Trace determination of Tamoxifen in Cancer patients using optimized Solvent bar microextraction and HPLC-UV

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Background: Tamoxifen (Soltamox) is an antineoplastic agent and an estrogen receptor antagonist with the indications for breast cancer, with severe side effects such as hot flashes, vaginal discharge, etc. Dose monitoring is a necessity for optimum treatment, free of severe adverse effects.

Method: The solvent bar microextraction method (SBME) was used for preconcentration and microextraction coupled with High-performance liquid chromatography-ultraviolet (HPLC-UV) analysis of tamoxifen in this study.

Results: The limit of detection and limit of quantification were 13.3 and 40 μgL^{-1}, respectively. The linearity range was between 40 to 10000 μgL^{-1} with a correlation coefficient of 0.999. The enrichment factor was 169 and the relative standard deviation Within-day and Between day were 3.6 and 4.0, respectively.

Conclusion: The use of trend and sensitive SBME method coupled with HPLC-UV analysis for detection of tamoxifen at trace level was successful, offering a desirable preconcentration factor and cost-effective and green set up for determining the rate of elimination in cancer patients and purification of wastewater.

Keywords: Tamoxifen, solvent bar, HPLC-UV, chemometrics, real sample

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Introduction

Tamoxifen (Soltamox) is an antineoplastic agent and an estrogen receptor antagonist with the indications for breast cancer, ductal carcinoma, breast cancer prevention and gynecomastia [1]. Tamoxifen has a half-life of 7-14 hr, and a peak plasma time of 3-6 hr, which makes it metabolized by hepatic P450 enzyme CYP2C9, CYP2D6, and CYP3A4. The metabolites are N-desmethyl tamoxifen and endoxifen. The route of excretion is through feces (65%) and urine (9%). It can also be protein bound by 99%. In addition to this, the peak plasma concentration for tamoxifen is 40 ng/mL. The most frequent adverse effects include hot flashes (64%), vaginal discharge (30%), amenorrhea (16%), and menstrual changes (13%) [2].

According to the aforementioned detrimental effects, and almost narrow therapeutic window for tamoxifen, the dose monitoring becomes a necessity for optimum treatment, free of severe adverse effects [1]. Considering the pharmacogenetic factors, especially CYP2D6 [3], the therapeutic dose could be affected, depending on the drug which is coadministered. Considering all of these factors, it can be worthwhile to trace determine this agent, not only in biological fluids but also in wastewater.

It was previously shown that there are several approaches for determination of tamoxifen using HPLC [4], Micellar liquid chromatography [5], liquid-liquid extraction, high-performance liquid chromatography with fluorescence detection [4], micelle to solvent stacking in non-aqueous capillary electrophoresis [6], and other sensitive techniques [7]. These techniques are already accurate. However, in order to increase the sensitivity of these methods, a great preconcentration and sample treatment is required.

Sample treatment can be done using hollow fiber, which was proposed by Pederson Bejdgard [8] for the first time, and a modified version of this method, the solvent bar microextraction method (SBME) [9] was used for preconcentration and microextraction of tamoxifen in this study. SBME, when coupled with HPLC analysis, can offer trace determination of tamoxifen. To our best knowledge, tamoxifen has been never determined using the use of SBME-HPLC-UV method. This study aims to determine tamoxifen at trace levels under the optimized condition. In order to address the optimized condition and accurate statistical analysis, Minitab was used.

Experimental

Reagent and chemicals

Tamoxifen was donated by the ministry of health (Tehran, Iran), HPLC grade K_2HPO_4, methanol, and acetonitrile purchased from Merk (Darmstadt, Germany), sodium hydroxide, sodium chloride, and orthophosphoric acid all purchased from Sigma–Aldrich. Moreover, the ultrapure water purified with an apparatus Youngling by Millipore (Madrid, Spain) was used in the HPLC mobile phase and preparation of all sample solutions.

The PPQ3/2 polypropylene hollow fiber which was employed in the microextraction procedure were purchased from Membrana

(Wuppertal, Germany) with an inner diameter of 0.6 mm, a wall thickness of 200 µm, and a pore size of 0.2 µm.

The sample solutions were stirred using an MR Hei-standard magnetic stirrer from Heidolph(Schwabach, Germany). The sample solution and the acceptor phase pH were adjusted by the means of GPHR 1400 digital pH meter from Greisinger (Regenstauf, Germany).

