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Utilization of carbon disulphide for the analytical determination of betahistine hydrochloride and captopril in their pharmaceutical preparations

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Abstract

Spectrophotometric, atomic absorption spectrometric and high performance liquid chromatographic (HPLC) procedures have been developed for the determination of betahistine hydrochloride and captopril. The three procedures are based on the reaction of the drugs with carbon disulphide in alkaline medium with the formation of the dithiocarbamate or the trithiocarbonate derivative of betahistine (BHT) and captopril (CAP), respectively, then subsequent chelation with divalent metals. The absorbance measurement of the formed chelates or of the metal moiety of chelates was used as the basis for the spectrophotometric and atomic absorption spectrometric determinations. The formed complexes have been used as pre-column derivatizing procedure for the HPLC determination of the two drugs. The different experimental conditions were optimized. The calibration graphs were linear over the applicable concentration ranges. The proposed procedures were applied successfully for the determination of the two investigated drugs in their tablets dosage form.

Keywords: Atomic absorption spectrometry; Betahistine; Captopril; Chelates; Dithiocarbamate; High performance liquid chromatography; Spectrophotometry; Trithiocarbonate

1. Introduction

Betahistine (BHT) (*N*-methyl-2-(2-pyridyl)-ethylamine) is a histamine analogue indicated in Menière's disease [1]. Betahistine mesilate is official in BP 98 [2] and EUP 97 [3]. Very few publications have been described for the drug determination. In its pharmaceutical dosage forms, BHT was analyzed colorimetrically through its reaction with chloranil in alkaline medium [4] or by a high performance liquid chromatographic (HPLC) technique [5].

Captopril (CAP) (1-[(2S)-3-Mercapto-2-methyl-propionyl]-L-proline) is an angiotensin converting enzyme inhibitor, used for management of hypertension and congestive heart failure [6]. The drug and its tablet are official in the USP 23 [7]. The analytical profile of CAP has been reviewed [8].

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Several analytical procedures have been described for its determination in pharmaceutical formulations. These include amperometry [9], polarography [10], gas chromatography [11] and fluorometry [12]. Captopril has been determined spectrophotometrically using absorbance difference (ΔA) [13] and derivative spectro-photometry [14]. The literature revealed numerous colorimetric methods, which are based on complexation [15–18] and Redox reaction [19,20]. CAP has been analyzed also using HPLC technique [21–23].

In basic media, amines (primary or secondary) and mercaptans are known to react readily with carbon disulphide to yield the corresponding dithiocarbamate (DTC) and trithiocarbonate (TTC), respectively, which can form complexes with many metal ions. Metal–DTC complexes have been used in the spectrophotometric analysis of some secondary amines [24], through the formation of their dithiocarbamate derivatives followed by the complexation with copper(II). However, this sensitive reaction has found little application to the analysis of pharmaceutically important compounds viz., determination of β-adrenergic blocking drugs [25–27] and ephedrine hydrochloride [28,29]. Some secondary amino-

Table 1
Optimum conditions for the color development

Item	(BHT)		(CAP)		
	Copper(II)	Nickel(II)	Cobalt(II)	Palladium(II)	Nickel(II)
Volume of carbon disulphide (ml)	1.5	1.5	3	3	3
Volume of ammonia buffer (ml)	2	2	2	2	2
Volume of metal ion (ml)	2.5	2.5	3	2	3
Volume of acetic acid (ml)	3	3	3	4	4
Wavelength (nm)[a]	430	390	390	445	505
Wavelength (nm)[b]	324.8	232	240.7	–	232

[a] For spectrophotometric measurements.
[b] For atomic absorption spectrometric measurements.

Table 2
HPLC conditions[a] for the quantitation of metal–betahistine–dithiocarbamate and metal–captopril–trithiocarbonate complexes

Item	Mobile phase	Flow rate (ml min^{-1})	λ (nm)	Retention time R_t, min)
Betahistine–dithiocarbamate				
Copper(II)	Acetonitrile	4	300	0.802
Nickel(II)	Acetonitrile	4	300	1.803
Captopril–trithiocarbonate				
Cobalt(II)	Acetate buffer–acetonitrile (70:30)	3	390	1.087
Nickel(II)[b]	Acetate buffer–acetonitrile (80:20)	2	450	8.271
Palladium(II)	Acetate buffer–acetonitrile (70:30)	3	364	1.010

[a] All of the chromatographic runs were carried out at ambient temperature on a 10 μm Hypersil C18 column (250 × 4.6 m I.D.) (Shandon Scientific, Cheshire, UK). The mobile phases were degassed for 15 min in an ultrasonic water-bath and by bubbling helium gas for 10 min before use. The analysis was usually started after passage of 20–30 ml of the mobile phase to reach equilibrium.
[b] Nickel–dithizone complex.

