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Development and Validation of RP-HPLC method for the determination of *p*-Toluene sulphonic acid and Ethyl-*p*-Toluene sulphonate in Perindopril *tert*-Butylamine drug substance

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ABSTRACT

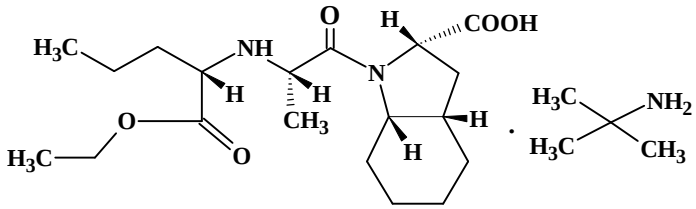
A simple and reliable reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the quantification of p-Toluenesulfonic acid and Ethyl-p-Toluene sulfonate in Perindopril tert-Butylamine drug substance. The separation of p-Toluenesulfonic acid and Ethyl-p-Toluene sulfonate was achieved from the related impurities of the drug on an Inertsil ODS-3V (250 x 4.6mm) 5µm column. The gradient LC method employs solution A and solution B as mobile phase. The solution A contains 0.2% OPA and solution B contains Acetonitrile. The developed RP-HPLC has been subsequently validated to prove its performance characteristics by demonstrating specificity, limit of detection and limit of quantification, linearity, accuracy, precision and robustness. The drug substance was subjected to stability studies using optimized method condition to enhance low level of detection with minimum acquisition time indicating the accuracy of the optimized HPLC method by citing ICH guideline requirement.

Keywords: Liquid Chromatography, *p*-Toluene sulfonic acid, Ethyl-*p*-Toluene sulfonate, Perindopril *tert*-Butyl amine, validation.

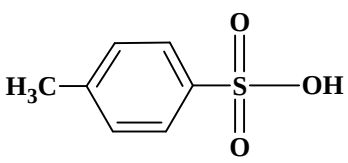
INTRODUCTION

Perindopril *tert*-Butylamine is long lasting angiotensin converting enzyme (ACE) inhibitor, used in the treatment of cardiovascular disease especially in the treatment of hypertension and congestive heart failure and diabetic nephropathy [1-3] and it acts through its active metabolite perindoprilat [4]. Perindopril *tert*-Butylamine is chemically known as (2*S*, 3a*S*, 7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl) butyl] amino]-propanoyl] octahydro-1*H*-indole-2-caroxylatic acid, *tert*-Butylamine and can be represented by the formula in Fig1 (a). The molecular formula is $C_{23}H_{43}N_3O_5$ and molecular weight is 441.6. Perindopril *tert*-Butylamine is official in the European pharmacopeia [5]. It is available as 2mg, 4mg and 8mg tablets for oral administration and marketed under the trade name "ACEON". *p*-Toluenesulfonic acid is used as counter-ions for basic drugs during the synthesis of the drug substance in pharmaceutical industry because of its strong acidic and hydrophilic properties [6-7] as well as the catalyst system is being an organic in character [8]. It has broad application towards, oxidative degradation [9], transesterification of an ester [10], esterification of carboxylic acid [11] and reductive amination of aldehydes and ketones [12] as well as reaction mediator [13]. The utilization of alcohols from synthetic reaction or in the salt formation steps may result in the formation of corresponding alkyl tosylates, which are potential and known to be genotoxic impurities [14]. The preparation of the subjected drug substance involves solvent ethanol and *p*-Toluenesulfonic (PTSA) as reagent in salt formation step. Hence it is possible to have one genotoxic impurity Ethyl-*p*-Toluenesulfonate (EPTS) and traces of PTSA in the drug matrix. EPTS is potential genotoxic impurities while PTSA having no such data, but prolonged exposure can produce target organ damage. Based on the safety assessment data and regulatory requirement the

