Determination of norfloxacin, cipfloxacin, levofloxacin and moxifloxacin in urine by HPLC with a two-channel fluorescence detector

Nguyen Xuan Truong[2,*], Nguyen Thi Minh Diep[1], Vu Anh Tuan[2]

[1]Faculty of Science, Phu Tho Medical and Pharmacy College, Vietnam
[2]School of Chemical Engineering, Ha Noi University of Science and Technology, Vietnam
[*]truong.nguyenxuan@hust.edu.vn

Abstract

A sensitive HPLC-FLD method with two-channel fluorescence detection was established and validated for quantification of four fluoroquinolones (norfloxacin, ciprofloxacin, levofloxacin and moxifloxacin) in urine samples. The fluorescent detector was set at two-channel excitation and emission wavelengths: 280/445 nm (for NOR, CIP) and 290/500 nm (for LEVO, MOXI). The measurement protocol was in compatible with both of the sample treatment procedures including the liquid-liquid extraction (LLE) and solid-phase extraction (SPE). The method was well-validated with the limit of quantification: 0.027; 0.034; 0.024; 0.020 µg/mL (for LLE), and 0.028; 0.033; 0.024; 0.020 µg/mL (for SPE) for NOR, CIP, LEVO, MOXI, respectively. The method has been proved to be precise and accurate, with the relative standard deviation lower than 3% and the recovery ranging from 98.0%÷102.0%, for all fluoroquinolones. The proposed HPLC-FLD method provides an alternative approach for the simultaneous analysis of fluoroquinolones in urine.

Received 20/12/2022
Accepted 17/04/2023
Published 29/12/2023

Keywords

two-channel excitation and emission, HPLC-FLD, fluoroquinolones, urine

1 Introduction

The fluoroquinolones, including norfloxacin, ciprofloxacin, levofloxacin and moxifloxacin, are classified as second-, third- and fourth-generation quinolones. They are active against both Gram-positive and Gram-negative bacteria through the inhibition of their DNA gyrase and used in the treatment of many diseases, including urinary, respiratory, and gastrointestinal tract infections [1-3]. Monitoring the concentration of the fluoroquinolones (FQs) is important for routine analyses and pharmacokinetic studies, which provides the effective pharmacotherapy of bacterial diseases and thus prevents bacterial resistance and lack of therapy efficacy. It is worth noting that fluoroquinolones are a class of antibiotics with intrinsic fluorescence properties. Therefore, several analytical procedures have been reported for determination of fluoroquinolones in biological fluids including high-performance liquid chromatography (HPLC) with a fluorescence detector (FLD) [4-7], spectrofluorimetry [8-11], flow-injection based on chemiluminescence [12, 13], chemiluminescence [14], and terbium-sensitized luminescence [15].

The aim of this work is to establish a sensitive alternative approach to conventional HPLC-FLD methods using two-channel fluorescence detector for the determination of norfloxacin, ciprofloxacin, levofloxacin and moxifloxacin (Fig. 1) in urine samples. The proposed method has been validated in accordance with the procedure of sample treatment such as liquid-liquid extraction (LLE) or solid-phase extraction (SPE) which were commonly used for analysis of urine samples.

To the best of our knowledge, this is the first report of an HPLC-FLD method using two couples of excitation and emission wavelengths for simultaneous determination of four fluoroquinolones in urine

samples. The analytical procedure developed herein could also be useful in the area of routine analyses and pharmacokinetic studies.

