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# Stability-indicating HPLC and PLS chemometric methods for the determination of acemetacin in presence of its degradation products and impurities

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ABSTRACT

Two stability-indicating methods were developed and validated for the quantitative determination of acemetacin (ACM) in presence of its degradation products and impurities. The first method was based on separation of ACM from its degradation products and impurities by RP-HPLC on Inertsil $C_8$ column (150 × 4.6 mm i.d) using a mobile phase composed of 0.02 M phosphate buffer: methanol (35:65, *v*:*v*, pH = 6.5). The flow rate was adjusted at 1 mL/min and quantification was achieved with UV detection at 245 nm using meloxicam as internal standard. The second method was based on multivariate spectrophotometric analysis using partial least square regression model. The drug was subjected to acid, base, oxidative and thermal stress conditions and the degradation products were identified. The developed methods have the requisite accuracy, selectivity, sensitivity and precision to assay ACM in presence of its degradation products and impurities either in bulk powder or in pharmaceutical dosage form. The results obtained for the analysis of ACM in its pure form by the proposed methods were statistically compared to those obtained by applying a reported HPLC method. The statistical comparison showed that there is no significant difference between the proposed methods and the reported one with respect to accuracy and precision.

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## 1. Introduction

Stability-indicating methods provide useful information about the degradation pathways and degradation products that can be formed during storage. The information thus obtained will facilitate pharmaceutical development in areas such as formulation development, manufacturing, and packaging, where knowledge of chemical behavior can be used to improve the quality of drug product.

Acemetacin is a glycolic acid ester of indomethacin, which is a non-steroidal anti-inflammatory drug (NSAID) (Figure 1). Its pharmacological activity is due to both ACM and its major metabolite indomethacin. ACM is used in rheumatoid arthritis, osteoarthritis, low back pain, postoperative pain and inflammation [1-3]. The efficacy of ACM is similar to that of indomethacin in the treatment of inflammatory and rheumatic disorders but it is generally better tolerated than Indomethacin; in particular it produces fewer gastrointestinal side effects [4-9].

Several methods have been reported for the analysis of ACM, including spectrophotometric [10-14], chromatographic [12,14-26] and electrochemical methods [27-29] for the determination of ACM either alone or in combination with other NSAIDs in biological fluids and pharmaceutical formulations. Stability indicating HPTLC and spectrophotometric methods have been reported for ACM [30] but the degradation pathway was different from the proposed one.

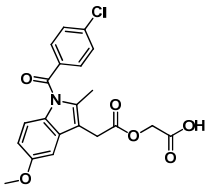


**Figure 1.** Chemical structure of acemetacin.

HPLC is one of the most useful and widely used analytical techniques. Compared to other separation procedures, HPLC is unique in possessing almost universal applicability and remarkable assay precision. Chemometrics is a chemical discipline that uses mathematics, statistics and formal logic to design or select optimal experimental procedures, to provide maximum relevant information by analyzing chemical data and to obtain knowledge about chemical systems [31,32]. No


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stability indicating HPLC or chemometric methods have been reported for the determination of ACM in presence of its degradation products and impurities. Consequently, the aim of the present work was to develop fast, simple and accurate methods for the assay of ACM in presence of its degradation products and impurities. Moreover, the degradation products obtained were identified by comparing their retention times against a standard solution of each using HPLC.

## 2. Experimental

### *2.1. Instrumentations and software*

#### *2.1.1. HPLC*

HPLC analysis was performed using Agilent HPLC instrument (1100 series) Japan, equipped with an isocratic pump model G1310A, connected with a UV detector model G1314A. The injector was a manual Rheodyne injector (Model G1328B, USA) equipped with a 20 µL injector loop and a 100 µL Agilent syringe. The instrument was connected to an IBM compatible PC bundled with Agilent Chemstation Chromatography data station software HPLC septum manager and an HP 5700 Deskjet printer. pH meter (Jenway-3510, UK). Sonicator (Soniclean-120 T, Australia). Column (Inertsil $C_8$-150 × 4.6 mm, 5 µm, Japan).

#### *2.1.2. Multivariate analysis*

Measurements were carried out on a Shimadzu 1650 double beam UV-PC spectrophotometer connected to a computer loaded with Shimadzu software UV probe 2.10, (Hiroshima, Japan). UV spectra were recorded using a 1 cm quartz cell. Scans were carried out in the range of 200-400 nm with 0.1 nm intervals. The computations were done using Matlab® 7.0.1 software [33]. PLS routine work was carried out using "PLS Toolbox" [34].

