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Rapid determination of amlodipine besylate and valsartan in pharmaceutical preparations by stability-indicating ultra performance liquid chromatography

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ABSTRACT

A stability- indicating UPLC method was developed for the simultaneous quantitative determination of amlodipine besylate and valsartan in pharmaceutical dosage forms in the presence of degradation products. It involved a 50 mm x 4.6 mm, 1.8 µm C-18 column. The separation was achieved on simple isocratic method. A mixture of potassium dihydrogen phosphate buffer (pH 3.2, 0.01M)- acetonitrile (55:45, v/v) was used as the mobile phase. The flow rate was 0.7 ml/min and the detection wavelength was 237 nm. The retention times of amlodipine besylate and valsartan were 0.8 min and 1.3 min; respectively. The total runtime was 2 min within which both active compounds and degradation products were separated. Amlodipine besylate and valsartan were subjected to different ICH prescribed stress conditions. Degradation was found to occur in hydrolytic stress conditions and some extent in oxidative stress conditions, while drugs were stable to photolytic and thermal stress. The drugs were particularly labile under acidic stress conditions. The drugs were subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The method developed was successfully applied to the simultaneous determination of amlodipine besylate and valsartan in pharmaceutical preparations. The developed RP-UPLC method was validated with respect to linearity, accuracy, precision, robustness and ruggedness.

KEYWORDS

Amlodipine besylate and valsartan;
Method development;
Validation;
UPLC;

INTRODUCTION

Present drug stability test guidance Q1A (R2) issued by international conference on harmonization (ICH)[1] suggest that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the

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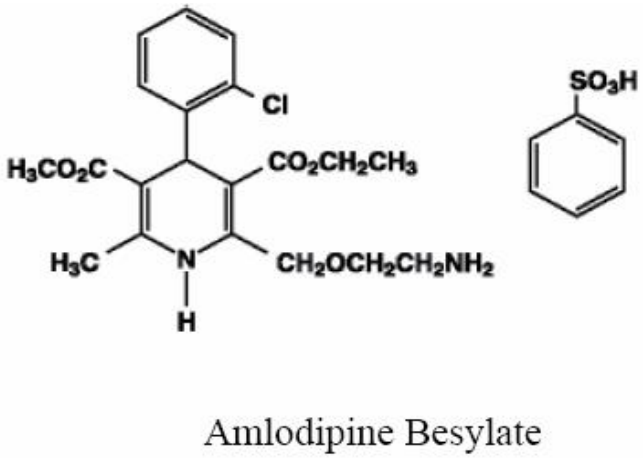
suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated.

Accordingly, the aim of the present study was to establish inherent stability of Losartan potassium, Atenolol, and Hydrochlorothiazide through stress studies under a variety of ICH recommended test conditions[1-3] and to develop a stability-indicating assay[4-6].

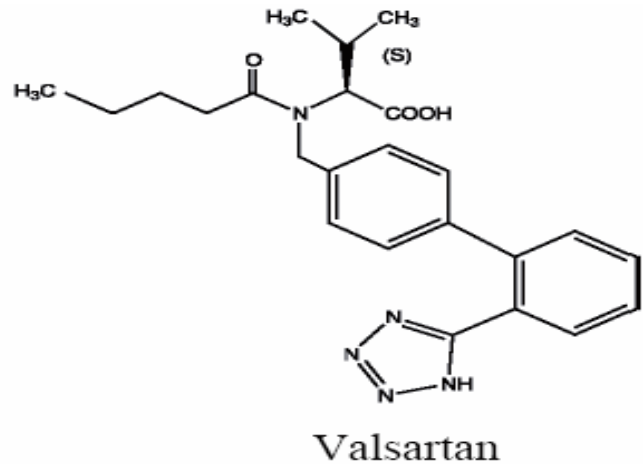
Amlodipine besylate is chemically described as 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate. It is indicated for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents Figure 1.

Valsartan is chemically described as N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl)1,1'-biphenyl]-4-yl]methyl]-L-valine. It is indicated for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents Figure 1.



