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Nano graphene oxide as solid phase extraction adsorbent coupled with dispersive liquid-liquid microextraction to determine ultra-trace quantities of propranololfrom urine samples

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Abstract: *In this research, a new nano graphene oxide based solid phase extraction followed by Dispersive Liquid-Liquid Microextraction was applied as simple, rapid and sensitive determination of trace amounts of Propranolol(PRO) in urine samples with HPLC-UVD. Several factors influencing the extraction of PRO, such as pH, adsorbent amounts, extraction time, organic solvent type and the composition of solvent and desorption conditions were studied and optimized. Under optimum condition, the limit of detection (LOD) and limit of quantification (LOQ) of the proposed method were 2 ng $mL^{-1}$and 6.6 ng/mL, respectively. Good linear behaviour over the investigated concentration ranges (2-2000 ng mL^{-1}) and good correlation coefficient of 0.9901(r^2) were obtained. The relative standard deviations (RSDs) based on three determinations at 2, 20, 200 ng ml^{-1} levels of PRO was less than 9.7 %.The findings of the present study may provide clinical and diagnostic laboratories.*

Keywords: *Propranolol (PRO);Nano Grapheme oxide(NGO);Solid Phase Extraction(SPE);High Performance Liquid Chromatography(HPLC); Dispersive Liquid-Liquid microextraction(DLLME); Urine analysis.*

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I. Introduction

Propranolol(PRO), one of the most widely prescribed –blockers in the long-term treatment of hypertension and cardiovascular diseases is usually taken orally, although an intravenous form is available for acute administration[**1**]. Figures 1 showed the chemical structure and properties of PRO.It is used in the treatment or prevention of many disorders including acute myocardial infarction, arrhythmias, angina pectoris, hypertension, hypertensive emergencies, hyperthyroidism, migraine, pheochromocytoma, menopause, and anxiety. It is chemically described as in (RS)-1-(1 -methylethylamino)-3-(1 -naphthyloxy) and its molecular formula is C16H21NO2 [**2-4**].

Recent years, the application of nanomaterials in extraction procedures, as stationary phases or adsorbents, has undergone rapid growth[**5,6**]. Graphene (G), a new allotropic member of carbon, due to its excellent physical and chemical properties, has been studied world-wide for several purposes since its discovery in 2004[**7-10**].The layered structure of graphite and GO is the same, but the plane of carbon atoms in graphite oxide is heavily decorated by oxygen containing groups. Since the reason,graphene(G) and graphene oxide (GO) has attracted attention from many researchers[**7**].

Blockers (known also as adrenergic antagonists) due to their intensive use for the treatment of various cardiovascular disorders, poor degradability and inefficient removal by wastewater treatment processes[**11-12**]able to affect the balance of ecological systems, even at low concentrations. It is easyto understand the necessity for theirdetermination in the environment.Various extraction techniques, such as membrane extraction [**13**], solid–phase extraction, [**14-15**]solid–phase microextraction [**16-19**],liquid–phase microextraction[**11,13,16,20-22**] were used to extract these classes of compounds from aqueous matrices. All these techniques possess pros and cons making each one more preferable than the others under different circumstances.

Dispersive liquid–liquid microextraction is an alternative tothe classical liquid–liquid extraction and includes extractant volumes, at microliter levels [**23**]. Briefly, a mixture of an extractingand a dispersing solvent is rapidly injected in an aqueous sample and a cloudy solution is formedforthe analysis of PRO in pharmaceutical and biologicalsamples including spectrophotometry[**17-18**], spectrofluorometry[**24**],chromatography [**20-21**], chemiluminescence combined withelectrochemical and electroanalytical methods [**25-28**].

In the present study, GO used forthe adsorption/removal of PROfrom urine sample.Afterwards, a simple, rapid and sensitive HPLC methodwith UV detection(HPLC-UVD), adequate sensitivity and short elution timeis described for determination of PRO in these samples.