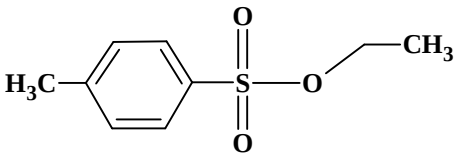
criteria for their acceptance up to certain limits calculated based on threshold of toxicological concern (TTC) concept and ICH guideline [15]. A TTC value was estimated to be 1.5µg/person/day intake of a genotoxic impurity is considered to be associated with an acceptable risk for most pharmaceuticals as per EMEA guideline on the limit of genotoxic impurities [CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006] as well as risk assessment literature [16, 17]. Based on the maximum daily dose (MDD) of the selected drug substance used in this study the proposed limit for EPTS and PTSA are 90ppm and 0.1%w/w respectively. The drug dosage form available in the market is directly consumed by human being based on the prescription, so it should be of good quality and highest purity. So monitoring of PTSA and EPTS in the drug substance is essential for preserving the desired quality of active moiety of the compound. The literature survey reveals that various analytical techniques have been reported in the literature for the determination of PTSA and EPTS. The reported works are HPLC determination by Nageswari [18], LC/MS by Taylor [19], trace level determination by Dokladalova et.al [20], coupled CE/MS method by Agilent [21], mass spectral determination by Jianguo et.al [22], NMR analysis by Shao et.al [23] and gas chromatography determination by Nardillo et.al [24]. But no specific publication is available for the determination of PTSA and EPTS in subjected drug substance. Therefore, we have developed a rapid and economical method for the determination of PTSA and EPTS in Perindopril *tert*-Butylamine drug substance without coelution of related impurities of sample under investigation.



(a). Perindopril *tert*-Butylamine



(b). *p*-Toluenesulfonic acid



(c). Ethyl-*p*-Toluenesulfonic acid

Fig.1 Structure of (a).Pernidopril *tert*-Butylamine drug substance, (b). *p*-Toluene sulfonic acid, (c). Ethyl-*p*-Toluene sulfonate

EXPERIMENTAL SECTION

Chemicals and Reagents

Reference samples of Perindopril *tert*-Butylamine and its related impurities including Ethyl-*p*-Toluene sulfonate (Figure 2) were synthesized and characterized by LC-MS, NMR and IR in the laboratory. The *p*-Toluene sulfonic acid was obtained from Sigma Aldrich Limited. All chemicals and reagents were of analytical purity grade unless stated otherwise. Water obtained from Milli-Q purification system. Orthophosphoric acid and LC grade Acetonitrile purchased from Merck Limited (Darmstad, Germany). The LC system consists of quaternary gradient pumps with auto sampler and auto injector (Alliance 2695, Waters, Milford, USA) controlled with Empower software for data acquisition (Waters). The reference samples obtained from Aurobindo Pharma Limited, Hyderabad, India.

Preparation of standard solution

Accurately weighed 500mg of *p*-Toluene sulfonic acid (PTSA) and 45mg of Ethyl-*p*-Toluene sulfonate (EPTS) reference samples was transferred to a 100mL volumetric flask, added about 70mL of diluent and sonicated to dissolve, diluted up to the mark with diluent and mixed. Further 10mL of this solution was diluted to 100mL with diluent and further 1mL of the resulting solution was diluted to 100mL with diluent. This solution contains 5µg/ mL of *p*-Toluene sulfonic acid and 0.45µg/ mL of Ethyl-*p*-Toluene sulfonate respectively.

Preparation of sample solution

Accurately weighed 50mg of Perindopril *tert*-Butylamine was transferred to 10mL volumetric flask and added about 35mL of diluent and sonicated to dissolve, diluted up to the mark with the diluent and mixed.

Preparation of impurities stock solution:
Accurately weighed 1.0 mg of each impurities of Perindopril *tert*-Butylamine namely Impurity-I, Impurity-II, Impurity-III, Impurity-IV, impurity-V, Impurity-VI and Impurity-VII was transferred to 10mL volumetric flask individually and added about 5mL of diluent and sonicated to dissolve, diluted up to the mark with the diluent and mixed.

Chromatographic condition
The gradient reverse phase LC method employs solution A and B as mobile phase. The solution A contains aqueous 0.2%v/v Ortho-phosphoric acid and LC grade Acetonitrile as solution-B. The flow rate of the mobile phase was 1.5 mL/min. The chromatographic separation was achieved on a GL sciences column, 250 x 4.6mm, Inertsil ODS-3V, 5µm particle. The HPLC gradient programme set as time (minutes)/ % solution B (v/v): $T_{0.01}$/35, T_{10}/65, T_{15}/65, T_{16}/35, T_{25}/35. The acquisition time was 15 minutes with a post run time of 10 minutes. The column temperature was maintained at 25°C and the detection was monitored at a wavelength of 220nm. The injection volume was 50µl. The degassed mixture of Acetonitrile and water in the ratio 75:25v/v was used as diluent.

