Simultaneous Determination of Trace Ofloxacin, Ciprofloxacin, and Sparfloxacin by Micelle TLC–Fluorimetry

Yu-Lin Feng[1,*] and **Chuan Dong**[2]

[1]The Institute of Modern Chemistry and [2]Department of Chemistry, Shanxi University, Taiyuan 030006, P.R. China

Abstract

The method of simultaneous determination of ofloxacin (OFLX), ciproflxacin (CPLX), and sparfloxacin (SPLX) by thin-layer chromatography is established, with micelle solutions as mobile phases. It is found that the optimum molar ratio of sodium dodecyl sulfate (SDS) to ethylene diamine tetraacetic acid is 0.01:0.1. On the polyamide thin-layer sheet, OFLX, CPLX, and SPLX are separated from each other, and the corresponding R_f values are 0.72, 0.55, and 0.32, respectively. The fluorescence spots are scanned with a spectrodensitometer at the excitation wavelength of 282 nm. The cut-off filter is set at 400 nm. The detection limits are 2×10^{-6} mol/L for OFLX, 1.5×10^{-6} mol/L for CPLX, and 1.6×10^{-6} mol/L for SPLX, and the respective linear ranges correspondingly fell in the concentration of 1×10^{-5} to 4×10^{-4}mol/L for OFLX, 1×10^{-5} to 4.5×10^{-4} mol/L for CPLX, and 1×10^{-5} to 4.2×10^{-4} mol/L for SPLX. For all the three components, the relative standard deviations are in the range of 1.12–5.82%, and the recoveries are found to be 96.7–104.2% in urine and serum samples.

Introduction

Surfactants and cyclodextrins are the corresponding mobile phase components in micelle and inclusion chromatography, which bring out the excellent selectivity to finish separation and analysis of many similar compounds in structure. Armstrong et al. (1) first investigated micelle thin-layer chromatography (TLC) in 1979, and since then many developments have been made in this field to conduct trace analysis. Using surfactant aqueous solution of greater than its critical micelle concentration (CMC) to develop such kinds of chromatography not only makes the separation of several compounds with analogous structure and low aqueous solubility better, but avoids the use of some potentially hazardous and easily flammable reagents for determination of many organic compounds as well (2–5).

Ofloxacin (OFLX), ciprofloxacin (CPLX), and sparfloxacin (SPLX) are antibacterial medicines widely used in clinics at present, and several determination methods including fluorescence, high-performance liquid chromatography, and TLC–fluorimetry (F) have reportedly been used for their determination (6–13). However, few research results were found that used micelle aqueous solution as mobile phases in TLC for the separation of norfloxacin compounds. The simultaneous determination of OFLX, CPLX, and SPLX has been herein described in urine and serum. This was achieved using the two-component developer of sodium dodecyl sulfate (SDS) and sodium salt of ethylene diamine tetraacetic acid (EDTA) aqueous solution of different molar ratios as the mobile phase and polyamide as the stationary phase. It showed that this method is simple, rapid, and accurate for determination of norfloxacin and related compounds. The recovery results satisfactorily met the needs for the analysis of real samples like urine and serum.

Experimental

Reagents

OFLX, CPLX, and SPLX were purchased from the Chinese Inspection Institute of Biological Products with the purity of +99.5%. Chemical-grade SDS was recrystallized from 95% ethanol (Shenyang Reagent Factory, Shenyang, China). Analytical-reagent-grade sodium EDTA was purchased from Xi'an Chemical Reagent Factory (Xi'an, China). Polyamide thin-layer sheets (10 × 10 cm) were obtained from Siqing Biochemical Material Factory (Zhejiang, China); they consisted of a 0.1-mm-thick coating of Nylon 66 on terylene. All the working solution was prepared by double distilled water.

Instrument

All fluorescence spectra were measured with a CS-9000 dual wavelength TLC flying spot scanner (Shimadzu, Kyoto, Japan). Quantitative micropipettes were purchased from Drummond

* Author to whom correspondence should be addressed: email ylfeng@sxu.edu.cn.

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission.

Scientific Instruments (Broomall, PA). A UV-1 UV ray meter (Shanghai Photoelectrosity Instrument Factory, Shanghai, China) was used for observing the fluorescence spots by illuminating the thin-layer sheets.

Samples

OFLX, CPLX, and SPLX were dried to a constant weight at 100°C shortly before use. The stock ethanol solution of 5×10^{-3} mol/L was resolved with several drops of NaOH solution of 0.1 mol/L and diluted to its final volume with 95% ethanol.

