Journal of Pharmacreations

PharmaCreations

Pharmacreations | Vol.3 | Issue 4 | Oct- Dec- 2016

Journal Home page: www.pharmacreations.com

Research article ***Open Access***

A novel validated RP-HPLC method for the determination of Balsalazide in Pure and in Pharmaceutical Dosage forms

[*1]M. Swapna[*], [2]M.Vijay Prakash, [3]M.Sunil Kumar

*[*1]Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad- 79.*

[2]Care College of Pharmacy, Ogulapur, Warangal.

[3]Hetero Labs Limited, Jeedimetla, Hyderabad-055

***Corresponding author: M. Swapna**

Email: ravi_sunil85@yahoo.com

SUMMARY

A simple reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for determination of balsalazide in bulk and in capsule dosage forms. An isocratic elution technique was employed on a Phenomenax Luna C_{18} column (150×4.6mm i.d., 5µ) at ambient temperature with a mixture of Acetonitrile: Methanol: Triethylamine buffer in the ratio of 40:30:30% (v/v) as mobile phase at a flow rate of 0.7 mL min^{-1} UV detection was performed at 254 nm. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The developed and validated method was successfully used for quantitative analysis of Balacol capsules. Total chromatographic analysis time per sample was approximately 6 min with balsalazide and nifedipine (internal standard) eluting with retention times of 3.42 and 5.07 min, respectively. Validation studies revealed the method is specific, rapid, reliable, and reproducible. A calibration plot was linear over the concentration range of 10 – 50 µg mL^{-1}. The LOD was found to be 0.01643 µg mL^{-1} and the LOQ was found to be 0.04960 µg mL^{-1}. The high recovery and low relative standard deviation confirm the suitability of the method for determination of balsalazide in bulk and in capsule dosage forms.

INTRODUCTION

Balsalazide (BSZ), (E) - 5 - [[- 4 - [[2 - carboxyethyl) amino) carbonyl) phenyl) azo] - 2 - hudroxybenzoicacid, disodium salt, dehydrate (Fig. 1). BSZ is used in the treatment of ulcerative colitis. BSZ disodium is delivered intact to the colon where it is cleaved by bacterial azoreduction to release equimolar quantities of mesalamine, which is the therapeutically active portion of the molecule and 4-aminobenzoyl - (beta) - alanine [1-7]. Hitherto there is only one analytical method was reported for estimation of BSZ. The determination of BSZ in small volumes of plasma by HPLC with fluorescence detection was described [8]. This method is complicated, costly, time consuming rather than a simple HPLC with UV detection. So it is unsuitable to use these highly sensitive methods for the routine quantitative assay of BSZ in bulk and in capsules where the content of active pharmaceutical ingredient is high in the formulation. Because HPLC methods have been widely used for routine

quality control assessment of drugs, because of their sensitivity, repeatability, and specificity. A simple and specific RP-HPLC method was developed for determination of BSZ in bulk and in pharmaceutical dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed HPLC–UV method was validated in accordance with International Conference on Harmonization (ICH) guidelines [9, 10], by assessing its selectivity, linearity, accuracy, precision, and limits of detection and quantitation.

EXPERIMENTAL

Materials

BSZ standard sample was provided by Aurobindo Pharma Limited (Hyderabad, India). The internal standard Nifedipine, (Fig.1) was obtained from Jubilent Pharma Limited (Mumbai, India). Balacol® capsules commercial formulation selected and it belongs to Torrent pharmaceuticals (New Delhi, India). The commercial sample was supplied as capsule dosage forms containing 750 mg of BSZ for oral administration. Acetonitrile (ACN), methanol (MeOH) and water of HPLC grade and Triethylamine (TEA) of AR grade were obtained from Qualigens fine chemicals (Mumbai, India).

