Acta Poloniae Pharmaceutica – Drug Research, Vol. 66 No. 1 pp. 3–9, 2009
ISSN 0001-6837
Polish Pharmaceutical Society

ANALYSIS

SPECTROPHOTOMETRIC DETERMINATION OF KETOPROFEN AND ITS APPLICATION IN PHARMACEUTICAL ANALYSIS

ZHOLT KORMOSH[a],*, IRYNA HUNKA[a] and YAROSLAV BASEL[b]

[a] Department of Analytical Chemistry, Volyn National University, Lutsk, Ukraine
[b] Department of Analytical Chemistry, P.J. Šafárik University, Košice, Slovakia

Abstract: A new simple rapid and sensitive spectrophotometric method has been developed for the determination of ketoprofen in pharmaceutical preparations. The method is based on the reaction of ketoprofen with an analytical reagent – Astra Phloxin FF – at pH 8.0 – 10.8 and followed by the extraction of formed ion associate in toluene with spectrophotometric detection (it has an absorption maximum at 563 nm, $\varepsilon = 7.6 \times 10^4$ $L \times mol^{-1} \times cm^{-1}$). The calibration plot was linear from 0.8 – 16.0 $\mu g \times mL^{-1}$ of ketoprofen, and the detection limit was 0.037 $\mu g \times mL^{-1}$.

Keywords: ketoprofen determination, Astra Phloxin FF, spectrophotometry

Currently over 50 pharmaceuticals are known that, though different in their chemical structure, are classified as non-steroidal anti-inflammatory drugs (NSAIDs) (1). They are widely used in medical practice and are important components in the treatment of rheumatoid arthritis (2) and osteoarthritis (3). The incidence of rheumatoid arthritis is high; yearly diagnostics add some 9.000 new patients, of which 67 – 68% are of able-bodied age (4).

Ketoprofen (Ket), (Figure 1. A) [2-(3-benzoylphenyl)propionic acid] is a nonsteroidal anti-inflammatory and analgesic agent. The positive qualities of ketoprofen are based on optimal physico-chemical and structural characteristics, its ability to penetrate into and accumulate in the inflammation centers and compatibility with other classes of drugs.

The determination of small amounts of ketoprofen in pharmaceutical preparations is very important for medical and pharmaceutical needs where it is used for the treatment of various diseases. Therefore, it is crucial to develop a simple, selective and cost-effective method of determination of micro amounts of ketoprofen in different pharmaceutical formulations (compliance to the specifications: specimen quantity, sample homogeneity, biodecomposability, mechanical impurities etc.).

Several types of analytical procedures have been proposed for the analysis of ketoprofen in pharmaceutical formulations. These procedures include potentiometry (5), chromatography (6-10), spectrophotometry (11-13) and partial least squares regression (14-16).

This paper describes a method of extraction-photometric determination of ketoprofen in pharmaceuticals as an ion associate with a basic dye Astra Phloxin FF (Figure 1 (B)).

EXPERIMENTAL

Apparatus

A SF-2000 spectrophotometer (LOMO, Russia) was used to obtain spectra, with 0.3 cm matched glass cells used to perform analyses. All pH

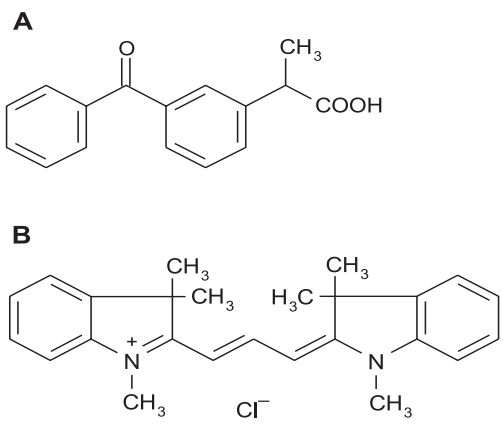

Figure 1. Structure of ketoprofen (A) and Astra Phloxin FF (B).

* Corresponding author: kormosh@univer.lutsk.ua

measurements were made with an I-160 Model pH meter (Gomel, Belarus).
All experiments were performed at room temperature, maintained at 20 ± 1°C.

Reagents

All chemicals were of analytical reagent grade. Distilled water was used to prepare all solutions and in all experiments. Astra Phloxin FF was used as a 1×10^{-3} mol×L^{-1} aqueous solution.

