99.70	99.82
6	90	200.60	100.15	99.70
Mean	–	–	99.82	99.78
%R.S.D.	–	–	0.50	0.56

[a] Each result is the average of three experiments.

concentrations for the three methods. Inter-day precision and accuracy of the proposed methods were evaluated by assaying freshly prepared solutions in triplicates for 3 days (Table 3).

3.4.2. Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences (degradation products, related substances, excipients). Specificity was checked by adding all the known degradation products to pure AC samples and the response of the analyte in the mixture was evaluated by the proposed densitometric and HPLC methods showing accurate and precise results while on applying the official method it showed unacceptable results (Table 4). Specificity was also checked by stressing pure samples under stress conditions such as acid hydrolysis (dissolve 100 mg in 20 ml ethanol and reflux with 1 N H_2SO_4 for 1 h) and applying the proposed methods, resolution of AC from its degradation products was attained. In the application of the proposed methods to pharmaceutical formulation no interference of the excipients peaks with the peaks of interest appeared, hence the proposed methods is applicable for quantitative determination of AC in pharmaceutical dosage forms applying standard addition technique to ensure the accuracy of the proposed methods (Table 5).

3.4.3. Sensitivity

The proposed methods were capable to determine AC at low concentration level up to 1 ug per spot for densitometric method and 20 ug ml^{-1} for both HPLC and spectrophotometric methods.

3.4.4. Stability

Analysing commercial formulation kept at room temperature on laboratory bench or in the refrigerator showed no degradation products. Under stressed conditions both degradation products were separated with the proposed chromatographic methods. Using the mobile phase of either of the proposed methods did not cause degradation of the drug.

3.4.5. Limit of detection and limit of quantitation

Limit of detection (LOD) represents the concentration of analyte that would yield a signal-to-noise ratio of three [11]. LOD for AC in HPLC and densitometric methods is 1.7 ug ml^{-1} and 78 ng per spot, respectively. The limit of quantitation

Table 5
Application of the proposed methods in the determination of pharmaceutical preparation

	Bristaflam tablets recovery% ± %R.S.D.	Standard addition ± %R.S.D.
Spectrophotometric method	98.81 ± 0.64	99.75 ± 0.51
Densitometric method	99.68 ± 0.48	100.01 ± 0.74
HPLC method	99.50 ± 0.38	99.50 ± 0.64

(LOQ) represents the concentration of analyte that would yield a signal-to-noise ratio of 10 [11]. LOQ for AC in HPLC and densitometric methods is 5.7 ug ml^{-1} and 260 ng per spot, respectively.

4. Conclusion

Three methods for determination of AC in both pure form and pharmaceutical formulation were proposed. The first method is spectrophotometric, it is not stability indicating, yet it has the advantages of simplicity, low-cost availability of equipment, it is more sensitive than the official method [3]. The other two methods are HPLC and densitometric methods, both methods have the advantage of being stability indicating and capable of differentiating between AC and its two degradation products, also they are more accurate and less intra- and inter-day variations than the reported HPLC method [6]. The proposed methods showed no interference with excipients in the dosage form. The results obtained from the three methods are compared statistically with those obtained using the official method indicating no significant difference in determination of intact pure drug between them, however, unaccepted results were obtained on applying the official method in the presence of degradation products. Validity was proved by standard addition where the percentage recovery was about the same as the pure substance.

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