Apparatus

The chromatographic conditions were adjusted using an HPLC system (Younglin, YL9100; Seoul, Korea) with a Quaternary9110 HPLC pump (Seoul, Korea) and a mixing valve with 4 channels of a 10 µL sample loop, a YL 9120 UV-Vis detector and a YL9101 vacuum degasser. Younglin Auto Chro 3000 software was used for analyzing and recording the chromatographic data.

A C_{18} column (150 mm × 4.6 mm, with a particle size of 5 µm) was employed for separation and analysis with a mobile phase of 10 mM phosphate buffer (pH:3) and acetonitrile (65:35), under isocratic condition. The flow rate of the HPLC-UV was adjusted ta 10 min with an injection volume of 10 µL and UV detection wavelength at 254 nm.

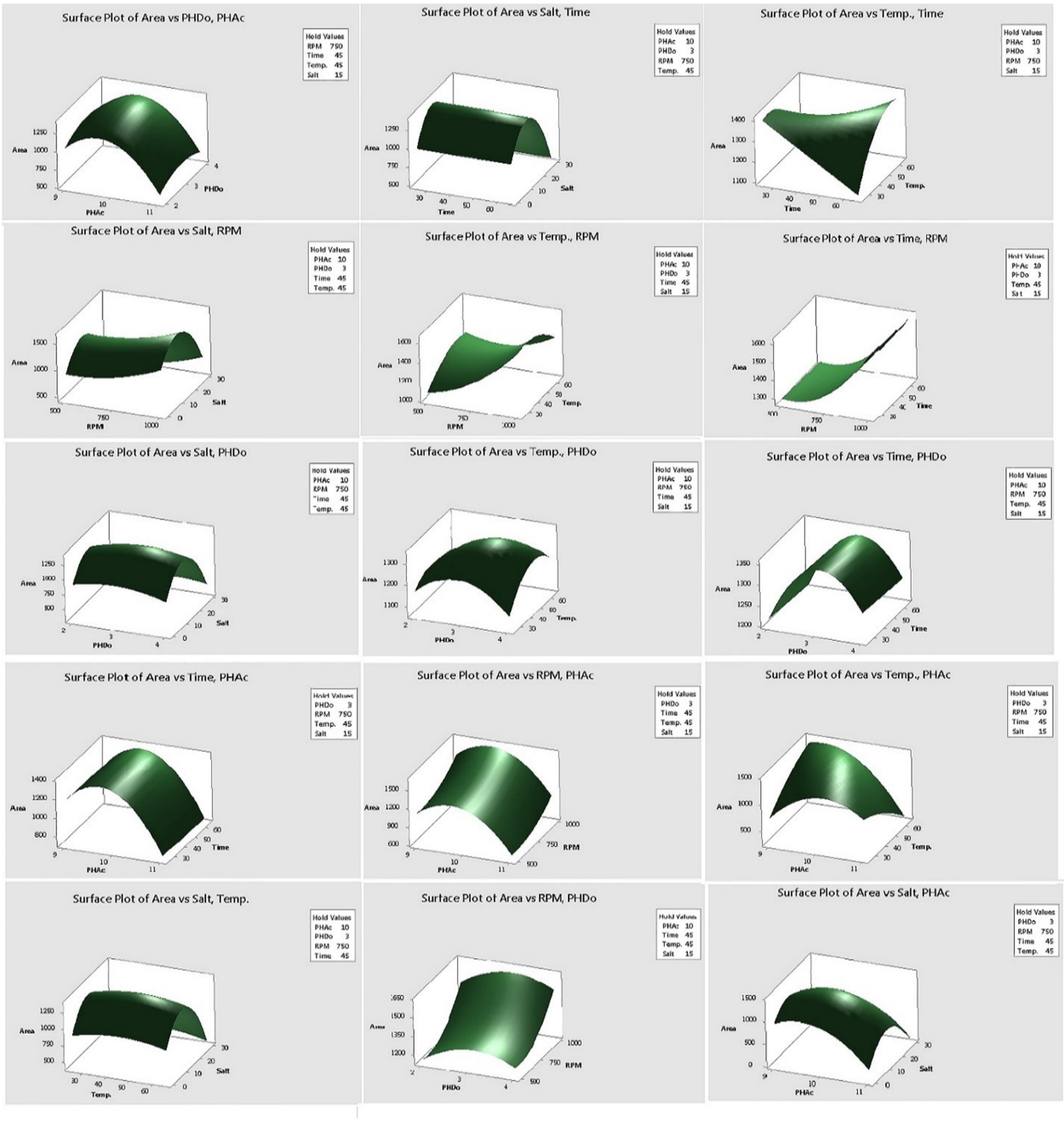

Figure 1. The response surface methodology used for analyzing the interaction of the parameters affecting the microextraction of tamoxifen