Procedure

Using a 1-µL quantitative micropipette, the sample and standard solutions of equal volume were spotted on the same polyamide thin-layer sheets. A chromatographic chamber was equilibrated with the mobile phase of SDS (0.01 mol/L) and EDTA (0.1 mol/L) for 15 min prior to use. All the sheets were of 8 × 10 cm, and, in an airtight container, the front part of the mobile phase reached 9 cm of every sheet. The developed sheets were then taken out and air dried. The position of blue (OFLX and CPLX) and green (SPLX) fluorescent spots could be observed by illuminating the TLC sheets with the UV-ray meter at 254 nm. All the fluorescent spots on the sheet were measured at the excitation wavelength of 282 nm, and a cut-off filter of 400 nm in the TLC–F spectrodensitometer was used.

Results and Discussion

Selection of surfactant concentration

Generally speaking, R_f values are relevant with the concentration of surfactant in the mobile phase. The higher proportion the surfactant, the larger the corresponding R_f values are. The experimental results indicated that the 0.01 mol/L of SDS was the most appropriate concentration for the separation of OFLX, CPLX, and SPLX. Therefore, the concentration of SDS was set at 0.01 mol/L, which was nearly close to its CMC value, for this separation.

Selection of the optimal ratio of SDS to EDTA

OFLX, CPLX, and SPLX have very strong tendency to form complexes with some transition metal ions, which cause lower separation performance because of spots tailing. Therefore, EDTA was added to the developer solution to eliminate interference of transition metal ions. The experiment results revealed that the best separation was obtained through the addition of EDTA to the mixture solution. The molar-ratio-selection results of the three components are listed in Table I, and the optimal ratio was set at the solution with SDS of 0.01 mol/L and EDTA of 0.1 mol/L.

Table I. R_f Values of Compounds in Different Proportions of the Developer

	SDS–EDTA (n/n)					
Compounds	0.01:0.01	0.01:0.03	0.01:0.05	0.01:0.1	0.01:0.15	0.01:0.2
OFLX	0.80	0.77	0.78	0.72	0.72	0.73
CPLX	0.59	0.56	0.57	0.55	0.55	0.57
SPLX	0.36	0.35	0.34	0.32	0.32	0.32

Effect of acidity on separation

Following the selection of optimum ratio of SDS to EDTA, the pH effect on the separation of norfloxcins was investigated. From the spread spots in the polyamide matrix, serious tailing was observed when the pH value was greater than 7, and the system had poor repeatability when the pH value was less than 4. Thus, the optimum pH range of the developer solution was adjusted to 4–6, and, as a result, 3 norfloxacin components were well separated without the appearance of tailing.

To elucidate the previously mentioned results, the relevant processes were listed as follows: first, on the weaker acidity, the fact that OFLX, CPLX, and SPLX were easily separated from each other might be related to the improved adsorptive interaction, which happened between the polyamide sheet and norfloxacin compounds and the details of which are still being explored in our laboratory. Second, the complexation capacity of EDTA with metal ions was reduced with decreasing pH values, resulting in poorer separation when the pH was less than 4. Finally, hydrogen bonding played a vital role in the entire separating process, and the increasing pH values would reduce the interaction between the polyamide and norfloxacin to decrease the separation. Thus, the optimum acidity was at pH 5 for the best separation resulting in regular and clear fluorescence spots. The scan of the separation performed on the polyamide TLC plate is shown in Figure 1.

Selection of excitation wavelength and emission filter

The fluorescence excitation spectra of OFLX, CPLX, and SPLX

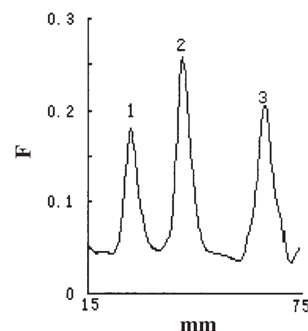

Figure 1. Chromatograms of OFLX (1), CPLX (2), and SPLX (3).

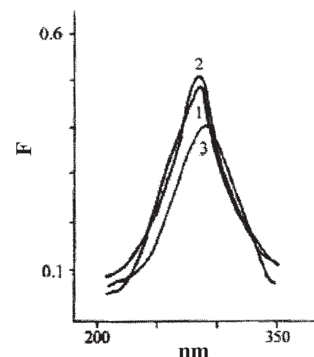

Figure 2. Excitation spectra of OFLX (1), CPLX (2), and SPLX (3).

are shown in Figure 2. The highest signal-to-noise ratio was obtained at the excitation wavelength of 282 nm and a cut-off filter of 400 nm (No. 2).

