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Quantitative determination of residual hydrazine content in cilazapril by ion chromatography

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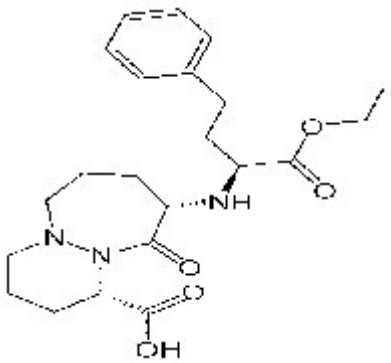
ABSTRACT

A simple & economic Ion chromatographic (IC) method with conductivity detector has been developed for quantitative determination of residual hydrazine content in cilazapril. The chromatographic separation was archived on a Metro Sep C2, 150x4.6x7µm. column. The limit of detection was 1.53µg mL^{-1} and limit of quantification was 4.63 µg $mL^{-1.}$ The developed method was validated according to ICH guide lines for Accuracy, Precession and Linearity and is sensitive to detect and quantify the Hydrazine as trace element, also highly precise and accurate. The calibration curves were linear with correlation co-efficient of >0.999.

Key words: Ion Chromatography (IC), Conductivity Detector, Cilazapril, Hydrazine, Method validation**.**

INTRODUCTION

Cilazapril is a novel drug which is being evaluated for the treatment of essential hepertension and congestive heart failure. Cilazapril is rapidly hydrolysed by non-specific esterases to the active acid metabilite cilazaprilat, which is a potent inhibitor of angiotension converting enzyme(ACE). Cilazapril is a pyridazine angiotensin-converting enzyme inhibitor (ACE inhibitor).Cilazapril works by causing blood vessels to relax. This lowers blood pressure by decreasing production of a strong chemical in the body. It helps the heart work more effectively. It improves blood flow and increases the supply of blood and oxygen to the heart. ACE inhibitors may be used for the treatment of several heart related problems & helps to decrease the risk of heart attacks.



Cilazapril

Determination of hydrazines in the environmental and food samples is an important area of research because of their high toxicity and health hazard. Nowadays, application of

chromatographic separation is preferable for analysis of environmental and food samples due to its high selectivity. Different techniques have been used for the determination of unsymmetrical dimethylhydrazine. Gas chromatography with prior derivatization with 4-nitrobenzaldehyde,[1-2] 2-nitrobenzaldehyde,[3-5] salicylaldehyde[6,7] or pentafluorobenzoyl chloride[8,9] has been widely used for determination of hydrazine and 1,1-dimethylhydrazine. Reversed phase HPLC with prior derivatization with salicylaldehyde using UV and amperometric[10] detection has been reported too. All procedures based on hydrazine formation suffer from not only increasing time of analysis and addition of labor-intensive step but low speed of reaction[11] what causes low yield of derivative.

Direct determination of hydrazines using liquid chromatography with amperometric detection has been used as an alternative approach. The detection limits on mol level were demonstrated in some reports.[12-16]. However reversed phase separation seems to cause overlaying of peaks of hydrazines and unretained substances in real samples due to low capacity factors of hydrazines. Ion chromatography seems to be preferable from this point of view, because Ion chromatography is a powerful technique for determining low concentrations of ions. It involves the retention of analyte molecules from the sample being retained based on ionic interactions. Fiala and Kulakis[17] reported the separation of hydrazine, methylhydrazine and dimethylhydrazines on cation-exchange column packed with Aminex A-5.[18]

IC represents a universal analytical technique for the separation and quantitative determination of specific ion species. Complex mixtures of anions or cations can be separated to the level of specific ions and then quantified in a relatively short time.

The main applications of IC methods are in the determination of trace anions in ultra pure water in the pharmaceutical industry, electronics, power plants, pulp and paper production[19].

The IC method can detect and quantify substances that cause color, smell and slime in the production process, as well as salts and other corrosive substances.These disturbing substances include volatile organic acids (acetic, formic,lactic and butyric) and inorganic salts present as anions: chloride, fluoride, sulfate, nitrate, etc [20-27].

A number of spectrophotometric methods were reported for the determination of Cilzapril in its binary mixtures[28,29]. And also HPLC methods for the specific determinations of Cilazapril along with other angiotensin-converting enzyme inhibitors is reported by[30,31]. A Validated method for the determination of Cilzapril and its Metaboletes in presence of other enzyme inhibitors are also reported [32] Shalini Joshi reported a method for formulations by TLC,HPLC and RPTLC[33].

The aim of this work was to develop and validate IC method for the determination of Hydrazine content in final sample of Cilazapril. The final samples of Cilzapril from production system were analysed by IC method with conductivity detector and Metro Sep C2, 150x4.6x7µm. column. It was examined that the developed IC method was able to determine residual hydrazine content as a trace element.

