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Preconcentration and Determination of Cyproheptadine in Biological Samples by Hollow Fiber Liquid Phase Microextraction Coupled with High Performance Liquid Chromatography

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Abstract**—**In this study, a liquid phase microextraction by hollow fiber (HF-LPME) combined with high performance liquid chromatography-UV detector was applied to preconcentrate and determine trace levels of Cyproheptadine in human urine and plasma samples. Cyproheptadine was extracted from 10 mL alkaline aqueous solution (pH: 9.81) into an organic solvent (n-octnol) which was immobilized in the wall pores of a hollow fiber. Then was back-extracted into an acidified aqueous solution (pH: 2.59) located inside the lumen of the hollow fiber. This method is simple, efficient and cost-effective. It is based on pH gradient and differences between two aqueous phases. In order to optimize the HF-LPME some affecting parameters including the pH of donor and acceptor phases, the type of organic solvent, ionic strength, stirring rate, extraction time and temperature were studied and optimized. Under optimal conditions enrichment factor, limit of detection (LOD) and relative standard deviation (RSD(%), n=3) were up to 112, 15 $\mu g.L^{-1}$ and 2.7, respectively.

Keywords**—**Biological samples, Cyproheptadine, hollow fiber, liquid phase microextraction.

I. Introduction

CYPROHEPTADINE hydrochloride (CPH), 4-(5H Dibenzo[a,d]cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride, a piperidine derivative, is a sedating antihistamine with antimuscarinic, serotonin-antagonist, and calcium-channel blocking actions[1]. Mean peak plasma levels of CPH obtained after an oral single dose is 30.0 $\mu g.L^{-1}$ [2].

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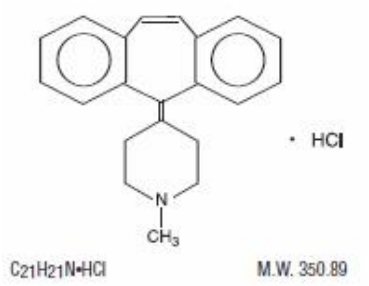


Fig. 1 Chemical structure of CPH

It is used as for the symptomatic relief of allergic conditions, management of migraine, appetite disorders, angina pectoris, carcinoid syndrome, serotonin syndrome, and sexual dysfunction induced by SSRIs [1]. Several analytical methods such as liquid chromatography tandem mass spectrometry [3]-[5], and Spectrophotometric determination [6] have been reported for the determination of CPH in biological fluids or in pharmaceutical formulations. Nevertheless, most of these techniques do not have an adequate limit of detection (LOD) proper for monitoring CPH at low levels in biological samples. Therefore, owing to low concentration of CPH in biological samples, using effective methods that have the ability to separate and elicit the analyte of interest is generally required prior to determination of trace levels of the drug by analytical instruments. Various extraction and preconcentration methods such as liquid–liquid extraction (LLE) [7], [8], solid phase extraction (SPE) [9], [10], liquid phase microextraction (LPME) [11], [12], and solid phase microextraction (SPME) [13], [14] are generally used for separation and sample preparation. LLE and SPE both require large amounts of organic solvents which are toxic in either human body or environment. In addition LLE and SPE are laborious, intensive and very costly [15]. Pedersen-Bjergaard and Rasmussen introduced hollow fiber liquid phase microextraction (HF-LPME) to ameliorate the stability and reliability of liquid–phase microextraction (LPME). In this methodology, disposable porous hollow fiber is used. Sample vial is filled with aqueous solution as donor phase. During HF-LPME procedure, target analytes that exist in donor phase are extracted into organic solvent which is immobilized in pores of the hollow fiber walls and further into the accepter phase inside the lumen of the hollow fiber. Acceptor phase can be

organic or aqueous solution resulting in two-phase extraction system compatible with capillary gas chromatography, and three-phase extraction system compatible with high-performance liquid chromatography or capillary electrophoresis, respectively. HF-LPME may be a good alternative for traditional extraction and pre-concentration techniques due to excellent features including good recovery and enrichment factors, high precision, accuracy, and clean-up sample capability [16], [17]. This paper describes an efficient and simple HF-LPME method in combination with HPLC-UV for preconcentration and determination of CPH in biological fluids. Several factors that affect the microextraction efficiency, such as donor and acceptor pH, organic solvent type, stirring rate, ionic strength, extraction time and temperature were optimized. To our knowledge, this is the first report of the use of HF-LPME combined with HPLC-UV for the trace analysis of CPH in human urine and plasma.

