Development and validation of HPTLC method for simultaneous estimation of cetirizine hydrochloride and phenylpropanolamine hydrochloride in tablet dosage form

P. S. Dhongle*[1], S. J. Sahare[2], S. S. Dhongle[3], A. S. Mundhey[1], S. P. Wate[1]

[1]*Sharad Pawar College of Pharmacy, Nagpur*
[2]*JL.Chaturvedi College of Pharmacy, Nagpur*
[3]*Hislop College, Nagpur*

_____

ABSTRACT

A Simple, rapid, accurate and precise high performance thin layer chromatography method has been developed and validated for simultaneous estimation of Cetirizine hydrochloride and Phenylpropanolamine hydrochloride in tablet dosage form. The method employed TLC aluminium plates precoated with silica gel 60F254 as stationary phase. The solvent system consisted of ethyl acetate: methanol: formic acid (7.5:1.5:0.5 v/v/v). This system gives well resolved spots for Cetirizine hydrochloride (R_f value 0.34) and Phenylpropanolamine hydrochloride (R_f value 0.45). Spectrodensitometric scanning integration was performed at a wavelength of 254nm.The method was validated by various parameters as per ICH and USP requirement. The calibration curve was found to be linear in range of 10-35µg/mL for Cetirizine hydrochloride and 4-25µg/mL for Phenylpropanolamine hydrochloride.

Key words: Cetirizine hydrochloride, HPTLC, Phenylpropanolamine hydrochloride, Spectrodensitometric.

_____

INTRODUCTION

Cetirizine is carboxylated metabolite of hydroxyzine and has high specific affinity for histamine H_1 receptor. Cetirizine hydrochloride is chemically known as [2-[4-[(4-chlorophenyl)phenylmethyl]-1piperazinyl] ethoxy acetic acid hydrochloride **(Fig.1)** . It is a white or almost white powder, freely soluble in water, ethanol, methanol, practically insoluble in acetone and dichloromethane [1]. Cetirizine is a highly potent long-acting peripheral H1-receptor antagonist which acts both on the early and late allergic response [2]. Cetirizine hydrochloride is official in Indian Pharmacopoeia. Phenylpropanolamine is sympathomimetic agent and acts as

111

_____

adrenergic alpha agonist. Phenylpropanolamine hydrochloride is chemically known as 2-amino-1-phenylpropan-1-ol hydrochloride **(Fig.2)**. It is a white or almost white crystalline powder**,** soluble in water and ethanol (96%), practically insoluble in dichloromethane [3]. Phenylpropanolamine is an orally active sympathomimetic amine and exerts a decongestant action on the nasal mucosa [2]. Phenylpropanolamine hydrochloride is official in British Pharmacopoeia.

Cetirizine hydrochloride with Phenylpropanolamine hydrochloride is indicated for the relief of symptoms of allergic rhinitis. As per literature survey, no HPTLC method has been reported for the simultaneous estimation of Cetirizine hydrochloride and Phenylpropanolamine hydrochloride in combined dosage form. Although several UV [4-12] and HPLC [13-19], HPTLC [20] method have been reported for individual drug or in other combination.

Thus, focus of present study is to develop and validate a simple, rapid, accurate, precise HPTLC method for simultaneous estimation of Cetirizine hydrochloride and Phenylpropanolamine hydrochloride in tablet dosage form.

MATERIALS AND METHODS

Cetirizine hydrochloride (CET) and Phenylpropanolamine hydrochloride (PPA) standards were procured as a gift sample from Cipla Ltd. Mumbai. All chemicals and reagents used were of analytical grade and obtained from Loba chemie. Marketed formulation (Alerid D, Cipla India Ltd.) containing Cetirizine hydrochloride 5mg and PPA 25mg was procured from local market and used for analysis.

The instrument used in analysis was Camag HPTLC system, comprising of Camag Linnomate V automatic sample applicator with Hamilton syringe (100µl), Camag TLC plate heater, Camag Reprostar 3, Camag TLC scanner 3, Camag twin trough chamber (20×10cm) and Camag Win CATS software.

