European Journal of Chemistry

Journal webpage: www.eurjchem.com

Ultra-performance liquid chromatography coupled to electrospray ionization-tandem mass spectrometric method for simultaneous determination of acemetacin in presence of its metabolite indomethacin and degradation products

Maha Mahmoud Abou-El Alamin

Analytical Chemistry Department, Faculty of Pharmacy, Helwan University, 11795, Cairo, Egypt

** Corresponding author at: Analytical Chemistry Department, Faculty of Pharmacy, Helwan University, 11795, Cairo, Egypt. Tel.: +2.02.25541601. Fax: +2.02.25541601. E-mail address: dr.maha.alamin@gmail.com (M.M.A. El-Alamin).*

ARTICLE INFORMATION

DOI: 10.5155/eurjchem.7.2.213-216.1434

Received: 02 April 2016
Received in revised form: 23 April 2016
Accepted: 29 April 2016
Published online: 30 June 2016
Printed: 30 June 2016

KEYWORDS

UPLC
Validation
Acemetacin
Indomethacin
Tandem mass
Stability indicating

ABSTRACT

Selective and sensitive ultra-performance liquid chromatography-tandem mass spectrometric technique (UPLC-MS/MS) was investigated for simultaneous determination of acemetacin (ACM) in presence of its metabolite (indomethacin) and degradation products in 3 min run time. The column employed was a Hypersil gold 50 mm × 2.1 mm (1.9 µm) with an isocratic mobile phase consisted of 0.1% formic acid aqueous solution and acetonitrile (10:90, *v*:*v*) with flow rate 250 µL/min. Detection of the cited drug was carried out using multiple reaction monitoring (MRM) mode on a triple quadrupole mass spectrometer coupled with electrospray ionization (ESI) *m/z* 416.44 → 139.24 for ACM and *m/z* 256.31 → 167.00 for diphenhydramine internal standard. Various parameters were studied; mobile phase composition, flow rate, rate of fragmentation, rate of collision and mode of ionization. Good linear relationship was obtained in concentration range 8.0-500.0 ng/mL (r = 0.9994). The method was validated (linearity, range, precision, accuracy, limit of quantification and limit of detection) according to ICH guidelines and there is no significance difference between the proposed method and the reference HPLC method regarding accuracy and precision. The simplicity and sensitivity of this method allows its use as stability indicating method.

Cite this: *Eur. J. Chem.* **2016**, *7(2)*, 213-216

1. Introduction

Acemetacin ACM, (2-[2-[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl] acetyl]oxyacetic acid) is a non-steroidal anti-inflammatory drug, used for the treatment of osteo-arthritis and rheumatoid arthritis (Figure 1). It is a glycolic acid ester of indometacin, acts as a prodrug; in the body, it is metabolized to indomethacin, which then acts as an inhibitor of cyclooxygenase, producing the anti-inflammatory effects. An advantage of acemetacin is that it reduces gastric damage when compared to indomethacin [1].

Literature survey reveals that there are several methods have been investigated for the determination of ACM, such as spectrophotometric [2-4], chromatographic [2-6] and voltammetric methods [7,8]. The British Pharmacopoeia [9] described indomethacin and *p*-chlorobenzoic acid (PCBA) as impurities for ACM. *p*-Chlorobenzoic acid and 5-methoxy-2-methyl-3-indole acetic acid are reported as the degradation products under specific alkaline and acidic stress conditions [5]. These degradation products obtained were confirmed by their *m/z* using UPLC-MS/MS.

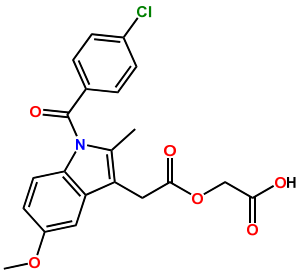

Figure 1. Structure of acemetacin.