II. Experimental

2.1. Chemicals and reagents

All of the reagents used were of analytical grade. PRO was purchased from daroupakhsh (Tehran,Iran). The chemical structure properties of PRO is shown in Fig. 1. HPLC-grade acetonitrile, methanol, sodium dilydrogen phosphate, sodium hydroxide and hydrochloric acid were all purchased from Merck (Darmstadt, Germany). Nano Graphen Oxide(NGO) were provided from Sigma Aldrich Company (Steinheim, Germany). The diameter of NGO were less than 50 nm . The reagent water used was purified with a Milli-Q system from Millipore (Bedford, MA, USA). Stock standard solution of PRO (1000 mg L−1) was prepared by dissolving in 5mL methanol and then diluted with reagent water. The working solutions were prepared by proper dilution of the standard solution in the reagent water.

2.2. Apparatus

The HPLC-UVD operating mode was isocratic, injection volume was 20 μL and column temperature was adjusted to room temperature. The chromatography column was a HPLC column Waters Symmetry C8 15 cm × 4.6 mm, 3.5 μm. The mobile phase used was a combination mixture of phosphate buffer with pH=2.5 and Methanol (50:50 V/V). The flow rate was 1 mL min−1. The mobile phase was filtered through a (0.45 μm) pore size filter (Merck Millipore, Billerica, Massachusetts, USA) and degassed by vacuum prior to use. The UV-visible detector was adjusted to 290 nm.

A 40kHz and 0.138 kW ultrasonic water bath with temperature control (Tecno-GazSpA Italy) was applied for ultrasonication of the samples. All of the pH measurements were performed with a Jenway model 3320 pH meter (Staffordshire ST15 0SA, England) supplied with a combined glass electrode. A Stuart CB162 motor-stirrer (StaffordshireST15 0SA, England) was applied to stirsolutions by a magnet.

2.3. Solid phase extraction-dispersive liquid-liquid microextraction procedure

15 mg of NGO was dispersed in a 30 mL sample in a 50 mL beaker andthen sonicated for about 2 min .The mixture was shaken for about 10 min and then the sorbent was separated from the solution by centrifugation (4000 rpm, 5 min). The preconcentrated target analytes were eluted from the isolated sorbent with 1.0 mL(2×0.5 ml)acetic methanol for about 5 min. Afterwards, eluted analyts collected into a 10-mL screw cap glass test tube with conical bottom afterwards, 100 μL chloroform (as the extraction solvent) were added to the test tube. A 5.0 mL of deionized water was quickly injected into the test tube through a 5.00-mL gastight syringe from Hamilton (Reno, NV, USA). A cloudy solution, resulting from distribution of fine chloroform droplets in the aqueous solution, was formed in the test tube. At this stage, the analyte were extracted into the fine droplets of chloroform in a few seconds. Next, the solution was shaken for about 5 min and centrifuged at 4000 rpm for 5 min. The organic layer was then transferred into a 4 mL conical vial and evaporated under a stream of nitrogen. The residue was redissolved in 100 μL of a mobile phase, finally, a 20 μL portion of this solution was injected into the HPLC-UVD system. All experiments were runin triplicates, and the mean values were exploited foroptimization.

2.4. Sample preparation of real samples

In order to study the feasibility of the proposed SPE method for extraction and determination of PRO in the real samples, the developed technique was applied for the extraction of PROin urine samples. In order to reduce the matrix effect, the spiked urine sample were diluted to 1:10 using ultra-pure water without further treatment. The pH of urine samples were adjusted to 4 and then centrifuged for 15 minutes until a white lipidic solid sedimented in the bottom of the tube [**29**] .Then the supernatant was transferred into a clean tube and spiked with PRO. All samples were stored at 4ºC and directly used for MSPE.

III. Result and discussion

3.1. Optimization of Nano graphene based-SPE parameters

To evaluate the effects of the operating parameters on the extraction recovery of the selected analytes and based on preliminary studies, four main variables (adsorption time,sorbent mass,desorption time, pH of sample, organic solvent type and the composition of solvent used for drug desorption) were chosen.The other variables and interactions were insignificant and were therefore neglected in further studies.

