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Short communication

Sulfamethoxazole-imprinted polymer for selective determination of sulfamethoxazole in tablets

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Abstract

A sulfamethoxazole (SMO)-imprinted polymer (MIP) was prepared in acetonitrile using the mixture of acrylamide and 4-vinylpyridine as functional monomers. The molecular recognition properties of the polymer was evaluated in both acetonitrile and aqueous acetonitrile mobile phases. SMO contents in two kinds of tablets were determined satisfactorily using the MIP packed HPLC column with aqueous mobile phase.

Keywords: Pharmaceutical analysis; Molecular imprinting; Sulfamethoxazole; Organosulfur compounds

1. Introduction

The sulfonamide class of antibiotics is a diverse class of chemically related compounds. A number of them have found widespread use in animal husbandry and, to a lesser extend, in the treatment of human infections such as bronchitis and urinary tract infection. Widespread use of sulfonamide drugs in veterinary without proper withdrawal period led to accumulation of sulfonamides in meat, eggs and milk as well as in fish [1–4]. Because of the possible risk of resistance development in human, the legal concentration limits for sulfonamides were set to 100 μg/kg in edible animal tissue and 10 μg/l in milk [valid for the European Union (EU) and the USA]. The low concentration limits led to development of fast and sensitive method to screen foodstuffs for sulfonamide drugs. On the other hand, pharmaceutical and veterinary products containing sulfonamides are often used in conjunction with other compounds in order to increase their activities such as an expectorant and/or a pulmonary balsamic-antiseptic agent: bromhexine (BRO) and guaiacol (GUA). These compounds are called potentiators (Fig. 1). A number of methods utilizing HPLC with UV, fluorescence and electrochemical detectors have been used for the determination of these chemicals in various animal foodstuffs [5–8]. Gas chromatography–mass spectrometry (GC–MS) method was developed for detecting sulfonamide after derivatization [9–11]. In the last few years, HPLC–MS and HPLC–MS–MS [12–14], as well as capillary electrophoresis [15,16] became to be favored methods.

Most of these methods require an extensive clean-up step, with the extent of sample pretreatment being dependent upon the analytical goal as well as the selectivity and sensitivity of the detection system. Molecularly imprinted polymers (MIPs) are attractive materials that enable the selective extraction of small molecules from complex mixtures. MIPs show antibody-like affinities toward the templates and can, therefore, be used for highly selective recognition and analysis of template chemicals in complex matrix. However, the majority of MIP-based methods are in aprotic and low polar organic solvents, often the one used in the polymerization process. In such systems, specific hydrogen bonds are stabilized and non-specific hydrophobic interactions are suppressed. Due to a common lack of selectivity in aqueous media, the successful application of MIPs to the selective recognition and thus analysis of analytes from water containing samples is often not possible. We have reported a sulfamethoxazole (SMO)-imprinted polymer, using the mixture of acrylamide and 4-vinylpyridine as functional monomers, which can effectively recognize its template in aqueous media [17]. Present work is to use this polymer for selective analysis of SMO in pharmaceutical tablets.

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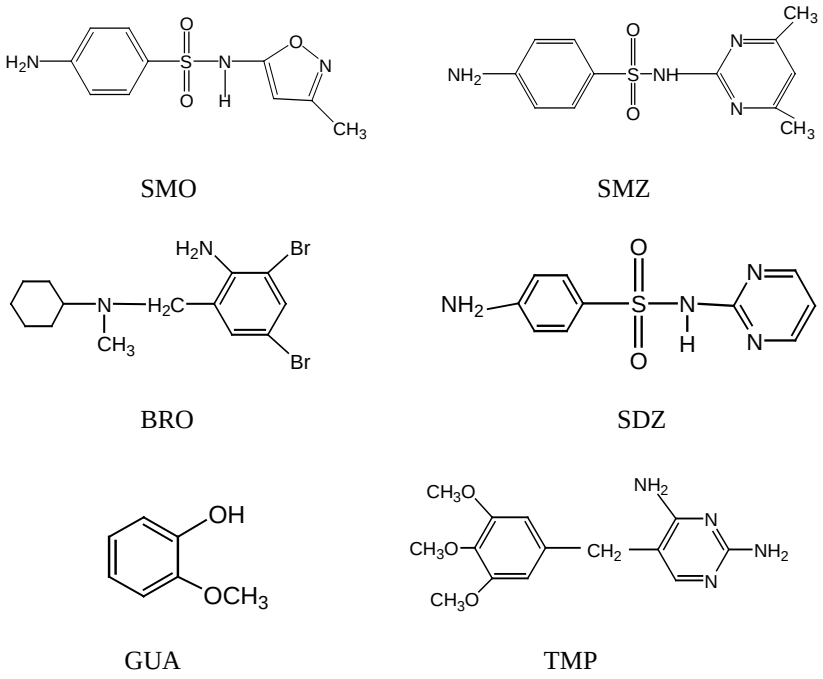