To our knowledge, there is no validated UPLC-MS/MS method was developed for the determination of ACM in presence of its metabolite (indomethacin) and degradation products. Therefore, the aim of this paper was to develop a validated, sensitive, selective and rapid UPLC-MS/MS method for the simultaneous analysis of the cited drug in pure form and pharmaceutical dosage form, also in presence of its degradation products and/or metabolite. Different chromato-

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.7.2.213-216.1434

graphic parameters and mass spectrometric conditions were investigated to select the optimum conditions for the separation. This technique couples high resolution chromatographic separation with sensitive and specific mass spectrometric detection, which is clearly advantageous, particularly because many compounds with similar or identical retention characteristics have quite different mass spectra and can therefore be differentiated [10].

2. Experimental

2.1. Materials

Acemetacin (99.73%) and indomethacin (100.36%) were kindly supplied by the National Organization for Drug Control and Research (NODCAR), Cairo, Egypt. Pharmaceutical dosage forms; Ost-Map capsules (60 mg ACM/capsule) manufactured by Multi-Apex Pharmaceutical Industries, Badr City, Egypt.

2.2. Chemicals and reagents

All chemicals used were of analytical grade and solvents were of HPLC grade. Diphenhydramine (IS), methanol, acetonitrile and formic acid were purchased from Sigma-Aldrich, Germany. Sodium hydroxide, hydrochloric acid (32%, *w*:*v*) were purchased from El-Nasr Company, Egypt. Pure deionized water was obtained by Elga Labwater, Prima 7, UK.

2.3. Instrumentations

The analysis was achieved using a TSQ Quantum Access MAX triple stage quadrupole mass spectrometer, Thermo Scientific, New York, USA, equipped with an electrospray ionization (ESI) source. The control of the LC-MS/MS system, acquisition and analysis of the data were performed utilizing Xcalibur software version 2.2. Chromatography was carried on Accela U-HPLC system which was composed of Accela 1250 quaternary pump and Accela open autosampler, New York, USA (operated at 25 °C).

2.4. Chromatographic and mass spectrometric conditions

Chromatographic separation was performed on HypersilGold column (C18-bonded ultrapure silica based column) 50 mm × 2.1 mm (1.9 µm) preceded by a C_{18} security guard cartridge Gemini 5 µm C18 (Phenomenex) 4 × 3 mm). Isocratic elution was achieved using mobile phase consisting of 0.1% formic acid aqueous solution and acetonitrile (10:90, *v*:*v*) and flow rate of 250 µL/min, where elution was performed at room temperature. The injection volume was 10 µL and the total run time for each sample was 3 min. The mass spectrometric detection method was carried out in the positive-ion mode in case of ACM, indomethacin and 5-methoxy-2-methyl-3-indole acetic acid and negative one for PCBA utilizing electrospray ionization (ESI) and multiple reactions monitoring (MRM). Samples are individually tuned for each target analyte by direct injection of the individual solution followed by data acquisition and processing. The optimized parameters are: auxiliary gas of 2 psi, sheath gas of 20 psi, capillary temperature of 270 °C, turbo ion spray temperature of 400 °C, capillary offset 35 and ion spray voltage of 3600 V. The quadrupole mass spectrometer was operated at the MRM mode, monitoring the transition of molecular ions to the product ions for ACM *m/z* 416.44 → 139.24 and IS *m/z* 256.31 → 167.00. The collision energies were 22 and 25 eV for ACM and IS, respectively.

2.5. Standard Solutions

Standard stock solutions of 100 µg/mL for ACM and indomethacin were prepared separately in methanol and stored in refrigerator. Sample degradation: ACM pure sample was subjected to forced degradation under different stressed conditions as, alkaline (0.1 N NaOH for 30 min at room temperature) or acidic (reflux with 0.1 N HCl for 3 hrs in boiling water bath) then neutralized as reported [5].

2.6. Procedures

2.6.1. Calibration curve

Working standard solutions of ACM (8.0-500.0 ng/mL) were prepared by serial dilutions of aliquots of the standard stock solution with methanol. 5 ng/mL of IS was added for each solution then 10 µL of each solution was injected into the LC-MS/MS system. The relative peak areas of the drug were plotted versus the concentrations of drug in ng/mL and the corresponding regression equation was derived.

