Original Article

Pharm Sci Asia 2018; 45 (2), 77-92
DOI : 10.29090/psa.2018.02.077

Synthesis and content determination of impurity A of terazosin for use to establish a reference standard for terazosin drug quality control

D.T. Thanh-Thuy[1], T.X. Nguyen[1], H.N. Nguyen[1], C.S. Doan[2].*
[1] Department of Pharmaceutical Chemistry, Hanoi University of Pharmacy, Hanoi, Vietnam
[2] National Institute of Drug Quality Control, Hanoi, Vietnam

ARTICLE INFO
Article history:
Received 30 September 2017
Received in revised form
12 December 2017
Accepted 22 December 2017

KEYWORDS:
Impurity A, terazosin, synthesis, terazosin related compound A

ABSTRACT

Terazosin, a quinazoline derivative is an alpha-1-selective adrenoceptor blocking agent commonly used for the treatment of symptomatic benign prostatic hyperplasia. To control quality of material and finished products of terazosin, it is required to have some terazosin related compound reference standards including impurity A of terazosin (IAT). This study aims include the synthesis of IAT to establish a reference standard for drug quality control and investigation of an HPLC method to determine the content of the obtained IAT. The chemicals for synthesis and analysis were purchased from Sigma Aldrich and Merck (Germany). IAT was synthesized from 2-chloro-6,7-dimethoxyquinazoline-4-amino and piperazine, using isoamyl alcohol as a solvent. IAT's purification was carried out by column chromatography. Spectroscopic methods including IR, MS, NMR were used to characterize and confirm the structure of the product. The HPLC method, which was investigated to determine the content of IAT was validated according to the ICH guidelines. The total yield of synthesis and purify process of IAT was 71.1%. The content of the purified IAT was determined by comparison to USP Terazosin Related Compound A RS and its content achieved 97.28% calculated on the basis (99.47% calculated on the dried basis). Our results proposed the new synthesis, purification procedures for IAT and the determination method for the content of this product. The IAT obtained can be used to establish a reference standard substance for terazosin drug quality control.

1. INTRODUCTION

Terazosin hydrochloride (1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(tetrahydro-2-furoyl) piperazine monohydrochloride), (Figure 1), a quinazoline derivative is an alpha-1-selective adrenoceptor blocking agent. It is a highly selective potent α_1 adreno-receptor antagonist, an effective drug for hypertension by relaxing veins and arteries, and for the symptomatic treatment of urinary obstruction caused by benign prostatic hyperplasia (BPH) by relaxing the muscles of the bladder and prostate. It is common that a patient with symptomatic BPH also has hypertension. So terazosin is often a convenient choice for these kind of patients to target both diseases[1-3]. Currently terazosin is being marketed in Asean and many countries in the world with many trade names such as Hytrin, Teraz, Apo-Terazosin, Teradip, Terazosabb, Terazosine EG and Terazosine Sandoz. To control the quality of material and finished products of terazosin, it is required to have some terazosin related compound reference standards including

***Corresponding author:** dothanhthuy75@gmail.com

impurity A of terazosin (IAT: 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)piperazine, dihydro chloride), a degradation product of terazosin[4, 5] (Figure 2) for related substances test. So, we decided to investigate the synthesis and purification of IAT. In our research, IAT was synthesized, purified and its purity was determined by HPLC method, which was built and validated according to the ICH guidelines. The results demonstrate that our product could use as material to establish reference standard substance for drug quality control.

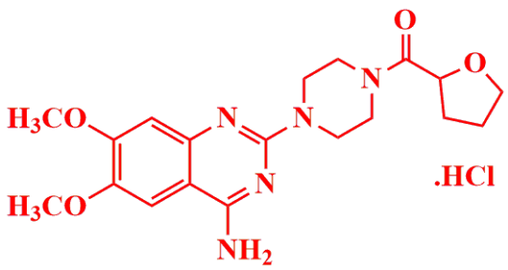


Figure 1. Structure of terazosin hydrochloride

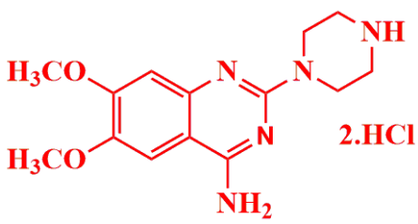


Figure 2. Structure of IAT

2. MATERIALS AND METHODS

2.1. Materials

2-Chloro-6,7-dimethoxyquinazoline-4-amino (ACDQ) was purchased from Sigma Aldrich (99.7%). USP Terazosin Related Compound A RS (IAT USPRS, lot FOC245, content 90.0%) was purchased from Pham Nguyen Trading copany (Vietnam). Terazosin was synthesized according to our reported procedures[6], Thin layer chromatography silica gel 60 $F_{254,}$ silica gel 60 (0.063 – 0.200 mm) for column chromatochraphy and other chemicals for synthesis were purchased from Merck (Germany). Methanol, acetonitrile and water were HPLC grade. Other reagents for analysis were of analytical grade.

2.2. Methods

2.2.1. Synthesis of IAT

The impurity A of terazosin (IAT) was synthesized from ACDQ and piperazin following the Figure 3.

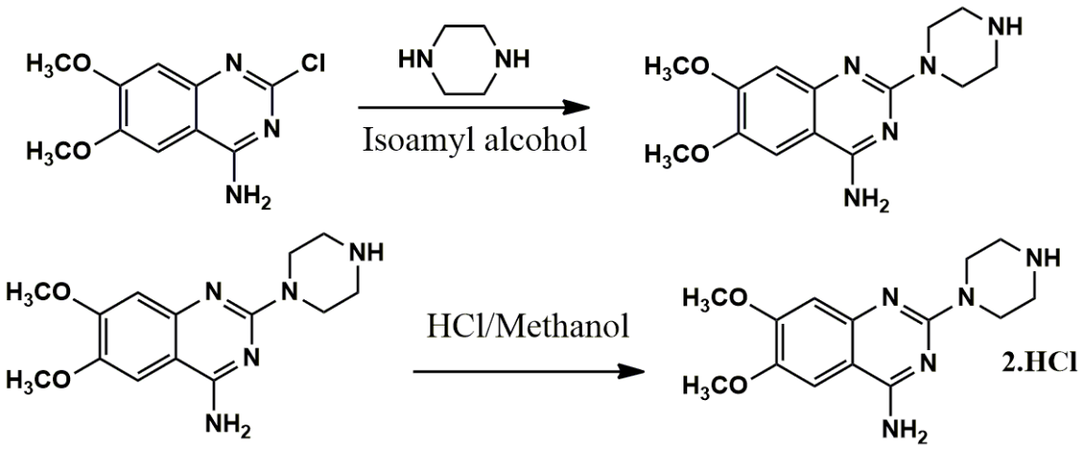


Figure 3. Synthetic pathway for IAT

2.2.2. The IR spectrum of the purified IAT was recorded with Thermo – Nicolet NEXUS 670 FTIR infrared spectrometer (USA). The purified IAT and IAT USPRS were mixed intimately with potassium bromide. Record the spectra of the test specimen and the standard specimen over the range from about 4000 cm^{-1} to 500 cm^{-1}.

