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Removal of potentioally genotoxic impurity from fluroxamine maleate crude drug by molecularly imprinted polymer

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Abstract−The present study describes the synthesis and preliminary testing of molecularly imprinted polymers (MIPs) as scavenger resins for removal of the genotoxic impurity (GTI) (2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl) phenyl] pentylidene] amino] oxy] ethyl] amino] butanedioic acid from active pharmaceutical ingredients (API). To compare the performance of this polymer, a control polymer or non-imprinted polymer (NIP) was prepared under the same conditions without the use of template molecule. The synthesized polymers were characterized by FT-IR spectroscopy. The results of the selectivity of the molecularly imprinted polymer for absorption GTI impurity through adsorption experiments reviews were compared with the adsorption of impurity by NIP. Various parameters were optimized, such as time, pH, type of eluent for elution of impurity from polymer, concentration of sample and saturation of polymer. The proposed method was applied for removal of this genotoxic impurity from Fluvoxamine maleate tablet.

Keywords: Molecularly Imprinted Polymer, Genotoxic Impurities, Fluvoxamine Maleate, Solid Phase Extraction

INTRODUCTION

Fluvoxamine is an antidepressant which functions as a selective serotonin reuptake inhibitor (SSRI). It is used for the treatment of major depressive disorder (MDD), obsessive compulsive disorder (OCD) [1], and anxiety disorders such as panic disorder and post-traumatic stress disorder (PTSD) [2]. Fluvoxamine CR (controlled release) is approved to treat social anxiety disorder [3]. It is one of only two SSRIs (along with alaproclate) to have a monocyclic structure [4,5].

(2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl] pentylidene] amino] oxy]ethyl] amino] butanedioic acid is one of the effective impurities in the drug fluvoxamine maleate.

Based on the British Pharmacopoeia Commission recommendation, the peak of this impurity in HPLC with UV-Vis detector should not be more than three-times the area of the principal peak in the chromatogram obtained by 2 mg L^{-1} of Fluvoxamine maleate (0.3 per cent) [6].

The need for efficient methods for sample preconcentration and clean up in medical, food and environmental analyses is constantly increasing. The advantages of SPE over liquid-liquid extraction (LLE) are that it is faster and more reproducible, cleaner extracts are obtained, emulsion formation is not an issue, solvent consumption is minimized and smaller sample sizes are required. Moreover, SPE can be easily incorporated into automated analytical procedures [7,8].

Polymeric network materials capable of recognizing target molecules by molecular imprinting technique are available. This is a process where functional and cross-linking monomers are copolymerized in the presence of a target analyte (the imprint molecule), which acts as a molecular template. The functional monomers initially form a complex with the imprint molecule, and following polymerization, their functional groups are held in position by the highly cross-linked polymeric network. Subsequent removal of the imprint molecule reveals binding sites that are complementary in size and shape to the analyte. In this way, a molecular memory is introduced into the polymer, which is now capable of selectively rebinding the analyte [9-12].

The need for separation of specific compounds from complex mixtures, industrial or biological, has led to an increase in the synthesis and use of molecularly imprinted polymers (MIPs), which in fact act as biomimetic materials. MIP-SPE has been used to extract the target analyte from blood plasma and serum [13,14], urine [15-17], bile, liver extract, [18] chewing gum, environmental water and sediment, [19-21] plant tissue [22,23], etc [24,25].

In the present work, molecularly imprinted polymer was synthesized using methacrylic acid (MAA) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as cross-linking agent and (2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl] pentylidene] amino] oxy] ethyl] amino] butanedioic acid as template.

This study was performed to ascertain the optimum conditions for maximum recovery of (2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl] pentylidene] amino] oxy] ethyl] amino] butanedioic acid from Fluvoxamine maleate hydrochloride using a molecularly imprinted polymer as solid phase extraction adsorbent, and the MISPE-eluate fractions were analyzed by spectrophotometry. Different experimental conditions, such as adsorption time, the type of eluting solvent, the effect of pH and concentration of sample have been investigated.

