

Simultaneous separation and determination of quinolones in pharmaceuticals by micellar liquid chromatography

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Abstract

A rapid and simple liquid chromatographic procedure using micellar mobile phases is reported for the separation and determination of four quinolones (pipemidic acid, levofloxacin, norfloxacin and moxifloxacin) in pharmaceuticals.

This purpose was achieved without any previous pretreatment step in a C18 column using a micellar mobile phase of 0.15 M sodium dodecyl sulphate, 2.5% propanol and 0.5% triethylamine at pH 3, with retention times below 12 min. For detection, the diode-array UV-Vis set at 276 nm was used. The limits of detection and quantification were between 8-51 and 28-171 ng/mL, respectively. This method was validated in terms of intra-day and inter-day precision and accuracy, and robustness. Calibration curves over the concentration range of 0.1-50 µg/mL were linear ($r^2 > 0.9997$) and. Good claim percentages (96–106 %) were obtained in the analysis of pharmaceutical formulations. The results show that the procedure is suitable for the routine analysis of drugs.

Key words: Quinolone, Fluoroquinolone, Micellar mobile phase, SDS, Direct injection, Pharmaceutical formulation

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1. Introduction

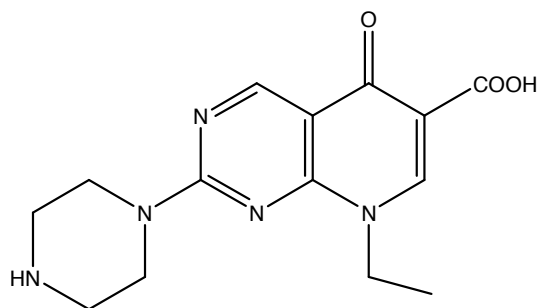
Quinolones and fluoroquinolones are synthetic antibiotics whose action is based on their anti-DNA activity. Since nalidixic acid was discovered [1], a number of structure modifications to the quinolone nucleus have been performed to increase antimicrobial activity and to enhance the pharmacokinetic performance of these drugs. The general structure consists of a 1-substituted-1,4-oxopyridine-3-carboxylic moiety combined with either an aromatic or heteroaromatic ring. Fluoroquinolones are quinolones with a fluorine atom at position 6 of the quinolone naphthyridine or benzoaxazine ring systems, and belong to the second generation of quinolones. They are characterised by their greater effectiveness against bacterial activity [2], and are used in both human and veterinary medicine. In humans, they are used to treat an extensive range of diseases, including urinary, respiratory and gastrointestinal tract infections [3].

The analysis of quinolones has traditionally been performed using microbiological methods. However, this technique is time-consuming and offers poor precision and specificity. Other non-routine techniques, such as terbium (III)-sensitised luminescence [4], capillary electrophoresis [5- 7] or immunoaffinity chromatography [8], have also been applied.

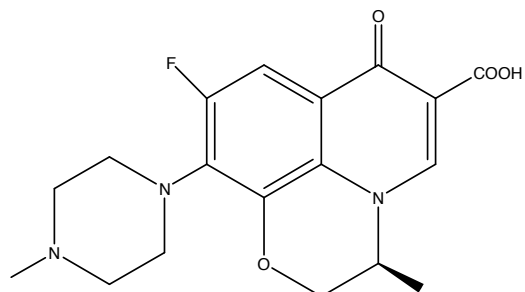
Last generation LC-MS-(MS) equipments has also been used [9-11], although this equipment is very expensive and only a few laboratories can afford such instrumentation. High-performance liquid chromatography (HPLC) has become an important tool for the analysis of single and various combinations of quinolones in biological fluids, foods, environmental samples and pharmaceutical preparations using either UV or fluorescence as the detection method [12-27].

Micellar chromatographic (MLC) methods offer the advantages of the direct injection of samples with no pretreatment other than filtration and the low toxicity of the mobile phases employed [28]. MLC has proved to be a useful technique in the determination of diverse groups of drugs, such as thiazide diuretics [29, 30], furosemide [31] and trazodone [32] in pharmaceutical formulations.

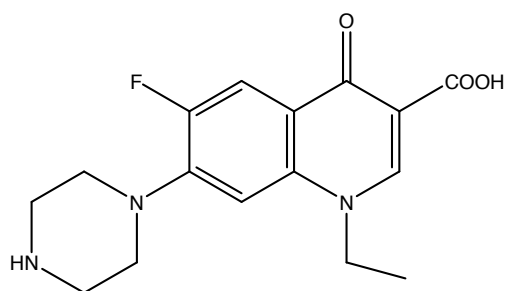
In this work, a simple chromatographic procedure with micellar mobile phases of SDS for the simultaneous determination of pipemidic acid, levofloxacin, norfloxacin and moxifloxacin (Figure 1) has been developed and applied to control numerous pharmaceuticals in several dosage forms, and validated according to the ICH harmonised tripartite guideline [33]. Although these compounds are not administered together, the proposed method allowed the determination and quantification of the four quinolones in a single chromatographic run without modifications being not necessary a chromatographic system for each compound separately, which make the proposed method more economic and faster.