Amlodipine Besylate

Figure 1a : Chemical structures of (a) amlodipine besylate



Valsartan

Figure 1b : Chemical structures of (b) valsartan.

Amlodipine and valsartan is indicated for the treatment of hypertension. It may be used in patients whose blood pressure is not adequately controlled on either monotherapy. It may also be used as initial therapy in patients who are likely to need multiple drugs to achieve their blood pressure goals.

In the literature there are limited methods has been reported. A LC method has been reported for the determination of amlodipine in combination with other drugs[7-8]. A protein precipitation and liquid chromatography method has been reported for rapid determination of valsartan in human plasma[9]. A reversed-phase HPLC method has been reported for the simultaneous determination of valsartan and hydrochlorothiazide in tablets[10]. A first-derivative UV spectrometry and LC method has been reported for the simultaneous determination of valsartan and Hydrochlorothiazide in tablets[11]. A validated HPLC method has been reported for simultaneous analysis of A and V for liver perfusion studies[12]. Whereas no single LC method has been reported for the simultaneous quantitative determination A and V from pharmaceutical dosage form. Hence, it is necessary to develop a rapid, accurate and validated stability indicating LC method for the simultaneous determination of A and V from combined dosage form.

Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows about ninefold decrease in analysis time as compared to the conventional HPLC system using 5µm particle size analytical columns, and about threefold decrease in analysis time in comparison with 3µm particle size analytical columns without compromise on overall separation[13-14].

A rapid simple isocratic reproducible Ultra performance liquid chromatography method was developed for simultaneous quantitative determination of A and V in pharmaceutical dosage forms in the presence of degradation products.

EXPERIMENTAL

Chemicals

Standards were supplied by Dr. Reddy's labo-

ratories limited, Hyderabad, India. Commercially available tablets (10 mg of amlodipine besylate and 160 mg of valsartan) were purchased. The HPLC grade acetonitrile, analytical grade triethyl amine and ortho phosphoric acid were purchased from Merck, Darmstadt, Germany; water was prepared by using Millipore MilliQ Plus water purification system

Equipment

The Waters UPLC Acquity system we used consists of a binary solvent manager, a sample manager and a UV detector. The out put signal was monitored and processed using empower software, water baths equipped with MV controller (Julabo, Seelbach, Germany) were used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (MACK Pharmatech, Hyderabad, India).

Chromatographic conditions

The chromatographic column used was a Zorbax C-18, 50 mm x 4.6 mm i.d with 1.8 µm particles. A mixture of potassium dihydrogen phosphate buffer (pH 3.2, 0.01M)- acetonitrile (55:45, v/v) was used as the mobile phase. The flow rate of mobile phase was 0.7 ml/min and the detection was monitored at a wavelength 237 nm. The column temperature was maintained at 40 ºC and injection volume was 3 µL.

Preparation of stock solutions

A stock solution of A and V standard and sample (0.2 mg/ml of A and 3.2 mg/ml of V) was prepared by dissolving an appropriate amount in mobile phase. Working solutions 0.02 mg/ml of A and 0.32 mg/ml of V were prepared from above stock solution in mobile phase for assay determination.

Preparation of sample solution

Twenty tablets were weighed and transferred into a clean and dry mortar, grinded well. Then an equivalent to 20 mg of A and 320 mg of V was transferred to a 100 mL volumetric flask, 70 mL mobile phase was added and sonicated for 20 min and diluted to 100 mL (0.2 mg/ml of A and 3.2 mg/ml of V). About 5 mL of supernant solution was taken and diluted to 50 mL with mobile phase. This was filtered using 0.45 µ (Nylon 66-membrane) filter.

System suitability solution criteria

The system suitability was assessed by five replicate analyses of the drugs at concentrations of 0.02 mg/ml of A and 0.32 mg/ml of V. The acceptance criteria was not more than 2.0% for the RSD for the peak areas of A and V and not less than 10.0 for resolution between A and V peaks.

