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A liquid chromatographic method for determination of para toluene sulphonic acid (PTSA) in Ramipril

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ABSTRACT

A liquid chromatographic method was developed for the quantitative determination of Para Toluene Sulphonic Acid in Ramipril bulk drug used as Antihypertensive. The chromatographic separation was achieved on Inertsil ODS-3,250×4.6 mm, 5-µm. The LC method employs consists in gradient elution, mobile phase-A is 2.0g sodium perchlorate in a mixture of 0.5mL of Triethylamine and 900mL of water and adjusted to the pH of the solution is 3.60 and add 100mL of Acetonitrile and mix. And mobile phase-B is 2.0g sodium perchlorate in a mixture of 0.5mL of Triethylamine and 300mL of water and adjusted to the pH of the solution is 2.60 and add 700mL of Acetonitrile and mix. Mobile phase-A use as diluent.The flow rate was 1.0mL $min^{-1}$and the detection wavelength was 210 nm. The developed HPLC method was validated with respect to Specificity, Linearity, accuracy, precision and Intermediate Precision.

KEYWORDS

Liquid chromatography;
Ramipril;
Validation.

INTRODUCTION

Ramipril [2*S*,3a*S*,6a*S*]-1-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid is used for treating high blood pressure or decreasing the risk of heart attack, stroke, and death in certain patients. It may be used alone or with other medicines. It is used along with other medicines to manage heart failure and improve survival after a heart attack.

PTSA limit arrival

Occupational exposure limits[1]

TLV not established.
MAK not established.

Inhalation risk

Evaporation at 20^0C is negligible; a harmful concentration of airborne particles can, however, be reached quickly when dispersed, especially if powdered.

Effects of short-term exposure

The substance is corrosive to the eyes, the skin and the respiratory tract. Corrosive on ingestion. Inhalation of aerosol may cause lung oedema (see Notes). Medical observation is indicated.

The symptoms of lung oedema often do not become manifest until a few hours have passed and they are aggravated by physical effort. Rest and medical observation are therefore essential. Immediate administration of an appropriate inhalation therapy by a doctor

or a person authorized by him/her, should be considered.

Limits calculation

No literature evidence/guidance on limiting in Pharmaceutical products. Taking the risk based approach TTC approach [guideline on the limits of genotoxic impurities -chmp guideline][2] was adopted and limit was calculated as below

Concentration limit (ppm) = TTC [µg/day]/dose(g/day]

1.6 / 0.01 = 160 ppm

10mg - MDD for Ramipril.

Accordingly LOQ of 20 ppm was established for PTSA.

EXPERIMENTAL

Chemicals

Samples of Ramipril are manufactured at Hetero labs Limited India. Acetonitrile was purchased from Merck, Germany. Triethylaniline was purchased from Merck, Darmstadt, Germany. Para Toluene Sulphonic acid was purchased from Merck, Darmstadt, Germany. High pure water was prepared by using Millipore Milli Q plus water purification system.

Equipment

The LC System, used for method development, and method validation were Shimadzu 2010 and Agilent 1100 model Systems with Diode Array Detector. The output signal was monitored and processed using LC-Solution and Chemstation software on Pentium computer.

Chromatographic conditions

The Liquid chromatographic column used was Inertsil ODS-3,250 4.6 mm 5µm.

The Gradient time Program was followed as below.

Time interval (min)	Mobile phase-A (%v/v)	Mobile phase-B (%v/v)
0	90	10
5	90	10
7	65	35
20	50	50
30	20	80
35	20	80
40	90	10
45	90	10
Programme Run time	: 45 minutes.	

Preparation of solutions

Stock solution of PTSA having the concentration 0.0009mg/mL.

Preparation of Ramipril standard solutions

Ramipril standard solution is having the concentration 0.05mg/mL.

Preparation of Standard solution

Take 2.0mL of PTSA stock solution and 0.8mL of Ramipril working standard solution into a 10mL volumetric flask and make-up to the volume with diluent.

Test solution Preparation

Prepared the test solution having the concentration 4mg/mL.

Method validation[3-9]

Specificity

Specificity is the ability of the method to measure the analyte (PTSA) response in the presence of Impurity-A, Impurity-B, Impurity-C, Impurity-D and Ramipril.

The specificity of the developed LC method for PTSA determination.

Limit of quantification (LOQ)

The LOQ for PTSA was estimated at a signal-to-noise ratio of 10:1 respectively, by injecting a series of dilute solutions with known concentration. The precision study was also carried out at the LOQ level by injecting six individual preparations of PTSA and calculated the % RSD for the area of PTSA.

Linearity

Linearity test solutions for LC method were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at seven concentration levels from 20ppm (LOQ level) to 90ppm level. The correlation coefficient, slope and Y-intercept of the calibration curve were reported.