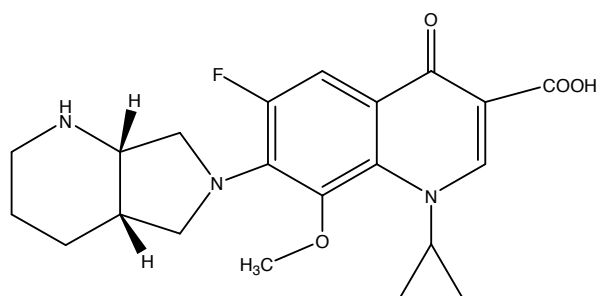
Log P 0.18; pKa₁ 5.5
Pipemidic Acid (PIP)



Log P 1.26; pKa₁ 6.05 pKa₂ 8.22
Levofloxacin (LEV)



Log P 1.64; pKa₁ 6.3 pKa₂ 8.8
Norfloxacin (NOR)



Log P 2.03; pKa₁ 6.4 pKa₂ 9.5
Moxifloxacin (MOX)

Figure 1. Structures, octanol-water partition coefficient ($\log P$) and acid-based constants of the studied quinolones.

2. Experimental

2.1. Reagents and samples

Pipemidic acid (PIP) and norfloxacin (NOR) were purchased from Sigma (St. Louis, MO, USA), moxifloxacin (MOX) from Bayer (Leverkusen, Germany) and levofloxacin (LEV) from Fluka (Milan, Italy). Distilled-deionised water was used throughout. Sodium dodecyl sulphate (SDS), sodium dihydrogen phosphate and methanol were obtained from Merck (Darmstadt, Germany). Propanol was purchased from Scharlab (Barcelona, Spain). Hydrochloric acid and triethylamine were acquired from J.T Baker (Deventer, the Netherlands).

2.2. Instrumentation

The pH of the solutions was measured with a Crison GLP 22 (Barcelona), equipped with a combined Ag/AgCl/glass electrode. The balance used was a Mettler-Toledo AX105 Delta-Range (Greifensee, Switzerland). The vortex shaker and sonification unit were acquired from Selecta (Barcelona). The chromatographic system was an Agilent Technologies Series 1100 (Palo Alto, CA, USA) equipped with a quaternary pump, an autosampler and a UV-Visible detector.

2.3. Chromatographic conditions

A reversed-phase Kromasil C18 column (150 mm×4.6 mm, 5 µm particle size) (Scharlab) was used. The selected mobile phase was 0.15 M SDS, 2.5% (v/v) propanol and 0.5% triethylamine at pH 3. The flow rate and injection volume were 1 mL/min and 20 µL, respectively. Experiments were carried out at room temperature and detection was performed at

276 nm. Chromatographic signals were acquired and processed with an Agilent ChemStation (Rev. A.10.01).

2.4. Mobile and standard solutions preparation

The micellar mobile phase was prepared using SDS and 0.5% (v/v) of triethylamine, which was buffered with sodium dihydrogen phosphate 0.01 M at pH 3 using HCl 0.1 M and, lastly, propanol was added to obtain the desired concentration.

Stock solutions of 50 µg/mL of each compound were prepared. Drugs were dissolved in ethanol with the help of an ultrasonic bath and topped up to the mark on the volumetric flask with a 0.1 M SDS solution buffered with phosphate at pH 3. For the analysis of the drugs, several standard solutions were prepared in the 1-25 µg/mL range. Fresh solutions were prepared periodically.

2.5. Pharmaceutical sample preparation

The pharmaceuticals analysed were tablets and coated capsules. The average weight per tablet was calculated from ten units. Tablets were ground and reduced to a fine homogeneous powder in a mortar. Several portions of this powder were accurately weighed and sonicated in the presence of ethanol (5% v/v of the final content) in an ultrasonic bath. Then 0.1 M SDS solution at pH 3 was added to favour the extraction of the analyte, and the ultrasonic bath was used again. The excipients in the tablets were not soluble in the micellar medium. Therefore, sample solutions were filtered before being injected into the chromatograph. However, filtration was always performed directly into the autosampler vials through 0.45 µm nylon membranes (Micron Separations, MA, USA).

3. Results and discussion

3.1 Mobile phase selection

Preliminary studies were carried out to select an efficient method for the analysis of four quinolones. Parameters, such as detection wavelength, mobile phase composition, percentages, and optimum pH, have been thoroughly studied.