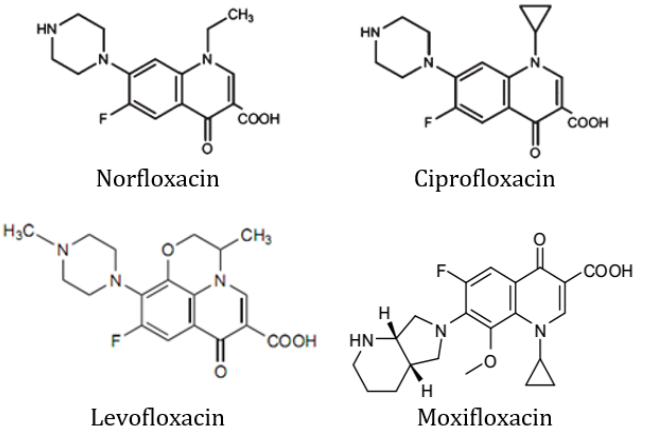

Fig. 1 Structures of the studied fluoroquinolones

2 Materials and methods

2.1 Chemicals and reagents

Standard of fluoroquinolones: norfloxacin (NOR, lot no. QT068050316, 99.5%), ciprofloxacin (CIP, lot no. QT012120318, 98.3%), levofloxacin (LEVO, lot no. QT165080417, 98.2%), moxifloxacin (MOXI, lot no. QT187041117, 97.0%) were purchased from Institute of Drug Quality Control, Ho Chi Minh City, Vietnam. HPLC-grade solvents (Merck, Germany) included chloroform, methanol (MeOH), dichloromethane (DCM), acetonitrile (ACN), ethyl acetate (EA) and triethylamine (TEA). ACS-reagent phosphoric acid (H_3PO_4) and hydrochloric acid (HCl) were purchased from Acros Organics (USA). Deionized (DI) water (18.2 MΩ.cm) was obtained with a Millipore Milli-Q water purification system.

2.2 Standard and sample preparation

Standard solutions: Primary stock standards of each antibiotic NOR, CIP, LEVO and MOXI 1000 µg/mL were prepared in MeOH/0.1 M HCl 1:1 (v:v). Working standard solutions of 100, 10 and 1 µg/mL were prepared by diluting the stock solution appropriately with methanol. From these solutions, working standard mixtures from each antibiotic were prepared and stored at −20 °C for 30 days.

Sample preparation: The blank urine samples were collected from healthy volunteers who did not take the drugs, at a general clinic in Phu Tho province, Vietnam. The spiked sample was prepared by taking a known volume of a standard mixture solution, evaporating the solvent to collect the residue (temperature 40 °C, N_2 gas), diluting the residue in the blank urine, and vortexing for 5 minutes. The blank urine and spiked samples were stored at −20°C for less than one month and were immediately thawed to room temperature before the assay treatment.

2.3 Sample treatments

Liquid-liquid extraction (LLE): The solvents (chloroform, DCM, EA, DCM/MeOH) and their volume used for the extraction were investigated. The procedural detail is described as following and comments are reported in Section 3.1.

Firstly, 0.5 mL of the urine sample and 5 mL of DCM/MeOH 8:2 (v:v) solution were added into a polypropylene tube. Then, the mixture was vortexed for 3 min at 2000 rpm and centrifuged for 15 min at 6000 rpm. The organic portion was separated, evaporated under a nitrogen stream, reconstituted with 2.0 mL of mobile phase and then was filtered through a 0.45 µm Nylon filter before analysis [16].

Solid-phase extraction (SPE): Three SPE cartridges (Chromfilter C18, Agilent Bond Elut PLEXA and Water Oasis HLB 500 mg, 6 mL), washing and eluting solutions used for the extraction were studied. The procedural detail is described as following and comments are reported in Section 3.1.

Before loading the sample (1.0 mL), the cartridge was conditioned with 5 mL of methanol, 3 mL of DI-water and 6 mL of DI-water adjusted to pH 3 with H3PO4. After the sample was loaded through a cartridge using a vacuum system, the solid phase was washed with 5 mL of DI-water and then dried for 5 min. Subsequently, FQs were extracted with 5.0 mL of DCM at a flow rate of 1 mL/min. The extracted portion was evaporated under a gentle nitrogen stream, reconstituted with 2.0 mL of mobile phase and then was filtered through a Nylon filter (diameter of 0.45 µm) before analysis [17].

2.4 Chromatographic system

The chromatographic analysis was performed on HPLC system equipped with an automatic sampler Sil-20AC XR, a thermostatic column oven CTO-20A and a fluorescent detector RF-20A (Shimadzu, Japan). The separation was achieved on a C18 YMC-pack ODS-A column (150 × 4.6 mm, 5µm). The mobile phase was a mixture of 0.025 M phosphoric acid (pH 3, adjusted with TEA) aqueous solution (mobile phase A) and MeOH (mobile phase B). The following gradient

program (with respect to mobile phase B) was used: 0-2.0 min, 15% B; 2.0-10.0 min, 15-35% B; 10.0-13.0 min, 35-80% B; 13.0-15.0 min, 80-100% B; >15.0 min, 100% B. The flow rate was 1.0 mL/min. The injection volume was 10 µL. The column oven was maintained at 30 ℃. Detection was performed with a fluorescent detector set at two-channel excitation and emission wavelengths: 280/445 nm for NOR, CIP and 290/500 nm for LEVO, MOXI.

