Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

# Validated RP-HPLC Method for Simultaneous Amoxicillin and Meloxicam Determination in Combined Dosage Forms

**T. Dao-Thanh[1], Juan José García-Rodriguez[2], C. Álvarez-Álvarez[1, 3], P.M. De La Torre-Iglesias[1, 3*]**

[1]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Complutense University, 28040 Madrid, SPAIN.*
[2]*Department of Parasitology, Faculty of Pharmacy, Complutense University, 28040 Madrid, SPAIN.*
[3]*Institute of Industrial Pharmacy, Complutense University, 28040 Madrid, SPAIN.*

**Received on: 06-11-2015; Revised and Accepted on: 18-11-2015**

## ABSTRACT

*A simple and sensitive reversed-phase high-performance liquid chromatographic method (RP-HPLC) has been developed and validated for the simultaneous determination of meloxicam (MLX) and sodium amoxicillin (AMX) in their combined dosage forms. Both drugs were separated on a $C_{18}$ column (250 mm × 4.6 mm, 5 µm). The mobile phase used was acetonitrile: buffer (30:70, v/v) (pH 6), at a flow rate of 1.0 mL/min, with UV detection at 270 nm. The method was validated in the samples concentration ranges of 0.5-15 µg/mL for MLX and 3-50 µg/mL for AMX, where it demonstrated good linearity, accuracy and precision. And the method showed the specificity for the determination of MLX and AMX in the presence of the excipients used in the pharmaceutical formulations and their degradation products under stress conditions. The proposed method can be used for the quality control of formulated products containing both drugs.*

***Keywords:*** *RP-HPLC; Meloxicam; Sodium Amoxicillin; Pharmaceutical Formulations.*

## INTRODUCTION

Sodium amoxicillin (AMX) is a semi-synthetic penicillin which exhibits a broad spectrum activity against Gram-positive and Gram-negative bacteria and displays a bactericidal mode of action [1, 2]. AMX is used extensively in the treatment of bacterial infections of animals and is approved in a variety of formulations for veterinarian use [3, 4]. Meloxicam (MLX) is a non-steroidal anti-inflammatory drug (NSAID) and a selective cyclooxygenase-2 (COX-2) inhibitor, used to treat mastitis and acute infections as an adjunctive treatment with antibiotics in veterinarian medicine [5-8]. To meet the clinical needs, a new formulation of a combination of an NSAID with an antibiotic was developed to propose a better treatment of acute infections in veterinary and then an analytical method was also developed for the quality control of the formulations of this combination.

Several HPLC methods have been reported for the determination of AMX [9-14] and MLX [15, 20] individually. To our knowledge, no method for analysis of both drugs combined has been yet published. RP-HPLC assay of MLX is relatively straightforward, while for AMX there are particular difficulties mainly due to its high polarity causing it to elute among other polar substances [21].

This article introduces a simple, specific, precise and robust HPLC method which has been developed and validated using the ICH guideline Q2 (R1) to measure simultaneously the amount of both drugs in order to study the stability of new formulations.

## MATERIALS AND METHODS

**Chemicals and reagents:**

All experiments were performed with pharmaceutical-grade MLX (Fagron Iberica, Spain) and AMX (Panreac S.A, Spain), and analytical-grade reagents. HPLC-grade solvents were employed for analyses. HPLC-grade water was obtained using a Milli-Q water purification system (Millipore, Milford, MA, USA) and used for preparation of all aqueous solutions.

