www.sphinxsai.com

PharmTech
International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.1, pp 89-96, Jan-Mar 2010

Identification of Ketoprofen in Drug Formulation and Spiked Urine Samples by Micellar Thin Layer Chromatography and its Quantitative Estimation by High Performance Liquid Chromatography

A. Mohammad*[1], S. Sharma[2] and S.A. Bhawani [3]

[1, 2] Analytical Research Laboratory, Department of Applied Chemistry, Faculty of Engineering & Technology, Aligarh Muslim University, Aligarh-202002, INDIA

[3] School of Industrial Technology, University Sains, Plau Penang, Malaysia

*Corresponding author: alimohammad08@gmail.com

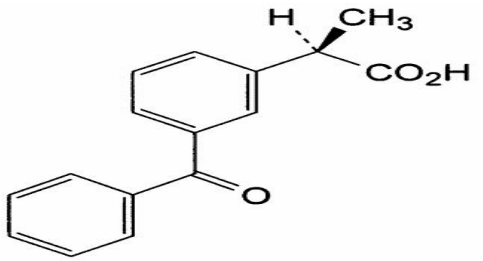
Abstract: A simple, selective and economical micellar thin layer chromatographic method for on-plate identification of ketoprofen from pure, pure, formulated and spiked urine samples was developed. The proposed method involves use of amino acid impregnated silica gel layers as stationary phase with mixed micelles (0.5% aqueous solutions of sodium dodecyl sulphate plus Triton X-100 and acetone (8:5:1.5, v/v) as mobile phase. The nature as well as the concentration of surfactant influences the mobility of ketoprofen. The interference study was carried out using various organic and inorganic metabolites, usually found in human urine. The HPLC determination of ketoprofen (formulated and spiked urine) samples carried out at λ=270 nm with mobile phase comprising of acetonitrile: double distilled water: acetic acid (1:1:1, v/v). The correlation coefficient was 0.99 and the recoveries of ketoprofen (formulated and spiked urine) were within range of 94.0-100.2% with relative standard deviation ranging from 0.6-0.86%.
Keywords: Ketoprofin, TLC, Surfactant, Urine

1. Introduction

Ketoprofen (Fig: 1) [RS-2-(3-benzoyl- phenyl) propionic acid], is a non steroidal anti-inflammatory drug[1]. It is used in musculoskeletal, joint disorders, dysmenorrheal, postoperative pains and gouts[2]. Ketoprofen has been analysed by different techniques viz: HPLC[3,4], HPFA[5], GCMS [6,7] and spectrophotometry[8] But these techniques require highly specified analytical samples, due to which these techniques are not found too appropriate in the analysis of drugs from biological samples[9].

Thin layer chromatography (TLC) enables a simple fast and effective separation of the complex mixtures present in various biological samples enzymes[10], porphyrins[11], alkaloid and drugs[12] from urine samples. Surfactant-modified thin layer chromatography (TLC) has found wider applications in separation studies[13-16]. It provides enhanced selectivity as a result of difference in the degree of binding of separated mixture components with mobile and stationary phases. The selective solubilization of mixture components with micelles is caused by complex electrostatic, hydrophobic, donor-acceptor and polarization interactions. All types of surfactants (cationic, anionic and nonionic) have been used in the mobile phase for the successful separation of vitamins[17], amino acids[18]. TLC proved its applicability during successful separation of bio-active amines, penicillin's and steroids[19].

Fig. 1: Structure of ketoprofen.