### *2.2. Materials*

#### *2.2.1. Pure standard materials*

Acemetacin standard material was supplied by Multi-Apex Pharma, D plot 21, Misr Suez Desert Rd., Industrial Zone, Badr City, Cairo, Egypt and it was certified to contain 99.92% *w*:*w* according to the manufacturer's method. Indomethacin standard material was supplied by AUG Pharma, 6th Industrial Zone, 6 October City, Egypt. Meloxicam standard material was supplied by Misr Pharmaceutical Industries, 92 Matareya St., El Matareya, Cairo, Egypt. *p*-Chlorobenzoic acid was supplied by Merck-Schuchardt, Dr. Tu. Schuchardt & Co. D-85662, Hohenbrunn, Germany. 5-Methoxy-2-methyl-3-indole acetic acid was supplied by Sigma-Aldrich Co., 3050 Spruce Street, St. Louis, MO, 63103, USA.

#### *2.2.2. Pharmaceutical formulations*

Ost-Map® capsules (Batch No. MT2760712) labeled to contain 60 mg of Acemetacin per capsule were manufactured by Multi-Apex for Pharmaceutical Industries, Societe Anonyme Egyptienne (S.A.E.), Badr City, Egypt.

#### *2.2.3. Chemicals and reagents*

All chemicals and reagents used throughout this work were of analytical grade and solvents were of HPLC grade: Sodium hydroxide (Adwic, Egypt), potassium dihydrogen phosphate (Adwic, Egypt), *o*-phosphoric acid (Adwic, Egypt), hydro-chloric acid (Sigma-Aldrich, Germany), hydrogen peroxide (Adwic, Egypt) and methanol (Sigma-Aldrich, Germany).

### *2.3. Solutions*

#### *2.3.1. Stock standard solutions*

*Acemetacin stock standard solution*: ACM standard solution (100 µg/mL) was prepared by accurately weighing 10 mg of ACM into 100 mL volumetric flask, dissolve in and complete to volume with methanol.

*Indomethacin stock standard solution*: Indomethacin standard solution (100 µg/mL) was prepared by accurately weighing 10 mg of indomethacin into 100 mL volumetric flask, dissolve in and complete to volume with methanol.

*Meloxicam stock standard solution*: Meloxicam standard solution (100 µg/mL) was prepared by accurately weighing 10 mg of meloxicam into 100 mL volumetric flask, dissolve in and complete to volume with methanol.

*p-Chlorobenzoic acid (PCBA) stock standard solution*: PCBA standard solution (100 µg/mL) was prepared by accurately weighing 10 mg of PCBA into 100 mL volumetric flask, dissolve in and complete to volume with methanol.

*5-Methoxy-2-methyl-3-indole acetic acid stock standard solution*: 5-Methoxy-2-methyl-3-indole acetic acid standard solution (100 µg/mL) was prepared by accurately weighing 10 mg into 100 mL volumetric flask, dissolve in and complete to volume with methanol.

#### *2.3.2. Degradation products stock solution*

##### *2.3.2.1. Alkaline degradation*

An accurately weighed amount of pure ACM (10 mg) was dissolved in 10 mL 0.1 N NaOH in a beaker and left for 30 min at room temperature. The solution was then neutralized with 0.1 N HCl and transferred quantitatively to 100 mL volumetric flask. The volume was then completed using methanol to produce concentration equivalent to 100 µg/mL. The degradation process was followed every 5 minutes and complete degradation was confirmed by HPLC using methanol: 0.02 M phosphate buffer (65:35, *v*:*v*), pH was adjusted to 6.5 using *o*-phosphoric acid.

##### *2.3.2.2. Acid degradation*

An accurately weighed amount of pure ACM (10 mg) was dissolved in 20 mL methanol, 10 mL 0.1 N HCl were added and refluxed for 3 hours in boiling water bath. The solution was neutralized with 0.1 N NaOH, transferred quantitatively to 100 mL volumetric flask and completed to volume using methanol to give final concentration equivalent to 100 µg/mL. The degradation process was followed every 15 min and complete degradation was confirmed by HPLC using methanol: 0.02 M phosphate buffer (65:35, *v*:*v*) at pH = 6.5.

##### *2.3.2.3. Oxidative degradation*

An accurately weighed amount of pure ACM (10 mg) was dissolved in 20 mL methanol in a beaker, 10 mL 3% $H_2O_2$ were added and left at room temperature for 3 hours. The solution was then transferred quantitatively to 100 mL volumetric flask and completed to volume with methanol to give final concentration equivalent to 100 µg/mL. The degradation process was followed every 15 minutes by HPLC using methanol: 0.02 M phosphate buffer (65:35, *v*:*v*), pH was adjusted to 6.5 using *o*-phosphoric acid.