Extraction procedure

A 100 mg L^{-1} stock solution of tamoxifen was prepared in methanol and standard working solutions were prepared by the spiking proper amount of the stock solution in pure water. Hollow fiber was cut into 4.5 cm pieces, washed in acetone using an ultrasonic device, and dried at room temperature, accordingly. Using Hamilton syringe, Acceptor phase with a pH of 9 was injected into the hollow fiber, which was immersed in n-octanol. After removing the excess amount of the acceptor phase, both ends of the hollow fiber was sealed mechanically using a few small aluminum foil pieces. Consequently, the hollow fiber was placed in a beaker containing 10 ml donor solution containing tamoxifen. The beaker was placed on a stirrer after putting a magnetic stirrer bar in the beaker. The temperature was set to 65°C. After 65 min, the solvent bar was unsealed, and the acceptor solution was drawn into a Hamilton syringe for injection to HPLC-UV for detection [10].

Experimental design and optimization

A total of 27 experiments were designed using Minitab and 6 parameters were considered. The Response Surface Methodology (RSM) [11] was used to study the interaction of the considered parameters on the area obtained by the HPLC-UV.

Real sample analysis

Urine samples were taken from patients taking tamoxifen and stored at -20 ºC prior to use. Urine samples were pretreated according to the following process; 5 mL urine sample was diluted at the ratio of 1:1 with HPLC-grade water and then centrifuged at 4000 rpm for 3 min.

Experimental

Calculation of PF, Relative recovery, and extraction recovery

The preconcentration factor was calculated using Equation 1 and

$$PF = \frac{Cf,a}{Ci,s} \quad (1)$$

$C_{f,\ a}$: final analyte concentration in the acceptor phase

$C_{i,\ s:}$ initial concentration of the analyte

Extraction recovery (*ER*) was calculated based on Equation 2 and 3.

$n_{f,\ a}$: the number of moles of analyte which was extracted to the acceptor phase

$n_{i,\ s}$: the number of moles of analyte originally present in the sample solution

$V_{\mathrm{i,\ s}}$: volume of sample solution

$V_{\mathrm{f,\ a}}$: volume of acceptor phase

$$ER = \frac{n_{f,\,a}}{n_{i,\,s}} \times 100 = \frac{n_{f,\,a} \times V_{f,\,a}}{n_{i,\,s} \times V_{i,\,s}} \times 100 \qquad (2)$$

$$ER = (\frac{V_{f,\,a}}{V_{i,\,s}}) PF \times 100 \qquad (3)$$

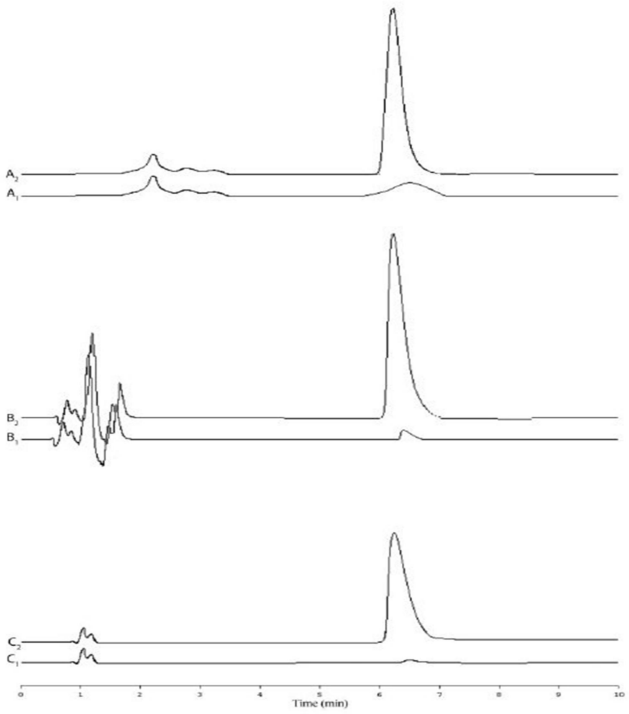

Figure 2. The chromatograms obtained from HPLC-UV before and after application of SBME to real human samples

