Detection of Endocrine Disruptors in Water around Landfills

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Abstract [**Objectives**] This study was conducted to explore the occurrence levels of endocrine disruptors (EDCs) in rural areas around a county landfill in Tongren City. [**Methods**] The water around the landfill was sampled and analyzed. A solid-phase extraction and high performance liquid chromatography-tandem mass spectrometry (SPE-UPLC-MS/MS) method was established for the determination of 27 EDCs. After the HLB solid-phase extraction column was activated, a water sample, which was adjusted with phosphoric acid to a pH of 2 (±0.5) and added with 500 mg of disodium EDTA, was loaded, and 5 ml of water and 20% methanol water was added for washing. Next, 10 ml of elution solution was added for elution, and the collected eluate was evaporated under reduced pressure at 40 ℃ to near dryness, and 1 ml of reconstitution solution was added to a constant volume. An ACQUITY UPLC BEH C18 (100×2.1 mm, 2.6 μm) chromatographic column was adopted for LC separation by gradient elution with pure water solution-acetonitrile as the mobile phase. For MS detection, the MRM mode was adopted for collection, and the positive and negative ion modes were switched for simultaneous determination, and the internal standard method was used for quantification. [**Results**] The correlation coefficient R2 was greater than 0.99 in the linear range of each target substance. The limits of quantitation in the method were between 0.05 and 2.00 ng/L, and the recoveries ranged from 75.3% to 105.7%. [**Conclusions**] The method has high sensitivity, good accuracy and strong practical value.

Key words Landfill; Endocrine disruptor; Solid phase extraction; High performance liquid chromatography-tandem mass spectrometry

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Endocrine disrupting chemicals (EDCs), also known as environmental hormones, are exogenous chemicals that interfere with the endocrine system[1-3]. The substances that exist in the environment can interfere with the endocrine system of human beings or animals and cause abnormal effects. They do not directly bring abnormal effects to organisms as toxic substances, but act like estrogens on organisms, through various ways such as intake and accumulation. Even if the quantity is very small, they can make the endocrine imbalance of organisms and cause various abnormal phenomena. Such substances can cause reproductive disorders, abnormal behavior, decreased reproductive capacity, death and even extinction of animals and human beings. At present, environmental hormone pollution has become a global ecological problem and has attracted widespread attention from the society. Studies have shown that environmental hormones (environmental endocrine disruptors) are a type of organic compounds with high stability, high persistence and difficult biodegradation. Therefore, their harm is enormous and far-reaching. This study mainly introduced the origin, meaning, characteristics, types and action mechanisms of environmental hormones and the impact of environmental hormone pollution on the environment and ecology, as well as possible countermeasures for environmental hormone pollution[4-12].

In this study, with the water bodies around a landfill site in a certain county of Tongren City as the object of study, the occurrence levels of endocrine disruptors (EDCs) in the water around the landfill site were elucidated. A rapid ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method for simultaneous analysis of 27 EDCs was established by focusing on optimizing the enrichment of pH value during solid-phase extraction, chromatographic conditions and mass spectrometry parameters. The method has wide linear range, good sensitivity and high accuracy. The method was successfully applied to the analysis of water samples around the actual landfill site, which provides another reliable analysis method for simultaneous determination of various endocrine disruptors in the water around landfill sites, which has strong practical value.

Materials and Methods

Experimental instruments and reagents

Instrument: Ultra-high performance liquid chromatography-tandem mass spectrometry (Waters XEVO-TQS micro, Waters, USA); MFV-24 nitrogen blowing instrument (Guangzhou Detai Instrument Technology Co., Ltd.); solid-phase extraction device (Oasis MCX, 500 mg, 6cc, Waters, USA); Milli-Q ultra-pure water instrument (Millipore, USA); TG16W high-speed centrifuge (Changsha Pingfan Instrument and Apparatus Co., Ltd.); 0.45 μM microporous filter membrane and solid-phase extraction small column (Dikma Technologies).

