Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(10):609-615

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

A novel HPLC method for simultaneous determination of trimebutine, methylparaben and propylparaben in peroral medicines

Vladimir N. Zaitsev[1,2], Oleksandr A. Syrotchuk[2,3] and Iryna R. Didukh[3]

[1]*Pontifícia Universidade Católica do Rio de Janeiro, Rua Marquês de São Vicente 225, Rio de Janeiro, RJ - Brasil*
[2]*Taras Shevchenko National University of Kyiv, Volodymyrska, Kyiv, Ukraine*
[3]*Central Laboratory for Quality Control of Medicines and Medical Products, Kudryavska 10 G, Kyiv, Ukraine*

ABSTRACT

A simple high performance liquid chromatographic method has been developed and validated for the simultaneous determination of trimebutine (TB), methylparaben (MP) and propylparaben (PP) in oral medical suspensions. It was demonstrated that successful separation of the analytes can be achieved in isocratic condition, using 0.1 M aqueous monosodium phosphate buffer solution in acetonitrile (55:45, v/v) as a mobile phase on stationary with immobilized pentafluorophenyl groups. The separation is observed due to specific π-π interaction of immobilized groups with analytes. The method allows to separates TB, MP, and PP in less than 15 min with good resolution, peak shapes and minimal tailing. Linearity ranges for TB, MP and PP are 192–288, 22–52, 9–21μg/mL, respectively. The method was validated with respect to linearity, precision, accuracy, selectivity, and robustness. The method was found to be applicable for routine analyzes (assays and stability tests) of bioactive compound (TB) and the preservatives (MP and PP).

INTRODUCTION

Trimebutine (TB) belongs to the class of medications termed *spasmolytics*. It is used to treat irritable bowel syndrome[1]. TB also is known as a drug with antimuscarinic and weak opioid antagonist effects [2]. TB is available in many pharmaceutical forms such as tablets, injectable solutions and oral liquids. A great number of assay methods for determination of TB in pharmaceutical formulations and biological fluids based upon HPLC [3-10]and differential pulse voltammetry at a glassy carbon electrode[11] have been reported. For HPLC determination with UV-VIS [3-5,7-10] or mass-selective detector[6]C18 stationary phases are commonly used in a gradient regime or with alkylsulphonatesas ion pairing agents[3-5,7,8]. In TB-containing oral liquids the presence of supplement antimicrobial agents such as parabens, a group of alkyl esters of p-hydroxybenzoic acid (PHBA), is required. Parabens have a broad spectrum of antimicrobial activity against yeasts and molds, andare effective over a wide range of pH and so is widely used as an antimicrobial preservative in cosmetics and pharmaceutical products. For simultaneous determination of various parabens different HPLC assay methods has been developed. For example HPLC analysis of methylparaben (**MP**) and propylparaben (**PP**) using spectrophotometric[12], electrochemical[13] and fluorometric[14] detection has been reported. Columns with C18 and C8 [12-18]as well as CN[19,20]stationary phases has been reported.

It is reported [21]that conversion of parabens into 4-hydroxy benzoic acid which possess a weaker preservative activity may be observed in presence of microbes in solutions of parabens. Degradation of parabens results in loss of the prescription quality.

Because of common combination of TB with parabens in oral liquids, and possible degradation of parabens in such prescriptions, we have developed a coherent HPLC method for simultaneous determination of active ingredient, preservatives as well as impurities resulted from their degradation shall be developed. We are not aware of any

method been reported for the simultaneous determination of TB, parabens and products of their degradation in oral liquids.

