

Determination of the Common Antibiotic OFL in the Water

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Abstract. This paper conducts a study on the detection method of common antibiotics ofloxacin(OFL) in different water. With high performance liquid chromatography- diode array detector(HPLC-DAD) as the detecting method, the chromatographic detection condition and pretreatment method of the samples are in the study range. The chromatographic detection condition includes DAD detection wavelength, the type of mobile phase and flow rate; the pretreatment method study includes the two parts of simple filtering with direct injection and water sample distillation. The experiment result shows that the linearly dependent coefficient of the external standard curve is 0.996, the detection limit is 0.025 mg·L⁻¹, the recovery rate of the four water samples is 101.0 %-122.8 %.

Introduction

The antibiotics in the environment mainly come from medical antibiotics, livestock breeding, aquaculture and factory emission. The antibiotics accessed in the human body can not be absorbed completely, and quite a number of it is discharged in the form of active compound and metabolin by urine and excrement which gets into the urban sewage treatment plant(STPs). Besides the antibiotics in surface water would not only accumulate in the bottom mud, but also get into the aquatic organism body such as fish and shrimp and get gathered by the food chain. In the aquaculture a lot of antibiotics is plunged into water directly, polluting the surface water, and the antibiotics gets deposited in the bottom mud and gets degraded hardly. The antibiotics getting into the landfill can get into the surface water and underground water by sewage discharge and rainwash and into the drinking water finally.

Ofloxacin (OFL) is the common medicine for both human and animal in quinolone antibiotics, exiting in drinking water, water source, reclaimed water and sewage. Commonly it is detected with liquid chromatograph and mass spectrum (MSD), fluorescence detector(FLD), diode array(DAD)combination.

The detection of OFL is the main indicator of monitoring the distribution, migration and transformation of antibiotics in water. Currently the antibiotics pollution of municipal sewage, surface water and tap water has been put into more attention, especially the sewage. Therefore this paper studies the pretreatment of OFL and liquid chromatography detection method in these water samples.

Experimental

Equipment, reagent and sample.

Agilent HPLC 1100(DAD, Agilent Zorbax SB-C18);

Ofloxacin (OFL): crystallized, $\geq 99.0\%$ (LC, Sigma);

All the water is Wahaha purified water;

Carbinol(LC);

Formic acid(AR):Tianjin Guangfu Fine Chemical Research Institute.

Chromatographic analysis condition.

Stationary phase: C18 chromatographic column (Agilent Zorbax SB-C18); mobile phase: acetonitrile and 0.1% methanoic acid solution(12:88); flow rate: 1.5 mL·min⁻¹; injection volume: 5μL; chromatographic column: 25°C; DAD detection wavelength: 295nm, the time of 8.429 is Ofloxacin(as Fig. 1 shows).

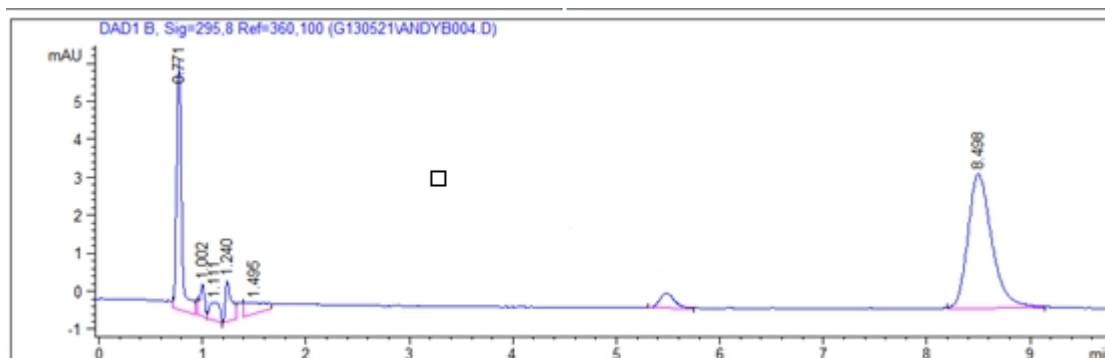


Fig. 1 OFL standard HPLC chromatogram

Standard curve.

Inject the standard solution dilute it to the constant volume with 0.1% methanoic acid solution, conduct 3 parallel analysis with HPLC(as table 1 shows).