RESULTS AND DISCUSSION

Chromatographic method optimization
The scope of the chromatography method was to separate PTSA and EPTS from Perindopril *tert*-Butylamine and its related impurities namely Impurity-I, Impurity-II, Impurity-III, Impurity-IV, Impurity-V Impurity-VI and Impurity-VII. The impurities were co-eluted with PTSA and EPTS using different makes of stationary phases like C8, C18 and phenyl as well as with different composition of mobile phases and organic modifiers. During the evaluation of pH study some of the impurities having acidic in nature, therefore it requires higher composition of organic modifier. Hence, 0.2%v/v Ortho-phosphoric acid was chosen as buffer solution to rule out the precipitation of aqueous salt buffers with combination of higher organic modifier ratios. During the evaluation of various column chemistries, ODS has shown better resolution. The retention of EPTS was critical and conditions were optimized as mentioned under section "chromatographic condition". In the optimized chromatographic condition the PTSA and EPTS were well separated with a resolution greater than 10. The retention time of PTSA and EPTS was found to be 5min and 11 min respectively. The system suitability results are given in Table 1, and the developed method was found to be specific for PTSA and EPTS in Perindopril *tert*-Butylamine drug substance (Fig.2).

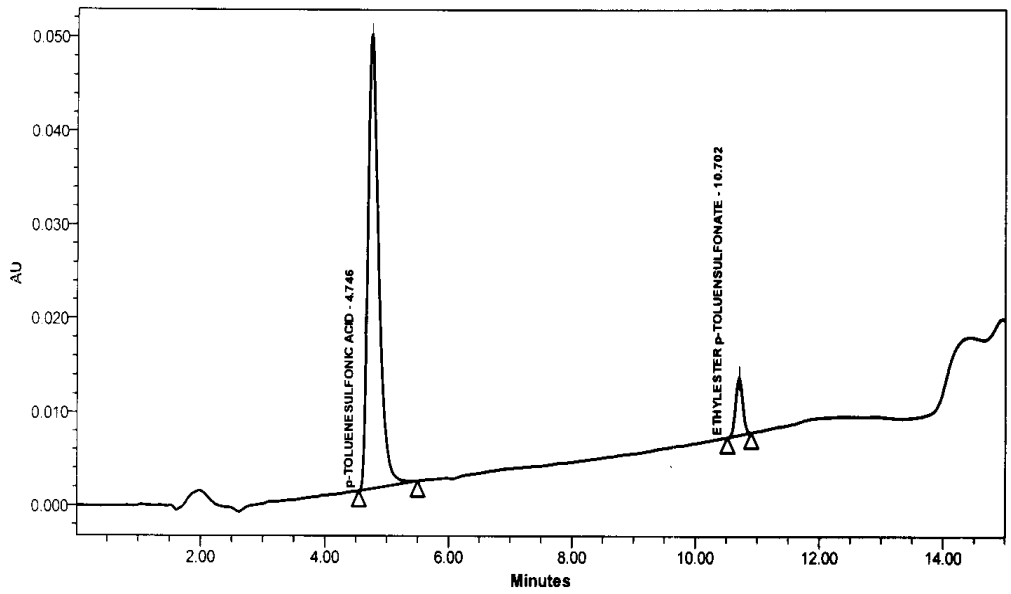


Fig.2 Zoomed chromatogram of PTSA and EPTS standard

Method Validation
The proposed HPLC method was validated [25] for selectivity, sensitivity, linearity, accuracy, limit of detection and limit of quantification, intermediate precision, sample solution stability, forced degradation and stability studies.