As it was reported [3-10], for successful determination of TB in medical prescriptions gradient of ion-pair regime of HPLC analysis is required. These regimes decrease life-time of the columns and the method robustness. A stationary phase with immobilized pentafluorophenyl moieties (PFP) in addition to its high hydrophobic character demonstrates Lewis acidity[22-24],since the electronegative fluorine atoms produce an electron deficient of the phenyl ring. Compounds can be separated on a PFP phase due to hydrophobic interactions and specific interactions based on steric recognition, charge transfer or π-π interactions[22]. Consequently PFP phases can be used as alternative to traditional C18 or C8 phases in reversed-phase mode particularly when additional selectivity towards π-donor aromatic compounds is required. Parabens and TB have π-donor aromatic rings and so might be expected specific affinity to PFP phase. The goal of this work was investigation of the applicability of PFP stationary phase for simultaneous HPLC determination of TB, MP and PP as well as the products of their possible degradation in medical oral liquids. In order to increase the method simplicity and robustness we avoided gradient elution and application of ion-pair reagents.

EXPERIMENTAL SECTION

Materials

Methyl 4-hydroxybenzoate and propyl 4-hydroxybenzoate (standard substances of European Pharmacopoeia), gallic acid (purity 99.7 %, Merck), 4-hydroxybenzoic acid (purity 99.0 %, Merck) and Trimebutine base (purity 99.7 %, working standard)were used for standard solutions and model solutions preparation. Deionized water (Milli-Q system, Millipore), acetonitrile (HPLC grade, Sigma-Aldrich) and monosodium phosphate (analytical grade, Merck) were used for solutions and mobile phases preparation. TB containing oral liquid was prepared according to manufacturer instruction from dry commercial prescription Tribudat, oral suspension manufactured by Amoun, Egypt (about 24 mg of TB in each 5 ml of oral liquid). MP and PP stock solutions were prepared from 70 mg of MP and 30 mg of PP diluted in 100.0 ml of water/acetonitrile mixture (60:40, v/v).

Sample preparation

Standard solution for HPLC analysis, containing TB, MP and PP was prepared according next procedure: to 24 mg of TB in 100.0 ml volumetric flask, 5 ml of 0.1 M HCl solution was added for dissolution of TB. Then 5.0 ml of the MP and PP stock solution was added to the flask and resulted solution was diluted with water/acetonitrile mixture (60:40, v/v).

Sample of oral liquid for HPLC analysis was prepared by dilution of 5.0 ml of the oral liquid with mixture of water and acetonitrile (60:40, v/v) in 100.0 mL volumetric flask. Model solution No.1 was obtained by mixing of 24 mg of TB, 4 mg of MP, 2 mg of PP, 15 mg of gallic acid, and 2 mg of 4-hydroxybenzoic acidin 100.0 mL volumetric flask with water: acetonitrile mixture (60:40, v/v).All model stressed stock solutions were prepared from 5.0 ml of MP and PP stock solutions by adding 5 ml of 1.0 M HCl (No. 2.1), 0.1 M NaOH (No. 2.2) or 1.0 ml of 30 % H_2O_2 (No.2.3). Then the solutions were shacked for 12 h at room temperature, neutralized by 0.1 MNaOH(2.1), 0.1 M HCl (2.2) or boiled for12 h (2.3) and then diluted to 100.0 ml with water/acetonitrile mixture (60:40, v/v)and diluted to100 ml with water/acetonitrile mixture (60:40, v/v).

For standard additions method 3.000 g of the oral liquid was weighed into five 100 ml volumetric flasks. To each of them was added 5.0, 7.5, 10.0, 12.5, 15.0 ml of TB solution (1.2 mg/ml) and 1.0, 1.5, 2.0, 2.5, 3.0 ml of MP and PP stock solution respectively.

Equipment/chromatographic system

HPLC analysis was performed on HP 1100 (Agilent Technologies, Germany)chromatographic system, equipped with diode array detector, gradient elution pump with degassing device and mixer, thermostated autosampler and column thermostat. Such a function of the diode array detector and Agilent software Chemstation A.8.3 as spectrum extraction and peak purity control was used to verify detection wavelength (270 nm). HPLC column Curosil PFP 250*4.6 5 μ (Phenomenex) was used for method development. The pH values of the mobile phase were adjusted using pH-meter Metrohm 713.