Table 1 data of ofloxacin (OFL) standard substance triple parallel experiments

concentration n/(mg·L ⁻¹)	0.050	0.100	0.500	1.000	5.000	10.000	15.000	20.000
Parallel	3.412	3.910	7.477	14.852	56.916	124.969	237.077	322.989
peak	3.414	3.924	7.478	14.851	56.922	124.973	237.084	323.003
area/(PA*S)	3.407	3.926	7.479	14.859	56.916	124.968	237.079	323.008

According to the average peak area(y) in table 1 and concentration(x) draw the external standard curve($y=0.01577x+0.5348$, $R^2=0.996$).

Result and discussion

DAD wavelength option.

Scan the ofloxacin(OFL) with DAD in full wavelength, and it is confirmed that the maximum absorption wavelength lies between 294-296nm, thus the DAD detection wavelength for HPLC is 295nm.

Mobile phase option.

(1) Mobile phase type option

When taking methyl alcohol and water, acetonitrile and water as the mobile phase, the chromatographic peak is trailing badly, while taking methyl alcohol and water(0.1% formic acid solution), acetonitrile and water(0.1% formic acid solution) as the mobile phase, the data is shown in table 2.

Table 2 The data comparison of the two mobile phases

mobile phase	mobile phase rate	t _R	W	S	n
MeOH:H ₂ O(0.1%HCOOH)	20:80	9.342	0.2910	0.790	16490
	30:70	2.907	0.1110	0.839	10974
CH ₃ CN:H ₂ O(0.1%HCOOH)	15:85	4.232	0.1315	0.846	16571
	10:90	14.786	0.3935	0.766	22590

According to the plate theory, calculate the number of theoretical plates with Eq. 1 As the indicator of measuring the column efficiency, the bigger n is , the higher column efficiency is , the more appropriate chromatographic condition is.

$$n=16(t_R /w)^2 \quad (1)$$

According to the number of theoretical plates, it is confirmed that the mobile phase is acetonitrile and water(0.1 %formic acid).

(2)Mobile phase rate option

With acetonitrile and water (0.1% formic acid) as mobile phase, experiment with different rates, the result shown in table 3.

Table 3 experiment data with different mobile phase rates

mobile phase rate	S	W	t_R	n
14:86	0.778	0.1602	5.309	17572
13:87	0.767	0.1923	6.634	19042
12:88	0.761	0.2433	8.549	19755
11:89	0.752	0.3098	11.262	21144

As table 3 shows, when the mobile phase rate of acetonitrile: water (0.1% formic acid) is 12:88, the retaining time is appropriate and in the meantime the column efficiency is high, the chromatograph peak has a good symmetry. Thus it is confirmed that the mobile phase is acetonitrile: water (0.1% formic acid) = 12:88.

Water sample analysis result.

Filter the three water samples of municipal sewage, South Lake water and tap water with OCEAN filter (0.45 μ m water filter membrane) respectively, collect the filtrate into the sample bottle and detect them, and keep the resolution (Eq. 2) $R \geq 1.5$, no target object is found in all the samples.

$$R=2 (t_2-t_1) / (w_1+w_2) \quad (2)$$

Adding standard recovery.

Carry on an adding standard experiment on the three samples of OFL(result shown in Fig.2, Fig.3, Fig.4 and table 4) , the recovery lies in 101.0-122.8%.