Selectivity
The sample solutions of impurities, sample and standard were prepared at proposed concentration based on Perindopril *tert*-Butylamine and injected into the chromatographic system to identify the retention time. The

retention time of PTSA and EPTS was found to be about 4.7 minutes and 10.7 minutes respectively. The sample was found to contain PTSA and EPTS at very low level, and therefore, the sample was spiked with PTSA at 0.1% w/w level and EPTS at 0.009%w/w level (Control sample) and sample spiked with known related impurities of Perindopril *tert*-Butylamine including PTSA and EPTS (spiked sample). It is confirm that no co-eluting peak was observed due to other known related impurities of Perindopril *tert*-Butylamine drug substance with the analyte peak under investigation, thereby indicating that the method is selective for determining the content of PTSA and EPTS. In view, Fig.3 describes the representative chromatogram obtaind from Diluent, As such sample, sample spiked with PTSA and EPTS (Control sample) and sample spiked with PTSA and EPTS along with known related impurities of Perindopril *tert*-Butylamine (Spiked sample).

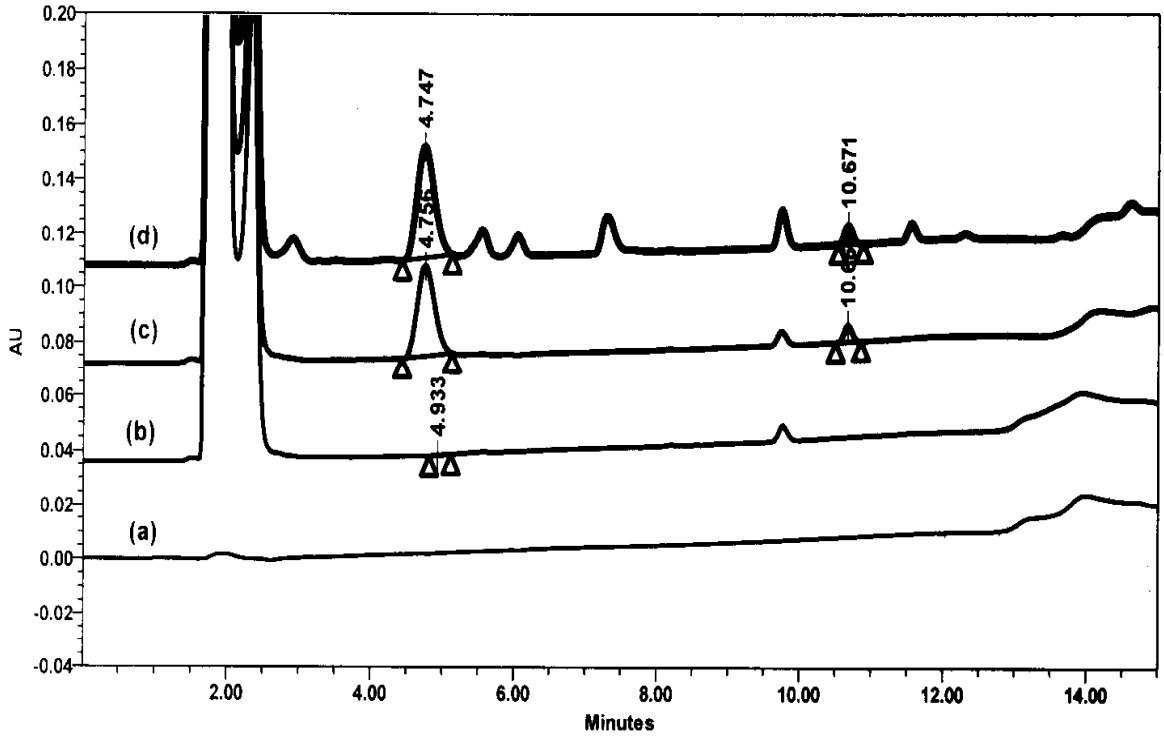


Fig. 3 Representative chromatogram obtained from Diluent, Standard, As such sample, Control sample and Spiked sample.

Table 1 System suitability and selectivity experimental summary data

Components	**PTSA**			**EPTS**
System suitability [a]				
Retention Time (R_T)	4.8 minutes			10.7 minutes
Peak Tailing	1.44			1.36
Plate counts	3456			4051
Selectivity [b]				
	PA	PT	PA	PT
Standard	0.412	0.544	0.521	1.541
Control sample	0.376	0.555	0.540	1.714
Spiked sample	0.365	0.485	0.746	1.637

[a] *Average experimental observation,*
PA stands for Purity angle and PT stands for Purity Threshold
[b] *Criteria for peak purity: Purity angle should be less than purity threshold*

A system suitability rule has been established from the above experiment for the following parameters that, the retention time of PTSA is about 4.5 minutes and about 11.0 minutes for EPTS respectively, peak tailing should not

be more than 1.5 and plate counts should not be less than 3000 for both the peaks. Therefore, the Table 1 summarized the system suitability and peak purity results obtained from the above experiment.