Stress studies

Specificity is the ability of the method to measure the analyte response in the presense of its potential impurities[4]. Stress testing of a drug substance can help to identify the likely degradation products, which can in tern help establish the degradation pathways and the intrinsic stability of the molecule.

All stress decomposition studies were performed at an initial drug concentration 0.02 mg/ml of A and 0.32 mg/ml of V. Acid hydrolysis was performed in 0.1N HCl at 60 ºC for 12 h. The study in basic solution was carried out in 0.1N NaOH at 60 ºC for 12 h. For study in neutral solution, drugs dissolved in water was heated at 60 ºC for 12 h. Oxidation studies were carried out at room temperature in 1% hydrogen peroxide for 12 h. Photo degradation studies were carried out at according to option 2 of Q1B in ICH guidelines[3]. Samples were exposed to light for an overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200 watt hm2. The drug product was exposed to dry heat at 80 ºC for 12 h. Samples were withdrawn at appropriate time and subjected to UPLC analysis after suitable dilution (0.02 mg/ml of A and 0.32 mg/ml of V).

Method validation

Method validation was performed as per ICH guidance for simultaneous determination of A and V in the formulations. The following validation characteristics were addressed: Systemsuitability, linearity, detection limit, quantification limit, precision, accuracy, robustness, ruggedness and specificity.

Note

System suitability

The system suitability test solution was injected and the chromatographic parameters like relative standard deviation for five replicate injections of A and V and the USP resolution factor between A and V peaks were evaluated for proving the system suitability.

Precision

The precision of the assay method was evaluated by carrying out six independent assays of A and V (0.02 mg/ml of A and 0.32 mg/ml of V) test samples against qualified reference standard. The percentage of RSD of six assay values was calculated. Different analyst from the same laboratory evaluated the intermediate precision of the method.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for A and H were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentration.

Linearity

Linearity solutions were prepared from stock solution at five concentration levels from 50% to 150% of analyte concentrations (10 to 30 µg/ml for A and 160 to 480 µg/ml for V). The slope, Y-intercept and correlation coefficient were calculated.

Accuracy

The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 50%, 100% and 150% of target test concentration (0.2 mg/ml of A and 3.2 mg/ml of V) in tablets. The percentage of recoveries were calculated.

Specificity and selectivity

The specificity of the method was established through study of resolution factors of the drug peaks from nearest resolving peak, and also among all other peaks.

Solution stability and mobile phase stability

The solution stability of A and V was carried out by leaving the test solution in tightly capped volumetric flask at room temperature for 24 h. The same sample solution was assayed for a 6 h interval up to the study period against freshly prepared standard solution of A and V. The mobile phase stability was also carried out by assaying the freshly prepared standard solution for 12 h interval up to 24 h. The mobile phase preparation was kept constant during the study period. The percentage of RSD of assay of A and V was calculated for the study period during mobile phase and solution stability experiments.

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method the experimental conditions were deliberately altered and assay, USP resolution factor between A and V and % R.S.D. results for assay were evaluated.

The mobile phase flow rate was 0.7-1. This was changed by 0.1to 0.6 and 0.8-1 and the effect was studied. Similarly, the effect of column temperature was studied at 35 and 45°C instead of 40°C. The effect of mobile phase composition was studied by use of potassium dihydrogen phosphate buffer (pH 3.2, 0.01M)- acetonitrile 57:43 and 53:47 (v/v). The effect of mobile phase buffer pH was studied at pH 3.1 and pH 3.3 instead of pH 3.2

RESULTS AND DISCUSSION

Stress studies

Degradation in acidic solution

In 0.1HCl at 60 ºC for 12 h, drug underwent degradation, forming degradation products at RRTs of 0.76, 0.89 and 1.38 (with respect to A). The total degradation was about 12% Figure 2b.

Degradation in basic solution

In 0.1NaOH at 60 ºC for 12 h, drug underwent degradation, forming degradation products at RRTs of 0.76, 0.89 and 1.38 (with respect to A). The total degradation was about 1.9% Figure 2c.