Fig. 1. The structures of sulfonamides and potentiators.

2. Experimental

2.1. Materials

Sulfamethoxazole (SMO), sulfamethazine (SMZ), sulfadiazine (SDZ), potassium salt of guaiacol (GUA) and bromhexine hydrochloride (BRO) are Sigma products. Sulphonamide tablet containing SMO, SDZ and trimethoprim (TMP) (sample 1) is a product of Beijing Shuanghe Pharmaceutical SMO tablet (sample 2) is a product of Beijing Shuguang Pharmaceutical ethylene glycol dimethacrylate (EGDMA) was purchased from Tokyo Kasei Kogyo (Japan). Acrylamide (AA) was from Sangon (Shanghai, China). 4-Vinylpyridine (4-Vpy) was from Merck (Darmstadt, Germany). Azo-bis-isobutyronitrile (AIBN) was obtained from Nankai Chemical Factory (Tianjing, China). EGDMA and 4-Vpy were distilled under vacuum to remove stabilizers before use. AIBN was re-crystallized from ethanol before use.

2.2. Preparation of imprinted sorbent

The preparation of sorbents was carried out according to the procedure described in [17]. SMO (1 mmol), AA (2 mmol), 4-Vpy (2 mmol) and EGDMA (20 mmol) were dissolved in 12 ml acetonitrile. Then, 48 mg of AIBN was added, the mixture was transferred to a borosilicate glass test tube and it was purged with nitrogen for 10 min. The tube was sealed under vacuum at a liquid nitrogen temperature. The polymerization was initiated at 60 °C and the reaction was allowed to continue for 24 h at the same temperature. Non-imprinted polymer was produced under exactly the same conditions in the absence of template. Bulk polymers were manually ground and sieved to collect 30–54 μm polymer particles. The fine particles were removed by repeated sedimentation from acetone.

2.3. Chromatographic evaluation of imprinted sorbent

Chromatography was carried out using a HPLC1100 system from Hewlett-Packard Company (USA) with a UV detector. The polymer particle was manually packed into a 50 mm × 4.6 mm stainless steel column. The column was washed on-line with methanol–acetic acid (9:1, v/v) to remove the template until a stable baseline was achieved. Unless specified, all HPLC experiments for polymer evaluation were performed at room temperature by injecting a 10 μl volume of standard sample (0.4 mM). The column was run at a flow rate of 1 ml min^{-1} using different contents of water in acetonitrile as mobile phase. The UV detector was set at 269, 277 and 249 nm for sulfonamides, GUA and BRO, respectively. Acetone was used as the void marker. The capacity factor (k') was calculated according to standard chromatographic procedures [18].

