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Validation of RP-HPLC method for the quantification of N-Bromosuccinimide in Angiotensin II receptor antagonists in Pharmaceuticals

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ABSTRACT

The present paper describes the development of a reversed-phase high performance liquid chromatographic (RP-HPLC) method for N-Bromosuccinimide (NBS) determination in group of "sartans" pharmaceuticals. The gradient LC method employs solution A and solution B as mobile phase. The solution A contains 0.01%v/v Ortho-phosphoric buffer and Acetonitrile as Solution B. Successful chromatographic separation were achieved on a Zorbax XDB C_{18}, 250mm x 4.6mm, 5µm particle size column without co-elution of drugs and its process related impurities and degradation products. The developed RP-HPLC method was validated with respect to specificity, linearity, accuracy, precision and sensitivity with detection limits and quantification limits are 0.007 mg/ml and 0.022 mg/ml respectively [1]. To the best of our knowledge, a rapid LC method for the determination of NBS in pharmaceuticals, disclosed in this investigation was not published elsewhere.

key words: RP-HPLC, NBS, Candesartan, Telmisartan, Irbesartan, Valsartan, Validation.

INTRODUCTION

Angiotensin II receptor antagonists, also known as angiotensin receptor blockers (ARBs), AT_1-receptor antagonists or sartans, are a group of pharmaceuticals which modulate the renin-angiotensin-aldosterone system. Their main use is in high blood pressure (hypertension), kidney damage due to diabetes (diabetic nephropathy) and congestive heart failure [2]. Drug substances which includes tetrazole group and one or two imidazole groups like Candesartan (CAN), Telmisartan (TEL), Irbesartan (IRB) and Valsartan (VAL) were selected. In Figure-1, the chemical structures of these drug substances are given including that of N-Bromosuccinimide.