Linearity:
By measuring area responses at different levels of PTSA and EPTS over the range of 5% to 150% of analyte concentration the linearity data were validated. Required concentrations of solutions were prepared from stock solution for different level of 0.050-1.488 µg mL^{-1} for PTSA and 0.005-0.147 µg mL^{-1} for EPTS respectively. The statistical parameters slope, intercept, residual standard on deviation response and correlation co-efficient values were calculated in Table 2.

Table 2 Summary of Linearity experimental data

Component	PTSA	EPTS
Calibration range (µg mL^{-1})	0.050 – 1.488	0.005 – 0.147
Calibration Points	7	7
Slope	591886	498653
Intercept	792	347
Correlation co-efficient (CC)	1.0000	0.9999
Residual sum of square (r^2)	0.9999	0.9999

The area and concentration were treated by least square linear regression analysis plot [Area count in terms of Area count (AU) at Y-axis Vs Concentration (µg mL^{-1}) at X-axis] as shown in Fig 4 (a) for PTSA and (b) EPTS respectively.

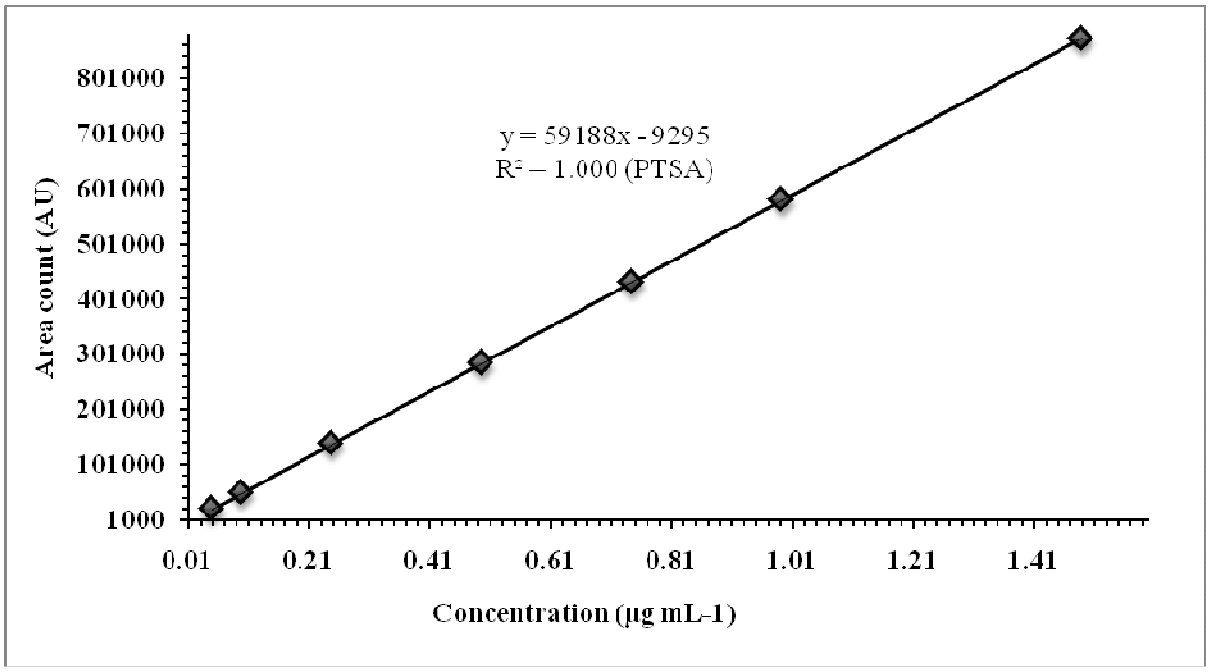


Fig.4(a) Regression plot for PTSA obtained from linearity experiment.