2.2.3. The mass spectrum of the purified IAT was recorded with LTQ ORBITRAP XL™ mass spectrometer 5989B-MS instrument (USA) at 70 eV.

2.2.4. The NMR spectra of the purified IAT were recorded with Bruker 500 MHz Ascend Nuclear Magnetic Resonance spectrometer (Germany), using DMSO-*d6* as solvent.

2.2.5. The thermogravimetric analysis (TGA) of IAT was performed using STARAM thermo-gravimetric analyzer Labsys TG1600 (France) in a air atmosphere with rate at 2.5 L/hour. The mass loses of sample and heat response of the change of the sample were measured from room temperature up to 105°C. The heating rate was 2°C/min. The temperature at 105 °C was held in 4 hours.

2.2.6. The chromatographic procedure was performed using the Shimadzu HPLC system was equipped the Lab-solutions software and composed of the LC-20AD gradient pump with four channel multisolvent delivery system DGU-2A5 degasser, a column oven model CTO-10AS VP, a diode array detector model SPD-M20A and an auto sampler model SIL-20AC HT (Japan). A new Supelco column (250 x 4.6 mm; 5.0 µm) packed with octylsilyl silica gel for chromatography was used as stationary phase, a mixture of 16 volumes of acetonitrile and 84 volumes of citrate buffer solution pH 3.2 was used as the mobile phase with a flow rate of 1.0 ml per minute, a detection wavelength of 245 nm.

2.2.7. pH 3.2 citrate buffer

6.0 g of sodium citrate dihydrate and 14.25 g of anhydrous citric acid were dissolved in 950 ml of water. The pH was adjusted with anhydrous citric acid or sodium citrate to 3.2 ± 0.1 if necessary. This solution was diluted with water to 1000 ml, mixed and filtred by a 0.45 µm filter. The solutions were filtred by a 0.45 µm filter before injecting into the HPLC system.

2.2.8. Selection of wavelength for assay

Wavelength with maximum absorbance of IAT solution in mobile phase.

2.2.9. Chromatographic system suitability

A solution of 15 µg/ml of USP Terazosin Related Compound A RS was injected six times and the variation in number of retention time, peak area, theoretical and tailing factor were calculated.

2.2.10. Specificity

The specificity was validated on blank solution (mobile phase), test solution, standard

solution and resolution solution. In addition, the peak purity index was also calculated.

2.2.11. Linearity

The linearity evaluation was perform with standard solutions of IAT USPRS at the concentration range from 50 to 150% of assay concentration. The data of peak area versus concentration of IAT was treated by linear regression analysis.

2.2.12. Accuracy

The accuracy of the method was tested by analyzing samples of IAT USPRS at various concentration levels including 80%, 100% and 120% of assay concentration, each level performed 3 times. The recovery was calculated by comparison tested value with theoretical value.

2.2.13. Precision and intermediate precision

The precision of method was determined by measuring the repeatability (intra-day precision) and intermediate precision (inter-day precision) of content of IAT in the purified IAT. (Test was replicated 6 times). The intra-day variability was performed by the same analyst on one day, while inter-day presicion was carried out by another independent analyst on other day. Precision was reported as % of relative standard deviation (% RSD).

2.2.14. Stability of the analyte

Both standard solution and test solution were analyzed at intervals of 0, 1, 2, 6, 12, 24 hours, storing the samples at room temperature. Relative standard deviation of data gave an estimate of the stability of the analyte.

3. RESULTS

3.1. Synthesis IAT

The synthesis condition such as reaction temperature, reaction time, the molar ratio of the reaction substances were studied.

3.1.1. The reaction temperature

Carried out synthesis reaction at different temperatures, the results showed that, at temperature of less than 115^{o}C IAT was not produced. At temperature of more than 125^{o}C, piperazine was decomposed and the reaction mixture became black brown. Only at 120^{o}C ±5^{o}C the IAT base was obtained.

3.1.2. The molar ratio of ACDQ and piperazine

The molar ratios of ACDQ and piperazine were studied at 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6. The results showed that at the ratio 1:5 and 1:6 the yield was maximal but at the ratio 1:6 a large amount of piperazine remained as impurity. So, the molar ratio between ACDQ and piperazine of 1:5 was selected.

3.1.3. The reaction time

The reaction time was studied in 2 stages. Stage 1 with the reaction temperature was 120^{o}C and stage 2 with the reaction temperature was 70^{o}C.

In stage 1, the synthesis reaction was carried out for 1 hour, 2 hours, 3 hours, 4 hours, 5 hours and 6 hours.The results showed that for 5 hours and 6 hours. The yields were similar and maximal. So, the reaction time of 5 hours at stage 1 was selected.

In stage 2, the synthesis reaction was carried out for 5 hour, 10 hours, 15 hours, 20 hours. The results showed that at 15 hours and 20 hours the yields were similar and maximal. So, the reaction time of 15 hours at stage 2 was selected.

3.1.4. The obtained synthesis process of IAT

A 100-ml round bottom flask fitted with a stirrer, condenser, and thermostat was charged with 8 ml of isoamyl alcohol, 0.5 g (0.002 mol) of ACDQ and 0.89 g (0.01 mol) of piperazin. The reaction mixture was stirred and refluxed at 120^{o}C for 5 hours, and it was cooled to 70^{o}C and continue stirred and refluxed for 15 hours. The precipitated white color solid (IAT base) that was obtained was filtered and washed by hot isoamyl alcohol and dried.

In other 250-ml round bottom flask fitted with a stirrer, condenser, and thermostat were charged with 100 ml of methanol and the IAT base, add 4 ml of strong hydrochloric acid, stirred and refluxed at 60^{o}C for 1 hour. After that, the reaction mixture was evaporated in vacuo and the residue was dissolved in 400 ml

of water and filtered. The filtrate was concentrated under reduced pressure to give the residue, which was IAT hydrochloride (the yield was 78.7%).