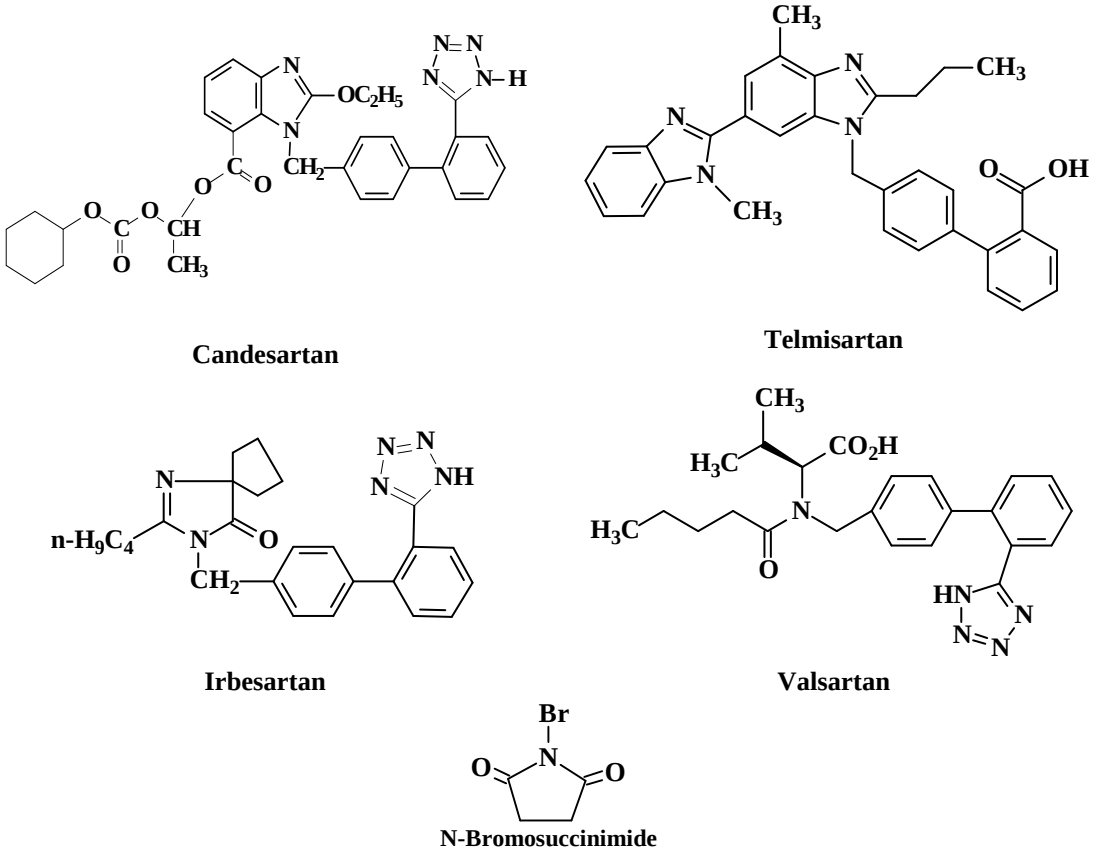


Figure-1

NBS is a brominating and oxidizing agent that is used as source for bromine in radical reaction like allylic brominations and various electrophilic additions. The brominations of NBS for substrate such as alcohol and amines, followed by elimination of Hydrogen bromide in the presence of a base, leads to the product of net oxidation in which no bromine has been incorporated [3-5]. During the preparation of the drug, active mechanism of NBS is disclosed in Figure-2.

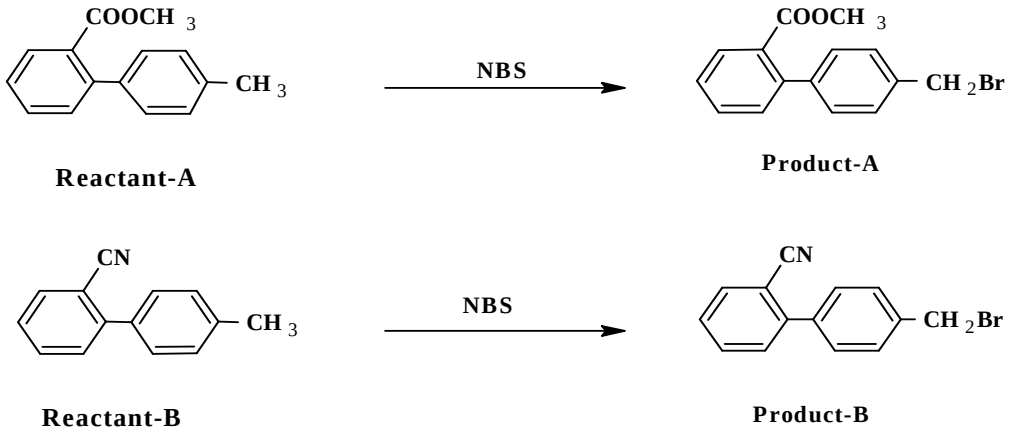


Figure-2

Monitoring of NBS in these drug substances is essential for preserving the desired quality of active moiety of the compound as bromine toxicity destroys thyroid and metabolism [6-8]. In view of that the impurities can arise during the manufacturing process and storage of the drug substance and the criteria for their acceptance up to certain limits are based on pharmaceutical studies or known safety data [9]. There are so many literatures available for determination of

Valsartan, Irbesartan, Telmisartan and Candesartan drug. Several techniques have been reported for the determination of drugs using NBS by Spectrophotometric and titrimetry [10-11].The aim of this study was to develop a rapid, economical, selective and easy liquid chromatographic method for the determination of NBS in these drugs. By employing simple gradient HPLC method [12], the elution pattern was established without co-elution of other impurities.

MATERIALS AND METHODS

Reagents and Chemicals:
All reagents used were of analytical reagent grade unless stated otherwise, Reference standard of NBS were procured from chemical lab, (Gujarat, India), Ortho-phosphoric acid were procured from (E.Merck Limited, Mumbai, India), LC grade methanol and Acetonitrile purchased from Merck (Mumbai, India) and water obtained from Milli-Q purification system. The investigation samples were gifted by research laboratory of Aurobindo Pharma Ltd, Hyderabad, India.

Standard stock and sample solution:
Standard solution was prepared by dissolving accurately weighed 20mg of NBS in 100ml volumetric flask to this 30ml of Mill-Q water were added sonicated to dissolve, make up to the mark with the same solvent. Diluted 1ml of the resulting solutions was taken into 20ml volumetric flask make up to volume with same solvent (10 µg ml^{-1}) as stock solution. Further, 1ml of this solution were diluted to 10ml volumetric flask containing 1ml of Methanol, mixed well then make up to volume with Mill-Q water (1 µg ml^{-1}) as standard solution, through 0.45µm Millex HV membrane filter.