Accuracy

The accuracy of the L C method was evaluated in triplicate at 20ppm (LOQ level), 45 ppm and 90 ppm level in bulk sample and drug product. The percentages of recoveries were calculated.

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TABLE 1 : LOQ study

Replicates	Area of PTSA
1	1608
2	1625
3	1623
4	1615
5	1628
6	1617
Mean	1619
%RSD(NMT 15.0%)	0.46

The study was carried out in triplicate at each level. The percentage of recoveries for PTSA was calculated.

Precision

Injecting six standard solutions checked the precision of the LC method. The % RSD of area of PTSA was calculated.

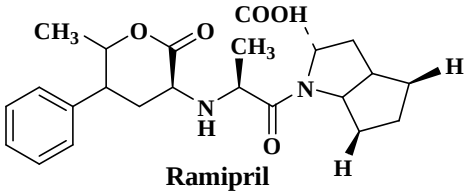
The precision study was also carried out by injecting six individual preparations of at the LOQ level Spiking in 100% test sample. Calculated the % RSD for the content of PTSA.

Intermediate precision

Intermediate precision study done by another analyst, changing column and also changing chemicals in the same laboratory conditions. Injecting six standard solutions checked the precision of the LC method. The % RSD of area of PTSA was calculated. and also the Intermediate precision study was carried out by injecting six individual preparations of at the LOQ level Spiking in 100% test sample. Calculated the % RSD for the content of PTSA.

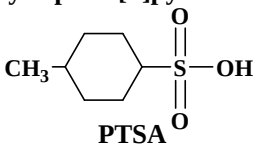
RESULTS AND DISCUSSION

A Liquid chromatographic method was developed for the quantitative determination of Para Toluene Sulphonic Acid in Ramipril bulk drug used as Antihypertensive. The chromatographic separation was achieved on Inertsil ODS-3,250×4.6 mm,5-µm. The LC method employs consists in gradient elution mobile phase-A is 2.0g sodium perchlorate in a mixture of 0.5mL of Triethylamine and 900mL of water and adjust pH of the solution is 3.60 and add 100mL of Acetonitrile and mix. And mobile phase-B is 2.0g sodium perchlorate in a mixture of 0.5mL of Triethylamine and 300mL of water and adjust pH of the solution is 2.60 and add 700mL of Acetonitrile and mix. Mobile phase



Ramipril

[2S,3aS,6aS]-1-[(2S)-2-[[(1S)-1-(Ethoxycarbonyl) -3-phenylpropyl]amino]-1-oxopropyl] octahydrocyclopenta[b]pyrrole-2-carboxylic acid



PTSA

Figure 1: Chemical structures and labels of Ramipril and PTSA

use as diluent.The flow rate was 1.0mL $min^{-1}$and the detection wavelength was 210 nm. The developed HPLC method was validated with respect to linearity, accuracy, precision and Intermediate Precision.

Analysis was performed for different batches of bulk drug samples (n=3) and for pharmaceutical dosage forms (n=3).

Method validation

Precision

The %RSD of PTSA during system precision study was within 5.0%, and the %RSD of PTSA content in Method precision was within 10.0%. Confirming good precision of the method.

Limit of detection and limit of quantification

The limit of quantification of PTSA was 20ppm (of analyte concentration, i.e. 4.0 mg mL^{-1}) respectively for 10µL injection volume.Obtained Signal to Noise ratio of PTSA from LOQ solution was 15.08. The precision at LOQ concentration for PTSA was below 5.0%.

Linearity

Linear calibration plot for PTSA was obtained over the calibration ranges tested, i.e. 20ppm to 90ppm, specification level and the correlation coefficient obtained was greater than 0.998. The results show that an excellent correlation existed between the peak area and concentration of the analyte. The slope and Y-intercept of the calibration curve were 77.71830 and 63.35510 respectively.

TABLE 2 : Linearity study

Level	Con (ppm)	Mean area	Response calculated through trend line equation	Residual	Residual square	Sensitivity
Level-1	20	1619	1617.721	1.279	1.636	80.9500
Level-2	30	2470	2394.904	75.096	5639.409	82.3333
Level-3	40	3196	3172.087	23.913	571.832	79.90000
Level-4	45	3549	3560.679	-11.679	136.399	78.8667
Level-5	63	4765	4959.608	-194.608	37872.274	75.6349
Level-6	90	7164	7058.002	105.998	11235.576	79.6000
Regression coefficient	0.99856		Residual sum of square		55457.126	
Slope	77.71830	10% of 100% concentration response is 354.9		90% of 100% Sensitivity		70.9800
Intercept	63.35510			110% of 100% Sensitivity		86.7533
% y-Intercept	1.78515	y = 77.718x+63.355				

