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HPLC-MS/MS 测定城市废水中的 10 种药物与个人护理用品

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摘　要：采用固相萃取技术对水样进行预处理，结合液相色谱 - 串联质谱分析方法（HPLC-MS/MS），建立了同时检测城市废水中包括扑热息痛、萘普生、磺胺甲恶唑、磺胺二甲嘧啶、三氯生、双氯芬酸钠、三氯卡班、盐酸四环素、盐酸土霉素、吉非罗平在内共计 10 种药物与个人护理用品（PPCPs）的分析检测方法。采用中性条件萃取水样，控制上样流速为 3—5 mL·min^{-1}，用甲醇溶液洗脱。纯水的平均加标回收率为 40.8%—104.5%，相对标准偏差为 5.0%—25.5%（n=3）。应用所建立的分析方法，对西安浐河表层水进行了分析。结果表明：该方法可用于城市废水 PPCPs 的检测。10 种目标物质中，共检测到 4 种，其含量为 1.4—15.0 ng·L^{-1}。

关键词：固相萃取；高效液相色谱 - 串联质谱；药物与个人护理品；城市废水

Determination of 10 pharmaceuticals and personal care products in waste water by HPLC-MS/MS

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Abstract: ***Background, aim, and scope*** Pharmaceuticals and personal care products (PPCPs) represent a variety of chemical, widely used by consumers on a daily basis which include prescription and non-prescription drugs, cosmetics, cleansers, detergents and fragrance produces. PPCPs are considered potentially hazardous compounds because some are ubiquitous, persistent, and biologically active compounds with recognized endocrine disrupting functions (Daughton and Ternes, 1999). These compounds have been widely detected in various environmental matrices throughout the world including rivers (Glen et al, 2003; Tixier et al, 2003; Yu and Chu, 2009; Zhang et al, 2011; Wu et al, 2014), lakes (Buser and Theobald, 1998; Glen et al, 2003; Tixier et al, 2003; Blair et al, 2013; Ferguson et al, 2013; Zhu et al, 2013), oceans (Weigel et al, 2002; Del Rosario et al, 2014), groundwater (Barnes et al, 2008), waste and drinking water (Carmona et al, 2014) and food (Wu et al, 2012; Baron et al, 2014). Detection methods of PPCP include spectrophotometry, gas chromatography, liquid chromatography and electrophoresis. The recent advances in analytical instrumentation have allowed the unequivocal identification and confirmation of the presence of any compound at very low levels using LC-MS^2.

