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# Simultaneous Determination of Bromhexine and Amoxicillin in Pharmaceutical Formulations by Capillary Electrophoresis

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**Abstract.** A simple and time-efficient method, based on capillary zone electrophoresis, was developed for the simultaneous determination of bromhexine (BMX) and amoxicillin (AMX) in pharmaceutical formulations. The optimized electrophoretic conditions comprise 50 mM sodium phosphate plus 50 mM citric acid as running buffer (pH 3.0), cartridge temperature 25°C, hydrodynamic injection (5 s. at 0.5 psi), 30 kV separation voltage, and UV detection at 214 nm. The analytes were separated in less than 5 min. Formulation samples were processed by successive treatment with methanol, hydrochloric acid, filtering and dilution in the running buffer. Loperamide was used as internal standard for quantitation. A linear detection range of 10-85 μg/mL for BMX ($R^2$ = 0.997) and 250-2010 μg/mL for AMX ($R^2$ = 0.995) was observed, with LOD for BMX as low as 2 μg/mL. The developed method enabled high analyte recoveries (99-104%) and excellent run-to-run reproducibility. Moreover, it was successfully employed for the determination of BMX and AMX in several pharmaceutical formulations, demonstrating its applicability to the routine quality control.
**Keywords:** Capillary electrophoresis, amoxicillin, bromhexine, loperamide, oral suspension.

**Resumen.** Se desarrolló un método simple y rápido, basado en la electroforesis capilar de zona, para la determinación simultánea de bromhexina (BMX) y amoxicilina (AMX) en formulaciones farmacéuticas. Las condiciones electroforéticas optimas comprenden una mezcla de fosfato sódico 50 mM y acido cítrico 50 mM como búfer de corrida, inyección hidrodinámica (5 s a 0.5 psi), voltaje de separación 30 kV, temperatura del capilar 25ºC y detección UV a 214 nm. Los analitos se separan en menos de 5 min. Las formulaciones muestra fueron procesadas empleando un tratamiento sucesivo con metanol, acido clorhídrico, filtración y dilución con el búfer de corrida. Se empleó loperamida como estándar interno para la cuantificación. El intervalo lineal de detección se observó de 10-85 μg/mL para BMX ($R^2$ = 0.997) y de 250-2010 μg/mL para AMX ($R^2$ = 0.995), con un LOD para BMX de 2 μg/mL. El método desarrollado presentó recobros altos (99-104%) y excelente reproducibilidad. Además, se utilizó exitosamente para la determinación de BMX y AMX en diversas formulaciones farmacéuticas, demostrando su aplicabilidad para control de calidad rutinario.
**Palabras clave:** electroforesis capilar, amoxicilina, bromhexina, loperamida, suspensión oral.

## Introduction

Pharmaceutical industry is a regulated and controlled sector of the Mexican Department of Health. Strict regulations are necessary to ensure quality of drug products. Therefore, appropriate analytical methodologies are required to control pharmaceutical formulations. The modern analysis of antibiotic drugs comprises the estimation of content and purity of active compounds, typically, by high performance liquid chromatography (HPLC) as a separation technique [1]. However, some drug products present a challenge due to specific properties (degradation, solubility, etc.) and HPLC limitations, calling for alternative techniques which can offer faster and more efficient and reliable analysis at low cost. Recently, capillary electrophoresis (CE) approach has been progressively introduced in the pharmaceutical industry, enabling simple and inexpensive quality control and representing a powerful alternative to HPLC [2-5].

In Mexico, a number of drug products on the market contain amoxicillin and bromhexine, and most of them are infant dosage forms. The Mexican United States Pharmacopeia (abbreviated in Spanish as FEUM) [6] or the USP [1] do not contain a comprehensive analytical procedure to quantify bromhexine (BMX) in pharmaceutical formulations, in spite of being present in different dosage forms as an active compound.

Amoxicillin (D-(-)-alpha-amino-p-hydroxybenzyl penicillin) is a semi-synthetic penicillin that belongs to the class of the β-lactam antibiotics. This class contains a β-lactam ring in the basic structure, responsible for the antibacterial activity, and variable side chains that account for the differences in chemical and pharmacological properties. Amoxicillin (AMX) is a white powder, water-soluble and insoluble in methanol.