To overcome errors caused by the uneven thickness on the different TLC plates as well as other environmental and operational factors, all of the standard and sample solution were spotted in the same polyamide sheet. It was found that there was a better repeatability in the same sheet, as shown in Table II.

Stability

During the period of 4 h, the stability of the fluorescent spots of OFLX, CPLX, and SPLX was studied eight times, and there were no obvious changes in the peak areas, as shown in Figure 3. Based on this investigation results, the quantitative scanning determination can be finished within 1 h.

Table II. Effects of Accuracy Experiment*

RSD (%)	Different sheets	Same sheets	Same spots
OFLX	4.2	2.3	1.1
CPLX	5.1	1.9	0.9
SPLX	3.9	3.1	2.3

* Average values of five determinations.

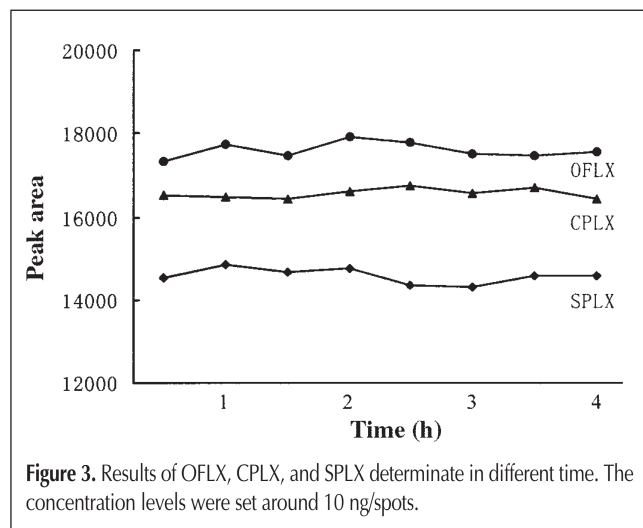

Figure 3. Results of OFLX, CPLX, and SPLX determinate in different time. The concentration levels were set around 10 ng/spots.

Calibration curve of OFLX, CPLX, and SPLX

Under the same conditions, the calibration working curves of OFLX, CPLX, and SPLX was drawn. The results showed that the linear dynamic ranges were 1×10^{-5} to 4×10^{-4} mol/L for OFLX, 1×10^{-5} to 4.5×10^{-4} mol/L for CPLX, and 1×10^{-5} to 4.2×10^{-4} mol/L for SPLX with the correlation coefficients 0.9987, 0.9995, and 0.9992, respectively. It also revealed that the linear regression equations were, for OFLX:

$$C = 8.3 \times 10^{-3} \text{ Area} - 2.14 \quad \text{Eq. 1}$$

for CPLX:

$$C = 6.5 \times 10^{-3} \text{ Area} - 1.67 \quad \text{Eq. 2}$$

for SPLX:

$$C = 9.8 \times 10^{-3} \text{ Area} - 3.20 \quad \text{Eq. 3}$$

When the signal-to-noise ratio was 3, the respective detection limits were 2.0×10^{-6}, 1.52×10^{-6}, and 1.5×10^{-6} mol/L.

Determination of OFLX, CPLX, and SPLX

First, the spiked serum was added to a volumetric flask of 5 mL, and then appropriate amounts of OFLX, CPLX, and SPLX were added. The protein in the serum samples was deposited with the addition of trichloroacetic acid, and the samples were vortexed for 1 min and then centrifuged for 5 min at the speed of 4000 rpm. Finally, the supernatant liquor was added, and the solution was diluted with 95% ethanol to the final volume.

For the standard recovery experiments, the standard OFLX, CPLX, and SPLX solution of different concentrations were simultaneously added to the 5-mL flask and then diluted to the final volume with fresh urine samples. The final results are listed in Table III. The nine determination results were satisfactory.

Determination of urine samples

The urine samples were collected from two healthy men who had 200 mg of OFLX or CPLX at the same time (4 h prior to sampling procedure). It was not necessary to pretreat the samples for the fluorescence measurements. On the same polyamide plate, standard and working solution were spotted for comparison. Based on the five experimental determinations, the average values were 9.8 and 10.4 µg/mL, and the relative standard deviations were found to be 3.7% and 4.8%, respectively.