Standards: Dienestrol, diethylstilbestrol, estrone, trenbolone, nandrolone, androstenedione, boldenone, testosterone, metandienone, methyltestosterone, durabolin, testosterone propionate,

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progesterone, 21α-hydroxyprogesterone, 17α-hydroxyprogesterone, medroxyprogesterone, megestrol acetate, chlormadinone acetate, medroxyprogesterone acetate, hexestrol, estradiol, levonorgestrel, norethindrone, estradiol benzoate, stanozolol, estriol, ethinylestradiol, 17β-estradiol-13C2, methyltestosterone-D3, progesterone-D9, diethylstilbestrol-D8, estrone-D2, levonorgestrel-D6 and medroxyprogesterone-D3, all purchased from Shanghai Anpel Laboratory Technologies Co., Ltd.; methanol (chromatographic purity) and acetonitrile (chromatographic purity), purchased from Merck, Germany; ultrapure water.

Preparation of solutions

Preparation of standard stock solution: First, a 0.005 0 g of solid standard sample was accurately weighed with an electronic balance and dissolved with methanol. Next, the obtained solution was added in a 5 ml brown glass bottle to prepare a standard stock solution with a concentration of 1 mg/ml, which was sealed and stored in a refrigerator at 4 ℃ for later use.

Preparation of standard intermediate solution: First, 100 μl of 1 mg/ml standard stock solution was pipetted into a 10 ml volumetric flask with a pipette of 1 – 100 μl. Next, the transferred solution was diluted with methanol to prepare a standard solution with a concentration of 10 μg/ml.

Preparation of standard working solution: Different volumes of the 10 μg/ml standard intermediate solutions of the 27 target compounds were accurately transferred into different 10 ml volumetric flasks. Next, mixed standard solutions with different concentrations were prepared by diluting with methanol.

Elution solution: Methanol: ethyl acetate: ammonia (150 : 150 : 6).

Reconstitution solution: Water: methanol: acetonitrile: formic acid (40 : 5 : 5 : 0.05).

Phosphate buffer solution: 19.3 g sodium phosphate monohydrate +10 ml phosphoric acid, with pH adjusted to 2.

Collections of samples

In July 2022, 13 surface water samples were collected from the vicinity of the urban landfill site in Dejiang County, Tongren City. At each sampling point, a 1 L brown hard glass bottle was rinsed three times, and then groundwater (shallow groundwater) 10 – 30 cm below the water surface was collected in the monitoring well. The pH value of the water sample was adjusted to a value equal to or lower than 3 with concentrated sulfuric acid, and it was transported to the laboratory for refrigeration at 1 – 5 ℃ in the dark. All samples were treated and analyzed within 48 h.

Pretreatment of samples

Extraction: Each water sample was stood still, and a 500 ml of water sample was added with 50 μl of internal standards and adjusted with phosphoric acid to a pH of (2 ± 0.5). Next, 500 mg of disodium EDTA was added.

Purification: An HLB column (500 mg, 6 ml) was placed in a solid-phase extraction device, and 6 ml of methanol and 6 ml of water were added. After discarding the effluent, a liquid to be purified was added into the small column, which was then added with 5 ml of water and 20% methanol water for washing. Next, 10 ml of elution solution was added for elution, and the eluate was collected. The collected eluate was evaporated under reduced pressure at 40 ℃ to near dryness, and 1 ml of reconstitution solution was added to a constant volume. The obtained solution was filtered through a microporous filter membrane to get a filtrate for analysis by instruments.

Conditions of instruments

An ACQUITY UPLC BEH C18 (100 × 2.1 mm, 2.6 μm) column was used to separate the target objects. The separation was carried out with a column temperature at 35 ℃, a sample injection volume of 10 μl, and a flow rate at 0.3 ml/min. The mobile phase A was pure water, and the mobile phase B was acetonitrile. The gradient elution conditions are shown in Table 1.