3.1.1. Effect of sample's pH

The pH of the sample influenced the reaction between PRO and NGO. The analyte is electrically neutral, so it can be efficiently adsorbed and desorption is unaffected by the charges on the surface of the adsorbent. The pH of the spiked urine samples were adjusted from 2 to 8 by 0.1 M HCl and NaOH (Fig.2).PRO is secondary amines with acidity constants (pKa=9.5) and therefore it is present in solution primarily in positively charged form at operating pH conditions (pH 2 –8).Thus,at strong acidic conditions(pH=4) where the highest drug removal was observed.

3.1.2. Effect of the adsorbent amount

Amount of adsorbent seems to affect drug extraction efficiency; therefore, the amount of NGO was optimized in the range of 5-25 mg (Fig. 3). The addition of increasing amounts of adsorbent up to 15 mg possibly helped the analyte and adsorbent reaction by providing an adequate surface for drug adsorption, but in high amounts of solid phase, low extraction efficiency was obtained. This is probably a result of NGO aggregation and a decrease in the effective adsorption surface area. Therefore, the remaining experiments were carried out with 15 mg of NGO.

3.1.3.Selection of proper eluent

Desorption of the analyte from MSPE adsorbent was carried out using various organic solvents such as acetic methanol, acetic acetonitrile and equal mixture of acetonitrile and methanol. Among these solvents, acetic methanol showed the highest peak area compared with the others. Therefore, acetic methanol was chosen for the rest of the experiments. Methanol is a stronger polar solvent than acetonitrile. Moreover, NGO can be dispersed in polar solvents because of the presence of polar groups on its surface. Maximum extraction efficiency in methanol resulted from the high dispersion of NGO in acetic methanol, which provided the maximum surface for the drug and adsorbent interaction (Fig. 4).

3.1.4. Effect of extraction and desorption time

Extraction time is an important parameter in the MSPE procedure, because maximum extraction efficiency depends on the time it takes the extraction to reach equilibrium. A time range of (1-15 minutes) was investigated for spiked urine samples. The highest peak area was achieved in 10 min, but extraction efficiency remained constant with subsequent increases in time. This phenomenon may be an expression of extraction equilibrium achievement in 10 minutes (Fig. 5a). Desorption time is another important factor in the MSPE method; it seems to affect the amount of drug desorbed from the NGO surface. In the time range of (2-20 minutes), the amount of desorbed drug was studied. Extraction efficiency increased with a time up to 5 min, and then peak areadecreased (Fig. 5b). Therefore, 5 min was selected for the rest of the experiments.

3.2.Optimization of DLLME parameters

On the otherhands, for optimization of DLLME stage,under optimum conditions of nano graphene oxide based SPE stage, effect of type ofextraction and disperser solvents and also their volumes wereinvestigated and optimized.The result obtained from articles shows that some important parameters such as type and volumes of extraction and disperser solvents, pH, salt effect and sample volume aresignificant effect on efficiency and acceptable separation occurs when disperser/extraction solvents are acetonitrile/chloroform, respectively[**30**].

3.3. Method validation

The quantitative analytical characteristics of linear range, correlation coefficient (r^2), limit of detection (LOD), limit of quantification (LOQ), and relative standard deviation (RSD) are shown in Table 1. The calibration curve was constructed by plotting the mean peak area of five concentrations, each in three measurements. The blank urine sample was spiked with PRO at different concentrations (2,20,200 ng/mL) and analyzed using the proposed procedure. Accuracy and precision experiments were performed at three concentrations covering the calibration range (Table 2). Considering the complexity of the biological matrix, these results can be regarded as satisfactory.

3.4. Application of* NGO-based SPE-DLLME method*procedure to real sample

In order to study the feasibility of the proposed MSPE method for extraction and determination of PRO in the real sample, the developed technique was applied for the extraction of drug from the urine sample (Table.3). The application of the proposed method was investigated using positive urine samples. Fig. 6 presents the blank urine and positive urine chromatograms. For reducing the matrix effect, the spiked urine sample were diluted to 1:10 using ultra-pure water without further treatment.