***Corresponding author:**
***Paloma Marina De La Torre-Iglesias***
*Department of Pharmaceutical Technology,*
*Faculty of Pharmacy, Complutense University, Madrid, Spain. Avda. Complutense s/n. 28040 Madrid. (SPAIN).*
*Tel + 34 91 394 16 20; Fax + 34 91 394 17 36.*
**E-Mail: pmtorre@ucm.es*

**Instrumentation and chromatographic conditions:**

The separations were performed with a modular Jasco (Tokyo, Japan) system consisting of a PU-1580 Intelligent HPLC Pump (Jasco, Tokyo, Japan), 1575 UV-Vis detector (Jasco, Tokyo, Japan) at the working wavelength of 230 mn, and 231 XL automatic sampling injector (Gilson, Middleton, USA) with a 100 µL loop. The chromatograms were recorded employing Borwin 1.5 software (JMBS Developments, USA). Compounds were separated on a 250 mm × 4.6 mm $C_{18}$ column of 5 µm particle size (Mediterranea, Barcelona, Spain). The mobile phase was 30:70 (v/v) mixture of acetonitrile and 170 mM sodium acetate buffer (pH 6), pumped at a flow rate of 1.0 mL/min.

**Preparation of stock and working standard solutions:**

The stock solution of MLX (1000 mg/L) was prepared in a 50 ml volumetric flask by dissolving in acetonitrile 50.0 mg of accurately weighted MLX. The stock solution of AMX (1000 mg/L) was prepared in a 50 ml volumetric flask by dissolving an accurately weighed amount of AMX in the mobile phase. The working standard solutions were produced by diluting the stock solutions with mobile phase to obtain solutions containing MLX and AMX in the concentration ranges of 0.3-15.0 µg/mL and 5.0-50.0 µg/mL, respectively.

**Preparation of sample solutions:**

Two developed formulations were evaluated. In each case, the content of five ampoules of 5 mL were taken and weighed. An accurately weighed portion of the powder equivalent to 12.5 mg of AMX was transferred to a 25.0 mL volumetric flask and volume was made up to mark with water. A 1 mL aliquot of this solution was transferred to a 25 mL volumetric flask and diluted to the mark with mobile phase. The solutions were filtered through a 0.45 µm nylon membrane filter before analysis.

**Preparation of formulations:**

Two combined formulations of MLX and AMX, named MLX:AMX:CD and MLX:AMX:Saccharine, were obtained by a freeze-drying method. An accurately weighed amount of MLX (25 mg) was dissolved in 5 ml of a 0,033 M glycine- sodium hydroxide buffer (pH 8.8). Then a 7.5 % (w:w) of 2-hydroxypropyl- β-cyclodextrin for formulation MLX:AMX:CD and a 0.9% of saccharine (w:w) for formulation MLX:AMX:Saccharine were dissolved in the same volume. Both solutions were shaken on a mechanical shaker to ensure complete solubilization of MLX and then 500 mg AMX were added to both formulations. The aqueous solutions of MLX and AMX

obtained were sterilized by filtration through 0.2 µm disposable membrane filter (Sartorius, Germany). The resulted solutions were frozen at -40ºC and lyophilized in a Telstar® Cryobloc® 764 (Tarrasa, Spain). After the freeze-drying process, the vials were capped within 5 min and stored at room temperature (22-24 °C) in a desiccator containing silica gel.

Placebo formulations were obtained by the same preparation method used for both freeze-dried formulations but without any drug.

***Validation criteria:*** The developed method was validated as per ICH guidelines[22] in terms of accuracy, precision, linearity, specificity, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability.

***Specificity:***

Specificity is defined as the ability of method to measure the analyte accurately and specifically in the presence of the components in the sample matrix and other compounds produced by forcedly degraded powder samples. The analysis of chromatograms of drug-free and drug-added placebo formulation was determined to see if any interference from the excipients were achieved. In addition, forced degradation studies were performed to provide an indicator of the stability of the proposed method. Intentional degradation was achieved by exposing the formulation products to high temperature (80 ºC), acid (3 M HCl), base (3 M NaOH) and oxidative conditions (3% $H_2O_2$) for 24 hours in order to test the ability of the proposed method to separate the active components from degradation products.

***Linearity:***

Linearity was determined by preparing three calibration curves with five concentration levels each. The calibration curves were obtained by plotting the peak area as a function of the analyte concentration using least-squares linear regression analysis.

***Accuracy:***

Accuracy of an analytical method is defined as the similarity of the results obtained by the analytical method to the true value. It was performed by recovery study of drug-spiked placebo formulations of three concentrations by triplicate on a single day assay. Accuracy is expressed as the relative standard error (R.S.E (%)).