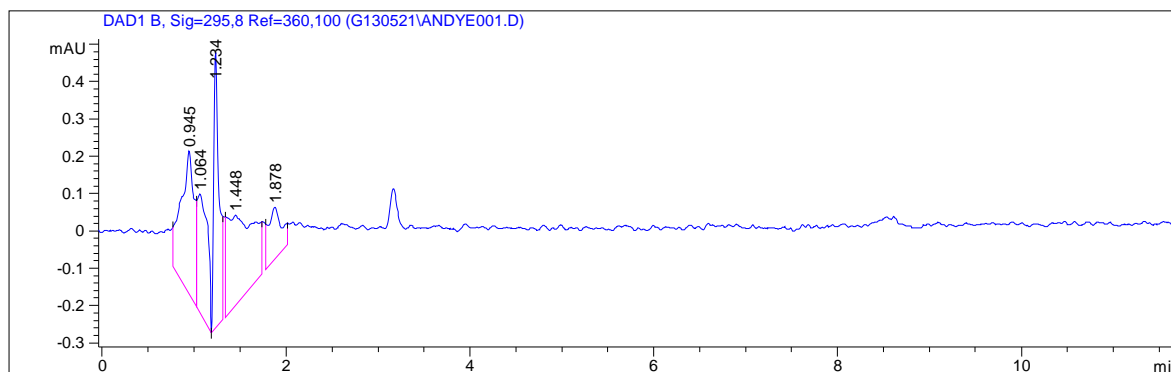


Fig.2 The test map of Urban sewage

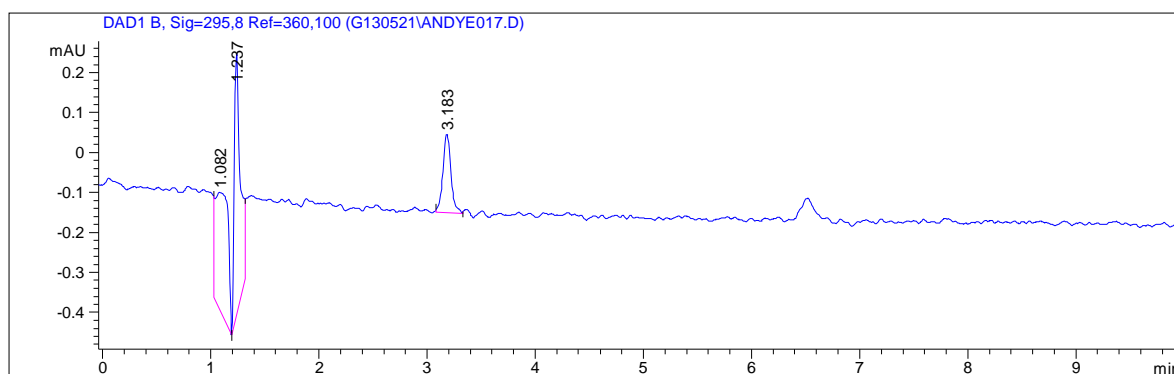


Fig.3 The test map of South lake

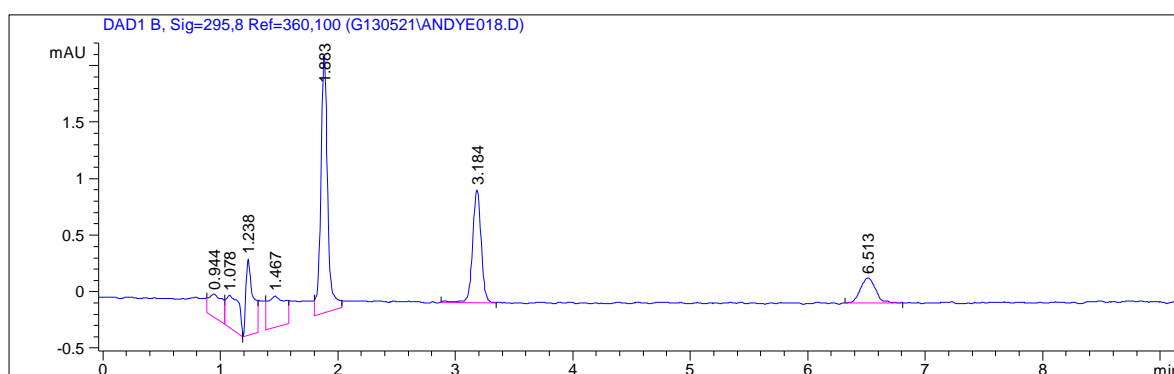


Fig.4 The test map of Tap water

Table 4 adding standard recovery experiment data

Sample name	background values /(mg·L ⁻¹)	adding standard mount/(mg·L ⁻¹)	estimated value /(mg·L ⁻¹)	recovery/%
municipal sewage	0	1.000	1.228	122.8
South Lake water	0	1.000	1.208	120.8
tap water	0	1.000	1.010	101.0

Detection limi.

Dilute the ofloxacin(OFL) standard solution stepwise, when the concentration is 0.025 mg·L⁻¹, the corresponding signal to noise ratio (S/N) is 4.0, thus the detection limit is 0.025 mg·L⁻¹(S/N=4.0).

Conclusion

This paper studies the method of detecting common antibiotics OFL in municipal sewage, surface water and tap water with HPLC-DAD, and confirms an external standard quantitative method with the linearity range of 0.500-20.000 mg·L⁻¹ and the linearly dependent coefficient of 0.996, and the adding standard recovery and the limit of detection can both satisfy the detection requirement of OFL in the three water samples.

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