Components identification in some hospital wastewater during traditional treatment process

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Abstract. The trace medicine metabolites in some hospital wastewater during treatment process were determined and characterized by gas chromatography - mass spectrometry. Analysis results identified the present of various polycyclic and heterocyclic refractory compounds in the hospital wastewater. This study indicated the medicine metabolites could not be effectively removed by the traditional treatment process, and it was irraditative for the technology improvement in the wastewater treatment plants receiving hospital effluents.

1. Introduction
In current China, most hospitals have their own wastewater treatment plant (WWTP) to pretreat their discharge. These WWTPs were usually equipped with traditional wastewater treatment process, such as anaerobic-anoxic-aerobic reactor, due to its low treatment cost, simple operation and maintenance [1]. However, ammonia nitrogen, chemical oxidation demand and other regulated conventional parameters of wastewater are the usual evaluation indexes for water quality, and they can roughly represent the pollution level of medicine metabolites since they do not give the detailed information about the pollutants. Hence, analysis of organic pollutants was performed to identify the changes of the compounds during the traditional treatment process. In this study, compounds in wastewater were detected and characterized by gas chromatography - mass spectrometry.

2. Materials and methods
2.1. Chemicals and reagents
Methanol and dichloromethane were chromatographic grade purchased from Sigma-Aldrich (Louis, USA). Ultrapure water was purified by a Milli-Q purification system. C18 Solid phase extraction column (SPE, 500 mg, 6 mL) was supplied by Steema science limited in Hong Kong. All glass vessels, prior to application, were immersed in chromic acid lotion for at least 6 h, and rinsed with ultrapure water.

2.2. Sample collection
The selected WWTP located in a tumor hospital in northeast China. The capacity of the WWTP is 20000 m³d⁻¹, where the pretreated hospital wastewater accounted for 60%-70%. The flowchart of the treatment process was briefly described in Figure 1. The wastewater was sampled over a 24h period in
terms of composite sampling methods [2] in June 2019. The sampling sites were chosen at the outlets of the regulating tank, anaerobic-anoxic-aerobic tank, disinfection tank and ultrafiltration tank. All samples were filtered with 0.22 μm filter, and stored in the airtight brown glass bottles at 4 °C for chemical analysis.

![Flowchart of treatment process and sampling scheme. A: regulating tank effluent; B: anaerobic-anoxic-aerobic tank effluent; C: disinfection tank effluent; D: ultrafiltration tank effluent](image)

2.3. Extraction
For the experiment, 250 mL sample was loaded onto SPE cartridges, which had been activated with 5 mL of methanol and 5 mL of water. The cartridges were then washed with 10 mL water, and dried for 30 min. Analytes were then eluted with 5 mL methanol. The eluent was dried at 30 °C with nitrogen stream, and the residue was dissolved in 1mL dichloromethane, and transferred to the autosampler vial. Finally, 1 μL sample was injected into the Gas Chromatography-Mass Spectrometer.

2.4. Organic chemicals analysis
The specific organic components in wastewaters from each stage of the WWTP was determined on a 7890B-5977B Gas Chromatography-Mass Spectrometer (GCMS, Agilent, USA). The GCMS was equipped with EI source and a HP-5ms capillary column (30 m × 0.25 mm × 0.25 μm). Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mL/min. Splitless mode was used for injection, and the temperature of the injector, transfer line and the ion source were kept at 250, 280 and 230 °C, respectively. The GC oven was programmed as 60 °C for 1 min, followed by a ramp of 30 °C min\(^{-1}\) to 180 °C (held for 5 min), a ramp of 7 °C min\(^{-1}\) to 240 °C (held for 1 min), a ramp of 1 °C min\(^{-1}\) to 245 °C (held for 2 min), and a final ramp of 30 °C min\(^{-1}\) to 300 °C (held for 5 min). The detected compounds were preliminarily identified with reference to the National Institute of Standards and Technology (NIST) mass spectral library database. For qualitative analysis, Mass selective detector was operated at scan mode (m/z 50 ~ 500) with 4 min solvent delay. Semi-quantification was performed for all sample analyses, and their concentrations were present in the form of calibrated peak area, which approach is proved appropriate since the response signals and the concentrations of the compounds were of linear relationship [3].
3. Results and discussion
The identified compounds of each sample were summarized in Table 1, and their detailed information were given in Table 2. It can be seen that eight kinds of organic compounds are identified in the regulating tank, and most of them are polycyclic and heterocyclic compounds with aromatic rings and short-chain alkyl functional groups. Specifically, they are most probably medicine or medicine metabolites. For example, 1,8-Diazaclotetradecane-2,9-dione is one pharmaceutical intermediate, and ifosfamide is an antineoplastic agent. Despite that the direct toxicity of these compounds was limited, similar compounds had been proved acutely toxic to multiple organisms [4]. When the wastewater was treated by the anaerobic/anoxic/aerobic tank, some compounds still remained, and new compounds was detected. After the last treatment process disinfection or ultrafiltration, kinds of compounds still remained, and was different, which indicated that compounds were selectively, not entirely, removed by the both last treatment processes.

