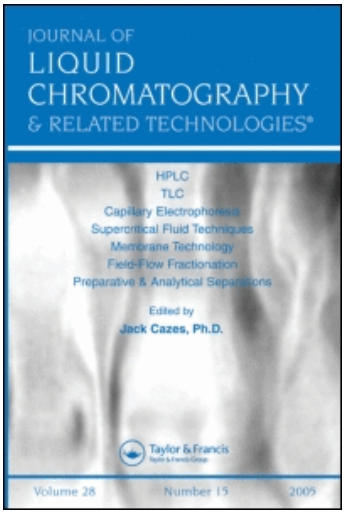

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Ahmed, S. , Rizk, M. , Belal, F. , Ibrahim, F. and Sheribah, Z. A.(2006) 'Stability-Indicating HPLC Method for Captopril Through Pre-Column Derivatization with Pd(II)', Journal of Liquid Chromatography & Related Technologies, 29: 4, 521 — 532
To link to this Article: DOI: 10.1080/10826070500478999
URL: http://dx.doi.org/10.1080/10826070500478999

Journal of Liquid Chromatography & Related Technologies®, 29: 521–532, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070500478999

Stability-Indicating HPLC Method for Captopril Through Pre-Column Derivatization with Pd(II)

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Abstract: An accurate, sensitive, and specific reversed phase high performance liquid chromatographic (HPLC) method was developed for the determination of captopril (CPT). The proposed method depends on the complexation ability of CPT with Pd(II) using a pre-column derivatization technique. Reversed phase chromatography was conducted using a Luna 5 μ phenyl-hexyl [250 × 4.6 mm i.d.] stainless steel column at ambient temperature with specific photometric-detection at 380 nm. A solution containing 0.025% w/v of Pd(II) chloride in a mixture of acetonitrile-methanol-water containing 10 mM Britton-Robinson buffer [BRb] of pH 4.0 and 0.25 M KCl solution [1 : 4 : 5 v/v/v] was used as a mobile phase,which was pumped at a flow rate of 0.75 mL min^{-1}. The method showed excellent linearity in the range 2–32 $\mu g \cdot mL^{-1}$ with a limit of detection [S/N = 2] of 0.18 $\mu g \cdot mL^{-1}$ [8.28×10^{-7} M]. The suggested method was successfully applied for the analysis of CPT in bulk, single and combined dosage forms with average % recoveries of 99.66 ± 0.70, 99.63 ± 0.68, and 99.32 ± 0.77, respectively. Hydrochlorothiazide, which is frequently co-formulated with CPT, did not interfere. The proposed method could be used successfully for the determination of CPT in the presence of its disulphide dimer in pure form and in pharmaceutical preparations. This finding proved the stability-indicating character of the method. The results obtained were favorably compared to those obtained by the official method.

Keywords: Stability-indicating, HPLC, Captopril, Pre-column derivatization, Pd(II)

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INTRODUCTION

Captopril (CPT) is an orally active antihypertensive agent.[1] It can be used alone or in combination with thiazide diuretics or digoxin for patients with moderate heart failure.[2] It acts as a potent and specific inhibitor of angiotensin converting enzyme, one of the most important components in the renin-angiotensin system.[1,2]

There is a need to quantify and measure the therapeutic concentrations of CPT. Unfortunately, the properties of the –SH group which make it an important biochemical moiety also cause several difficulties in its reliable estimation.[3]

A number of approaches to the assay of CPT now exists. The USP[4] and BP[5] recommended HPLC and titrimetric methods, respectively, for its determination. A comprehensive monograph dealing with the analytical profile of the drug up to 1982 has been published.[6] Several methods were later reported for the quantitative determination of CPT, either per se in dosage forms, or in biological fluids. These include: titrimetric,[7,8] spectrophotometric,[9–15] spectrofluorometric,[16,17] voltammetric,[18–20] HPLC,[3,21–26] methods, and flow injection analysis.[27,28]

Pd(II)-CPT complex formation was used for the spectrophotometric and atomic absorption determination of CPT.[10–12] Until now, there are no reports on the use of Pd(II) ion as a derivatizing reagent for the HPLC determination of CPT.

The aim of the present work was to develop a simple HPLC method for the quantitation of CPT in the presence of its disulphide dimer in pure materials and pharmaceutical preparations in single or combined formulations. Pre-column derivatization with Pd(II) was adopted and the results obtained were promising.