II. Experimental

A. Chemicals and Materials

Cyproheptadine reference standard was kindly donated by Food and Drug Organization (Tehran, Iran). HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). All the other chemicals were of reagent grade or of the highest purity available. Ultrapure water (resistivity, 18.2 MΩ cm^{-1}) was produced by an Aqua Max (Youngline, Korea), and was used throughout for the preparation of solutions. Phosphate buffers were prepared from phosphoric acid and their appropriate salts (Merck Chemical Co.). Frozen, drug-free human plasma was obtained from Iranian Blood Transfusion Organization (Tehran, Iran), and the urine sample was collected from healthy volunteers (males mean 30 years old). The polypropylene hollow fiber (200μm wall thickness, 600μm internal diameter, 0.2μm pore size) was purchased from Membrana (Wuppertal, Germany).

B. Apparatus and Chromatographic Conditions

Chromatographic analysis was performed by Youngline (Korea) HPLC system equipped with a quaternary YL9110 pump and a UV/VIS YL9120 detector. Chromatographic data were recorded and analyzed using Younglin Autochro 3000 software. The chromatographic separation was carried out at room temperature (about 20°C) on a C18 analytical column (4.6 mm × 150 mm, 5 μm) with a C18 guard column (4.0 mm × 10 mm, 5 μm) from Teknokroma (Barcelona, Spain). The injector was a Rheodyne manual injection valve fitted with a 10 μL sample loop. The mobile phase was phosphate buffer acetonitrile (35:65, v/v) with a flow rate of 1.2 mL $.min^{-1}$. The phosphate buffer was prepared freshly by adding 6.12 g NaH_2PO_4 to 900 mL deionized water and then the pH was adjusted to 4.5 with phosphoric acid. At the end it was diluted up to 1000 mL. The analytes were detected at 230 nm. All of the pH measurements were performed with a GPHR 1400A pH meter (Germany). Stirring of the solution was carried out with a Heidolph MR 3001 K magnetic stirrer (Schwabach, Germany) and a 7 mm × 1.5 mm magnetic stirring bar.

C. Preparation of Standard Solutions and Biological Samples

The stock standard solution of CPH (100 $mg.L^{-1}$) was prepared in methanol and stored in refrigerator (4◦C). All of the working standard solutions were freshly prepared by proper dilution of the stock standard solution with ultrapure water to the required concentration. The blood plasma samples were diluted three times using deionized water up to 10 mL. 10 mL urine sample was subjected to the analytical process without any dilution.

D. HF-LPME Procedure

Hollow fibers were cut into 4 cm pieces, washed with acetone in an ultrasonic bath and dried. The internal volume of the hollow fibers was approximately 10 μL. 10 mL of the aqueous sample containing the drug which was made alkaline (pH=9.81) using NaOH was poured into a 20 mL sample vial having a 7 mm × 1.5 magnetic stirring bar. The sample vial was placed on a magnetic stirrer. Subsequently, 10 μL of the acceptor phase (pH=2.59) was withdrawn in the microsyringe and then its needle was inserted into the hollow fiber. The hollow fiber was immersed in the organic solvent for about 10 s to impregnate the pores with the solvent. After solvent impregnation, the fiber was put in a water bath for 10 s to remove excess of the solvent. Then, the solvent in the syringe was injected completely into the hollow fiber. The microsyringe was then fixed by a stander so that the hollow fiber was immersed into the sample solution together with the microsyringe needle. During the extraction, the sample solution was continuously stirred at room temperature with a magnetic stirrer at 750 rpm for 30 min. After extraction, the solvent in the hollow fiber was retracted back into the microsyringe for HPLC analysis, and the hollow fiber was discarded.

III. Results and Discussion

A. Optimization of HF-LPME Procedure

Several parameters affect the extraction by HF-LPME method. In order to achieve the maximum efficiency, following parameters were studied and optimized.