3.2. Purification of IAT

The purification of IAT was carried out by column chromatography using silica gel (0.04 – 0.063 mm) as the stationary phase and eluting with a mixture of dichloromethane and methanol with different ratios: (9:2), (7:4), (5:6) . The obtained fraction were analyzed by TLC (methanol/ ethyl acetate/ strong ammonium solution 8:2:0.5), using 100 µl of each fraction for one spot and 5 µl of 2 mg/ml of solution of IAT USPRS as reference. Visualization was performed as UV light at 254 nm. Fractions showing only one spot with position and colour similar to that of the reference were combined and evaporated to dryness on to give purified IAT (the yield was 90.3%, the melting point was 219.2 ^{o}C).

The total yield of synthesis and purify process was 71.1%.

3.3. Confirmation of the structure of IAT

3.3.1. Identification of HCl in IAT

10 mg of IAT was dissolved in 5 mL of distill water, acidified with diluted nitric acid and added 0.4 mL of silver nitrate 0.1 N, shaked, and allowed to stand. A curdled, white precipitate was formed. This precipitate was insoluble in nitric acid but soluble in a slight excess of 6 N ammonium hydroxide.

3.3.2. Infrared absorption spectrum

Infrared absorption spectrum of IAT showed 3435.75 (-NH_2), 3317.01 (-NH), 3166.17 (C-H aren), 2943.67 (CH_2), 1650.56 (C-C- aren), 1265.92 (CH_3O-), 833.55 (-C-H- aren), (Figure 4). In addition, the IR spectrum of the purified IAT was concordant with the infrared absorption spectrum of the IAT USPRS , with match factor was 99.38.

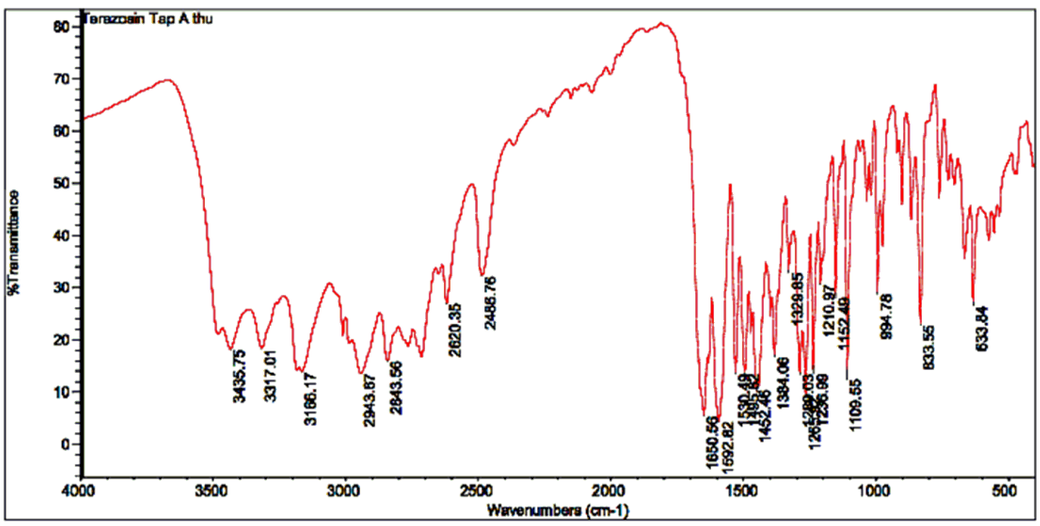


Figure 4. The IR spectrum of IAT

3.3.3. Mass spectrometry (MS)

The MS showed the molecular peak at m/z 289.1600 corresponding to molecular mass of IAT formula: $C_{14}H_{19}N_5O_2$, (Figure 5). The molecular peak corresponded to the mass of IAT (289.1539).

3.3.4. Nuclear magnetic resonance spectrometry

The ^{1}H-NMR, ^{13}C-NMR, and HSQC spectra were measured for the purified IAT. The results are shown in Table 1, Figure 6, Figure 7, Figure 8.

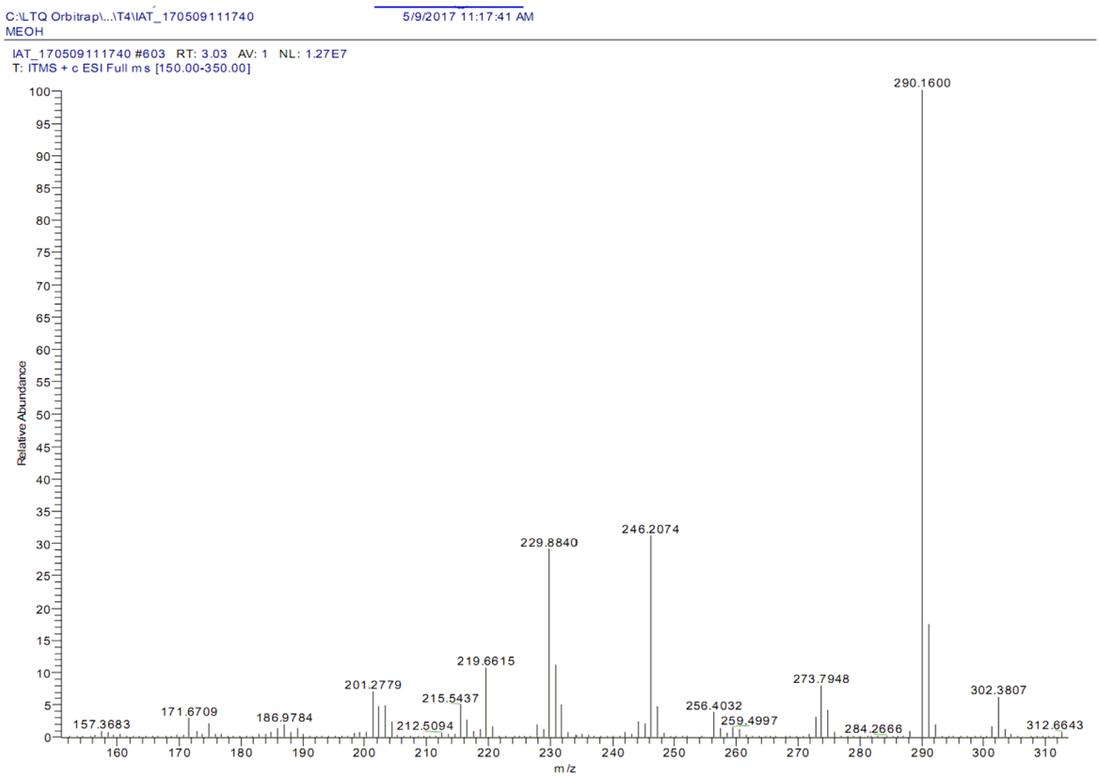
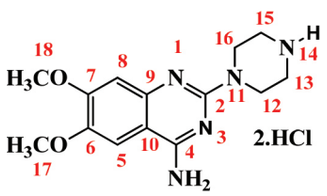