Table 3
Optical characteristics and statistical data of the regression equations for the analysis of betahistine hydrochloride and captopril using the spectrophotometric method

Item	BHT		CAP		
	Copper(II)	Nickel(II)	Cobalt(II)	Palladium(II)	Nickel(II)
Beer's law limit (μg ml^{-1})	6–14	10–28	16–48	40–120	30–80
Apparent molar absorptivity (l mole^{-1} cm^{-1})	1.16×10^{4}[a]	5.90×10^{3}[a]	3.00×10^{3}	1.85×10^{3}	1.30×10^{3}
Sandell's sensitivity (μg ml^{-1} per 0.001 A)	1.64×10^{-2}	3.48×10^{-2}	6.90×10^{-3}	0.131	0.164
Regression equation (A):					
Intercept (a)	4.71×10^{-2}	9.57×10^{-3}	2.02×10^{-2}	-5.75×10^{-2}	5.99×10^{-3}
tS_a[b]	2.42×10^{-2}	2.14×10^{-2}	1.82×10^{-2}	4.27×10^{-2}	1.70×10^{-2}
Angular coefficient (b)	5.55×10^{-2}	2.82×10^{-2}	1.38×10^{-2}	1.19×10^{-2}	6.00×10^{-3}
tS_b[c]	2.44×10^{-3}	1.34×10^{-3}	5.30×10^{-4}	5.17×10^{-4}	2.95×10^{-4}
Correlation coefficient (r)	0.9995	0.9993	0.9996	0.9990	0.9994
Linearity ($S_{b\ rel\ (\%)}$)[d]	1.584	1.943	1.383	2.193	1.769
Variance (S_o^2)	3.68×10^{-5}	4.53×10^{-5}	2.38×10^{-5}	1.42×10^{-4}	1.97×10^{-5}
Detection limit (μg ml^{-1})	0.271	0.541	0.878	3.479	1.838

[a] Calculated on the basis of the molecular weight of the hydrochloride salt of betahistine.
[b] Confidence intervals of the intercept ($P = 0.05$).
[c] Confidence intervals of the slope ($P = 0.05$).
[d] Relative standard deviation of the slope.

Table 4
Statistical data of the regression equations for the betahistine hydrochloride and captopril analysis using AAS method

Item	BHT		CAP	
	Copper(II)	Nickel(II)	Cobalt(II)	Nickel(II)
Concentration range (μg ml^{-1})	1.25–5	2.5–7.5	5–30	8–20
Regression equation (A):				
Intercept (a)	-4.73×10^{-3}	1.42×10^{-3}	3.29×10^{-4}	-9.43×10^{-3}
tS_a	2.03×10^{-3}	2.78×10^{-3}	5.18×10^{-3}	1.52×10^{-3}
Angular coefficient (b)	7.00×10^{-3}	4.68×10^{-3}	1.43×10^{-3}	2.45×10^{-3}
tS_b	6.25×10^{-4}	5.19×10^{-4}	2.55×10^{-4}	1.09×10^{-4}
Correlation coefficient (r)	0.9988	0.9982	0.9983	0.9997
Linearity ($S_{b\ rel\ (\%)}$)	2.805	3.488	4.159	1.396
Variance (S_o^2)	3.67×10^{-7}	6.67×10^{-7}	1.20×10^{-6}	1.09×10^{-7}
Detection limit (μg ml^{-1})	0.238	0.651	2.699	0.371