2.6.2. Analysis of ACM pure sample

An aliquot of 10 µL of different ACM working standard solutions covering the concentration range (8.0-500.0 ng/mL) was injected into UPLC-MS/MS and measured by the proposed method. The percentage recoveries were calculated by means of the regression equation or from the calibration graph.

2.6.3. Analysis of laboratory prepared mixtures containing different concentrations of ACM, indomethacin and its degradation products

Aliquots of ACM working standard solutions were mixed with indomethacin and degradation products and determined by the proposed method. The concentrations of ACM were calculated from the regression equation.

2.6.4. Analysis of pharmaceutical dosage form

The contents of ten capsules of Ost-Map were weighed and mixed well. An accurately amount claimed to be 10 mg ACM was mixed with 100 mL methanol then sonicated for 30 min. The solution was filtrated and completed as under Section 2.6.1. The concentrations of ACM were calculated from the corresponding regression equation.

3. Results and discussion

The proposed UPLC-MS/MS method permits the quantification of ACM in pure material and in presence of its metabolite (indomethacin) and two degradation products. The method suggests high sensitivity and selectivity for ACM.

3.1. Selection and optimization of chromatographic condition and mass spectrometric detection

To achieve the best chromatographic conditions: mobile phase composition and flow rate were carefully studied and optimized to provide sufficient selectivity and sensitivity in a short separation time. So, isocratic elution using mobile phase consisting of 0.1% formic acid aqueous solution and acetonitrile (10:90, *v*:*v*), flow rate of 250 µL/min and injection volume 10 µL, performed at room temperature allowed high resolution and separation of each analyt (ACM, indomethacin, degradates and IS).

For mass spectrometry, different parameters were studied as, sheath gas pressure, spray voltage, collision energy, rate of fragmentation, mode of ionization, etc. The selected conditions (under Section 2.4) permitted good separation in short time (less than 1 min). The optimized MRM transitions (precursor ion *m/z* → product ion *m/z*) are ACM *m/z* 416.44 → 139.24 and IS *m/z* 256.31 → 167.00, where, full scan for PCBA *m/z* 156.32 → 156.32, 5-methoxy-2-methyl-3-indole acetic acid

m/z 220.30 → 220.30 and indomethacin *m/z* 358.35 → 358.35 (Figure 2). ACM was fragmented into different product ions *m/z* 139.24, 111.28 and 174.30, but we selected 139.24 because it gave high intensity (Figure 3 and 4).

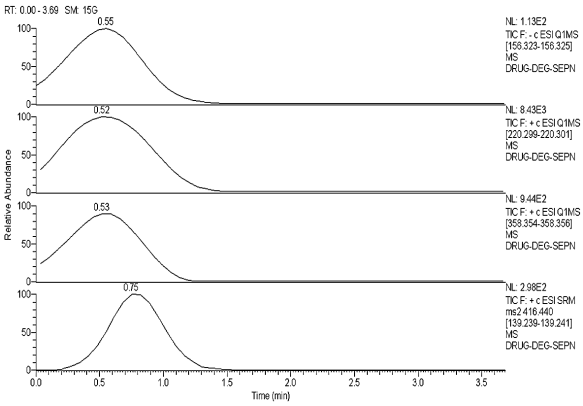

Figure 2. Chromatographic separation of PCBA, 5-methoxy-2- methyl -3-indole acetic acid, indomethacin and ACM, respectively, by the proposed UPLC-MS/MS.

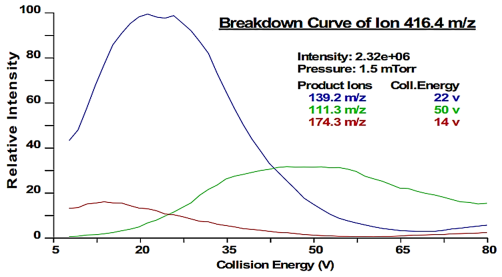

Figure 3. Breakdown curve of ACM *m/z* 416.44 → 139.24, 111.3 and 174.3.