Figure 5. The MS spectrum of IAT

Table 1. The NMR spectroscopic data of the purified IAT

Struture	**^{1}H-NMR** (500 MHz, DMSO-d_6, ppm)	**^{13}C-NMR** (125 MHz, DMSO-d_6, ppm)
	3.27 (4H, s, H13, H15) 3.86 (3H, s, H18) 3.88 (3H, s, H17) 4.18 (4H, s, H12, H16) 7.71 (1H, s, H8) 7.81 (1H, s, H5) 8.72 (1H, s, NH) 9.06 (1H, s, NH) 9.71 (2H, s, NH_2) 12.86 (1H, s-br, NH)	42.25 (C_{13}) 42.38 (C_{15}) 49.06 (C_{12}, C_{16}) 56.56 (C_{18}) 56.79 (C_{17}) 99.69 (C_8) 102.32 (C_9) 105.45 (C_5) 136.71 (C_{10}) 147.42 (C_7) 151.84 (C_2) 155.74 (C_6) 161.84 (C_4)

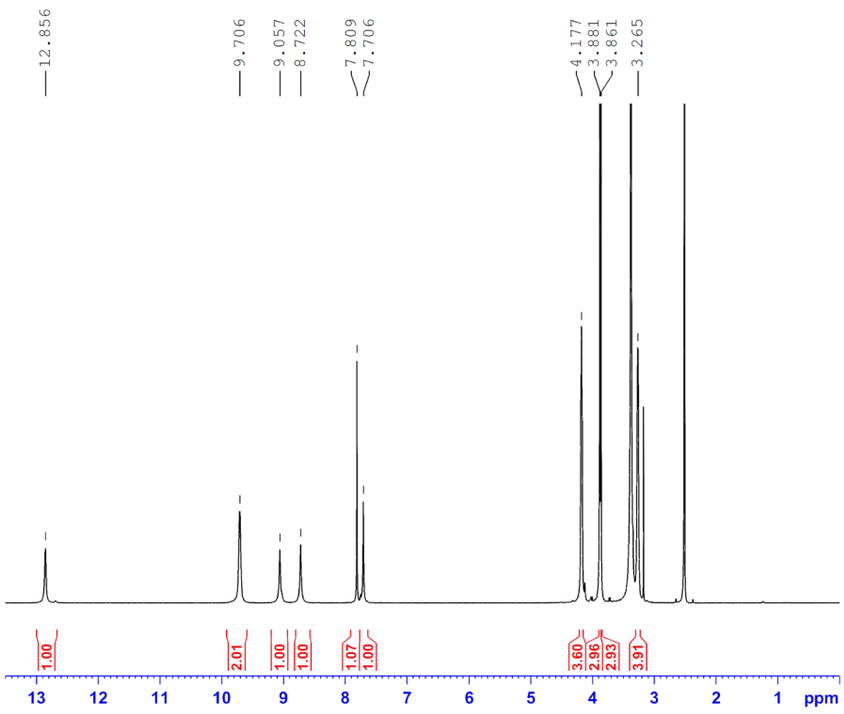


Figure 6. The ^{1}H-NMR spectrum of IAT

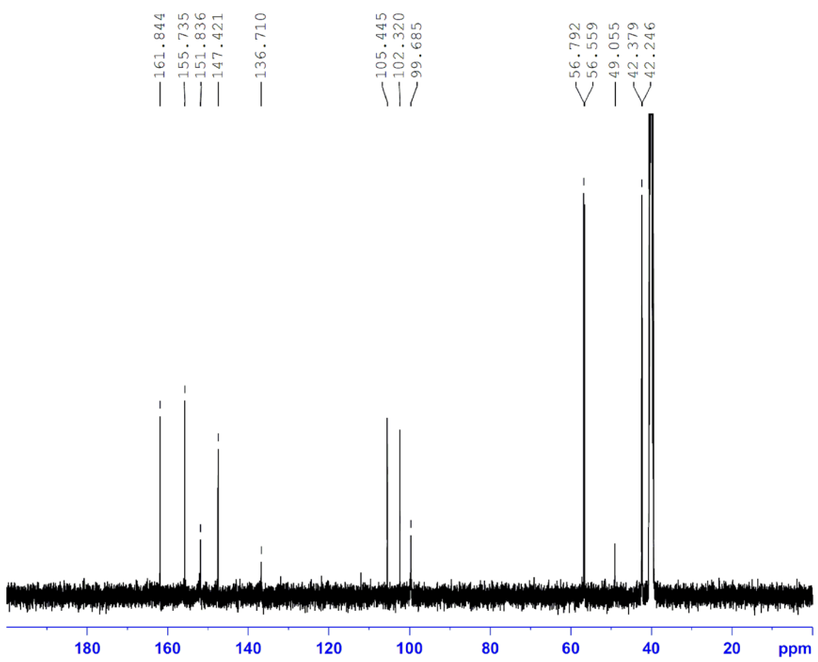


Figure 7. The ^{13}C-NMR spectrum of IAT

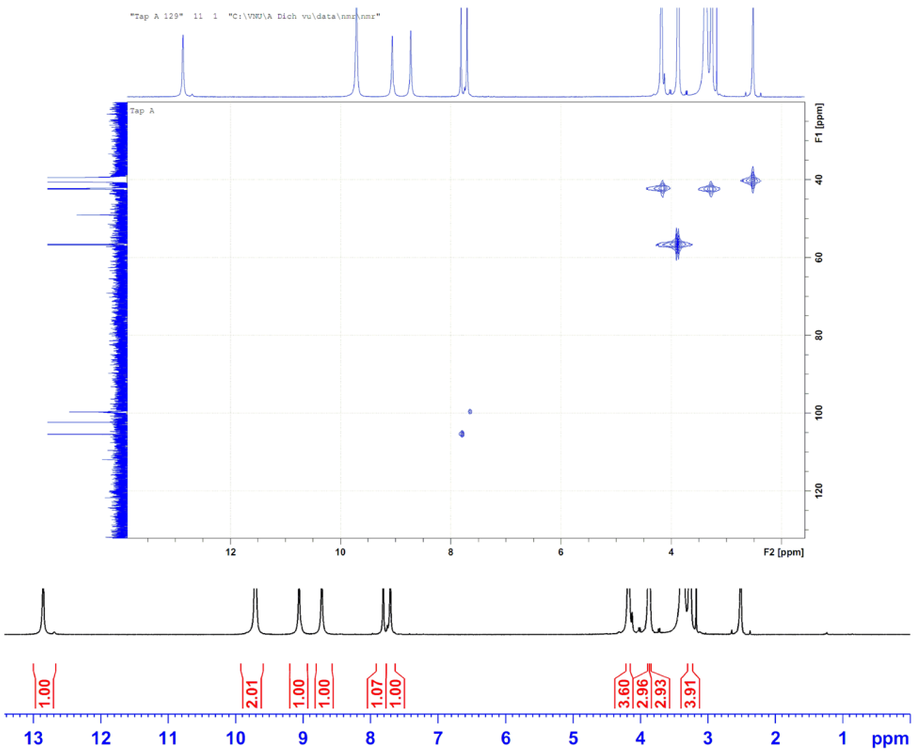


Figure 8. The HSQC spectrum of IAT

3.4. Development of the assay method for the IAT by HPLC

3.4.1. Development of the assay method

Based on the references[4,7-12] we studied and proposed a method to assay for the purified IAT, which was mentioned in the methods. Liquid chromatography was carried out using 20 µl of the following solutions. The standard solution contains about 15 µg/ml of IAT USPRS in mobile phase. The test solution contains about 15 µg/ml of the purified IAT in mobile phase. The resolution solution contains 15 µg/ml of IAT USPRS, 30 µg/ml ACDQ and 30 µg/ml terazosin hydrochloride in mobile phase.

3.4.1.1. Selection of wavelength for assay

The UV spectrum of the solution of 5 µg/ml of IAT in the mobile phase was recorded in 1-cm cells, over the spectral range from 200 to 400 nm, using mobile phase as blank. The result showed the UV maximum absorbance of IAT was 245 nm, (Figure 9).