2.5 Method validation

The method validation was carried out according to the ICH (the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) and the AOAC (the Association of Official Analytical Chemists) guidelines [18, 19]. The HPLC dual-wavelength fluorescence detection method was validated for selectivity, sensitivity, linearity, precision and accuracy.

Selectivity

The selectivity was assessed by examining peak interference from a blank urine and a spiked sample of mixture at a concentration 1 µg/mL of each drug.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were determined by gradually reducing the standard mixture of each drug (0.02 µg/mL) to a blank urine sample. LOD was obtained at the concentration in which height of peak signal to noise (S/N) equals to 3. LOQ was accepted as LOQ = 3.3×LOD. From these LOD and LOQ values, the method detection limit (MDL) and quantification limit (MQL) values were estimated by taking the dilution factor (of 4 with LLE or 2 with SPE) into account during the urine sample treatment.

Linearity

The calibration samples were prepared in blank urine over a range of 0.02-20 µg/mL (0.02, 0.025, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0 µg/mL). The standard calibration curve was constructed by least square linear regression using peak areas. The linearity of the standard curves was assessed with its intercept, slope and correlation coefficient (R2).

Precision and accuracy

The precision and accuracy of the method were evaluated using three quality control (QC) spiked urine samples (0.1, 1.0 and 5.0 µg/mL). The precision was expressed as the relative standard deviation (RSD%) of the measured QC samples, each QC sample was performed in sextuplicate. The accuracy was determined as a relative error bias. The results were calculated from the calibration curves by means of the external standard method. The procedure for the sample preparation was described in Sections 2.2 and 2.3.

3 Results and discussion

3.1. Investigation on extraction procedure

In addition to the measurement protocol, the sample preparation is an important factor, especially in analysis of biological fluids. In this study, two extraction procedures including LLE and SPEwere investigated for the proposed analytical assay.

Several organic solvents/mixtures in combination with urine sample at different volume ratios were tested during LLE process (Tables 1-3). The result indicated that the best solvent for LLE process was achieved by using a mixture of DCM/MeOH 8:2 (v:v). This could be explained by the polarity of the mixture is suitable for dissolving FQs and separating out of liquid phase.

Table 1 The effect of solvent/mixture on the response of peak area for the four FQs (10 µg/mL)

Solvent	Peak area			
	NOR	CIP	LEVO	MOXI
Chloroform	14546666	11349826	24302248	14094218
Dichloromethan	21013794	15925073	22220182	14364416
Dichloromethane/MeOH (9:1)	22796541	16876392	24365605	16245895
Ethylacetate	4855887	3861418	15229775	9640201

Table 2 The effect of the DCM:MeOH (v:v) ratio on the response of peak area for the four FQs (10 µg/mL)

DCM:MeOH (v:v)	Peak area			
	NOR	CIP	LEVO	MOXI
9:1	22792341	16856345	24367805	16205651
8:2	**23473877**	**19014775**	**24091843**	**17755478**
7:3	21726816	18384530	22163729	17302903

Table 3 The effect of mixture ratio to urine sample (0.5 mL) on the response of peak area for the four FQs (10 µg/mL)

Mixture volume	Peak area			
	NOR	CIP	LEVO	MOXI
3 mL	9453040	8953299	9969632	8016207
4 mL	10022324	10532192	11146284	8204721
5 mL	**13550283**	**11404927**	**12748936**	**9129573**
6 mL	11046974	10163224	11263253	9001494

On the other hand, three types of cartridges and different parameters evaluated during SPE process were the composition and volume of the washing solution as well as the eluting solution, and the

breakthrough volume of the SPE cartridges. It was found that the HLB cartridge containing hydrophillic-lipophillic sorbent allowed to obtain the highest FQs recovery (Table 4). Water and DCM were chosen as the washing and the eluting solutions during the SPE performance, respectively.

Table 4 The extraction recovery of the four FQs (10 µg/mL) from urine sample with the different SPE cartridges

SPE cartridge*	Recovery			
	NOR	CIP	LEVO	MOXI
C18	80.1	82.4	89.0	85.1
Oasis HLB	**90.2**	**91.0**	**98.3**	**98.9**
Plexa	78.7	84.0	82.9	88.1
*SPE cartridge: 500 mg, 6 mL				

3.2 Chromatographic characteristics

Fig. 2 showed chromatograms of the FQs recorded with the two-channel excitation and emission wavelengths fluorescence detector. It was readily realized that the signals (fluorescent intensities) were significantly enhanced at the analytical wavelengths of 280/445 nm for NOR and CIP while those of 290/500 nm for LEVO and MOXI. It means that the sensitivity of the proposed method which ultilized a dual-channel fluorescence detection was improved compared to the conventional HPLC-FLD methods in which the detector was set at singular mode.