##### *2.3.2.4. Thermal degradation*

An accurately weighed amount of pure ACM (10 mg) was kept in oven at 140 °C for 3 hours then dissolved in methanol and transferred to a 100 mL volumetric flask. The solution was completed to volume with methanol to produce concentration

equivalent to 100 µg/mL. The degradation process was followed every 15 minutes by HPLC using methanol: 0.02 M phosphate buffer (65:35, *v*:*v*), pH was adjusted to 6.5 using *o*-phosphoric acid.

### *2.4. Procedure*

#### *2.4.1. HPLC method*

##### *2.4.1.1. Calibration of standard solutions*

Different aliquots (0.01-5.00 mL) of ACM standard solution (100 µg/mL), equivalent to 0.1-50.0 µg/mL, were transferred into a series of 10 mL volumetric flasks and the volumes were adjusted with the mobile phase. An aliquot of 20 µL of each solution was injected into the chromatographic system and processed according to the previously described conditions.

##### *2.4.1.2. Accuracy*

The Accuracy was checked by applying the proposed method for the determination of different ACM samples within the linearity range. The concentrations were obtained from the corresponding regression equation. The percentage recoveries and RSD values were then calculated.

##### *2.4.1.3. Precision*

###### *2.4.1.3.1. Repeatability*

Three concentrations of ACM (10, 20 and 30 µg/mL) were analyzed in triplicates three times intra-daily using the previously mentioned procedure under calibration of standard solutions and RSD values were calculated.

###### *2.4.1.3.2. Intermediate precision*

The previously mentioned ACM concentrations under repeatability were analyzed in triplicates on three successive days using the procedure mentioned under calibration of standard solutions and RSD values were calculated.

##### *2.4.1.4. Robustness*

Robustness of the method was tested by applying deliberate variations in flow rate, pH of mobile phase buffer and organic composition of mobile phase. The RSD values were then calculated.

##### *2.4.1.5. System suitability parameters*

System suitability was checked by calculating different parameters such as capacity factor, tailing factor, column efficiency (N), selectivity and resolution factors.

#### *2.4.2. PLS chemometric method*

##### *2.4.2.1. Calibration and validation sets*

For the construction of calibration and validation sets, twenty five mixtures of ACM, indomethacin, PCBA and 5-methoxy-2-methyl-3-indole acetic acid in different ratios were prepared using five level four factor experimental design where different volumes were transferred from their corresponding stock solutions (100 µg/mL) into a series of 10 mL volumetric flasks and the volumes were completed with 0.01 N HCl. From this mixed set, eight samples were randomly chosen and used for external validation (validation set) and the rest of the samples were used for construction of the regression model (calibration set).

##### *2.4.2.2. Spectral characteristics of ACM, indomethacin, PCBA and 5-methoxy-2-mehyl-3-indole acetic acid*

The absorption spectra of calibration and validation samples were recorded in the range of 200-400 nm. The recorded spectra were then transferred to MATLAB® 7.0.1 for subsequent data analysis.

#### *2.4.3. Application to pharmaceutical formulations (Ost-Map® capsules)*

Ten capsules (labeled to contain 60 mg ACM) were emptied and accurately weighed. An accurate weight equivalent to 10 mg ACM was transferred into a 100 mL volumetric flask, extracted with 50 mL methanol in an ultrasonic bath for 15 minutes and diluted to volume with the same solvent. The solution (100 µg/mL) was sonicated again for 10 minutes in the ultrasonic bath and filtered. An aliquot (1 mL) was transferred into a 10 mL volumetric flask and the volume was completed with the mobile phase for HPLC method and with 0.01 N HCl for the PLS chemometric method to produce solutions of concentrations equivalent to 10 µg/mL of ACM. The procedure was completed as described under calibration step. For HPLC method, the concentration of ACM was calculated from the corresponding regression equation. For PLS model, the zero-order spectra of the prepared solution were recorded in the range of 200-400 nm. Then the recorded spectra were transferred to MATLAB® 7.0.1 for subsequent data analysis.

## 3. Results and discussion

ACM was subjected to forced degradation under different stress conditions: Acidic (reflux with 0.1 N HCl for 3 hours in boiling water bath), basic (0.1 N NaOH for 30 min at room temperature), oxidative (3% $H_2O_2$ for 3 hours at room temperature) and thermal stress conditions (140 °C for 3 hours).