EXPERIMENTAL

Materials and Reagents

Captopril (CPT) was kindly provided by Squibb and Sons, Princeton, NY, USA, and was used as received. Tablets containing CPT were obtained from commercial sources in the local market.

Palladium chloride (Merck, Darmstadt, Germany). An 0.1% aqueous solution was prepared by dissolving 0.1 g in 5 mL of deionized distilled water containing 0.1 mL of concentrated hydrochloric acid and warming the mixture using a water-bath. The solution was cooled and diluted with water in a 100 mL volumetric flask.

Acetonitrile and methanol, HPLC grade, Hipersolv. (Merck, Darmstadt, Germany).

A stock solution containing 1 mg · mL^{-1} of CPT was prepared in distilled water. This solution was found to be stable for at least two weeks without alteration when kept in the refrigerator.

Working standard solutions were prepared daily from the stock solution by serial dilutions with water to contain 20–320 μg · mL^{-1}. These solutions remain stable during the actual time of the analysis within the same day.

Instrumentation and Chromatographic Conditions

Chromatographic analyses were carried out using E. Merck Hitachi Chromatograph L-7100 injector valve with a 20 μL loop and an E. Merck Hitachi L-7400 UV detector (E. Merck, Darmstadt, Germany), set at 380 nm. Retention times, peak areas and UV-spectra were recorded on an E. Merck Hitachi L-7500 integrator. The study of the recommended procedure was performed on a stainless steel column [250 × 4.6 mm], 5 μm Luna phenyl-hexyl column combined with guard column [E. Merck, Darmstadt, Germany]. The analysis was achieved isocratically using a mobile phase containing 0.025% w/v Pd(II) chloride in a solvent consisting of acetonitrile-methanol-water containing10 mM Briton Robinson buffer [BRb of pH 4] and 0.25 M KCl solution [1 : 4 : 5 v/v/v].The eluent was filtered through 0.45 μm membrane filter [Gelman Instrument Co.], degassed using E. Merck Solvent L-7612 degasser and pumped at a flow rate of 0.75 mL · min^{-1} at ambient temperature.

Construction of Calibration Curve

Aliquots of the standard solution containing 20–320 μg · mL^{-1} of CPT were transferred into a series of 10 mL volumetric flasks and diluted with the mobile phase containing 0.025% Pd(II) to the mark. 20 μL aliquots were injected (triplicate) and eluted with the mobile phase under the reported chromatographic conditions. The area under the curve was plotted against the concentration to obtain the calibration graph. Alternatively, the corresponding regression equation was derived.

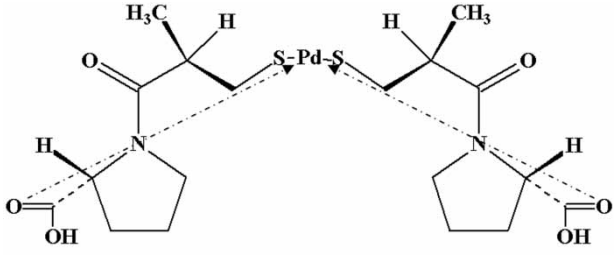
Procedure for Tablets

Twenty tablets were weighed and pulverized. An accurately weighed amount of the powder equivalent to 100 mg of CPT was extracted with 3 × 25 mL of methanol by sonication for 5 min. The extract was filtered into a 100 mL volumetric flask, completed to the mark with the same solvent, and mixed well. A working solution of 10 μg · mL^{-1} was prepared by suitable dilution. The procedure in the preceeding section was applied. The nominal contents

of the tablets were calculated using either the calibration graph or using the corresponding regression equation.

RESULTS AND DISCUSSION

The spectrophotometric studies confirmed the instantaneous formation of Pd(II)-CPT complex with a molar ratio of 1 : 2.[10,11] The following structural formula is proposed for the reaction product:



Proposed structural formula of Pd(II)-CPT complex

The complex formation can be achieved immediately, especially after the establishment of the optimum conditions. These facts potentiate the adaptation of a pre-column derivatization LC method in an attempt to develop a sensitive, specific, stability-indicating method for the analysis of CPT in bulk and tablet formulations.

Chromatographic Performance

A well-defined symmetrical peak was obtained upon measuring the UV-response of the eluate under the optimum experimental parameters (Fig. 1). A thorough experimental investigation was conducted and can be summarized as follows.