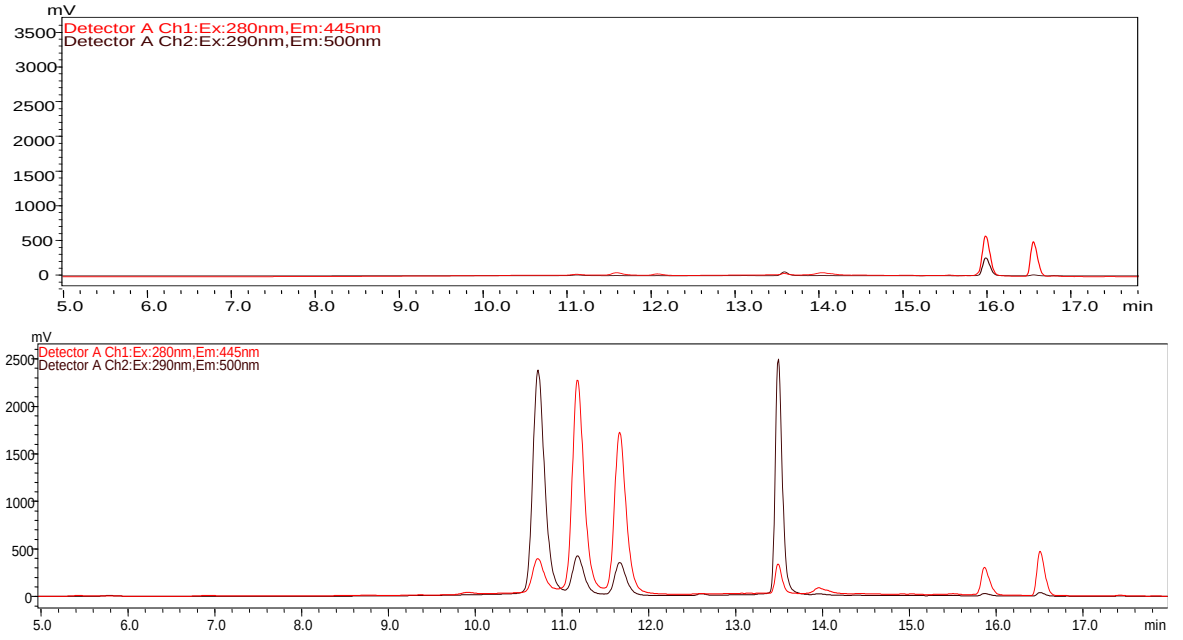

Fig. 2 Chromatograms of a blank urine sample (a) and a spiked sample (1.0 µg/mL) in urine (b) recorded with λex/λem = 290/500 nm (black curve) and λex/λem = 280/445 nm (red curve)

3.3 Method validation

Assay selectivity

Blank urine samples were analyzed and no interference was observed at the retention time of LEVO, NOR, CIP or MOXI. Representative chromatograms of blank urine and spiked samples wereshown in Fig. 2.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ values for the determination of the FQs were in the range of 0.006 – 0.010 and 0.020 – 0.034 µg/mL, respectively (Table 5). By considering the procedure of the urine sample treatment, the method detection limit (MDL) and quantification limit (MQL) values were also reported in Table 5.

Table 5 Validation parameters of the method for the analysis of the fluoroquinolones in urine

Extraction method	FQs	Regression equation	R^2	Linearity (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	MDL (µg/mL)	MQL (µg/mL)
LLE	NOR	y = 15313397.95x – 332379.90	0.9997	0.025 - 10.0	0.008	0.027	0.032	0.108

	CIP	y = 10452676.06x – 82095.41	0.9999		0.010	0.034	0.041	0.136
	LEVO	y = 14307738.59x – 356669.60	0.9997		0.007	0.024	0.029	0.096
	MOXI	y = 7752335.92x + 25104.75	0.9999		0.006	0.020	0.024	0.080
SPE	NOR	y = 11842918.06x + 853994.31	0.9998	0.020 - 25.0	0.009	0.028	0.017	0.057
	CIP	y = 11573367.29x + 354372.18	0.9999		0.010	0.033	0.020	0.065
	LEVO	y = 12082493.02x – 255541.28	0.9996		0.007	0.024	0.014	0.047
	MOXI	y = 10080269.14x + 680658.66	0.9997		0.006	0.020	0.012	0.039

y: peak area; x: concentration of the FQ in urine; R^2: correlation coefficient.