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The multiple reaction monitoring (MRM) allows monitoring two transitions between precursor and product ions. It is possible to quantify and confirm the presence of PPCPs at very low concentration levels. However, due to the absence of official monitoring protocols, there is an increasing demand of analytical methods that allow the determination of those compounds in order to obtain more information regarding their behavior and fate in the environments. Therefore, we proposed here a method for the determination of ten pharmaceuticals and personal care products, including acetaminophen, naproxen, diclofenac, sulfamethoxazole, sulfadimidine, triclosan, triclocarban, tetracycline hydrochloride, oxytetracycline and gemfibrozile, in waste water using high performance liquid chromatography-tandem mass spectrometry. The aim of this work is to develop an efficient method for determination of various PPCPs in water. ***Materials and methods*** Filtered water samples were extracted using solid-phase extraction cartridges (SPEs) extraction. HLB SPEs (Poly-sery HLB, 6 mL/500 mg, CNW) were conditioned with 3×5 mL of water. Water samples were allowed to pass through the cartridges at a flow rate of approximately 5—10 $mL \cdot min^{-1}$. After sample loading, the cartridges were then subsequently dried for 30 min under full vacuum, and subsequently the ten pharmaceutical compounds were eluted with 10 mL of methanol. The residue was dissolved in 0.5 mL of methanol and transferred into vials for analysis. The PPCPs were analyzed using liquid chromatography-mass spectrometry (LC-MS) with a Shimadzu 8030 system equipped with an autosampler and Labsolutions manager software. ***Results*** Electrospray ionization (ESI) was used as LC-MS interfaces since it is the most frequently used ionization mode which is a soft ionization technique, suitable for polar and moderately non-polar compounds. Fragmentor, collision energy, and other source parameters were optimized by injecting individual standard solutions into mass spectrometer by flow injection analysis (FIA). After that, two different MRM transitions were selected for each compound: one for quantification and one for qualification. These ions were monitored under time scheduled MRM conditions. The analysis was done with electrospray ionization in negative mode (ESI^-) for TCC, NPX, TCS, DF, GF and in positive mode (ESI^+) for the ACT, SMX, SMT, TC, OTC. The initial mobile phase proportion was 20% A and 80% B where A=methanol and B=formic acid:water 1:9999, held for 10 min. A was then increased linearly to 90% in 25 min. The MRM transitions for different PPCPs are as follows: ACT *m*/*z* 151/110; NPX *m*/*z* 229/185; SMX *m*/*z* 254/156; SMT *m*/*z* 279/186; TCS *m*/*z* 287/35; DF *m*/*z* 294/250; TCC *m*/*z* 313/60; TC *m*/*z* 445/410; OTC *m*/*z* 461/426; GF *m*/*z* 249/121. ***Discussion*** The linearity of the MS-MS detector was tested with matrix extracts containing PPCPs at concentration between 1 $\mu g \cdot L^{-1}$ and 250 $\mu g \cdot L^{-1}$ for ACT, NPX and DF, between 1 $\mu g \cdot L^{-1}$ and 100 $\mu g \cdot L^{-1}$ for SMX and SMT, between 2.5 $\mu g \cdot L^{-1}$ and 100 $\mu g \cdot L^{-1}$ for TCS and TCC, between 2.5 $\mu g \cdot L^{-1}$ and 250 $\mu g \cdot L^{-1}$ for TC, OTC and GF. The average recoveries of the target compounds in the spiked pure water samples ranged from 40.8%—104.5% with the relative standard deviations ranged from 5.0%—25.5% (n=3). The waste water sample collected from Chanhe River in Xi'an was investigated as a case study. Among the 10 PPCPs, 4 PPCPs were detected and the concentrations ranged from 1.4 $ng \cdot L^{-1}$ to 15.0 $ng \cdot L^{-1}$. ***Conclusions*** A method using HPLC-MS/MS has been developed and validated for determination of 10 PPCPs (TCC, NPX, TCS, DF, GF, ACT, SMX, SMT, TC and OTC) in water. Subsequently, the method was successfully applied to analysis of the investigated chemicals in water samples collected from Chanhe River in Xi'an. ***Recommendations and perspectives*** The complexity of the biological matrices and the low concentration levels of these compounds make necessary the use of advanced sample treatment procedures, sample clean-up, to remove potentially interfering matrix components, as well as the concentration of analytes. Increased attention would have to be paid to metabolites generated in the organisms and released into the environment as well as to metabolites generated in the environment itself by biodegradation, photolytic or oxidation reactions.

Key words: solid phase extraction; HPLC-MS/MS; pharmaceuticals and personal care products (PPCPs); waste water

药物与个人护理品（pharmaceuticals and personal care products，PPCPs）是一类新型的环境污染物，主要是指各种药物（如抗生素、类固醇、消炎药、镇静剂、抗癫痫药、避孕药、神经兴奋剂等）以及个人护理品（如化妆品中的合成香料、显影剂、遮光剂、驱蚊剂、消毒杀菌剂等）等。研究认为：水环境中残留的 PPCPs 可能会导致人类致癌或过敏性反应，并可能使细菌产生耐药性及抗生素抗性基因的传递和扩散，干扰天然细菌的生态系统，从而威胁人类健康（Daughton and Ternes，1999）。一系列监测研究表明：药物与个人护理品已经成为环境中广泛存在的一类新兴污染物，在河流（Glen et al，2003；Tixier et al，2003；Yu and Chu，2009；Zhang et al，2011；Wu et al，2014）、湖泊（Buser and Theobald，1998；Glen et al，2003；Tixier et al，2003；Blair et al，2013；Fergusonet al，2013；Zhu et al，2013）、海洋（Weigel et al，2002；Del Rosario et al，2014）、地下水（Barnes et al，2008）、城市污水（Carmona et al，2014）、饮用水（Carmona et al，2014）、食品（Wu et al，2012；Baron et al，2014）等样品中被检出。由于其本身的特殊性质，它们在环境中的残留及潜在风险引起了人们越来越多的忧虑。