Recently the usefulness of mixed surfactants systems containing ionic - nonionics or mixed-ionic micelles has been realized by physical chemists[20,21]. Mixed surfactants often exhibit synergism in their physicochemical properties and sometimes offer superior performance compared to the pure surfactant components. Thus the coupling of anionic and nonionic surfactants will provide a novel thin layer chromatographic system for excellent resolution of ketoprofin from human urine as well as drug formulations. The human urine analysis for identification of selected drugs and their metabolites has emerged as an important investigation tool in forensic drug analysis[22]. This report is an attempt in the direction of developing a simple and reliable method for on plate identification and quantification of ketoprofen in pharmaceutical formulations as well as from human urine samples, for this purpose, several approaches have been adopted to develop new sorbent phases to realize a desired separation with a particular mobile phase. Bhushan and Parshad,[23] have achieved separation of two isomers of ketoprofen from drug samples on silica gel layers impregnated with L-arginine. Literature survey revealed that no information is available on the use of differentially charged amino acids impregnated silica gel layers with mixed micellar solvent systems for analysis of ketoprofen from formulated and human urine spiked samples. On the basis of above facts it was consider as worthwhile to study the mixed surfactant promoted TLC for identification of ketoprofen drug from formulations and spiked human urine samples

2. Experimental

2.1 Apparatus

A TLC applicator (Toshiwal, India) was used for coating silica gel on 20cm× 3cm glass plate. High Performance liquid chromatograph by Able & Jasco with PU-1580 Pump, UV-1575 detecting module, Rheodyne injector, Neulosil 150 C-18, 5µm, 150×4.6 mm chromatographic column. A glass sprayer was used to locate the position of spot of analyte. A pH meter (Elico, India) was used.

2.2 Chemical and Reagents

Silica Gel 'G', t- octyl phenoxydacaethoxy ethanol (TX-100), sodium dodecyl sulfate (SDS), methanol, ethanol, K_4FeCN_6, $FeCl_3$ and acetonitrile (HPLC-grade) were obtained from Merck Reagents, Mumbai, India. Iodine, isoamyl-alcohol and ethylene dichloride were from CDH India. Ketoprofen pure and formulation were from Lark Labs, Bihwadi, India.

2.3. Preparation of Test Solution

Ten tablets equivalent to 50 mg of the ketoprofen were powdered and transformed into a 50 mL standard flask and dissolved with 50 mL of acetonitrile l followed by stirring. The solution was filtered with Wattman No. 41 and the volume was make upto mark with acetonitrile. The solution contains 1mg/ mL of drug.

2.3.1. Extraction of ketoprofen from human urine samples

2.3.2. Preparation of spiked drug urine samples –

A sample of Urine was taken from a healthy person. Take 50 mL of this urine sample and adjusted to pH 7-8 with 2% bicarbonate solution. Add 50 mg of ketoprofen drug powder to the urine sample. The resulting mixture was kept for shaking (20 minutes) at room temperature (15 - 20^{0}C) for achieving complete dissolution of drug in urine.

2.3.3. Isolation of drug from spiked drug urine samples

The 50 ml of spiked urine sample was filtered with whatman filter paper (No-41) and then the filtrate was mixed with 200 mL of ethylene dichloride containing 10% of isoamyl alcohol and 10 % diethyl ether which was kept for shaking for 15 min. After this the organic layer was separated and evaporated to obtain ketoprofen drug as residue. The residue was diluted with 50 ml acetonitrile. The chromatography of the extracted drug (ketoprofen) was performed on best proved chromatographic system and the R_F value of ketoprofen from urine is compared with R_F value of pure ketoprofen drug.

2.3.4. Chromatography

2.3.5. Mobile phases

The following solvent systems **(Table 1)** were used for the chromatography.

2.4. Stationary phase

The following **(Table 2)** were used as a stationary phases.

2.5. Detector

Mixture of 5% aqueous solution of K_4FeCN_6 and $FeCl_3$ (1:1, v/v/) were used as detector.

2.5.1. Preparation of TLC plates

(a) Plain Silica gel TLC plates-

The TLC plates were prepared by mixing silica gel G with double distilled water in 1:3 ratio by weight with constant shaking to obtain homogeneous slurry. The resultant slurry was applied on the glass plates with the help of a manual applicator to give a 0.25 mm-thick layer. The plates were dried at room temperature and then activated at 100 ± 2 ^{0}C by heating in an electrically controlled oven for one hr. The activated

plates were stored in a close chamber at room temperature until used.