Chromatographic conditions: Column: Curosil PFP 250*4.6 5 μ; mobile phase: 0.025 – 0.1 M $NaH_2PO_4$with pH range 2.0 – 6.8 in water/acetonitrile (50:50 and 55:45 (v/v)) mixture; flow rate: 1.5 ml/min, column temperature 25-45˚C, injection volume: 10 μl. Concentration of the analytes was determined with UV-VIS detector using 270 nm absorption line.

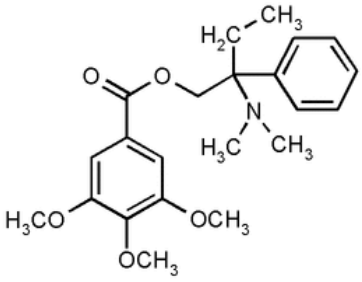
Linearity range for TB determination was examined using 80%, 90%, 100%, 110%, 120% from target level (0.24 mg/ml), linearity range for MP and PP were checked using 60%, 80%, 90%, 100%, 110%, 120%, 140% from target level (0.04 mg/ml and 0.02 mg/ml respectively).

The precision of the method was assessed by determining the relative standard deviation (RSD) values of the analysis (n = 6) of the sample (100 %) by two analysts, using a different chromatographic apparatus. The accuracy of the determination was controlled using methods of standard additions. The robustness of the method was proven by changing parameters of the analysis (column temperature, salt concentration, pH, % acetonitrile).

3. Results and discussion

3.1.Investigation of the pH effect on chromatographic behavior of the compounds

The model solution No.1 containing TB, MP and PP in common for oral liquid concentrations, together with representatives of possible degradation products (gallic and benzoic acids) was used to determine PFP-phase characteristics depending from the solution pH. Isocratic elution was performed in water/acetonitrile = 1/1 solution with sodium phosphate as a mobile phase modifier. In selected conditions separation of all five compounds from the model solution No.1 was achieved in pH range 4.0 – 5.5, Fig.1. At the pH > 6.0 p-hydroxybenzoic and gallic acids cannot be separated. Contrary, picks from TB and MP overlaps in the pH interval 2.1 – 3.0, Fig.1.



As it can be seen from Fig.1 the retention time of PP and MP is constant in all studied pH range and indicates pure hydrophobic interaction of those analytes with the immobilized phase. In contrast, retention of basic (TB) and acidic(p-hydroxybenzoic and gallic acids) compounds is pH-dependent. This chromatographic behavior of the chemicals from the model solution No.1 can be explained by protolytic properties of the analytes and chemical nature of the immobilized layer. Indeed, for acidic compounds such as p-hydroxybenzoic acid and gallic acid the retention time at low pH's (2-4) is constant due to hydrophobic interaction of the stationary phase with molecular forms of the acids. At pH>5 the retention time for those compounds is decreased due to ionization of the acids and repulsion between analyte and negatively charged deionized residual silanols on stationary phase surface. Weak interaction of basic TB (see scheme) with selected stationary phase at low pHs can be explained by similar model - due to repulsion between positively charged surface and quaternary ammonium salt of TB. Contrary, sharp increasing of the retention time for TB at pH > 5.5 cannot be explained by its simple hydrophobic interaction with stationary phase. It is more likely that such strong interaction between TB and immobilized pentafluorophenyl groups is the result of charge-transfer interaction. Indeed, deprotonation of TB at pH > 5 increases donor-acceptor interaction between amine group of TB molecule and perfluorinated immobilized groups and resulted in corresponded increasing of the analyte retention time. Because of pH dependence of retention time, we studied chromatographic behavior of the PFP-stationary phase over a wide pH range (2.1 – 6.8) and salt concentration (0.025-0.1 M).

As it can be seen from Fig.1, the best separation of TB, PP and MP was achieved at neutral pH, therefor further experiment was performed at pH=6.7.

3.2 Investigation of the salt concentration.

The influence of salt concentration to chromatographic behavior of the analytes was studied at optimum pHof mobile phase determined above, for model solution No.2, while salt concentration in the mobile phasewas altered in the range of 0.025 – 0.1 M withstep of 0.025 M.The results are presented in the Table 1 in form of peak purity and column efficiency.