传统的 PPCPs 的检测方法有微生物检测法，分光光度检测法、气相色谱、液相色谱、电泳分析法等。自 20 世纪 90 年代以来，随着新型接口技术，如电喷雾离子化（ESI）、大气压化学电离（APCI）、大气压光电离（APPI）等的成熟，液相色谱 - 质谱（HPLC-MS）技术在 PPCPs 检测研究中得到了广泛的应用（Tixier et al，2003；Barnes et al，2008；Blair et al，2013；Zhu et al，2013；Carmona et al，2014）。高分辨率或串联质谱（MS-MS）可提供目标物质结构的详细信息，具有高选择性和高灵敏性，非常适用于环境样品中痕量药物的分析检测，已成为环境样品中 PPCPs 化合物的分析检测的一个强有力的工具。据文献报道，我国水环境 PPCPs 化合物在环境水样中检出的质量浓度通常是 ng·L^{-1} 到 μg·L^{-1} 级水平（Yang et al，2011；王丹等，2014），目前报道出来的环境中检测到的 PPCPs 的种类多达 160 多种，因此，开展快速、灵敏、可靠的 PPCPs 化合物集成定量检测方法尤为重要。不同于以往文献中报道的药物检测方法多数仅适用于同类药物，本文选取包括抗生素类（磺胺甲恶唑、磺胺二甲嘧啶、盐酸四环素、盐酸土霉素），消炎止痛类（扑热息痛、萘普生、三氯生、双氯芬酸钠、三氯卡班）以及血脂调节剂（吉非罗平）等在内的共 10 种药物与个人护理用品为目标物，基于高效液相色谱串联质谱联用技术，采用固相萃取技术对样品进行前处理，建立了城市废水中 PPCPs 的分析方法，并以西安一城市废水为代表，对这一新方法进行了考察。

1　实验部分

1.1　仪器与试剂

PPCPs 标准品（扑热息痛（ACT）、萘普生（NPX）、吉非罗平（GF）、磺胺甲恶唑（SMX）、磺胺二甲嘧啶（SMT）、三氯生（TCS）、双氯芬酸钠（DF）、三氯卡班（TCC）、盐酸四环素（TC）、盐酸土霉素（OTC（购自 Dr. Ehrenstorfer 公司（德国）；罗红霉素 -d7（RTM-d7）购自于 terc 公司。甲醇、甲酸为 HPLC 级，其余药品或试剂均为分析纯。

1.2　样品前处理

用 47 mm 的玻璃纤维滤纸（GF/F，Whatman）过滤水样（400 mL），过滤后的水样，加入 200 μL 内标混合液，调节 pH=7.0。固相萃取时，先用 3×5 mL 高纯水通过 SPE 小柱（Polyseryl HLB，6 mL/500 mg，CNW）进行活化和平衡，而后将含有内标的过滤液以 5—10 mL·min^{-1} 的流速通过 SPE 小柱。接着将 5 mL 高纯水通过 SPE 小柱，以清洗小柱，并继续抽真空 30 min，除去水分。再以 1 mL·min^{-1} 的流速用甲醇洗脱小柱，洗脱液收集于 10 mL 具塞玻璃刻度离心管中。最后以高纯氮吹扫洗脱液（水浴温度 35℃）至刚好吹干，移入自动进样样品瓶并用甲醇定容至 0.5 mL，待色谱分析（流程见图 1）。

2　结果与讨论

2.1　HPLC-MS/MS 条件优化

根据目标化合物的理化性质，选择 ESI 为离子源。首先配制 1 μg·L^{-1} 的 10 种 PPCPs 化合物标准液，之后根据化合物性质，结合文献报道，选取 ESI^{+} 或者 ESI^{-} 模式，以流动注射分析（Flow Injection Analysis，FIA）的方法分别确定各目标抗生素的特征离子对。利用获取的全部特征离子及离子间的丰度比进行定性分析，以丰度最高的特征离子响应与浓度的关系进行定量分析。10 种目标化合物优化的质谱参数见表 1。

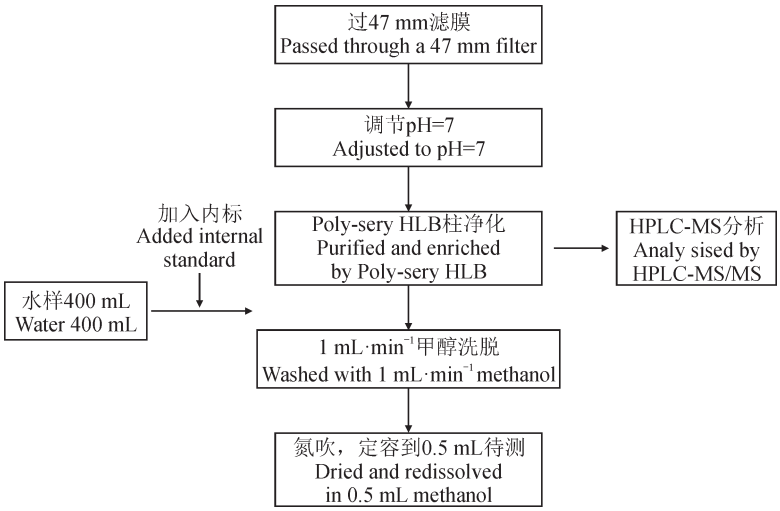

图 1 水中 PPCPs 分析方法流程
Fig.1 Flow chart for determining the PPCPs in water

表 1 10 种目标化合物的详细信息及 HPLC-MS/MS 参数
Tab.1 Details of the 10 target compounds and operating parameters of HPLC-MS/MS