3.4.2. Validation of the IAT assay method

The liquid chromatography method to assay IAT was assessed for chromatographic system suitability, specificity, linearity, accuracy, precision, intermediate precision, and stability of the analyte.

3.4.2.1. Chromatographic system suitability

The standard solution was injected six times and the variation in number of peak area, retention time, theoretical plates and tailing factor were calculated. The resuls showed that RSD of the retention time of peaks is 0.15%, RSD of peak areas is 0.27%. The tailing factor is from 1.435 to 1.441. The value of the average threotical plates is 2538. So, the chromatography system was suitable to assay IAT (Table 2).

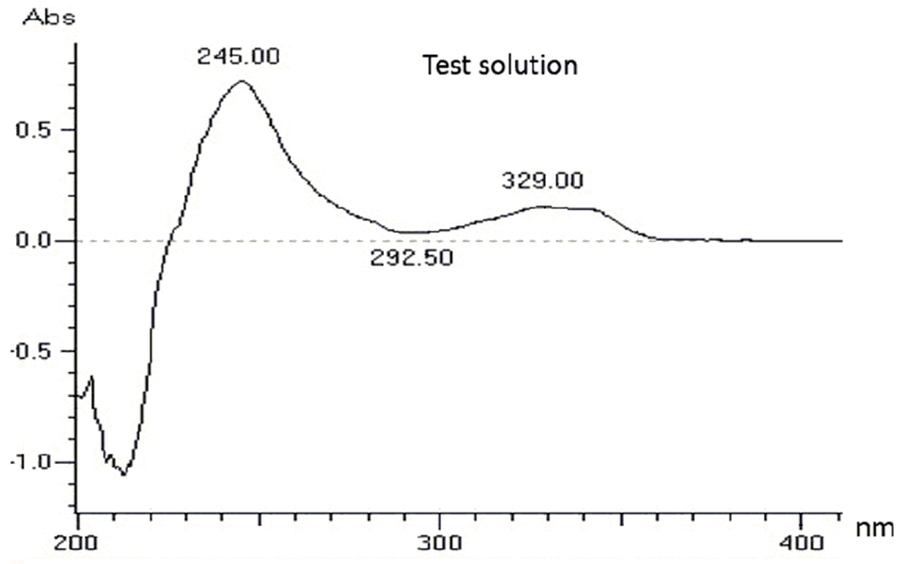


Figure 9. The UV spectrum of IAT

Table 2. The obtained data of liquid chromatography system

Times	Retention time (minutes)	Peak area (mAU.s)	Theoretical plates	Tailing factor
1	3.440	2946199	2556	1.435
2	3.431	2955706	2539	1.439
3	3.430	2962020	2523	1.437
4	3.441	2942591	2552	1.436
5	3.432	2943633	2531	1.441
6	3.429	2956042	2524	1.438
Average	3.434	2951032	2538	
RSD (%)	0.15	0.27		

3.4.2.2. Specificity

In the chromarogram of the blank solution, there was no any peak (Figure 10A). The retention time of the peak in the chromatogram of the test solution (t_R = 3.435 minutes) (Figure 10B) corresponded to that of the standard solution (t_R = 3.440 minutes), (Figure 10C). In the chromarogram of resolution solution, the resolution between peaks were more than 10 (Figure 10D). Besides the peak purity index was 1.0000 (Figure 11).