Sample solution was prepared by dissolving accurately weighed 50 mg of each drug substance in 50 ml volumetric flask, to this 5 ml of methanol were added sonicated to dissolve, make up to volume with Milli-Q water filtered through 0.45 µm Millex HV membrane filter.

Chromatographic condition:
A HPLC system Waters alliance 2695 separation module equipped with a 2996 photodiode array detector along with Empower software for data acquisition and processing was used (Waters, Milford, USA), using Zorbax XDB C_{18} column, 250mm x 4.6mm, 5µm particle size, (Agilent Technologies, USA). 1ml of Ortho-phosphoric acid is taken into a 1000ml of water, as mobile phase-A and LC grade Acetonitrile as Mobile phase-B. Flow rate was set to 1.0 ml min^{-1} and injection volume of 50µl. The analysis was carried out under the gradient conditions are as follows,

Time (minutes)/A(v/v):B(v/v); $T_{0.01}$/98:2, T_8/98:2, T_{10}/12:88, T_{16}/12:88, T_{17}/98:2, T_{25}/98:2. The peak homogeneity was expressed in terms of peak purity values using Empower software, 2996 Photodiode array detector at 205nm.

RESULTS AND DISCUSSION

Method optimization and technique development summary
The main objective of this chromatographic technique was to determine content of NBS present in Valsartan, Irbesartan, Candesartan and Telmisartan without co-elution of other impurities of these drug substances. NBS co-eluted with some unknown impurities in other stationary phases like C_8, phenyl and Cyano as well as different mobile phases. In the described experiment, effect of organic modifier on retention and elution were studied with respect to drug concentration. Based on the solubility nature methanol was found to be the best suit as diluent, but NBS is

freely soluble in water and Telmisartan is insoluble in both the media. Table-1, describes the selectivity of diluent for each of the selected drug substance during sample preparation.

Table 1: Diluent selectivity

Diluent	Drug substance observation				
	Valsartan	Irbesartan	Candesartan	Telmisartan	Observation
Methanol (MeOH)	Clear solution	Clear solution	Clear solution	Insoluble solution	Peak shape affected
Water (H_20)	White curdy precipitate	White curdy precipitate	White curdy precipitate	White curdy precipitate	Drug is insoluble
MeOH :H_20 (1:1v/v)	Clear solution	Clear solution	Clear solution	Insoluble solution	Peak shape affected
MeOH :H_20 (9:1v/v)	White curdy precipitate	White curdy precipitate	White curdy precipitate	White curdy precipitate	Peak shape is good
MeOH :H_20 (1:9v/v) &100µl 0.1M NaOH	Clear solution	Clear solution	Clear solution	Clear solution	Stability is low

From the above table, it is concluded that the last two combinations of diluents is suitable. In the case of second last diluent composition, precipitation of the drug substance was observed in addition to insoluble nature of Telmisartan. So it is concluded that the last diluent of the above table is suitable for quantification of NBS. But the stability of sample solution of the drugs shows low stability for NBS in the last diluent of the above table. Methanol and water (1:9 v/v) is selected as diluent for this study. The method was optimized, using simple phosphoric acid buffer and Acetonitrile as organic solvent.