From the date presented it can beconcluded that peaks of interest is recognized as "pure" only in case of using 0.1 M solution of NaH_2PO4. This concentration of the modifier was used for further investigations.

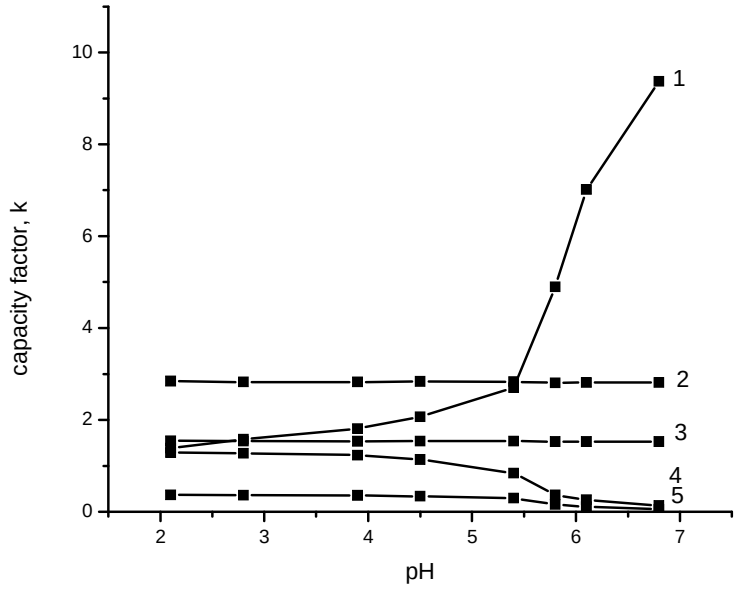


Fig. 1 Effect of the mobile phase pH on capacity factor of compounds in model solution no.1: 1) TB, 2) PP, 3) MP, 4) p-hydroxybenzoic acid, 5) gallic acid

Table 1.Peak purity and column effectiveness of TB, MP and PP depending on NaH_2PO_4 concentration

	0.025M		0.05 M		0.075		0.1 M	
	Purity	N	Purity	N	Purity	N	Purity	N
MP	866.8	13379	999.9	12874	947.1	14063	999.6	13761
PP	840.6	15352	896.6	14871	949.1	15712	999.3	16056
TB	999.0	11201	999.3	9829	999.6	14824	999.1	14829

3.3 Column temperature effect

The optimal mobile phase composition, pH and salt concentrationshown above were used as the chromatographic conditions for investigation of the column temperature influence on resolution, retention, asymmetry, and effectiveness of the column for the model solution 2. Variations in temperature have no significant effect on the asymmetry factor for any compound. Effectiveness of MP and PP does not change significantly with temperature rises but it has been observed effectiveness rising for TB with temperature dropping. Capacity factor for MP and PP decreases as it was expected but increases for TB. It may be explained by pH dependence of TB capacity factor and pH rising of the mobile phase with temperature dropping. Resolution between PP and impurity in front on the peak non-linearly increased from 0.91 to 1.55with temperaturedropping, Table 2. In order to obtain maximum resolution for PP and impurity temperature 30˚C was selected for further chromatographic separation.

Table 2.Peak purity and column effectiveness of TB, MP and PP depending on temperature

	Temperature (0.1 M NaH2PO4-CH3CN=55:45 pH=6.7) v=1.5 ml min^{-1}, 270 nm				
	25	*30*	*35*	*40*	*45*
TB					
k	*9.5*	*9.9*	*10.3*	*10.6*	*10.8*
As	*1.085*	*1.055*	*1.055*	*1.053*	*1.065*
N	*14596*	*15416*	*16048*	*16161*	*16800*
MP					
k	*2.12*	*2.05*	*1.90*	*1.78*	*1.70*
As	*1.20*	*1.188*	*1.187*	*1.194*	*1.213*
N	*13250*	*13460*	*13372*	*13596*	*13218*
PP					
k	*4.18*	*4.06*	*3.75*	*3.52*	*3.33*
As	*1.14*	*1.117*	*1.124*	*1.132*	*1.15*
N	*15217*	*15612*	*15983*	*15768*	*15454*
*R**	*1.55*	*1.57*	*1.36*	*1.16*	*0.91*

R – resolution between PP and impurity on the front of the peak*
k – capacity factor, As – a peak symmetry, N – theoretical plates number.

