

Quantification of Captopril using Ultra High Performance Liquid Chromatography

Glenn Zammit*, Ralph Cassar, Mark Pace

Department of Science, Malta College of Arts, Science and Technology, Poala, Malta

Article History:

Submitted: 10.01.2022

Accepted: 24.01.2022

Published: 31.01.2022

ABSTRACT

We develop an efficient, sensitive, accurate, cost effective, and greener liquid chromatography method for the determination of a quantification method concerning Captopril in pharmaceutical dosage forms by using Ultra High Performance Liquid Chromatography (UHPLC) equipment. Satisfactory resolution was able to be achieved using a Water Aquity UHPLC BEH C18 1.7 $\mu\text{m} \times 2.1 \text{ mm} \times 50 \text{ mm}$ column and an isocratic mobile phase composition of Methanol, Milli-Q water and Trifluoroacetic acid in the ratio of 55:45:0.05 v/v/v respectively at a flow rate of 0.1 mL/min and a wavelength detection of 220 nm. The retention time for Captopril was found to be 1.744 minutes while for Captopril disulfide it was found to be 2.657 minutes. The method was validated based on the ICH and USP guidelines.

Keywords: Captopril disulphide, UHPLC, ACE, Hypertension drugs, Analytical chemistry.

Abbreviations: UHPLC: Ultra High Performance Liquid Chromatography; ACE: Angiotensin-Converting Enzyme; GMP: Good Manufacturing Practices; ICH: The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; USP: United States Pharmacopeia; CPT: Current Procedural Terminology; CPT-DIS: BEH: Ethylene Bridged Hybrid; PUDF: Public Use Data File; RSD: Relative Standard Deviation; GZ: Gliclazide; MP: Mobile Phase.

Correspondence:

Glenn Zammit

Department of Science, Malta College of Arts, Science and Technology, Poala, Malta,

E-mail glenn.zammit.a101215@mcast.edu.mt

INTRODUCTION

Captopril ((2S)-1-[(2S)-2-Methyl-3-sulfanylpropanoyl] pyrrolidine-2-carboxylic acid), molecular formula $\text{C}_9\text{H}_{15}\text{NO}_3\text{S}$ is a popular well-known drug which subjects in the class of drugs called Angiotensin-Converting Enzyme (ACE) inhibitors [1]. Captopril is the first orally active inhibitor of ACE inhibitor in which the enzyme is responsible for the conversion of inactive angiotensin I to the potent pressure peptide angiotensin II. ACE inhibitors are mainly used for the treatment of high blood pressure but they also contain some other medical properties such as vasculo-protective and antithrombotic activities which plays a favourable role in terms of cardiovascular morbidity [2]. Captopril, has established a position in the medical treatment for hypertension and congestive heart failure. Amongst the hypertension drugs, Captopril is a preferred drug amongst doctors, it is prescribed to patients who are chronically ill and require a long-term treatment, due to its therapeutic benefits and because of its effectiveness, low price and low toxicity [3-6]. Captopril disulfide (1,10-[Disulfanediybis[(2S)-2-methyl-1-oxopropane3,1-diyl]] bis[(2S)-pyrrolidine-2-carboxylic acid], molecular formula $\text{C}_{18}\text{H}_{28}\text{N}_2\text{O}_6\text{S}_2$ is the major degradation impurity of Captopril. Captopril disulfide is also referred to as impurity A [7]. The Captopril molecule starts to degrade after eight hours when in solution and also when being exposed to air by means of oxidation [8,9]. The Captopril molecule was analytically tested for stability under different stress conditions [10]. The stability testing was performed under various pH and temperature conditions, in the presence of strong oxidizing agents and under both natural light and Ultra-Violet (UV) light exposure; this was done in order to distinguish it from other types of impurities that may be present in the final product. Although approved in 1981 in the USA, Captopril is still widely used today [11] (Fig. 1).

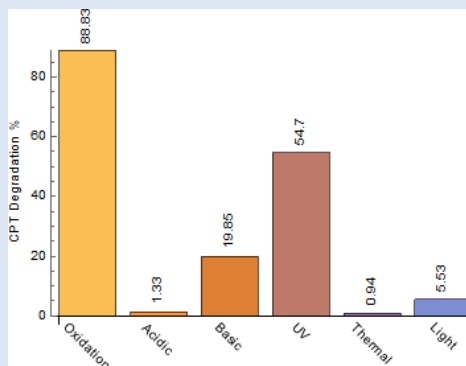


Figure 1: The degradation rate of Captopril (%) under different forced degradation conditions [10].