Sensitivity:
Limit of detection (LOD) and limit of quantification (LOQ) values of individual contents of interest were predicted from signal to noise ratio data. These predicted concentrations were verified for precision by preparing the solutions at about these predicted concentrations and injected each solution six times into the HPLC by following the developed method conditions. The limit of detection and quantification predicted was found to be 0.0004 %w/w and 0.0012 %w/w for PTSA and 0.008 µg mL^{-1} and 0.003µg mL^{-1} for EPTS respectively, by using the calculation 3.3*Standard concentration/Signal to noise ratio of standard (for LOD) and 10* Standard concentration/Signal to noise ratio of standard (for LOQ). The percentage relative standard deviation for six replicate measurements at predicted LOD and LOQ concentration levels was found to be 10.8 and 2.2 for PTSA and 11.3 and 8.8 for EPTS respectively, verifying the predicted values.

Precision

The method was assessed by six replicate injections of PTSA and EPTS standard solution (5 µg mL^{-1} for PTSA and 0.45µg mL^{-1} for EPTS) into chromatographic system, and the percentage relative standard deviation of response for six replicate measurements was found to be 0.9 and 1.3 for PTSA and EPTS respectively proves the repeatability of the system. Reproducibility of the method (Method precision) was demonstrated by preparing six replicate sample preparations by spiking known concentration 1.0µg mL^{-1} of PTSA and 0.09µg mL^{-1} of EPTS in random selection of one batch of Perindopril *tert*-Butylamine drug substance. The samples were analyzed as per method, and the content of PTSA and EPTS were determined. The values obtained from the above experiment were found to be 1.01µg mL^{-1} with %RSD value of 1.8 for PTSA and 0.093µg mL^{-1} with %RSD value of 2.6 for EPTS has shown good repeatability for analytical experiment. The degree of reproducibility is known as ruggedness, obtained by the analysis of the same sample concentration (which is used in the method precision) under a variety of conditions using different series of column, with different user on different day by using new standard also found to be 0.99 µg mL^{-1} with %RSD value of 2.9 for PTSA and 0.089 µg mL^{-1} with %RSD value of 2.2 for EPTS respectively also proves that the method is rugged for the determination of PTSA and EPTS under the experimental conditions.

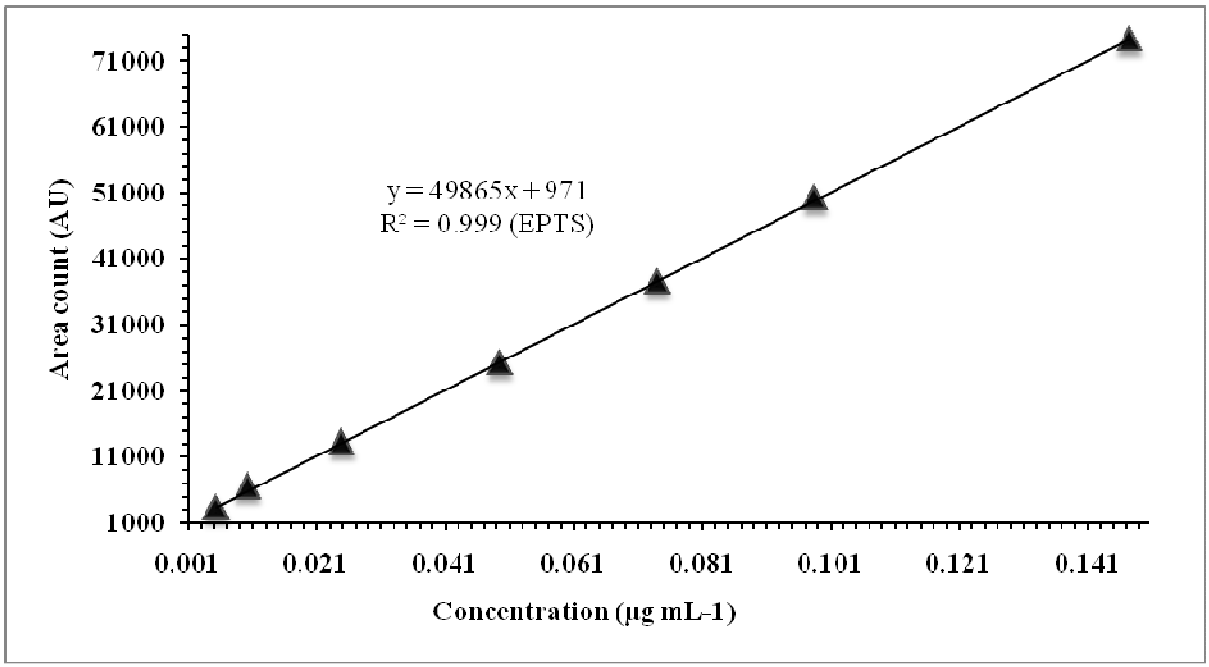


Fig.4(b) Regression plot for EPTS obtained from linearity experiment.