Table 1 Elution conditions

Time	Flow rate//ml/min	A	B
0.0	0.3	95	5
1.0	0.3	95	5
3.0	0.3	5	95
5.0	0.3	5	95
5.1	0.3	95	5
7.0	0.3	95	5

Mass spectrometry parameters: The time window of mass spectrometry was divided by the MRM mode to collect positive and negative ions. An electrospray ionization source (ESI) was used. The temperature of desolventizing gas was 350 ℃, and the ion source temperature was 150 ℃. The flow rate of carrier gas was 650 L/h, and the flow rate of collider was 50 L/h. Specific parameters are shown in Table 2.

Results and Analysis

Optimization of chromatographic conditions

In this study, the separation effects of ACQUITY UPLC BEH C18 (50 × 2.1 mm, 1.7 μm) and ACQUITY UPLC BEH C18 (100 × 2.1 mm, 2.6 μm) with different lengths were compared. The results showed that the 50 mm column was too short, and the resolution of endocrine disruptors was not good, while the 100 mm chromatographic column was better. In order to obtain chromatographic peaks well separated with good peak shapes, the separation effects using acetonitrile and methanol as mobile phases were compared in this study. When methanol was used as the mobile phase B, the resolution and peak shapes of some substances were not good, and the sensitivity of some substances was reduced, but the above situation did not occur in acetonitrile. Comparing the gradient elution conditions at column temperatures of 30 and 45 ℃ under a flow rate of 0.3 ml/min, the target compounds were basically separated. Considering the service life of the chromatographic column, the final temperature of the column was determined to be 35 ℃, at which all 27 EDCs were separated, showing a chromatogram with good peak shapes, strong signals and good separation effects.

Table 2 MS parameters of 27 EDCs

Compound	Parent ion (*m/z*)	Daughter ion (*m/z*)	Declustering potential // v	Collision energy // ev	Capillary voltage // kv	Ionization mode
Dienestrol	264.9	236.1	50	20	1.5	ESI⁻
	264.9	249.1*	50	25		
Diethylstilbestrol	266.9	237.1	50	25	1.5	ESI⁻
	266.9	251.1*	50	25		
Hexestrol	269.2	119.1*	50	38	1.5	ESI⁻
	269.2	134.1	50	10		
Estrone	268.9	145.1*	50	35	1.5	ESI⁻
	268.9	183.1	50	40		
Estradiol	271.2	145.1	35	40	1.5	ESI⁻
	271.2	183.2*	35	40		
Estriol	287.1	145.1	50	38	1.5	ESI⁻
	287.1	171.1*	50	35		
Ethinylestradiol	295.1	145.1*	50	35	1.5	ESI⁻
	295.1	159.1	50	30		
Trenbolone	271.1	199.1	45	20	1.5	ESI⁺
	271.1	253.2*	45	15		
Nandrolone	275.2	239.2*	45	10	1.5	ESI⁺
	275.2	257.2	45	10		
Boldenone	287.2	121.1	45	20	1.5	ESI⁺
	287.2	135.2*	45	10		
Testosterone	289.2	97.2	45	20	1.5	ESI⁺
	289.2	109.1*	45	22		
Norethindrone	299.2	109.1*	40	25	1.5	ESI⁺
	299.2	231.2	40	15		
Levonorgestrel	313.2	109.1	45	30	1.5	ESI⁺
	313.2	245.2*	45	12		
Progesterone	315	97.1*	40	25	1.5	ESI⁺
	315	109.1	40	25		
Stanozolol	329.4	81.1*	45	40	1.5	ESI⁺
	329.4	121.1	45	35		
17α-Hydroxyprogesterone	331.2	97.1*	45	25	1.5	ESI⁺
	331.2	109.1	45	25		
Medroxyprogesterone	345.3	97.1	45	30	1.5	ESI⁺
	345.3	123.1*	45	25		
Estradiol benzoate	377.2	105.1*	45	25	1.5	ESI⁺
	377.2	135.1	45	10		
Megestrol acetate	385.2	224.2*	45	25	1.5	ESI⁺
	385.2	267.2	45	20		
Medroxyprogesterone acetate	387.2	123.1	45	25	1.5	ESI⁺
	387.2	327.3*	45	10		
Chlormadinone acetate	405.2	301.2*	45	20	1.5	ESI⁺
	405.2	309.3	45	12		
Durabolin	407.2	105.2	50	25	1.5	ESI⁺
	407.2	257.2*	50	15		
Androstenedione	287.2	97.1*	45	20	1.5	ESI⁺
	287.2	109.1	45	25		
Metandienone	301.2	121.1*	45	25	1.5	ESI⁺
	301.2	149.2	45	12		
Methyltestosterone	303.1	97.1*	45	20	1.5	ESI⁺
	303.1	109.1	45	25		
21α-Hydroxyprogesterone	331.2	97.1*	45	25	1.5	ESI⁺