Ketoprofen (Sigma Aldrich): a freshly prepared 1×10^{-3} mol×L^{-1} aqueous solution was used as a standard solution for analytical purposes. It was dissolved in the minimum volume of 0.1 mol×L^{-1} sodium hydroxide and diluted to the mark with distilled water.

2 mol×L^{-1} Na_2SO_4 solution was prepared by dissolving 142 g of Na_2SO_4 and diluting to 500 mL with distilled water.

The following organic solvents: toluene, benzene, *o*-xylene (Special Cleanness, Merck, Germany) were studied for the extraction procedure.

Universal buffer solution (pH = 9) was prepared by mixing of calculated volumes of 0.04 mol×L^{-1} H_3BO_3, 0.04 mol×L^{-1} CH_3COOH, 0.04 mol×L^{-1} H_3PO_4 and 0.20 mol×L^{-1} NaOH.

General procedure

The amounts of 3 to 60 μg of ketoprofen were introduced into 10 mL test tubes, and 0.5 mL of universal buffer solution of pH 9 and 1.5 mL of 1×10^{-3} mol×L^{-1} Astra Phloxin FF and 3.0 mL of 2 mol×L^{-1} solution of Na_2SO_4 were added. The solution was mixed well and diluted to 5 mL with water. Toluene (5 mL) was added to the flask and was shaken for exactly 1 min. The absorbance of the separated toluene layer was measured at 563 nm against that of the blank test.

Procedure for real samples

An amount of the tablet powder, capsule powder or gels equivalent to 50 mg of the anti-inflammatory agent was weighed (or measured) accurately and treated as described above for the standard drug solution. Filtration was performed when insoluble matter remained during preparation of the sample solutions.

RESULTS AND DISCUSSION

Investigation of the Astra Phloxin FF – ketoprofen interaction

The investigation of the Astra Phloxin FF in aqueous solutions shows what they have intensive coloring (ε = 1.43×10^5 L×mol^{-1}×cm^{-1}) with a maximum of absorption at 538 nm (Figure 2) in neutral media. The coloring of the dye fades at high acidity or alkalinity of media. Such transformations are caused by protolysis processes that can be expressed as:

$$\begin{aligned} R^+ + H_2O &\leftrightarrow ROH + H^+ \\ R^+ + H_3O^+ &\leftrightarrow RH^{2+} + H_2O \end{aligned} \qquad (1)$$

Applicable protolysis constants equal:

$$K_1 = \frac{[R^+] \cdot [H_3O^+]}{[RH^{2+}]} \qquad (2)$$

and

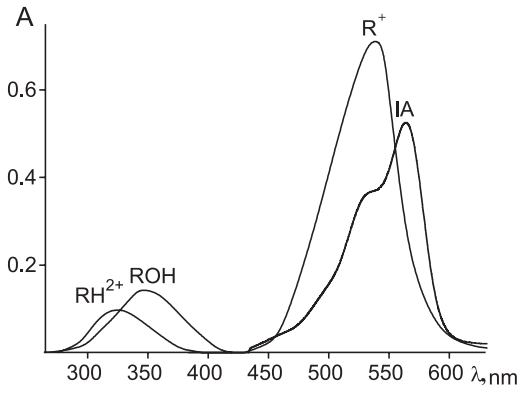

Figure 2. The absorption spectra of: Astra Phloxin FF 5×10^{-6} mol ×L^{-1} aqueous solution in different forms and ion associate of ketoprofen (2×10^{-5} mol×L^{-1}, 3.0×10^{-4} mol×L^{-1} Astra Phloxin FF, pH 9) with Astra Phloxin FF in toluene.

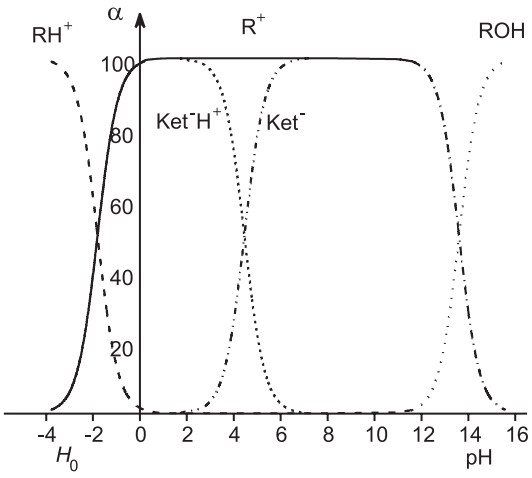

Figure 3. Distribution diagram of Astra Phloxin FF and ketoprofen.