***Precision:***

Precision is a measure of the method variability it can be expressed as intra-day and inter-day precision. Precision was determined by replicate analysis of three levels of concentrations MLX (0.5, 5, 15 µg/mL), AMX (3, 15, 50 µg/mL) within 1 day or on 3 consecutive days.

***Limit of detection (LOD) and Limit of quantification (LOQ):***

LOD and LOQ for each analyte were determined based on signal to noise concept, as the lowest concentrations in which signal to noise ratio is 3:1 and 10:1, respectively, with defined precision and accuracy under the experimental conditions given.

***Robustness:***

Robustness of the method was determined by deliverately varying certain parameters like flow rate (altered by ±0.1 mL/min), mobile phase composition (acetonitrile ±2%), buffer pH (altered by ±0.2). One factor at a time was changed to estimate the effect. The assay was carried out in triplicate at 3 different concentration levels, i.e, 0.5, 5 and 15 µg/mL for MLX and 3, 5 and 50 µg/mL for AMX.

***System suitability:***

System suitability tests were performed to confirm that the equipment was adequate for the analysis to be performed. Tests were carried out by injecting 5 replicates of a standard solution containing 2 µg/mL and 40.0 µg/mL of MLX and AMX, respectively.

## RESULTS AND DISCUSSION

**Method development:**

The UV spectra of MLX and AMX in the mobile phase are shown in Fig. 1. In their pharmaceutical combination, AMX is nominally 20 times more concentrated than MLX, but the latter having better absorbance characteristics in the UV region. As observed, MLX has a significant absorption throughout the UV region, but AMX exhibits a maximum absorption at 272 nm. Hence, detection was performed at 272 nm where both analytes have a reasonable absorption.

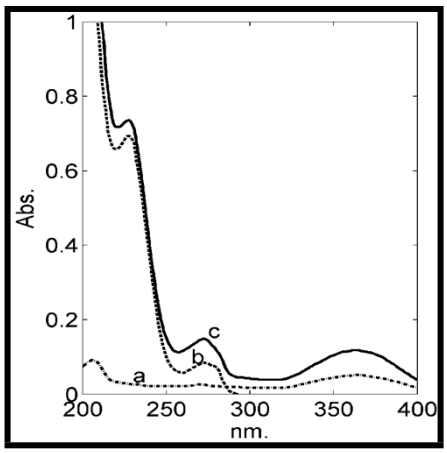


**Fig. 1: UV spectra of: 1 µg mL$^{-1}$ of MLX (a),20 µg mL$^{-1}$ of AMX (b) and the mixture of 2 µg mL$^{-1}$ of MLX and 40 µg mL$^{-1}$ of AMX (c) in a 30:70 mixture of acetonitrile and 170 mM sodium acetate buffer (pH 6).**

The difficulty during the development method consisted on the different retention times of MLX and AMX. MLX need a high amount of organic solvents (>25% v/v) in the mobile phase to be eluted due to its amphoteric structure. But AMX, with its high polarity, elutes among polar substances. If the organic solvent in the mobile phase is increased, the retention time of MLX decreases, but then AMX elutes too fast to detect it. After a series of preliminary tests, the composition of mobile phase with more than 25% of organic solvent produced satisfactory separations. Among the most common solvents used for HPLC: methanol and acetonitrile, the use of acetonitrile as organic solvent gave better peak shapes. Taking into account the instability of AMX in the strong acidic and alkaline conditions, the range of buffers pH is from 4 to 7. The best choice for buffer is acetate. It was observed that the retention time of MLX decreases slowly with an increase of the pH and decreases rapidly with increments in the ionic strength of buffers. On the other side, AMX was barely affected with these changes.

Finally, the mixture of acetonitrile and 170 mM sodium acetate buffer (30:70 v/v) was employed for the simultaneous determination of both drugs. The method produced symmetric peak shape, good resolution and reasonable retention time 10.7 and 2.4 min for MLX and AMX, respectively (Fig. 2).