Model solutions were analyzed under different stress conditions.As it can be seen from Table 3 no degradation of analytes was observed under acidic stress condition (stress stock solution No.1). Stress stock solution No. 2 and No. 3 showed different influence on analytical signals of each component,Table 3.

Table 3.Degradation of the compounds of interest

ID of stress solution	Stress Condition	Treatment	MP, Area, % of initial	PP, Area, % of initial	TB, Area, % of initial
1	Acid	1 N HCl	98	99	98
2	Base	1 N NaOH	6	39	95
3	Oxidative	30 % H_2O_2	88	91	26

Particularly, basic conditions causedstrong degradation of MP and PP. Oxidative conditions caused degradation of TB.

3.4Specificity and selectivity

One of the ways to studyapplicability of the proposed method for routine analysis of pharmaceuticals is to compare the results of the analyte determination in stressed solution that contains productsof the analyte degradation. The results of such determination are presented onFig. 2. It can be clear seen that the peaks of TB, MP and PP are well resolved from the additionally observed peaks from degradation products.

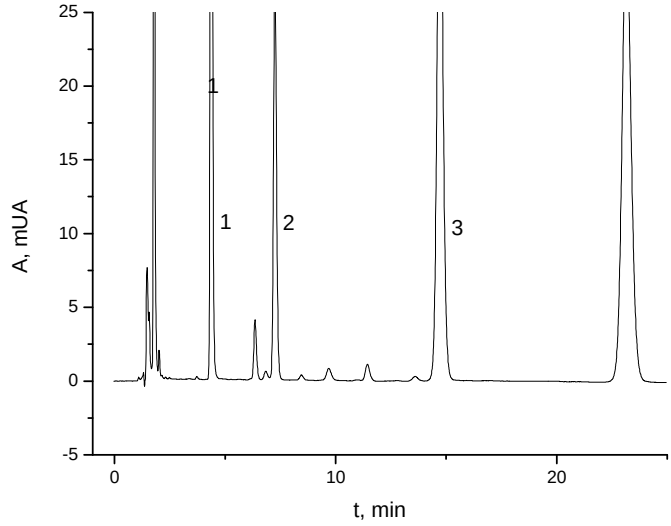


Fig.2HPLC chromatogram of the stressed solutions No.2 and No.3 (1:1, v/v), containing MP (1), PP (2) and TB (3)

3.5. Method validation

3.5.1. Linearity/range

Five solutions were prepared for establishing linearity at varying concentrations of TB in the range of 80–120% of theoretical value. Seven solutions were prepared containing differing concentrations of MP and PP respectively in the range of 60–140% of theoretical values. The DL and QL were calculated in accordance with 3.3·SD/b and 10·SD/b criteria, where SD is standard deviation of a response and b is the slope of the calibration plot for each compound, Table 4.

Table 4. Linearity/range characteristics of method developed.

	TB	MP	PP	Acceptance Criteria [25]
Linearity range, μg/ml	192.1 – 288.1	22.4 – 52.3	9.0 – 20.9	
Quantification limit, μg/ml	16.9	2.2	0.9	
Detection limit, μg/ml	5.6	0.7	0.3	
Y intercept	-7.36	-3.86	-0.35	
SD of Y-intercept	17.09	5.36	2.02	≤ 2·SD
Y intercept/St100%, %	-0.30	-0.48	-0.12	≤ 2.0%
Slope	10126.7	24051.6	21458.9	
SD of slope	70.47	139.4	131.2	
RSD of slope, %	0.70	0.58	0.61	
Coefficient of regression	0.9999	0.9998	0.9998	≥ 0.998

3.5.2. System precision

The system precision was examined by analyzing six determinations of the same test concentration 100% (TB 0.24 mg/ml, MP 0.038 mg/ml and PP 0.015 mg/ml). The relative standard deviation of the areas of each peak was found to be less than 0.9% (Table 5).

