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DETERMINATION OF SECONDARY AMINO DRUGS AS THEIR METAL DITHIOCARBAMATE COMPLEXES BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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SUMMARY

To assay various secondary amino drugs (sympatomimetic, β-blocking, anti-arrythmic agents), they were converted to metal (copper or nickel) dithiocarbamate complexes by means of a pre-column derivatization method. The electrochemical properties of the complexes were studied. They were chromatographed on a reversed-phase column (LiChrosorb RP-18) with mixtures of acetate buffer (pH 5.8) and organic solvents (methanol, acetonitrile, ethanol or dichloromethane) as mobile phases. The complexes were detected by amperometry (applied potential of +0.7 V *vs.* SCE) or by UV spectrophotometry. The procedure has great sensitivity (10^{-12} mole for each injected compound) and good selectivity for the more substituted amino drugs.

INTRODUCTION

The determination of amino drugs in pharmaceutical or biological mixtures by high-performance liquid chromatography (HPLC) often requires a derivatization step to improve selectivity and sensitivity. Our aim is the development of a derivatization procedure for drugs that will convert them into electroactive species. These species can then be detected by electrochemical methods, which are very sensitive. Recently, we reacted primary amino drugs with *o*-phthalaldehyde and obtained adducts with electro-oxidative properties[1]. In this paper we describe the formation of metal dithiocarbamate complexes of secondary amino drugs and their separation on a reversed-phase (RP) HPLC system with electrochemical detection (ED).

In HPLC, metal chelates have been used previously to determine metal traces with normal-phase[2–4] and RP[5–11] columns and spectrophotometric[2–4,7–11] or electrochemical[5,6] detectors. These methods seem to have general application and compare favorably with atomic absorption spectroscopy[5,7]. In the field of drug analysis, secondary amino compounds were converted to metal dithiocarbamate complexes so that they could be determined by colorimetry[12,13]. Recently, this method was applied to the precise quantification of eniantiomeric impurities in ephedrine preparations[2,11]. We extended this derivatization method to various drugs. A voltammetric

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study of the compound obtained by reaction of the amino drugs with carbon disulphide and of the metal complexes subsequently formed, permitted establishment of the conditions optimal for their ED.

EXPERIMENTAL

Chemicals

All chemicals used were of analytical reagent grade. The solvents were distilled twice before use.

The amino drugs (Table I) were kindly donated by various pharmaceutical manufacturers.

Synthesis of metal dithiocarbamate complexes

Stock solutions of standard drugs (0.01 *M*) were prepared and then appropriately diluted with doubly distilled water. To the drug solution (1.0 ml) were added 0.1 *M* aqueous nickel or copper sulphate solution (1.0 ml), 20% (w/v) aqueous ammonia solution (1.0 ml) and 2% (v/v) carbon disulphide in chloroform (5.0 ml). After vigorous shaking for 1 min, the organic layer was washed three times with distilled water (2.0 ml each) and filtered through a phase-separation paper. The filtrate was evaporated to dryness under a gentle stream of nitrogen. The residue was redissolved in the appropriate mobile phase (1.0 ml). An aliquot of this solution (10 μl) was injected into the chromatograph. For voltammetric studies, the dithiocarbamate was synthesized from the amino drug and carbon disulphide in benzene, as previously described[14].

Voltammetric study

A polarographic analyzer (Model 174 A; E.G. & G. Princeton Applied Research, Princeton, NJ, U.S.A.) was used in conjunction with a classical three-electrode stationary cell and an *X–Y* recorder (Model RE 0074, Omnigraphic; Houston Instrument, TX, U.S.A.) to obtain the current–potential curves. The working electrode was a rotating glassy carbon or platinum unit (Model Edi + Controvit; Tacussel, Villeurbanne, France; 3 mm disk diameter). The saturated calomel reference electrode (SCE) was placed into a compartment separated by a porous bridge from the measuring cell. The cell and the compartment were filled with the same solution. A platinum wire served as the auxiliary electrode.

Voltammograms were obtained in a 0.1 *M* $LiClO_4$ solution in acetonitrile. The solutions were degassed with nitrogen prior to measurements. Recordings in direct current (d.c.), differential pulse (d.p.) and cyclic modes were made at scan rate of 2, 5 and 200 mV/sec, respectively. In d.p. voltammetry, the pulse height was 10 mV and the pulse repetition 0.5 sec. The rotating speed of the working electrode in the d.c. and d.p. modes was 600 rpm. The working electrode was stationary in the cyclic mode. The electroactivity of the ligand was studied at a concentration of 10^{-3} *M*. An excess of the metal ion was added to the solution of the ligand to form the metal complexes.