Column

Three types of columns were utilized for performing the investigations, including: Maxil 5 μ C_{18} column (250 × 4.6 mm), Luna 5 μ phenyl-hexyl column (250 × 4.6 mm), and Bondclone 10 μ phenyl column (300 × 3.9 mm). The experimental studies revealed that the phenyl-hexyl column was the column of choice, since the complex was eluted in the form of a highly symmetrical peak and at a reasonable time (5.52 min), as shown in Fig. 1. In the case of the Maxil column, the peak was eluted after a longer retention time; additionally, the eluted peak was small and not symmetrical. In the case of the phenyl column, the peak was early eluted and overlapped with the solvent front.

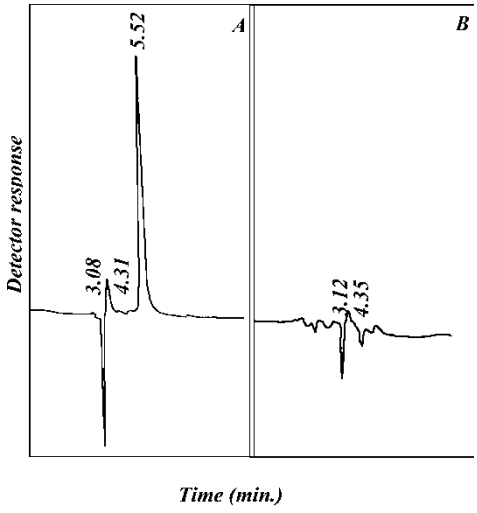


Figure 1. Typical chromatograms of (A) Pd(II)-CPT complex ($10\,\mu g \cdot mL^{-1}$ CPT, 5.52 min); (B) captopril disulphide ($50\,\mu g \cdot mL^{-1}$) in presence of 0.025% Pd(II).

Detection

The formed complex was detected at 380 nm. Although there were two more peaks at 246 and 315 nm, the measurements were performed at 380 nm to avoid the interference resulting from the absorbance of excess of Pd(II) at the same wavelengths.

Mobile Phase

Different mobile phase systems were investigated to achieve the highest sensitivity with reasonable retention time. Mobile phase consisting of acetonitrile or methanol with water at different ratios were investigated. The results obtained showed that Pd(II) in mixtures of methanol and water was reduced to black Pd°. Also, the peak of the formed complex was eluted overlapped with the solvent front when a mixture of acetonitrile and water was used.

Mobile phases of acetonitrile, methanol, and water, in different ratios, were used to investigate the effect of the organic modifier on the development and elution of the peak. A mobile phase consisting of acetonitrile-methanol-water [1 : 4 : 5, v/v/v] was chosen, since the formed complex was separately eluted from the solvent front but the eluted peak still had low sensitivity. So, the effect of addition of buffer system instead of water ought to be investigated.

Effect of Buffer Type and pH

The formed peak, using mobile phase containing 50% (v/v) organic modifier and 50% water was separately eluted from the solvent front at a reasonable retention time; however, it was unstable and of low sensitivity. So, the effect of addition of buffer instead of water was investigated. Firstly, three different types of buffers were used: phosphate, acetate, and BRb at pH 4 (Table 1). From this study, BRb was selected, since the eluted peak was the most symmetrical and gave the highest peak area.

Further, BRb series, 0.08 M solutions, covering the pH range of 3–6, were used to investigate its effect on the stability, sensitivity, and elution time of the formed complex. It was found that pH 4 was the optimal pH for this purpose, as the calculated values of K′ and T were 0.54 ± 0.02 and 1.0 ± 0.1, respectively (Table 1).

Effect of Ionic Strength

Increasing the ionic strength of the buffer over the range 4–24 mM (final concentration) was found to affect the sensitivity, retention time, and the baseline of the eluted peak. It was revealed from this study that 10 mM BRb (125 mL of 0.08 M/100 mL mobile phase) was the optimal concentration (Fig. 2). Also, the effect of KCl as a measure of ionic strength was investigated (Table 2). The study revealed that the presence of KCl was essential to increase the stability of the formed complex since, in the absence of KCl, the formed complex has a very small peak area upon changing the concentration of KCl over the range 0.125–1 M. It was found that the optimal concentration was 0.25 M.

Flow Rate

The effect of flow rate ($0.5–2\,mL \cdot min^{-1}$) on the formation and elution of Pd(II)-CPT complex was studied; a flow rate of $0.75\,mL \cdot min^{-1}$ was optimal for elution within a suitable retention time.