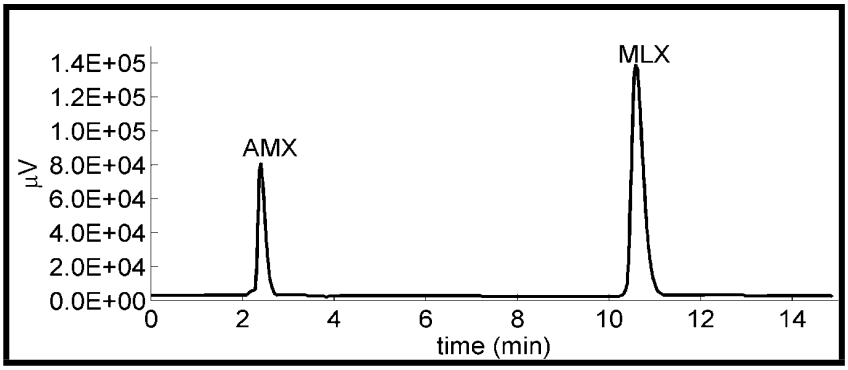


**Fig. 2: Typical chromatogram of a sample solution: 40 µg mL$^{-1}$ of AMX ($t_r$=2.413) and 2 µg mL$^{-1}$ of MLX ($t_r$=10.77) peaks.**

***Specificity:***

Fig. 2 shows chromatogram for both drugs indicating no interference between them at 272 nm. No interfering peaks of excipients were observed in the placebo samples. In addition, forced degradation studies were carried out to provide an indication of the stability property for the proposed method. Fig. 3A-D shows chromatograms of the mixture of 2 drugs under the stress conditions in A-thermal condition, B-acidic condition, C-base condition and D-oxidative condition. Samples were degraded to levels where the content of MLX and AMX was reduced to less than 90% of the original level. Both drugs suffered significant degradation due to their instability nature, especially AMX. Two major peaks corresponding to parent drugs can be found in Fig. 3A. In Fig 3B-D it can be seen that significant decreased peaks appear with more new peaks. This suggests the poor stability of the both drugs under acidic, base and oxidative conditions. Fig 3A-D shows that AMX and MLX peaks amongst degradation products peaks were found resolved.

The ability of the method to separate the drugs from their degradation products and the non-interference from the matrix indicates the good specificity of the developed method.