B. Selection of Organic Extraction Solvent

The type of organic solvent to be immobilized within the pores of the HF is a crucial step to ensure proper analyte preconcentration. The selected organic solvent should be easily immobilized within the pores of the fiber, have a high selectivity for the target analyte and low tendency to extract the interferences which exist in the donor phase, be low soluble or immiscible with water to avoid dissolution, and be nonvolatile to avoid solvent loss during the extraction. In this study, the effect of different organic solvents such as n-octanol, n-hexane, n-heptane, n-decanol, isobutyl methyl ketone (IBMK) were tested on the extraction efficiency of CPH. As it can be seen in Fig. 2, n-octanol can give the best extraction performance due to much higher enrichment factor and selectivity and low solvent loss. Therefore, n-octanol was selected for further optimization.

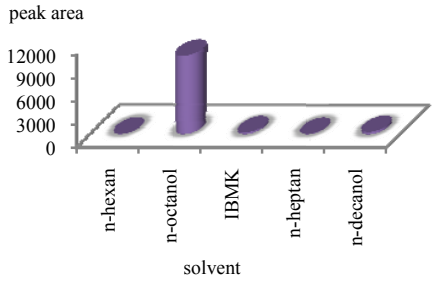


Fig. 2 Effect of different extraction solvent on HF-LPME of CPH. Conditions: Donor and acceptor pH, 9.81 and 2.59, respectively; Stirring speed, 500 rpm; extraction time, 20 min

C. The pH of Donor and Acceptor Phase

The pH of both the donor phase (sample solution) and acceptor phase influence the extraction performance. For basic drugs such as CPH (pKa=8.05), the donor phase should be strongly alkalized to efficaciously deionize the analytes and therefore reduce their solubility within the sample, while the acceptor phase should be acidized to prompt dissolution of the basic analytes. The effects of donor and acceptor pH in the range of 8-12 and 1.5-3, respectively, were tested on the extraction efficiency of CPH. All the experiments were conducted for the duration of extraction of 20 min at a stirring rate of 500 rpm with n-octanol as the solvent for impregnation of the HF pores. According to Fig.s 3 and 4, the obtained results showed that the best extraction efficiency for CPH was at pH 9.81 and 2.59 for the donor phase and acceptor phase, respectively.

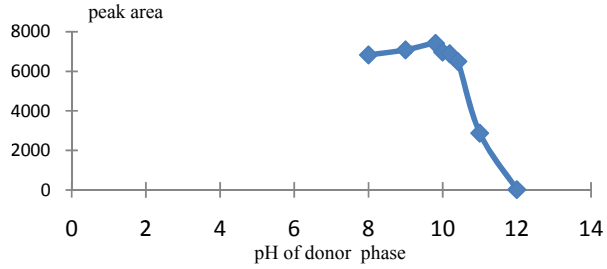


Fig. 3 Effect of pH of donor phases on the extraction efficiency. Condition: organic extraction solvent, n-octanol; acceptor phase pH, 3; stirring speed, 500 rpm; extraction time, 20 min

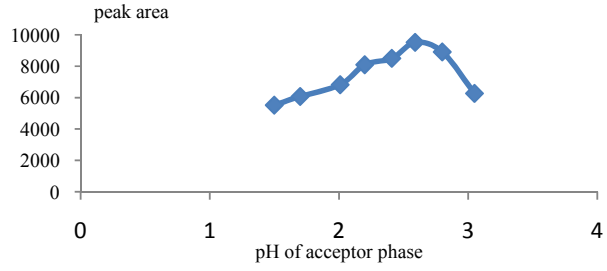


Fig. 4 Effect of pH of acceptor phases on the extraction efficiency. Conditions: organic extraction solvent, n-octanol; donor phase pH, 9.81; stirring speed, 500 rpm; extraction time, 20 min

D. Effect of Stirring Rate

Agitation of the sample is routinely applied to hasten the extraction kinetics. Facilitating the diffusion rate of the drug from the aqueous sample to organic phase is obtained by increasing the stirring speed of aqueous phase. In this study, the effect of stirring rate on the extraction of target drug was investigated by agitating of 10 mL sample solution at different stirring rates (250-1000 rpm) using magnetic stirrer. The achieved results indicate that the extraction efficiency of the drug increased by rising the stirring rate up to 750 rpm. However, high stirring rates made some problems such as production of air bubbles on the surface of the hollow fiber. Hence, in order to prevent air bubble formation, 750 rpm was selected as the optimum stirring speed for the rest of the experiments.