The degradation process was monitored by HPLC ($C_8$ column, mobile phase: methanol:0.02 M phosphate buffer (65:35, *v*:*v*), pH adjusted to 6.5 using *o*-phosphoric acid and UV detection at 245 nm). It was found that ACM is highly unstable in alkaline medium where it undergoes complete degradation under mild conditions giving two degradation products. In acidic conditions, ACM is degraded but under stress conditions (reflux for 3 hours in boiling water bath) giving the same degradation products formed under alkaline hydrolysis, Figure 2. ACM shows significant stability under oxidative and thermal stress conditions.

### *3.1. Identification of ACM degradation products*

ACM is liable to alkali and acid hydrolysis through cleavage of the amide and the ester linkages giving two degradation products. By reviewing the reported stability-indicating methods for indomethacin [35-37] which possesses almost similar structure to ACM, the expected degradation products were *p*-chlorobenzoic acid and 5-methoxy-2-methyl-3-indole acetic acid. Consequently, standard solutions of the expected degradation products were prepared and injected under the same chromatographic conditions. Elution of the standard solutions at the same retention times confirmed the presence of *p*-chlorobenzoic (PCBA) acid and 5-methoxy-2-methyl-3-indole acetic acid as degradation products under both alkaline and acidic stress conditions. Indomethacin appeared as an intermediate degradation product in acid hydrolysis, Figure 3. The expected degradation pathway can be represented in Scheme 1.

By referring to the British Pharmacopoeia [38], PCBA and indomethacin are reported as impurities for ACM.

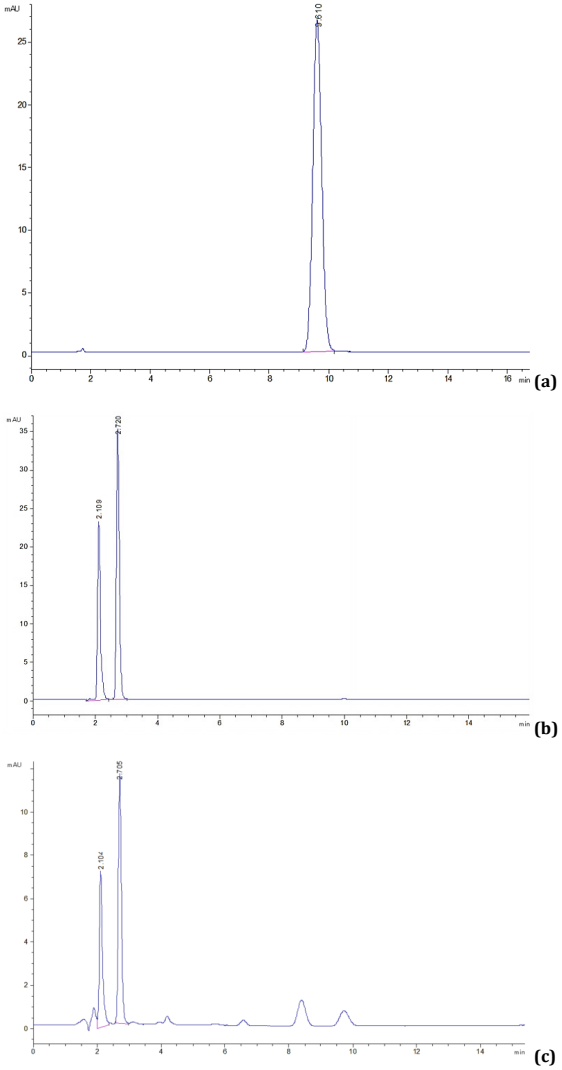



**Figure 2.** (a) HPLC chromatogram of pure acemetacin 10 µg/mL ($t_R$ = 9.610 min), (b) HPLC chromatogram of alkaline-induced degradation products equivalent to 10 µg/mL ($t_R$ = 2.109 and 2.720 min), (c) HPLC chromatogram of acid-induced degradation products equivalent to 10 µg/mL ($t_R$ = 2.104 and 2.705 min).

PCBA is also a degradation product and a minor metabolite, while indomethacin is considered as a major metabolite [39]. Furthermore, indomethacin is used as an intermediate in the synthesis of ACM [40], which represents a valuable modification of indomethacin due to its better tolerability and less gastric damage [41,42]. Hence, it's important to detect the presence of indomethacin as impurity.

Finally, by applying the chromatographic conditions mentioned above, it was possible to determine ACM in the presence of its degradation products and impurities using meloxicam as internal standard, Figure 4.

### *3.2. Optimization of chromatographic conditions*

In order to optimize the proposed chromatographic conditions, several trials were carried out to obtain good and optimum separation of ACM from its degradation products and impurities.