Chromatographic conditions
The optimal conditions are as follows
Stationary phase   : Precoated Silica Gel 60F254 TLC plate
Format   : 10×10cm
Thickness   : 200µm
Mode of application : Band
Band length   : 8mm
Sample volume   : 5µl
Mobile phase   : Ethyl acetate: methanol: formic acid (7.5:1.5:0.5v/v/v)
Separation mode   : Ascending
Developing chamber : Twin trough glass chamber (20×10cm)
Saturation time   : 10min with mobile phase and spotted plate.
Migration distance   : 50mm
Detection   : UV Densitometric scanning
Measurement mode   : Absorption-reflectance
Scanning wavelength : 254nm
Slit dimension   : 6.00×0.45mm
Scanning speed   : 20mm/sec

_____

To carry out HPTLC analysis [21], first TLC plate was prewashed with methanol. Activation was done in oven at 50˚c for 10 min then activated plates were spotted by means of Linnomat V sample applicator. Spotted plates were dried on plate heater at 60˚c for 5min. For development twin trough chamber along with mobile phase and spotted plate was saturated for 10min. After development a plate was further dried on TLC plate heater at 60˚c for 5min. and finally plate was scanned at 254 nm in scanner.

Standard and Sample Preparation:
A. Standard preparation
a) Standard Solutions:
i) Solution A (CET):
An accurately weighed quantity of 10 mg of CET was dissolved in methanol and volume was made up to 10 mL with same solvent. A 0.1 mL of resultant solution was diluted to 10 mL with methanol (Conc.10 µg /mL).

ii) Solution B (PPA):
An accurately weighed quantity of about 10 mg of PPA was dissolved in methanol and diluted to 10 ml. A 0.5 mL of resultant solution was diluted to 10 mL with methanol (Conc.50 µg /mL).

b) Mixed standard stock solution C:
An accurately weighed quantity of CET (10 mg) and PPA (50 mg) were transferred to 50 mL volumetric flask and dissolved in about 25 mL methanol and the volume was made up to the mark with methanol.

i) Working mixed standard solution C1:
From the above solution 0.5 mL was further diluted to 10 mL by methanol to get concentration of 10 µg /mL and 50 µg /mL for CET and PPA respectively.

B. Sample preparation:
Twenty tablets were weighed and finely powdered. An accurately weighed quantity of tablet powder equivalent to about 5 mg of CET and 25 mg of PPA was transferred to 50 mL volumetric flask, sonicated with 25 mL methanol for 15 min. The volume was made up to the mark with methanol and filtered through whatman filter paper. Further dilutions were carried out to get final concentration 10 µg/mL of CET and 50 µg/mL of PPA was used as sample solution.

Analysis of Standard Laboratory Mixture:
Two bands of std. solution and six band of laboratory mixture C1 of equal volume (5µL) were applied on TLC plate and it was developed and scanned as per chromatographic conditions. Typical densitogram is shown in **Fig.3**

Analysis of Marketed Formulation:
Two bands of std. solution and six band of marketed formulation of equal volume (5µl) were applied on TLC plate and it was developed and scanned as per chromatographic conditions. Typical densitogram shown in **Fig.4**

_____

Validation [22, 23]

The developed method was validated as per ICH and USP requirement in terms of linearity, accuracy, precision, limit of detection, limit of quantification, ruggedness.

Linearity

Linearity evaluates the analytical method ability (within a given range) to obtain a response that directly proportional to concentration (amount) of analyte in the sample

Preparation of Calibration Curve

From the standards solution, final concentration of 10-35 µg/mL for CET and 4-25 µg/mL for PPA were prepared. Each concentration applied on plate three times and it is developed as per optimal chromatographic condition. The graph was plotted between concentrations of drug Vs peak area. **(Fig.5, 6)** Calibration curve was obtained in range of 10-35µg/mL for CET and 4-25 µg/mL for PPA.

Limit of Detection

The limit of detection (LOD) was calculated using following formulae:

LOD = 3.3(SD)/S

Where

SD=standard deviation of response (peak area)

S= average of the slope of the calibration curve.

Limit of Quantitation

The limit of quantitation (LOQ) was calculated using following formulae:

LOQ = 10 (SD)/S

Where

SD=standard deviation of response (peak area)

S= average of the slope of the calibration curve.

Accuracy

Accuracy of proposed method was ascertained on the basis of recovery studies performed by standard addition method at different levels of labeled claim (i.e. 80 to 120 % of labeled claim). The percent recovery was calculated by using following formula:

$$\% \text{ Recovery} = \frac{A - B}{C} \times 100$$

Where,

A   =   Total amount of drug estimated, mg

B   =   Amount of drug contributed by tablet powder, mg

C   =   Amount of pure drug added, mg

Precision

Precision of an analytical method is expressed as S.D. and % R.S.D. of series of measurement. It was ascertained by replicate estimation of both drugs by proposed method.