Table 5.System suitability characteristics and precision of method developed.

	Parameters	TB	MP	PP	Acceptance criteria
System suitability					
	Capacity factor	2.2	4.2	10.5	≥ 2
	Peak asymmetry	1.09	1.20	1.15	0.8-1.5
	Theoretical plates	15028	13401	15557	≥ 2000
	RSD (areas)[a]	0.05	0.05	0.05	≤ 1.0 %
Precision					
Analyst 1	RSD (areas)[b]	0.9	0.3	0.4	≤ 2.0 %
Analyst 2		0.6	0.4	0.4	≤ 2.0 %
Analyst 1-2		0.8	0.4	0.4	≤ 2.0 %

[a] *n=5*, [b]*n=6*

Table 6.Results of the determination the proposed method accuracy and recovery

Analytes	Added	Found		Recovery, %
		With addition	Without addition	
TB	0	-	11.29	
	6.02	17.15	11.13	98.6
	9.03	20.27	11.24	99.6
	15.05	26.37	11.32	100.3
	18.06	29.72	11.66	100.7
Mean				99.8
MP	0	-	1.470	
	0.602	2.056	1.454	98.9
	0.903	2.365	1.462	99.4
	1.505	2.956	1.451	98.7
	1.806	3.250	1.444	98.2
Mean				98.8
PP				
	0	-	0.738	
	0.249	0.976	0.727	98.5
	0.374	1.106	0.732	99.2
	0.623	1.358	0.736	99.7
	0.747	1.471	0.724	98.2
Mean				98.9

3.5.4. Accuracy/recovery

The accuracy of the method was checked using standard additions method. Known amounts of TB, MP, PP were added to a given concentration of commercial suspension. The percentage recovery of all three substances was in the range 98.0-102.0 % as shown in Table 6.

3.5.5. Stability of analytical solutions

Sample and standard solutions were analyzed immediatelyafter preparation as well as after storage at room temperature and at 4˚ C with exclusion of light for 24 h. The response of the three substances over this period is shown in Table 7.

Table 7. Stability of analytical solutions

Analytes	Ambient temperature			4˚ C		
	6 h.	12 h.	24 h.	6 h.	12 h.	24 h.
Standard solution						
TB	0.4	0.3	0.7	0.1	0.0	0.2
MP	0.1	0.2	0.1	0.2	0.1	0.4
PP	0.2	0.1	0.1	0.2	0.2	0.4
Sample solution						
TB	1.3	4.1	7.1	0.4	0.6	0.6
MP	0.1	0.1	1.2	0.4	0.3	0.3
PP	0.3	0.2	1.1	0.3	0.2	0.2

3.5.7. Robustness

Several parameters of the method were selectively alteredin order to determine the robustness of the method. Parametersvaried (one at a time) included percent of organic component content in the mobile phase (± 2%); buffer pH in the mobilephase (± 0.2 pH units); column temperature (± 5˚C); flow rate (± 10%); salt concentration (± 10%). The effect of such variations on the capacity factor, the asymmetry factor, the theoretical plates,andpeak purity were evaluated. No significant effect on chromatographic performance and peak purity of MP, PP, and TB was observed.

3.5.8. Assay of TB

The method developed was used for analysisof commercial batch of Tribudat, oral suspension manufactured by Amoun, Egypt. The results obtained were 96.9 ± 1.0 % of TB, 82.6 ± 0.2 % of MP, and 98.5 ± 0.4 % of MP from labeled values.