Chromatographic System and Conditions

Analysis was performed with a Shimadzu (Japan) chromatograph equipped with an LC-10 AT vp solvent-delivery module, a SPD-10A UV–Visible detector, and a Rheodyne model 7161 injector valve with 20 µL sample loop (Rheodyne Inc.,Cotati, CA, USA), BSZ and the IS were separated on a Phenomenax Luna ODS analytical column (150 mm × 4.6 mm i.d., 5µ) under reversed-phase chromatographic conditions. The mobile phase was a mixture of ACN, MeOH and TEA buffer (10mM, pH 3.0 ± 0.05 adjusted with 85% phosphoric acid) in the ratio 40:30:30 (v/v). The flow rate was 0.7 mL min^{-1} .The analyte and the internal standards were monitored at 254 nm. The equipment was controlled by a PC workstation with Winchrom chromatography software installed. The system was used in an air-conditioned HPLC laboratory (20 ± 1.5°C). Before analysis the mobile phase was degassed by use of a Soltec sonicator (Soluzioni technologic and Luglio, Italy). Sample solutions were also filtered through a 0.2 µm filter. The system was equilibrated before each injection.

Choice of Internal Standard

To select the suitable internal standard for the analysis, several substances were examined. Among these, Nifedipine has been chosen as the most appropriate in the present analysis because it was stable during the analysis, readily available, was well resolved from BSZ (Rs 3.3), its peak shape was good (tailing factor 1.09). In the present study, it did not interfere with the matrix of pharmaceutical samples and it was well separated from BSZ.

Construction of Calibration Plots

Individual stock solutions of BSZ and IS were prepared by dissolving the drugs (25 mg, accurately weighed) in 25 mL mobile phase (final concentration 1 mg mL^{-1}). The stock solutions were stored at 4°C protected from light. From this stock solution, 100 µg mL^{-1} standards were freshly prepared on the day of analysis.

Calibration standards for analyte were prepared at concentrations of 10, 20, 30, 40 and 50 µg mL^{-1} for BSZ (1 - 5ml of 100 µg mL^{-1}) with a fixed concentration of the Nifedipine 30 µg mL^{-1}. Calibration plot was constructed by plotting BSZ/IS, peak area ratio against their respective concentrations. Unknown assay samples were quantified by reference to these calibration plots.

Assay Sample Preparation

For assay of BSZ in capsules, twenty capsules were weighed and their contents were mixed thoroughly. An amount of capsule powder equivalent to 25mg BSZ was accurately weighed and transferred to a 25 mL volumetric flask. A suitable quantity of IS was added and dissolved in mobile phase. This mixture was sonicated for 10 min. for complete extraction of the drugs and the solution was diluted to volume with mobile phase. The solution was centrifuged at 4000 rpm for 15 min, the clear supernatant was collected and filtered through 13 mm membrane syringe filter (pore size 0.2 µm). 3 ml of each BSZ (100 µg mL^{-1}) and nifedipine (100 µg mL^{-1}) solutions were transferred in to six 10 ml volumetric flasks and

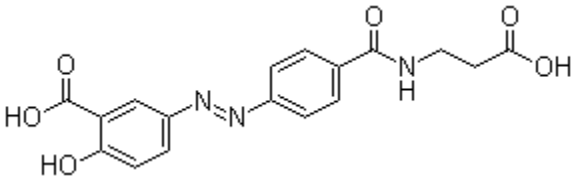
made up with mobile phase. 20 µL of the solutions were injected for HPLC analysis.

Content Sample Preparation

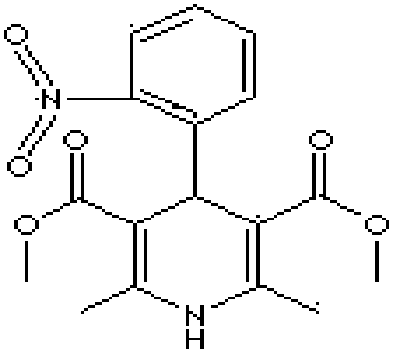
For assessment of drug-content uniformity in the capsules, the contents of ten capsules were separately transferred to 50-mL volumetric flasks. A suitable quantity of IS was added to each, then 25 mL mobile phase. The mixtures were sonicated then diluted to volume with mobile phase to furnish concentrations of 30 µg mL^{-1} for both BSZ and IS. The solutions were centrifuged, supernatant was collected and filtered and 20 µL of each solution was injected for HPLC analysis.

Statistical Calculations

Standard regression curve analysis was performed by use of Micro-soft Office Excel 2003 software (Microsoft, USA), without forcing through zero. Means and standard deviations were calculated by use of SPSS software version 9.5 (SPSS, Cary, NC, USA).