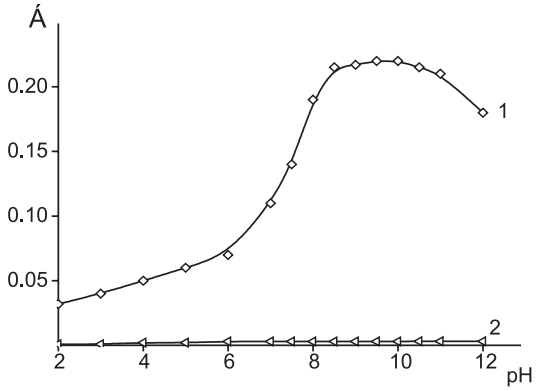

Figure 4. Influence of pH of aqueous phase on the IA formation and extraction (1 - A_k, 2 - A_o). 4×10^{-5} mol×L^{-1} ketoprofen, 3×10^{-4} mol×L^{-1} Astra Phloxin FF ; extractant - toluene, λ = 563 nm, l = 0.3.

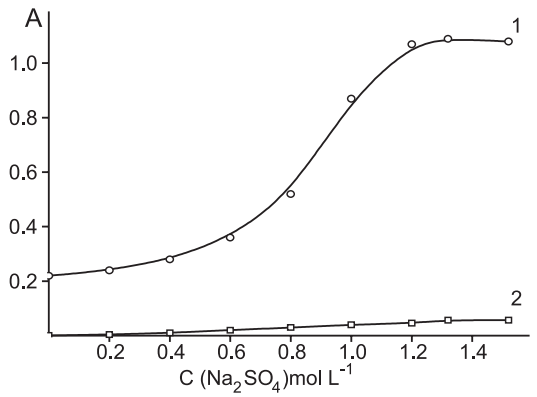

Figure 6. Influence of Astra Phloxin FF concentration on the IA formation and extraction (1 - A_k, 2 - A_o). pH 9; 4×10^{-5} mol×L^{-1} ketoprofen; 1.2 mol×L^{-1} Na_2SO_4; extractant - toluene; λ = 563 nm, l = 0.3.

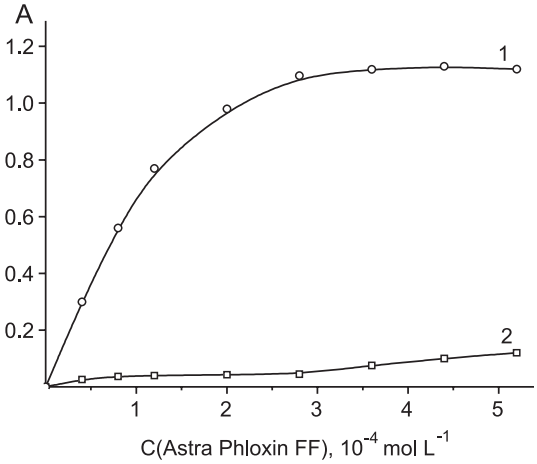

Figure 5. The effect of Na_2SO_4 on the IA formation and extraction (1 - A_k, 2 - A_o). 4×10^{-5} mol×L^{-1} ketoprofen, 3×10^{-4} mol×L^{-1} Astra Phloxin FF; pH 9; extractant - toluene, λ = 563 nm, l = 0.3

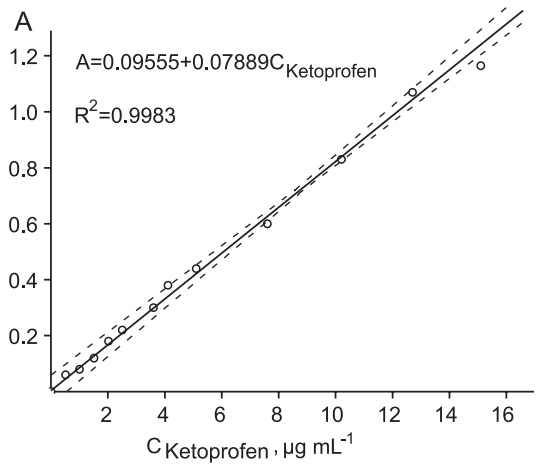

Figure 7. Calibration curve for the ketoprofen determination. pH 9, 3×10^{-4} mol×L^{-1} of Astra Phloxin FF, 1.2 mol×L^{-1} Na_2SO_4; extractant - toluene, λ = 563 nm, l = 0.3 cm.

$$K_1 = \frac{[ROH] \cdot [H_3O^+]}{[R^+]} \qquad (3)$$

The protolysis constants were determined by spectrophotometric method. Series of samples with different acidity of medium were prepared. Figure 2 shows that absorbance of Astra Phloxin FF solutions depends on the media acidity. Three maxima correspond to the different forms of a dye – ionic R^+ (538 nm), protonated HR^{2+} (325 nm) and hydrolyzed ROH (345 nm). The protolysis constants for Astra Phloxin FF are K_1 = –1.81 and K_2 = 13.60. Figure 3 shows the protonation of the Astra Phloxin FF.