Bromhexine (2-amino-3,5-dibromo-*N*-cyclohexyl-*N*-methylenzenemethane) is a weak base, highly soluble in methanol and slightly soluble in water; it precipitates at pH values above 6. BMX is a bronchosecretolytic and expectorant drug, typically used in a mixture with antibiotics to enhance their efficiency in the treatment of respiratory infections [7].

Different methods have been used to identify and quantify amoxicillin, including capillary zone electrophoresis (CZE) [8-9] and micellar electrokinetic capillary chromatography (MEKC) [10-12], in different samples. However, very few papers are related to bromhexine determination [13-15]. Moreover, simultaneous determination of BMX and β-lactam antibiotics in mixed formulations has not been reported until now.

Since BMX and AMX possess quite different physicochemical properties (Figure 1), having both analytes dissolved in the same medium in the presence of up to 9 excipients presented a challenging task.

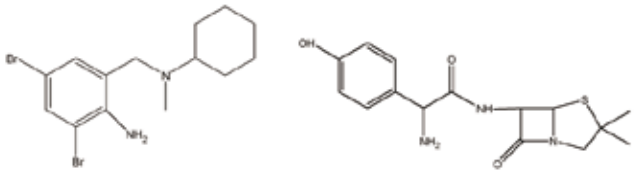

**Fig. 1.** Chemical structures of Bromhexine (left) and Amoxicillin (right).

The goal of this study was to develop an efficient procedure of sample pretreatment followed by CZE based analysis of BMX and AMX. The new proposed technique represents an attractive alternative to HPLC methods reported in FEUM.

## Results and discussion

### Method Development

Different parameters were studied to obtain final separation conditions of the analytes. Since BMX is insoluble above pH 6.0 [12] the analysis was conducted in 25 mM phosphate buffer at pH 6, 4.5 and 3. The best separation and shortest analysis time were achieved at pH 3. Further pH decrease resulted in significant increase of the analysis time due to either suppression of electroosmotic flow or change of electrophoretic mobility of AMX (Figure 2).

Other buffer systems were tested as background electrolytes (BGE). No analyte peaks were observed using 25 mM phthalate buffer, most likely due to the high absorption of the buffer itself. Using citrate buffer resulted into improved efficiency of the separation, shorter analysis time and lower current, compared to phosphate buffer. 50 mM concentration of the citrate buffer was found optimal for the separation efficiency and reproducibility.

Absorption spectra of analytes were obtained during CZE analysis and maximum absorption wavelengths were 210 and 220 nm for BMX and 230 nm for AMX. 214 nm as detection wavelength was selected based on the maximized signal-to-noise ratio for BMX, which is the minor component in pharmaceutical formulations that contain both active compounds.

Hydrodynamic (5 s at 0.5 psi) and electrokinetic (10 kV for 25 s) injections were evaluated, the former resulting in the higher and better resolved peaks. Longer injection times (8 and 10 s) were tested using hydrodynamic injection, but wider peaks and concomitant lower separation efficiency were observed.

Effect of the applied electric potential was investigated as well, and the highest voltage (30 kV) was found optimal for the analysis rendering faster separations and minimized peak broadening. Since this method is intended to be used in the pharmaceutical industry, RSD equal or less than 2% for peak area and migration time must be obtained. Rinsing the capillary between runs was quite important to achieve the necessary

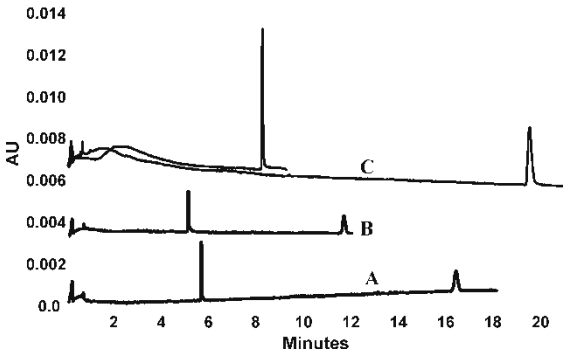


**Fig. 2.** Effect of pH in BMX and AMX separation: (A) 3.5, (B) 3.0 and (C) 2.5. Other conditions: 75 μm I.D. capillary and 40 cm total length, 25 mM phosphate buffer, hydrodynamic injection 5 s at 0.5 psi, 25 kV, 25°C, detection at 214 nm. First peak BMX, second peak AMX.