Balsalazide (BSZ)



Nifedipine (IS)

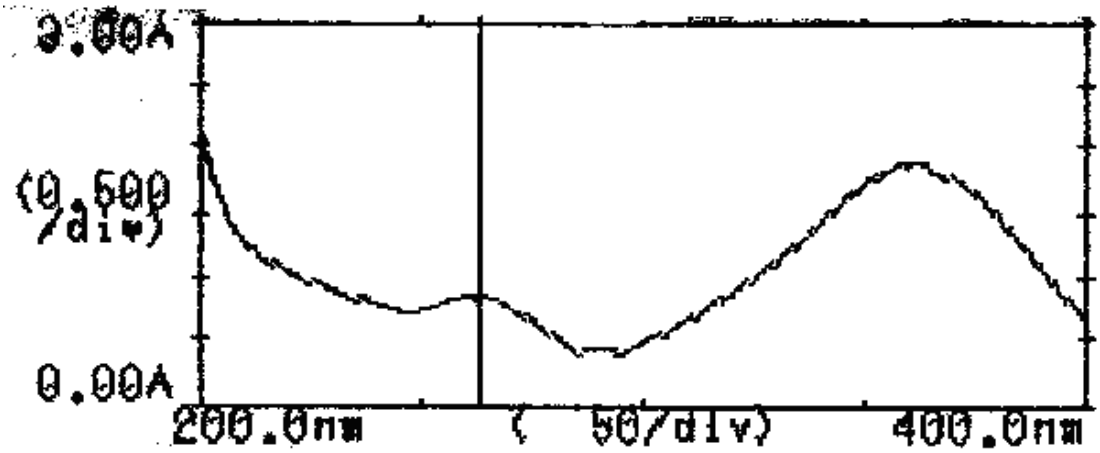


Fig.1. Chemical structures of BSZ and Nifedipine (IS).

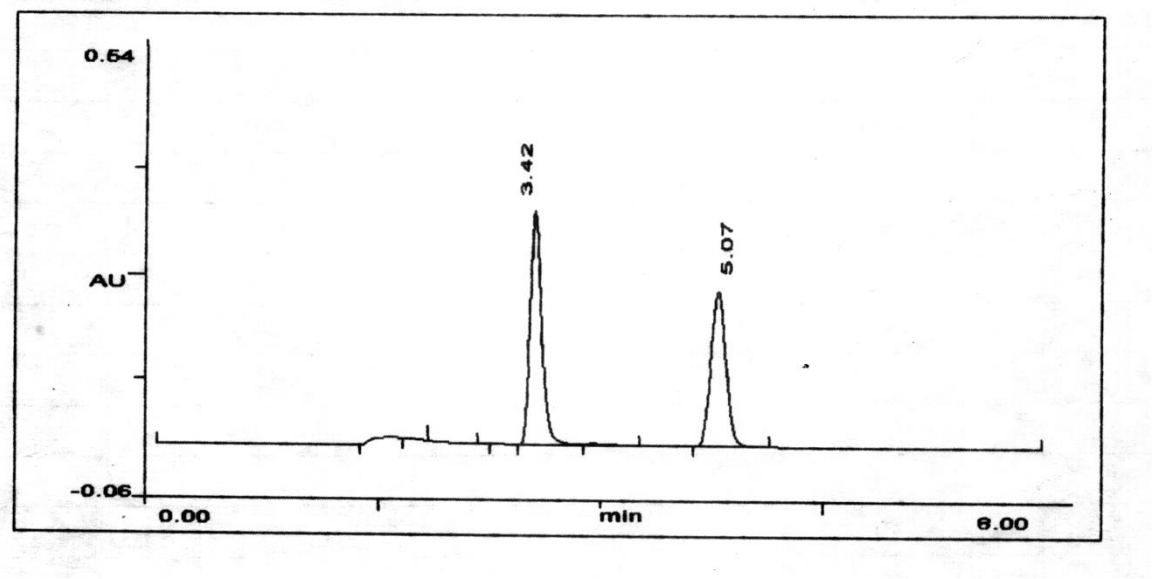


Fig. 3 Chromatogram obtained from sample solution (Balacol capsule)