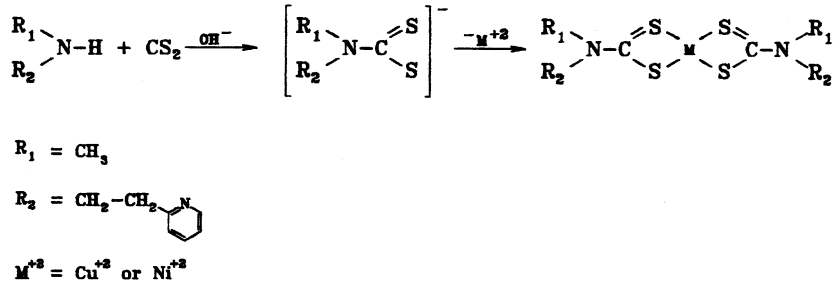
drugs were analyzed by conversion into their copper or nickel dithiocarbamate followed by HPLC determination [30]. No reports have been published for the use of trithiocarbonate–metal complexes for the determination of pharmaceutical compounds.

In view of the fact that few publications have been reported for the analysis of BHT, sensitive and precise method would greatly aid in its determination in bulk sample or pharmaceutical tablets form.. As CAP in aqueous solution undergoes free radical oxidation at its thiol to yield captopril disulphide [8], it is worthwhile to develop a method which depends on the presence of free sulph hydryl group (stability indicated assay).

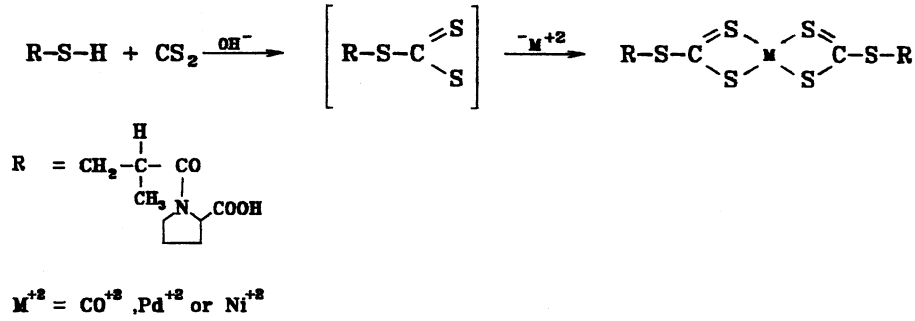
In the present study BHT, as a secondary amine, has been analyzed through the formation of its dithiocarbamate derivative with subsequent chelation with metal ions (copper(II) and nickel(II)). Furthermore, CAP, as sulph hydryl containing molecule, was determined through the formation of its trithiocarbonate derivative with subsequent chelation with cobalt(II), palladium(II) and nickel(II) ions. The absorbance measurement of the formed chelates was used as the basis for BHT and CAP, spectrophotometric and atomic absorption spectrometric determinations.

Table 5
Statistical data of the regression equations for the betahistine hydrochloride and captopril analysis using HPLC method

Item	BHT		CAP		
	Copper(II)	Nickel(II)	Cobalt(II)	Palladium(II)	Nickel(II)
Concentration range (μg ml^{-1})	2–8	5–12	3–16	4–12	4–12
Regression equation (A):					
Intercept (a)	6.20	−2.27	−1.82	−2.40	−4.60
tS_a	28.49	26.23	15.29	20.37	58.02
Angular coefficient (b)	516.09	249.08	36.33	39.85	55.75
tS_b	5.25	2.78	1.49	2.40	0.81
Correlation coefficient (r)	0.9998	0.9999	0.9998	0.9995	0.9996
Linearity ($S_{b\ rel\ (\%)}$)	0.366	0.402	1.290	1.893	1.61
Variance (S_o^2)	58.53	33.13	22.56	22.77	32.23
Detection limit (μg ml^{-1})	3.56×10^{-2}	5.55×10^{-2}	0.360	0.330	0.281



Scheme 1.



Scheme 2.