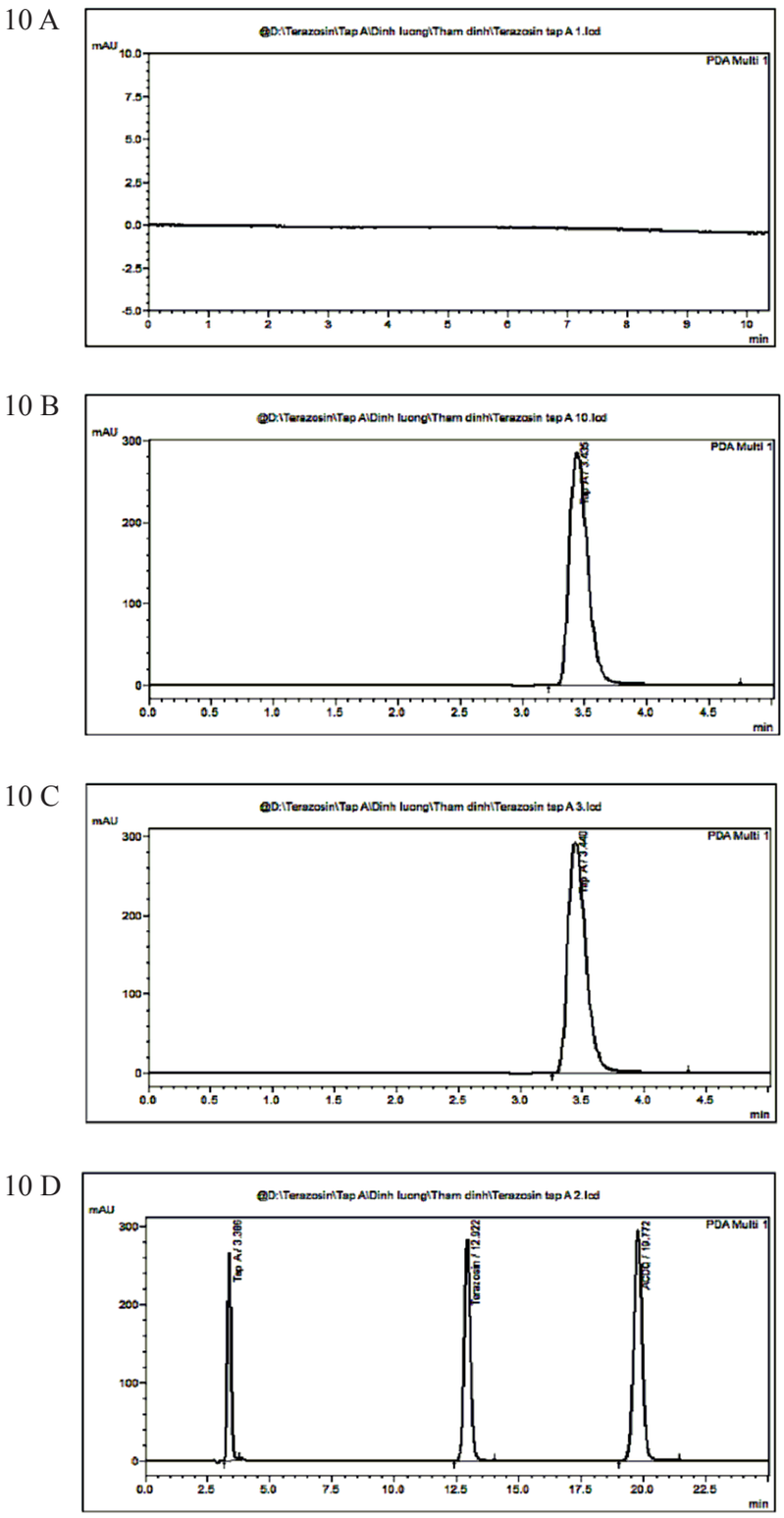


Figure 10. The HPLC chromatograms of the 10 A) Blank solution 10 B) Test solution 10 C) Standard solution 10 D) Resolution solution

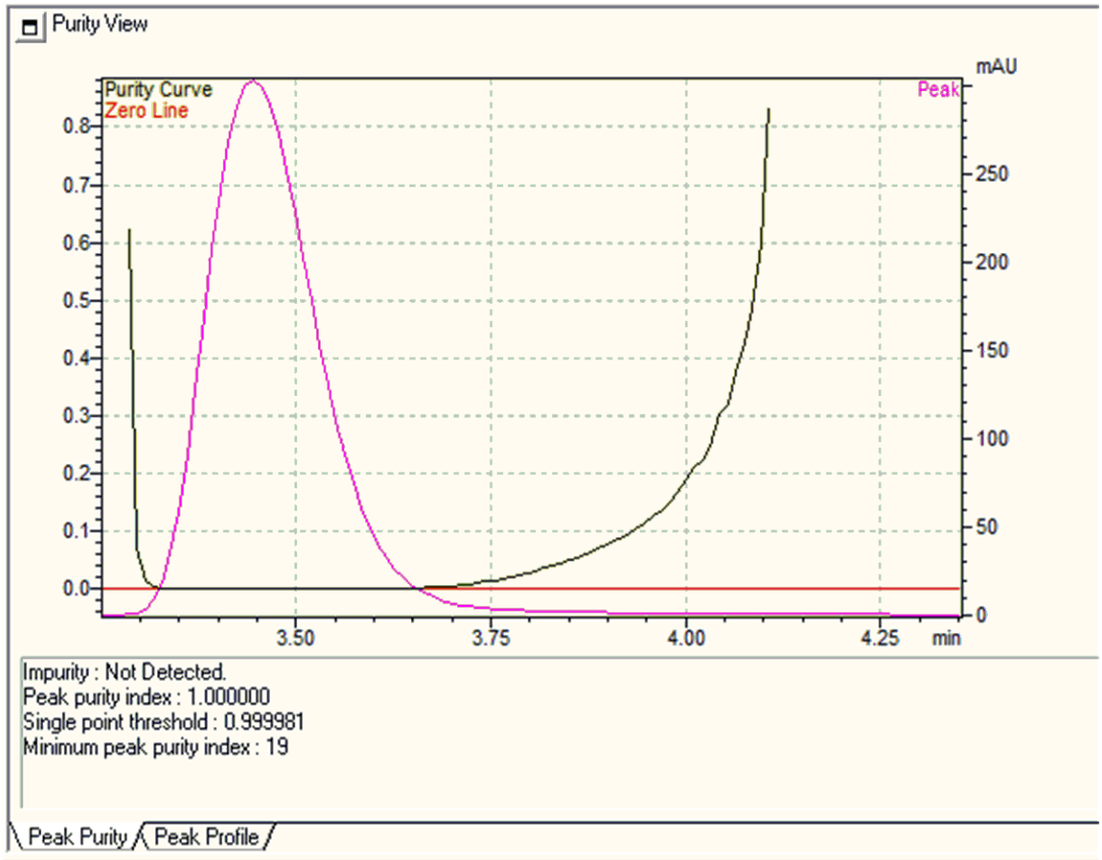


Figure 11. The peak purity of the purified IAT in test solution

3.4.2.3. Linearity

The standard solutions were prepared from IAT USPRS in mobile phase to obtain the concentration from 7.399 µg/ml to 22.198 µg/ml. These standard solutions were injected in the chromatographic system. The area of the peaks were calculated and the correlation between concentration and peak area was determined. The results showed that, in the concentration range from 7.399 to 22.198 mg of IAT per ml (50 to 150% of assay concentration), there is linear correlation between concentration and peak area. (Linear equation: $y = 198791.2410x + 875.5413$, correlation factor: $R = 1.0000$), (Table 3, Figure 12).