repeatability. RSD values for peak area and migration time were lower when 3 min wash time was employed, compared to shorter rinsing times.

### Sample treatment

Among the pharmaceutical formulations of AMX and BMX, the oral suspensions typically contain a number of excipients, e.g. Amoxibrom® has 9 excipients. This oral suspension was selected as a model sample to develop an adequate sample treatment to ensure complete dissolution of active ingredients in the mixture.

Different solvents were tested, including aqueous solutions at acidic pH, to determine the best system for each analyte. The best solubility was achieved using first methanol and then water solution of pH 1.0 (prepared as 0.1M HCl) for BMX and AMX, respectively.

A mixture of analytes, in the proportion presented in oral suspension (approximately 6 mg BMX and 180 mg AMX), was treated with the addition of 5 mL methanol followed by 20 mL 0.1M HCl. It was found that solvent addition order is important, and no complete dissolution of BMX was accomplished when HCl was added before methanol.

Solubility of the 9 excipients in methanol and HCl was determined. Using proportions established in the formulation, 5 mL of methanol were added to each component, followed by 20 mL 0.1 M HCl. Three excipients were found insoluble in the above solution, while the others dissolved to a certain extent. The excipients that showed some dissolution were analyzed by CE, and one fast migrating peak was observed preceding the BMX peak.

In order to determine BMX and AMX solubility in the presence of the 9 excipients, a mixture of excipients, BMX

and AMX, simulating the oral suspension formulation (fortified placebo), was prepared by adding 5 mL of methanol to 440 mg of the powder and stirring for 5 min followed by addition of 20 mL 0.1 M HCl and 10 min stirring. The electropherogram of the above mixture revealed a somewhat distorted BMX peak, while no matrix effect on electrophoretic migration of AMX was observed. The cause of the peak asymmetry was likely the high acidity of the sample solution (pH=1) relatively to the BGE at pH 3. Attempts were made to reduce the acidity. However, the best recovery was achieved using the combination of methanol, HCl and citrate buffer resulting in the low pH final solution. To circumvent this problem, an effort was made to improve efficiency of CE analysis and BMX peak shape by optimizing the separation conditions.

## CE Method Modifications

In order to buffer pH on CE separation, a higher concentration buffer was investigated. The mixed buffer of 50 mM phosphate and 50 mM citrate at pH 3.0 was found optimal, while 100 mM phosphate buffer generated a higher electrical current. To further reduce the Joule heating, a narrower 50 μm ID capillary was tested.

A short total capillary length (30 cm) was chosen to speed up the analysis of well resolved BMX and AMX peaks. Comparison of separations under different conditions is presented in Figure 3. As one can observe, separation efficiency is higher and the analysis time is shorter under optimized CE parameters. Proposed method enables simultaneous quantitative analysis of BMX and AMX in a single run.

## Statistical Parameters

Five calibration standard solutions in the range of 10-85 and 250-2010 μg/mL were used for BMX and AMX, respectively. Two replicates were prepared at each concentration level, and triplicate injections were performed, using a placebo as blank. Calibration curves were constructed considering peak area as a function of analyte concentration. Calibration parameters derived from the least-squared regression are listed in Table 1. Repeatability was determined over six replicate experiments on the same day. Relative standard deviations (RSD) for BMX and AMX migration time (0.67 and 2.06%) and peak area (1.79 and 3.52%) were obtained. Estimated limits of detection (LOD) and quantitation (LOQ) for BMX, the minor component in the mixture, were 1.94 and 5.9 μg/mL, respectively. These limits are well below from expected concentrations in pharmaceutical formulations prepared according to the proposed procedure.