Results and Discussion

Tamoxifen was successfully preconcentrated and microextraction using the SBME method. As shown in Figure 1, the effect of each parameter was assessed. As seen, increasing the duration of the experiment to the maximum (65 min) range increased the preconcentration. Due to bubble formation and solution loss, a range of 500-1000 rpm was chosen for the stirring rate, and 1000 rpm was used as the optimum speed. The pH gradient applied for this experiment was 9.24 for the acceptor phase, and 3.27 as the donor phase. As can be seen, since the drug was a weak acid, the donor solution had to be within 3-4. The pH level lower than 3 could harm the column of the HPLC, therefore, it was avoided. The optimum organic solvent was proven to be n-octanol based on previous studies. The salt addition had a major effect on the extraction of this drug, and 10.9% salt was proven to be effective on the preconcentration efficiency. The result of each experiment was shown in

Table 1. As shown, the method was applied to the real samples taken from the volunteers. Furthermore, the figures of merit can be seen in Table 2 and Table 3. The limit of detection and the limit of quantification were 13.3 and 40 μgL^{-1}, respectively. The linearity range was between 40 to 10000 μgL^{-1} with a correlation coefficient of 0.999. The enrichment factor was 169 and the relative standard deviation Within-day and Between day were 3.6 and 4.0, respectively. The chromatograms obtained after preconcentration and determination of tamoxifen in real urine samples are shown in Figure 2. Additionally, the chromatograms in this study showed less complexity and easier implementation in comparison with the other methods such as Micellar liquid chromatography [5]. While liquid-liquid extraction and high-performance liquid chromatography with fluorescence detection [13] provide good linearity and narrow detection ranges, the trace levels can be determined using the SBME method. Moreover, micelle to solvent stacking in non-aqueous capillary electrophoresis [6] was used for the quantitation of tamoxifen and offered desirable accuracy and precision. These methods have unique properties which suggest great approaches for tamoxifen analysis with considering the context, such as medical applications.

Table 1. The design of the experiment for the SBME of tamoxifen using Minitab

No.	pH of acceptor phase	pH of the donor phase	Stirring rate (rpm)	Time (min)	Temperature(°C)	Area
1	9	2	500	25	25	510
2	9	2	500	25	45	670
3	9	2	500	25	65	380
4	9	3	750	45	25	650
5	9	3	750	45	45	1100
6	9	3	750	45	65	370
7	9	4	1000	65	25	310
8	9	4	1000	65	45	1710
9	9	4	1000	65	65	615
10	10	2	750	65	25	980
11	10	2	750	65	45	210

12	10	2	750	65	65	1050
13	10	3	1000	25	25	2011
14	10	3	1000	25	45	780
15	10	3	1000	25	65	680
16	10	4	500	45	25	745
17	10	4	500	45	45	580
18	10	4	500	45	65	910
19	11	2	1000	45	25	440
20	11	2	1000	45	45	305
21	11	2	1000	45	65	450
22	11	3	500	65	25	235
23	11	3	500	65	45	150
24	11	3	500	65	65	245
25	11	4	750	25	25	186
26	11	4	750	25	45	231
27	11	4	750	25	65	156

Table 2. The relative recovery of tamoxifen in urine sample

Sample	C_{added}	$C_{founded}$	RSD	Recovery
urine	1	0.89	8.2	89.3%
urine	2	1.90	7.5	95%

Table 3. Figures of merit for SBME of tamoxifen in a urine sample

LOD (ng L^{-1})	LOQ (μgL^{-1})	(μgL^{-1}) Linearity	R^2	EF^d	RSD%	
					Within-day	Between day
13.3	40	40-10000	0.999	169	3.6	4.0

Conclusion

In this study, the aim was to use the trend and sensitive SBME method coupled with HPLC-UV analysis for the detection of tamoxifen at the trace levels, which was successful offering a desirable preconcentration factor, cost-effective, and green set up. This experiment could help trace determine tamoxifen, a cytotoxic agent, in wastewater and the extent of elimination in cancer patients.

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