#### *3.2.1. Effect of changing the organic modifier ratio*

The effect of changing the ratio of the organic modifier on the retention times was investigated. Different ratios of methanol were tried in the range of 60-90%. It was found that methanol in the ratio of 65% was optimum for separation as lowering the ratio of methanol below 65% resulted in very high retention times and increasing the methanol ratio above 65% resulted in faster elution but showed overlap between ACM and indomethacin.

#### *3.2.2. Effect of changing pH*

Different pH values were tried in the range of pH = 4.5 to 6.5. The low pH values resulted in faster elution but showed overlap between the peaks of ACM and indomethacin. Besides, the reported pka values for ACM (pKa = 2.9) and Indomethacin (pKa = 4.5) [43], prove that pH = 6.5 is suitable for the determination of both ACM and indomethacin.

#### *3.2.3. Effect of changing buffer*

Upon trying different buffers as phosphate and acetate buffers, no change was observed in the symmetry or the retention times of the separated peaks.

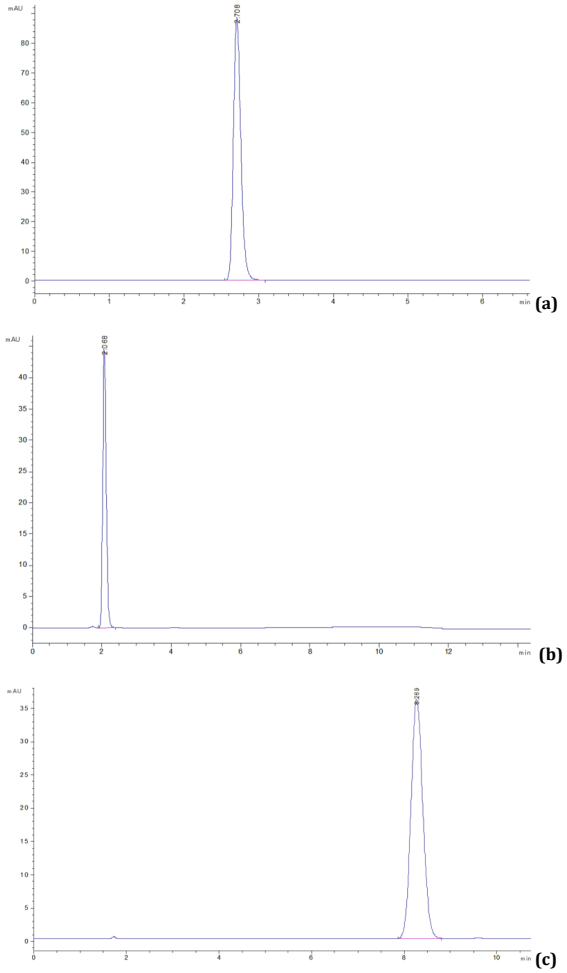
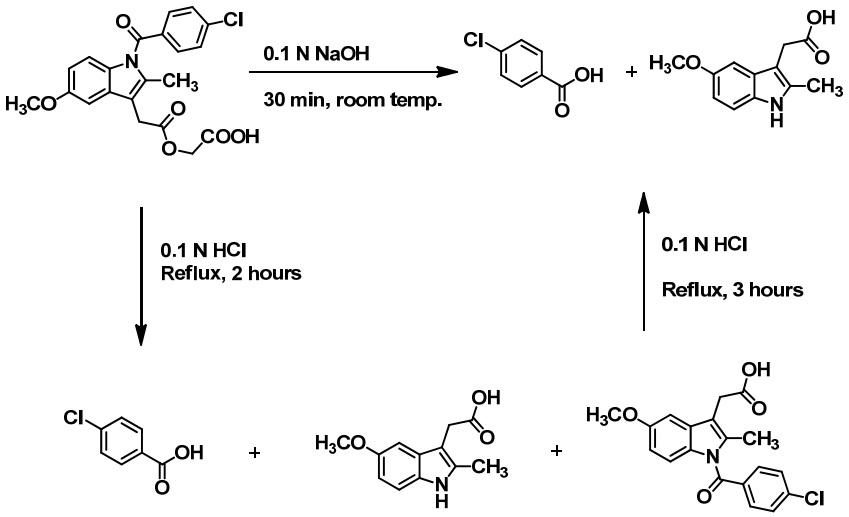



**Figure 3.** (a) HPLC chromatogram of standard *p*-chlorobenzoic acid 10 µg/mL ($t_R$ = 2.708 min), (b) HPLC chromatogram of standard 5-methoxy-2-methyl-3-indole acetic acid 10 µg/mL ($t_R$ = 2.068 min), (c) HPLC chromatogram of standard indomethacin 10 µg/mL ($t_R$ = 8.269 min).