Table 1. Effect of buffer type and pH on HPLC performance data of Pd(II)-captopril complex

Buffer type	pH	K′	T	N	HETP	R
Acetate	4	0.11	1.05	1332	0.188	0.86
Phosphate	4	0.47	1.64	601	0.416	2.48
BRb	3	0.59	2.1	986	0.254	2.98
	4	0.54	1.00	2218	0.113	3.59
	5	0.34	1.2	1308	0.191	1.32
	6	—	—	—	—	—

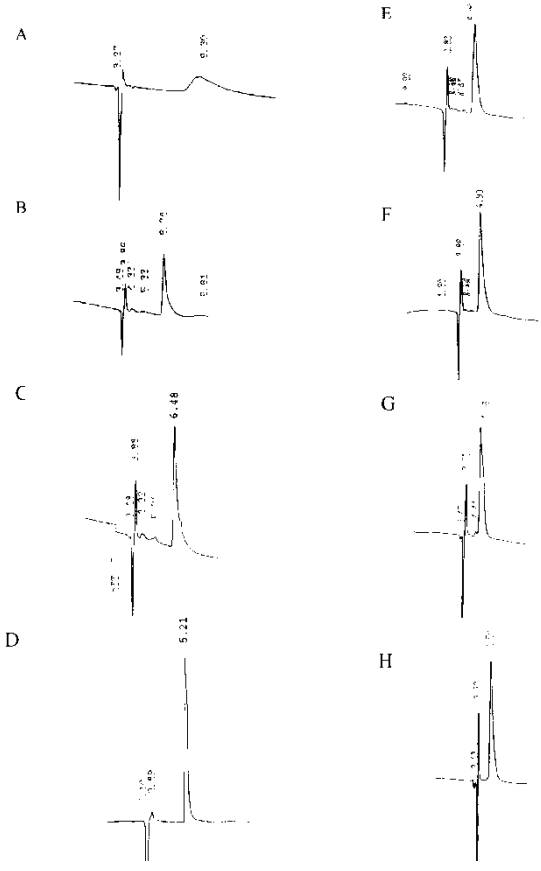


Figure 2. Effect of buffer concentration on the formation and stability of Pd(II)-CPT complex using BRb (pH 4) of (A) 0 mM, (B) 4 mM, (C) 8 mM, (D) 10 mM, (E) 12 mM, (F) 16 mM, (G) 20 mM, (H) 24 mM.

Table 2. Effect of potassium chloride on the HPLC performance data of Pd(II)-captopril complex

KCl (M)	Peak height	t_r (min)	K′	T	N	HETP	R
0	1708	9.3	—	—	—		
0.125	7963	7.36	1.24	2.20	9.60	0.260	3.86
0.25	15430	5.52	0.54	1.0	2218	0.113	3.59
0.5	10665	4.80	0.48	1.10	1480	0.169	2.58
0.75	8500	4.53	0.52	1.05	1322	0.189	1.96
1.0	6852	4.46	0.36	1.02	1162	0.215	1.22

K′= Capacity factor; T = tailing factor; N = number of theoretical plates; HETP = height equivalent to theoretical plate; R = resolution factor.

Effect of Pd(II) Concentration

The effect of the concentration of Pd(II) (0.005–0.05%) on the complete formation of Pd(II)-CPT complex was investigated. 0.025% (w/v) was the optimal concentration in the mobile phase. It is essential to prevent the hydrolysis of the formed complex on the column.

Validation

Linearity

The calibration curve of the Pd(II)-CPT complex vs. peak area was found to be rectilinear over the range 2–32 $\mu g \cdot mL^{-1}$. Linear regression analysis of the data gave the following equation:

$$P = 176 + 5308C \quad (r = 0.99994)$$

where, C is the concentration of CPT in $\mu g \cdot mL^{-1}$ and P is the peak area.

Statistical analysis of the data gave small values of the standard deviations of residuals, ($S_{x/y}$) 4.4×10^2, standard deviation of slope (S_b) 14.39, standard deviation of intercept, (S_a) 2.11×10^2 and % relative error, (%Er) 0.21%. These figures point out to the high precision of the method.[29]

Limits of Quantitation (LOQ) and Limit of Detection (LOD)

CPT can be quantified under these conditions with LOQ of 0.6 $\mu g \cdot mL^{-1}$ and LOD at (S/N = 2) was found to be 0.18 $\mu g \cdot mL^{-1}$ (8.28×10^{-7} M).