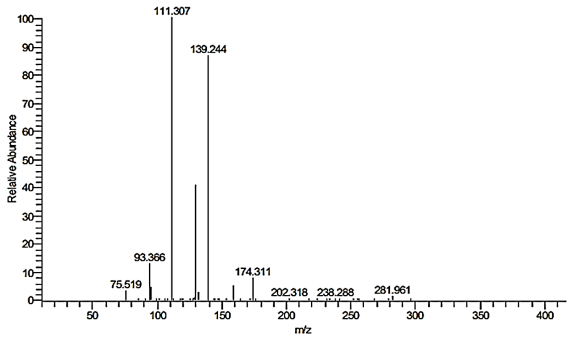

Figure 4. Product ion spectra of ACM $[M+H]^+$, *m/z* 139.2, 111.3 and 174.3.

3.2. Degradation

ACM under different stressed acidic (reflux with 0.1 N HCl for 3 hrs in boiling water bath) and basic (0.1 N NaOH for 30 min at room temperature) conditions undergo complete degradation, where it is stable under oxidative and thermal stress conditions as reported [5]. ACM was degradated into PCBA and 5-methoxy-2-methyl-3-indole acetic acid as reported [5], which confirmed by their molecular weights *m/z* 156.32, 220.30 using the proposed method (Full scan). So, by applying the proposed UPLC-MS/MS technique, it was probable to detect ACM in the presence of its metabolite and/or degradation products in short time (< 1 min) as shown in Figure 2.

3.3. Method validation

The method was validated according to ICH guidelines regarding linearity, range, limit of detection, limit of quantification, accuracy and precision [11].

3.3.1. Linearity and range

Under the above-described experimental conditions, linear relationships were established by plotting relative peak areas against ACM concentrations. The concentration range was found linear in 8.0-500.0 ng/mL. Linear regression analysis of the data gave the following equation (Table 1):

$$A = -1.044 + 0.6353 \times C \quad (r = 0.9994) \qquad (1)$$

where A is the relative peak areas and C is the concentration of drug in ng/mL and *r* is the regression coefficient. The high values of the correlation coefficients (> 0.999) indicate good linearity of the calibration graphs (Figure 5)

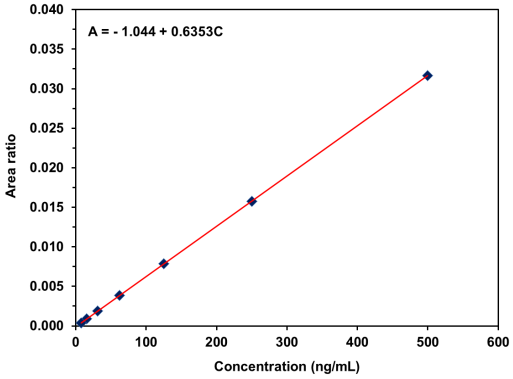

Figure 5. Calibration curve of ACM (8.0-500.0 ng/mL) by the proposed UPLC-MS/MS method.

3.3.2. Limit of quantitation (LOQ) and limit of detection (LOD)

The minimum level at which the ACM can be reliably detected (LOD) and quantified (LOQ) were determined and the data are presented in Table 1.

3.3.3. Accuracy and precision

The accuracy of the proposed method was evaluated by assay of ACM standard solutions by the proposed method and the results obtained were compared with those obtained using the reported one [5]. Statistical analysis of the results obtained by the proposed and reported methods using student´s *t*-test and variance ratio *F*-test, showed no significant difference between the two methods (Table 2).

Evaluation of the intra-day precision was made by replicate assay of the standard solutions of the studied drug on the same day, while the inter-day precision was evaluated through replicate the assay on three successive days (Table 2). The values of standard deviation (SD) were small what indicates that the repeatability of the proposed method is good and the suitability of the LC-MS/MS method, for the routine detection of ACM in presence of its metabolite and degradations products.

Table 1. Validation parameters for the determination of pure ACM by the proposed UPLC-MS/MS method.