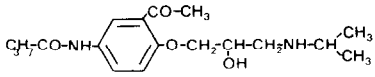
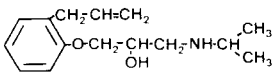
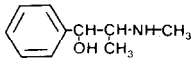
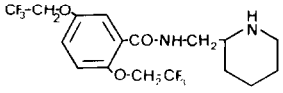
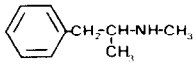
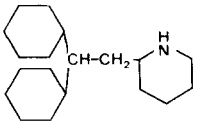
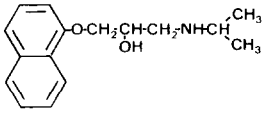
Chromatographic conditions

The HPLC system consisted of a ternary solvent delivery pump (Model SP 8700; Spectra-Physics, Santa Clara, CA, U.S.A.), an injection valve with a 10-μl sample loop (Model 7125; Rheodyne, Cotati, CA, U.S.A.) and a UV–visible detector

TABLE I

FORMULAE OF STUDIED SECONDARY AMINO DRUGS AND CAPACITY FACTORS OF THEIR METAL DITHIOCARBAMATES

+ = Hydrochloride; + + = monoacetate; + + + = maleate. For composition of mobile phases A, B and C see Experimental. – = No detection in 90 min following the injection. 0 = Elution in the dead-volume of the column.

Structure	*Capacity factors of metal complexes in mobile phase*					
	A		*B*		*C*	
	Ni	*Cu*	*Ni*	*Cu*	*Ni*	*Cu*
Acebutolol^{+} (MW = 336.4) 	2.48	2.66	0	0	0	0
Alprenolol^{+} (MW = 249.3) 	21.96	24.72	6.39	9.24	0	0
Ephedrine^{+} (MW = 201.7) 	3.07	4.00	0	0	0	0
Flecainide^{++} (MW = 474.4) 	2.93	3.86	0	0	0	0
Methamphetamine^{+} (MW = 185.7) 	9.67	11.94	0	0	0	0
Perhexiline^{+++} (MW = 393.5) 	–	–	–	–	3.28	4.68
Propranolol^{+} (MW = 295.8) 	21.96	26.65	5.86	8.33	0.26	0.37

(Model LC 871; Pye Unicam, Cambridge, U.K.). Reversed-phase columns were pre-packed with LiChrosorb RP-18 (Hibar E.C. 250-4, 7 μm; E. Merck). A pre-column (30 × 4 mm I.D.), packed with LiChrosorb RP-18 (40 μm) was used in-line for all chromatographic analysis.

Three different mobile phases were used in order to elute the complexes of the various drugs: (A) methanol–aqueous 0.02 *M* sodium acetate buffer, pH 5.8 (80:20, v/v); (B) acetonitrile–aqueous 0.02 *M* sodium acetate buffer, pH 5.8 (80:20, v/v); (C) ethanol–aqueous 0.02 *M* sodium acetate buffer, pH 5.8–dichloromethane (90:5:5, v/v). Solid $LiClO_4$ was added to make all mobile phases 0.005 *M* with respect to $LiClO_4$. They were filtered through a 0.6-μm microfilter (type BD; Millipore, Bedford, MA, U.S.A.). Flow-rates were 1.5 ml/min with mobile phases A and B and 1.0 ml/min with C.

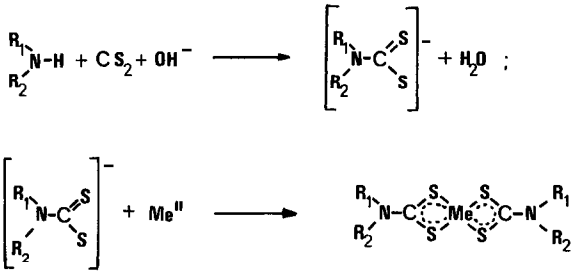
For amperometric detection, a thin-layer electrolytic cell (Model LCC 231; Merck-Clevenot, Nogent-sur-Marne, France), fitted with glassy carbon working and auxiliary electrodes and a reference SCE, was used in connection with an electronic control unit (Model E 230; Merck-Clevenot). The spectrophotometric detector was set at 325 nm for nickel chelates and 270 nm for copper chelates. The amperometric detector was operated at an applied potential of +0.7 V *versus* SCE. The dead volumes of the columns were measured for each mobile phase by injection of a sodium nitrite solution.

The chromatograms were recorded and all calculations made with an integrator (Model ICR-1; Intersmat, Courtry, France).

