ISSN 0027-1314, Moscow University Chemistry Bulletin, 2007, Vol. 62, No. 2, pp. 81–84. © Allerton Press, Inc., 2007.
Original Russian Text © I.Ya. Kamochkina, Ye.M. Rekharsky, A.P. Chukharkhina, A.G. Borzenko, 2007, published in Vestnik Moskovskogo Universiteta. Khimiya, 2007, No. 2, pp. 97–100.

Pefloxacin Determination in Urine by Synchronous Fluorimetry

I. Ya. Kamochkina, Ye. M. Rekharsky, A. P. Chukharkhina, and A. G. Borzenko

Analytical Chemistry Department; e-mail: borzenko@environment.chem.msu.ru

Received June 8, 2006

Abstract—The feasibility of using synchronous fluorescence spectra for the determination of fluoroquinolone antibiotics in biological liquids is demonstrated. Pefloxacin chelating with metal ions in a micellar medium increases the determination sensitivity.

DOI: 10.3103/S0027131407020058

Control over the fluoroquinolone concentration that enters the organism during the healing of soft tissues is a challenging problem. According to the existing regulations, the procedure of taking biosamples should not disturb the patient; in particular, taking blood from a vein is permissible only in case of danger to life. Uranalysis is very informative: most drugs are removed with urine.

Several factors complicate uranalysis. One factor is a change in pH with time due to the effect of ammonia-producing bacterial flora. Another factor is the presence in urine of low-molecular-weight products of amino acid metabolism and saccharometabolism (amines, carboxylic acids, and others), small amounts of peptides, saccharides, steroids, and urobilin (the latter makes urine yellow). Apart from organic compounds, urine contains significant levels of inorganic salts (chlorides, oxalates, urates, and others).

As a rule, the separation of the analyte from the biomatrix is the most important problem of sample preparation for analysis. Liquid–liquid extraction is the most widespread method for the separation of drugs from urine; liquid–solid extraction is used less frequently [1].

Gas chromatography and high-performance liquid chromatography are the major bioassay methods [2–5]. These methods, despite their evident advantages, require complex and expensive equipment and skilled staff; thus, they are not always available for use in ordinary analytical laboratories and monitoring control. Thanks to their stiff structure, most fluoroquinolones possess intrinsic fluorescence. Therefore, it is pertinent to use luminescence methods for their determination in urine: these methods are sufficiently rapid and sensitive; in some cases, they allow determination without preseparation of the matrix.

This work concerns the feasibility of using luminescence methods for the determination of fluoroquinolone antibiotics (e.g., pefloxacin) in urine without preconcentration.

EXPERIMENTAL

Reagents. The stock solution used in the work was a 1.0×10^{-4} M solution of pefloxacin hydrochloride (from Sigma). More dilute solutions were prepared by diluting the stock solution immediately before an experiment. The dilute solutions were stored in the dark for a period of no longer than 1 week to avoid the photochemical degradation of the compounds. Calcium chloride, magnesium nitrate, aluminum chloride, and iron(III) chloride solutions (1.0×10^{-3} mol/L, from Khimmed) were prepared by dissolving proper weights in distilled water.

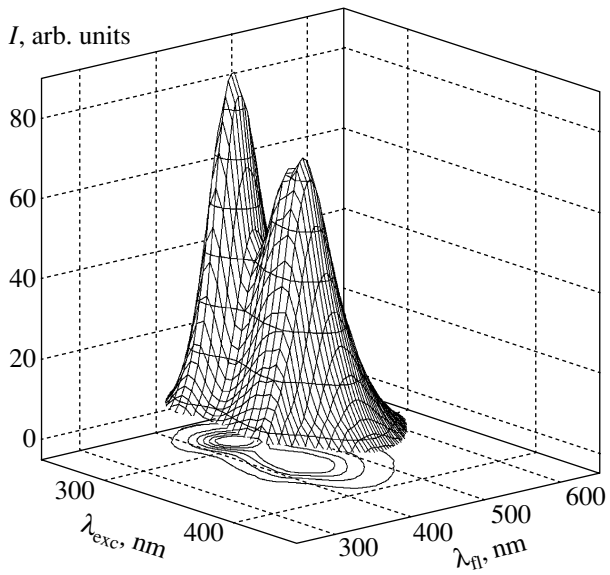

Fig. 1. Emission–excitation fluorescence spectrum of an aqueous pefloxacin solution.