Table 1. Organic compounds detected in the wastewaters from different sampling sites

| NO. | Chemical Name                                           | Chemical Formula | Sampling sites a | A | B | C | D |
|-----|--------------------------------------------------------|------------------|------------------|---|---|---|---|
| 1   | 1,8-Diazaclotetradecane-2,9-dione                      | C12H22N2O2       |                  | + | + | - | + |
| 2   | Ifosfamide                                             | C7H15Cl2N2O2P    |                  | + | + | - | + |
| 3   | 11H-Pyrido [3',2'-4,5] pyrrolo [3,2-c] quinoline      | C14H9N3          |                  | + | + | + | - |
| 4   | 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylengol         | C43H46O10        |                  | + | - | - | - |
| 5   | 17-(1,5-Dimethyloxy)-10,13-dimethyl-3-styrylhexadecahydrocyclopenta[a]phenanthren-2-one | C35H52O         |                  | + | - | - | - |
| 6   | 7,8-Epoxylanostan-11-ol, 3-acetoxy                    | C32H54O4         |                  | + | + | + | - |
| 7   | Acrylothiamide                                         | C19H19ClN2O2S    |                  | + | + | + | - |
| 8   | Demecolcine                                            | C21H25NO5        |                  | + | - | - | - |
| 9   | Olean-12-ene-3,15,16,21,22,28-hexol                   | C30H50O6         |                  | - | + | - | - |
| 10  | 1-(4-bromophenyl)-2-(piperazin-1-yl) propan-1-one     | C13H17BrN2O      |                  | - | + | + | + |
| 11  | 3-Pyridinecarboxylic acid, 2,7,10-tris(acetoxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1 | C32H39NO10 |                  | - | - | + | - |
| 12  | Benzenemethanol                                        | C13H12O          |                  | - | - | + | - |

“+” detected; “-” not detected.

a: refer to Figure 1 for the samples A-D.
Table 2. Detailed information of the identified chemicals by GC-MS

| NO. a | R.T. (min) | CAS            | Structure |
|-------|------------|----------------|-----------|
| 1     | 31.78      | 56403-09-9     | ![Structure](image) |
| 2     | 28.34      | 3778-73-2      | ![Structure](image) |
| 3     | 27.08      |                |           |
| 4     | 26.30      | 77495-84-2     | ![Structure](image) |
| 5     | 26.18      |                |           |
| 6     | 24.39      |                |           |
| 7     | 21.96      |                | ![Structure](image) |
| 8     | 21.56      | 77646-20-9     | ![Structure](image) |
| 9     | 30.42      | 15399-43-6     | ![Structure](image) |
| 10    | 13.45      | 109607-56-9    | ![Structure](image) |
| 11    | 27.15      | 51906-00-4     | ![Structure](image) |
| 12    | 21.6       | 91-01-0        | ![Structure](image) |
It should be noted that some identified compounds were found all through the treatment process. As shown in Figure 2, their abundances were presented in the form of peak area. The abundance of 1,8-Diazacyclotetradecane-2,9-dione and ifosfamide was not present in disinfection effluent, and was roughly the same in other sites, which implied that both of them could be effectively removed by disinfection process. Similarly for 11H-Pyrido [3',2'-4,5] pyrrolo [3,2-c] quinoline, it could be effectively removed by ultrafiltration process. 7,8-Epoxylanostan-11-ol, 3-acetoxy was of refractory and found all through the treatment process. 1-(4-bromophenyl)-2-(piperazin-1-yl) propan-1-one was metabolites of activated sludge and not easily removed.

Based on these results, traditional treatment process could not effectively remove medicine metabolites, and some compounds still remained in the effluent. Hence, the effluent is required to decrease its potential adverse impacts on the water-receiving environment. Advanced oxidation processes, such as catalytic ozonation [5] and Fenton [6], may be the feasible solution for further removal of refractory compounds since they can produce high oxidation species, hydroxyl radicals.

**Figure 2.** Semi-quantitative analysis of 5 organic compounds in the wastewater samples.

(Refer to Figure 1 for the samples A-D)

4. Conclusion
Twelve kinds of trace medicine metabolites were identified in some hospital wastewater during treatment process by gas chromatography - mass spectrometry. The trace medicine metabolites were refractory compounds, and could be effectively removed in the traditional treatment process. The effluent may present potential ecological risk. Advanced oxidation processes were suggested to be applied to make the traditional treatment process up.

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