RESULTS AND DISCUSSION

Electrochemical properties

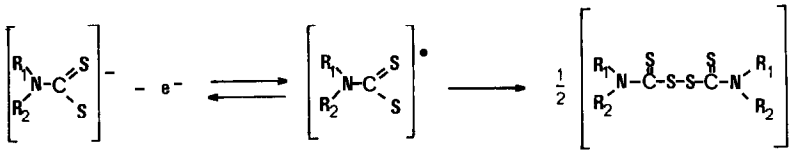
The electroactivity of drugs attributable to a secondary amino group has already been studied. These compounds have anodic oxidation waves at potentials higher than 1 V, well-defined only in non-aqueous media or in aqueous solutions at pH values above 10. This behaviour is characteristic of ephedrine and related compounds[15] and some β-blocking agents[18]. These conditions demanded by the electrochemical properties prevent the use of ED in a chromatographic system. We developed a derivatization procedure that produces easily electrooxidizable species. In the presence of a base (sodium hydroxide or ammonia), secondary amines react fast with carbon disulfide to give the corresponding N-disubstitued dithiocarbamates. These compounds easily form metal complexes (eqn. 1).



(1).

The metal complexes strongly absorb light in the UV–visible region (for instance, log $\varepsilon \approx 4$ at 434 nm for copper dithiocarbamates[12]). They are reduced and oxidized at various working electrodes[16,17]. Although the electrochemical properties of many metal dialkyldithiocarbamates are known and were used to quantitate metal ions at trace levels in HPLC[5,6], electrochemical data for metal drug–dithiocarbamate complexes were not available in the literature. For the detection of the drug complexes, the oxidation rather than the reduction process was chosen because the reduction waves may be obscured by oxygen, which is difficult to remove completely from an HPLC system, and by hydrogen ions in mobile phases.

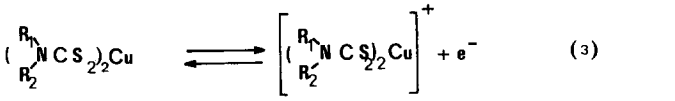
The current–potential curves obtained for the ligand alone in acetonitrile show two anodic waves (Fig. 1). The first wave of the dithiocarbamate is diffusion-controlled ($E_p = +0.07$ V) and well defined with no cathodic wave appearing in the cyclic mode. The process may be a one-electron oxidation followed by an irreversible dimerization, as previously noted for sodium diethyldithiocarbamate[16] (eqn. 2).



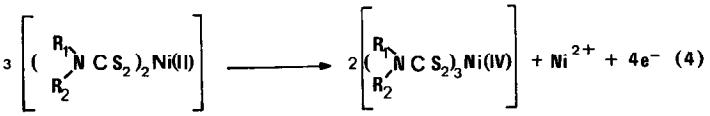
(2)

The second wave ($E_p = +0.89$ V) is distorted and might be caused by adsorption of the disulfide on the electrode. After addition of excess Cu(II) or Ni(II), the first oxidation wave appears at +0.33 V (Cu) and +0.57 V (Ni).

In the cyclic voltammograms obtained with both glassy carbon and platinum working electrodes, only the copper dithiocarbamates had cathodic peaks, indicating a reversible mechanism. When platinum was used, the cathodic wave is close to the anodic wave. With the glassy carbon electrode, the reduction peak is at a more negative potential. This great peak-to-peak separation may be explained by a slow electron exchange rate[6]. The electrochemical processes may proceed according to previously proposed mechanisms for the oxidation of metal diethyldithiocarbamate complexes[5,6] (eqns. 3 and 4).



(3)



(4)

Chromatographic study

The first chromatographic methods described for the separation of metal dithiocarbamates used silical gel columns and apolar solvents as mobile phases[2–4]. Such systems have poor reproducibility and are not suitable for coupling to an electro-