CONCLUSION

Method developed here has several advantages over other methods reported for TB determination. It allows symmetric peak of TB to be obtained without ion pair reagents through use of an alternative pentafluorophenyl column. This makes the method safer for chromatographic column compared to methods described earlier. Important advantage of the method is good chromatographic resolution of TB, MP, and PP as well as impurities and components of matrix. Achieved resolution of TB and TB impurity (2-(dimethyl amino)-2-phenylbutanol) is about 13.1 and it is much more than reported earlier. Mobile phase optimization allowed to determine both TB and parabens in presence of more the 10 products of sample degradation.

REFERENCES

[1] A Brayfield. Martindale: The Complete Drug Reference. *Emerg. Nurse*, Pharmaceutical Press, **2014**, 22,(5).
[2] H Kaneto; M Takahashi and J Watanabe. *J. Pharmacobiodyn.*, **1990**, 13,(7), 448–453.
[3] A El-Gindy; S Emara and GM Hadad. *J. Pharm. Biomed. Anal.*, **2003**, 33,(2), 231–241.
[4] M Lavit; S Saivin; H Boudra *et al.Arzneimittel-Forschung/Drug Res.*, **2000**, 50,(7), 640–644.
[5] E-H Joo; W-I Chang; I Oh *et al.J. Chromatogr. B Biomed. Sci. Appl.*, **1999**, 723,(1-2), 239–246.
[6] H Wang; H Zhou; S Horimoto *et al.J. Chromatogr. B*, **2002**, 779,(2), 173–187.
[7] Z LIN and M SONG. *J. Pharm. Anal.*, **2001**,(1), 25–28.
[8] Y Hashimoto; M Tanaka; H Kishimoto *et al.J. Pharm. Pharmacol.*, **2002**, 54,(10), 1323–8.
[9] R WANG; L ZHANG and S ZHANG. *J. SHANXI Med. Univ.*, **2006**,(7), 723–726.
[10] J Sanjiu; L Jinfang; Q Shuang *et al.Chinese J. Mod. Appl. Pharm.*, **2002**,(5).
[11] N Adhoum and L Monser. *J. Pharm. Biomed. Anal.*, **2005**, 38,(4), 619–23.
[12] S Ho Kang and H Kim. *J. Pharm. Biomed. Anal.*, **1997**, 15,(9-10), 1359–1364.
[13] Q Zhang; M Lian; L Liu *et al.Anal. Chim. Acta*, **2005**, 537,(1-2), 31–39.
[14] R Hájková; P Solich; M Pospíšilová *et al.Anal. Chim. Acta*, **2002**, 467,(1-2), 91–96.
[15] R Hájková; P Solich; J Dvořák *et al.J. Pharm. Biomed. Anal.*, **2003**, 32,(4-5), 921–927.
[16] DM Kreuz; AL Howard and D Ip. *J. Pharm. Biomed. Anal.*, **1999**, 19,(5), 725–735.
[17] MS Ali; RS Chaudhary and MA Takieddin. *Drug Dev. Ind. Pharm.*, **1999**, 25,(10), 1143–7.
[18] MX Kokoletsi; S Kafkala and M Tsiaganis. *J. Pharm. Biomed. Anal.*, **2005**, 38,(4), 763–7.
[19] E Sottofattori; M Anzaldi; A Balbi *et al.J. Pharm. Biomed. Anal.*, **1998**, 18,(1-2), 213–217.
[20] M Ma; T Lee and E Kwong. *J. Pharm. Sci.*, **2002**, 91,(7), 1715–23.
[21] N Valkova; F Lépine; L Valeanu *et al.Appl. Environ. Microbiol.*, **2001**, 67,(6), 2404–9.
[22] CH Lochmüller and CW Amoss. *J. Chromatogr. A*, **1975**, 108,(1), 85–93.
[23] N Tanaka; Y Tokuda; K Iwaguchi *et al.J. Chromatogr. A*, **1982**, 239,(C), 761–772.
[24] PC Sadek and PW Carr. *J. Chromatogr. A*, **1984**, 288,(C), 25–41.
[25] GA Shabir. *J. Chromatogr. A*, **2003**, 987,(1-2), 57–66.