(b) Amino acid impregnated TLC plates**:**

Impregnated TLC plates were prepared by mixing silica gel with aqueous amino acids solution in 1:3 ratios with constant stirring until homogeneous slurry was obtained. Thin layers of resultant slurry were prepared by following the method as described in above section (a).

2.5.2. Chromatographic procedure

Test solutions (10 µL) were applied on (15 × 3 cm) silica gel G thin layer plates with the help of micropipette at about 2 cm above the lower edge of the plates. The solvent ascent was fixed to 10 cm in all cases for the determination of R_F values of all individual drugs. Linear ascending development was carried out in a vapor equilibrated TLC twin trough chamber. The optimized chamber saturation time for the mobile phase was 15 min at room temperature (25 ± 1^0C). Subsequent to the development, TLC plates were dried at room temperature. The plates were then detected by using iodine vapors and dragondroff solution as chromogenic reagents and the drugs is visualized as colored spots. The R_F values of drug were determined by the

Following relation –

$$R_F = 0.5 (R_L + R_T)$$

Where $R_L = R_F$ of leading front.

$R_T = R_F$ of trailing front.

2.5.3. Interference

For investigating the interference of various organic and inorganic metabolites like sodium and potassium salts, urea, liquor ammonia nitrate and glucose normally found in human urine samples, on ketoprofen were studied. An aliquot (5 µL) of drug were spotted on silica gel TLC plate followed by spotting of 5µL of the interfering species (1mg/mL) on the same spot. The chromatography was performed on amino acid impregnated silica gel layer (S_3) with solvent system M_{14}. The spots were detected and the R_F values of drug were calculated and compared.

2.5.4. Quantitative determination of ketoprofen from spiked human urine samples

The HPLC technique was applied for the quantitative determination of on plate identified ketoprofen drug from spiked human urine samples. For this purpose 10 µL of ketoprofen solutions of different concentration (10-30 µg) were spotted on TLC plates. After complete drying of the spots, the TLC plates were developed with solvent system M_{14}. At the same time, a pilot plate was also run simultaneously to locate the position of spot. After development the area corresponding to spot was scarped from the plate. The scraped silica powder was mixed with 2 mL of acetonitrile for the extraction of ketoprofen from adsorbent. After that, it was filtered with Whatman filter paper no-41. In order to ensure complete extraction of drug the adsorbent was again washed with 3 mL of acetonitirile. All the filtrate was collected in a test tube and analyzed at λ=270 nm with mobile phase comprising of acetonitrile: double distilled water: acetic acid (1:1:1) by HPLC. A calibration curve was plotted between the Peak area vs Concentration of ketoprofen drug. The content of ketoprofen in the formulated and urine spiked samples was determined from the standard curve by five replicate readings under similar conditions.

3. Results and Discussion

The results obtained are summarized in Tables I-II. Initially the mobility of ketoprofen was examined on plain silica gel G layers with different concentrations of aqueous solutions of anionic (SDS), nonionic (TX-100) and anionic-nonionic mixed surfactants as mobile phases. The selected best proved mobile phase were utilized for optimization of experimental conditions on the basis of mobility of ketoprofen with various factors, such as charge and concentration of amino acids used as impregnates of stationary phase, nature and concentration of added alkanols, acetone and weak acid in the micellar mobile phases and interference effect due to various organic and inorganic metabolites normally found in urine samples.

3.1 Effect of type and concentration of surfactants

The chromatography of ketoprofen was performed on S_1 plain silica gel layers using distilled water and aqueous solutions of anionic, nonionic and mixed surfactants as mobile phase systems (M_1- M_{11}). The results of the effect of type and concentration of different classes of surfactants are presented in Table I. The following conclusions are drawn from the data listed in Table I.

(1) In double distilled water (zero concentration of surfactant), the drug ketoprofen was not detected

(2) The mobility of ketoprofen decreases with the increase in concentration of SDS in the solvent systems.