Several mobile phases were investigated using different alcohols and percentages. Propanol yielded better efficiencies but larger retentions than butanol. However, the peaks of the compounds could not be resolved with butanol. Thus, propanol was preferred to optimise the separation of the four drugs.

Quinolones have two ionisable functional groups: carboxylic acid and N₄ of the piperazine ring. The carboxylic group is normally a stronger acid than the ammonium group and has a pK_{a1} value ranging from 5.5 to 9.5 in water. The pK_a values and octanol-water partition coefficients ($\log P$) are shown in Figure 1 [34-36]. Among the different pH media tested, pH 3 was chosen because good retention times were obtained with narrow and well resolved peaks. Furthermore, efficiencies deteriorated when the pH of the mobile phase was increased.

Bonded silica phases are problematic from the point of view of pH stability and residual chemical activity of the unprotected silica support, which can induce tailing peaks and variable retention times for basic compounds. Using an amine, such as triethylamine (TEA), is common practice to protect the silanol groups of the stationary phase in order to increase peak efficiencies for basic compounds with amine groups. The addition of triethylamine (TEA) enhanced the efficiencies of the four quinolones. However, this amine behaved as another modifier and the

retention factors of the compounds lowered. For these reasons, the TEA concentration was limited to 0.5%.

3.2. Optimization strategy

An optimization study for the mixture of PIP, LEV, NOR and MOX was done. An adequate control of the concentrations of both the surfactant and modifier can lead to chromatograms presenting a good resolution and sufficient elution strength.

In order to optimize the mobile phase composition, the retention equation of the four quinolones was obtained using a reduced (five) and selected number of mobile phases – four located at the corners of a rectangular factor space and the fifth in its centre. The limits of the factor space (surfactant and alcohol) were in the 0.05M to 0.15M and 2.5 to 12.5% ranges for the concentration of SDS and the volume of propanol, respectively. The errors in the retention factors predicted with these equations were below 2% for all the compounds. The retention factors (k), efficiencies (N) and asymmetry factors (B/A) of the four compounds were measured and processed with the Michrom software [37, 38], which helps to model the compounds retention by taking into account the maximum resolution factor and the minimum analysis time. The equation used was:

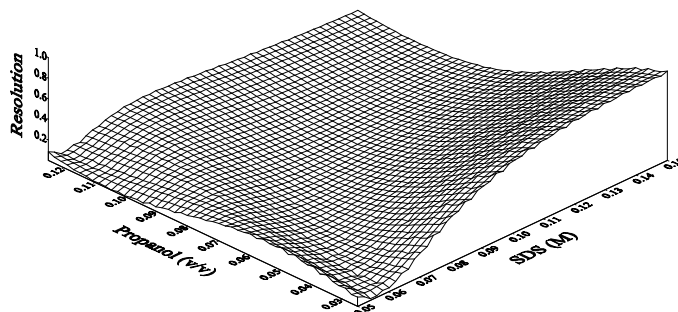
$$k = \frac{K_{AS} \frac{1}{1 + K_{AD}\varphi}}{1 + K_{AM} \frac{1 + K_{MD}\varphi}{1 + K_{AD}\varphi} [M]} \quad (1)$$

where $[M]$ and φ are the concentrations of the surfactant and modifier; K_{AS} and K_{AM} correspond to the equilibria between the solute in bulk water and the stationary phase or micelle, respectively; K_{AD} and K_{MD} measure the relative variation in the concentration of the solute in

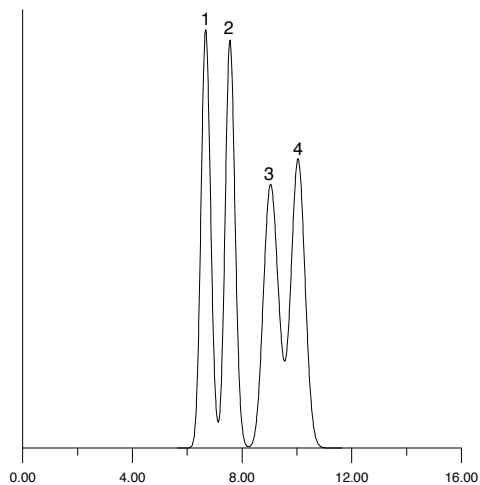
bulk water and micelles due to the presence of a modifier, as compared to a pure micellar solution (without a modifier).