2.4. Analysis of tablet pharmaceuticals

Ten tablets of sample 1 were weighed and ground to powder. The powder (30.0 mg) was dissolved in 50 ml of acetonitrile. After 20 min extraction in a sonicator the suspension solution was filtered through a 0.45 μm nylon membrane and washed with 30 ml of acetonitrile. The filtrate was brought

Table 1
Retention factors of SMO and potentiators on columns packed with imprinted and non-imprinted polymers in different mobile phases

Polymer	Mobile phase	k'_{SMO}	k'_{SMZ}	k'_{SDZ}	k'_{GUA}	k'_{BRO}
SMO-imprinted	MeCN	1.0	0.53	0.71	0.24	0.27
Non-imprinted	MeCN	0.27	0.24	0.30	0.18	0.21
SMO-imprinted	MeCN–water (2:8)	11	2.8	3.0	3.5	1.0
Non-imprinted	MeCN–water (2:8)	4.6	2.0	2.1	3.2	1.0

to 100 ml with acetonitrile. Sample 2 was prepared in the similar way with 15 mg power. For analysis, 10 μl of the sample prepared was injected and eluted with MeCN–water (2:8, v/v) with UV detection at 269 nm.

3. Results and discussion

3.1. Selectivity of SMO-imprinted polymers

Chromatographic retention behavior of sulfonamides and potentiators on the polymer was tested. Table 1 shows that in both organic and aqueous phases, the retention of SMO on imprinted polymer is the longest among all the compounds studied. Compared with others, the retention difference of SMO on imprinted and non-imprinted polymer is also the biggest. These are indications of the imprinting effect of SMO. The retention time of SMO on the imprinted polymer varied with the composition of mobile-phase. In aqueous phase, the retention difference of SMO is much larger than it in organic phase and SMO can be completely separated from the others in the mixture. A possible explanation for the uncommon high retention of SMO in aqueous solution is that SMO itself share both hydrophilic and hydrophobic characters. For MIP preparation, the main interaction is hydrogen bonding and monomer 4-vinylpyridine may play a more important role than acrylamide do. During the recognition step in aqueous solution the hydrogen bonding should be highly suppressed due to strong hydrogen bonding properties of water. However, the synergetic effect of cavity restriction and hydrophobic interaction might contribute to the selective and even stronger binding of SMO on the MIP. Here acrylamide as well as the crosslinker might be involved in hydrophobic interaction with SMO.

3.2. Determination of SMO in tablet pharmaceuticals

The standard calibration curve was drawn with 1.0–10 nmol (0.25–2.5 μg) of SMO prepared in acetonitrile. The linear equation was $A = 737C - 395$ ($r = 0.9968$), in which A was the peak area and C was the amount of SMO (nmol). The R.S.D. is 2.5% for determination of 1 μg of SMO (10 replicates). The chromatogram of sample 1 was shown in Fig. 2. The amounts found were based on standard calibration curve and recoveries were obtained by standard addition method. Clearly, the amount of SMO in each tablet is in good agreement with that claimed (Table 2). The HPLC column packed with the polymer can be continuously used for at least a month without evident changing in performance.

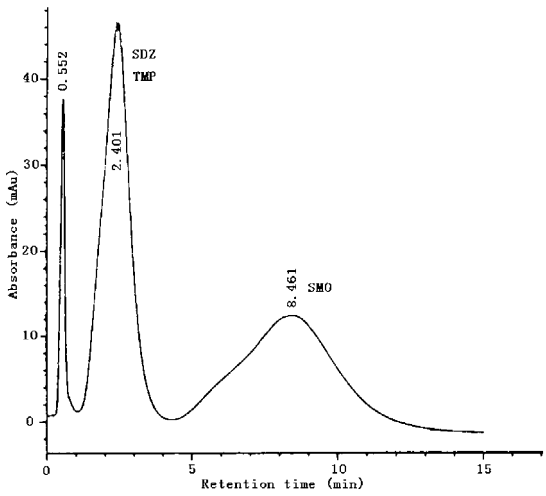

Fig. 2. Chromatogram of sample 1. A 10 μl solution was injected, mobile phase: acetonitrile–water (2:8, v/v).

Table 2
The results of sample analysis (mg per tablet)

Commercial	Claimed (mg)	Mean found (mg) ± S.D. ($n = 3$)	Recovery (%)
Sample 1	200	205 ± 6	97.1
Sample 2	400	409 ± 9	98.5

4. Conclusions

A HPLC method based on SMO-imprinted polymer with high selectivity for SMO in aqueous mobile phase was successfully used for direct determination of SMO in tablet pharmaceuticals.

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