EXPERIMENTAL

1. Reagents

The required materials included, methacrylic acid (MAA), 2,2′-azobisisobutyronitrile (AIBN), ethylene glycol dimethacrylate (EGDMA),

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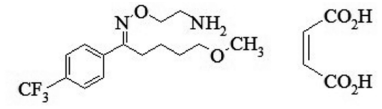
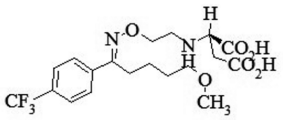


Fluvoxamine maleate



(2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl] pentylidene] amino] oxy] ethyl] amino] butanedioic acid

Fig. 1. The structure of Fluvoxamine maleate and its impurity.

chloroform, methanol, hydrochloric acid, sodium hydroxide, sulfuric acid, ethanol, acetic acid, potassium hydroxide, potassium dihydrogen phosphate, phosphoric acid and acetonitrile which, were obtained from Merck. Fluvoxamine maleate and (2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl) phenyl]pentylidene]amino]oxy]ethyl] amino]butanedioic acid were obtained from Sigma-Aldrich. All chemicals and reagents were of analytical grade and used without any further purification.

Buffer solution containing 1.1 g/*l* of potassium dihydrogen phosphate and 1.9 g/*l* of sodium pentanesulphonate in water, previously adjusted to pH 3.0 with phosphoric acid, was used. Mobile phase was prepared by mixing 370 volumes of acetonitrile and 630 volumes of a buffer solution.

For liquid chromatography, 50 mg of the substance was dissolved in the mobile phase and diluted to 25 mL with the mobile phase. It was diluted 1000 times before its use. The test solution was prepared immediately before use. Fig. 1 shows the structure of (2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl) phenyl] pentylidene] amino] oxy]ethyl] amino] butanedioic acid and Fluvoxamine maleate.

2. Apparatus

HPLC measurements were performed on Kenauer D-14163 (Berlin, Germany). The high-performance liquid chromatography (HPLC) system was composed of a D-14163 pump (Knauer Co., Germany), a reversed C18 column (4.6×150 mm i.d.; Knauer, Germany), a D-14163-detector (Knauer, Germany) set at 249 nm, and a reversed C18 guard column (4.6×15 mm; Knauer, Germany).

The flow rate was 1.2 mL min^{-1} at 25 ºC, and the injection volume was 20 µl. Quantification was carried out by the external standard method using a commercial standard sample of (2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl) phenyl] pentylidene] amino] oxy] ethyl] amino] butanedioic acid. Integration of the chromatograms was made with the Knauer EZChrom software package.

UV-VIS spectrophotometer (Lambda 25 Perkin Elmer, USA) was used for measuring impurities in standard solutions after contact with the polymer. Soxhlet extraction apparatus was used for removing the target molecule of the polymer network. A model 211 Hana pH-meter was used for the pH adjustments. The Fourier transform infrared (FTIR) spectra of template molecule ((2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl]pentylidene] amino] oxy]ethyl] amino] butanedioic acid), non-imprinted and molecularly imprinted polymers were obtained using a 6700 Thermo Nicolet FTIR spectrometer. IR spectra were recorded in the range 400-4,000 cm^{-1}.

3. Preparation of MIP and NIP

An MIP and a non-imprinted polymer (NIP) should be prepared in parallel and with identical compositions (except that template is to be omitted from the NIP). The procedure for the polymer synthesis was as follows:

The template ((2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl]pentylidene]amino]oxy]ethyl]amino]butanedioic acid--0.25 mmol or 0.54 g), functional monomer (MAA: 2 mmol or 0.17 mL), cross-linker (EGDMA: 10 mmol or 1.89 mL) and initiator (AIBN: 0.25 mmol or 0.042 g) in chloroform (100 mL)--were added to a three-necked round-bottom flask. The mixture was sparged with nitrogen for 10 minutes to remove dissolved oxygen, which can inhibit free radical polymerization. The polymerization was continued in a water bath at 60 ºC for 18 hours. After polymerization, a hard polymer monolith was obtained, which was crushed and ground into a fine powder with a mortar and pestle. Soxhlet extraction was performed to remove the template with 70/30 (V/V) methanol/acetic acid overnight. Then, the polymer was washed several times with pure methanol to remove the acetic acid and facilitate drying. The dried polymer was ready for testing. The method for preparation of NIP was exactly similar to the procedure for the synthesis of MIP with the exception that the template (imprint molecule) was omitted in the preparation of NIP.