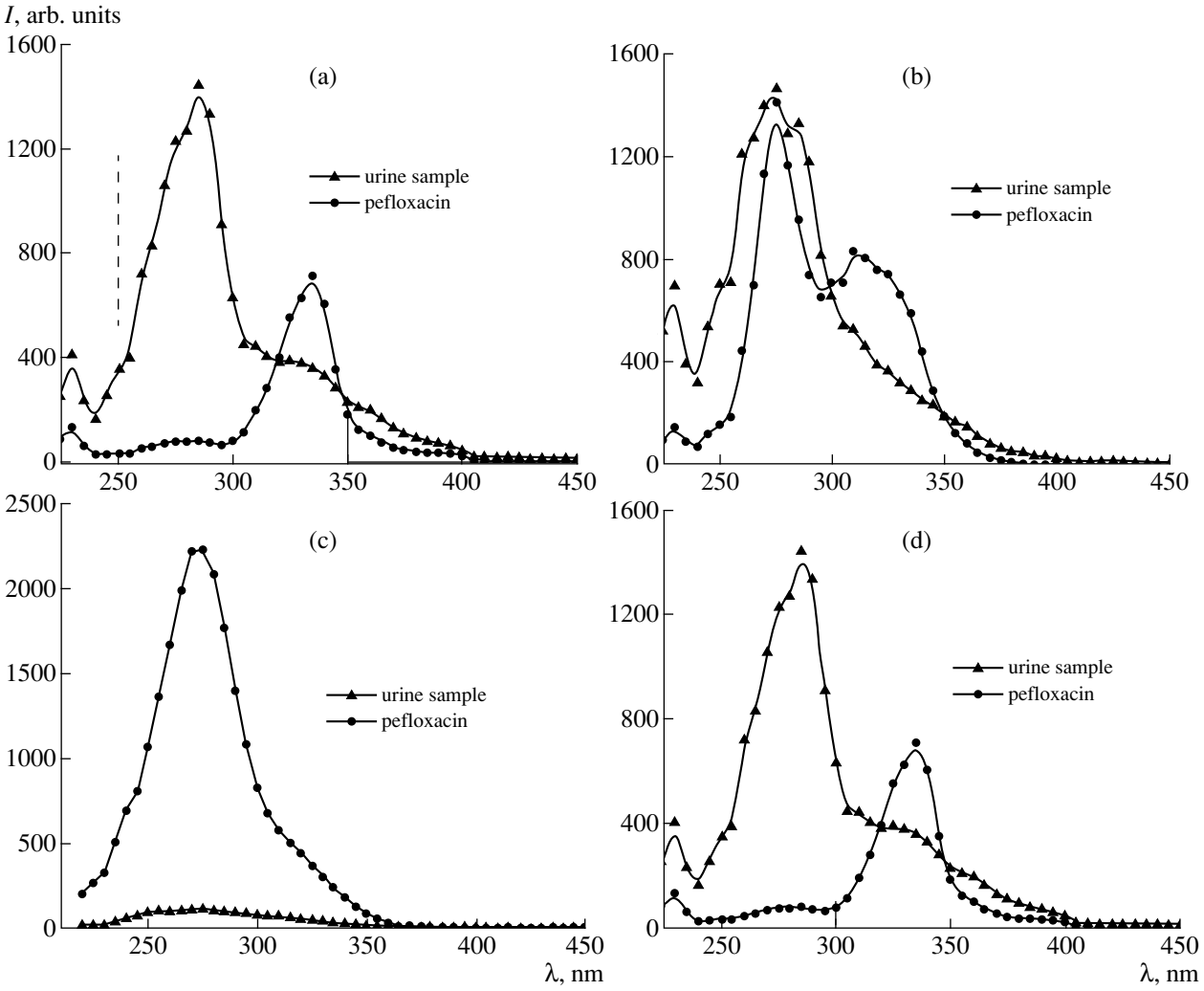

Fig. 2. Synchronous fluorescence spectra for pefloxacin and a urine sample measured at various Δλ (nm): (a) 60, (b) 110, (c) 150, and (d) 200.