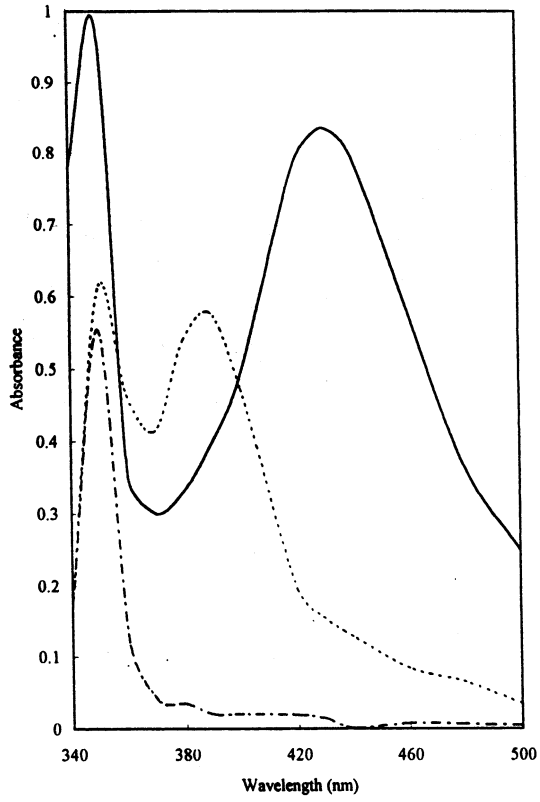


Fig. 1. Absorption spectra of BHT–dithiocarbamate complex with copper (—) and nickel (-----) [BHT = 14, 20 μg ml^{-1} in case of copper and nickel, respectively]. Absorption spectrum of chloroformic blank solution (–.–.–).

The procedure has been further extended to the use of the formed complexes as pre-column derivatizing procedures for the HPLC determination of the two drugs using a diode array detector.

2. Experimental

2.1. Instrumentation

The spectrophotometric measurements were carried out using a Perkin Elmer double beam spectrophotometer Model 550S, attached to a Hitachi recorder model 561, with a fixed slit width of 2 nm and using 1 cm quartz cells. The atomic absorbances were measured on a Perkin Elmer 2380 atomic absorption spectrophotometer equipped with hollow cathode lamp, was operated under the following conditions: air to acetylene ratio was 20: 40, slit width 0.2 nm for nickel and cobalt and 0.7 nm for copper. Air and acetylene flow rates were adjusted at standard conditions. A Hewlett-Packard Model 1090 liquid chromatograph equipped with UV diode-array detector and binary DR 5 solvent delivery system, was used. The chromatograms were recorded and the peaks were quantitated using HP Chem. Station for area integration.

2.2. Materials and reagents

- Betahistine hydrochloride was supplied by Solvay Duphar B.V. (The Netherlands) and certified to contain 99.0%.

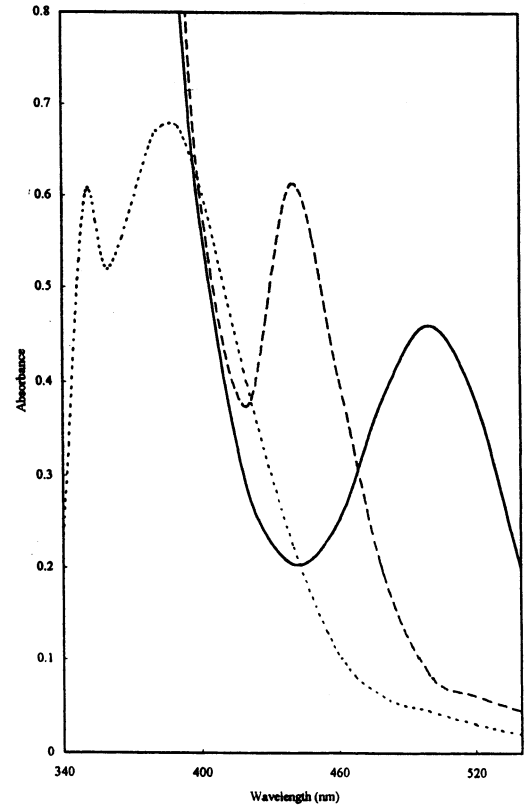


Fig. 2. Absorption spectra of CAP–trithiocarbonate complex with cobalt (. . . . .) or palladium (-----) [CAP = 48, 80 μg ml^{-1} in case of cobalt and palladium, respectively]. Absorption spectrum of nickel dithizone complex equivalent to 80 μg ml^{-1} CAP (—).