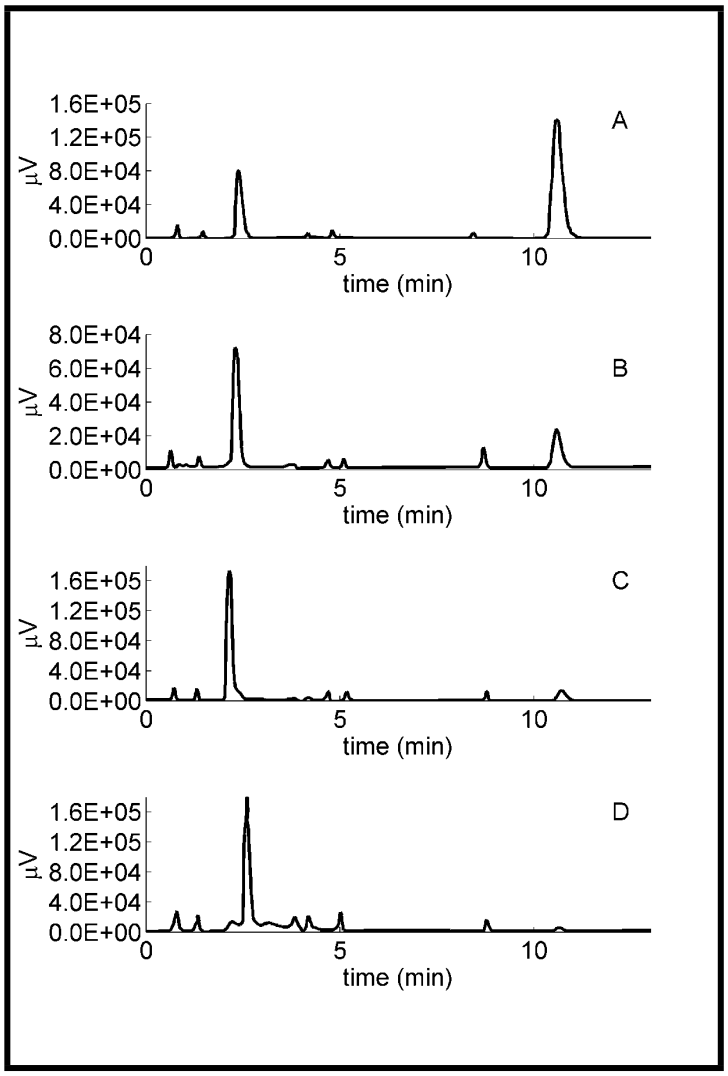


**Fig. 3A-D: Chromatograms of MLX and AMX a initial concentration 2 µg mL$^{-1}$ and 40 µg mL$^{-1}$ respectively and their degraded products: (A) thermal degradation; (B) acidic degradation; (C) alkaline degradation; (D) oxidative degradation.**

***Linearity:***

Table 1 shows the statistical analysis of calibration curves of MLX and AMX. The results for the determination of the coefficient demonstrate an excellent correlation between peak area and analyte concentration for both drugs. The relative standard error of slopes was < 2% for each curve.

***Limit of detection (LOD) and Limit of quantification (LOQ):***

The LOD values for MLX and AMX were 0.01 and 1.3 µg/mL, respectively, and the LOQ values for MLX and AMX were 0.02 and 2.3 µg/mL, respectively (Table 1).

***Precision:***

Table 2 shows the data obtained from the analysis of the samples in the same day and in consecutive days (n=9). The RSD values obtained were below 2% for both intra- and interday precision. These results indicate that the method is adequately precise.

***Accuracy:***

Percent recoveries were calculated from the peak areas obtained from the sample drug-spiked placebo. The two placebos correspondent to both formulations MLX:AMX:CD and MLX:AMX:Saccharine were used. The first formulations, MLX:AMX:CD, includes both drugs and 2-hydroxypropyl- β-cyclodextrin and the latter one, MLX:AMX:Saccharine, is composed of both MLX and AMX and saccharine. Table 3 shows the good recoveries obtained, from 98.75 to 101.60 %, which indicate that the method is accurate.

***Robustness:***

Robustness of the proposed method was examined in the range 5.8 – 6.2 of pH. The flow rate effect was evaluated between 0.9-1.1 mL/min. Finally, the change of the composition of mobile phase from 28:72 to 32:68 (v/v) of acetonitrile: sodium acetate was also studied. Table 4 shows that the method has a good robustness with the observed variations less than ± 2%.

***System suitability:***

The different parameters of system suitability, such as resolution, tailing factors and theoretical plates of the proposed method were described in Table 5. The corresponding values of RSD observed were in the range between 0.70% and 1.20% which were considered satisfactory, meeting the requirements of USP 36–NF 31 (RSD < 2%).

***Method application:***

The validated HPLC method was applied to the simultaneous determinations of AMX and MLX in both powders for injection developed in our laboratory (MLX:AMX:CD and MLX:AMX:Saccharine). Table 6 shows that the results of the amount of each drug in the formulations, expressed as a percentage of the values claimed by the label, were from 99.9-101.5 %.

A suitable analytical procedure refers to the way of performing the analysis with accuracy and precision. This developed method describes in detail the steps necessary to perform each parameter for validation. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The quality control laboratory requires analytical methods which are simple, robust, and rugged. Interpretation of results of validation parameters study shows that results of method is directly proportional to the concentration of both analytes within a given range shows linearity of method. Different environmental condition and minor change in chromatographic condition doesn't cause any significant change in results shows stability and reproducibility of developed method. There was no interference by excipients with both MLX and AMX peaks shows proposed method is specific for both analytes. As well as Recovery study shows the developed method is highly accurate.