(3) Reverse trend was observed in case of TX-100 as compared to SDS, the mobility of ketoprofen increases with the increase in concentration of TX-100 in solvent system.

(4) In case of mixed surfactant-mediated mobile phase systems (M_9 - M_{11}), initially the mobility of ketoprofen increases with the increase in concentration of surfactant but on further increase in concentration causes a slight decrease in mobility for ketoprofen drug.

It may be concluded from the present study that in case of SDS, the micellar thin layer chromatography is involved. The surfactant in the solvent system occurs in both the micellar and ionic forms. In this case

concentration of surfactants in the mobile phase leads to an increase only in the concentration of micelles in MMPs and the concentration in the stationary phase remains nearly constant. This may result in decrease in retention of adsorbates. While in case of SDS and TX-100, the ion-pair TLC situation is observed. The mobile phase in the system contains only ions of a surfactant. An increase in their concentration in the mobile phase increases the concentration of surfactant ions adsorbed on the stationary phase. As a result the retention of adsorbates increases[24].

3.2 Effects of different solvent system modifiers

The different alcohols (methanol and ethanol) were added to 0.5% mixed aqueous SDS and TX-100 mobile phases (M_{10}) for the chromatography of ketoprofen on plain silica gel layer. From the results presented in Table I, it is clear that the presence of traces of alcohol in mixed micellar mobile phase increases the mobility of ketoprofen drug but mobility decreases with increase in the chain length of alcohols. This may be due to the fact that addition of alcohols in the micellar solvent systems may result in the less population of surfactants molecules on the adsorbent and this may provide some free silanol groups on the silica surface for the adsorption of ketoprofen[25]. This may cause the increase in the retention of ketoprofen on the silica surface and hence decreases the mobility.

From Table I and Fig. 2, it is clearly concluded that acetone containing mixed aqueous surfactant solvent system (M_{14}) offers better chromatographic performance by providing more compact and well defined spots for ketoprofen drug at optimum R_F range (0.62). Thus it is selected as the optimum mobile phase for further studies on ketoprofen drug.

3.3 Effect of type and concentration of amino acids used as impregnants

In order to investigate variation in mobility pattern during chromatography of ketoprofen drug was performed on variably charged amino acids (alanine, arginine and glutamate) modified silica gel G layers (S_2-S_{10}) developed with M_{14} solvent system.

The results summarized in Table II, shows that-

(1) When L-arginine was used as an impregnant, a diffused and compact spot of ketoprofen drug was observed. This may be due to partial conversion of surface silianols to a new organic functional surface that acquires organophillic properties which enhances the surface adsorption[26].

(2) In case of glutamte impregnated silica gel layers no spot was detected for ketoprofen drug.

(3) No mobility was observed for ketoprofen drug when alanine impregnated silica gel layers were used during chromatography.

3.4 Identification of ketoprofen in presence of impurities

To widen the applicability of the developed TLC system (amino acid impregnated silica gel layer (S_3) with 0.5% mixed aqueous SDS and TX-100 mobile phases (M_{14}) for identification and isolation of ketoprofen from formulated drug and urine samples in presence of various metabolites normally found in urine are presented in Table III. It is clear that sodium and potassium salts do not affects the mobility of drug, but in case of urea and liquor ammonia a long trailing spot was observed. In case of nitrate and glucose no spot was detected possibly due to complex formation with drug.

3.4.1 Identification of ketoprofen from human urine samples

The proposed chromatographic method (arginine impregnated silica gel layers (S_3) with 0.5% mixed aqueous SDS and TX-100 mobile phases (M_{14}). successfully identified the ketoprofen drug extracted from the human urine samples on the basis of R_F value. The spots of the formulated and the urine extracted ketoprofen samples are shown in Fig. 2**.** Thus the proposed method is very sensitive for the identification of ketoprofen from urine samples.