E. Salt Effect

The effect of salt concentration was also tested by adding of NaCl to the aqueous sample in the range of 0–20% (w/v). It was found that the addition of salt has effect on the extraction efficiency of the drug. Therefore, HF-LPME was employed with 5 % (w/v) NaCl addition in the further work.

F. Effect of Extraction Time and Temperature

The effect of extraction time in the range of 10-50 min was investigated. The experimental results indicated that the extraction efficiency raised with increasing the extraction time form 10 to 30 min, but decreased thereafter. Generally, if extraction time is too long, solvent loss and air bubble formation may occur that both of them affect the extraction. In longer extraction time, air bubbles are created on the surface of the hollow fiber and decrease the transport rate of analytes from sample solution to organic solvent immobilized in the pores of the HF, and then reduce the amount of analytes extracted. Hence, 30 min was selected as the optimal extraction time and used in the remainder of the experiments. Temperature has a significant effect on both the kinetics and the thermodynamics of the extraction process. To study the effect of extraction temperature on HF-LPME, the extraction responses were tested over a temperature range of 4–40°C. Increasing temperature can promote the mass transfer coefficient which improves the extraction efficiency in shorter time. Also, solvent loss may occur due to increased solubility of the organic solvent at higher temperatures which causes to decrement the extraction efficiency. The obtained results showed that the peak area ratio of the analytes reached its maximum at about 20°C. Thus, room temperature (20°C) was adopted in the following studies.

G. Analytical Performance

Under the optimized conditions, the analytical performance of the developed method such as LOD, LOQ, linearity, RSD% was evaluated and summarized in Table I. The calibration curve was obtained after the standard series were subjected to the HF-LPME-HPLC. A broad dynamic linear range with good correlation coefficient (r) was achieved. Limit of detection (LOD) and limit of quantification (LOQ) were

calculated as the minimum concentration of an analyte giving peaks whose signal-to- noise ratio is 3 to 10, respectively. Enrichment factor (EF) was calculated based on the following equation:

$$EF = Cap/ Cdp \quad (1)$$

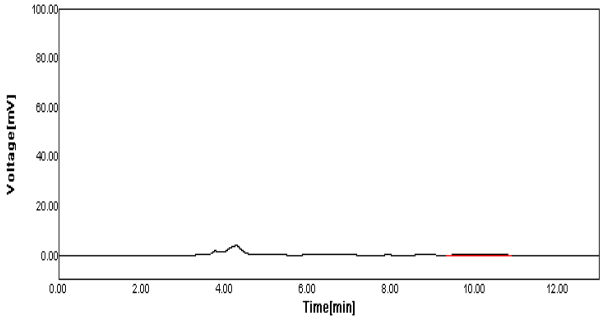
where Cap is the concentration of analyte in the acceptor phase after the extraction and Cdp is the initial concentration of analyte in the sample solution (donor phase) before the extraction.

TABLE I
ANALYTICAL PERFORMANCE OF HF-LPME-HPLC-UV ANALYSIS OF CPH

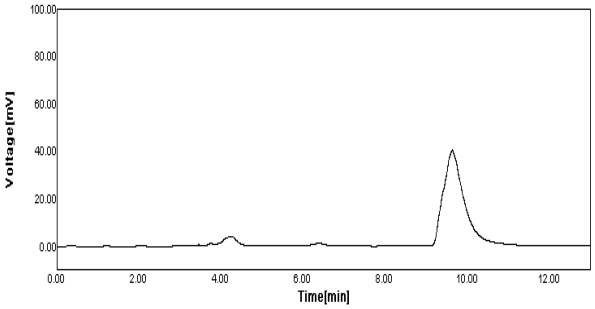
Symbol	Quantity
ef	112
lod	15 µg.L^{-1}
loq	45 µg.L^{-1}
R^2	0.998
ldr	45-5000 µg.L^{-1}
%rsd(intra day)	2.5
%rsd(inter day)	2.7