**Table No. 1: Results from regression analysis of the calibration curves for the determination of MLX and AMX by the proposed HPLC method, LOD and LOQ.**

| Analyte | Range (µg mL$^{-1}$) | Regression equation (Y= a + bC)[a] | r$^{2}$[b] | $S_a$[c] | $S_b$[d] | $Sb_{rel}$ (%)[e] | LOD (µg mL$^{-1}$) | LOQ (µg mL$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| **MLX** | 0.5-15 | Y= 2888.8 + 155718 *C* | 0.9997 | 6044.68 | 837.84 | 0.53 | 0.01 | 0.02 |
| **AMX** | 3.0-50 | Y= -8612.6 + 157797 *C* | 0.9998 | 2885.96 | 124.58 | 0.79 | 1.3 | 2.3 |

[a] Peak area values versus concentration of analyte in µg/mL (n=15); [b] r$^2$, determination coefficient; [c] $S_a$, standard deviation of the intercept of regression curve; [d] $S_b$, standard deviation of slope of regression curve; [e] $S_{b,rel}$ (%), relative standard error of the slope.

**Table No. 2: Intraday and interday precision studies for the determination of MLX and AMX by the proposed HPLC method.**

| | | Intraday precision[a] | | Interday precision[a] | |
|---|---|---|---|---|---|
| **Analyte** | **Added (µg mL$^{-1}$)** | **Measured ± SD (µg mL$^{-1}$)** | **RSD (%)** | **Measured ± SD (µg mL$^{-1}$)** | **RSD (%)** |
| **MLX** | **0.5** | 0.504 ± 0.009 | 1.79 | 0.494 ± 0.012 | 2.43 |
| | **5** | 4.944 ± 0.064 | 1.29 | 5.139 ± 0.086 | 1.67 |
| | **15** | 15.017 ± 0.113 | 0.75 | 14.96 ± 0.126 | 0.84 |
| **AMX** | **3** | 3.133 ± 0.051 | 1.42 | 3.09 ± 0.056 | 1.81 |
| | **5** | 4.860 ± 0.092 | 1.89 | 4.84 ± 0.098 | 2.02 |
| | **50** | 50.005 ± 0.711 | 1.63 | 50.67 ± 0.699 | 1.38 |

[a] n=9

**Table No. 3: Recovery studies for the determination of MLX and AMX in the proposed HPLC method.**

| Analyte | Added (µg mL$^{-1}$) | Formulation MLX:AMX:CD | | Formulation MLX:AMX:Saccharine | |
|---|---|---|---|---|---|
| | | Recovery ± SD (%) | RSD (%) | Recovery ± SD (%) | RSD (%) |
| **MLX** | **0.5** | 98.75 ± 1.236 | 1.25 | 101.31 ± 1.755 | 1.73 |
| | **5** | 100.51 ± 1.249 | 1.24 | 99.85 ± 1.125 | 1.13 |
| | **15** | 100.65 ± 1.49 | 1.47 | 100.65 ± 1.486 | 1.47 |
| **AMX** | **3** | 101.60 ± 1.550 | 1.53 | 98.78 ± 1.276 | 1.29 |
| | **5** | 99.0 ± 0.676 | 0.68 | 101.56 ± 1.853 | 1.83 |
| | **15** | 100.0 ± 0.873 | 0.87 | 100.66 ± 1.634 | 1.62 |

**Table No. 4: Robustness studies for the determination of MLX and AMX by the proposed HPLC method.**

| Parameter | Value | MLX | | AMX | |
|---|---|---|---|---|---|
| | | Mean recovery (%) | RSD (%) | Mean recovery (%) | RSD (%) |
| **pH** | 5.8 | 99.6 | 1.5 | 99.5 | 1.8 |
| | 6 | | | | |
| | 6.2 | | | | |
| **Flow rate (mL min$^{-1}$)** | 0.9 | 100.3 | 0.8 | 100.0 | 0.4 |
| | 1 | | | | |
| | 1.1 | | | | |

| | | | | | |
|---|---|---|---|---|---|
| **Mobile phase (organic:aqueous)** | 28:72 | | | | |
| | 30:70 | 101.2 | 1.3 | 99.8 | 0.9 |
| | 32:68 | | | | |