The aim of this research is to develop a cost effective, efficient and a greener liquid chromatography method for the determination of Captopril in pharmaceutical dosage forms using shorter runs and using less mobile phase than conventional liquid chromatography methods.

Greener instrumental analytical chemistry

The use of UHPLC methods in pharmaceutical analysis while reducing the time for analysis of batches for release to patients and customers, decreases cost of testing. Much less solvents are used. In fact this study shows that a significant reduction of 75% of mobile phase is achieved compared to the USP method for Captopril [12]. This means that the UHPLC method proposed is greener. UHPLC methods reduce energy consumption, reduce the use of solvents, and make waste management more manageable in terms of costs and environmental impacts [13]. The UHPLC method validated in this paper satisfies some

of the principles of green analytical chemistry as described in [14]. In particular using UHPLC rather than a conventional HPLC method generates much less waste, reduces analysis time and the use of energy. The number of samples to test for assay in the pharmaceutical industry is determined according to guidelines such as the ICH guidelines [15] and Good Manufacturing Practices (GMP) [16].

MATERIALS AND METHODS

Materials and reagents

USP grade CPT, USP grade CPT-DIS, captopril tablets, and necessary reagents.

Apparatus

Waters Acquity UHPLC and Aquity UHPLC BEH C18 1.7 μm \times 2.1 mm \times 50 mm column. ACQUITY H-CLASS UHPLC.

Chromatographic conditions

Both the sample cooler temperature and the column oven temperature were set at 25°C. The compounds were separated isocratically with a mobile phase composition of 550 mL methanol (Carlo Erba. Gold Ultragradient grade), 450 mL milli-Q water and 0.50 mL trifluoroacetic acid (Fisher, 99% HPLC Grade) having a flow rate of 0.1 mL/min with an injection volume of 0.8 μL . The wavelength detection for was set at 220 nm.

Preparation of stock solutions for system suitability criteria

Captopril standard solution was prepared in duplicate, labelled as 'standard solution 1' and 'standard solution 2', by dissolving 10 mg of Captopril reference standard in a 50 mL volumetric flask. Dissolved and diluted up to volume using mobile phase, filtered through a 0.45 μm Millipore Millex-HV Hydrophilic PVDF Filter discarding the first 6 mL of filtrate. This solution contains a concentration of 0.2 mg/mL of Captopril API, prepared fresh on the day of use.

Captopril-disulfide solution was prepared by dissolving 25 mg of CPT-Dis into a 50 mL volumetric flask. Dissolved and diluted up to volume using mobile phase. This solution contains a concentration of 0.5 mg/mL of Captopril-disulfide, prepared fresh on the day of use.

Resolution solution was prepared by dissolving 10 mg of Captopril reference standard into a 50 mL volumetric flask. 5 mL of Captopril disulfide solution (0.5 mg/mL) was added together with sufficient mobile phase. Dissolved and diluted up to volume using mobile phase, filtered through a 0.45 μm Millipore Millex-HV Hydrophilic PVDF Filter discarding the first 6 mL of filtrate. This solution contains a concentration of 0.2 mg/mL of captopril API and 0.05 mg/mL captopril disulfide, prepared fresh on the day of use.

Injection procedure

The UHPLC column was left to equilibrate with mobile phase, and blank runs were initially injected. These were followed by six repeated injections of Captopril 'standard solution 1' and a duplicate injection of 'standard solution 2'. The resolution solution was injected afterwards. The six injections were used to determine the % RSD and to calculate the % recovery with the average area of the duplicate injection of 'standard solution 2'. Resolution between Captopril and Captopril disulfide was determined and the tailing factor was recorded. Test solutions were bracketed with a duplicate injection of Captopril 'standard solution 1' and % RSD was calculated and compared with the six repeated injection of Captopril 'standard solution 1' for system suitability

Sample preparation

The contents of 20 Captopril 100 mg tablets were crushed and homogenized using a pestle and mortar. 96 mg of the Captopril crushed tablet sample were transferred to a 100 mL volumetric flask. About 40 mL of diluent were added, followed by sonication for 15 minutes. Dilution up to volume with diluent and filtration through a 0.45 μm Millipore Millex-HV Hydrophilic PVDF Filter followed, discarding the first 6 mL of filtrate. The final test solution had a concentration of 0.2 mg/mL of Captopril API (Figs. 2-4).