Table 3. The concentration and the peak area of standard solutions

Concentration of IAT (µg/ml)	7.399	11.839	14.799	17.758	22.198
Area (mAU.s)	1468717	2351784	2952211	3531377	4409449

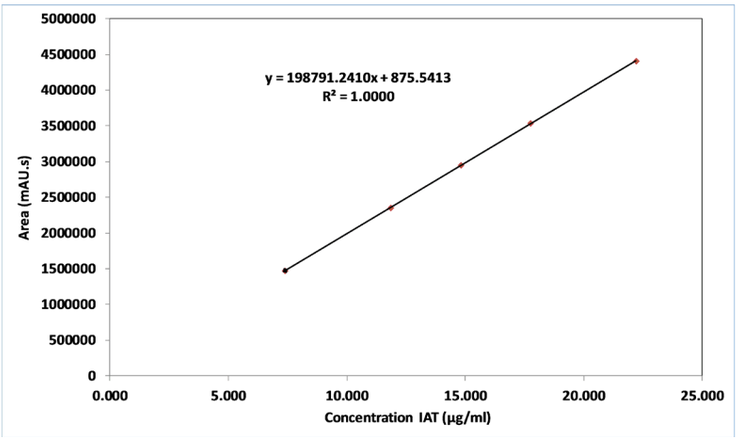


Figure 12. The calibration curve of IAT

2.4.2.4. Accuracy

Tests of accuracy were performed according to the method. The results showed that the recovery from 98.02 to 102.00% (Table 4). So, this HPLC method was accurate.

Table 4. The data of accuracy

Specimen	*Weight (mg)*	*Recovery weight (mg)*	*Recovery (%)*
1	4.111	4.175	101.56
2	4.125	4.207	101.98
3	4.001	3.921	98.02
4	4.999	4.913	98.29
5	5.216	5.260	100.85
6	5.449	5.558	102.00
7	6.121	6.239	101.94
8	6.290	6.416	102.00
9	6.142	6.264	101.99
Average			100.96

3.4.2.5. Precision and intermediate precision

The precision and intermediate precision was performed according to the methods. The results showed that the RSD of content of IAT was 0.48% and 0.54% (less than 2.0%) (Table 5). So, this HPLC method was precise.

Table 5. The data of precision and intermediate precision

Precision		Intermediate precision	
Test	Content of IAT calculated on the basis (%)	Test	Content of IAT calculated on the basis (%)
1	97.73	1	97.71
2	97.59	2	96.62
3	97.35	3	97.59
4	97.14	4	97.93
5	96.84	5	97.33
6	96.49	6	96.72
Average	97.19	Average	97.32
RSD (%)	0.48	RSD (%)	0.55

3.4.2.6. Stability of the analyte

A solution of 15 µg/ml of IAT and a solution of 15 µg/ml of IAT USPRS were analyzed at intervals of 0 and 24 hours, storing the samples at room temperature. Relative standard deviation (RSD) of retention time were 0.05% and 0.02%. RSD of peak area were 0.22% and 0.08%. So, the test solution and the standard solution were stable for 24 hours (Table 6).

Table 6. The data of stability of the analyte

Time (hours)	Standard solution		Test solution	
	Retention time (minutes)	Peak area (mAU.s)	Retention time (minutes)	Peak area (mAU.s)
0	3.442	3092681	3.444	3141438
1	3.444	3106290	3.446	3142288
2	3.443	3103159	3.444	3142800
6	3.440	3101127	3.444	3142536
12	3.440	3099587	3.444	3136127
24	3.440	3112851	3.444	3142186
Average	3.442	3102616	3.444	3141229
RSD (%)	0.05	0.22	0.02	0.08

3.5. Determine the content of water in IAT

The content of water in IAT was determined 3 times by TGA meter and calculated the averages. The results showed that the content of water in IAT was 2.20%.

3.6. Determine the content of the purified IAT

The content of IAT in the purified IAT was calculated based on the declared purity of IAT USPRS, Weights and the peak areas obtained in the chromatograms of IAT USPRS and IAT. The results were shown in Table 7.

Table 7. The content of the purified IAT and the statistical data

Solution	Weight (mg)	Rt (minutes)	Peak area (mAU.s)	Content of IAT calculated on the basis (%)	Content of IAT calculated on the dried basis. (%)
Standard 1 (Average of 6 injected times)	5.517	3.426	3002366	90.00	-
Standard 2 (Average of 6 injected times)	5.624	3.427	3105729	90.00	-
Test 1	4.981	3.416	2917732	96.87	99.05
Test 2	5.001	3.484	2951991	97.62	99.82
Test 3	5.259	3.423	3104760	97.64	99.83
Test 4	5.257	3.440	3146743	97.55	99.75
Test 5	5.481	3.417	3261499	96.98	99.16
Test 6	5.507	3.427	3277389	96.99	99.17
Average				97.28	99.47
S				0.36	0.39
SX				0.15	0.16
RSD (%)				0.37	0.39
ΔX				0.39	0.41
Confidence range (%) (p = 0.95)				97.28 ± 0.39	99.41 ± 0.41

Test 1, 2, 3 calculated on standard 1; Test 4, 5, 6 calculated on standard 2

4. DISCUSSIONS

There are very few publications on the synthesis of IAT. In United State Patent[13, 14] and Canadian Patent[15] or some reports[16] there were only procedures for the synthesis of derivatives with structure similar to IAT but not IAT. These derivatives were synthesized from ACDQ and derivatives of piperazine or from derivatives of ACDQ and piperazine. However, the synthesis of these derivatives were not described in detail. In the paper of Nageswara RR, et al[10], the synthesis procedures for IAT base with reaction time up to 72 hours was proposed to obtain IAT base with 97.4% of the purity. But in this paper, the authors did not give the purification procedures and purity determination method for IAT. In