MATERIAL AND METHODS

Chemicals & Reagents

All reagents were analytical grade and no further purification was required, Cilazapril sample used for analysis was synthesized by Hetero Drugs Limited Hyderabad, India. And Cilazapril chemically described as (1*S*,9S)-9-{[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}-10-oxo-octahydro-1*H*-pyridazino[1,2-][1,2]diazepine-1-Carboxylic acid.

Nitric acid and acetone are purchased from Merck, India. Water was deionised and purified on a Milli Q water purification system (Millipore, Bedford, MA, USA) and used to prepare all the solutions

Instrumentation

The Ion Chromatography system used is Metrohm compact IC, Model No. 761, through out this study equipped with 818 IC pump, Sample injector with 20 µl loop with conductivity detector. Quantitation was performed from the output signal, monitored and processed using software. Dilutions were accomplished with Hamilton precision pipettes.

Chromatographic conditions

The analysis carried out on a Metrosep C-2 (6.1010.220) 150 x 4.0 mm, 7µm column with a mobile phase of 5.0mM Nitric acid and acetone mixture 90:10 v/v at 25°C with a flow rate of 0.8 ml /min with the run time of each run 15 minutes and sample and standard injection volume is 20 µl.

Standard Preparation

A standard solution containing 0.406 gms of Hydrazine sulphate is dissolved in 100 ml diluent.(90:10 v/v 5.0mM nitric acid and acetone) to get the concentration 1000µg ml^{-1}.

Sample preparation

10.0 mg of sample was transferred to a 10.0 ml standard volumetric flask. The sample was dissolved in diluent. The solution was filtered through 0.2µm Millipore PVDF filter. Then 20.0 µl of this solution was injected in to the column and the chromatogram was recorded. As shown in figure 2 the retention time of hydrazine in cilazapril was found to be 5.38 min.

RESULTS AND DISCUSSION

Method Development and Optimized Conditions

The main objective of this Chromatographic method is to elute Hydrazine using different columns such as Metrosep C2 250,Metrosep C2 150mm as well as different Mobile phases Finally the Chromatographic separation was achieved on an Silica gel with Carboxylic groups 150 X 4.0mm,7 µm Column, the peak shape of Hydrazine was found to be symmetrical. The retention time was 5.38min. All calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas.

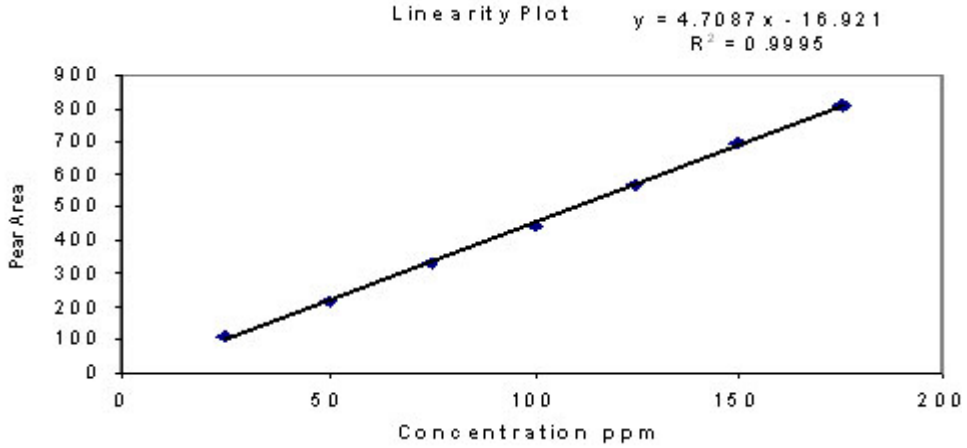
Method Validation

The method developed meets all the requirements of system suitability. Such as %RSD, Tailing Factor. %RSD was 0.7 and the tailing factor was 1.3%. The method is validated as per ICH guidelines. Limit of detection and quantitation values were determined at lowest concentrations. The method is linier over range of 25 – 175 µg $mL^{-1.}$

Linearity

The linearity of peak areas verses different concentrations was evaluated using seven levels of linearity solutions, prepared in the range of 25 – 175µg $mL^{-1.}$ from 1000 µg mL^{-1} of standard Hydrazine sulphate solution. The tests were carried out for three consecutive days in the same concentration range.

The results show that an excellent correlation existed between the peak area and concentration of the analyte, which is directly proportional to the concentration of the analyte in the sample. Correlation coefficient is 0.99974.