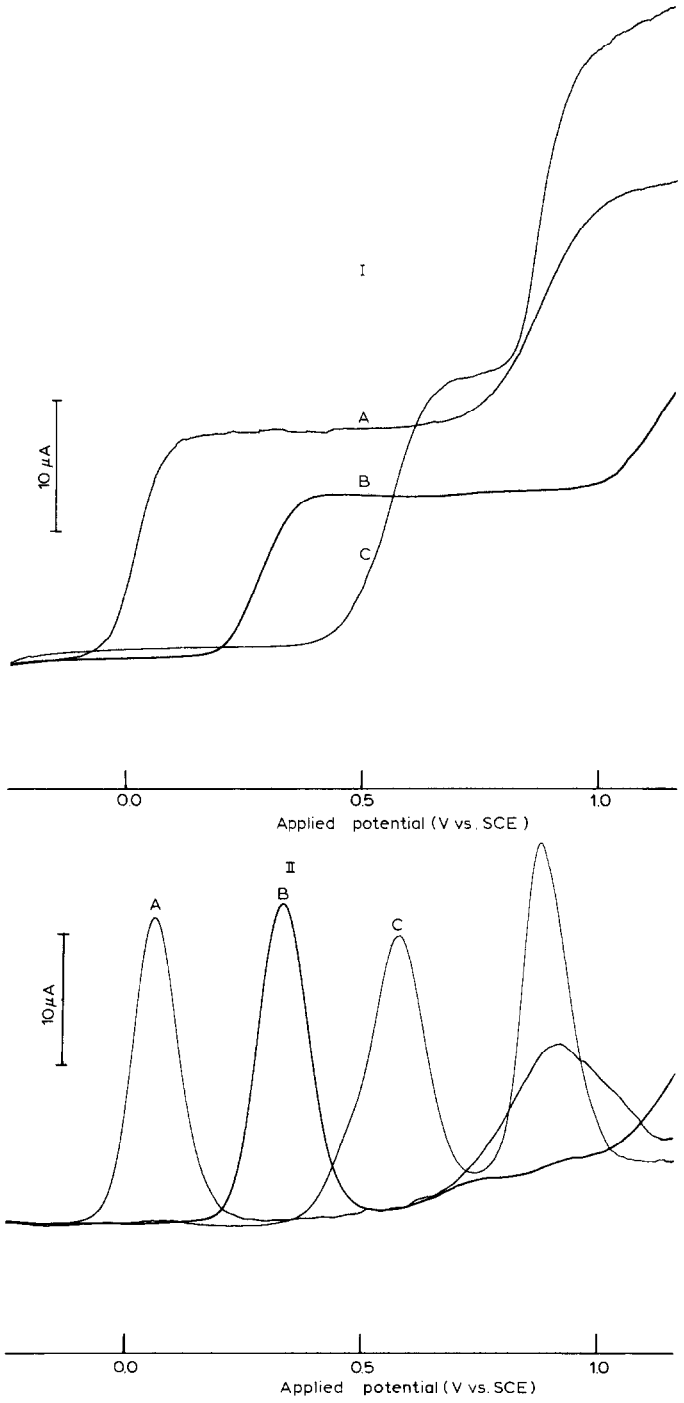


Fig. 1. Current–potential curves obtained in d.c. (I) and d.p. (II) modes for ligand alone (A), with Cu(II) added (B) or with Ni(II) added (C). For operating conditions, see Experimental section.

chemical detector because the commercially available electrochemical cells have aqueous reference electrodes. The use of several RP columns with buffered[5,6,11] or unbuffered[7–9,10] mobile phases was described. We employed eluents such as an aqueous/organic acetate buffer of pH 5.8, because the stability of metal dithiocarbamates is optimal at approximately pH 6[5,6]. The strongly hydrophobic C_{18} bonded silica stationary phase requires high concentrations of organic modifiers in the mobile phase for elution of the metal chelates. At least 70% of a mobile phase must consist of an organic solvent to assure dissolution of the metal dithiocarbamates. Hydrophilic phases such as diol or nitrile do not retain the complexes. The capacity factors of the nickel and copper dithiocarbamates derived from the drugs are listed in Table I. The copper complexes have higher capacity factors. Mobile phase B (acetonitrile–buffer) elutes the nickel complexes faster than mobile phase A (methanol–buffer) (Table I). The metal complexes derived from perhexiline, the most hydrophobic drug with alicyclic rings, were eluted only by a mobile phase containing some dichloromethane.

The electrochemical detector was operated at +0.7 V *vs.* SCE in order to take advantage of the oxidation process of the copper and nickel complexes. The hydrodynamic current–potential curves confirm the results of a previous voltammetric study. Quantities of each drug as small as $1 \cdot 10^{-12}$ mole may be detected. UV spectrophotometric detection is about a thousand-fold less sensitive. Typical chromatograms are shown in Fig. 2.