The MDL values of the proposed method were satisfied with quantification of the four FQs in urine samples which their concentration were approximately 50 µg/mL [5] and were even suffered a dilution of 500 to 1000-fold. Moreover, the information found in the literature reported that the FQs excreted mainly as the unchanged drug in urine were 30% for NOR, 40–60% for CIP, 80–85% for LEV and 20% for MOX [4, 20]. It is suggested that pharmacokinetic studies can be performed under the proposed method.

Linearity

The linearity of the four FQs in urine by the proposed method was evaluated. The calibration curves were linear over the range of 0.025 - 10.0 µg/mL for LLE and 0.020 - 20 µg/mL for SPE process (Fig. 3). The linear regression equations with corresponding correlation coefficients of the calibration curves were indicated in Table 5.

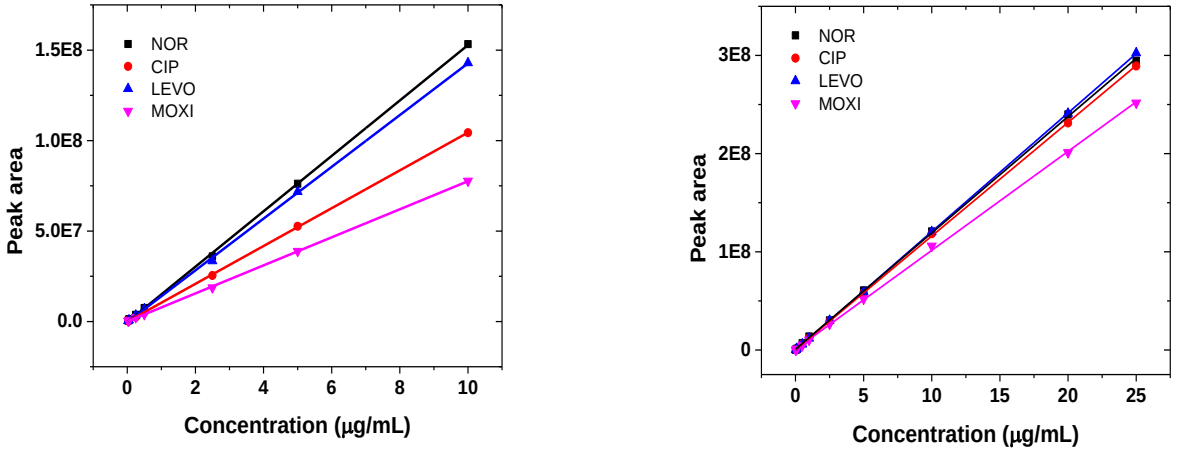

Fig. 3 Calibration curves for determination of the FQs in urine with (a) the LLE process and (b) the SPE process

Precision and accuracy

The precision and accuracy data of the analytical method were summarized in Table 6. For all four FQs, the precision was lower than 3% at all three concentration levels and the accuracy ranged from 98.0% to 102.0%. These results indicated that the proposed assay was precise and accurate [19].

Table 6 Precision and accuracy data of the method for the analysis of the FQs in urine

FQs	Added conc. (µg/mL)	Extraction method					
		LLE			SPE		
		Found conc. (µg/mL)	RSD % (n = 6)	Recovery (%)	Found conc. (µg/mL)	RSD % (n = 6)	Recovery (%)
Nor	0.100	0.101 ± 0.001	1.0	101.0	0.099 ± 0.001	1.0	99.0
	1.000	0.992 ± 0.006	0.6	99.2	0.982 ± 0.006	0.6	98.2
	5.000	5.100 ± 0.040	0.8	102.0	5.056 ± 0.013	0.3	101.1
Cip	0.100	0.099 ± 0.001	1.0	99.0	0.102 ± 0.001	1.0	102.0
	1.000	0.989 ± 0.008	0.8	98.9	1.002 ± 0.008	0.8	100.2
	5.000	4.883 ± 0.020	0.4	97.7	4.972 ± 0.070	0.2	99.4
Levo	0.100	0.102 ± 0.001	1.0	102.0	0.098 ± 0.001	1.0	98.0
	1.000	0.989 ± 0.007	0.7	98.9	1.012 ± 0.007	0.7	101.2
	5.000	4.875 ± 0.030	0.6	97.5	5.034 ± 0.050	1.0	100.7
Moxi	0.100	0.098 ± 0.001	1.0	98.0	0.100 ± 0.001	1.0	100.0
	1.000	0.978 ± 0.002	0.2	97.8	0.995 ± 0.005	0.5	99.5
	5.000	5.017 ± 0.090	1.8	100.3	4.984 ± 0.030	0.6	99.7