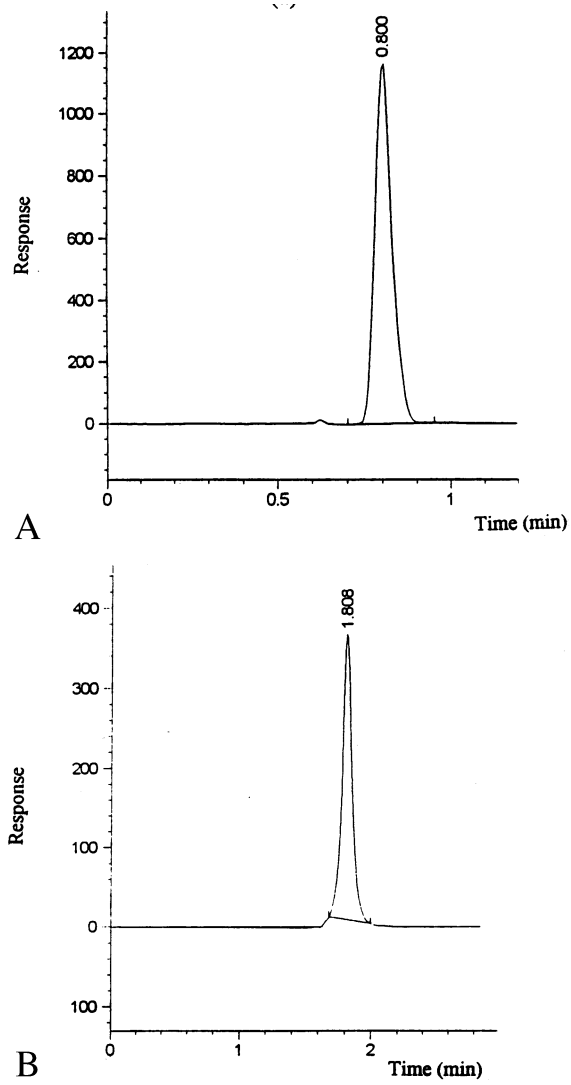


Fig. 3. Typical chromatogram for (A) copper; and (B) nickel–betahistine dithiocarbamate.

- Captopril was gifted from Pharco Pharm. (Alexandria, Egypt), certified and analyzed (USP 23) to contain 99.25%.
- Copper acetate (Fluka, Switzerland) solution was prepared in 2% acetic acid to obtain 0.1% m/v final concentration.
- Cobalt chloride (Rhone Poulenc, France) solution was prepared as 1% m/v aqueous solution.
- Palladium chloride (Sigma, Milwaukee, WI) solution was prepared as 0.1% m/v in water containing 1% v/v concentrated hydrochloric acid.
- Nickel sulphate (M&B, UK) solution was prepared as 0.1 and 1% m/v aqueous solution for the reaction with BHT and CAP, respectively.
- Dithizone (BDH, Poole, UK) solution, was prepared as 0.1% m/v in chloroform.
- Commercial tablets of Betaserc® (Pharco Pharm) under license from Solvay Duphar (The Netherlands)) and Microserc® (Amriya Pharm, Alexandria, Egypt) were labelled to contain 8 mg betahistine hydrochloride.
- Commercial tablets of Capoten® (Bristol-Myers Squibb, (Egypt)), Capotril® (EIPICO (Cairo, Egypt)), Farcopril® (Pharco Pharm) and Lotensine® (Kahira Pharm. Chem., Cairo, Egypt) were labelled to contain 25 mg captopril.

The tablets were purchased from the local market

2.3. Standard solutions

An accurately weighed amount of BHT and CAP was dissolved in water to provide solutions of final concentration of 500 μg ml^{-1} (solution A for spectrophotometric and HPLC procedures) and 125 μg ml^{-1} (solutions B for the atomic absorption procedure).

2.4. Sample solutions

A total of 20 tablets were weighed and powdered. An accurately weighed quantity equivalent to 8 or 25 mg of BHT or CAP, respectively, was transferred into a 50-ml volumetric flask. The flask was half filled with distilled water and shaken for 15 min. The volume was completed to the mark with the same solvent and the produced suspension was filtered. The final solution was further diluted to suit the application of the general procedures.