#### *3.2.4. Effect of changing wavelength*

Different wavelengths were tried as 228, 254 and 245 nm. At λ = 228 nm, good sensitivity and symmetry of the resolved peaks were observed but equilibration time was too long.




**Scheme 1**

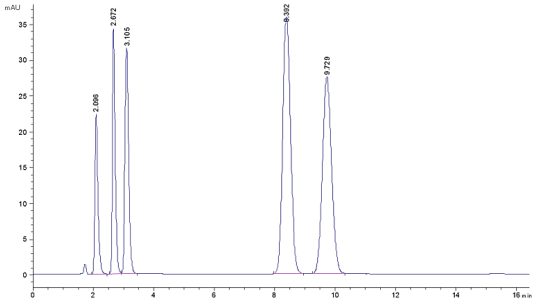


**Figure 4.** HPLC chromatogram of a resolved mixture of 10 µg/mL of *p*-chlorobenzoic acid ($t_R$ = 2.672), and 10 µg/mL of 5-methoxy-2-methyl-3-indole acetic acid ($t_R$ = 2.096), 10 µg/mL of meloxicam ($t_R$ = 3.105), 10 µg/mL of indomethacin ($t_R$ = 8.392) and 10 µg/mL of acemetacin ($t_R$ = 9.729), mobile phase is methanol: 0.02 M phosphate buffer (65:35, *v:v*, pH = 6.5, detection at 245 nm using meloxicam as internal standard).

By changing the wavelength to 254 nm, the sensitivity to the degradation products was reduced. Finally by adjusting the wavelength at 245 nm, high symmetry and sensitivity were observed. Thus it was selected as the optimum wavelength for detection and quantification, at which good detector response was obtained with symmetrical peaks.

Finally, it was found that methanol: phosphate buffer (pH = 6.5) in the ratio of (65:35, *v:v*) at a flow rate of 1 mL/min and UV detection at 245 nm were most suitable to get resolved and sharp peaks and meloxicam was used as internal standard. Calibration curves were constructed by plotting the relative peak areas against the corresponding concentrations and the regression equations were computed. The method was validated as per the ICH [44] guidelines. The regression and validation parameters of the proposed method are summarized in Table 1. System suitability was checked by calculating different parameters such as capacity factor, tailing factor, column efficiency (N), selectivity and resolution factors, where the system was found to be suitable relative to the reference values [45] as shown in Table 2. The accuracy of the proposed HPLC method was assessed by analyzing five different concentrations of ACM in its pure form and calculating its concentration from the obtained regression equation. The percentage recoveries, mean recovery and relative standard deviation were then calculated, Table 3.

**Table 1.** Validation parameters for the determination of pure acemetacin samples by the proposed HPLC method *.

| Parameter | Acemetacin |
|---|---|
| Range (µg/mL) | 0.1-50.0 |
| Slope | 0.1967 |
| Intercept | 0.0088 |
| SE of the slope | 0.0003 |
| SE of the intercept | 0.0103 |
| Correlation coefficient (r) | 0.9999 |
| LOD (µg/mL) | 0.023 |
| LOQ (µg/mL) | 0.070 |
| Robustness % | 1.44 |
| *Precision* | |
| Repeatability % | 0.206 |
| Intermediate precision % | 0.078 |

* SE: Standard error.

### *3.3. PLS chemometric method*

The absorption spectra of ACM, its degradation products and impurities (Indomethacin, PCBA, 5-methoxy-2-methyl-3-indole acetic acid) are shown in Figure 5. ACM and indomethacin show almost similar spectral features, which hinders the determination of ACM in presence of indomethacin by univariate spectrophotometric methods. Thus, multivariate PLS regression model was applied which enabled the determination of ACM in presence of indomethacin as well as PCBA and 5-methoxy-2-methyl-3-indole acetic acid in their laboratory prepared mixtures and pharmaceutical preparations.

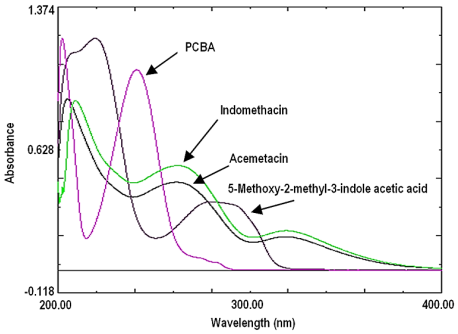



**Figure 5.** Absorption spectra of 10 µg/mL of each of acemetacin, indomethacin, *p*-chlorobenzoic acid (PCBA) and 5-methoxy-2-methyl-3-indole acetic acid in 0.01 N HCl.