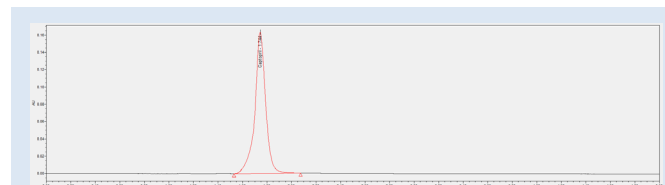


Figure 2: Chromatogram of USP Captopril standard solution using UHPLC.

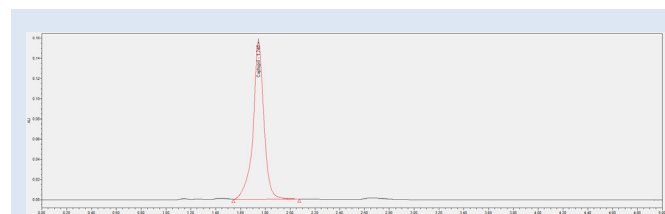


Figure 3: Chromatogram of Captopril in the test solution using UHPLC.

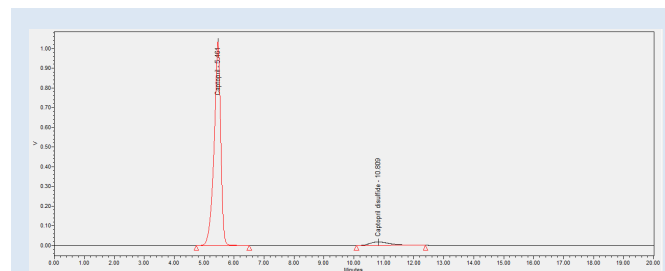


Figure 4: Chromatogram of a well resolved Resolution solution showing Captopril and Captopril disulfide using UHPLC.

RESULTS AND DISCUSSION

During this study trials were conducted in order to find the optimum chromatographic conditions. The objective of this study was to develop a chromatographic method, which is more environmentally friendly compared to an HPLC method, cost effective, yet following all regulations and achieving results according to the standards set out in ICH and USP guidelines. The chromatographic method was set to achieve a peak tailing factor between 0.8 and 1.2, a retention time between 1 to 2 minutes, along with a good resolution of >1.5 . A relative standard deviation of $\geq 2.0\%$ and a similarity factor between 'standard 1' and 'standard 2' injections between 98.0 and 102.0%. The peaks for Captopril molecule and its dimer, Captopril disulfide were well defined, resolved and free from tailing. The elution order was Captopril at 1.744 minutes and Captopril disulfide at 2.657 minutes for the UHPLC method developed, whilst for the USP HPLC method the elution order is Captopril at 5.461 minutes and Captopril disulfide at 10.809 minutes (Table 1).

Table 1: System suitability parameters.

System suitability parameters		
Parameter	Limit	Results
Resolution of CPT with CPT-DIS	>1.5	4.3
%RSD on 6 injections of STD 1	≤ 2.0%	0.5%
Tailing factor	0.8-1.2	0.9
Bracketing check	98.0-102.0%	99.5-99.8%
Similarity factor STD 1 and STD 2	98.0-102.0%	100.9%

Running a single batch for analyses using the HPLC and UHPLC methods consist of 17 runs which includes; 2 blank injections at the beginning, 6 injections of 'standard solution 1', 2 injections of 'standard solution 2' followed by a single resolution injection. Then another 2 'standard solution 1' injections, batch sample in duplicate and another 2 'standard solution 1' injections to close off the bracketing of standards. HPLC method: Running the above sample set, a mobile phase usage of 340 mL for the analysis of one batch. Developed UHPLC method: Running the same sample set using the UHPLC method parameters, a mobile phase usage of 8.5 mL for the analysis of one batch. On another note, the Captopril USP monograph declares that Captopril solutions have a stability of 8 hours, meaning that when using the conventional USP HPLC method only a total of 24 injections i.e., a maximum of 4 batch runs can be conducted when excluding the 2 blank injections at the beginning of the run. Whilst with the newly developed UHPLC method it is possible to run a total of 96 injections i.e., a maximum of 31 batches in a single 8-hour sample set, excluding the 2 blank injections at the beginning of the run. This clearly shows that the new greener UHPLC method is far more efficient and requires a much less solvent to carry out the quantification analysis (Tables 2 and 3).

Table 2: HPLC and UHPLC methods comparison.