(Continued)

(Table 2)

Compound	Parent ion (m/z)	Daughter ion (m/z)	Declustering potential // v	Collision energy // ev	Capillary voltage // kv	Ionization mode
	331.2	109.1	45	25		
Testosterone propionate	345.2	97.1 *	45	28	1.5	ESI^+
	345.2	109.1	45	25	1.5	ESI^+
Methyltestosterone-D3	306.2	109.2 *	45	25	1.5	ESI^+
Progesterone-D9	324.2	100.2 *	45	20	1.5	ESI^+
Diethylstilbestrol-D8	275.2	259.4 *	35	25	1.5	ESI^-
Estrone-D2	271.2	147.1 *	45	35	1.5	ESI^-
Levonorgestrel-D6	319.2	251.2 *	45	15	1.5	ESI^+
Medroxyprogesterone-D3	348.2	126.2 *	50	28	1.5	ESI^+
17β-Estradiol-13C2	272.9	147.1 *	30	35	1.5	ESI^-

The mark * stands for ion for quantification.

Table 3 Linear equations, limits of detection and limits of quantitation

Compound	Retention time // min	Linear equation	Correlation coefficient R^2	Limit of detection // ng/L	Limit of quantification // ng/L
Dienestrol	4.27	$y = 0.208\,449 * x - 0.551\,025$	0.992	0.10	0.30
Diethylstilbestrol	4.22	$y = 0.240\,772 * x - 0.405\,543$	0.994	0.10	0.30
Hexestrol	4.26	$y = 538.186 * x + 125.865$	0.993	0.05	0.20
Estrone	4.22	$y = 69.4835 * x - 660.886$	0.997	0.80	2.00
Estradiol	4.08	$y = 0.399\,9 * x + 3.011\,8$	0.996	0.80	2.00
Estriol	3.60	$y = 2.436\,99 * x + 20.566\,7$	0.993	0.20	0.80
Ethinylestradiol	4.15	$y = 0.545\,011 * x - 3.454\,64$	0.994	0.20	0.80
Trenbolone	3.78	$y = 1.703\,91 * x - 1.442\,03$	0.996	0.02	0.10
Nandrolone	3.89	$y = 0.179\,263 * x - 0.068\,805\,5$	0.995	0.20	0.80
Boldenone	3.83	$y = 3.295\,43 * x - 2.470\,1$	0.995	0.01	0.05
Testosterone	4.00	$y = 2.161\,94 * x + 2.547\,22$	0.995	0.01	0.05
Norethindrone	3.98	$y = 0.525\,784 * x + 3.711\,44$	0.992	0.03	0.10
Levonorgestrel	4.18	$y = 1.371\,09 * x + 0.068\,663\,8$	0.999	0.03	0.10
Progesterone	4.50	$y = 1.723\,77 * x + 0.686\,389$	0.999	0.02	0.08
Stanozolol	4.09	$y = 5.105\,06 * x - 2.149\,88$	0.998	0.01	0.05
17α-Hydroxyprogesterone	4.03	$y = 0.665\,01 * x + 2.110\,47$	0.992	0.01	0.05
Medroxyprogesterone	4.27	$y = 1.983\,7 * x + 0.198\,431$	0.998	0.03	0.10
Estradiol benzoate	4.01	$y = 0.003\,20 * x - 0.007\,363$	0.994	0.50	1.50
Megestrol acetate	4.29	$y = 0.786\,472 * x + 2.004\,48$	0.997	0.01	0.05
Medroxyprogesterone acetate	4.47	$y = 0.441\,416 * x + 1.039\,26$	0.997	0.08	0.30
Chlormadinone acetate	4.42	$y = 0.052\,097\,4 * x + 0.215\,627$	0.996	0.08	0.30
Durabolin	5.15	$y = 0.022\,33 * x - 0.004\,667$	0.994	0.50	2.00
Androstenedione	4.12	$y = 1.425\,07 * x - 1.034\,87$	0.997	0.03	0.10
Metandienone	3.93	$y = 8.316\,5 * x - 5.155\,42$	0.991	0.01	0.05
Methyltestosterone	4.10	$y = 2.015\,2 * x + 0.300\,123$	0.999	0.01	0.05
21α-Hydroxyprogesterone	4.48	$y = 0.669\,641 * x + 1.975\,15$	0.995	0.03	0.10
Testosterone propionate	4.28	$y = 1.420\,64 * x - 2.168\,84$	0.994	0.02	0.08