The global resolution diagram, and the simulated and real chromatograms for the optimum mobile phase obtained are depicted in Figure 2. As can be seen in Figure 2a, resolution values close to one (maximum value) can be obtained in a narrow region of SDS (0.12-0.15 M) and propanol (2.5-3.5 % (v/v)). The best resolution value was obtained for a composition of 0.15 M SDS – 2.5% propanol – 0.5% TEA- 0.01 M NaH₂PO₄ at pH 3 with an analysis time below 12 minutes. Thus this mobile phase was selected as optimum. Figure 2b and 2c show the simulated and experimental chromatogram for the mixture of the four quinolones in the optimum mobile phase. The agreement between both is excellent. The chromatographic parameters (*k*, *N* and *B/A*) obtained were: 6.6, 1900 and 1.1; 5.7, 1500 and 1.1; 9.1, 1700 and 1.0; and 8.1, 1100 and 1.2 for PIP, LEV, NOR and MOX, respectively.

(a)



(b)



(c)

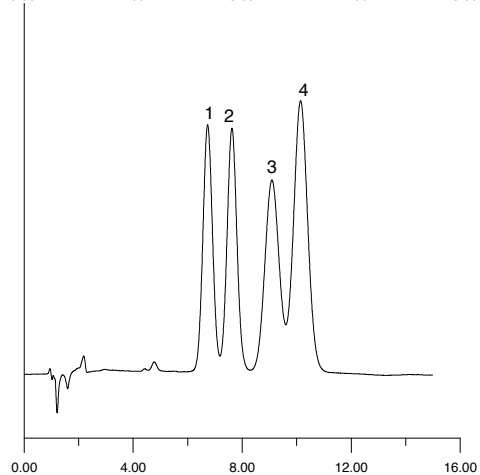


Figure 2. (a) Global resolution diagram, (b) simulated and (c) real chromatogram for a mixture of (1) PIP (1.25 $\mu\text{g}/\text{mL}$), (2) LEV (5 $\mu\text{g}/\text{mL}$), (3) MOX (10 $\mu\text{g}/\text{mL}$) and (4) NOR (2.5 $\mu\text{g}/\text{mL}$). Mobile phase: 0.15 M SDS - 2.5% 1-propanol - 0.5% thiethylamine - pH 3, flow rate: 1mL/min, UV detection at 276 nm.

4. Validation

The ICH harmonised tripartite guideline [33] was followed to validate the method.

4.1. Linearity

Under the selected chromatographic conditions, the linear range of the signal response for each drug was studied over the concentration range of 0.1–50 µg/mL. Seven different concentration levels (0.1, 0.5, 1, 5, 15, 25, 50 µg/mL) were obtained for each standard solution, and were conveniently diluted with 0.1 M SDS solution at pH 3. Each solution was injected into the chromatographic system (n=6), and the average value of the peak areas was plotted against the concentrations. Curves were adjusted for linear regression with the least mean squares method. All the calibration plots in the concentration range studied were linear and with correlation coefficients (r^2) higher than 0.9997, as shown in Table 1.

Table 1. Linear regression data and the limits of detection and quantification for PIP, LEV, NOR and MOX

Analyte	Slope \pm SD	Intercept \pm SD	R ²	LOD (ng/mL)	LOQ (ng/mL)
Pipemidic Acid	2.67 \pm 0.19	-0.27 \pm 0.16	0.9998	8	28
Levofloxacin	0.83 \pm 0.03	-0.149 \pm 0.021	0.9997	56	171
Norfloxacine	2.47 \pm 0.14	-0.09 \pm 0.07	0.9999	33	100
Moxifloxacin	0.405 \pm 0.024	-0.0199 \pm 0.016	0.9999	14	43

4.2. Precision and Accuracy

In order to determine the intra-day precision and accuracy of the method, four known concentrations (0.5, 5, 25 and 50 µg/mL) of each drug were analysed on the same day (n=6).

Inter-day precision and accuracy were also evaluated over five consecutive days by performing six successive injections each day of the same concentrations. The results are summarized in Table 2. The low variability and high precision of the results obtained in different days are evident, which indicate the usefulness of the method.