Comparing HPLC vs. UHPLC method when conducting one batch of quantification analysis			
Parameter	HPLC method	UHPLC method	Results in %
Run time (minutes)	20	5	UHPLC is 75% more efficient when compared to HPLC method
Mobile phase used (mL)	340	8.5	97.5% less mobile phase was used for the UHPLC analysis

Table 3: Batch runs times for HPLC and UHPLC methods.

Batch runs that can be conducted within 8 hours for stability tests		
HPLC method	UHPLC method	Percentage
4	31	775% more batches for the UHPLC method

Method validation results

Specificity:

The specificity tests, for any interference found in the excipients (placebo), mobile phase, blank, and degradation impurity with the main analyte Captopril. As chromatograms were over-layered together the test is said to comply because there was no interference from excipients and degradant impurity Captopril disulfide with the main analyte of interest, Captopril, retention time 1.744 minutes (Fig. 5).

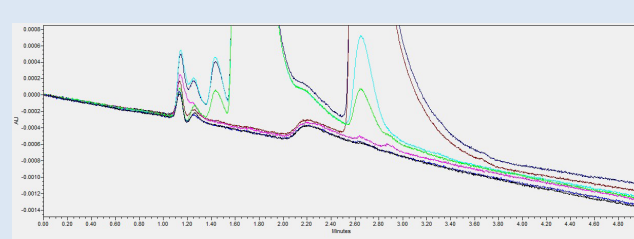


Figure 5: An overlay chromatogram showing the specificity test. Note: (—) Blank Inj:3, (—) Mobilephase, (—) STD Solution, (—) Placebo, (—) CPT Disulfide 0.05mg/ml, (—) Recovery Solution (Sample Spiked).

Linearity and range:

The linearity was determined by injecting a series of 5 standard solutions whose concentrations were at 0.05 mg/mL, 0.10 mg/mL, 0.15 mg/mL, 0.2 mg/mL (Target concentration, 100%), and 0.25 mg/mL. As shown in Fig. 6 a plot of area of response (UNITS) vs. linearity concentration levels in mg/mL was plotted. The correlation coefficient (R), range and the residual sum of squares graph were determined. All values comply with the ICH and USP guidelines. The values of the correlation coefficient were close to unity which indicates a good linearity and shows that the developed UHPLC method has a high sensitivity (Figs. 6 and 7) (Table 4).

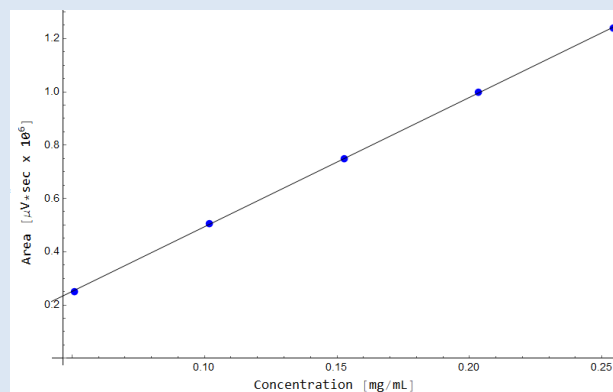


Figure 6: Graph of Area [$\mu\text{V} \cdot \text{sec} \times 10^6$] against Concentration [mg/mL].

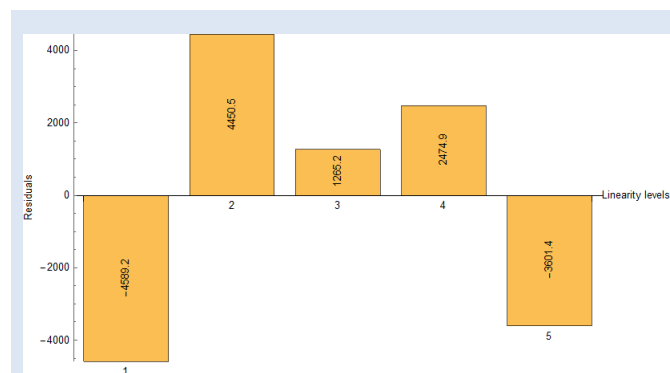


Figure 7: Residuals scattered plot.

Table 4: Results gained compared to ICH guidelines.