化合物 Coumpound	化学物质登录号 CAS	分子式 Molecular formula	分子量 Molecular weight	特征离子对 Transitions (*m/z*)	离子源 ESI mode
扑热息痛（ACT）	103-90-2	$C_8H_9NO_2$	151.16	152/110	ESI^+
萘普生（NPX）	22204-53-1	$C_{14}H_{14}O_3$	230.26	229/185	ESI^-
磺胺甲恶唑（SMX）	723-46-6	$C_{10}H_{11}N_3O_3S$	253.27	254/156	ESI^+
磺胺二甲嘧啶（SMT）	57-68-1	$C_{12}H_{14}N_4O_2S$	278.33	279/186	ESI^+
三氯生（TCS）	3380-34-5	$C_{12}H_7Cl_3O_2$	289.5	287/35	ESI^-
双氯芬酸钠（DF）	15307-79-6	$C_{14}H_{10}C_{12}NNaO_2$	318.13	294/250	ESI^-
三氯卡班（TCC）	101-20-2	$C_{13}H_9C_{l3}N_2O$	315.58	313/160	ESI^-
盐酸四环素（TC）	64-75-5	$C_{22}H_{24}N_2O_8HCl$	444.43	445/410	ESI^+
盐酸土霉素 (OTC)	2058-46-0	$C_{22}H_{24}N_2O_9·HCl$	496.89	461/426	ESI^+
吉非罗平（GF）	25812-30-0	$C_{15}H_{22}O_3$	250.33	249/121	ESI^-

2.2 HPLC-MS/MS 测试分析

采用高效液相色谱 - 串联质谱（岛津 HPLC-ESI-MS/MS 8030）对样品进行测定。流动相 A 为甲醇溶液，B 为含 0.01% 甲酸的高纯水溶液，流速为 0.35 mL·min^{-1}。梯度洗脱顺序为：0—10 min，20% A 和 80% B；10—35 min，90% A 和 10% B。柱温 40℃。进样量 10 μL。10 种 PPCPs 化合物的总离子流色谱图见图 2。

配制 1—250 μg·L^{-1} 6个不同浓度的标准溶液，以目标化合物的浓度为横坐标，不同浓度目标化合物的峰面积为纵坐标，做线性回归分析。10 种 PPCPs 化合物的工作曲线、相关系数和检出限（3 倍信噪比）见表 2。结果表明：目标化合物在两个数量级浓度范围内具有很好的线性关系。与文献报道相比，PPCPs 化合物的线性范围以及检出限均在合理范围内，考虑到目前我国水环境 PPCPs 化合物在环境水样中检出的质量浓度通常是 ng·L^{-1} 到 μg·L^{-1} 级水平，本方法可实际应用于环境中水样的检测。

以高纯水为介质，分别做 3 组平行试验，利用回收率和空白实验保证实验的准确性。取 400 mL 高纯水样，定量加入 500 μL 浓度为 25 μg·L^{-1} 的 PPCPs 混标溶液和 200 μL 内标溶液，按照图 1 所示流程对样品进行前处理和仪器分析。结果表明：纯水中的平均加标回收率为 40.8%—104.5%，相对标准偏差为 5.0%—25.5%（*n*=3）。