Schematic illustration of the process of preparing the GTI-MIP is shown in Fig. 2.

4. Batch Procedure

In 100 mL polyethylene bottles were added, in sequence, buffer solution, the GTI solution and synthesized imprinted polymer with shaking at 25 ºC. At a preset time, an aliquot of the supernatant was separated and the GTI was determined by UV-Vis spectrophotometer at 249 nm. The adsorbed GTI was eluted with 0.2 M HCl in methanol and the desorbed GTI was measured with UV-Vis spectrophotometer. The phase distribution ratio (K_d) and adsorption capacity (Q) were calculated by using the following equations:

$$K_d = \frac{C_i - C_f}{C_f} \times \frac{V}{W} \quad (1)$$

$$Q = \frac{(C_i - C_f)V}{W} \quad (2)$$

where Q represents the adsorption capacity (µmol L^{-1}), C_i and C_f represent the initial and equilibrium concentration of metoprolol in the aqueous phase (µmol L^{-1}), W is the weight of the polymer (g)

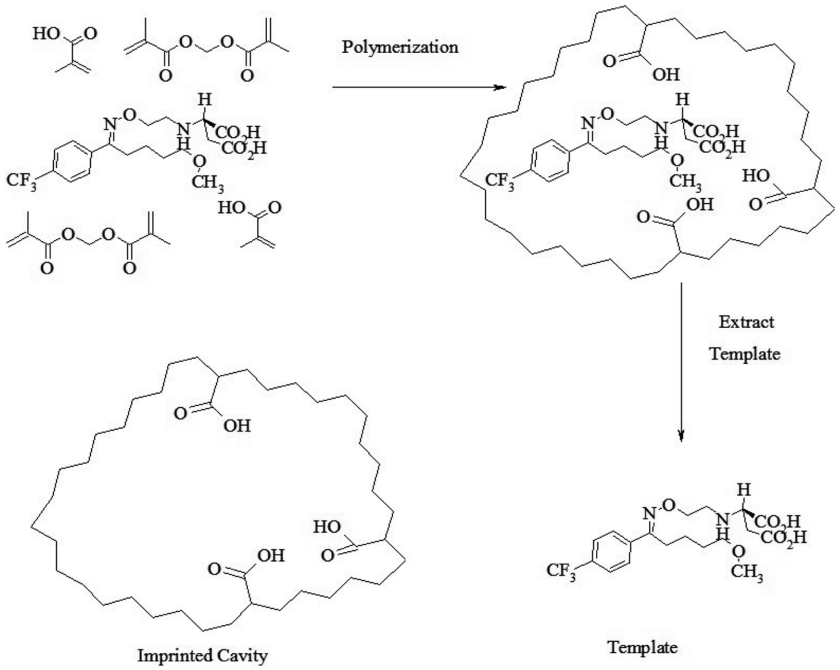


Fig. 2. Schematic illustration of the process of preparing the GTI-MIP.

and V is the volume of the aqueous phase (L). The percent extraction, E, was calculated using the following equation:

$$E = \frac{C_i - C_f}{C_i} \times 100 \quad (3)$$

RESULTS AND DISCUSSION

The aim of this work was to evaluate the feasibility of using an imprinted polymer as scavenger resins for the removal of the genotoxic impurity (GTI) (2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl]pentylidene]amino]oxy]ethyl]amino]butanedioic acid from active pharmaceutical ingredients (API).

1. FT-IR Spectra

Synthesized molecularly imprinted and control polymers were subjected to characterization by FT-IR. Both polymers have similar IR spectra, indicating a similarity in the backbone structure.

In the IR spectra, the absorptions due to carboxyl OH stretch (ca. 3,430 cm^{-1}), carbonyl group stretch (ca. 1,733 cm^{-1}), C-O stretch (ca. 1,260 cm^{-1}) and C-H vibrations (ca. 730, ca. 1,380, ca. 1,460, and ca. 2,941 cm^{-1}) were observed. Two important results that were also acquired from spectra include:

(1) The absorbances pertaining to GTI are not observed in the MIP spectrum. This difference proves that imprint molecule has been sufficiently leached from MIP in the Soxhlet extraction step.