Parameter	ICH guidelines limit	Results
Correlation coefficient (R)	>0.995	0.99995
The residual sum of squares should be randomly distributed and show no bias		Complies, distributed and show no bias
Residual Sum of Squares (RSS)		61563649.90
Range (Upper and lower intervals)		25 to 125%

Accuracy:

The accuracy of a method was determined by performing a recovery experiment. Concentration levels and preparations were done according to the ICH and USP guidelines. For 70% and 130% concentration levels, 3 preparations were done, and for the 100% concentration levels 6 preparations were prepared and injected. The percentage recovery i.e. the actual concentration divided by the theoretical concentration multiplied by a 100 was calculated. The percent recovery of each individual should be >98.0%. The % RSD was determined for each concentration level where the limit is <2.0%. None of the individual samples were less than 98.0% and the % RSD was found to be less than 2.0% for all concentration levels. This indicates a good repeatability and reliability of the proposed UHPLC method (Table 5).

Precision and intermediate precision:

The precision testing was performed by injecting six determinations of batch sample at a concentration level of 100% (0.2 mg/mL). It was imperative that the limits for the precision for individual values fall between 90.0 to 110.0% of the labelled amount and the % RSD on the six injections should be <2.0%. The intermediate precision tests were conducted by a different analyst, on different equipment and on a different day. These results were gathered from 6 injections of precision and 6 of intermediate combined together. The limits for intermediate precision is that of <2.0% (Table 6).

CONCLUSION

In this study we have developed a new method for quantifying captopril using UHPLC. The study shows that this may be a viable way forward for the pharmaceutical industry, given the increasing emphasis on environmental responsibility and efficient and cost-effective use of resources. This validation study of the new method was carried out according to the ICH and USP guidelines.

ACKNOWLEDGEMENTS

We would like to thank Combino Pharm Malta Ltd. for their invaluable contributions to this study.

AUTHOR'S CONTRIBUTIONS

GZ designed and built the apparatus and did the measurements and data analysis together with RC and MP. The manuscript was written by GZ and MP. RC supervised the project. All authors have read and approved the final manuscript.

REFERENCES

- Vidt DG, Bravo EL, Fouad FM. Captopril. *N Engl J Med* 1982; 306:214-19
- Bojarska J, Maniukiewicz W, Fruziński A, et al. Captopril and its dimer captopril disulfide: Comparative structural and conformational studies. *Acta Crystallogr C Struct Chem*. 2015 Mar 1;71(3):199-3.
- Amar M, Lam SW, Faulkenberg K, Perez A, Tang WW, et al. Captopril versus hydralazine-isosorbide dinitrate vasodilator protocols in acute decompensated heart failure patients transitioning from sodium nitroprusside. *J Card Fail*. 2021 May 26.
- Sigit ML, Rahman MA, Utamayasa IK. Comparison of the effects of giving captopril and valsartan in cases of heart failure for people with congenital heart disease. *Eurasia J Biosci*. 2020 Sep 23;14(2):3463-7.
- Brogden RN, Todd PA, Sorokin EM. Captopril. *Drugs*. 1988 Nov;36(5):540-600.
- Captopril-Digoxin Multicenter Research Group. Comparative effects of therapy with captopril and digoxin in patients with mild to moderate heart failure. *JAMA The Journal of American Medical Association*. 1988 Jan 22;259(4):539-44.
- Vasconcelos DL, Silva CB, Teixeira AM, et al. Structural and spectroscopic analysis of the Cis-Trans isomers of the captopril in the gaseous and aqueous phases. *J Mol Struct*. 2021 Jun 11:130872.
- Timmins P, Jackson IM, Wang YC. Factors affecting captopril stability in aqueous solution. *Int J Pharm*. 1982 Aug 1;11(4):329-36.
- Hillaert S, Van den Bossche W. Determination of captopril and its degradation products by capillary electrophoresis. *J Pharm Biomed Anal*. 1999 Oct 1;21(1):65-73.
- Cârje AG, Balint AL, Ion V, et al. HPLC-UV method approach for the analysis and impurity profiling of captopril. *Stud. Univ. Babeş-Bolyai Chem*. 2019 Jan 1;64:231-42.
- National Institutes of Health and others, "Livertox database," 2018.
- United States Pharmacopeia and National Formulary. United States Pharmacopeial Convention. 2021.
- Cielecka-Piontek J, Zalewski P, Jelińska A, et al. UHPLC: the greening face of liquid chromatography. *Chromatographia*. 2013 Nov;76(21):1429-37.
- Gałaszka A, Migaszewski Z, Namieśnik J. The 12 principles of green analytical chemistry and the significance mnemonic of green analytical practices. *Trends Analyt Chem*. 2013 Oct 1;50:78-84.
- Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005 Nov;1(20):05.
- Nally JD. Good manufacturing practices for pharmaceuticals. CRC Press; 2016 Apr 19.