IV. Conclusion

In this study, a new NGO-based SPE-DLLME–HPLC-UVD method wasdeveloped for determination of PRO inurine samples. The samples were initially extracted in via the NGO-based SPE, and then the eluents of this stage were exploited as disperser solvent for the following DLLME procedure for further purification and enrichment of the analytes prior to HPLC analysis.As a consequence, High extraction efficiency method was achieved, resulting in a low detection limit. The proposed method offers a simple, sensitive, inexpensive method for extraction and determination the presence of PRO in positive urine samples. Based on the obtained results and due to the commercial availability of NGO, it is anticipated that the proposed method has a great analytical potential in pretreatment of drugs from real samples in the same way. Furthermore, the findings of the present study may provide clinical and diagnostic laboratories with an improved analytical method for determining the presence of PRO.

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Figure captions

Fig. 1. Chemical structure of propranolol.

Fig. 2. Effect of pH on extraction efficiency. Conditions: Sample volume = 30 mL; concentration of the propranolol = 100 ng L^{-1}; 20 mg NGO; stirring time = 15 min; elution with 1mL (2×0.5ml) acetic methanol; desorption time = 4 min.

Fig.3. Effect of weight of NGO extraction efficiency. Conditions: Sample volume = 30 mL; concentration of the propranolol = 100 ng L^{-1}; sample's pH = 4.0; stirring time = 15 min; elution with 1mL (2×0.5ml) methanol; desorption time = 4 min.

Fig.4. Effect of various extraction solvent on extraction efficiency. Conditions: Sample volume = 30 mL; concentration of the propranolol = 100 ng L^{-1}; sample's pH = 4.0; 15 mg NGO ;stirring time = 15 min; elution with 1mL (2×0.5ml) methanol; desorption time = 4 min.

Fig. 5.(a) Effect of different time of drug extraction, (b) effect of various time of desorption, other extraction conditions are as mentioned in Fig. 4.

Fig.6. Chromatograms of blank urine (a) positive urine (b) samples.

Fig.1.

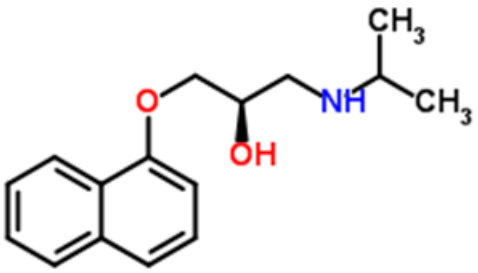


Fig.2.

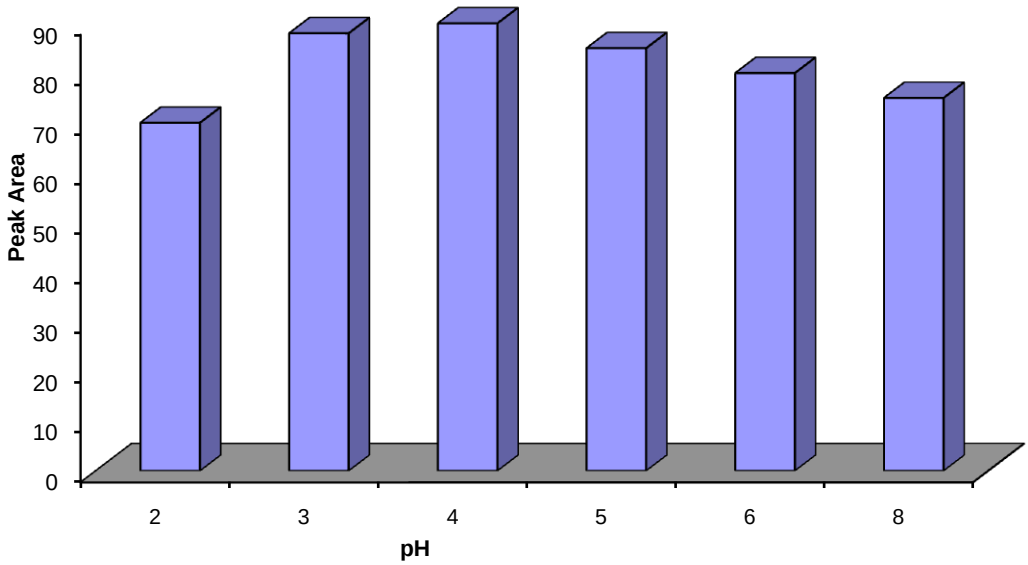


Fig.3.