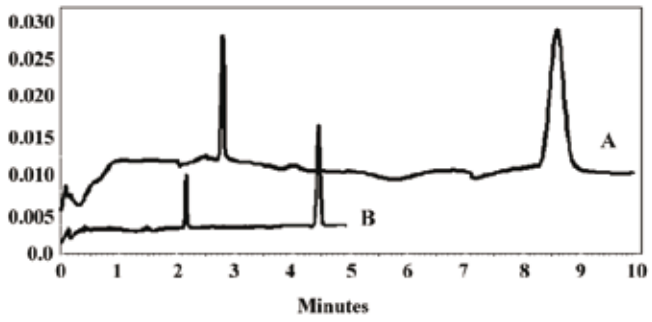


**Fig. 3.** BMX and AMX separation, pH 3.0, hydrodynamic injection 5 s at 0.5 psi, 30 kV, 25°C, detection at 214 nm. (A) 50 mM citrate buffer, 75μm I.D. capillary and 40 cm total length; (B) 50 mM citrate plus 50 mM phosphate as BGE, 50 μm I.D. capillary and 30 cm total length.

## Internal Standard

The internal standard was introduced to improve AMX analysis accuracy and compensate for possible injection and migration time variability. Among all the compounds tested (30), loperamide, migrating between BMX and AMX, was selected as a good internal standard based on its symmetrical peak and migration time similar to the analytes of interest. Calibration curves based on the relative corrected peak area as a function of analyte concentration and their parameters are presented in Table 1. Lower relative standard deviations (RSD) for BMX and AMX peak area (1.63 and 1.89%) using loperamide as internal standard were obtained, confirming the good reproducibility of the developed analytical approach.

## Application

Pharmaceutical formulations containing AMX and BMX for both adult and infant use have been commercialized as different drug products. To demonstrate the applicability of the above proposed methodology for the analysis of these different formulations, Amoxibrom®, oral suspension and capsules were analyzed. These formulations are used in adults to treat various infections. Three samples of Amoxibron® oral suspension (lot 001GF046) and Amoxibron® capsules (lot 002FG034) were analyzed in duplicates using the developed analytical procedure, recoveries between 98-102% were achieved for both compounds in the preparations which is in compliance with the values indicated for AMX (90-120%) by Mexican Pharmacopeia [6]. Other commercial drug formulations were also successfully analyzed employing the established methodology (Table 2). The content obtained for AMX (101-110%) and BMX (99-102%) were adequate. Thus, the applicability of the method for simultaneous quality control of the two active ingredients in pharmaceutical preparations, without any pre-concentration

**Table 1.** Calibration curve parameters.

| Analyte | Linearity range (μg/mL) | Linear equation | $R^2$ |
|---|---|---|---|
| BMX | 10-85 | 317.73 + 1158.1x | 0.9947 |
| AMX | 250-2010 | 231.0 + 21200.3x | 0.9937 |
| BMX-IS* | 10-85 | 0.0048 + 0.0182x | 0.9967 |
| AMX-IS* | 250-2010 | 0.0035 + 0.3310x | 0.9943 |

* Loperamide (200 ppm) as internal standard.