2.5. General procedure

Aliquots of the standard solutions were pipetted and transferred into series of 60 ml separatory funnels. The specified volume of carbon disulphide (Table 1) was added and followed by 2 ml ammonia buffer (pH 10) (0.1 N NH_4OH and

0.1 N NH_4Cl). The resulted two phase system was shaken for 5 min. The specified volume of metals solutions (Table 1) was added and the solution was acidified with acetic acid (96%) (Table 1). The formed complexes were extracted three times each with 7 ml chloroform.

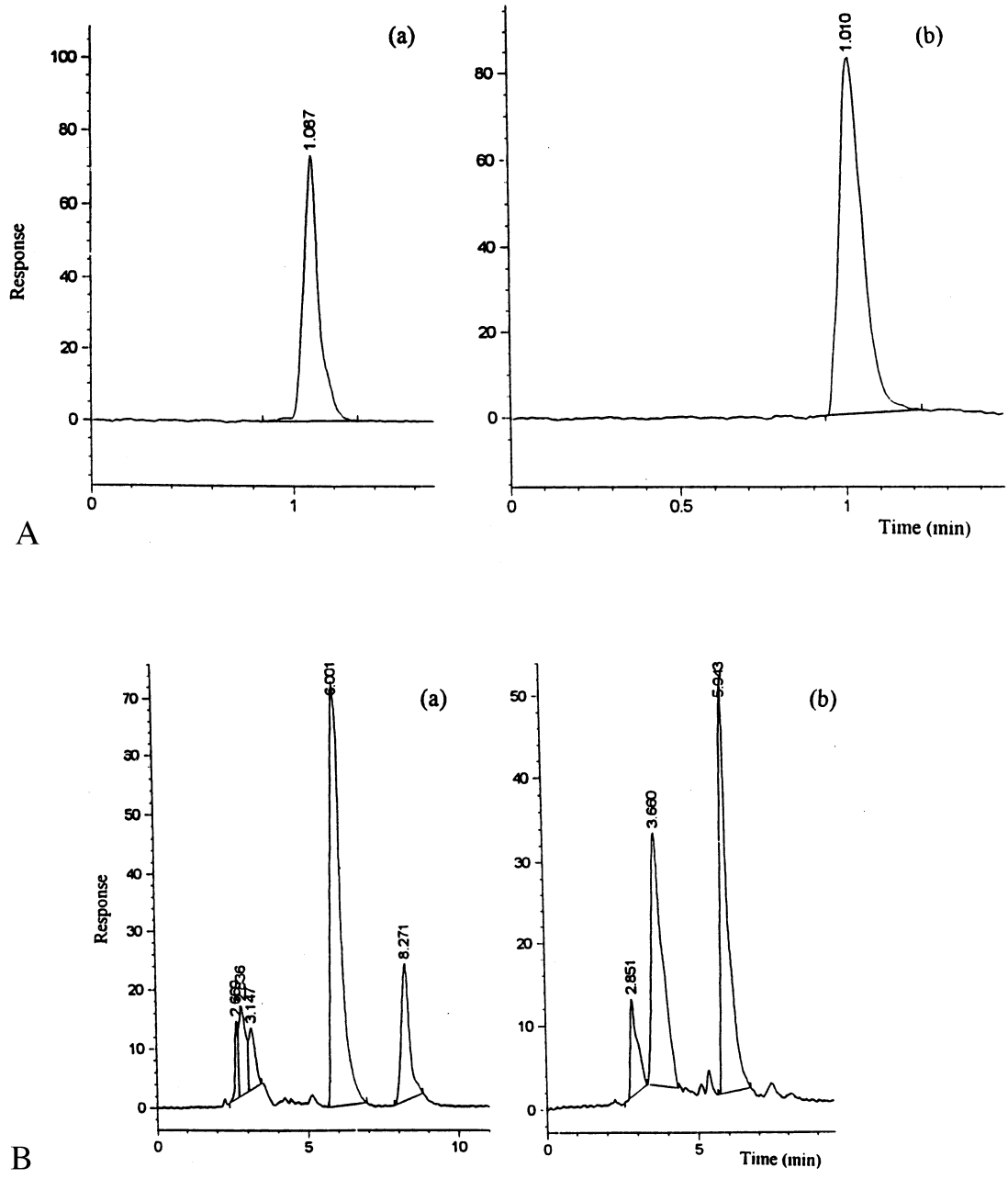


Fig. 4. (A) Typical chromatogram for: (a) cobalt; and (b) palladium–captopril trithiocarbonate. (B) Typical chromatogram for: (a) nickel dithizonate; and (b) Dithizon.

