A Fully Automated Multiple Injection On-line SPE-LC-MS/MS Method for Analysis of Trace Level Drug Residues in Water

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1. Introduction

With the development of new sensitive analytical techniques, numerous historically unknown environmental contaminants, including pharmaceutical compounds, endocrine disrupting chemicals (EDC) and algal toxins etc, have been discovered in types of water. LC-MS has been used to detect and quantify these chemical residues in water samples. Sample pre-concentration is a critical step for analysis of the trace level chemical residues. On-line solid phase extraction (on-line SPE) method with flow injection of a large volume of sample has been adopted and coupled to LC-MS system to enhance sensitivity and throughput. Here we report a new on-line SPE-LC-MS/MS method with multiple injection function using an auto-sampler, which enhances not only the throughput, but also the trapping efficiency and recovery of the analysis.

2. Experimental

A system established for this study is consisted of a typical configuration of LC/MS/MS based on Shimadzu LCMS-8030 and an automated on-line SPE kit with an auto-sampler (Figure 1). The system is capable to perform consecutive multiple injections to the on-line SPE column for sample extraction and concentration before switching to the analysis flow line for LC/MS/MS analysis. Fifteen pharmaceutical compounds reported in literature were investigated using this multiple injection on-line SPE LC-MS/MS system to evaluate trapping efficiency, recovery and sensitivity aiming at analysis of trace level compounds in water samples. Two MRM transitions were established for each compound and the higher intensity one was used for quantitation (Table 1). A MAYI-ODS column (4.6 mmID x 10 mmL) from Shimadzu was used as on-line SPE column. A Shim-Pack ODS column (2.1 mmID x 150 mmL, 5 um) was used for analysis of compounds eluted from the SPE column connected through a programmable switching valve.

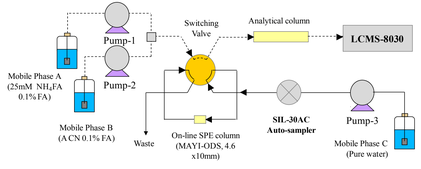

Figure 1. Schematic diagram of multi-injection on-line SPE LC-MS/MS system

3. Results and Discussion

3.1 MRM quantitative method

A quantitative MRM analysis method was established first on the LCMS-8030. Figure 2 shows the MRM chromatograms of the 15 pharmaceutical compounds. The method performance for quantitative analysis was evaluated. According to the detection sensitivity, the 15 compounds were classified into three groups: group 1 including 9 compounds (refer to Fig. 4) with LODs at about 0.2~1 ng/mL (S/N >3) and group 2 including five compounds with LODs at 0.5 ~ 5 ng/mL (S/N>3). Group 3 has only one compound, hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$). The detection limit of hydrochlorothiazide in negative MRM mode (296 -> 250) was 30 ng/mL (S/N >3). Calibration

Table 1. MRM and conditions of fifteen pharmaceutical compounds on LCMS-8030

Compound	RT	MRM	PreQ1	CE	PreQ3	Event & polarity
Salbutamol	1.912	240.20>148.10	-13	-20	-11	1:MRM(+)
Hydrochlorothiazide	2.254	296.00>205.05	21	25	22	11:MRM(-)
1,7-Dimethylxanthine	3.023	181.10>124.10	-14	-20	-26	2:MRM(+)
Sulfamethoxazole	4.011	254.10>92.15	-10	-30	-18	3:MRM(+)
Thiabendazole Pestanal	4.872	202.10>65.10	-14	-50	-25	4:MRM(+)
Furosemide	5.141	329.00>285.15	16	10	18	12:MRM(-)
Diphenhydramine	5.327	256.20>167.10	-26	-15	-12	5:MRM(+)
Ketoprofen	6.274	255.10>77.05	-13	-50	-27	6:MRM(+)
2-(p-Chlorophenoxy)-2-Methylpropionic acid	6.282	213.00>126.85	14	15	24	13:MRM(-)
Gemfibrozil	6.458	231.10>185.10	-11	-15	-14	8:MRM(+)
Warfarin Pestanal	6.523	309.10>163.05	-12	-15	-12	7:MRM(+)
Diclofenac Sodium	6.999	294.10>249.95	14	10	29	14:MRM(-)
3,4,4'-Trichlorocarbanilide	7.439	313.00>160.00	23	15	30	15:MRM(-)
Naproxen	7.486	251.10>83.15	-24	-15	-17	10:MRM(+)
Miconazole Nitrate salt	7.558	417.10>158.95	-13	-30	-12	9:MRM(+)

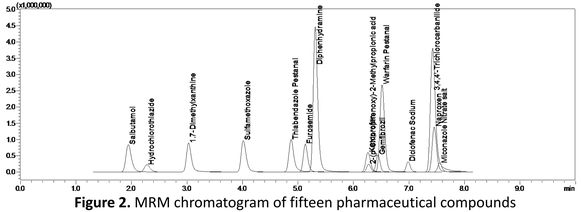

Figure 2. MRM chromatogram of fifteen pharmaceutical compounds

curves of these compounds are shown in Figure 3.

3.2 Recovery of on-line SPE method

Mixed standards were spiked in Millipore water and the spiked water samples were used to evaluate the recovery of on-line SPE method. First, samples of relatively high concentrations were injected into the on-line SPE-LC-MS/MS by single injection method. The recoveries are shown in Figure 4. Because the spiked concentrations of different compounds prepared were different according to their detection sensitivity, the recovery curves are shown in three groups, group 1, 2 and 3. Group 1 includes nine compounds, which exhibited higher detection sensitivity by MRM method. It can be seen that the recoveries of most compounds of this group were at 70%~120% for loading amount of 1.1ng ~ 5.5 ng on-column. However, the recovery of Salbutamol was lower than 20%. The recovery of Ketoprofen was higher than 100% always.