(2) It is clear that the absorbances attributed to the C-H stretch of methylene group (ca. 2,959.15 cm^{-1}), carbonyl group stretch (ca. 1,733.0 cm^{-1}), C-O stretch (ca. 1,254.0 cm^{-1}) and C-H bend of CH_2 (ca. 1,460.0 cm^{-1}) for the molecularly imprinted polymer are relatively stronger than for the non-imprinted polymer. From this comparison, it was found that presence of the imprint molecule ((2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl] pentylidene] amino] oxy] ethyl] amino] butanedioic acid) caused incorporation of the ethylene glycol dimethacrylate in the preparation of polymer to be increased.

2. Optimization of Impurity Adsorption on Polymer

2-1. Effect of Time on Adsorption of Impurity

Six portions of standard or sample solutions (100 mL) containing GTI (0.05 mg) were transferred into 250 mL beakers. Then exactly 1 g of MIP adsorbent was added to each beaker, and the mixtures were shaken vigorously for 20, 30, 40, 50, 60 and 70 minutes to facilitate adsorption of the (2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl] pentylidene] amino] oxy] ethyl] amino] butanedioic acid onto the imprinted polymer particles. After the solutions were centrifuged, the amount of unadsorbed GTI in the filtrate solutions was directly determined by spectrophotometry. Fig. 3 shows that an equilibration time of about 50 minutes was required for 70% adsorption. The amount of GTI bound to the polymer was calculated by subtracting the amount of unadsorbed substrate from the initial amount of template.

2-2. Effect of Sample pH on Adsorption of Impurity

The effect of different pHs on impurity uptake was investigated using the batch procedure. Six portions of standard or sample solu-

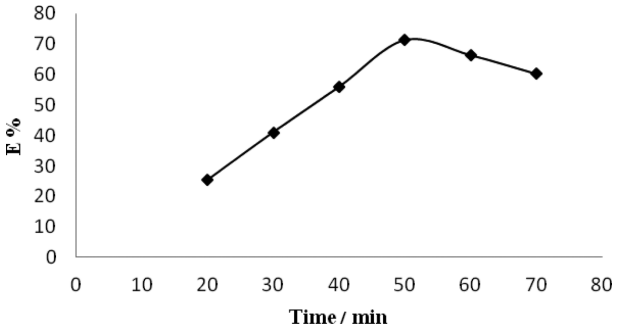


Fig. 3. Influence of adsorbing time on the extraction of impurity.

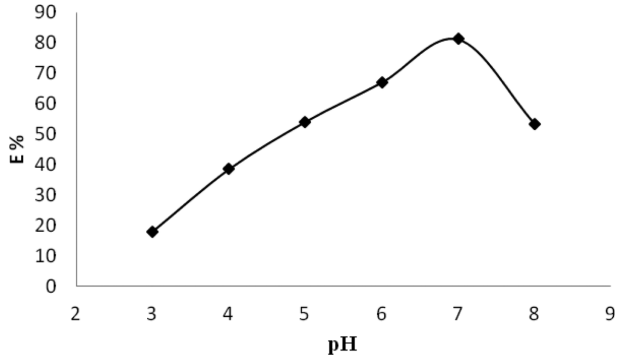


Fig. 4. Effect of pH on impurity uptake.

tions (100 mL) containing GTI (0.434 mg) were transferred into 250 mL beakers, and the pH value was adjusted in range 3-8 with 0.01 mol L^{-1}, HNO_3 or NaOH. Then exactly 1 g of MIP adsorbent was added to each beaker, and the mixtures were shaken vigorously for 50 minutes to facilitate adsorption of the GTI into the imprinted polymer particles. According to the results shown in Fig. 4, as the pH value increased the adsorption quantity of impurity increased and maximum adsorption was achieved at pH=7.

Thus, pH 7.0 was chosen for this experiment. The adsorption capacity of the polymer was decreased after pH 7.0.