4 Conclusion

The developed and validated HPLC-FLD method coupled with the LLE or SPE procedure is readily available for the simultaneous determination of the FQs, including, norfloxacin, ciprofloxacin, levofloxacin and moxifloxacin in urine samples. The proposed method exhibited an acceptable accuracy, and good reproducibility with the detection limit of 0.006-0.010 µg/mL. Although the applicability of the proposed method for analyzing real samples requires further investigation, this work contributes a sensitive and reproducible alternative HPLC-FLD measurement of fluoroquinolones in urine, and this could also be useful for therapeutic drug monitoring and pharmacokinetic study.

References

1. Czyrski, A. (2017). Analytical methods for determining third and fourth generation fluoroquinolones: A review. *Chromatographia*, *80*, 181-200.

2. Sousa, J., Alves, G., Fortuna, A., & Falcao, A. (2012). Analytical methods for determination of new fluoroquinolones in biological matrices and pharmaceutical formulations by liquid chromatography: a review. *Analytical and bioanalytical chemistry*, *403*, 93-129.

3. Van, T. T., Minejima, E., Chiu, C. A., & Butler-Wu, S. M. (2019). Don't get wound up: revised fluoroquinolone breakpoints for Enterobacteriaceae and Pseudomonas aeruginosa. *Journal of clinical microbiology*, *57*(7), 10-1128.

4. Rambla-Alegre, M., Esteve-Romero, J., & Carda-Broch, S. (2009). Validation of a MLC method with fluorescence detection for the determination of quinolones in urine samples by direct injection. *Journal of Chromatography B*, *877*(31), 3975-3981.

5. Espinosa-Mansilla, A., de la Pena, A. M., Gómez, D. G., & López, F. S. (2006). Determination of fluoroquinolones in urine and serum by using high performance liquid chromatography and multiemission scan fluorimetric detection. *Talanta*, *68*(4), 1215-1221.

6. Yıldırım, S., Karakoç, H. N., Yaşar, A., & Köksal, İ. (2020). Determination of levofloxacin, ciprofloxacin, moxifloxacin and gemifloxacin in urine and plasma by HPLC–FLD–DAD using pentafluorophenyl core–shell column: Application to drug monitoring. *Biomedical Chromatography*, *34*(10), e4925.

7. Van Toi, P., Pouplin, T., Tho, N. D. K., Phuong, P. N., Chau, T. T. H., Thuong, N. T. T., ... & Thwaites, G. E. (2017). High-performance liquid chromatography with time-programmed fluorescence detection for the quantification of Levofloxacin in human plasma and cerebrospinal fluid in adults with tuberculous meningitis. *Journal of Chromatography B*, *1061*, 256-262.

8. Espinosa-Mansilla, A., de la Peña, A. M., Salinas, F., & Gómez, D. G. (2004). Partial least squares multicomponent fluorimetric determination of fluoroquinolones in human urine samples. *Talanta*, *62*(4), 853-860.

9. Navalon, A., Ballesteros, O., Blanc, R., & Vílchez, J. L. (2000). Determination of ciprofloxacin in human urine and serum samples by solid-phase spectrofluorimetry. *Talanta*, *52*(5), 845-852.

10. Ocaña, J. A., Barragán, F. J., & Callejón, M. (2000). Spectrofluorimetric determination of moxifloxacin in tablets, human urine and serum. *Analyst*, *125*(12), 2322-2325.

11. González, J. A. O., Mochón, M. C., & de la Rosa, F. J. B. (2000). Spectrofluorimetric determination of levofloxacin in tablets, human urine and serum. *Talanta*, *52*(6), 1149-1156.