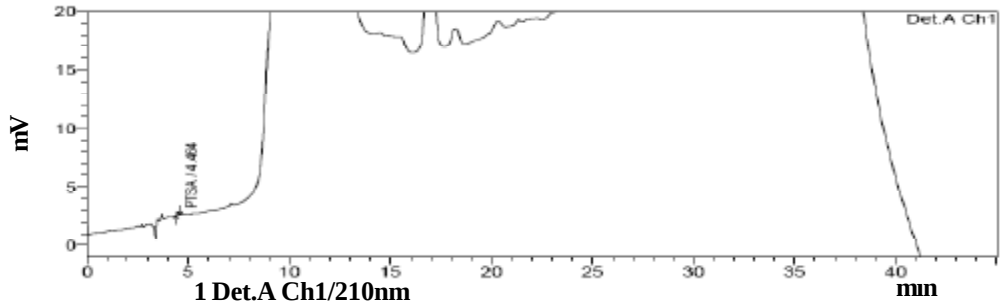


Figure 2: Typical chromatogram of standard solution

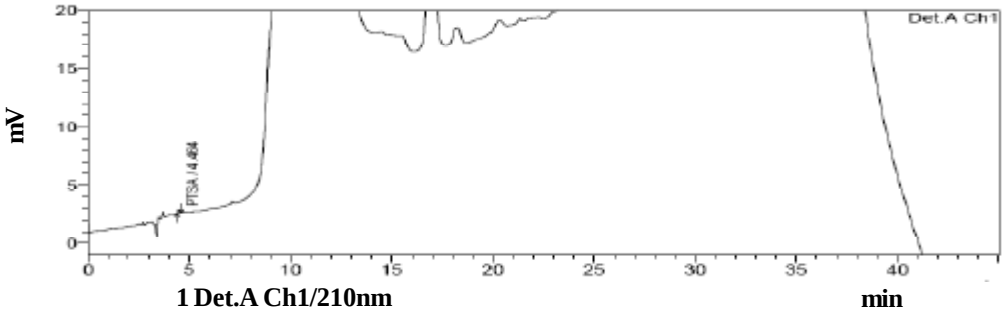


Figure 3: Typical chromatogram of LOQ level 20 ppm of para toluene sulphonic acid

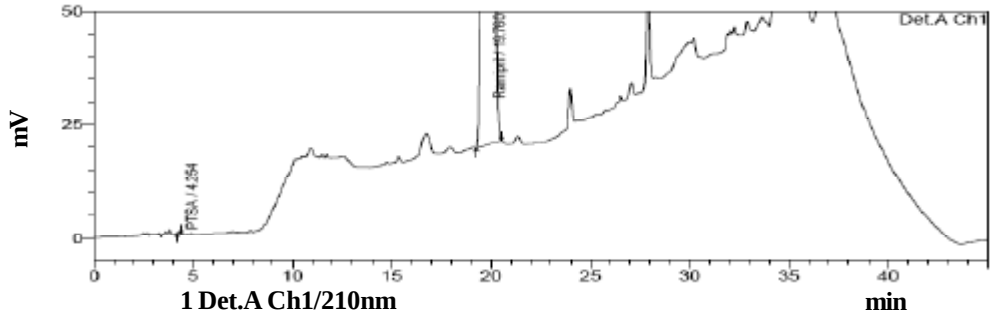


Figure 4: Typical chromatogram of Ramipril spiked with 20ppm of para toluene sulphonic acid

TABLE 3: Results of accuracy study for PTSA

Added (ppm) (n= 3)	% Recovery
20ppm	105.7
45ppm	100.7
90ppm	106.2

n =3, Number of determinations

Linear calibration plot for PTSA content method was obtained over the calibration ranges tested, i.e. LOQ (20ppm) to 90ppm for PTSA. The correlation coefficient obtained was greater than 0.998. Linearity was checked for LC method over the same concentra-

tion range. The percentage of R.S.D. values of the each level, slope and Y-intercept is within the limit. The results show that an excellent correlation existed between the peak area of PTSA and concentration.

Accuracy

The percentage recovery of PTSA in bulk drug samples ranged from 100.7 to 106.2 (TABLE 3). The percentage recovery of PTSA in bulk drugs samples ranged from 85 to 110. HPLC chromatogram of spiked sample at 20 ppm (LOQ level), 45 ppm and 90 ppm of specification level of PTSA in Ramipril bulk drug sample.

Intermediate precision

The %RSD of PTSA during system precision study was within 5.0%, and the %RSD of PTSA content in Method precision was within 10.0%.and cumulative %RSD found below 15.0% between precision study and Intermediate precision study.

CONCLUSIONS

The Liquid chromatograph method developed for quantitative and PTSA content determination of Ramipril in bulk drug samples is precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method can be used for the analysis of samples of Ramipril.

ACKNOWLEDGMENTS

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