Limit of Detection & Limit of Quantitation

LOD, LOQ were determined by injecting a series of dilute solutions with known concentrations[35] at which signal to noise ratio is 3 & 10 respectively under the experimental conditions used. LOD is 1.53 µg mL^{-1} and LOQ is 4.63µg $mL^{-1.}$

Precision

Method precision was checked for six different preparations of Cilzapril with Hydrazine standard at the LOQ level. The calculated %RSD of Hydrazine is 2.082, which is less than 15.0. The intermediate precision of the method was also evaluated by different analyst and on different day. Chromatograms attached as fig.1 & 2.

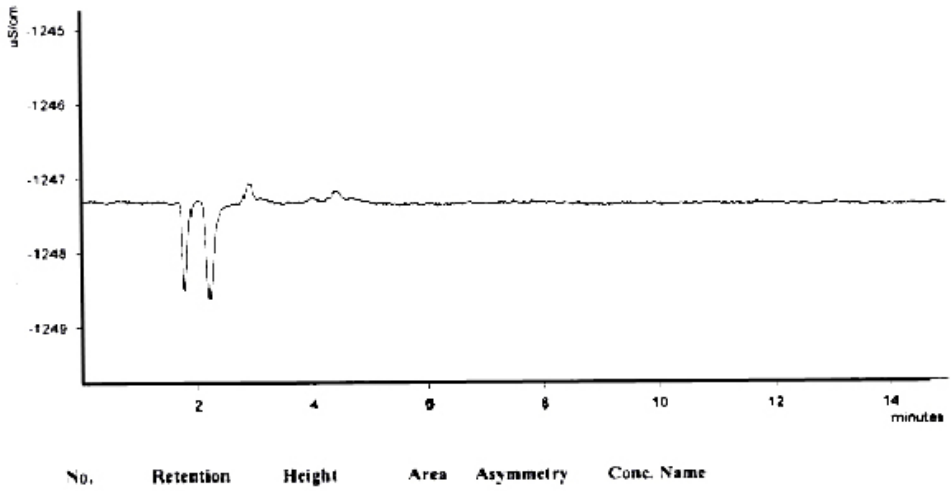


Fig. 1: Cilazapril- Blank Chromatogram

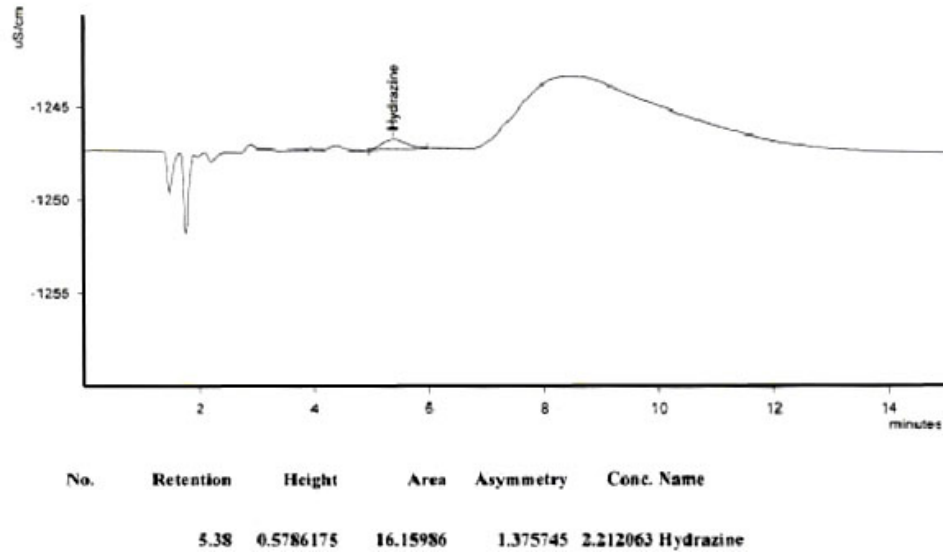


Fig. 2: Cilazapril-spiked with Hydrazine standard at LOQ level

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either a conventional true value or an accepted reference value and the expected value found[34]. Standard addition and recovery experiments were conducted to determine accuracy of the quantitation of Hydrazine content in Cilazapril. The study was carried out by addition of Hydrazine at 80%,100%120% of specification level. The % recoveries for hydrazine were calculated from the slope and y-intercept of the calibration curve obtained. It was observed that % recovery of Hydrazine was in between 80% – 120%

CONCLUSION

A simple, economic, environmental friendly ion Chromatographic method with isocratic elution was developed for quantitation of hydrazine in Cilazapril. This method was validated and found to be Linear, Precise and accurate for the detection and quantification of hydrazine in Cilazapril.

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