Table I Results from method development and optimisation studies

S.No.	Nature and composition of mobile phase	Flow rate (ml/min)	pH	Column	T	As	Resolution between Balsalazide and Nifedipine
1	(ACN: MeOH: TEA Buffer) 30: 30: 40	0.7	3	C_{18}	1.8	4.0	6.54
2	(ACN: MeOH: TEA Buffer) 30: 40: 30	0.7	3	C_{18}	1.5	2.2	1.85
3	(ACN: MeOH: TEA Buffer) 40: 30: 30	0.7	3	C_{18}	1.36	1.55	3.3

T, Tailing factor; As, Asymmetric factor;

Table II Results from system-suitability study

Parameters	**Values***	
	BSZ	**IS**
Retention time	3.42	5.07
Area ratio of BSZ/IS	1.104	1.104
Capacity factor	2.13	3.65
Asymmetrical factor	1.55	1.14
Tailing factor	1.36	1.09
Number of theoretical plates	5141	7939
Resolution	Between BSZ and IS 3.3	

* Mean of 10 observations

Table III Important calibration data

Validation Parameters	**Values***
Concentration range (µg/mL)	10 - 50
Number of concentration range	5
Regression equation	Y= 0.037272x + 0.008476
Slope(b)	0.037272
Standard deviation on intercept ($S_{a)}$	0.002865
Intercept (a)	0.008476
Standard deviation on intercept ($S_{a)}$	0.0002075
Determination coefficient	0.9998
Residual sum of square	0.000606
F-value	16026.54

* Mean from six determinations (n = 6)

Table IV Results from quantitative analysis of Balsalazide

Sample	**Amount Present (mg/ capsule)**	**Amount Found* (mg/ capsule)**	**Recovery* %**	**R.S.D. %**
Balacol	750	743 ± 0.106	99.09	0.107

* Standard deviation (n = 10)

Table V Intermediate precision of the RP–HPLC method

Drug	**Sample No.**	**Labeled amount (mg/cap)**	**Percentage obtained***		**S.D**		**% R.S.D.**	
			Intra day	**Inter day**	**Intra day**	**Inter day**	**Intra day**	**Inter day**
Balacol	1	750	100.25	98.56	0.8800	1.6266	0.8856	1.6280
	2	750	98.49	99.47				
	3	750	99.36	101.72				

* Standard deviation (n = 10)

Table VI Accuracy of the method

Sample No.	**Amount present (mg/ml)**	**Amount added (mg/ml)**	**Amount estimated* (mg/ml)**	**Amount recovered (mg/ml)**	**% Recovery***	**S.D**	**% R.S.D**	**S.E.**
1	14.67	7.37	22.04	7.34	99.59			
2	14.34	14.74	29.08	14.60	99.10	1.429	1.434	0.583
3	14.55	22.11	36.66	22.46	101.58			
4	14.74	29.48	44.22	28.95	98.20			

***** Standard deviation (n = 10)

RESULTS AND DISCUSSION

Method Development and Optimisation

Column chemistry, solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent(s) in the mobile phase), additive strength, detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimised so the peak from the first eluting compound did not interfere with those from the solvent, excipients, or plasma components. Other criteria, viz. time required for analysis, appropriate k range ($1 < k < 10$) for eluted peaks, assay sensitivity, solvent noise, and use of the same solvent system for extraction of drug from formulation matrices during drug analysis, were also considered. After each change of mobile phase the column was reequilibrated by passage of at least ten column volumes of the new mobile phase.

To investigate the appropriate wavelength for determination of BSZ and IS, solutions of these compounds in the mobile phase were scanned by UV–visible spectrophotometry (Shimadzu, Japan; model UV-1700PC) in the range 200–400 nm. From the overlaid UV spectra, it showed that at 254 nm, both BSZ and IS have marked absorbance. It was observed there was no interference from the mobile phase or baseline disturbance at 254 nm. It was, therefore, concluded that 254 nm is the most appropriate wavelength for analysis of BSZ (Fig. 2).

