A High Performance Liquid Chromatographic Method Using UV Detection for the Determination of Lisinopril

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A fast and simple HPLC method for the determination of lisinopril dissolved in pH 1.2 buffer was developed and validated. UV detection at 215 nm was used, on a HPLC Agilent 1200 system. Lisinopril was separated on a C8 column using a mobile phase consisting of a mixture of 0.125% sodium hexanesulphonate solution prepared in phosphate buffer pH 2 and acetonitrile (72:28, v/v), at a flow rate of 1 mL·min⁻¹. The injected volume was 20 μL. In these chromatographic conditions, the retention time of lisinopril was 1.9 minutes and the overall time of the analysis was 3 minutes. The proposed method was validated for a linear range of 0.6-60 μg·mL⁻¹ with correlation coefficient of r = 0.9991. The precision and accuracy were within 1.3 % for inter- and intra-day HPLC runs. The overall recovery for lisinopril was 99%. This assay can be used for the therapeutic drug monitoring of lisinopril.

Keywords: lisinopril, HPLC analytical method, validation.

Lisinopril [(S)-1-[N²-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate], is a derivative that belongs to nitrogen heterocycle compounds [1-4]. It is a drug of the angiotensin converting enzyme (ACE) inhibitor class. Several assay methods are available for the assay of lisinopril, including HPLC-UV [5-9]. The HPLC assays should be sensitive enough to measure lisinopril levels in biological fluids after oral administration of therapeutic doses to humans [10-27].

The present study reports a simple and sensitive HPLC method for the determination of lisinopril dissolved at pH 1.2. This assay can be used for the therapeutic drug monitoring of lisinopril.

Experimental part
All analyses were performed using the HPLC Agilent 1200 system. The system components included the Agilent 1200 Degasser, Agilent 1200 Binary Pump, Agilent 1200 Autosampler and Agilent 1200 UV Detector. The Agilent Chem 32 software was used for system control and data acquisition. An analytical scale Mettler-Toledo XP56, a Sigma 2-16 K centrifuge and a Vibramax 110 shaker were used. The separation was performed using a Lichrospher® 100 RP 8 (125 X 4.6), 5μM.

The standard lisinopril dihydrate used in this study was supplied by European Pharmacopoeia, with a purity of 100.00%. All solvents and other chemicals were HPLC grade provided by Merck, Germany.

The mobile phase consist in a mixture of pH 2 phosphate buffer containing 0.125% sodium hexanesulphonate and acetonitrile (72:28, v/v) with a flow rate of 1 mL/min.

A stock solution of lisinopril dihydrate with a concentration of 0.3 mg·mL⁻¹ was prepared by dissolving an appropriate quantity of lisinopril dihydrate reference substance in methanol:water (1:1). This solution was kept at 5°C. In these conditions, it was stable for at least 7 days.

Seven lisinopril solutions of various concentrations (0.6, 1.2, 9, 18, 24, 48, 60 μg·mL⁻¹) were prepared for the study of the linearity response in pH 1.2 buffer, covering the concentrations range of 0.6-60 μg·mL⁻¹.

The quality control samples with theoretical concentrations of 3 μg·mL⁻¹ (the low-QC1), 30 μg·mL⁻¹ (the medium–QC2) and 45 μg·mL⁻¹ (the high–QC3) were used to validate the analytical method.

Results and discussions
The method was validated according to The Guidance for Industry: Bioanalytical Method Validation [28]. The parameters examined in the validation process were selectivity/specificity, linearity, limit of quantification, accuracy and precision.

The study of the Selectivity/Specificity: a placebo mixture containing non-active ingredients usually found in lisinopril tablets was carried through the extraction procedure and chromatographed to determine the extent to which non active ingredients may interfere with the chromatographic analysis of lisinopril. No significant interferences were observed in 3 different extracted samples. Thus the analytical method proved to be selective.

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REV. CHIM. (Bucharest) • 64 • No. 3 • 2013
The study of linearity and lower limit of quantification: Calibration curves are found to be consistently accurate over 0.600 to 60.000 μg·mL⁻¹ calibration range. The coefficient of correlation ($r^2$) is higher than or equal to 0.99998 (fig. 1).

The lower limit of quantification or the lowest standard level with a coefficient of variation less than 20%, is 0.6 μg lisinopril·mL⁻¹. The analytical method proved to be sensitive, allowing a precise quantification of concentrations as low as 0.6 μg·mL⁻¹ (table 1).

Within-run accuracy and precision evaluations were performed by analyzing replicate concentrations of lisinopril. The within-run coefficient of variation ranged between 1.329 and 2.265%. The within-run percentages of nominal concentration ranged between 98.873 and 100.594%. Results are presented in table 2.

The between-run accuracy and precision were assessed by repeated analysis of quality control samples containing different concentrations of lisinopril on separate occasions. A single run consisted of a calibration curve and 6 replicates of the low (QC1), medium (QC2) and high (QC3) quality samples for lisinopril. The between-run coefficient of variation ranged between 0.33 and 0.74 %. The between-run percentages of nominal concentrations ranged between 97.833 and 101.112%. Results are presented in table 3.

Conclusions
A reversed phase HPLC method has been developed and validated for the determination of lisinopril dissolved in pH 1.2 buffer solution. This chromatographic assay fulfilled all the requirements of a reliable method, including accuracy and precision, linearity, selectivity/specificity. The assay has proven to be sensitive, specific and reproducible and it can be used for the therapeutic drug monitoring of lisinopril.
References

Manuscript received: 18.12.2012