**Table No. 5: System suitability parameters for determination of MLX and AMX by the proposed HPLC method.**

| Parameter | MLX | AMX |
|---|---|---|
| **Resolution** | 9.86 | — |
| **Tailing factor ($T_f$)** | 1.02 | 1.07 |
| **Theorical plates (N)** | 40162.5 | 3548.1 |
| **RSD (%)** | 0.78 | 1.2 |

**Table No. 6: Assay results obtained for the combined dosage form by using the proposed HPLC method.**

| Sample No. | *Formulation MLX:AMX:CD* | | *Formulation MLX:AMX:Saccharine* | |
|---|---|---|---|---|
| | MLX (%) | AMX (%) | MLX (%) | AMX (%) |
| **1** | 99.9 | 101.2 | 101.3 | 99.8 |
| **2** | 101.9 | 100.7 | 103.6 | 98.2 |
| **3** | 100.1 | 101.9 | 99.8 | 101.9 |
| **Mean** | 100.6 | 101.4 | 101.5 | 99.9 |
| **RSD (%)** | 1.13 | 0.56 | 1.89 | 1.86 |

## CONCLUSION

Hence the developed and validated HPLC method is lineal, accurate, robust, precise and specific for the simultaneous determination of MLX and AMX in the sterile powder for injection formulations. The proposed method can be used for routine analysis and for quality control of pharmaceutical formulations containing these two drugs.

## ACKNOWLEDGEMENTS

We thank the Complutense University and Madrid Community Administration and its research group number CC G07-UCM/BIO-2824. Thuy Dao Thanh has a grant from AECID. Assistance provided by Khanh Gia Nguyen and Jose Alberto Delgado Montes was greatly appreciated.