Oxidative conditions

The drug was exposed to 1% hydrogen perox-

ide at 60 ºC for 12. No major decomposition was observed.

Degradation in neutral aqueous solution

The drug was exposed to water at 60 ºC for 12 h. No major decomposition was observed.

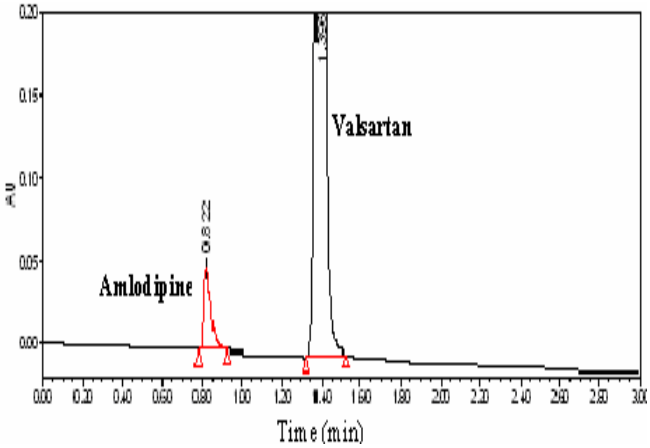


Figure 2a : A typical HPLC chromatogram of (a) amlodipine besylate.

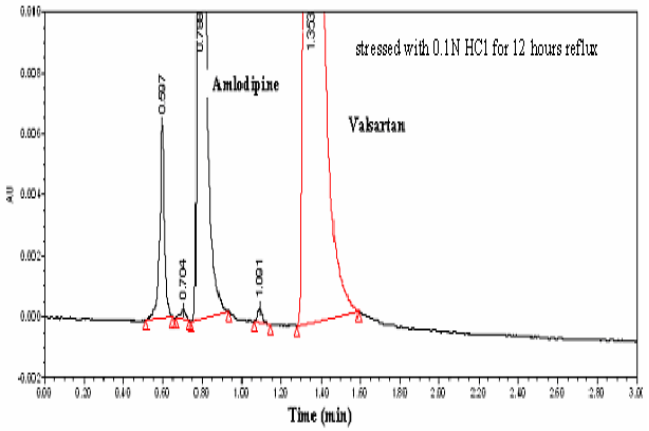


Figure 2b : A typical HPLC chromatogram of valsartan (b, c,) stressed amlodipine besylate and valsartan.

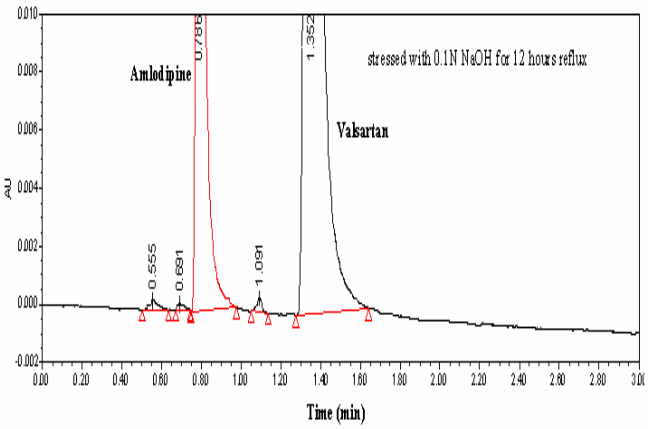


Figure 2c : A typical HPLC chromatogram of valsartan (b, c,) stressed amlodipine besylate and valsartan.

Photolytic conditions

The drug was stable against the effect of photolysis. When the powdered drug was exposed to light for an overall illumination of 1.2and an integrated near ultraviolet energy of 200in a photo stability chamber, no degradation was observed.

Thermal degradation

When the drug powder was exposed to dry heat at 80°C for 24 h, no decomposition was observed.

Mass balance (%assay + % degradents + % impurities) is calculated for stress sample. The mass balance of stressed sample was > 99% for all stress conditions.