The preferred existence ranges of the reactive species of the dye (singly-charged cation) and of ketoprofen (singly-charged anion) depend on many factors, particularly on the medium acidity. Taking into account the respective protolysis constants that determine the equilibria in the solutions of these compounds, the distribution diagram (Figure 3) of different forms of the dye and ketoprofen at different pH were calculated based on the Astra Phloxin FF protolysis constants and the pK of ketoprofen (4.45).

One can observe that in a range at 7–11, the singly-charged forms of the dye and of ketoprofen are preferred. This is the range of the formation of the IA of ketoprofen and Astra Phloxin FF.

Table 1. The main characteristics of extracted IA of Ket with Astra Phloxin FF (A_k is the absorbance of IA, A_0 is the absorbance of a blank test, R is the distribution coefficient)

Organic solvent	λ_{max} [nm]	$\varepsilon \times 10^{-4}$ $L \times mol^{-1} \times cm^{-1}$	A_k/A_o	R %
Toluene	563	7.6	12.4	98.9
Benzene	560	5.3	6.3	93.4
o-Xylene	563	6.4	8.7	97.6

Table 2. The influence of foreign ions and substances on the studied system.

Foreign ion or substance	Multiple quantity
Cl^-	2000
Br^-	250
I^-	interfere
NO_3^-	600
SO_4^{2-}	30000
PO_4^{3-}	10000
SCN^-	60
CH_3COO^-	200
glucose	150
glycine	150
histidine	130
aspirin	10
salicylate	5

Therefore, the necessary condition for the extraction of this IA into an organic phase is the pH range of the aqueous phase from 7 to 11.

Determination of optimal conditions of the formation and extraction of the ion associate of ketoprofen and Astra Phloxin FF

To determine the optimal conditions of the formation and extraction of the ion associate of ketoprofen and Astra Phloxin FF, the effect of acidity of the medium, of the dye concentration, of the solvent nature and other factors were investigated.

Effect of pH

One of the defining factors of the process of the formation of ion associates is the concentration of hydrogen ions in solution. It is clearly understood that the acidity of the aqueous phase substantially

Table 3. Comparison of main analytical parameters of various spectrophotometric methods for the determination of sodium ketoprofen

Analytical reagent	Organic solvent	pH	λ_{max} [nm]	ε, 10^{-4} $L \times mol^{-1} \times cm^{-1}$	Range of Beer's law [$\mu g \times mL^{-1}$]	Detection limit [$\mu g \times mL^{-1}$]	Disadvantages
Methylene Violet (11)	Chloroform	7.4–8.0	540	0.61	2.5 – 20	0.042	Low sensitivity
o-Chloranil (12)	Chloroform	–	530	–	10 – 80	< 10	Some important parameters are unknown
N-bromosuccinimide, 2,2 -diphenyl-1-picryl hydrazine (13)	–	< 7	520	0.315	5 – 80	–	Some important parameters are unknown
Proposed work with Astra Phloxin	Toluene	8.0– 10.8	563	7.6	0.8 – 16.0	0.037	–

Table 4. Determination of ketoprofen in different dosage forms (n = 5, average)

Sample	Form	Pharmaceuticals composition (auxiliary substances)	Label amount [mg]	Found by proposed method, [mg]	Recovery, %
Ketonal®	Tablet	Lactose	50	49.4 ± 0.6	98.8
	Tablet	Propylene glycol, benzyl alcohol, NaOH, purified water	100	99.4 ± 0.6	99.4
	Gel	Methylhydroxybenzoate, propylhydroxybenzoate, isopropyl myristate, with vaseline	50	49.8 ± 0.4	99.6
	Gel	Methylhydroxybenzoate, propylhydroxybenzoate, isopropyl myristate, with vaseline	25	24.7 ± 0.3	98.8
Ketonal Forte	Tablet	Lactose	100	99.7 ± 0.3	99.7
Ketonal retard	Tablet	Microcrystalline cellulose	150	149.3 ± 0.7	99.5
Ketonal duo	Capsule	Microcrystalline cellulose	150	149.4 ± 0.6	99.6
F-Gel	Gel	Carbopol 940 or carbopol 980 NF, lavender oil, nerol oil, C_2H_5OH 96%, nipagin, thrometamol, purified water	25	24.6 ± 0.4	98.4
Fastum® Gel	Gel	Carbopol 940, C_2H_5OH, nerol oil, lavender oil, triethanolamine, purified water	25	24.7 ± 0.3	98.8
Lipicard	Capsule	Polyoxile 40, starch, talcum, SiO_2	200	199.2 ± 0.8	99.6