Stability of sample solution

The Perindopril *tert*-Butylamine drug substance were spiked with known concentration of PTSA (1.0µg mL^{-1}) and EPTS (0.09µg mL^{-1}) with respect to sample concentration and the solution was stored at 25 ± 2°C temperature condition then injected into chromatographic system at different time intervals. The content of PTSA and EPTS was determined at each interval, the sample solution was found to be stable over a period of 15 hours. The % difference between the peak area obtained at initial and different time interval was found to be 4.9 for PTSA and 5.2 for EPTS. However, it is observed from the experimental condition the stability of the sample was found to be stable for at least 15 hour at room temperature (~25°C).

Accuracy

The recovery studies during the method was evaluated by preparing sample solution spiked with known amount of PTSA and EPTS at different concentration levels in the range between 50%, 100% and 150% with respect to Perindopril *tert*-Butylamine concentration. Each concentration of sample solution was prepared in triplicate and analyzed as per the method. The overall percent recoveries were found to be 99.7% and 99.0% for PTSA and EPTS respectively, when calculated against the known added amount, indicating that the method is accurate describes in the Table 3.

Robustness

To assess the robustness of the method, experimental conditions were deliberately altered for specified range for temperature (±5°C), flow of mobile phase (±10%) and wavelength (±5nm). The result obtained from the robustness indicated that, the experimental method parameters were tolerance limit with minor changes to optimize the method.

Table 3 Experimental summary for Accuracy analysis

Components	PTSA [c]			EPTS [d]		
	Level-I (50%)	Level-I (100%)	Level-I (150%)	Level-I (50%)	Level-I (100%)	Level-I (150%)
Added ($\mu g\ mL^{-1}$) [e]	0.04811	0.0983	0.15424	0.00452	0.00922	0.01321
Found ($\mu g\ mL^{-1}$) [e]	0.04826	0.0993	0.15339	0.00449	0.00909	0.01307
Recovery (%)	100.3	99.5	99.9	99.3	98.6	98.9
%RSD	1.4	1.3	0.8	1.8	0.9	1.1
Overall statistical data						
Mean Recovery (%)	99.7			99.0		
SD	0.4			0.6		
%RSD [f]	0.4			0.6		
95%CI (±)[g]	1.2			0.9		

[c] Calculated with respect to 0.1%w/w, [d] Calculated with respect to 90ppm,
[e] Average of n=3 determination, [f] Overall %RSD

Stability Studies

To present stability studies on Perindopril *tert*-Butylamine drug substance for the determination of *p*-Toluene sulfonic acid Ethyl-*p*-Toluene sulfonate 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) and refrigerated (5°C±3°C) storage condition [26]. The results obtained from the above storage conditions are found to be below the limit of quantification. Hence formation of *p*-Toluene sulfonic acid Ethyl-*p*-Toluene sulfonate in Perindopril *tert*-Butylamine 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 inter and intra day with other analyst and different chromatograph with different lot of the column shows the method is rugged for the determination of *p*-Toluene sulfonic acid Ethyl-*p*-Toluene sulfonate content.

Forced degradation studies

The experiment further studied on different degradation variables to comply the parameters as per guideline for the sample kept under 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 such degradation was observed on PTSA and EPTS peaks in Perindopril *tert*-Butylamine 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 72 hours and hydrolysis using aqueous media up to 10 hour at room temperature were conducted, the samples were analyzed as per the method. The result obtained from the chromatographic data shows higher percentage of degradation was observed at 105°C for the subjected drug substance, as the sample contains at very low level of PTSA and EPTS. Therefore the interference of other impurities of the subjected drug substance was studied with PTSA and EPTS peaks. No interference or co-elution was observed due to other impurities on either side of the both PTSA and EPTS peaks. In addition, wet stress condition also conducted using 30% H_2O_2, 5M Hydrochloric acid, 1M sodium hydroxide were added separately for sample taken into different reservoir and analyzed as per method condition, the observation shows about higher % of degradation with H_2O_2 as well as with base for the drug substance but no interference of other peaks with PTSA and EPTS peaks. The data reveals that the subjected drug substance is sensitive toward thermal and base contact. Hence, the method is found to be selective and stability indicating with respect to forced degradation data.