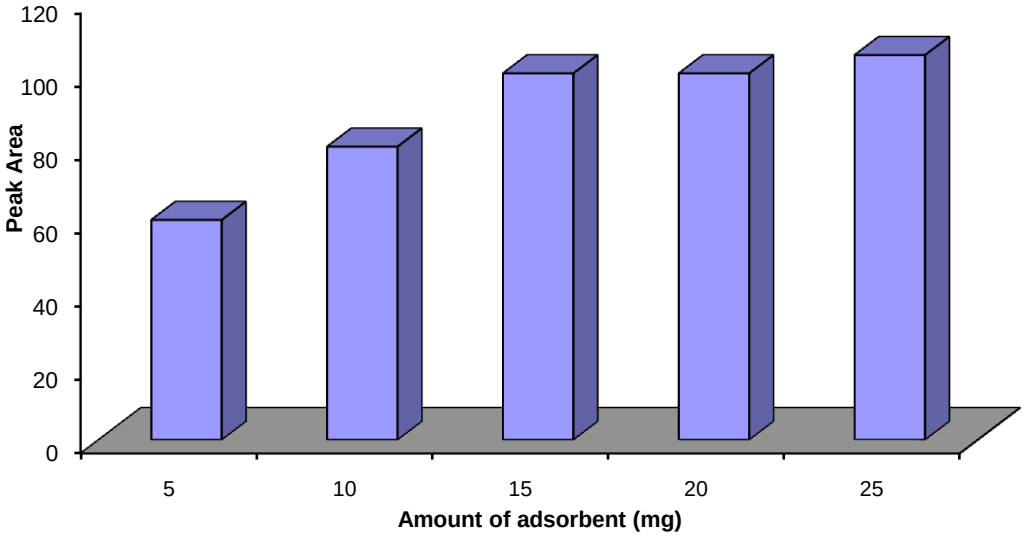


Fig.4.

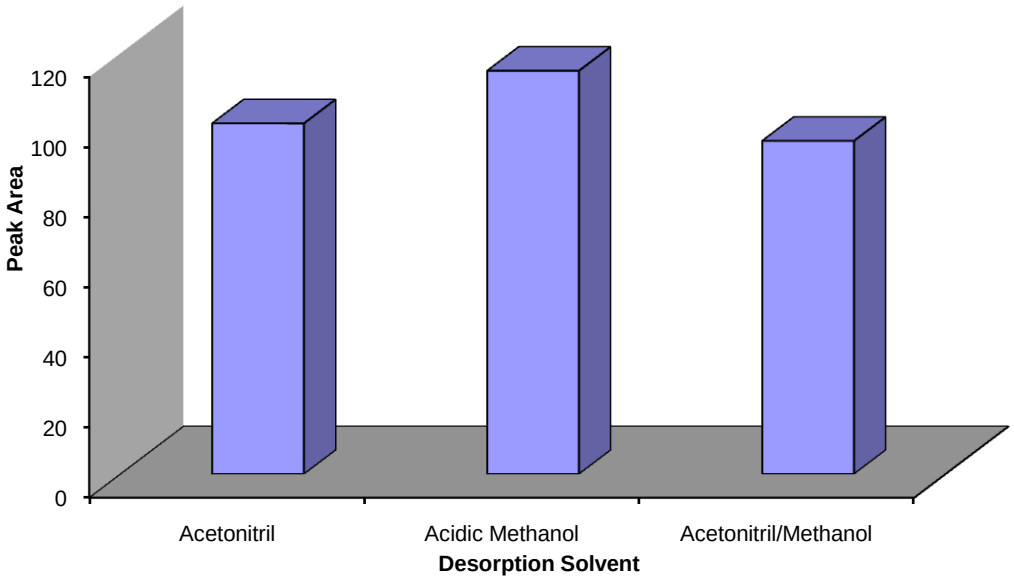


Fig.5(a).