**Table 2.** System suitability parameters of the proposed HPLC method.

| Parameters | 5-Methoxy-2-methyl-3-indole acetic acid | PCBA | Indomethacin | Acemetacin | Limit |
|---|---|---|---|---|---|
| Retention time ($t_R$) | 2.096 | 2.672 | 8.392 | 9.729 | |
| Resolution (R) | | 3.430 | 15.24 | 2.610 | Rs > 1.5 |
| Tailing factor (T) | 1.100 | 1.500 | 0.875 | 0.944 | T ≤ 2 |
| Capacity factor (K') | | | 4.000 | 4.830 | K' > 2 |
| Selectivity factor (α) | | | | 1.207 | α > 1 |
| Column efficiency (N) | 2458 | 4096 | 5098 | 4959 | N > 2000 |
| Height equivalent to theoretical plate (HETP) | 0.061 | 0.037 | 0.029 | 0.030 | As the HETP decreases, the column efficiency increases |

**Table 3.** Accuracy results of the proposed HPLC method for the determination of acemetacin pure samples.

| Acemetacin (µg/mL) | | Recovery % |
|---|---|---|
| Taken | Found | |
| 3.00 | 3.03 | 101.00 |
| 5.00 | 5.05 | 101.00 |
| 7.00 | 7.05 | 100.71 |
| 12.00 | 12.17 | 101.42 |
| 15.00 | 14.81 | 98.73 |
| 25.00 | 24.93 | 99.72 |
| 35.00 | 34.74 | 99.26 |
| 45.00 | 44.73 | 99.40 |
| Mean | | 100.15 |
| RSD% | | 0.9930 |

**Table 4.** Concentrations of acemetacin, indomethacin, *p*-chlorobenzoic acid and 5-methoxy-2-methyl-3-indole acetic acid in the calibration and validation sets *.

| Sample no | Acemetacin (µg/mL) | Indomethacin (µg/mL) | *p*-Chlorobenzoic acid (µg/mL) | 5-Methoxy-2-methyl-3-indole acetic acid (µg/mL) |
|---|---|---|---|---|
| 1 | 16 | 3.2 | 2.0 | 6.0 |
| 2 | 16 | 1.6 | 1.0 | 9.0 |
| 3 | 8 | 1.6 | 3.0 | 4.5 |
| 4 | 8 | 4.8 | 1.5 | 9.0 |
| 5 | 24 | 2.4 | 3.0 | 6.0 |
| 6 | 12 | 4.8 | 2.0 | 4.5 |
| 7 | 24 | 3.2 | 1.5 | 4.5 |
| 8 | 16 | 2.4 | 1.5 | 7.5 |
| 9 | 12 | 2.4 | 2.5 | 9.0 |
| 10 | 12 | 4.0 | 3.0 | 7.5 |
| 11 | 20 | 4.8 | 2.5 | 6.0 |
| 12 | 24 | 4.0 | 2.0 | 9.0 |
| 13 | 20 | 3.2 | 3.0 | 9.0 |
| 14 | 16 | 4.8 | 3.0 | 3.0 |
| 15 | 24 | 4.8 | 1.0 | 7.5 |
| 16 | 24 | 1.6 | 2.5 | 3.0 |
| 17 | 8 | 4.0 | 1.0 | 6.0 |
| 18 | 20 | 1.6 | 2.0 | 7.5 |
| 19 | 8 | 3.2 | 2.5 | 7.5 |
| 20 | 16 | 4.0 | 2.5 | 4.5 |
| 21 | 20 | 4.0 | 1.5 | 3.0 |
| 22 | 20 | 2.4 | 1.0 | 4.5 |
| 23 | 12 | 1.6 | 1.5 | 6.0 |
| 24 | 8 | 2.4 | 2.0 | 3.0 |
| 25 | 12 | 3.2 | 1.0 | 3.0 |

* The shaded samples are those of the validation set.

**Table 5.** Statistical parameters of the linear relationship between the calculated and the true concentrations of acemetacin in the validation set by the proposed PLS model.

| Statistical parameters | PLS |
|---|---|
| Slope | 0.9648 |
| SE of slope * | 0.0131 |
| Intercept | 0.2700 |
| SE of intercept | 0.2230 |
| Correlation coefficient (*r*) | 0.9997 |

* SE: Standard error.

The prepared samples were scanned in the range of 270-400 nm with 0.1 nm intervals, thus producing 1302 data points per spectrum. The produced spectral data matrix has 25 rows representing different samples and 1302 columns representing wavelengths (25 × 1302). Seventeen samples were randomly chosen and used for calibration and eight were used as an external validation set. The concentrations of ACM, indomethacin, PCBA and 5-methoxy-2-methyl-3-indole acetic acid in each mixture are shown in Table 4. In order to apply PLS model to the data, random subsets were applied as an internal cross validation method [46]. To choose the optimum number of LVs, F-statistics [47] was used in which the root mean squared error of calibration (RMSEC) values were determined. After the PLS model has been constructed, it was found that the optimum number of LVs was five, as shown in Figure 6.