_____

Specificity

The specificity studies were carried out by attempting deliberate degradation of the tablet sample with exposure to stress conditions like acidic (0.1 N HCl), alkaline (0.1 N NaOH), oxidizing (3% H_2O_2), and heat (60 ^{0}C).

Ruggedness:

The Ruggedness studies were carried out for different parameters i.e. different elapsed times (Intraday and Interday) and different analysts

RESULTS AND DISCUSSION

For HPTLC method development, both pure drugs were spotted on TLC plates and run in mobile phase consisting of ethyl acetate: methanol in varying ratio was tried. With these mobile phases diffused spot were obtained for both CET and PPA. When formic acid was added in this mobile phase the spots were found to improve. Hence ethyl acetate: methanol: formic acid (8:1:1v/v/v) was tried here again spot for PPA was slightly diffused. Decreasing the concentration of formic acid and ethyl acetate with increasing concentration of methanol improved the spot characteristics. Finally the mobile phase ethyl acetate: methanol: formic acid in the ratio 7.5: 1.5: 0.5 v/v/v gave good resolution of two components with R_f value 0.34 and 0.45 for CET and PPA. Optimum wavelength selected was 254 nm respectively.

The spot of pure drug were observed in the chromatogram of the drug sample extracted from the developed bilayered tablets. There was no interference from exicipient present in the tablet, as evidence from chromatogram of marketed formulation **(Fig.4).** The drug content was found to be 99.42 % for CET and 99.17% for PPA (**Table.1**) .The polynomial regression data for calibration plot showed good linear relationship over concentration range 10-35 μg/mL for CET and 4-24 μg/mL for PPA. CET and PPA showed good correlation coefficient (r^2 = 0.9992 for CET and 0.9988 for PPA) in given concentration range. Limit of detection was found to be 0.0219 μg/mL for CET and 0.0353 μg/mL for PPA, whereas Limit of quantitation was found to be 0.0605 μg/mL for CET and 0.1071 μg/mL for PPA. The results in **Table.2** revealed excellent accuracy and precision of assay method. The proposed method used for simultaneous determination of drug in combination from pharmaceutical dosage form after spiking with 80%, 100% and 120% of additional drug afforded recovery 99-100%. The ruggedness of the method was determined by intraday, interday and different analyst studies.
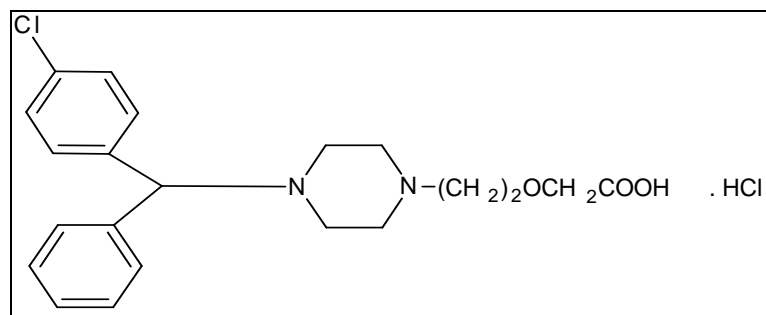

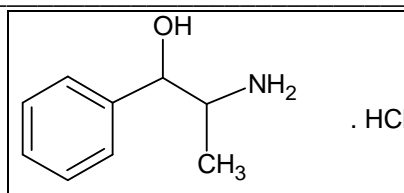**Fig .1: Structure of Cetirizine hydrochloride**