CONCLUSION

The proposed simple methodology for quantitative determination of *p*-Toluene sulfonic acid Ethyl-*p*-Toluene sulfonate in Perindopril *tert*-Butylamine drug substance is rapid, accurate, precise and selective .The method provided satisfactory validation data for the tested parameters as per the ICH guidelines. Hence the proposed method may be conveniently used in bulk manufacturing laboratories.

Acknowledgements

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REFERENCES

[1] P Lechat; SP Granham; P Desche. *Am Heart J*., **1993**, 126, 798-806.
[2] D Simpson; N Stuart; GL Karen. *Drugs*., **2002**, 62(9), 1367-1377.
[3] JR Hampten. *Drugs*., **2002**, 62(9), 1378-1379.
[4] JP Bounhoure; G Bottineau; P Lechat. *Clin Exp Hypertens.*, **1989**, A11, 575-586.
[5] European Pharmacopeia 7th edition, Perindopril *tert*-Butylamine, (01/2008:2019), 2692-2695.
[6] Armarego, Wilfred. Purification of Laboratory Chemicals, Elsevier Science, **2003**; 370.
[7] *Merck Index*, 11th edition., **1989**, 9459.
[8] K S Bhatt; *J. Chem.Pharm.Res.*, **2011**, 3(3):286-289.
[9] B Stoffler; G Luft . *Chemosphere*., **1999,** 38(5), 1035-47.
[10] R.Imwinkelried; M.Schiess; D.Seebach. *Organ, Synth*., **1993**, 8, 201.
[11] Furuta; K Gao; H Yamamoto, *Organ. Synth*., **1998**, 9, 722.
[12] S Ambati; PH Rao; TVMaruthikumar; N Sreenivasan; NBAmbati. *Der Chemica Sinica.*, **2011**, 2(6), 151-157.
[13] IT Phucho; S Tumtin; A Nongpiur; R Nongrum; RL Nongkhlaw; *J.Chem.Pharm.Res*., **2010**, 2(3):214-222
[14] KL Dobo; N Greene; MO Cyr; S Caron; WW Ku. *Regul Toxicol Pharmacol*., **2006**, 44, 282-293.
[15] International conference on harmonization, Draft revised Guidance on impurities in new drug substances, **2006**, Q3A (R2).
[16] R Kroes; J Kleiner; A Renwick. *Toxicological sciences.*, **2005**, 86(2), 226.
[17] R Kroes; AG Renwick; M Cheeseman; J Kleiner; I Mangelsdorf; A Piersma; B Schilter; J Schalatter; F Van Schothorst; JG Vog. *Food Chem Toxicol*, **2004**, 42(1), 65.
[18] A Nageswari; KVS Ramakrishna Reddy; K Mukkanti. *Scientia Pharmaceutica*., **2011**, 79, 865-876.
[19] Taylor GE; Gosling M; Pearce A. *J Chromatogr A.*, **2006**, 1119(1), 231-237.
[20] J.Dokladalova, R.P.Upton, Mikrochimica Acta., **1972**, 623-630.
[21] CE/MS analysis optimization guide, *Agilent Technologies.*, **2009**, 52-54.
[22] A Jianguo; S Mingjiang; B Lin; T Chen; DQ Liu; Alireza Kord. *Journal of Pharmaceutical and Biomedical Analysis.*, **2008**, 48(3), 2008, 1006-1010.
[23] G.Shao, R.Kautz, S.Peng, G.Cui, R.W.Giesse, *Journal of Chromatography A.*, **2007**, 1138 (1-2), 305-308.
[24] A M Nardillo; R C Castells; E L Arancibia; M L Casella. *Chromatographia*., **1988**, 25(7), 618-620.
[25] ICH harmonized tripartite guideline. Validation of Analytical procedures Text and methodology, **2005**, Q2 (R1).
[26] International Conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human use. ICH harmonized tripartite guideline, Stability testing of new drug substances and products, **2003**, Q1A (R2).