Table 2. Inter-day and intra-day precision and accuracy of analytes

Analyte	Concentration added ($\mu\text{g/mL}$)	Found ^a (mean \pm SD) ($\mu\text{g/mL}$)	Accuracy (%)	Intra-day C.V. (%)	Found ^b (mean \pm SD) ($\mu\text{g/mL}$)	Accuracy (%)	Inter-day C.V. (%)
Pipemidic Acid	0.5	0.505 \pm 0.008	1.6	2.1	0.501 \pm 0.007	0.3	3.0
	5	4.97 \pm 0.15	0.6	3.2	5.00 \pm 0.08	0.1	5.1
	25	24.75 \pm 0.13	1.0	0.5	24.7 \pm 0.3	0.4	3.9
	50	50.17 \pm 0.19	0.3	0.4	50.11 \pm 0.18	0.2	5.2
Levofloxacin	0.5	0.4888 \pm 0.0011	2.2	0.4	0.499 \pm 0.010	0.2	2.8
	5	4.88 \pm 0.08	2.3	1.7	5.03 \pm 0.09	0.6	4.9
	25	24.51 \pm 0.14	2.0	0.6	25.2 \pm 0.4	0.7	6.4
	50	50.3 \pm 0.6	0.6	1.0	50.16 \pm 0.13	0.3	6.3
Norfloxacin	0.5	0.505 \pm 0.006	1.0	0.1	0.498 \pm 0.006	0.3	2.4
	5	4.90 \pm 0.17	1.9	3.5	4.99 \pm 0.08	0.1	4.7
	25	25.20 \pm 0.06	0.8	0.3	24.9 \pm 0.5	0.4	3.9
	50	49.9 \pm 0.3	0.2	0.7	50.05 \pm 0.13	0.1	5.4
Moxifloxacin	0.5	0.495 \pm 0.007	1.0	1.4	0.496 \pm 0.007	0.6	4.6
	5	4.89 \pm 0.06	2.1	1.3	4.97 \pm 0.03	0.6	4.3
	25	24.4 \pm 0.3	2.3	1.2	24.85 \pm 0.14	0.6	4.4
	50	50.1 \pm 0.9	0.2	1.8	50.15 \pm 0.14	0.3	4.3

^a n = 6, ^b n = 5

4.3. Limits of detection and quantification

The limit of detection (LOD) and quantification (LOQ) for PIP, LEV, NOR and MOX (n = 10) were determined with the *3.3s criterion* and *10 criterion*, respectively, using a series of 10 solutions containing a low concentration. The results were based not only on the standard deviation of the response, but also on the slope of a specific calibration curve containing the

analyte. Both LODs and LOQs are summarised in Table 1 according to the ICH harmonised tripartite guideline [33]. The values of the limits obtained were in ng/ml range doing the procedure sensitive enough for routine analysis.

4.4. Robustness

The robustness of the method was evaluated in terms of SDS (M), percentage of 1-propanol (%) (v/v), pH, percentage of thiethylamine (%) (v/v) and the flow rate of the mobile phase by six replicate injections of a standard solution at 5 µg/mL. The RSD (%) of the retention times calculated from these variations is shown in Table 3 and was lower than 11.0%. Variation of the flow rate values (0.9, 1, 1.1 mL/min) had a stronger influence on the retention of the studied compounds than other parameters. However, the variations in all the parameters had no significant effect on resolution, peak area and peak shape.

Table 3. Evaluation of the robustness of the MLC method

Chromatographic changes	Level	PIP	LEV	NOR	MOX
A: Flow rate (mL/min)		t_R (min)	t_R (min)	t_R (min)	t_R (min)
0.9	-0.1	7.7	7.4	11.3	10.23
1	0	7.6	6.7	10.08	9.06
1.1	+0.1	6.7	6.10	9.18	8.24
Mean ± SD		7.7 ± 0.7	6.7 ± 0.7	10.2 ± 1.0	9.2 ± 1.0
RSD (%)		9.7	10.0	10.3	10.9
B: SDS concentration (M)					
0.145	-0.05	7.7	6.8	10.3	9.23
0.15	0	7.6	6.7	10.08	9.06
0.155	+0.05	7.6	6.7	10.06	9.08
Mean ± SD		7.64 ± 0.03	6.76 ± 0.08	10.17 ± 0.16	9.13 ± 0.09
RSD (%)		0.34	1.12	1.6	1.01
C: Percentage of butanol (v/v)					
2.4	-0.1	8.3	7.3	11.20	9.9
2.5	0	7.6	6.7	10.08	9.06
2.6	+0.1	6.07	6.7	10.0	9.3
Mean ± SD		7.8 ± 0.4	6.9 ± 0.3	10.4 ± 0.7	9.3 ± 0.5
RSD (%)		5.21	4.8	6.4	5.7
D: pH of mobile phase					
2.9	-0.1	7.7	6.7	10.19	9.14
3	0	7.6	6.7	10.08	9.06
3.1	+0.1	7.7	6.7	10.21	9.11
Mean ± SD		7.65 ± 0.03	6.734 ± 0.021	10.16 ± 0.07	9.10 ± 0.04
RSD (%)		0.3	0.3	0.7	0.4
E: Percentage of TEA (v/v)					
0.45	-0.05	7.7	6.7	10.18	9.10
0.5	0	7.6	6.7	10.08	9.06
0.55	+0.05	7.6	6.7	10.11	9.02
Mean ± SD		7.63 ± 0.03	6.721 ± 0.014	10.13 ± 0.05	9.06 ± 0.04
RSD (%)		0.4	0.2	0.5	0.5

4.5. Analysis of pharmaceutical formulations

The contents of nineteen pharmaceutical formulations, commercially available in Spain, were determined. Calibration curves were constructed by measuring the areas of the chromatographic peaks of the duplicate injections of the PIP, LEV, NOR and MOX solutions at five increasing concentrations in the 1-25 $\mu\text{g/mL}$ range. For each drug, six injections were performed using 10 $\mu\text{g/mL}$ of each compound. Figure 3 illustrates the chromatograms of the pharmaceuticals: pipemidic acid (a), levofloxacin (b), norfloxacin (c) and moxifloxacin (d). The excipients were eluted with the dead time or did not absorb at the measuring wavelength.