_____

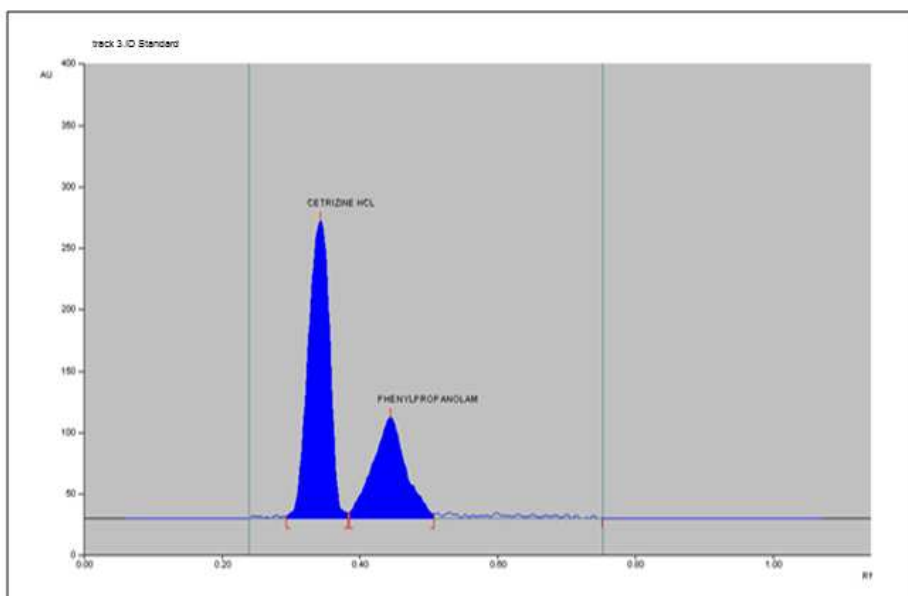**Fig. 2: Structure of Phenylpropanolamine hydrochloride**
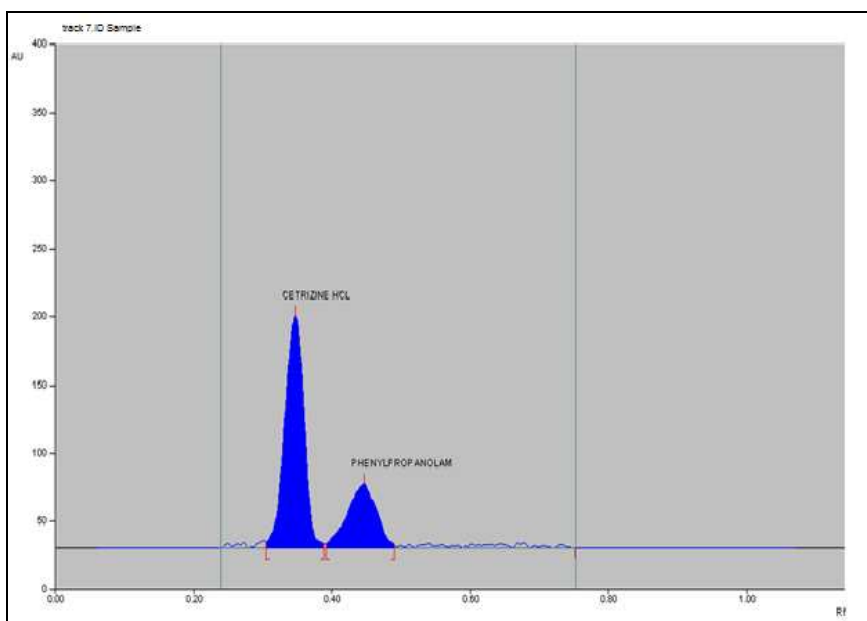
Fig. 3: Typical HPTLC Densitogram of Standard Solution
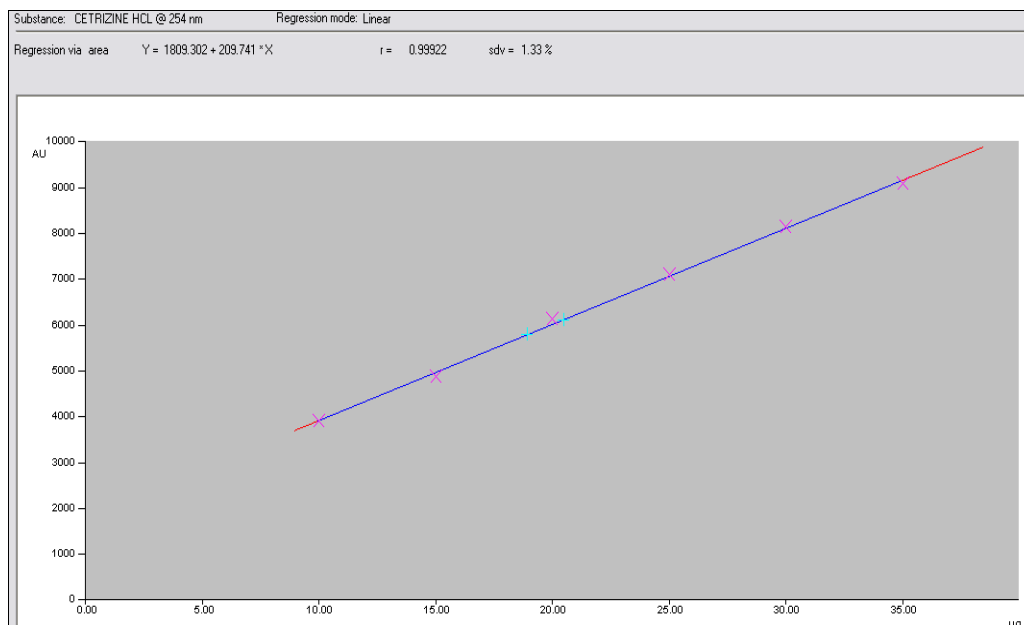
Fig. 4: Typical HPTLC Densitogram of marketed formulation