The peak purity test results derived from PDA (Photo Diode Array detector) confirmed that the A and V peaks were pure and homogeneous in all the analyzed stress. This indicates that the method is specific and stability indicating.

Method development and optimization of stability indicating assay method

The method was optimized to separate major degradation products formed under varies stress conditions. The main target of the chromatographic method is to get the separation for closely eluting degradation products, mainly the degradation products at 0.76, 0.89, 1.38 RRT (with respect to A). The degradation samples were run using different stationary phases like C18, C8, Cyano and Mobile phases containing buffers like phosphate, sulphate and acetate with different pH (2-7) and using organic modifiers like acetonitrile and methanol in the mobile phase. But the separation was satisfactory in the adopted chromatographic conditions only. It indicated that the isocratic with 45% acetonitrile as organic modifier in mobile phase was successful in separating drugs and all chromatographic degradation products.

Validation of developed stability-indicating method

System suitability

The system suitability test solution was injected and the chromatographic parameters like relative standard deviation for replicate injections

Note

of L, A and H and the USP resolution factor between the peaks A and V were evaluated. The relative standard deviation for replicate injections was 0.2% and 0.2% for A and V, respectively. The USP resolution factor between A and V was 12.5.

Precision (inter-day precision and intra-day precision)

The percentage RSD values for the precision study were 0.7%, 0.5% (inter-day precision) and 0.6%, 0.4% (intra-day precision) for A and V; respectively. This is confirming good precision of the method.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection was 0.02 and 0.3 µg/ml for A and V; respectively for 3 mL injection volume. The limit of quantification was 0.05 and 0.81 µg/ml for A and V; respectively for 3 mL injection volume.

Linearity

Linear calibration plot for this method was obtained over the calibration ranges tested, i.e. from 10 µg/ml to 30 µg/ml for A and from 160 µg/ml to 480 µg/ml for V and the correlation coefficient obtained was greater than 0.999 for both the drugs. The results show that an excellent correlation existed between the peak area and concentration of the analyte. The mean regressions equations were found as A = 0.000152 C -87059 (r2 = 0.9996, n = 5) and A =0.000157 C –21.0729 (r2 = 0.9995, n = 5) for A and V, respectively. A = aC + b, where A is the peak area ratio of the drugs, a is the slope, b is the intercept and C is concentration of the measured solution in µg mL-1. The results show that an excellent correlation existed between the peak area and concentration of the analyte.

Accuracy

The percentage recovery of A and V in pharmaceutical dosage forms ranged from 99.4 to 101.1% for A and 98.8 to 100.0% for V. Excellent recoveries were made at each added concentration.

Solution stability and mobile phase stability

The solution stability and mobile phase stability experiment data confirms that sample solutions and mobile phase used during the assay were stable up to 24 h.

Specificity and selectivity

Figure 2 shows that the method was sufficiently specific to the drugs. The resolution factor for the drug peaks was >1.5 from the nearest resolving peak (RRT ~0.89)

Intermediate precision was performed to confirm that separation between A and V satisfactory under conditions mentioned above. The resolution factor between the A and V was > 12, indicating that the method remains selective for all components under tested conditions.

Robustness

For robustness study, for all changes of conditions the sample was assayed in triplicate. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values. Assay of A and V for all deliberate changes of conditions was within 98.5–101.4 % and 98.7-101.8% for both A and V respectively. The USP resolution factor between the A and V was > 12 for all robustness conditions. The % R.S.D. of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust The complete results are shown in TABLE 3.

TABLE 1 : Precision results of amlodipine besylate and valsartan from tablets.

	Active Name	Pre-1 % Assay	Pre-2 % Assay	Pre-3 % Assay	Pre-4 % Assay	Pre-5 % Assay	Pre-6 % Assay	% RSD	% Mean
Inter-day precision	A	101.3	99.5	99.4	99.7	100.3	100.3	0.7	100.0
	V	99.1	99.4	99.9	99.5	100.1	100.3	0.5	99.6
Inter-day precision	A	99.7	99.8	99.3	100.0	99.7	101.1	0.6	99.7
	V	100.7	99.8	100.2	99.8	100.5	100.6	0.4	100.2

Pre = preparation

Note

TABLE 2 : Percentage recovery of amlodipine besylate and valsartan from tablets.