**Table 2.** Determination of AMX and BMX in pharmaceutical formulations.

| Medicament | Composition | AMX | | | BMX | | |
|---|---|---|---|---|---|---|---|
| | | *Nominal (mg)* | *Found (mg)** | Content (%)* | *Nominal (mg)* | *Found (mg)** | Content (%)* |
| Amoxibron® oral suspension | BMX, AMX, (9 excipients) | 250 | 231.1 ± 2.7 | 92.5 ± 1.1 | 8.0 | 8.0 ± 0.2 | 99.6 ± 2.6 |
| Amoxibron® Capsules | BMX, AMX, (1 excipient) | 500 | 493.4 ± 15.4 | 98.7 ± 3.1 | 8.0 | 8.2 ± 0.2 | 101.9 ± 2.5 |
| Lumoxbron-S® oral suspension | BMX, AMX | 250 | 270.3 ± 7.4 | 108.1 ± 2.9 | 8.0 | 8.07 ± 0.4 | 100.9 ± 5.2 |
| Tusibron® oral suspension | BMX, AMX, Oxolamine | 250 | 258.2 ± 2.7 | 103.3 ± 1.1 | 4.0 | 3.95 ± 0.2 | 99.4 ± 2.8 |
| Servamox CLV tablets | AMX, Clavulanic acid | 500 | 509.1 ± 2.4 | 101.8 ± 2.3 | — | — | — |
| Clavulin 12H tablets | AMX, Clavulanic acid | 875 | 886.3 ± 10.9 | 101.3 ± 1.2 | — | — | — |
| Ormocyn T-S capsules | AMX | 500 | 550.3 ± 4.4 | 110.1 ± 4.2 | — | — | — |
| Bisolvon Syrup | BMX | — | — | — | 4.0 | 3.87 ± 0.2 | 96.85 ± 2.4 |

* ± Standard deviation.

step or interference from different excipients or other actives, has been demonstrated (Figure 4).

## Conclusions

A simple and time-efficient method, based on capillary zone electrophoresis, was developed for the simultaneous determination of bromhexine (BMX) and amoxicillin (AMX) in pharmaceutical formulations. The developed method showed linearity (10-85 µg/mL BMX; 250-2010 µg/mL AMX) and high analyte recoveries (99 - 104 %) and was successfully employed for the determination of BMX and AMX in other pharmaceutical formulations, even in presence of other actives, such as clavulinic acid.

## Experimental

A P/ACE MDQ Beckman capillary electrophoresis system (Beckman Coulter Fullerton, CA., USA) equipped with a diode-array detector was used. The CE instrument was fully controlled by 32 KARAT software (Beckman Coulter). Polyimide coated bare fused silica capillaries (Polymicro Technologies,

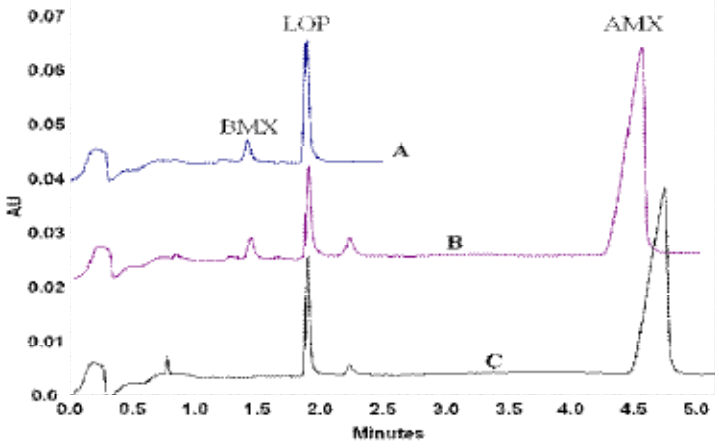


**Fig. 4.** Comparison of AMX and BMX separation, within some commercial medicaments. (A) Bisolvon® infant solution: BMX 80 mg/100 mL, (B) Lumoxbron-S Adult suspension: BMX 8 mg/5 mL, AMX 250 mg/5 mL, (C) Servamox® tablets: AMX 500 mg, Clavulanic acid 125

Arizona, USA) were utilized. The temperature of the capillary cartridge during electrophoresis was maintained at 25°C and UV detection was set to 214 nm. Samples were introduced by pressure injection at 0.5 psi for 5 s.

New capillaries were conditioned by rinsing with 1 M NaOH for 5 min, 0.1 M NaOH for 5 min and $H_2O$ for 5 min. Working capillaries were conditioned daily with 0.1M HCl at 20 psi for 5 min, deionized water for 7 min and running buffer for 15 min. Between runs, the capillary was flushed with buffer for 3 min.

The pH of the buffer solutions and samples was adjusted with an HI931401 pH/meter. The samples were weighed on analytical balance BOECO (Germany) with precision of 0.0001 g. A vacuum pump (Alltech, Benchtop Vacuum Station) was used for filtration.