3. Adsorption Capacity

Adsorption of impurity from sample solution was investigated in batch experiments. At this stage, the effect of sample concentration on the adsorption impurity was studied to obtain the best concentration for the sample solution. Solutions with 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} mmol L^{-1} concentrations of impurity were prepared, and the pH value was adjusted to 7.0 with 0.01 mol L^{-1}, HNO_3 or NaOH. Then exactly 1 g of MIP adsorbent was added to each beaker, and the mixtures were shaken vigorously for 50 minutes to facilitate adsorption of the GTI into the imprinted polymer particles. To reach saturation, the initial impurity concentrations were increased until the plateau values (adsorption capacity values) were obtained. The data are shown in Fig. 5. The average maximum adsorption capacity was 100 μmol g^{-1} for three replicate measurements.

4. Comparison of Impurity Adsorption on MIP and NIP

Two solutions were prepared at a concentration of 10^{-4} mol L^{-1} of impurity, and the pH value was adjusted to 7.0 with 0.01 mol L^{-1}, HNO_3 or NaOH. Then 1 g of MIP and NIP was added to the solutions. The mixtures were shaken vigorously for 50 minutes to facilitate adsorption of the GTI onto the polymers particles. Then, both the filtrate and the adsorption of the filter solution were measured with a spectrophotometer. The results in Table 1 show that MIP performed better adsorption than the NIP, which confirmed the accuracy of the molecular format.

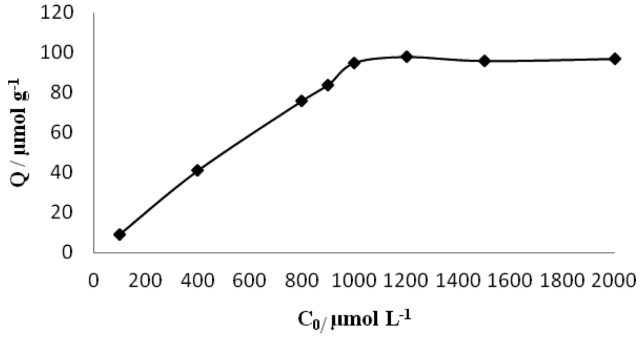


Fig. 5. The effect of GTI initial concentration on the adsorption quantity of synthesized polymer. Other conditions: 1 g of synthesized polymer, pH 7.0, shaking time 50 minutes, in a temperature of 25 °C.

Table 1. Comparison of MIP and NIP

Polymer type	Initial concentration/ μmol L^{-1}	Final concentration/ μmol L^{-1}	K_d	Extraction %
MIP	1000	58	1.62	94.2
NIP	1000	822	0.02	17.8

Table 2. Effect of type of eluent on extraction efficiency

Eluent	Recovery%
0.2 mol L^{-1} H_2SO_4	73.12
0.4 mol L^{-1} H_2SO_4	61.21
0.4 mol L^{-1} CH_3COOH	74.46
Methanol	52.14
Ethanol	37.89
0.2 mol L^{-1} KOH in methanol	7.34
0.2 mol L^{-1} H_2SO_4 in methanol	95.74

5. Efficient Eluent for Removal of Adsorbed Impurity

To choose a proper eluent for the retained GTI after the extraction of 0.1 mmol GTI from 100 mL of aqueous sample solution, the GTI was stripped with 5 mL of various concentrations of different organic and mineral acids. GTI was stripped with 5 mL of eluents. To choose the most efficient eluent, different organic solvents and various concentrations of different acids in organic solvents were tested. As shown in Table 2, acidic eluents were more effective for stripping of (2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl] pentylidene] amino] oxy] ethyl] amino] butanedioic acid from polymer.

Acidic eluent interacts, via hydrogen bonds, with the polymer to disrupt the interaction between GTI and polymer.