The labelled composition of the formulations, recoveries and CV (%) values are shown in Table 4. The label claim percentage values were in the 96-106% range and the coefficient of variation in the range of 0.2-1.8%. As observed, the results obtained are in accordance with the labelled values. Moreover, the excipients were eluted with the dead time or did not absorb at the measuring wavelength.

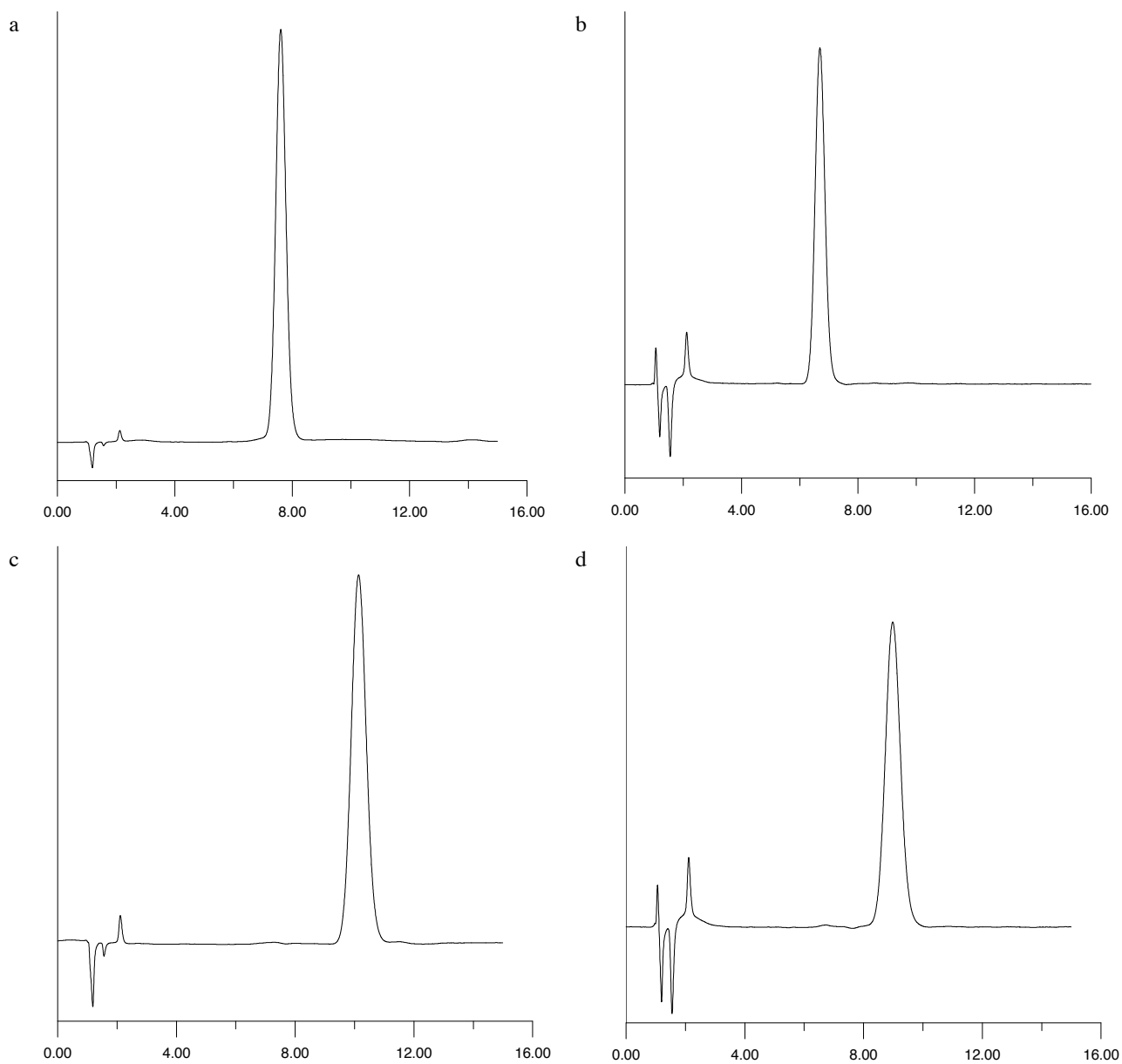


Figure 3. Chromatogram of (a) pipemidic acid (Nuril, 400mg), (b) levofloxacin (Stada, 500mg), (c) norfloxacin (Sadoz, 400mg) and (d) moxifloxacin (Actira, 400mg) pharmaceutical application (10 $\mu\text{g/mL}$). Mobile phase: 0.15 M SDS - 2.5% 1-propanol - 0.5% thiethylamine - pH3, flow rate: 1mL/min, UV detection at 276 nm.