In order to assess the predictive ability of the developed model, it was applied to an external validation set for the determination of ACM. Calibration graphs were constructed by plotting the predicted concentrations for ACM by the developed model versus the true concentrations. The statistical parameters of the linear relationship between the calculated and the true concentrations of ACM in the validation set are represented in Table 5. The recoveries, mean recoveries, relative standard deviation and RMSEP values are summarized in Table 6.

**Table 6.** Percent recoveries of acemetacin in the validation set by PLS regression model.

| Statistical parameters | Taken | PLS | |
|---|---|---|---|
| | | Found | Recovery % |
| 1 | 12.00 | 12.25 | 102.08 |
| 2 | 24.00 | 23.51 | 97.96 |
| 3 | 24.00 | 24.14 | 100.58 |
| 4 | 20.00 | 19.34 | 96.70 |
| 5 | 8.00 | 7.94 | 99.25 |
| 6 | 16.00 | 15.85 | 99.06 |
| 7 | 8.00 | 8.04 | 100.50 |
| 8 | 12.00 | 11.89 | 99.08 |
| Mean ± RSD% | | | 99.40±1.674 |
| RMSEP | | | 0.360 |

**Table 7.** Results obtained by applying the proposed methods for the determination of acemetacin in Ost-map® capsules.

| Product | HPLC method | | | PLS model | | |
|---|---|---|---|---|---|---|
| | Taken (µg/mL) | Found | Recovery % | Taken (µg/mL) | Found | Recovery % |
| Ost-Map® capsules | 10.00 | 10.14 | 101.40 | 10.00 | 10.04 | 100.40 |
| 60 mg Acemetacin/capsule | | 10.04 | 100.40 | | 10.06 | 100.60 |
| B.N. MT2760712 | | 10.09 | 100.90 | | 10.13 | 101.30 |
| | Mean | | 100.90 | Mean | | 100.77 |
| | RSD% | | 0.495 | RSD% | | 0.469 |

**Table 8.** Statistical comparison of the results obtained by the proposed methods and the reported HPLC method [24] for the analysis of acemetacin in pure form.

| Parameter | HPLC method | PLS model | Reported HPLC method ** |
|---|---|---|---|
| Mean | 100.15 | 99.40 | 99.39 |
| SD | 0.995 | 1.664 | 1.013 |
| n | 8 | 8 | 8 |
| Variance | 0.990 | 2.770 | 1.026 |
| Student's t-test | 1.521 (2.145) * | 0.014 (2.179) * | |
| F-test | 1.037 (3.787) * | 2.699 (3.787) * | |

* The values in the parentheses are the corresponding tabulated values at $p = 0.05$.
** HPLC method (C-18, using 0.02 M phosphate buffer (pH = 4.2): methanol in the ratio of 29:71 (*v:v*) at a flow rate of 1 mL/min and detection at 254 nm).

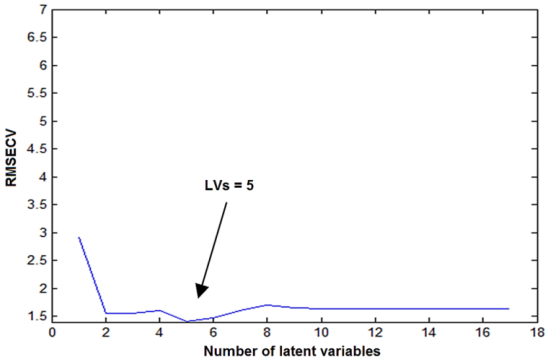



**Figure 6.** RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PLS model.

The proposed methods were successfully applied for the determination of ACM in Ost-Map® capsules with good recoveries, Table 7.

The results obtained by the proposed methods were statistically compared to those obtained by applying the reported HPLC method [24] and no significant difference has been observed between the proposed methods and the reported one with respect to accuracy and precision, Table 8.

## 4. Conclusion

The suggested methods are simple, accurate and rapid. Application of the proposed method to the analysis of ACM in the presence of its degradation products, impurities and in pharmaceutical formulations showed that neither the degradation products, impurities nor the excipients interfere with the determination of ACM, indicating that the proposed method could be applied as a stability indicating one. The proposed methods are simple precise and could be applied in quality control laboratories for the routine analysis of the studied drug either in bulk powder or in pharmaceutical dosage form.

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