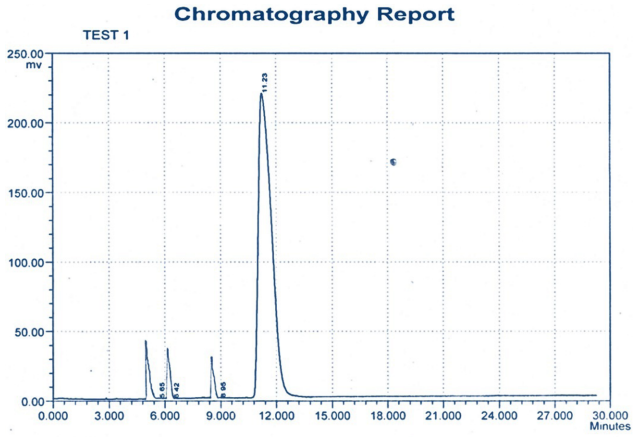
From the data given in Table 2, it is clear that 5 mL of 0.2 mol L^{-1} H_2SO_4 in methanol can strip the retained (2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl] pentylidene] amino] oxy] ethyl] amino] butanedioic acid almost quantitatively. Thus, this eluting solvent was chosen for further studies.

6. Purification Procedure

Ten g fluvoxamine maleate was recrystallized in the presence of MIP. A solution containing 10 g crude fluvoxamine maleate and 1 g of MIP in 30 mL deionized water was heated for 50 minutes at a temperature of 40 °C. Then, the MIP was filtered. Finally, fluvoxamine maleate gradually recrystallized, and fluvoxamine maleate was filtered, washed with cold water (5 °C) and dried at 50 °C.

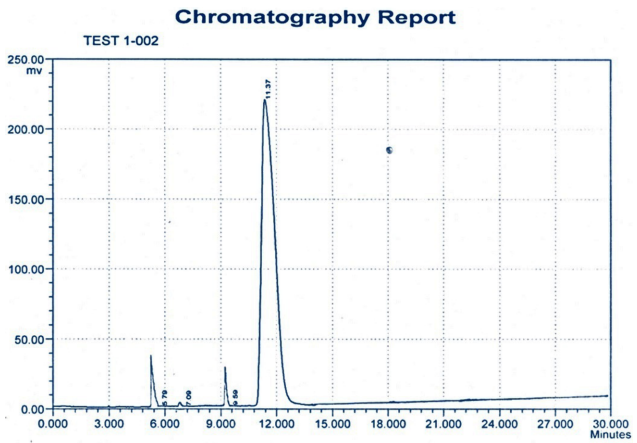
To evaluate the performance of synthesized MIP for absorption of impurity from the real sample solutions of active pharmaceutical ingredients, before and after purification were separately injected to HPLC column. The conditions for HPLC separation were mentioned in the apparatus section.

The obtained results are shown in Figs. 6 and 7. The peak cor-



ID	Name	Retain.T	Area	Conc
1	IMP F	5.647	30435.3	0.284
2	IMP C	6.420	26288.4	0.245
3	IMP B	8.947	22587.9	0.210
4	FLUVOXAMINE	11.227	10654826.0	99.261
	Sum:		10734137.7	100.0000

Fig. 6. Chromatogram of fluvoxamine maleate tablet before of purification.



ID	Name	Retain.T	Area	Conc
1	IMP F	5.793	28387.2	0.265
2	IMP C	7.093	3089.6	0.029
3	IMP B	9.593	21163.3	0.197
4	FLUVOXAMINE	11.373	10664448.7	99.509
	Sum:		10717088.8	100.0000

Fig. 7. Chromatogram of fluvoxamine maleate tablet after of purification.

responding to the impurity c appeared after 7 minutes. The results showed that the peak intensity of impurity c is reduced after purification. In addition, it demonstrated the selectivity of the polymer to the target molecule rather than fluvoxamine maleate, and it proved the performance of molecularly imprinted polymer as scavenger resins for removal of the genotoxic impurity.

CONCLUSION

Novel molecularly imprinted polymers have been designed that can recognize (2RS)-2-[[2-[[[(1E)-5-methoxy-1-[4(trifluoromethyl)phenyl] pentylidene] amino] oxy] ethyl] amino] butanedioic acid as a pharmaceutical impurity. These MIPs have the potential to purify APIs as selective adsorbents due to high affinity binding sites. Rebinding tests indicate that high-affinity interactions are present between the binding sites of the hosts and the target impurity, which results in high efficiency purification. It is suitable for repeated use without considerable loss of adsorption capacity. The new MIPs that were developed answer to the industrial call for systems that provide a high and selective binding between impurity and adsorbent.

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