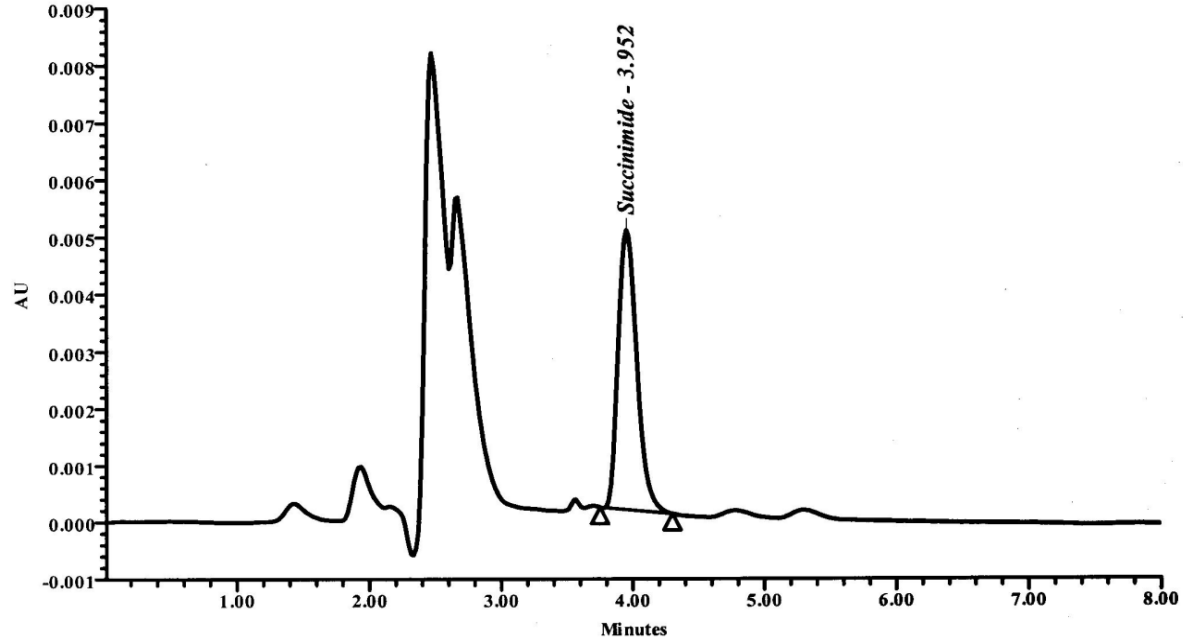


Fig-3., Typical chromatogram of Standard

The initial data acquisition up to 8 minutes of chromatogram is considered for NBS determination, the gradient time after 8 minutes taken as injection delay, because in this investigation the peak of interest is NBS. But in the case of specificity analysis about 25 minutes run time is considered to check the retention time for all known related impurities of each drug substances. The effect of column oven temperature also studied at variable points. Slight decreases in resolution of NBS with unknown impurities were noticed towards lower side when the temperature rises. Hence, it is concluded that the best result were achieved when the temperature is ambient. From overall chromatographic conditions determination of NBS was

established by using 0.01%v/v H_3PO_4 and Acetonitrile with gradient chromatographic condition with column oven temperature of 25°C. NBS has shown ionization into succinimide with the liberation of free bromine was confirmed by injecting pure Succinimide and Hydro bromide solution into the chromatograph. Hence in this investigation NBS is quantified as succinimide. The Figure-3 represents typical chromatogram of NBS standard.

The proposed HPLC method was validated as per guideline [10] for specificity, linearity, accuracy, limit of detection and limit of quantification, precision and stability of sample solution.

Validation of the method:

Selectivity

The sample solutions of impurities, sample and standard were prepared at 0.1%w/w concentration based on the selected drug like CAN, TEL, IRB and VAL and injected into the chromatographic system to identify the retention time. The retention time of NBS was found to be about 3.952 min. However, the known related impurities and concentrated main analyte of the respective drugs were eluting in the gradient programme. The sample was found to contain NBS at very low level, and therefore, the samples were spiked with NBS at 0.1% w/w level along with other known impurities of these drugs, thereby indicating that the method is selective for determining the content of NBS. Table-2 describes the peak purity results for these drugs as per above selectivity.

Table 2, Specificity experimental data of N-Bromosuccinimide in drug matrix

Drug substance	Control [a]		Spiked [b]		Retention time (min)
	PA	PT	PA	PT	
CAN	0.196	0.500	0.240	0.557	15.348
TEL	0.231	0.362	0.362	0.398	13.982
IRB	0.235	0.495	0.492	0.654	12.378
VAL	0.143	0.311	0.262	0.301	13.204

[a] *drug substance spiked with NBS at 0.1% level*

[b] *drug substance spiked with all known impurities and NBS at 0.1% level*

In view of the above selectivity result, overlay chromatogram of standard, diluent and sample chromatogram of respective drug substances spiked with NBS is enclosed as Figure-4.

Linearity:

By measuring area responses at different levels of NBS over the range of 5% to 150% of drug concentration, the linearity data were validated. The solutions were prepared from stock solutions of required concentration for six different levels of 0.050, 0.099, 0.249, 0.497, 0.746, 0.994, 1.491 and 1.988µg ml^{-1}, the calculated values for 8 Calibration points in µg ml^{-1} are slope (35537), STEY X (79), Y-intercept (35) and correlation co-efficient (r) 1.0000. The area and concentration treated were treated by least squares linear regression analysis plot [Area counts (AU) at Y-axis Vs Concentration (µg ml^{-1}) at X-axis] as Figure-5.