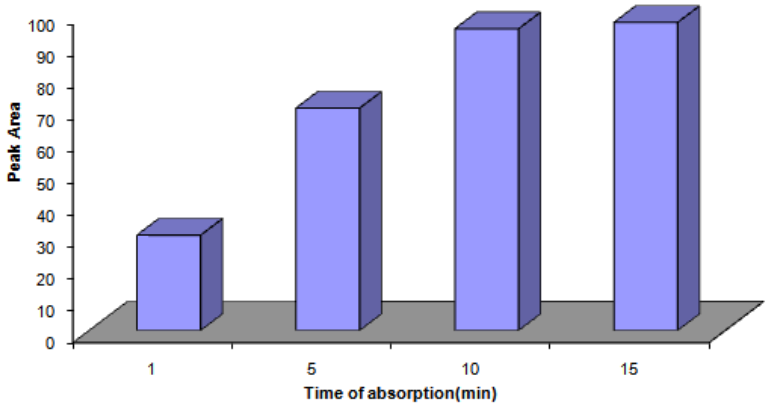


Fig.5b.

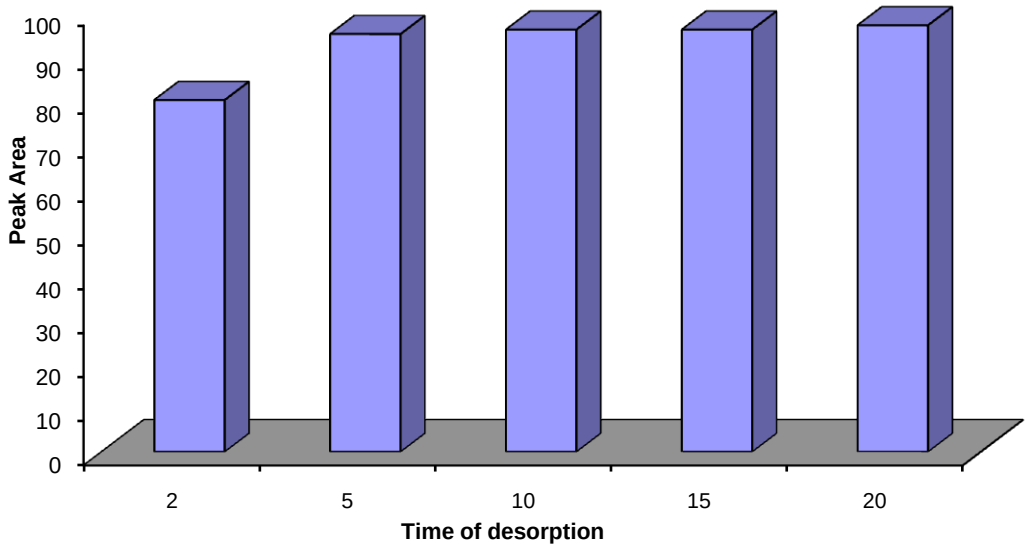
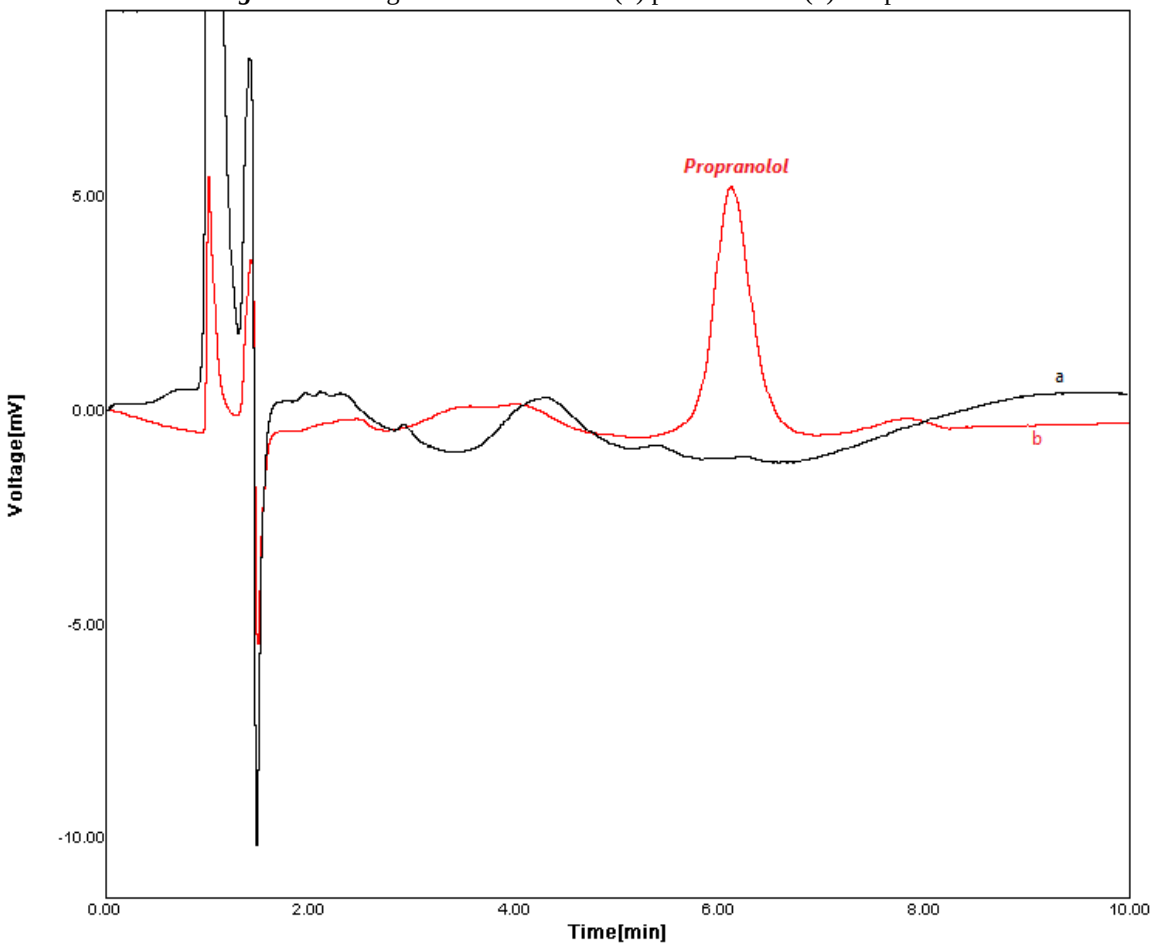


Fig.6.Chromatograms of blank urine (2) positive urine (1) samples.



Tables

Table 1. Table of figures of merit for MSPE extraction of propranolol.

Analyte	Concentration range(ng/ml)	Linearity (r^2)	LOD (ng/ml)	LOQ (ng/ml)	RSD (%)
Propranolol	2-2000	0.9901	2	6.6	**3.5**

Linearity is described by the correlation coefficient for the calibration curve.
Limit of Detection (LOD): S/N=3
Limit of Quantification (LOQ) :S/N=10
Relative Standard Deviation:RSD

Table2 . Result of method validation of MSPE extraction.

Analyte Concentration (ng/ml)	Intra-day (n=3)		Inter-day (n=3)	
	Precision (RSD[a])	Accuracy (bias)	Precision (RSD[a])	Accuracy (bias)
2	5.1	0.58	6.1	0.39
20	7.6	0.3	5.2	0.7
200	9.7	0.9	8.3	0.95

[a]Relative Standard Deviation

Table3 . Result of method validation of GO-based SPE-DLLME–HPLC-UVD method.

Analyte	Recovery (%)
Subject 1	96.13
Subject 2	97.15

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