To determine the stability constants of chelates, the salt concentrations were varied in the range from 2.0×10^{-6} to 2.0×10^{-5} mol/L. The pH was adjusted with 0.1 M solutions of potassium hydroxide and hydrochloric acid. The 0.1 M stock solution of sodium dodecyl sulfate (SDS; from Acros) was prepared by dissolving the salt in distilled water on an ultrasonic bath. The urine sample used to determine pefloxacin in the biological liquid was obtained from a healthy man.

Equipment. Fluorescence spectra were measured on a Panorama spectrofluorimeter (from Lumex). Silica glass cells with $l = 1$ cm were used in the measurements.

RESULTS AND DISCUSSION

Analysis of pefloxacin fluorescence spectra. The stiff structure of fluoroquinolones allows one to obtain well-resolved electron-vibrational phosphorescence spectra at room temperature. The underlying idea of the spectral selection of the luminescence technique is to use the difference between the major spectral parameters of the analyte and matrix, namely, between the excitation and emission wavelengths and luminescence intensities. Such problems are solved on the basis of the three-dimensional representation of the fluorescence characteristics, called the emission–excitation spectra (EES) or emission–excitation matrix (EEM). This representation carries the maximal amount of information about the luminophore, including absorption and emission characteristics and the direction of maximal signal change, whose knowledge is necessary for the implementation of synchronous scanning. In this case, it is much easier to choose optimal excitation and recording wavelengths for individual compounds.

The fluorescence emission–excitation spectrum of an aqueous pefloxacin solution is displayed in Fig. 1. There are two excitation peaks (283 and 330 nm) in the

Pefloxacin determination in urea ($n = 3$, $P = 0.95$)

Added, μg/mL	Found, μ/mL
1.7	1.6 ± 0.2
3.3	3.4 ± 0.2
16.7	16.5 ± 0.2
33.3	33.5 ± 0.2

pefloxacin spectrum. The emission peak appears at 446 nm. Our spectrum agrees with the literature [6].

Synchronous fluorescence spectra of pefloxacin and a urine sample. Inasmuch as urobilin (a pigment in urine) has quite an intense signal, conventional fluorimetry is of low efficiency for the spectral determination of fluoroquinolones. In addition, the spectral ranges of fluorescence for pefloxacin and urobilin significantly overlap (350–500 nm).

Synchronous fluorimetry is a convenient tool in this case. This method consists of measuring the fluorescence spectrum with simultaneously changing the excitation and measurement wavelengths. The difference between the energies of the excitation and measurement radiations is fixed during scanning. Synchronous scanning substantially simplifies the spectral pattern recorded. Evidently, this approach is very promising for bioassay.

We measured synchronous fluorescence spectra for a 100-fold dilute urine sample and a pefloxacin solution at various shifts Δλ from 5 to 200 nm in 10-nm steps. Figure 2 displays the synchronous spectra of urine and pefloxacin samples measured at various Δλ.

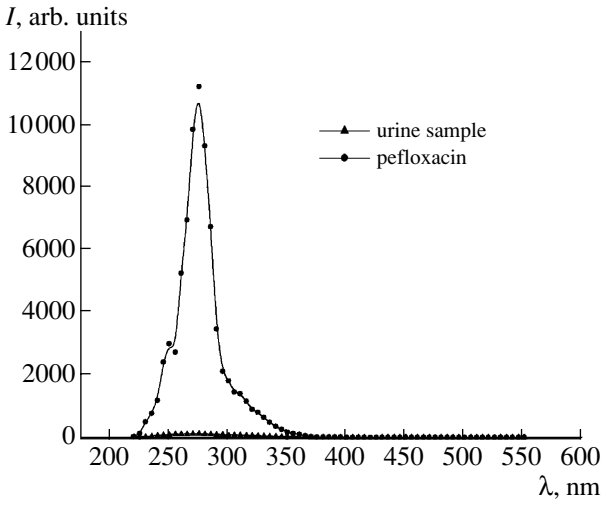

Fig. 3. Synchronous fluorescence spectra for pefloxacin and a urine sample measured at Δλ = 150 nm in the presence of SDS ($c = 2.5 \times 10^{-2}$ mol/L) and aluminum chloride ($c = 1.5 \times 10^{-4}$ mol/L).