Recovery level	mg added		mg found		Recovery (%) (*n* = 3)	
	A	V	A	V	A	V
50%	10.03	159.77	9.96	157.82	99.4	98.8
100%	20.02	320.00	20.03	319.09	100.1	99.7
150%	30.29	480.53	30.62	480.38	101.1	100.0

n = 3 determinations

TABLE 3 : Robustness study results.

Condition	Assay % (*n* = 3)		USP resolution between A andV	% R.S.D. of results	
	A	V		A	V
Flow rate (±0.1 ml/min of the optimum flow)					
0.6 mL min^{-1}	100.7	100.8	12.7	0.19	0.08
0.8 mL min^{-1}	99.7	98.7	12.4	0.79	0.75
Mobile phase composition (±2% of optimum organic modifier concentration)					
43	101.4	101.8	13.0	0.48	0.30
47	100.4	100.9	12.5	0.45	0.26
Temperature (±5° of optimum temperature)					
35 °C	98.5	99.3	12.8	0.49	0.62
45 °C	99.7	99.9	12.7	0.45	0.26
pH (±0.1 of optimum pH)					
3.1	99.8	100.0	12.7	0.09	0.24
3.3	100.0	99.6	12.8	0.23	0.50

***n* = 3 determinations**

CONCLUSIONS

A novel isocratic UPLC method prove to be simple, linear, precise, accurate, robust, rugged and specific. The total runtime was 2 min within which both the drugs and their degradation products were separated. The method was completely validated showing satisfactory data for all the method validation parameters tested. The Developed method is stability indicating and can be used for simultaneous quantitative determination of the drugs A and V in presence of degradation products in stability by the industry.

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REFERENCES

[1] CH-Q1A (R2); Stability Testing of New Drug Substances and Products International Conference on Harmonization, IFPMA, Geneva.
[2] S.Singh, M.Bakshi, Pharm.Tech.Online, **24**, 1-14 **(2000)**.
[3] ICH-Q1B; Photo Stability Testing of New Drug Substance and Products International Conference on Harmonization, IFPMA, Geneva.
[4] M.Bakshi, S.Singh; J.Pharm. Biomed.Anal., **28,** 1011-1040 **(2002)**.
[5] S.Singh, B.Singh, R.Bahuguna, L.Wadhwa, R.Saxena; J.Pharm.Biomed.Anal., **41,** 1037-1040 **(2006)**.
[6] J.T. Carstensen, C.T.Rhodes; (Eds); Drug Stability Principles and Practices, 3rd Edition; **(2000)**.
[7] B.G.Chaudhari, N.M.Patel, P.B.Shah; Chem.Pharm.Bull., **55**, 241-246 **(2007)**.
[8] K.Raghu Naidu, N.Kale, S.ShingareMurlidhar; J.Pharm.Biomed.Anal., **39,** 147-155 **(2005)**.
[9] J.Macek, J.Klima, P.Ptacek; J.Chromatogr., **832**, 169-172 **(2006)**.
[10] D.F.Tian, X.L.Tian, T.Tian, Z.Y.Wang, F.K.Mo; Indian.J.Pharm.Sci., **70,** 372-374 **(2008)**.
[11] E.Satana, S.Altinay, N.G.Goger, S.A.Ozkan, Z.Senturk; J.Pharm.Biomed.Anal., **25**, 1009-1013 **(2001)**.
[12] M.Celebier, M.S.Kaynak, S.Altinoz, S.Sahin; Hacettepe University Journal of the Faculty of Pharmacy, **28**, 15-30 **(2008)**.
[13] S.Wren S, P.Tchelitcheff; J.Pharm.Biomed.Anal., **48**, 120-126 **(2008)**.
[14] L.Novakova, L.Matysova; P.Solich, Talanta, **68,** 908-918 **(2006)**.