affects the reaction ability of reaction participants, both the basic dye and ketoprofen. Therefore the favorable formation and extraction of IA to the organic phase requires the conditions of the domination of the reactive species of the dye cation (R^+) and of the organic anion (Ket^-) (Eq. 4-6).

$$R^+ + Ket \leftrightarrow R^+ \times Ket^- \quad (4)$$

$$R^+Cl^- \leftrightarrow R^+ + Cl^- \quad (5)$$

$$Ket^- + R^+ \leftrightarrow (Ket^-) \times (R^+)\downarrow \quad (6)$$

To establish the optimum pH range, ketoprofen was mixed with Astra Phloxin FF in aqueous solution with pH values from 2 to 12, and the IA extract absorbances were measured. Figure 4 shows that the absorbance increases and reaches a maximum plateau at 8.0 – 10.8 pH range. Hence, a pH of 9 was used in all the subsequent experimental work. As the shape of the absorption curve and the position of the absorption maximum do not vary with pH, it was assumed that only one type of complex is formed within this pH range.

We theoretically predicted the possibility of the extraction of the ion associate of ketoprofen and Astra Phloxin FF for pH range 7 – 11 (Figure 3). Experimental data illustrate the necessary pH range 8.0 – 10.8 (Figure 4) which agrees well with the theoretical data. Somewhat narrower experimental pH region may be probably explained by the influence of the extraction process itself.

Effects of salting-out agent

Salting-out agents are known to improve extraction by organic solvents (17). The influence of Na_2SO_4 as salting-out agent on the IA formation was studied. The result shows that Na_2SO_4 indeed exhibits salting-out effect in this system (Figure 5).

Effect of Astra Phloxin FF concentration

To establish the optimum Astra Phloxin FF content, the solution absorbance was plotted as a function of Astra Phloxin FF concentration. The absorbance of the system increased in the concentration range of $(0.0 – 5.0)\times10^{-4}$ mol×L^{-1} and was practically constant in $(3.0 – 5.0)\times10^{-4}$ mol×L^{-1} range. The dependence is described by the saturation curve (Figure 6). Thereafter, 3.0×10^{-4} mol×L^{-1} of Astra Phloxin FF solution was used as the optimum concentration.

Effects of organic solvents

An apt selection of the organic solvent is very important for the extraction due to the direct relation between certain solvent properties and their extraction (dissolving) ability (18). Additionally, the solubility of the substance in the organic solvent should be taken into account, this depends on the nature of the substance. However, the effect of the solvent is different for each system, and there are no reliable criteria for the extraction solvent selection for various systems. Extraction ability of a solvent can be related to the presence of certain functional groups in the solvent molecule.

Aliphatic and aromatic (and halogen-substituted) hydrocarbons and certain acetic acid esters were investigated as extracting solvents. The absorption spectra of the extracted IA colored complex of ketoprofen with Astra Phloxin FF in toluene, benzene, *o*-xylene were measured over 400 – 750 nm. The complex shows the maximum absorbance at 560 – 563 nm which can therefore be used for analytical purposes. This wavelength was used for all subsequent measurements. The absorption spectra were recorded under optimal complex formation and extraction conditions. The absorption spectra from the dye and from the ion associate of ketoprofen practically do not differ. Changes in the position of the maximum help to explain the solvatochromic effect observed which provides evidence of the formation of complex compounds of the ion associate type. The maximum production and extraction of complex was attained after 1 min. The best results were obtained for toluene.

Table 1 shows the main spectrophotometric characteristics of these systems.

A successful process of the ion pair extraction requires also the knowledge of the chemistry of complex formation, the study and the prediction of spectrophotometric and extraction properties of the products formed in the chemical reaction. The photometry reduces such problems to the determination of the composition of the compounds extracted, of their stability and spectrophotometric characteristics. The IA composition was determined by two methods: isomolar series and equilibrium shift. The results obtained by these methods agree well. It was determined that the molar ratio ketoprofen : Astra Phloxin FF in the formed IA is 1:1. This means that the ion associate contains singly-charged ketoprofen and dye cations.