The maximal difference between the fluorescence intensity of pefloxacin and the fluorescence intensity of urobilin was observed at Δλ = 150 nm. The pefloxacin peak in the synchronous spectrum had the minimal width. The pefloxacin signal was several tens of times the background signal, which was due to the intrinsic fluorescence of the biomatrix. This wavelength shift was chosen for all further measurements.

Pefloxacin chelating with aluminum(III). Fluoroquinolones are known to form chelates with various metal ions [7, 8]. The fluorescence of these chelates exceeds the intrinsic fluorescence of the test compounds. A significant increase in the fluorescence intensity of chelates compared to fluoroquinolones can be assigned to the stiff structure of the six-membered ring formed by the carbonyl and carboxyl groups of the compounds and a metal cation. Fluoroquinolone chelation is affected by the size and charge of the metal. Two-charged and three-charged metal ions, such as Ca^{+2}, Mg^{+2}, Al^{+3}, and Fe^{+3}, can change the signal intensity. Here, we studied pefloxacin chelation with calcium, magnesium, aluminum, and iron(III) ions. We showed that the most stable pefloxacin complex (with $\log K = 5.7$) is formed with aluminum ion in weakly acid solutions.

Pefloxacin determination in urine. In order to maximally increase the fluorescence signal, we carried out pefloxacin determination in urine in the presence of optimal aluminum chloride and SDS concentrations [9]. It is known that micelle-stabilized florescence is based on the ability of surfactant micelles to concentrate particles, decrease their vibrational energy, and mitigate the effect of quenchers, thus increasing the fluorescence signal. The optimal aluminum chloride and SDS concentrations are 1.5×10^{-4} and 2.5×10^{-2} mol/L, respectively. The use of these reagents for the synchronous fluorimetric determination of pefloxacin in urine increases the pefloxacin signal intensity by a factor of more than 6.5 (Fig. 3). The background signal from the fluorescing components of urine remains practically unchanged under these conditions. This allowed us to determine pefloxacin in urine without preseparation of the matrix.

The added/found technique was used to verify the validity of the fluorimetric determination of pefloxacin in urine. A set of solutions was prepared with pefloxacin concentrations in urine ranging within 1.7–33.3 μg/mL. The optimal measurement conditions were ensured by addition of the appropriate amounts of all required reagents. The pefloxacin detection limit was 0.1 μg/mL. The results of pefloxacin determination in urine samples are complied in the table.

In summary, we have demonstrated the feasibility of using synchronous fluorimetry to determine trace pefloxacin in urine without separating it from the biomatrix.

REFERENCES

1. Clarke, E.G.C., *Clarkes Isolation and Identification of Drugs*, London: Pharmaceutical Press, 1985.
2. Samanidou, V.F., Demetriou, C.E., and Papadoyannis, I.N., *Anal. Bional. Chem.*, 2003, vol. 375, p. 623.
3. Rizk, M., Belal, F., Aly, F.A., and El-Enany, N.M., *Talanta*, 1998, vol. 46, p. 83.
4. Kamberi, M., Kamberi, P., and Nakano, S., *J. Chromatogr. B*, 2000, vol. 741, p. 295.
5. Immanuel, C. and Hemanth, KumarA.K., *J. Chromatogr. B*, 2001, vol. 760, p. 91.
6. Albini, A. and Monti, S., *Chem. Soc. Rev.*, 2003, vol. 32, p. 238.
7. El-Kommos, M.E., Saleh, G.A., El-Gizawi, S.M., and Abou-Elwafa, M.A., *Talanta*, 2003, vol. 60, p. 1033.
8. Djurdjevic, P.T., Jelikic-Stankov, M., and Stankov, D., *Anal. Chim. Acta*, 1995, vol. 300, p. 253.
9. Perez-Ruiz, T., Martinez-Lozano, C., Tomas, V., and Carpena, J., *Analyst*, 1999, vol. 122, p. 705.