Accuracy and Precision

As shown in Table 3, the repeatability of the assay was found to be within 0.45–0.79% (n = 5) at 5, 15, and 25 $\mu g \cdot mL^{-1}$. The reproducibility of the assay at the same concentration levels was found to be within 0.46–0.77% (n = 3). The accuracy, calculated as % error, was found to be within 0.20–0.35% for both the intra-day (n = 5) and inter-day (n = 3) measurements.

Robustness

The assay parameters were optimized in order to increase the robustness of the method. The effect of organic modifier, type and pH of buffer, ionic strength, and Pd(II) concentration on K′, T, N, HETP, and R values were comprehensively investigated (Tables 1, 2).

Table 3. Accuracy and precision data for captopril using the proposed method

		Concentration of captopril ($\mu g \cdot mL^{-1}$)		
Regimen	Parameters	5	10	15
Intra-day		97.98	98.32	99.12
		98.88	94.12	98.52
		100.21	99.45	99.21
		99.36	100.12	96.64
		99.99	99.85	99.99
	Mean ($\bar{x}$)	99.26	99.37	99.40
	$\pm$S.D.	0.785	0.701	0.451
	%RSD	0.791	0.705	0.454
	%Er	0.354	0.315	0.203
Inter-day	1st day	98.28	99.02	99.98
	2nd day	99.32	99.98	99.28
	3rd day	99.92	99.46	100.02
	Mean ($\bar{x}$)	99.25	99.49	99.48
	$\pm$S.D.	0.767	0.481	0.455
	%RSD	0.772	0.483	0.457
	%Er	0.346	0.216	0.204

Each result is the average of three separate determinations.

Applications

Application of the Proposed Method to the Analysis of the Studied Drug in Bulk

Applying the method to pure sample of CPT proved the validity of the method. The results show good agreement with those obtained by an official method,[4] regarding accuracy and precision, as revealed by the t-test and the F-test, respectively.[29]

Application of the Proposed Method to the Analysis of the Studied Drug in Tablet Formulations

The proposed method was successfully applied to the assay of CPT, both in single tablet formulation and co-formulated with other drugs. As shown in Table 4 the presence of hydrochlorothiazide in combination with CPT does not interfere with its accurate quantitation, since Pd(II)-hydrochlorothiazide complex was not detected at 380 nm.

Table 4. Assay of captopril and its commercial tablets using the proposed and official methods

	Recovery (%)	
Material	Proposed method	Official method[4]
1- Captopril (raw material)	98.79	
	99.82	
	99.18	
	100.56	
	98.38	
	99.45	
	100.21	
	99.99	
	100.01	
	100.24	
$\bar{x} \pm$ S.D.	99.66 $\pm$ 0.697	100.12 $\pm$ 0.984
Student's t-test	1.275	(2.179)
F-test	1.99	(6.104)
2-Capoten tablets[a]	99.86	
(25 mg captopril/tablet)	99.62	
	98.46	
	100.21	
	99.98	
$\bar{x} \pm$ S.D.	99.63 $\pm$ 0.684	100.12 $\pm$ 0.982
Student's t-test	1.08	(2.31)
F-test	1.655	(6.39)
3-Capozide tablets[a]	97.98	
(50 mg captopril + 25 mg hydrochlorothiazide/tablet)	99.12	
	98.58	
	99.38	
	100.06	
$\bar{x} \pm$ S.D.	99. 32 $\pm$ 0.766	100.46 $\pm$ 0.929
Student's t-test	2.486	(2.31)
F-test	1.470	(6.39)

Each result is the average of three separate determinations.

[a]Products of Bristol-Myers Squibb Egypt.

Figures in parentheses are the tabulated t and F values, respectively at $p = 0.05$.

Stability Indication of the Method

Captopril disulphide dimmer, which is reported to be formed during the analysis or in the raw materials, did not interfere with the assay under the

studied conditions. Only one peak, corresponding to Pd(II)-CPT complex, was eluted (Fig. 1). It lost the ability to form complexes, since the site of complexation was logged. This finding confirmed the stability indicating character of the method.

CONCLUSION

The proposed method is characterized by high sensitivity for the analysis of CPT; concentrations as low as $2\,\mu g \cdot mL^{-1}$ could be accurately quantified with LOD at $(S/N = 2)$ of $0.18\,\mu g \cdot mL^{-1}$ (8.28×10^{-7} M). Furthermore, it is stability indicating. Also, the method is specific, since it permits the separation and quantitation of CPT when co-formulated with hydrochlorothiazide.

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Received September 10, 2005
Accepted October 15, 2005
Manuscript 6715