H.Analysis of Plasma and Urine Samples

Under the optimized conditions, the developed HF-LPME-HPLC technique was applied to preconcentration and determination of CPH in plasma and urine samples. In order to validate the proposed method, recovery experiments were also conducted by spiking the samples with different amounts of CPH. The recoveries for the spiked samples varied from 84% to 87%. Table II shows the results of the analysis of samples gained by the proposed method and the added amount of CPH are in satisfactory agreement. HPLC-UV chromatograms of plasma and urine sample without and with spiked with 0.2 mg.L^{-1} and 1 mg.L^{-1} of CPH after the HF-LPME are shown in Figs. 5 and 6, respectively.

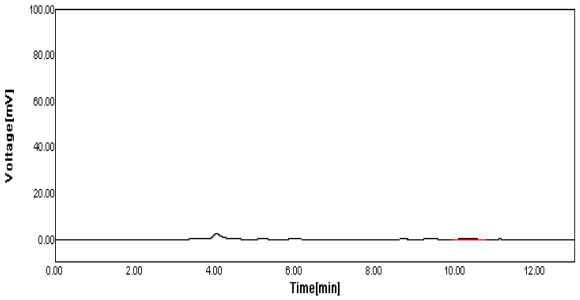


(a)

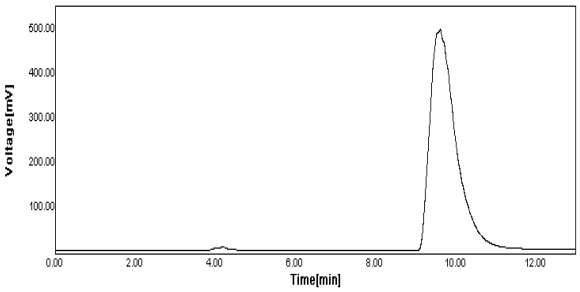


(b)

Fig. 5 HPLC-UV Chromatogram of (a) human blank plasma and (b) human plasma sample spiked with 0.2 mg.L-1 of CPH after HF-LPME at optimum conditions



(a)



(b)

Fig. 6 HPLC-UV Chromatogram of (a) human blank urine and (b) human urine sample spiked with 1 mg.L-1 of CPH after HF-LPME at optimum conditions

TABLE II
RESULTS FOR DETERMINATION OF CPH IN SPIKED PLASMA AND URINE SAMPLES SUBJECTED TO THE HF-LPME AND ANALYZED USING HPLC

Sample	C_{added}(mg.L^{-1})	C_{found}(mg.L^{-1})	R(%)	RSD%(n=3)
plasma	0.2	0.168	84	3.2
urine	1	0.87	87	3.8

A comparison between the analytical characteristics of the developed method and those of the published methods was shown in Table III. As can be seen, the LOD of this method is comparable with those obtained in the previous studies, and even lower than some of those reported in the literatures. The developed method has less sensitivity compared with that reported in references of [3]-[5]; however, these methods are

more expensive to purchase and use, and the instrumentation is also not available in most laboratories. In addition, due to the simplicity and low cost of the extraction device, the HF can be discarded after each extraction to avoid carryover and cross-contamination. Finally, it is concluded that this method is an effective and simple technique for the preconcentration and determination of CPH in plasma and urine samples.

TABLE III
COMPARISON OF THE CHARACTERISTICS FOR THE METHODS DESCRIBED IN THE LITERATURE WITH THE PROPOSED METHOD FOR THE DETERMINATION OF CPH

Method	Sample	LOD(μg.L^{-1})	RSD%	Ref.
LLE-LC-MS/MS	Human plasma	0.05	-	[3]
SPE-LC-MS/MS	Bovine urine	0.48	20.4	[4]
LC–MS/MS	Pharmaceutical syrup formulations	0.86	-	[5]
Spectrophotometry	Bulk drug and tablet formulations	40	-	[6]
HF-LPME-HPLC-UV	Human plasma Human urine	15	2.7	This work

IV. CONCLUSION

The present study developed HF-LPME-HPLC-UV method for the preconcentration and determination of trace amounts of CPH in plasma and urine samples. The developed method proved to be a fast, simple, low-priced and sensitive with minimal usage of organic solvents. Three phase hollow fiber liquid phase microextraction (HF-LPME) as a clean-up method decreased the matrix effect and produced relatively high enrichment factors in the extract of the target drug. This method has been successfully applied to the analysis of CPH in plasma and urine samples, indicating that HF-LPME-HPLC-UV is a promising combination for analysis of basic drugs present at low levels in biological matrix.