Table 6
Evaluation of the precision of the proposed methods for the determination betahistine hydrochloride and captopril

Item	(BHT)		(CAP)		
	Copper(II)	Nickel(II)	Cobalt(II)	Palladium(II)	Nickel(II)
Spectrophotometry					
SD[a]	0.045	0.167	0.439	1.095	0.996
RSD%[a]	0.484	1.174	2.207	1.941	1.994
AAS					
SD[a]	0.041	0.050	0.338		0.116
RSD%[a]	1.043	0.900	2.350		0.870
HPLC					
SD[a]	0.046	0.091	0.073	0.033	0.191
RSD%[a]	0.819	1.220	0.978	0.558	2.778

[a] Each calculated from solutions containing three different concentrations of the drug and analyzed in five replicates.

2.5.1. For the spectrophotometric procedure

The chloroformic extracts were transferred quantitatively into 25-ml volumetric flasks and the flasks were completed to mark with the same solvent. The absorbance of the resulting solutions were measured at their λ_{max} against similarly treated blank (Table 1).

2.5.2. For the atomic absorption procedure

The combined chloroformic extracts were evaporated to dryness and the residues were dissolved in 10-ml 1 N nitric acid and aspirated into the flame. The absorbance of the metals was measured at their specified wavelengths (Table 1).

2.5.3. For the HPLC procedure

The chloroformic extracts were transferred quantitatively into 25-ml volumetric flasks and the volumes were completed with the same solvent. Before the injection in the HPLC, all solutions were subjected to filtration through a membrane filter of 0.2 μm. A 20 μl of the chloroformic chelate solutions were injected to the HPLC, in triplicate, under the conditions specified in Table 2.

In case of the Ni(II)–CAP–Trithiocarbonate complex and due to its instability, chloroformic solution of dithizone was added to form a more stable Ni(II)–dithizone complex. The dithizone solution was added to both blank and test solutions in dropwise manner until the test solution acquired a faint green color (excess dithizone). The formed Ni(II)–dithizone complex was measured either spectrophotometrically or using HPLC.

2.5.4. Construction of calibration graphs

The calibration graphs were prepared by applying the general procedure to the standard solutions of BHT and CAP in concentration ranges stated in Tables 3–5.

3. Results and discussion

The dithiocarbamate and trithiocarbonate derivative of BHT and CAP, respectively, are rapidly and quantitatively generated by simply adding carbon disulphide to the drug solution in presence of ammonia buffer. The formed derivatives are able to form stable colored chelates with divalent metal ions. The formed complexes were extractable in chloroform according to Schemes 1 and 2

For the quantitation of the formed chelates three procedures were suggested;

(1) Spectrophotometric procedure: The absorbance of the chelates was directly measured at their λ_{max} (Table 1) (Figs. 1 and 2) against similarly treated blank. The CAP–trithiocarbonate–

Ni (II) complex was unstable in order to obtain stable measurable extractable chelate, dithizone was added and the formed Ni(II)–dithizone complex (which is equivalent to the CAP–trithiocarbonate Ni(II) complex) was measured against simultaneously prepared blank at wavelength of 505 nm (Fig. 2).

(2) Atomic absorption spectrometric procedure: The absorbance of the metal moiety of the complexes was determined at their recommended λ_{max}. Unfortunately, we can not determine the CAP trithiocarbonate–palladium(II) complex by this procedure due to the unavailability of palladium lamp.

(3) HPLC procedure: The complexation of the different metals with the dithiocarbamate or trithiocarbonate derivatives provides a simple pre-column derivatizing procedure for quantitation of

Table 7
Determination of betahistine hydrochloride (a) and captopril (b) in their pharmaceutical tablet dosage forms[a]