Parameter	ACM
Range (ng/mL)	8.0-500.0
Slope	0.6353
Intercept	-1.044
Correlation coefficient (r)	0.9994
LOD (ng/mL)	2.52
LOQ (ng/mL)	7.64

Table 2. Accuracy and precision of the proposed UPLC-MS/MS method for the determination of pure ACM.

Parameter	Proposed UPLC-MS/MS	Reported HPLC [5]
Mean±SD	101.10±0.719	100.62±0.818
Variance	0.517	0.669
N	6	6
Student´s *t*-test (2.228) *	0.790	
F-test (5.050) *	1.294	
Intra-day precision	100.64±1.430	
Inter-day precision	101.29±1.931	

* The values in the parentheses are the corresponding tabulated values at $p = 0.05$.

Table 3. Application of the proposed method for the determination of ACM in Ost-Map capsules.

Parameter	Proposed UPLC-MS/MS	Reported HPLC [5]
Mean±SD	99.669±3.068	98.031±1.978
Variance	9.413	3.912
N	6	6
Student´s *t*-test (2.228) *	0.769	
F-test (5.050) *	2.406	

* The values in the parentheses are the corresponding tabulated values at $p = 0.05$.

3.3.4. Robustness of the method

The robustness of analytical method measures the capacity of it to restrain small but deliberate changes in method parameters. Evaluation of the robustness of the proposed method was done for the chromatographic parameters as well as, the mass parameters ,e.g. flow rate of mobile phase (±10 µL/min), vaporizer temperature or transfer capillary temperature (±5 °C), collision energy (±2 V) and sheath gas pressure (±5 psi) did not show significant changes in the values of peak areas of ACM.

3.4. Application of the proposed method

The proposed UPLC-MS/MS method was successfully applied for the determination of ACM in its dosage form with satisfactory recovery. The obtained results are listed in Table 3.

4. Conclusion

Specific and sensitive ultra-performance liquid chromatography coupled to tandem mass spectrometric technique (UPLC-MS/MS) was developed for simultaneous determination of acemetacin (ACM) in presence of its metabolite (indomethacin) and degradation products. Different chromatographic parameters and mass spectrometric conditions were investigated to select the optimum conditions for the separation. The method was validated according to ICH guidelines and successfully applied for the determination of cited drug in dosage form.

Acknowledgements

The author thanks department of Analytical Chemistry, Faculty of Pharmacy, Helwan University for providing instruments and National Organization for Drug Control and Research (NODCAR), Cairo, Egypt for supplying the standard materials during this work.

References

[1]. Acemetacin, Wikipedia, the free encyclopaedia, https://en.wikipedia.org/wiki/Acemetacin
[2]. Ozbakan, H.; Palabiyik, I. M.; Caglayan, M. G.; Onur, F. *Turk. J. Pharm. Sci.* **2009**, *6*, 163-176.
[3]. Naguib, I. A.; Abdel-Aleem, E. A.; Zaazaa, H.; Hussein, E. A. *Eur. J. Chem*. **2014**, *5*, 219-226.
[4]. *Walash, M. I.; Belal, F.; Tolba, M. M.; Halawa, M. I.* Sep. Sci. Tech. ***2015***, 50, *1403-1412.*
[5]. Kessiba, A. M.; Hegazy, M. A. M.; Abdelkawy, M. M.; El Gendy, A. E. *Eur. J. Chem*. **2015**, *6(4)*, 422-429.
[6]. Ban, E.; Cho, J. H.; Jang, D. J.; Piao, X. L.; Kim, C. K. *J. Liq. Chrom. Rel. Tech.* **2005**, *28*, 1593-1604.
[7]. Reguera, C.; Arcos, M. J.; Ortiz, M. C. *Talanta* **1998**, *46*, 1493-1505.
[8]. Reguera, C.; Ortiz, M. C.; Arcos, M. *J. Electroanal*. **2002**, *14*, 1699-1706.
[9]. British Pharmacopoeia; British Pharmacopoeia Commission; The stationary Office, 2012.
[10]. Mowaka, S.; Mohamed, D. *RSC Adv*. **2015**, *5,* 60467-60481.
[11]. ICH, Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2(R1), Parent Guidelines on Methodology, 2005.