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REFERENCES

[1] Sean C Sweetman: Martindale The Complete Drug Reference. 36th ed. RPS; 2009:575.
[2] Gunja N, Collins M, Graudins A. A comparison of the pharmacokinetics of oral and sublingual cyproheptadine. Clinical Toxicology. 2004; 42(1):79-83.
[3] Mendes GD, Arruda A, Chen LS, Almeida Magalhães JC, Alkharfy KM, De Nucci G. Quantification of cyproheptadine in human plasma by high performance liquid chromatography coupled to electrospray tandem mass spectrometry in a bioequivalence study. Biomedical Chromatography. 2012;26(1):129-36.
[4] Fente CA, Regal P, Vazquez BI, Feas X, Franco CM, Cepeda A. Development and validation of an LC-MS/MS confirmatory method for residue analysis of cyproheptadine in urine of food-producing animals. Journal of agricultural and food chemistry. 2009;57(6):2595-8.
[5] Feás X, Ye L, Hosseini SV, Fente CA, Cepeda A. Development and validation of LC–MS/MS method for the determination of cyproheptadine in several pharmaceutical syrup formulations. Journal of pharmaceutical and biomedical analysis. 2009;50(5):1044-9.
[6] Basavaiah K. Application of bromate-bromide mixture and methyl orange in the titrimetric, spectrophotometric and kinetic assay methods for cyproheptadine in pharmaceuticals. Indian journal of chemical technology. 2006; 13(4):360.
[7] Trivelin LA, Rohwedder JJR, Rath S. Determination of pentavalent antimony in antileishmaniotic drugs using an automated system for liquid–liquid extraction with on-line detection. Talanta. 2006; 68(5):1536-43.
[8] Billard I, Ouadi A, Gaillard C. Liquid–liquid extraction of actinides, lanthanides, and fission products by use of ionic liquids: from discovery to understanding. Analytical and bioanalytical chemistry. 2011; 400(6):1555-66.
[9] Lindberg R, Jarnheimer P-Å, Olsen B, Johansson M, Tysklind M. Determination of antibiotic substances in hospital sewage water using solid phase extraction and liquid chromatography/mass spectrometry and group analogue internal standards. Chemosphere. 2004;57(10):1479-88.
[10] Zou L, Tong Z, Dong X-Z. Determination of carbamates and organophosporus pesticides in vegetables and fruits by solid-phase extraction and high performance liquid chromatography. Basic Sciences Journal of Textile Universities. 2011;4:021.
[11] Pedersen-Bjergaard S, Rasmussen KE. Liquid-phase microextraction and capillary electrophoresis of acidic drugs. Electrophoresis. 2000;21(3):579-85.
[12] Zhao L, Lee HK. Liquid-phase microextraction combined with hollow fiber as a sample preparation technique prior to gas chromatography/mass spectrometry. Analytical chemistry. 2002; 74(11):2486-92.
[13] Prosen H, Zupančič-Kralj L. Solid-phase microextraction. TrAC Trends in Analytical Chemistry. 1999;18(4):272-82.
[14] M Ligor, T Ligor, A Bajtarevic, C Ager, M Pienz, M Klieber, et al. Determination of volatile organic compounds in exhaled breath of patients with lung cancer using solid phase microextraction and gas chromatography mass spectrometry. Clinical Chemistry and Laboratory Medicine. 2009;47(5):550-60.
[15] Pawliszyn J: Applications of solid phase microextraction. Royal Society of Chemistry; 1999:213.
[16] Sarafraz-Yazdi A, Amiri A. Liquid-phase microextraction. TrAC Trends in Analytical Chemistry. 2010;29(1):9-11.
[17] Lee J, Lee HK, Rasmussen KE, Pedersen-Bjergaard S. Environmental and bioanalytical applications of hollow fiber membrane liquid-phase microextraction: a review. Analytica chimica acta. 2008;624(2):253-68.