3.4.2 TLC-HPLC of ketoprofen:

The analytical parameters for the HPLC determination of ketoprofen by the proposed method are given in Table IV. The linear response of the detector obtained for the concentrations are between 0.1 and 0.005 mg/mL, with a correlation coefficient of 0.9997. These values suggested that the proposed method is very sensitive for the determination of ketoprofen from formulation as well as spiked urine samples. The accuracy and validity of the proposed method were ascertained by performing recovery studies. The recovery studies of pure, formulated and urine spiked ketoprofen samples at different concentrations indicates that the recovery was good. The percentage recovery values were found in the range 97.0 -100.2 % with relative standard deviation of less than 2%.

4. Application

The practical utility of proposed method was examined on commercially available formulation and spiked human urine samples and it was concluded that the present method works well in identification of ketoprofen in pharmaceutical formulations as well as from human urine samples.

Table I: R_F value of ketoprofen on silica gel G layers developed with mobile phases M_1- M_{18}.

Code	Mobile phase	R_F value	
		Ketoprofen Pure	Ketoprofen Fomulation
M_1	Double distilled water	ND	ND
M_2	0.2% Aqueous SDS	ND	ND
M_3	0.5% Aqueous SDS	0.43(T)	0.44 (T)
M_4	1.0% Aqueous SDS	0.40 (T)	0.38 (T)
M_5	0.2% Aqueous TX-100	0.56 (T)	0.59(T)
M_6	0.5% Aqueous TX-100	0.61	0.60
M_7	1.0% Aqueous TX-100	0.64	0.62
M_8	M_2 + M_5 (1:1)	0.66	0.64
M_9	M_3 + M_6 (1:1)	0.69	0.69
M_{10}	M_4 + M_8 (1:1)	0.73	0.75
M_{11}	M_{10} + Acetone (9:1)	0.59	0.57
M_{12}	M_{10} + Acetone (8.5:1.5)	0.62	0.63
M_{13}	M_{10} + Acetone (8:2)	0.64	0.68
M_{14}	M_{10} + Methanol (9.5:0.5)	0.75	0.71
M_{15}	M_{10}+ Ethanol (9.5:0.5)	0.79	0.80
M_{16}	M_{10} + Formic acid (9.5:0.5)	0.57 (T)	0.58 (T)
M_{17}	M_{10} + Acetic acid (9.0.5:0.5)	ND	ND
M_{18}	M_{10} + Formamide (9.5:0.5)	0.39 (T)	0.41 (T)

ND = Not detected , T = Trailing

Table II: R_F value of ketoprofen on silica gel layers (S_1-S_{10}) developed with mobile phases M_{14}.

Code	Stationary phase	R_F value	
		Ketoprofen Pure	Ketoprofen Fomulation
S_1	Plain silica gel 'G'	0.63	0.59
S_2	Silica gel 'G' slurry in 0.2% arginine	0.57	0.61
S_3	Silica gel 'G' slurry in 0.6% arginine	0.61	0.63
S_4	Silica gel 'G' slurry in 1.0% arginine	0.64	0.60
S_5	Silica gel 'G' slurry in 0.2% alanine	0.06 (T)	0.02
S_6	Silica gel 'G' slurry in 0.6% alanine	0.00	0.00
S_7	Silica gel 'G' slurry in 1.0% alanine	0.00	0.00
S_8	Silica gel 'G' slurry in 0.2% glutamate	ND	ND
S_9	Silica gel 'G' slurry in 0.6% glutamate	ND	ND
S_{10}	Silica gel 'G' slurry in 1.0% glutamate	ND	ND

ND = Not detected ,T = Trailing

Table III: Effect various organic and inorganic metabolites found in urine on mobility (R_F) of ketoprofen drug.