12. Sun, H. W., Li, L. Q., & Chen, X. Y. (2006). Flow-injection enhanced chemiluminescence method for determination of ciprofloxacin in pharmaceutical preparations and biological fluids. *Analytical and bioanalytical chemistry*, *384*, 1314-1319.

13. Liang, Y. D., Song, J. F., & Yang, X. F. (2004). Flow-injection chemiluminescence determination of fluoroquinolones by enhancement of weak chemiluminescence from peroxynitrous acid. *Analytica Chimica Acta*, *510*(1), 21-28.

14. Aly, F. A., Al-Tamimi, S. A., & Alwarthan, A. A. (2001). Chemiluminescence determination of some fluoroquinolone derivatives in pharmaceutical formulations and biological fluids using [Ru (bipy) 32+]–Ce (IV) system. *Talanta*, *53*(4), 885-893.

15. Ocaña, J.A., M. Callejón, and F.J. Barragán, Terbium-sensitized luminescence determination of levofloxacin in tablets and human urine and serum, *Analyst*, 125(10): 1851-1854, 2000.

16. Pea, F., Qual, E. D., Cusenza, A., Brollo, L., Baldassarre, M., & Furlanut, M. (2003). Pharmacokinetics and pharmacodynamics of intravenous levofloxacin in patients with early-onset ventilator-associated pneumonia. *Clinical pharmacokinetics*, *42*, 589-598.

17. Lemoine, T., Breilh, D., Ducint, D., Dubrez, J., Jougon, J., Velly, J. F., & Saux, M. C. (2000). Determination of moxifloxacin (BAY 12-8039) in plasma and lung tissue by high-performance liquid chromatography with ultraviolet detection using a fully automated extraction method with a new polymeric cartridge. *Journal of Chromatography B: Biomedical Sciences and Applications*, *742*(2), 247-254.

18. Guideline, I. H. T. (2005). Validation of analytical procedures: text and methodology. *Q2 (R1)*, *1*(20), 05.

19. International, A. (2016). Guidelines for Standard Method Performance Requirements, *AOAC Official Methods of Analysis*, Appendix F, 1-18.

20. Novelli, A., & Rosi, E. (2017). Pharmacological properties of oral antibiotics for the treatment of uncomplicated urinary tract infections. *Journal of Chemotherapy*, *29*(sup1), 10-18.

Xác định Norfloxacin, Cipfloxacin, Levofloxacin và Moxifloxacin trong mẫu nước tiểu bằng phương pháp HPLC ghép nối detectơ huỳnh quang hai kênh

Nguyễn Xuân Trường[2,*], Nguyễn Thị Minh Diệp[1], Vũ Anh Tuấn[2]
[1]Khoa Khoa học, Trường Cao đẳng Y Dược Phú Thọ, Việt Nam
[2]Trường Hóa và Khoa học sự sống, Đại học Bách Khoa Hà Nội, Việt Nam
[*]truong.nguyenxuan@hust.edu.vn

Tóm tắt Trong nghiên cứu này, phương pháp HPLC-FLD detectơ huỳnh quang hai kênh độ nhạy cao đã được phát triển và xác nhận để định lượng bốn fluoroquinolone (norfloxacin, ciprofloxacin, levofloxacin và moxifloxacin) trong mẫu nước tiểu. Detectơ huỳnh quang với hai kênh kích thích và phát xạ ở bước sóng: 280/445 nm (đối với NOR, CIP) và 290/500 nm (đối với LEVO, MOXI), tương ứng. Phương pháp phân tích phù hợp với cả hai quy trình xử lí mẫu bao gồm chiết lỏng-lỏng (LLE) và chiết pha rắn (SPE). Phương pháp phân tích đã được đánh giá và xác nhận giá trị sử dụng, giới hạn định lượng NOR, CIP, LEVO, MOXI tương ứng là: 0,027; 0,034; 0,024; 0,020 µg/mL (với LLE) và 0,028; 0,033; 0,024; 0,020 µg/mL (với SPE). Độ lệch chuẩn tương đối < 3% và độ thu hồi từ 98,0 ÷ 102,0%, đối với tất cả các fluoroquinolone. Phương pháp HPLC-FLD phát triển trong nghiên cứu này có thể được sử dụng để phân tích đồng thời một số fluoroquinolone trong mẫu nước tiểu.

Từ khóa hai kênh kích thích và phát xạ, HPLC-FLD, fluoroquinolones, mẫu nước tiểu.