Substance: CETRIZINE HCL @ 254 nm	Regression mode: Linear

Regression via area	Y = 1809.302 + 209.741 * X	r = 0.99922	sdv = 1.33 %

Fig. 5: Calibration Curve of Cetirizine hydrochloride (CET)

Substance: PHENYLPROPANOLAM @ 254 nm	Regression mode: Linear

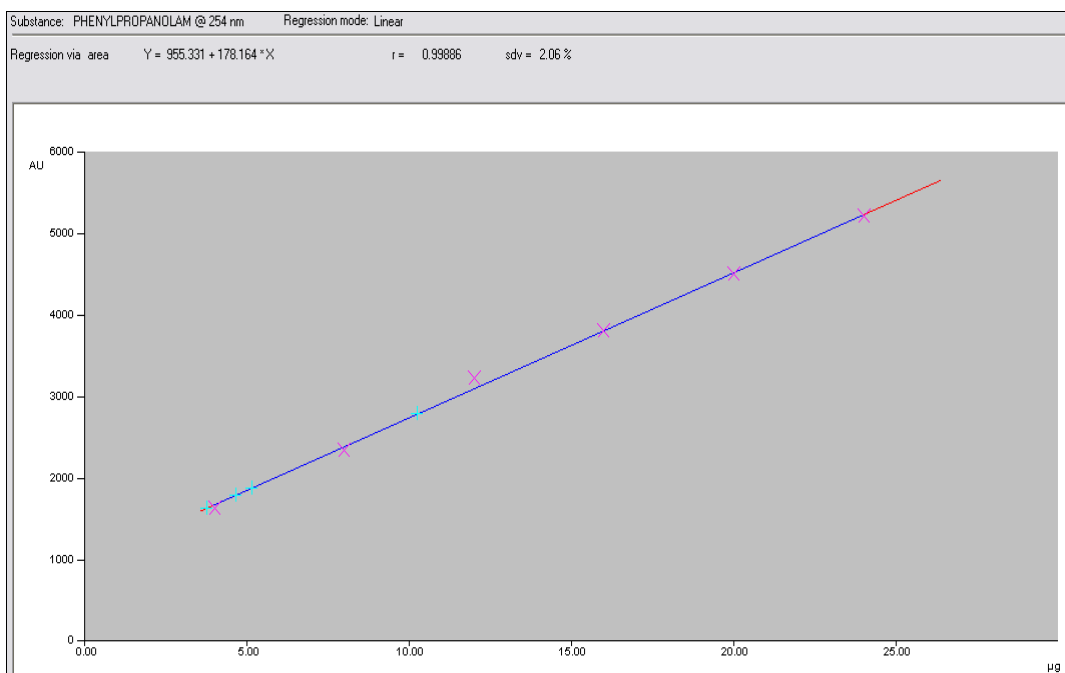| Regression via area	Y = 955.331 + 178.164 * X	r = 0.99886	sdv = 2.06 %

Fig. 6: Calibration Curve of Phenylpropanolamine hydrochloride (PPA)

_____

Table 1: analysis of marketed formulation

| Sr. No. | Sample | Particulars | Estimation (%) | |
			CET	PPA
1.	Alerid-D	Mean (n=5)	99.42	99.17
		SD	0.299	0.430
		RSD (%)	0.0029	0.0043