Table III. The Results for the Determination of OFLX, CPLX, and SPLX*

Compounds		Added (ng/µL)		Found (ng/µL)		Average recovery (%)		RSD (%)	
Urine	OFLX	7.22	14.4	7.30 ± 0.56	14.48 ± 0.38	101.1	100.6	2.86	2.36
	CPLX	6.64	13.2	6.56 ± 0.44	13.22 ± 0.22	98.0	100.2	4.07	1.12
	SPLX	7.84	15.7	7.61 ± 0.41	16.31 ± 0.33	97.1	104.0	4.51	3.51
Serum	OFLX	7.22	14.4	7.28 ± 0.49	14.34 ± 0.34	101.2	99.6	5.82	3.61
	CPLX	6.64	13.2	6.46 ± 0.51	13.17 ± 0.43	97.2	99.8	3.85	2.65
	SPLX	7.84	15.7	7.58 ± 0.62	15.24 ± 0.43	96.7	97.2	4.03	3.87

* Average values of nine determinations.

Conclusion

Instead of the potential harmful or toxic solvents, the selected developer of SDS and EDTA was used in the simultaneous determination of OFLX, CPLX, and SPLX by TLC. The proposed method has high precision and is a simple procedure. For instance, no previous treatment for thin-layer sheets was regarded, and the R_f values of every spot were dependent on the proportion of

the surfactant additive. Furthermore, the "apparent polarity" for the micelle mobile phase can be adjusted simply by means of altering the surfactant concentration, which might be an aid for some specific separation purposes. It was found that the fluorescent spots were clearly and regularly separated, and the micelle solution aided in increasing and stabilizing the fluorescence intensity of the samples. Additionally, micelle solutions are cheap enough to conduct the analytical characteristics for OFLX, CPLX, and SPLX. Based on our investigation, it was suggested that the micelle TLC–F is a practical method for the determine some biological samples.

Acknowledgments

This work was financially supported by the Natural Science Foundation Shanxi Province in China (No. 20021011). The authors also thank Prof. Wei-Jun Jin and Master Jian-Jun Wu for helpful discussions.

References

1. D.W. Armstrong and R.Q. Terrlll. Thin layer chromatographic separation of pesticides, decachlorobiphenyl, and nucleosides with micellar solutions. *Anal. Chem.* **51:** 2160–63 (1979).
2. T.H. Zhao. Application of micellar thin layer chromatographic. *Chin. J. Chromatogr.* **6:** 209–11 (1988).
3. D.W. Armstrong and G.Y. Stine. Selectivity in pseudophase liquid chromatographic. *Anal. Chem.* **55:** 2317–20 (1983).
4. Z.C. Ge, H.G. Lin, and Z.L. Li. Survey and development of micellar and inclusion chromatography. *Chin. J. Anal. Chem.* **19:** 1092–99 (1991).
5. C.F. Poole and N.C. Dias. Practiotioner's guide to method development in thin-layer chromatography. *J. Chromatogr. A* **892:** 123–42 (2000).
6. J.A.O. Gonzalez, M.C. Mochon, and F.J.B. Rosa. Spectrofluorimetric determintion of levofloxacin in tablets, human urine and serum. *Talanta* **52:** 1149–56 (2000).
7. P. Diurdjevic, M. Todorovic, M.J. Stankov, and J. Odovic. Spectrofluorimetric determination of ciprofloracin in serum using iron (III) ion as chromogenic agent. *Anal. Lett.* **33:** 657–65 (2000).
8. D.H. Kim, S.K. Lee, Y.H. Park, and D.W. Lee. High performance liquid chromatographic separation and determination of ciprofloxacin and its metablites in urine. *J. Liq. Chromatogr. Relat. Technol.* **23:** 1069–81 (2000).
9. A. Navaion, O. Ballesteros, R. Blanc, and J.Z. Vilchez. Determination of ciprofloxacin in human urine and serum samples by solid-phase spectrafluorimetry. *Talanta* **52:** 845–52 (2000).
10. P.L. Wang, Y.L. Feng, and L. Chen. Simultaneous determination of trace norfloxacin, pefloxacin, and ciprofloxacin by TLC-fluorescence spectrodensitometry. *J. Microchemical.* **56:** 229–35 (1997).
11. S.Q. Wu, Q. Cao, Y.K. Zhao, W.J. Zhang, and X.G. Chen. The fluorescence characteristic of Tb^{3+}-ciprofloxacin and determination of ciprofloxacin, *Chin. J. Anal. Chem.* **28:** 1462–1466 (2000).
12. Q.J. Gong, J.L. Qiao, L.M. Du, C. Dong, and W.J. Jin. Recognition and simultaneous determination of ofloxacin enatiomers by simple synchronous 1st derivative fluorescence spectroscopy. *Talanta* **53:** 359–65 (2000).
13. Y.L. Feng. Determination of fluroxacin and sparfloxacin simultaneously by TLC-fluorescence scanning densitometry. *Anal. Lett.* **34:** 2963–71 (2001).

Manuscript accepted September 16, 2004.