Because the compounds of interest are predominantly polar and of low molecular mass, a reversed-phase column C_{18} Phenomenax (150 mm × 4.6 mm i.d., 5-µm particles) is used. Several binary mobile phases containing TEA solution (pH 3.0; 10 mM) and ACN (30–40% v/v) were evaluated with the C_{18} column. The retention times of the solutes increased with increasing concentration of organic modifier. It is well known that multiple-component mobile phases result in better separation efficiency than binary mobile phases, because with these solvent strength and selectivity can be varied simultaneously to obtain the retention times desired [11, 12]. A third component, MeOH, was therefore included in the mobile phase and ternary mixtures of ACN, MeOH and TEA buffer solution (pH 3.0; 10 mM) in the proportions 30:30:40, 30:40:30 and 40:30:30 (v/v) were tried. Use of the last of these resulted in a quality separation in terms of peak symmetry, optimum resolution, reasonable run time, and acceptable k values (Table I). No further improvement in peak symmetry was observed when a higher TEA concentration (25 mM) was used. With the optimized mobile phase, increasing the flow rate from 0.6 to 1.2 mL min^{-1}reduced the run time of BSZ. The optimised chromatographic conditions were, therefore, use of the C_{18} column with ACN: MeOH: TEA solution (pH 3.0; 10 mM), 40:30:30 (v/v) as mobile phase at 0.7 mL min^{-1}. This method was therefore validated in accordance with ICH guidelines.

Method Validation

System suitability

The resolution factor between BSZ and IS, in the developed method, was above 2. The %R.S.D. of peak area ratios of BSZ to that of IS and retention times for both drug and IS were within 2% indicating the suitability of the system [13, 14] (Table II). These results indicate the applicability of this method to routine with no problems, its suitability being proved. The system suitability parameter like capacity factor, asymmetric factor, tailing factor, HETP and Number of theoretical plates also calculated. It was observed that all the values are within the limits.

The statistical evaluation of the proposed method revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of BSZ in tablet formulation.

Linearity

Five points calibration graphs were constructed covering a concentration range 10-50 µg mL^{-1}. Six independent determinations were performed at each concentration. Linear relationships between the ratio of peak area signal of BSZ to that of IS versus the corresponding drug concentrations were observed, as shown by the results presented in Table III. The standard deviations of the slope and intercept were low. The determination coefficient (r^2) exceeded 0.999.

The calibration plots for BSZ was constructed by plotting peak area ratio against their respective

concentrations and the method was evaluated by determination of the correlation coefficient and intercept, calculated in the corresponding statistical study (ANOVA; $P < 0.05$). R^2 values >0.999 and intercepts very close to zero confirmed the good linearity of the method.

LOD and LOQ

Calibration plots were constructed in the very low concentration region 10 to 50 µg mL^{-1} for BSZ for calculation of the Limit of detection (LOD) and Limit of quantification (LOQ) using eqs (1) and (2), respectively.

$$LOD = \frac{3.3\sigma}{S} \quad (1)$$

$$LOQ = \frac{10\sigma}{S} \quad (2)$$

Where σ is the residual standard deviation of the regression line and S is the slope of the standard plot. The values obtained for LOD was 0.01643 µg mL^{-1} , the LOQ was 0.04960 µg mL^{-1}.

Precision

The repeatability study (n=6) carried out showed a R.S.D. of 0.107 for the peak area ratio of BSZ of IS obtained, thus showing that the equipment used for the study worked correctly for the developed analytical method and being highly repetitive. These results were summarized in Table IV. For the intermediate precision a study carried out by the same analyst working on 3 consecutive days (n=3) indicated a R.S.D. of 0.8856 and 1.6280% for Intra day and Inter day, respectively. Both values were far below 2%, the limit percentage set for the precision and indicated a good method precision. These results were summarized in Table V.

Accuracy

The data for accuracy were expressed in terms of percentage recoveries of BSZ in the real samples. These results are summarized in Table VI. The mean recovery data of BSZ in real sample were within the range of 98.20 and 101.58% for Balacol. The % R.S.D. was found to be 1.434, satisfying the acceptance criteria for the study.

Specificity

The HPLC chromatogram recorded for the mixture of the drug excipients revealed no peak within a retention time range of 6 min. The results showed that the developed method was specific as none of the excipients interfered with the analytes of interest (Fig.3).