Table 2: Validation Parameter

Sr.no	Method characteristics	CET	PPA
1	**Linearity performance parameter**		
	Linearity range	10-35 μg/ mL	4-24 μg/ mL
	Correlation coefficient	0.9992	0.9988
	LOD	0.0219 μg/ mL	0.0353 μg/ mL
	LOQ	0.0605 μg/ mL	0.1071 μg/ mL
2	**Accuracy (% recovery)**		
	80%	99.95	100.02
	100%	99.68	99.97
	120%	99.63	100.11
3	**Precision (% RSD)**		
	Repeatability of peak area	0.0028	0.0070
4	**Ruggedness (%)**		
	Intraday	99.96	100.27
	Interday	100.96	101.11
	Different analyst	100.26	100.62
5	**Specificity (%)**		
	Normal	100.06	100.53
	0.1NHCl	72.92	32.89
	0.1N NaOH	55.61	50.68
	3% H_2O_2	66.25	74.83
	Thermal	47.46	62.75

CONCLUSION

The developed HPTLC method is simple, precise, specific and accurate and from statistical data, it is found that method is reproducible and selective for analysis of Cetirizine hydrochloride and Phenylpropanolamine hydrochloride in tablet dosage form.

REFERENCES

[1] Indian Pharmacopoeia, vol. 2, controller publication New Delhi **2007**; 896.
[2]. www.http//alerid_d tablet.//.org.in

118

---

[3]. British Pharmacopeia, vol.1, Int. Ed. Published on the recommendation of the medicine comission pursuant to medicine act 1968,**1993**; 511.

[4]. MY Khuhawar; FM Arind; Rajpar A. *J Food Drug Anal*, **2005**, 13, 4, 388-391.

[5]. A Trivedi; L Banerjee. *J Pharm Res,* **2010**, 3, 6, 1398-1401.

[6]. AFM Walily; MA Korany; A Gindy; MF Bedair. *J Pharm Biomed Anal*, **1998**, 17, 435-442.

[7]. SK Panda; AK Sharma; LK Sahu. *Indian J Pharm Sci*, **2002**, 64, 6, 540-544.

[8]. P Trivedi, A Sachan. *East Pharm,* **1999**, 42, 496, 107-112.

[9]. AK Sahu; LK Sahu. *Indian J Pharm Sci*, **2000**, 62, 3, 205-209.

[10]. CN Nalini; K Kavitha; M Nappinnai; RS Cherian. *Indian drugs*, **2003**, 40, 11, 658-660.

[11]. NM Bhatia; SK Ganbavale; HN More. *Asian J Pharm*. **2008**, 159-162.

[12].PR Patel; FA Mehta. *J Anal Chem*, **2010**, 1, 1-9.

[13]. PS Selvan; R Gopinath; VS Saravanan; N Gopal. *Indian J Chem*, **2006**, 18, 4, 2591-2596.

[14]. S Karakus, I Kucukguzel, SG Kucukguzel. *J Pharm Biomed Anal*, **2008**, 46, 295-302.

[15]. N Badwe; A Garg; D Eapen; Seth PD. *East Pharm*. **1995**, 38, 452, 179-180.

[16]. B Raman; GV Kanumular; IC Bhoir. *Indian drug* **2001**, 38, 6, 294-298.

[17]. AMY Jabera; HA Sherifeb; MM Omarib; AA Badwanb. *J Pharm Biomed Anal*, **2004**, 36, 341-350.

[18]. SS Zarapkar; UP Halkar; SH Rane. *Indian Drugs* **1998**, 35, 10, 658-661.

[19]. AS Birajdar; SN Meyyanathan; RB Raja; N Krishnaveni; B Suresh. *Acta Chromatgraphica* **2008**, 20, 8, 411-421.

[20]. SN Makhija; PR Vavia. *J Pharm Biomed Anal*, **2001**, 26, 663-667.

[21]. PD Sethi. HPTLC Quantitative Analysis of Pharmaceutical Formulation.1st ed.CBS publishers and distributor, New Delhi, **2001**; pp. 3-60.

[22]. ICH, Q2B, Validation of Analytical Procedure: Methodology. Geneva: International Conference on Harmonization, **Nov 1996**.

[23]. United States Pharmacopoeia, vol.2, Asian Edition NF27, The Official Compounds of Standards **2007**; 680-683.