Drawing of working curves

The mixed standard solutions of endocrine disruptors at the third level were diluted with methanol and prepared into different concentrations based on the response of each substance. The concentrations for curves of dienestrol, diethylstilbestrol, estron, trenbolone, nandrolone, androstenedione, boldenone, testosterone, metandienone, methyltestosterone, durabolin, testosterone propionate, progesterone, 21α-hydroxyprogesterone, 17α-hydroxyprogesterone, medroxyprogesterone, megestrol acetate, chlormadinone acetate and medroxyprogesterone acetate were 2.5, 5.0, 10, 25, 50 and 100 ng/ml. The concentrations for curves of hexestrol, estradiol, levonorgestrel, norethindrone, estradiol benzoate and stanozolol were 5.0, 10, 20, 50, 100 and 200 ng/ml. The concentrations for curves of estriol and ethinylestradiol were 10, 20, 40, 100, 200 and 400 ng/ml. The concentrations for internal standards were as follows: 17β-estradiol-13C2 200 ng/ml, and methyltestosterone-D3, progesterone-D9, diethylstilbestrol-D8, estrone-D2, levonorgestrel-D6 and medroxyprogesterone-D3 100 ng/ml. Each working curve was drawn taking the area of quantitative ion mass spectrum peak of corresponding EDC as the ordinate and the

mass concentration as the abscissa. The limit of detection (LOD) of the method was calculated according to 3 times of the signal-to-noise ratio (S/N), and the limit of quantitation (LOQ) of the method was calculated according to 10 times of the signal-to-noise ratio. The linear equations, regression coefficients, and limits of detection and limits of quantification for the method are shown in Table 3. From the table, it can be seen that there was a good linear relationship between the peak areas and mass concentrations of the 27 target substances. The linear correlation coefficient R^2 of each substance was higher than 0.99, and the limits of detection ranged from 0.01 to 0.50 μg/L, and the limits of quantitation were between 0.05 and 2.00 μg/L.

Recovery test

The mixed standard solutions of 27 endocrine disruptors were accurately added to 500 ml of ultra-pure water and Watsons pure water, respectively, to carry out a recovery test, and the recoveries and precision of the method were investigated. The spiked concentration levels were set as low (5.00 ng/L), medium (20.00 ng/L) and high (100.00 ng/L), and the recoveries ranged from 75.3% to 105.7%, which met the requirements of methodology. The results of recovery data are shown in Table 4.