Sensitivity:

The solutions were prepared from known stock concentration to predict the limit of detection (LOD) and limit of quantification (LOQ).The values were predicted using slope (S) and residual standard deviation (S.D) obtained from a linear regression line performed at lower concentration levels. The predicted limit of detection and quantification for NBS was found to be 0.002%w/w and 0.001%w/w respectively, by using the calculation 3.3*STEY.X/SLOPE*100/Sample concentration (for LOD) and 10*STEY.X/Slope*100/Sample concentration (for LOQ), and each predicted level was verified for precision by analyzing six replicate measurements.. The

percentage relative standard deviation for six replicate measurements at predicted LOD and LOQ concentration levels was found to be 10.2 and 2.8 respectively, verifying the predicted values.

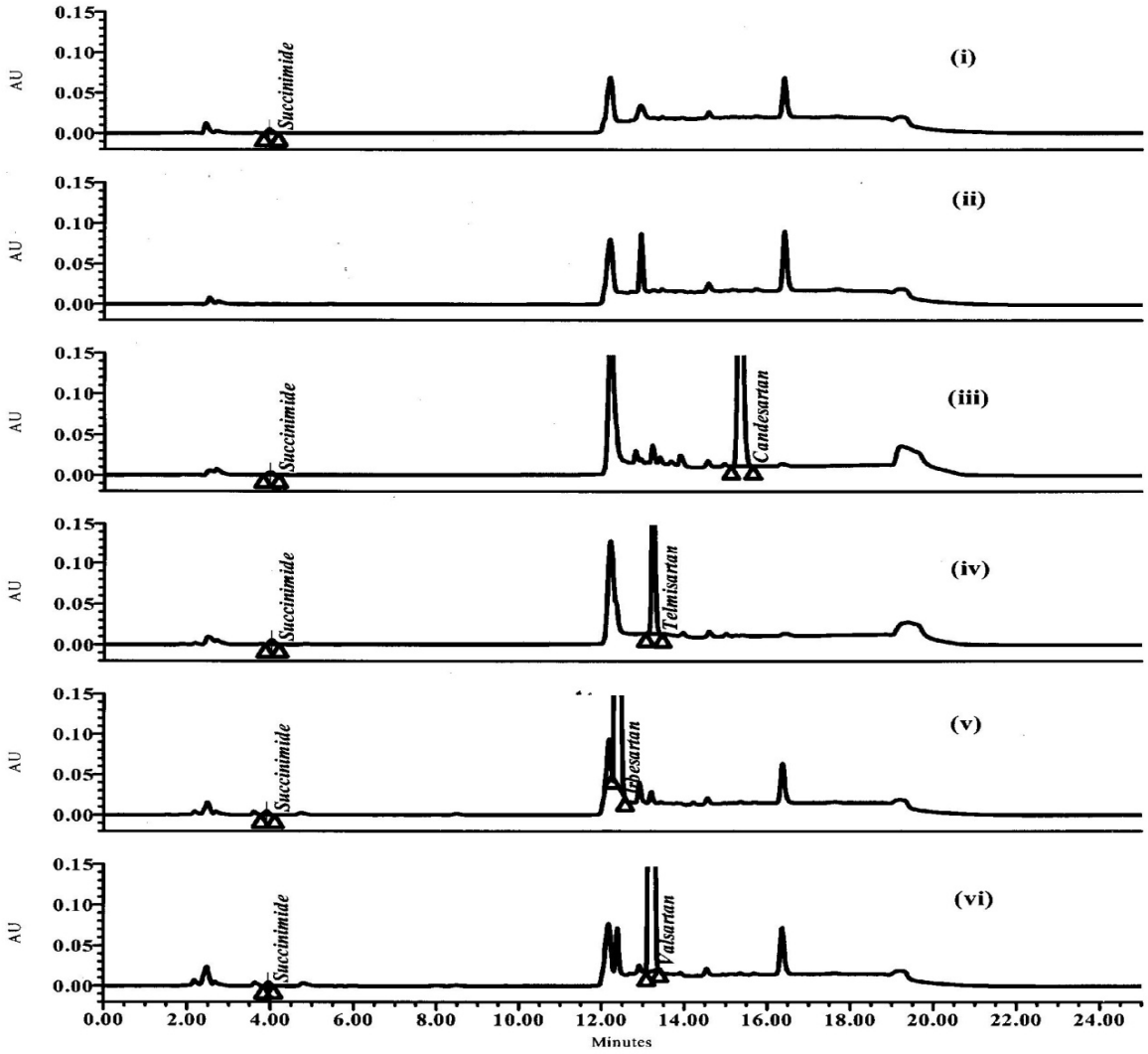
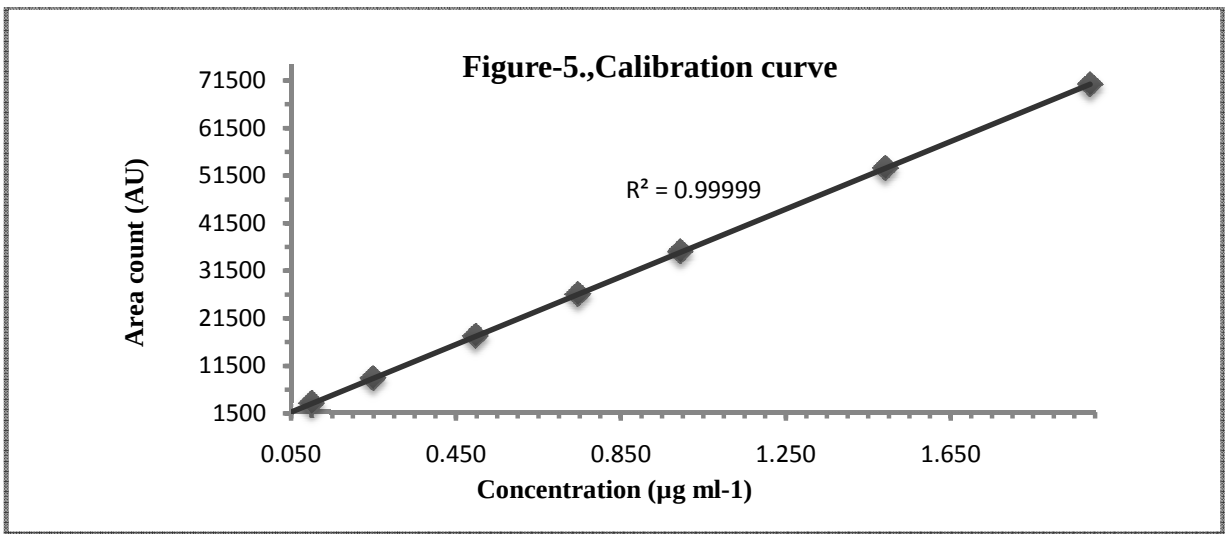