## REFERENCES:

1. Craven, N. Efficacy and financial value of antibiotic treatment of bovine clinical mastitis during lactation. A review, British Veterinary Journal, **1987**; 143: 410-422.
2. Prescott J.F. and Baggot J.D. Antimicrobial therapy in veterinary medicine. 18, Bovine mastitis, Blackwell Scientific Pub. Ox. UK, **1988**; p. 321-331.
3. Taponen S, Jantunen A, Pyörälä E, Pyörälä S. Efficacy of targeted 5-day combined parenteral and intramammary treatment of clinical mastitis caused by penicillin-susceptible or penicillin-resistant *Staphylococcus aureus*, Acta Veterinaria Scandinavica, **2003**; 44: 53-62.
4. Ziv G. and Sulman F. G. Permeability of the Mammary Gland to Large Antibiotic Molecules, Zentralblatt für Veterinärmedizin Reihe A, **1973**; 20: 388–394.
5. Georgoulakis I. E, Petridou E, Filiousis G, Alexopoulos C., Kyriakis S.C, Papatsas I. Meloxicam as adjunctive therapy in treatment and control of porcine respiratory disease complex in growing pigs, Journal of Swine Health and Production. **2006**; 14(5): 253-257.
6. Hirsch A.C, Philipp H. and Kleemann R. Investigation on the efficacy of meloxicam in sows with mastitis–metritis–agalactia syndrome, Journal of Veterinary Pharmacology and Therapeutics, **2003**; 26: 355–360.
7. McDougall S, Bryan MA, Tiddy RM. Effect of treatment with the nonsteroidal antiinflammatory meloxicam on milk production, somatic cell count, probability of re-treatment, and culling of dairy cows with mild clinical mastitis, Journal of Dairy Science, **2009**; 92(9): 4421-31.
8. Friton GM, Cajal C, Ramirez-Romero R. Long-term effects of meloxicam in the treatment of respiratory disease in fattening cattle, Veterinary Record, **2005**; 156(25): 809-11.
9. Hoizey G, Lamiable D, Frances C, Trenque T, Kaltenbach M, Denis J, et al. Simultaneous determination of amoxicillin and clavulanic acid in human plasma by HPLC with UV detection, Journal of Pharmaceutical and Biomedical Analysis, **2002**; 30(3): 661-6.
10. Muth P, Metz R, Beck H, Bolten WW, Vergin H. Improved high-performance liquid chromatographic determination of amoxicillin in human plasma by means of column switching, Journal of Chromatography A, **1996**; 729(1-2): 259-66.
11. Shan-Ying C, Chang-Qin H, Ming-Zhe X. Chromatographic determination of high-molecular weight impurities in amoxicillin, Journal of Pharmaceutical and Biomedical Analysis, **2003**; 31(3): 589-96.
12. Tavakoli N, Varshosaz J, Dorkoosh F, Zargarzadeh MR. Development and validation of a simple HPLC method for simultaneous *in vitro* determination of amoxicillin and metronidazole at single wavelength, Journal of Pharmaceutical and Biomedical Analysis, **2007**; 43(1): 325-9.
13. Wiesner JL, de Jager AD, Sutherland FC, Hundt HK, Swart KJ, Hundt AF, et al. Sensitive and rapid liquid chromatography-tandem mass spectrometry method for the determination of meloxicam in human plasma, Journal of chromatography B; Analytical technologies in the biomedical and life sciences, **2003**; 785(1): 115-21.
14. Xie K, Jia L, Xu D, Guo H, Xie X, Huang Y, et al. Simultaneous determination of amoxicillin and ampicillin in eggs by reversed-phase high-performance liquid chromatography with fluorescence detection using pre-column derivatization, Journal of Chromatographic Science, **2012**; 50(7): 620-4.
15. Dasandi B, Shivaprakash, Saroj H, Bhat KM. LC determination and pharmacokinetics of meloxicam, Journal of Pharmaceutical and Biomedical Analysis, **2002**; 28(5): 999-1004.
16. Joseph-Charles J, Bertucat M. Simultaneous high performance liquid chromatographic analysis of non-steroidal anti-inflammatory oxicams in pharmaceutical preparations, Journal of Liquid Chromatography & Related Technologies, **1999**; 22(13): 2009-2021.
17. Sane R.T, Surve V, Francis M. Reversed phase high performance liquid chromatographic determination of meloxicam from human plasma using an internal standard, Indian Drugs, **2000**; 37(5): 251-254.
18. Sultana N, Arayne MS, Ali KA, Nawaz M. Simultaneous determination of clopidogrel and aspirin by RP-HPLC from bulk material and dosage formulations using multivariate calibration technique, Journal of Chromatographic Science, **2011**; 49(2): 165-9.
19. Velpandian T, Jaiswal J, Bhardwaj RK, Gupta SK. Development and validation of a new high-performance liquid chromatographic estimation method of meloxicam in biological samples, Journal of Chromatography B: Biomedical Sciences and Applications, **2000**; 738(2): 431-6.
20. Vignaduzzo SE, Castellano PM, Kaufman TS. Method development and validation for the simultaneous determination of meloxicam and pridinol mesylate using RP-HPLC and its application in drug formulations, Journal of Pharmaceutical and Biomedical Analysis, **2008**; 46(2): 219-25.
21. Charles B, Chulavatnatol S. Simple analysis of amoxycillin in plasma by high performance liquid chromatography with

internal standardization and ultraviolet detection, Biomedical Chromatography, **1993**; 7(4): 204-7.

22. ICH, International Conference on Harmonisation (ICH) Q2 (R1): Validation of analytical procedures: Test and methodology, Geneva, Switzerland, **2005**.

**How to cite this article:**

P.M. De La Torre-Iglesias et al.,: Validated RP-HPLC Method for Simultaneous Amoxicillin and Meloxicam Determination in Combined Dosage Forms, J. Pharm. Res., 2015; 4(11): 355-360.

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Source of support: Nil**