our research, the synthesis condition such as reaction temperature, reaction time, ratio of the reaction substances were studied. Our synthesis of IAT base had two stages with different reaction temperature. In the stage 1 the result showed that, at the temperature less than 115°C, there was not IAT produced. But at the temperature more than 125°C, piperazine was decomposed and the reaction mixture became black brown. Only at 120°C the product was obtained and the reaction reached balance. In the stage 2, the reaction temperature was 70°C. This temperature was suitable to obtain precipitate of IAT base with maximum yield. The molar ratios of ACDQ and piperazine was studied at 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6. The results showed that at the ratio 1:5 and 1:6 the yield was maximal and similar but at the ratio 1: 6 in the production there was piperazine. So, the reaction molar ratio between ACDQ and piperazine of 1:5 was selected. The reaction time was studied in 2 stages. In stage 1, carried out synthesis reaction for 1 hour, 2 hours, 3 hours, 4 hours, 5 hours and 6 hours and selected the reaction time at stage 1 for 5 hours because reaction for 5 hours and 6 hours the yield was maximal and similar. In stage 2, carried out synthesis reaction for 5 hours, 10 hours, 15 hours, 20 hours. The results showed that at 15 hours and 20 hours the yield was maximal and similar, so the reaction time of 15 hours at stage 2 was selected. The total reaction times for the 2 synthesis states of IAT base were 20 hours. To obtain IAT dihydrochoride like IAT USPRS, we performed reaction between the obtained IAT base and HCl in methanol. After that, the purification of product was carried out by column chromatography using silica gel (0.04 – 0.063 mm) as the station phase and mixture of dichloromethane and methanol with different ratios as eluting solvent. The purified IAT was structurally confirmed by spectroscopic methods including IR, MS, NMR. Besides, the purity of our product was determined by HPLC method was 97.28% calculated on basis (confidence range 97.28 ± 0.39%; $p = 0.95$) and 99.47% calculated on dried basis (confidence range 99.47 ± 0.41%; $p = 0.95$) while the purity of IAT USPRS is 90.00% calculated on basis. The assay method, which was validated according to ICH guide lines, has high specificity, high accuracy (the recovery of IAT was from 98.02% to 102.00% of the theoretical value), high precision (RSD = 0.48%), high intermediate precision (RSD = 0.55%), and shows linear correlation between concentration and peak area of IAT area in the concentration range from 7.399 to 22.198 µg of IAT per ml (50 to 150% of assay concentration). In addition, the analytes were stable in 24 hours. So our result of assay of the purified IAT is confident. The purified IAT can be used to establish a working standard substance for quality control of material and finished products containing terazosin.

5. CONCLUSIONS

Our research proposed the new synthesis, purification procedures for IAT from ACDQ and piperazine with the molar ratios of ACDQ and piperazine was at 1:5. The reaction procedures has 2 stages. Stage 1 with the reaction temperature was 120°C for 5 hours and stage 2 with the reaction temperature was 70°C for 15 hours. The total yield of synthesis and purify process of IAT was 71.1%. The structure of the purified IAT which was confirmed by spectroscopic methods including IR, MS, NMR. The HPLC method using to determine the content of IAT was validated according to ICH guidelines. The purity of our product was 97.28% calculated on basic and 99.47% calculated on dried basic.

6. ACKNOWLEDGEMENTS

The authors thank National Institute of Drug Quality Control in Vietnam for assistance in analytical instruments for this research.

REFERENCES

1. Oh EY, Bae SK, Kwon JW, You M, Lee DC, Lee MG. Pharmacokinetic and pharmacodynamic consequences of inhibition of terazosin metabolism via CYP3A1 and or 3A2 by DA-8159, an erectogenic, in rat. Br J Clin Pharmacol. 2007;151:24-34.
2. Tehranchi A, Razaei Y, Khalkhali H, Rezaei M. Effects of terazosin and tolterodine on ureteral stent related symptoms: a double -

blind placebo controlled randomized clinical trial. Int Braz J Urol. 2013; 39(6):832-40.
3. Yong Y, Xiao-feng Z, Han-zhong LI, Wei W, Yong Z, He X, et al. Efficacy and safety of combined therapy with terazosin and tolteradine for patients with lower urinary tract symptoms associated with benign prostatic hyperplasia: a prospective study. Chin Med J. 2007;120(5):370-4.
4. Bauer JF, Krogh SK, Chang ZL, Wong CF. Determination of minor impurities in terazosin hydrochloride by high-performance liquid chromatography. J Chromatogr A. 1993; 648(1):175-81.
5. Ojha T, Bakshi M, Chakraborti AK, Singh S. The ICH guidance in practice: stress decomposition studies on three piperazinyl quinazoline adrenergic receptor-blocking agents and comparison of their degradation behavior. J Pharm Biomed Anal. 2003;31 (4):775-83.
6. Nam NH. Research on synthesis of terazosin hydrochloride. Vietnamese Pharmaceutical journal. 2010;50(11):35-9.
7. Council of Europe. European pharmacopoeia 7.0; 2008. p. 3045-7.
8. Korea Food and Drug Administration. Korean pharmacopoeia X. 2013 p. 1140-3.
9. Kormány R, Molnár I, Fekete J. Renewal of an old European Pharmacopoeia method for Terazosin using modeling with mass spectrometric peak tracking. J Pharm Biomed Anal. 2017;135:8-15.
10. Nageswara RR, Nagaraju D, Das AK, Jena N. Separation, characterization, and quantitation of process-related substances of the anti-hypertensive drug doxazosin mesylate by reversed-phase LC with PDA and ESI-MS as detectors. J Chromatogr Sci. 2007;45(2): 63-9.
11. The British medicines commission. British pharmacopoeia. 2014;2:982-5.
12. The United States Pharmacopeial Convention. United States Pharmacopoeia 37. 2014; 4871-4.
13. Campbell SF, Plews RM, inventor; Pfizer Inc, New York, N.Y, assignee. 4-Amino-6, 7-dimethoxy-2-(4-heteroaryl-piperazino) quinazoline antihypertensives. U.S 4,483, 857. 20 Nov 1984.
14. Yokoyama K, Kato K, Kitahara T, Ohno H, Nishina T, Kumakura M, et al, inventor; Mitsui Petrochemical Industries, Ltd., Tokyo, Japan, assignee. Quinazoline compouds and antihypertensives. U.S 4,734,418. 29 Mar 1988.
15. Campbell SF, Plews RM, inventor; Pfizer Corporation, Panama, assignee. Antihypertensive quinazolines. CA 1165318 A; 10 Apr 1984.
16. Naik PP. Design and synthesis of some multitargeted ligands as potential antihypertensive agents. Ph.D. [dissertation]. India: Sayajirao University of Baroda; 2011.