Table 4. Recoveries of pharmaceutical formulations (n=6)

Pharmaceutical (laboratory)	Composition (mg)	Found (mg)	Label claim (%)	C.V. (%)
	Per capsule:			
Urisan	Pipemidic acid (400), excipients	415 ± 7	103.7	1.7
	Per capsule:			
Galusan	Pipemidic acid (400), excipients	420 ± 8	104.9	1.8
	Per capsule:			
Nuril	Pipemidic acid (400), excipients	421 ± 4	105.3	0.8
	Per tablet:			
Normon	Levofloxacin (500), excipients	500.0 ± 1.7	100.0	0.3
	Per tablet:			
Stada	Levofloxacin (500), excipients	485 ± 3	96.8	0.6
	Per tablet:			
Tavanic	Levofloxacin (500), excipients	483.7 ± 1.0	96.7	0.21
	Per capsule:			
Amicrobin	Norfloxacin (400), excipients	417.64 ± 1.3	104.4	0.3
	Per tablet:			
Stada	Norfloxacin (400), excipients	407.0 ± 1.8	101.7	0.4
	Per tablet:			
Nalion	Norfloxacin (400), excipients	423.7 ± 1.9	105.9	0.4
	Per tablet:			
Uroctal	Norfloxacin (400), excipients	422.18 ± 1.3	105.5	0.3
	Per tablet:			
Noroxin	Norfloxacin (400), excipients	383.85 ± 1.7	96.0	0.4
	Per tablet:			
Norflok	Norfloxacin (400), excipients	420.3 ± 0.7	105.1	0.17
	Per tablet:			
Normon	Norfloxacin (400), excipients	393.5 ± 0.6	98.4	0.6
	Per tablet:			
Sadoz	Norfloxacin (400), excipients	393 ± 4	98.3	1.14
	Per tablet:			
Bexal	Norfloxacin (400), excipients	421.0 ± 1.5	105.3	0.4
	Per tablet:			
Esclabin	Norfloxacin (400), excipients	384.7 ± 1.8	96.2	0.5
	Per tablet:			
Octegra	Moxifloxacin (400), excipients	394.4 ± 1.9	98.6	0.5
	Per tablet:			
Proflox	Moxifloxacin (400), excipients	406.8 ± 1.6	101.7	0.4
	Per tablet:			
Actira	Moxifloxacin (400), excipients	413 ± 6	103.3	1.3

5. Conclusions

The analytical method developed can be used to simultaneously separate and quantify an antibiotic mixture consisting of pipemidic acid, levofloxacin, norfloxacin and moxifloxacin. Good sensitivity, linearity and robustness were obtained. RSD values were lower than 2% and 6.4% for intra- and inter-day analyses, respectively. The recoveries in the pharmaceutical samples were within a range of 96-106%, and no interferences from excipients were observed.

The proposed method is fast, precise, accurate, sensitive and efficient, and the tablet formulation of the individual antibiotics studied in this research work can be routinely analysed.

Acknowledgements

This work was supported by projects P1-1B2006-12 and CTQ2007-64473/BQU, funded by Foundation Caixa Castelló-Bancaixa-Universitat Jaume I and the Ministerio de Educación y Ciencia (MEC), respectively. Maria Rambla-Alegre also wishes to thank the MEC for the FPU grant.