Fig-4., Overlay chromatogram of (i) Standard (ii) Diluent, (iii) Candesartan, (iv) Telmisartan, (v) Irbesartan and (vi) Valsartan



Precision:

The system precision for the method was assessed by six replicate injections of NBS standard solution (1 mg ml^{-1}) into chromatographic system, and the percentage relative standard deviation of response for six replicate measurements was found to be 0.9. Repeatability of the method (Method precision) was demonstrated by preparing six replicate sample preparations by spiking known concentration (0.1% w/w) of NBS in each selected drug substance. The samples were analyzed as per method, and the content of NBS was determined. Intermediate precision of the method (Ruggedness) was performed in the same way as described in method precision, however, by employing different analyst on other day using another lot of chromatograph. The content of NBS was determined in each preparation, and the percentage relative standard deviation for six replicate measurements determined for each preparation of selected drug substances. Table-3, describes experimental results are summarized with system suitability parameters and precision.

Table-3., Summary of system suitability and precision experiment

Precision [c]					
	Method Precision		Ruggedness		System Precision
	Amount (%w/w)	%RSD	Amount (%w/w)	%RSD	%RSD
Candesartan	0.098	4.1	0.096	3.3	0.9
Telmisartan	0.099	2.2	0.100	4.3	
Irbesartan	0.098	3.1	0.097	4.4	
Valsartan	0.098	2.3	0.098	4.5	

System suitability [d]			
Retention time (min)	Peak Tailing	Plate count	%RSD
~ 4.0	Not more than 1.3	Not less than 3800	Not more than 1.2

[c] *n=6, Average of six determinations.*
[d] *Average experimental observation*

Stability of sample solution:

The sample solution prepared by spiking known concentration (0.1% w/w) of NBS with respect to sample concentration was stored at 25 ± 2°C temperature conditions, and was injected into chromatographic system at different time intervals. The content of NBS was determined at each interval, the sample solution was found to be stable over a period of 12 hours in the case of Valsartan but in other three drugs some precipitations was observed after 2 to 3 hours but slight variations in % area for NBS was observed . Based on the diluents selectivity the sample solution stability also varies with respect to time.