Stability

The stability of BSZ in standard and sample solutions containing IS determined by storing the solutions at ambient temperature (20±1°C) protected from light. The solutions were checked in triplicate after 3 successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 97%. This denotes that BSZ is stable in standard and sample solutions for at least 2 days at ambient temperature, protected from light and is compatible with IS.

Capsule Assay and Content Uniformity

The method developed in this study was used for determination of the BSZ content of Balacol capsules. Total chromatographic analysis time per sample was 6 min with BSZ and IS eluting at retention times of 3.42 and 5.07 min, respectively (Fig. 3). Assay results from three replicate analysis of Balacol capsules showed recovery was in the range of 98.20 and 101.58% and the %RSD was found to be 1.434.

The results from the content uniformity experiment revealed that the BSZ content of the ten capsules examined was in the range 99.36–101.06% and %RSD was found to be 1.543. This indicates that distribution of the drug in the capsules is uniform without significant variation. According to US pharmacopoeia the acceptance limits for drug-content uniformity and RSD are 85–115% and <6%, respectively.

There was good agreement between assay results and the label claim of the product. %RSD values both for capsule potency and for content uniformity testing were <3%. This is indicative both of a well-controlled manufacturing process and a precise analytical method.

CONCLUSION

A simple isocratic RP-HPLC method with UV detection has been developed for estimation of BSZ in pure and in capsule dosage forms. The method was validated for accuracy, precision, specificity, and linearity. The run time is relatively short (6 min), which enables rapid quantification of many samples in routine and quality control analysis of capsules. The method also uses a solvent system with the same composition as the mobile phase for dissolving and extracting drug from the matrices, thus minimizing noise. Therefore, it is suitable for the routine analysis of BSZ in pure and in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC-MS that is complicated, costly and time consuming rather than a simple HPLC-UV method. Hence the proposed method could be useful for the national quality control laboratories in developing countries.

REFERENCES

[1]. Gita Chauraria, D. Bhadra, S. Bhadra, and N.K. Jain, Indian J. Pharm. Sci., **66**, 2005, 606
[2]. J. R. Green, C. H. Swan, and A. Rowlinson, Aliment.Pharmacol. Therap., **427**, 2000, 647.
[3]. M. H. Giaffer, C. D. Holdworth, and J. E. Lennard Jones, Aliment.Pharmacol. Therap., **425**, 2000, 479
[4]. J. R. B. Green, A. J. Lobo, and C. D. Holdsworth, Gastroenterology, **86**, 2000, 114
[5]. F. N. Hussain, R. A. Ajjan, and S. A. Riley, Br. J. Clin. Pharmacol., **4**, 2000, 323.
[6]. W. J. Sandborn, S. B. Hanauer, and A. Buch, Aliment.Pharmacol. Therap., **418**, 1998, 1089
[7]. P. B. McIntyre , C A. Rodrigues , J. E. Lennard Jones , I. G. Barrison , J. G Walker, J. H. Baron and P.C. Thornton , Clin. Pharmacokinet, **10**, 1985, 285.
[8]. B. N. Suhagia, H. M. Patel, S. A. Shan, I. S. Rathod, and B. P. Marolia, Indian J. Pharm. Sci., **84**, 2005, 634.
[9]. International Conference on Harmonization (ICH), Q2A: Text on Validation of Analytical Procedures: Definitions and Terminology, Vol. 60, US FDA Federal Register, 1995
[10]. International Conference on Harmonization (ICH), Q2B: Validation of Analytical Procedures: Methodology, Vol. 62, US FDA Federal Register, 1997
[11]. D. Eberle, R. P. Hummel, and R. Kahn, J. Chromatogr. A, **759**, 1997, 185.
[12]. Q. B. Cass, A. L. G. Degani, N. M. Cassiano, and J. Pedrazolli Jr., J. Chromatogr. B, **766**, 2001, 153.
[13]. L. R. Snyder, J. J. Kirkland, and J. L. Glajch (Eds), Practical HPLC Method Development, Wiley Interscience, New York, 1988, 685-695
[14]. The United States Pharmacopeia 28/National Formulary 23, United States Pharmacopeia Convention, Rockville, MD, 2004, 1620-1623.