Group 2 includes five compounds and their MRM sensitivity was about 5-10 times lower than the compounds in group 1. The recovery of these compounds deceased with the on-column loading amount. The recovery of Miconazole was below 30%. It was found that hydrochlorothiazide, the only compound in group 3, could not be extracted by the MAYI-ODS column used. We also tested longer MAYI-ODS (4.6 x 30 mm) and Oasis HLB on-line SPE cartridge (20 mmLx2.1 mm i.d) and observed the same phenomenon, i.e., hydrochlorothiazide not being extracted at all.

3.3 Recovery of multiple injection on-line SPE method

In order to load and concentrate from a large volume of water sample, flow injection

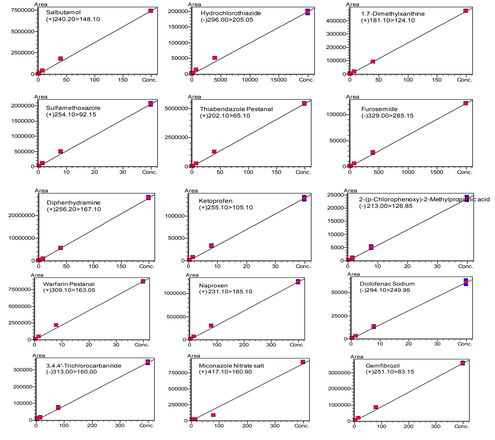

Figure 3. Calibration curves of fifteen pharmaceutical compounds by MRM method

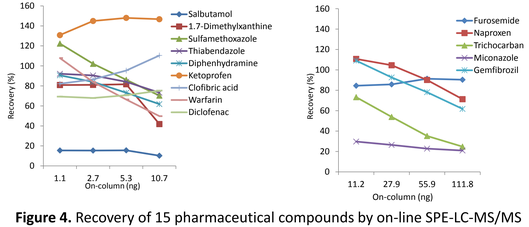

Figure 4. Recovery of 15 pharmaceutical compounds by on-line SPE-LC-MS/MS (Left: group 1; Right: group 2)

method has been used widely in on-line SPE LC-MS/MS analysis. The advantage of flow injection is no limit in extraction volume of water sample. However, flow injection on-line SPE operation may face problem such as cross contamination and tedious washing procedure of pump, flow line and valves etc. An alternative way is to use auto-sampler inject water samples multiple times to achieve a large volume of sample introduction to SPE column. In this study, we investigated multiple injection method using an analytical scale auto-sampler (50 uL sample loop). A preparative LC auto-sampler, which is capable of injection of 5 mL sample each time, is used for enlarged injection volumes in multi-injection mode (data not shown in this report).

Five to fifty consecutive injections with each injection volume of 50 uL were carried out for low concentration spiked water samples. The loading amounts onto the SPE column were kept same in order to investigate the effect of injection number on the recovery of the compounds. The details of the experiments are shown in Table 2.

Table 2. Details of multi-injection on-line SPE experiments (* Group 1 compounds)

Sample	Conc. (pg/uL)*	Vol per inj (uL)	No of injection	Total Vol (uL)	On-column amount (pg)
M10ppb	14.93	50	5	250	3733
M5ppb	7.47	50	10	500	3733
M2.5ppb	3.73	50	20	1000	3733
M1ppb	1.49	50	50	2500	3733

The recovery results of above multiple injection experiments for on-line SPE are shown in Figure 5. It can be seen that the recoveries of most compounds of group 1 and 2 increased or remained at same levels with increasing the number of injection. It is worth to note that each injection and SPE trapping process took 2 min, which means that the total sample loading time for a 50 multi-injection experiment lasting for 100 min during which period water was kept flowing through the SPE column at 2 ml/min. This result indicates clearly that multiple injections did not cause any loss of compounds or decrease of recoveries. However, compared to the results shown in Figure 4, the recoveries of 1,7-dimethylxanthine and sulfamethoxazole were significantly lower than that by single injection with higher concentration samples. This is believed to be related to the concentration effect. In general, the recovery of SPE extraction decreases with decrease of concentration at trace levels. It is interested to note that, for instance, the recovery of sulfamethoxazole decreased from 120% to 70% when sample loading amount increased from 1.1 ng to 10.7 ng in single injection experiment (Figure 4). While, its recovery increased from 5% to near 60% for same sample loading amount (3.7 ng), but injection number increased from 5 to 50. This result suggests that for trace level sample, multiple injection method can enhance the recovery.

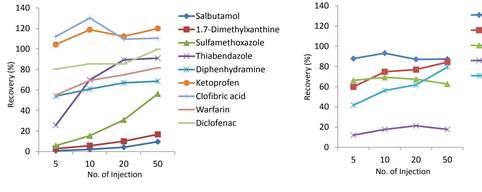

Figure 5. Recovery of 15 pharmaceutical compounds by multi-injection on-line SPE LC-MS/MS (Left: group 1; Right: group 2)

4. Conclusions

The results of this study show that multi-injection method for on-line SPE LC-MS/MS analysis of trace level water samples does not cause any loss of trapped compounds. Enhanced recoveries were obtained by more consecutive injections of low concentration water samples compared to less number of injections of higher concentration water sample. This preliminary finding suggests that multi-injection method is potentially useful for on-line SPE LC-MS/MS method for analysis of trace level pharmaceutical compounds in water samples as well as other applications.