Table 4 Recoveries of EDCs in spiked water samples

Compound	Spiked concentration 5.00 ng/L	Spiked concentration 20.00 ng/L	Spiked concentration 100.00 ng/L
Dienestrol	88.5	85.6	101.8
Diethylstilbestrol	89.4	99.8	75.3
Hexestrol	86.2	96.4	83.5
Estrone	88.5	87.7	79.7
Estradiol	75.8	104.6	97.7
Estriol	89.8	105.7	77.5
Ethinylestradiol	87.4	85.2	89.6
Trenbolone	101.6	79.8	101.3
Nandrolone	88.3	102.5	86.7
Boldenone	78.7	95.6	76.9
Testosterone	79.6	93.4	86.5
Norethindrone	79.3	94.4	85.2
Levonorgestrel	99.7	84.9	92.8
Progesterone	90.5	87.8	94.3
Stanozolol	97.3	98.2	101.3
17α-Hydroxyprogesterone	84.6	84.8	91.2
Medroxyprogesterone	83.5	79.4	101.8
Estradiol benzoate	87.1	95.6	101.9
Megestrol acetate	86.5	97.1	82.3
Medroxyprogesterone acetate	95.6	87.3	83.0
Chlormadinone acetate	103.3	74.2	95.5
Durabolin	77.2	77.3	80.3
Androstenedione	84.4	102.2	89.2
Metandienone	74.7	95.7	83.8
Methyltestosterone	90.4	88.7	88.0
21α-Hydroxyprogesterone	90.3	87.3	79.4
Testosterone propionate	79.9	78.5	87.3

Actual sample analysis

The determination method established in this study was applied to the analysis of EDCs in samples from 13 locations around the landfill site in Dejiang County, Tongren City. Testosterone was detected at 2 locations, but not in others. The results showed that this analysis method has good practical value for the determination of EDCs in water samples around landfill sites.

Conclusions and Discussion

In this study, an analytical method for simultaneous determination of 27 kinds of EDCs in the water around landfill sites was established. The limits of detection ranged from 0.01 to 0.50 g/L, and the limits of quantitation were between 0.05 and 2.00 g/L, and the recoveries ranged from 75.3% to 105.7%. The method was successfully applied to the analysis of EDCs in the water around the landfill site in Dejiang County, Tongren City. The results indicated that the method has high sensitivity and good accuracy. This study provides a rapid, accurate and reliable analysis method for the determination of endocrine disruptors in the water around landfill sites, which has strong practical value.

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Biofacies and biomass analysis After the operation of the aerated biofilter stabilized, it was found that bacteria, fungi, protozoa and micro metazoa were present in the biofilm attached to the filter material. Protozoa mainly include amoeba, bean shaped worms, small mouthed bell worms, *etc.*, while micro metazoa mainly include rotifers, nematodes, *etc.* The microbial population in the aerated biofilter was abundant, forming a functionally stable ecosystem.

The surface and internal biomass of porous ceramic particles were 289.35 and 17.69 nmol P/g of filter material, respectively, with a total amount of 307.04 nmol P/g of filter material. The surface and internal biomass accounted for 94.24% and 5.76% of the total amount, respectively. It can be seen that the biological load of preparing porous ceramsite using fly ash as raw material is relatively large.

Conclusions

(1) Using fly ash to prepare porous ceramsite, its apparent density was 1.21 g/cm^3, apparent porosity was 51.03%, and specific surface area was 4.26 m^2/g. Porous ceramic particles had a rough surface and well-developed pores, making them environmentally friendly materials for wastewater treatment.

(2) The fly ash-based porous ceramsite aerated biofilter had a good operating effect in treating wastewater. The removal rates of COD_{Cr}, NH_4^+-N, and TN were 85.46%, 96.13%, and 32.31%, respectively. The removal volume loads were 5.23, 0.98, and 0.35 kg/(m^3 · d), respectively.

(3) The surface and internal biomass of porous ceramic filter media were 289.35 and 17.69 nmol P/g respectively, and the microbial population in the aerated biofilter was abundant.

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