Impurity	R_F Value
NaCl	0.61
KCl	0.63
Urea	0.66 (T)
Liquor Ammonia	0.69 (T)
Nitrate	ND
Glucose	ND

T = Trailing, ND = Not detected

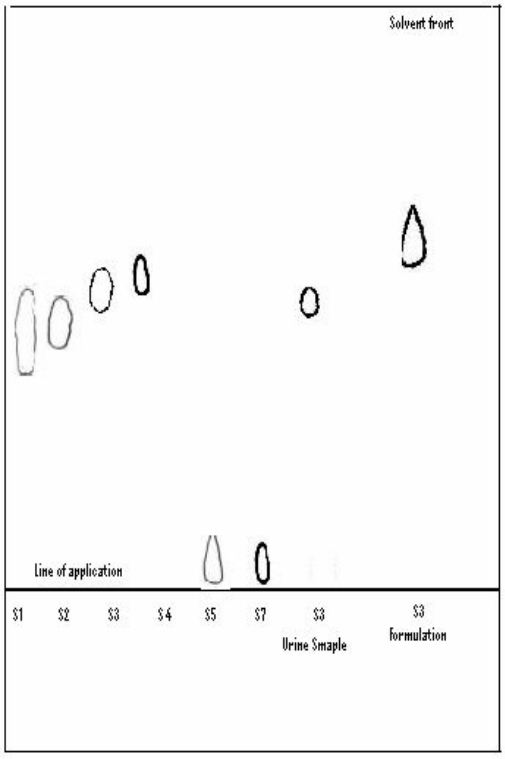
Table IV: Statical data for HPLC analysis of Ketoprofen drug.

Limit of detection (mg/mL)- 0.00003				
Limit of quantification (mg/mL)- 0.00056				
Recovery				
(1) Formulation	Amount taken	Amount found	Percent	RE
	0.004	0.0397	99.25	0.37
(2) Urine sample	0.04	0.0379	94.75	0.62
Concentration of Ketoprofen (mg/mL)		Peak area (mAU/ sec)		RSD %

Limit of detection (mg/mL)- 0.00003				
Limit of Quantification (mg/ mL)- 0.00056				
Recovery				
	Amount Taken	Amount found	Percent	RE
(1) Formulation -	0.04	0.0397	99.25	0.37
(2) Urine sample-	0.04	0.0379	94.75	

Concentration of ketoprofen (mg/mL)	Peak area (mAU/sec)	RSD %
0.005	551918	0.6
0.01	1116049	0.86
0.02	2300671	0.46
0.05	5585673	0.82
0.1	11142927	0.66

Fig. 2: Chromatogram showing identification of ketoprofen in formulated and spiked urine samples on (S_3) silica gel layers developed with mobile phase M_{14}.



5. Acknowledgment

The author's are thankful to the Chairman, Department of Applied Chemistry for providing research facilities to perform the work and also to Lark Labs India Ltd for providing gift samples of ketoprofen and HPLC analysis facility at their WHO certified analytical research lab. One of the authors (S. Sharma) is also thankful to the University Grant Commission, New Delhi for providing financial assistance.

References

[1] British Pharmacopea, 2007. Vol 2nd, Deptt of health and public safety, U.K.

[2] Parfitt K., Martinadele (the extra Pharmacoepia) 32nd ed, The Pharmaceutical Press, London, (1999), 48-49.

[3] Zakeri Milani P., Barzegarjalai M., Tajerzadeh H, Azarmi, Valizadeh Y, Simultaneous determination of naproxen, ketoprofen and phenol red in samples from rat intestinal permeability studies: HPLC method development and validation, J. Pharma-Biomed Anal., 2005, 39, 624- 630.

[4] Chawla S., Ghosh S, Shorkar V, Nellore R, Kumar R, Srinivas N.R., High-performance liquid chromatography method development and validation for simultaneous determination of five model compounds, antipyrine, metoprolol, ketoprofen, furosemide and phenol red, as a tool for the standardization of rat in situ intestinal permeability studies using timed wavelength detection, J. Biomed. Chromatogr. 2005, 20 349-357.

[5] Qiao M.X., Guao X.J., Li F.M., Sepu. 2001, 19, 329-331.

[6] Kosjek T., Heath E., Krbavcic A., Determination of non-steroidal anti-inflammatory drug (NSAIDs) residues in water samples, Environ Int., 2005, 3, 579-585.