AMX trihydrate (88.7%) and BMX hydrochloride (99.9%) standards (Sigma Aldrich, USA) and raw materials were donated by Grimman Laboratories. Methanol (HPLC grade) and analytical grade reagents (sodium hydroxide, sodium tetraborate, citric acid, sodium phosphate monobasic and hydrochloric acid) were purchased from J.T. Baker (Xalostoc, Mexico). Water (18.2 $M\Omega \cdot cm^{-1}$) was deionized on a Milli-Q plus system (Millipore, Bedford, MA).

Amoxibron®, oral suspension and capsules, were kindly supplied by Grimann Laboratories.

Other samples were bought directly from retail pharmacies.

The optimized running buffer, 50 mM phosphate and 50 mM citrate (pH 3), was prepared by mixing equal amounts of 100 mM phosphate and 100 mM citrate adjusted to pH 3 with NaOH or HCl, as appropriate.

The stock solutions of AMX, BMX and internal standard (loperamide) were prepared daily by dissolving the appropriated amount of bromhexine hydrochloride, amoxicillin trihydrate and loperamide in methanol, 0.1 M HCl and running buffer, respectively, to a final concentration of 5000 μg/mL AMX, 210 μg/mL BMX and 1000 μg/mL loperamide. Separate stock solutions were prepared for calibration curves and further diluted with running buffer.

Amoxibrom® Oral Suspension: a portion of the powder equivalent to 2 mg of BMX and 62.5 mg of AMX was dissolved in 10 mL of methanol and shaken for 15 min, followed by addition of 25 mL of 0.1N HCl and shaking for 15 min. The solution was filtered and diluted to 50 mL with running buffer (AMX 1250 μg/mL and BMX 40 μg/mL). For quantitation 200 μg/mL loperamide was added as an internal standard.

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## References

1. United States Pharmacopeia, USP32, NF27, U.S.A., 2009.
2. Altria, K. D.; Elder, D. *J. Chromatogr. A* **2004**, *1023*, 1-14.
3. Anastos, N.; Barnett, N. W.; Lewis, W. S. *Talanta* **2005**, *67*, 269-279.
4. Hong, W.; Thompson, R. A. *Drug Discovery Today: Technologies* **2005**, *2*, 171-178.
5. Thanh, P. T.; Hoogmartens, J.; Van Schepdael, A. *J. Pharm. Biomed. Anal.* **2006**, *41*, 1-11.
6. Farmacopea de los Estados Unidos Mexicanos, Secretaría de Salud, Comisión Permanente de la FEUM. 9ª ed., México, **2008**.
7. Rosestein Ster, E. Compendio del Diccionario de Especialidades Farmacéuticas, 49 ed., Thomson, PLM, **2003**.
8. Hernandez, M.; Borull, F.; Calull, M. *J. Chromatogr. B* **1999**, *731*, 309-315.
9. Santos, S. M.; Henriques, M.; Duarte, A. C.; Esteves, V. I. *Talanta* **2007**, *71*, 731-737.
10. Li, Y. M.; Schepdael, A.; Zhu, Y.; Roets, E.; Hoogmartens, J. *J. Chromatogr. B* **1998**, *812*, 227-236.
11. Pajchel, G.; Pawlowski, K.; Tysli, S. *J. Pharm. Biomed. Anal.* **2002**, *29*, 75-81.
12. Bailon, M. I.; Cuadros, L.; Cruces-Blanco, C.; *J. Pharm. Biomed. Anal.* **2007**, *43*, 746-752.
13. Perez-Ruiz, T.; Martinez-Lozano, C.; Sanz, A.; Bravo E. *J Chromatogr. B* **1997**, *692*, 199-205.
14. Berzas, J. J.; Castañeda, G.; Guzmán, F. J. *J. Chromatogr. A* **2001**, *918*, 205-210.
15. Okamoto, H.; Nakajima, T.; Ito, Y.; Aketo, T.; Shimada, K.; Yamato, S. *J. Pharm. Biomed. Anal.* **2005**, *37*, 517-528.