Accuracy:

The accuracy of the method was evaluated by preparing sample solution spiked with known amount of NBS at different concentration levels in the range between 20%, 50%, 100% and 150% with respect to each drug concentration. Each concentration of sample solution was prepared in triplicate and analyzed as per the method. The percent recoveries of NBS were calculated against the known added amount, indicating that the method is accurate for Valsartan. Table-4, describes the accuracy data for other selected drugs.

Robustness

To assess the robustness of the method, experimental conditions were deliberately altered. The experimental condition altered by changing the flow of mobile phase (0.9-1.1 ml min^{-1}), temperature (20°C-30°C) and wavelength (200-210nm). The result obtained from the robustness indicated that, the experimental method parameters were tolerance limit with minor changes to optimize the method.

Table-4, Experimental summary of Accuracy

Drug	level [e]	Added [f] (%w/w)	Found [f] (%w/w)	% Recovery	Overall statistical data			
					% Mean Recovery	SD	% RSD	95% CI(±)[g]
CAN	20	0.0198	0.0193	97.6	97.9	1.26	1.0	3.1
	50	0.0496	0.0489	98.5				
	100	0.0991	0.0968	97.7				
	150	0.1487	0.1454	97.8				
TEL	20	0.0198	0.0191	96.6	97.9	1.99	2.0	4.9
	50	0.0496	0.0487	98.3				
	100	0.0991	0.0987	99.6				
	150	0.1487	0.1445	97.2				
IRB	20	0.0198	0.0192	97.1	97.8	1.90	2.0	4.7
	50	0.0496	0.0487	98.1				
	100	0.0991	0.0973	98.2				
	150	0.1487	0.1451	97.6				
VAL	20	0.0198	0.0198	100.0	99.6	2.16	2.0	5.4
	50	0.0496	0.0488	98.4				
	100	0.0991	0.1001	101.0				
	150	0.1487	0.1473	99.0				

[e] 0.1% level of the target, [f] Average of three determinations,
[g] 95%CI stands for 95%Confidence interval.

Stability Studies

To present stability studies on Valsartan , Irbesartan, Candesartan and Telmisartan drug substance for the determination of NBS content, the analysis were conducted on samples from variable sources of temperature and humidity storage of accelerated (40°C/75%RH), long term (25°C/60%RH) storage condition [13]. The results obtained from accelerated storage condition of 6 months samples were shown to be below detection limit. Where as in the case of sample stored for long-term period also shows similar result were obtained. Hence formation of NBS in each drug substances resulting as process related impurity, in view of that the sample shows no degradation profile with respect to storage at different conditions of temperature and humidity. The experimental condition shows precise results with good repeatability on different days with other analyst, shows the method is rugged for the determination of NBS

Forced degradation studies

The degradation study conducted on different condition of light, thermal and humidity for dry exposure. Liquid phase degradation using acid, base and peroxide of different concentration also conducted to prove the method is stability indicating shows no peak generation on either side of NBS for the above selected drug substance. The UV light exposed up to 200 watts/sq.mtr, fluorescent light exposed up to 1.2 million lux hour, Humidity exposure at 25°C / 92%RH, thermal exposure at 105°C and 60°C with the time duration of 144 hours and hydrolysis using aqueous media up to 12 hour at room temperature were performed to these condition. No generation of other peaks was observed, on either side of succinimide retention time of the sample chromatogram. Hence, the method is found to be selective and stability indicating with respect to forced degradation data.

CONCLUSION

The proposed new reversed phased-HPLC method developed for quantitative determination of N-Bromosuccinimide in Valsartan, Irbesartan, Candesartan and Telmisartan drug substance is accurate, precise and selective. The method has produced satisfactory validation data for the tested parameters as per the ICH guidelines. The proposed method is simple and cost effective as

it uses commonly used C_{18} column under gradient elution, with moderate run time. Hence the proposed method is conveniently used for the determination of NBS during bulk manufacturing of Valsartan, Telmisartan, Irbesartan and Candesartan in quality control laboratories.

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