[7] Lin W.C., Chen H.C., Ding W.H., Determination of pharmaceutical residues in waters by solid-phase extraction and large-volume on-line derivatization with gas chromatography-mass spectrometry, J. Chromatogr., 2005, 1065, 279- 285.

[8] Pomykalski A., Hopakala H., simulataneous determination of levomepromazine hydrochloride in pharmaceutical formulations, Acta- Pol Pharm, 2005, 62, 171-176.

[9] Raza A., Mahmood T.A., and Rehman A., Spectrophotometeric determination of lisinopril in pure and pharmaceutical formulations, J. Chin. Chem. Soci., 2005, 52, 1055- 1059.

[10] Wadman S.K., De Bree P.K., Duran M., De Jonge H.F., Detection of inherited adenylosuccinase by two dimensional TLC of urinary imidazoles, Adv. Exp. Med. Biol., 1986, 195, 21-25.

[11] Henderson M. J., Thin layer chromatography for diagnosis of porphyries, Clin. Chem., 1989, 35, 1043-1044.

[12] Tyrpien K., Bodzek P. and Manka G., Application of planar chromatography to the determination of cotinine in urine of active and passive smoking pregnant womens, J. Biomed. Chromatogr., 2001, 15, 50-56.

[13] Medina Hernandez M.J., Garcia Alvarez-Coque M.C., Solute-mobile pahse and solute-stationary phase interaction in micellar liquid chromatography, Analyst, 1992, 117, 831-837.

[14] Shtykov S.N., Sumina E.G., Smushkina E.V., Tyurina N.V., Dynamic and static modifications of stationary phases with surfactant in TLC: A comparative study, J. Planar Chromatogr., 2000, 13, 182-186.

[15] Sumina E.G., Shtykov S.N., Tyurina N.V., Surfactant in thin layer chromatography, J. Anal. Chem. 2003, 58, 720-730.

[16] Mohammad A., Iraqi E., Khan I.A., Use of non-ionic poly (ethylene glycol) p-isooctyl-phenyl ether (Triton X-100) surfactant mobile pahse in the thin layer chromatography of heavy metals cations, Chromatogr. Sci., 2002, 40, 1-8.

[17] Kartsova L.A., Koroleva O.A., Simulataneous determination of water and fat soluble vitamins by high performance thin layer chromatography using an aqueous micellar mobile phase, J. Anal. Chem. 2007, 62, 255-259.

[18] Kartsova L.A., Strel nikova E.G., Separation of exogenous and endogenous steroids hormones by micellar thin layer chromatography, J. Anal. Chem., 2007, 62, 872-874.

[19] Jain R., Gupta S., Rapid thin layer chromatographic separation of some aromatic amines using surfactants, J. Indian Chem. Soc., 1994, 71, 709-710.

[20] Claesson P.M., Kjellin M., Rojas O.J., Stubenrauch C., J. Phy. Chem. 2006, 8, 5501-5514.

[21] Morisada S., Shinto H., Higgashitani K., Relationship between chain length, disorder, and resistivity in polypyrrole, J. Phys. Chem., 2005, 109, 11762-11769.

[22] Bladek J., Neffe S., Application of TLC in cilinical Chem., Seprat. & Purifi. Reviews, 2005, 33, 60-120.

[23] Bhushan R., Parsad V., Sepration of S (-) and

R (+) ibuprofen by TLC, J. Chromatogr., 1996, 721, 369-372.

[24] Pramauro E., Nad Plizzeti E., Surfactant in analytical chem.., application of organized amphilic media, Elsevier, 1996, 1**,** 145-167.

[25] Dorsey J.G, Etchegaray De MT, Landy J.S, Cetylpyridinium chloride (DDPC) as micellar mobile phase modifiers, Anal. Chem., 1983, 55, 924- 928.

[26] Pyell U., Stark G., Fresenius, A comparative study of mixed micelles as the stationary phase, J. Anal Chem., 1992, 1, 342-347.

*****