Commercial product	Copper chelate			Nickel chelate			Palladium chelate		Reference method
	Spectro	AAS	HPLC	Spectro	AAS	HPLC	Spectro	HPLC	Found%
(a) Betaserc tablets									
Recovery (%)[a]	96.96	96.90	97.71	97.15	97.41	97.69			97.29
CV%	0.575	0.794	0.399	0.596	0.893	0.368			0.803
T	0.78	0.80	1.08	0.33	0.22	1.02			
F	1.96	1.03	4.02	1.82	1.24	4.71			
Microserc tablets									
Recovery (%)[a]	99.21	99.10	99.10	99.80	99.21	99.02			99.52
CV%	0.600	0.845	0.478	0.575	0.669	0.777			0.879
T	0.65	0.76	0.93	0.60	0.62	0.96			
F	2.16	1.09	3.41	2.33	1.74	1.29			
(b) Capoten tablets									
Recovery (%)[a]	100.02	100.50	99.86	101.25	100.15	100.38	100.18	101.00	100.69
CV%	1.197	0.596	0.650	0.743	0.654	1.01	0.968	0.522	0.905
T	0.99	0.39	1.66	1.06	1.07	0.51	0.86	0.67	
F	1.73	2.31	1.97	1.47	1.94	1.24	1.13	2.98	
Farcopril tablets									
Recovery (%)[a]	99.09	98.37	98.28	98.84	98.04	98.62	99.04	98.28	98.66
CV%	0.566	0.873	0.667	0.802	0.640	0.812	0.820	1.132	0.984
T	0.86	0.50	0.73	0.325	1.20	7.26×10^{-2}	0.68	0.57	
F	2.99	1.28	2.19	1.50	2.40	1.47	1.43	1.31	
Lotensine tablets									
Recovery (%)[a]	99.29	99.78	99.51	99.52	99.66	98.99	99.41	99.26	98.99
CV%	1.141	1.005	1.159	0.912	0.739	0.688	0.275	0.698	0.526
T	0.55	1.56	0.93	1.15	0.82	5.19×10^{-3}	1.60	0.71	
F	4.73	3.71	4.90	3.03	1.96	1.71	3.63	1.77	
Capotril tablets									
Recovery (%)[a]	102.17	102.20	101.51	102.57	101.03	102.74	101.12	102.57	101.69
CV%	1.022	0.604	1.081	0.889	1.057	0.849	1.341	0.859	1.258
T	0.65	0.80	0.24	1.26	0.88	1.51	0.69	1.27	
F	1.50	4.29	1.36	1.97	1.43	2.15	1.12	2.11	

[a] Each value is the mean of five measurements. Theoretical values for t and F at $P = 0.05$ are 2.31 and 6.39, respectively.

both drugs. The typical chromatogram are shown in Figs. 3 and 4.

3.1. Optimum conditions for complexes formation

The effect of volume of carbon disulphide, ammonia buffer (pH 10) and acetic acid (96%), the metal concentration and the stability of the developed colors were investigated with respect to the maximum sensitivity and obedience to Beer's law. The optimum results are summarized in Table 1.

3.2. Interference

Betahistine is formulated only as single component tablets, while captopril is dispensed as a single component and as a binary mixture with hydrochlorothiazide; fortunately, the hydrochlorothiazide is practically insoluble in water, so no interference occurs. The effect of tablets excipients (starch, talc, lactose, manitol, citric acid, aerosil, avicel, acacia, magnesium stearate or microcrystalline cellulose) was studied and no interference were observed. The influence of captopril disulphide (related substance in USP 23) was investigated, no interference was observed..

3.3. Calibration graphs and statistical analysis

Linear regression equations were obtained over the concentration ranges stated in Tables 3–5. The angular coefficients, intercepts, correlation coefficients, variances and detection limits obtained by the least square treatment of the results are listed in Tables 3–5. The good linearity of the calibration graphs is clearly evident by the values of variances of the slope [31].

In order to determine the precision of the proposed procedures, solutions containing three different concentrations of the drug were prepared and analyzed in five replicates. The analytical results obtained from the investigation (Table 6) can be considered to be satisfactory.

3.4. Commercial preparation analysis

The commercial tablets containing BHT or CAP were analyzed by the proposed procedures and reference methods in order to confirm the accuracy of the proposed methods. The HPLC method supplied by Solvay Duphar and the USP 23 HPLC method [7] were used as reference methods for the analysis of BHT and CAP, respectively. Typical results given in Table 7 showed good agreement with those obtained by the reference methods (t- and F-tests), as the calculated values did not exceed the theoretical ones.

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