Selectivity

The interference of possible coexisting substances was studied. The sample containing 2×10^{-5} mol×L^{-1} ketoprofen and various concentrations of foreign substances were added into the system. The tolerance limit was taken as the concentration causing an error of not more than ± 5% in the determination of ketoprofen. The results obtained are shown in Table 2. One can see that the proposed method is selective in the case of excipients commonly used in pharmaceuticals, the components of human urine and other common drugs assayed.

Linearity

A calibration plot was obtained under the optimum experimental conditions (pH 9, 3.0×10^{-4} mol×L^{-1} Astra Phloxin FF, 1.2 mol×L^{-1} Na_2SO_4; extractant – toluene; λ = 563 nm, l = 0.3). The Beer's law is obeyed in relation to the concentration of ketoprofen over the range of 0.8 – 16.0 μg×mL^{-1}. The calibration equation is A = 0.09555 + 0.07889C (R = 0.9983; RSD = 0.0237), where A and C are absorbance and the concentration ketoprofen (μg×mL^{-1}), respectively (Figure 7).

Data of Table 3 show that the method reported here has higher sensitivity than other spectrophotometric methods (11-13).

Application

To demonstrate the applicability of the proposed method to the determination of ketoprofen, the method was applied to the analysis of ketoprofen in some samples of pharmaceutical preparations.

Table 4 shows the results of ketoprofen determination in pharmaceutical preparations by spectrophotometric method with the reagent Astra Phloxin FF.

Acknowledgments

This work has been supported by the Ministry of Education and Science of Ukraine (Project 0108U003119); International Vishegrad Fund (No. 997015) and Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences (Grant VEGA No. 1/4450/07) and Slovak Research and Development Agency (Project APVV SK-UA-0002-07).

REFERENCES

1. Gentili A.: Anal. Bional. Chem. 387, 1185 (2007).
2. Kazymyrko V.K., Ivanytska L.M., Davydov I.A.: Ukr. Rheumat. J. 4, 34 (2000).
3. Shuba N.M., Kazymyrko V.K., Ivanytska L.M., Davydov I.A.: Ukr. Rheumat. J. 2, 36 (2000).
4. Kovalenko V.N., Bortkevych O.P., Prostenko H.A.: Ukr. Rheumat. J. 23, 17 (2006).

5. Lenik J., Wardak C., Marczewska B.: Acta Pol. Pharm. Drug Res. 63, 239 (2006).
6. Fan Y, Feng Yu-Qi , Da Shi-Lu Wang, Zhong-Hua: Talanta 65, 111 (2005).
7. Herraez-Hernandez R., Merbel N.C., Brinkman U.A.Th.: J. Chromatogr. B 666, 127 (1995).
8. Martin M.J., Pablos F., Gonzalez A.G.: Talanta 49, 453 (1999).
9. Dvořák J., Hájková R., Matysová L., Nováková L., Koupparis M.A., Solich P.: J. Pharm. Biomed. Anal. 36, 625 (2004).
10. Özlü C., Basan H., Ştana E., Ertaş N., Göğer N.G.: J. Pharm. Biomed. Anal. 39, 606 (2005).
11. Sastry C.S.P., Prasad Tipirneni A.S.R., Suryanarayana M.V.: Analyst 114, 513 (1989).
12. El-Sadek M., El-Adl S., Afiou-Kull M., Sabb S. M.: Talanta 40, 585 (1993).
13. Emara K. M., Ali A.M.M., Maalin A.E.: Talanta 41, 639 (1994).
14. El-Saharty Y.S., Metwally F.H., Refaat M., El-Khateeb S.Z.: J. AOAC Int. 90, 102 (2007).
15. Blanco M., Coello J., Iturriaga H., Maspoch S., Alaoui-Ismaili S.: Fresenius J. Anal. Chem. 357, 967 (1997).
16. Hu J.H., Zhu Y., Xue P.H.: Acta Pharm. Sinica 32, 542 (1997).
17. Morrison G. H., Freiser H.: Solvent extraction in analytical chemistry. MIR, Leningrad, 1960 (in Russian).
18. Korenman I. M.: Extraction in the Organic Substance Analysis. Khimija, Moscow, 1977 (in Russian).

Received: 01. 04. 2008