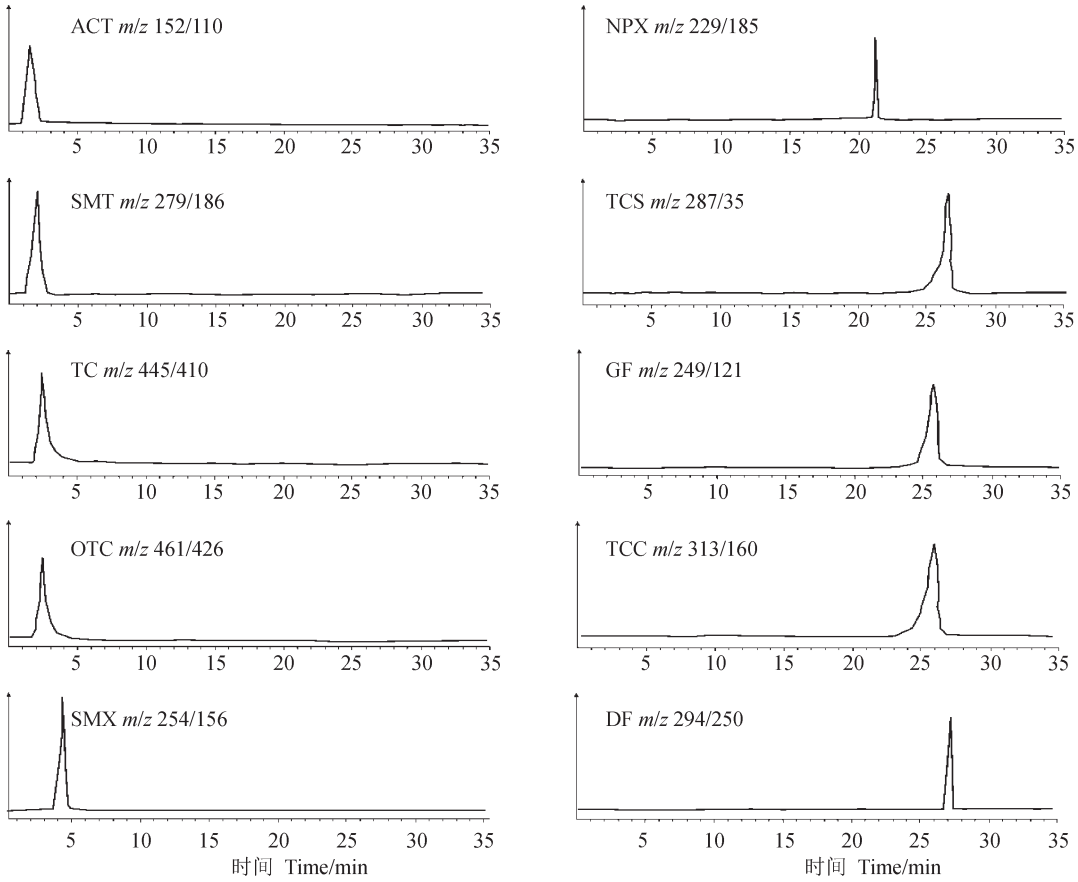

图 2　10 种 PPCPs 的总离子流色谱

Fig.2　Total ion chromatograms of the 10 PPCPs

表 2　10 种 PPCPs 化合物的线性回归方程、相关系数及检出限

Tab.2　Regression equations, correlation coefficients (r^2) and method detection limits of the 10 PPCPs

化合物 Coumpound	回归方程 Regression equation	相关系数 r^2	线性范围 Linear range/(μg·L^{-1})	方法检测限 MDL/(μg·L^{-1})
扑热息痛（ACT）	$y=241391x-152568$	0.999	1—250	0.20
萘普生（NPX）	$y=19995x+21867$	0.993	1—250	0.50
磺胺甲恶唑（SMX）	$y=154991x-72857$	0.994	1—100	0.01
磺胺二甲嘧啶（SMT）	$y=436490x+121320$	0.990	1—100	0.01
三氯生（TCS）	$y=12734x-17251$	0.998	2.5—100	1.25
双氯芬酸钠（DF）	$y=217247x+560471$	0.999	1—250	0.01
三氯卡班（TCC）	$y=262001x-313821$	0.999	2.5—100	0.20
盐酸四环素（TC）	$y=6357.9x+291209$	0.990	2.5—250	0.20
盐酸土霉素 (OTC)	$y=4953.1x+185900$	0.991	2.5—250	0.20
吉非罗平（GF）	$y=183378x-198447$	0.998	25—250	0.50

2.3　实际水样分析

采集西安市区浐河表层水，采集时间为 2015 年 10 月。按照图 1 所示流程进行样品处理和 HPLC-MS/MS 分析，结果表明：10 种 PPCPs 样品中，共检测到 4 种 PPCPs，分别为 ACT（1.4 ng·L^{-1}），NPX（1.4 ng·L^{-1}），TCC（4.8 ng·L^{-1}）以及GF（15.0 ng·L^{-1}），其他 6 种 PPCPs 未检测到或者浓度低于方法检测限。

3　结论

结合文献报道以及实验室实测结果，本文建

立了利用 HPLC-MS/MS 分析水样中 10 种痕量 PPCPs 化合物的检测分析方法，并利用该方法对西安浐河水样进行了 PPCPs 化合物的检测分析。由于环境中的 PPCPs 化合物种类繁多，各类化合物的性质差别较大，对样品前处理以及仪器检测方法均提出了更多的挑战，仅用一种方法无法实现所有 PPCPs 化合物的同时检测分析。因此，今后将进一步优化前处理条件，建立更多类别的 PPCPs 化合物检测分析方法，并进一步加强注重迁移转化规律及环境风险方面的研究。

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