References

- [1] Leshner G.Y., Froelich E.D., Gruet M.D., Bailey J.H., Brudage R.P.; *J. Med. Pharm. Chem.* 1962, 5, 1063-1068.
- [2] Jackson L.C., Machado L.A., Hamilton M.L.; *Acta Medica* 1998, 8, 5-8.
- [3] American Hospital Formulary Service, Drug Information, American Society of Health-System Pharmacists, Bethesda, MD, 1988.
- [4] Hernández-Arteros J.A., Companó R., Ferrer R., Prat M.D.D; *Analyst* 2000, 125, 1155-1158.
- [5] Fan Y., Gan X., Li S., Qin W.; *Electrophoresis* 2007, 28, 4101-4107.
- [6] Sun H., Zhoro W., He P.; *Chromatographia* 2008, 68, 425-429.
- [7] Ferdig M., Kaleta A., Vo T.D.T., Buchberger W.; *J. Chromatogr. A* 2004, 1047, 305-311.
- [8] Holtzapple C.K., Buckley S.A., Stanker L.H.; *J. Agric. Food Chem.* 1999, 47, 2963-2968.
- [9] Yue Z., Lin X., Tang S., Chen X., Ji C., Hua H., Liu Y.; *Chin. J. Chromatogr. (Se Pu)* 2007, 25, 491-495.
- [10] Hernando M.D., Mezcuca M., Suárez-Barcena J. M., Fernández-Alba A.R.; *Analytica Chimica Acta* 2006, 562, 176-184.
- [11] Romero-González R., López-Martínez J.C., Gómez-Milán E., Garrido-Frenich A., Martínez-Vidal J.L.; *J. Chromatogr. B* 2007, 857, 142-148.
- [12] Hermo M.P., Nemitlu E., Kir S., Barrón D., Barbosa J.; *Analytica Chimica Acta* 2008, 613, 98-107.
- [13] Cañada Cañada F., Espinosa Mansilla A., Muñoz Peña A.; *J. Sep. Sci.* 2007, 30, 1242-1249.
- [14] Santoro M.I.R.M., Kassab N.M., Singh A.K., Kedor Hackman E.R.M.; *J. Pharm. and Biomed. Analysis* 2006, 40, 179-184.

- [15] Shervington L.A., Abba M., Hussain B., Donnelly J.; *J. Pharm. and Biomed. Analysis* 2005, 39, 769-775.
- [16] Espinosa Mansilla A., Muñoz de la Peña A., Salinas F., González Gómez D.; *Talanta* 2004, 62, 853-860.
- [17] Vílchez J.L., Araujo L., Prieto A., Navalón A.; *Anal. Chim. Acta* 2004, 516, 135-140.
- [18] Vybíralová Z., Nobilis M., Zoulova J., Kvetina J., Petr P.; *J. Pharm. and Biomed. Analysis* 2005, 37, 851-858.
- [19] Tatar Ulu S.; *J. Pharm. and Biomed. Analysis* 2007, 43, 320-324.
- [20] Barbato F., di Martino G., Grumetto L., la Rotonda M.I.; *European J. Pharm. Sciences* 2007, 30, 211-219.
- [21] Christodoulou E.A., Samanidou V.F., Papadoyannis I.N.; *J. Sep Sci.* 2008, 31, 119-127.
- [22] Zhao S.J., Jiang H.Y., Ding S.Y., Wang G.Q., Li C., Shen J.Z.; *Chromatographia* 2007, 65, 539-544.
- [23] Christodoulou E.A., Samanidou V.F., Papadoyannis I.N.; *J. Chromatogr. B* 2007, 859, 246-255.
- [24] Marazuela M.D., Moreno-Bondi M.C.; *J. Chromatogr. A* 2004, 1034, 25-32.
- [25] Huang J.F., Lin B., Yu Q.W., Feng Y.Q.; *Anal. Bioanal. Chem.* 2006, 384, 1228-1235.
- [26] Rodriguez Diaz RC., Aguilar Caballos MP., Gomez Hens A.; *Analytical Letters* 2004, 37, 1163-1175.
- [27] Seifrtová DeM., Pena A., Lino C.M., Solich P.; *Anal. Bioanal. Chem.* 2008, 391, 799-805.
- [28] Berthod A., García-Álvarez-Coque M.C., *Micellar Liquid Chromatography*, Marcel-Dekker, New York, 2000.

- [29] Carda Broch S., Esteve Romero J., García-Álvarez-Coque M.C.; *Analyst* 1998, 123, 301-306.
- [30] Ruiz Ángel M.J., Gil Agustí M.T., Esteve Romero J., Carda Broch S.; *LC-GC Europe* 2004, 18, 32-40.
- [31] Carda Broch S., Esteve Romero J., García-Álvarez-Coque M.C.; *J. Pharm. and Biomed. Analysis* 2000, 23, 803-817.
- [32] Carda Broch S., Gil-Agustí M.T., Monferrer Pons Ll., Esteve Romero J.; *J. Chromatogr. A* 2007, 1156, 254-258.
- [33] Validation of Analytical Procedures: Text and Methodology Q2 (R1). ICH Harmonised Tripartite Guideline (.2005). <http://www.ich.org/cache/compo/363-272-1.html#Q2A> (30.04.09)
- [34] Clarke's Analysis of Drugs and Poisons, Pharmaceutical Press, London (UK), 2004.
- [35] Moxifloxacin, *Tuberculosis* 2008, 88, 127-131.
- [36] Levofloxacin, *Tuberculosis* 2008, 88, 119-121.
- [37] Torres Lapasió J.R., García-Álvarez-Coque M.C., Baeza-Baeza J.J.; *Anal. Chim. Acta* 1997, 348, 187-196.
- [38] Torres Lapasió J.R.; Michrom Software, Marcel-Dekker, New York, USA, 2000.