We used this method to determine amino drugs in pharmaceutical preparations, such as cough syrups containing ephedrine. We observed no interferences from

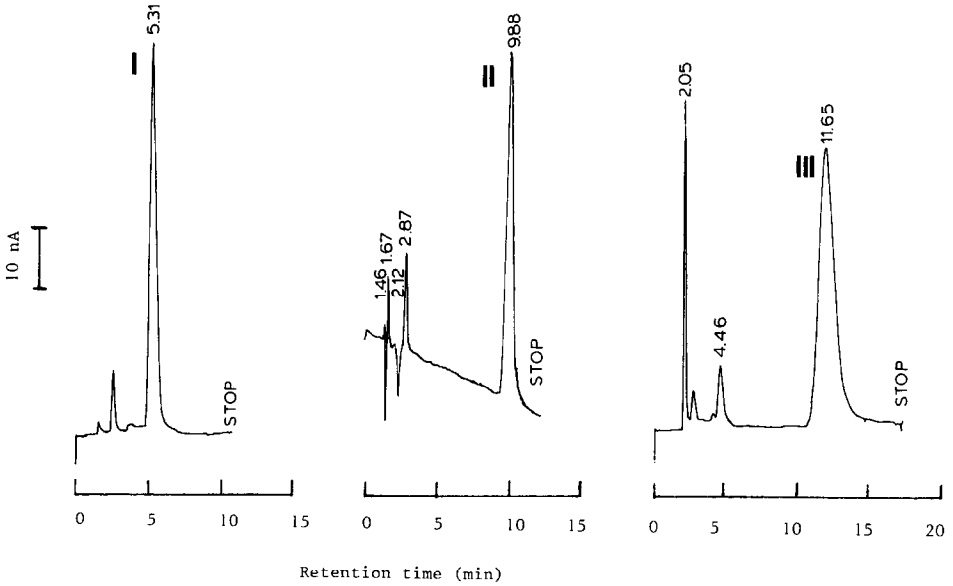


Fig. 2. Chromatograms of metal drug–dithiocarbamates derived from: (I) atenolol and nickel (elution performed with mobile phase A); (II) propranolol and nickel (elution with mobile phase B); (III) perhexiline and copper (elution with mobile phase C). For other chromatographic conditions see Experimental section. About 10^{-11} mole of each chelate was injected.

other compounds (tinctures, products with tertiary and quaternary amino groups, preservatives, vehicles). The linearity of the response *versus* concentration of ephedrine converted to nickel dithiocarbamate was studied in the range $5 \cdot 10^{-7}$–$5 \cdot 10^{-3}$ *M*. The sample measurements were carried out by injecting into the HPLC system an amount equivalent to $5 \cdot 10^{-11}$ mol of ephedrine. The accuracy of the method, including the derivatization and chromatographic steps, was calculated for ten replicate determinations; the coefficient of variation was 1.9%.

Limiting factors and prospects

The conversion of amino drugs into N-disubstituted dithiocarbamates and the complexation of these ligands with metals in a single extraction step can be used for fast "pre-column" derivatization. However, this method has the disadvantage of producing mixed complexes when several amino drugs are present in the initial solution[2,3,11]. The interpretation of the chromatograms becomes difficult when the number of drugs increases. This is illustrated in Fig. 3 for nickel dithiocarbamates derived from ephedrine and methamphetamine. To overcome this difficulty we are investigating different chromatographic procedures. For instance, the uncomplexed dithiocarbamates can be separated chromatographically and the complexes formed in a post-column reaction. The direct electrochemical detection of the ligand may also be possible. Initial results are promising.

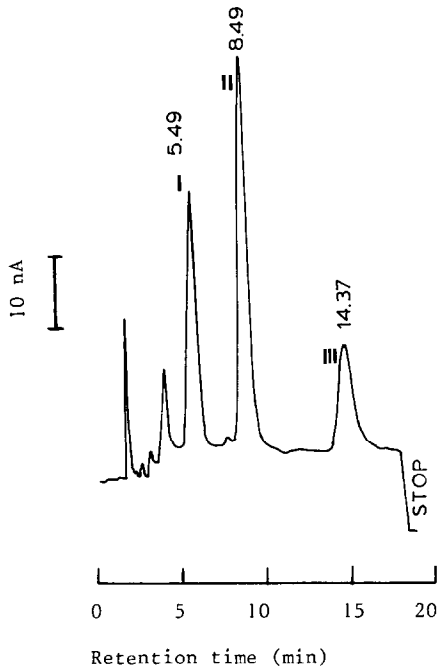


Fig. 3. Chromatogram of a mixture of ephedrine and methamphetamine nickel dithiocarbamates: (I) Ni[CS_2: ephedrine]$_2$; (II) Ni[CS_2: ephedrine] [CS_2: methamphetamine]; (III) Ni[CS_2: methamphetamine]$_2$. The elution was performed with mobile phase A.

CONCLUSIONS

The electrochemical oxidative properties of copper and nickel N-disubstituted dithiocarbamates derived from secondary amino drugs were determined. The easy formation of these complexes in a pre-column derivatization step and their electrochemical detection after HPLC separation can serve as a basis for a rapid, selective and sensitive method for the identification of secondary amino drugs in spite of the formation of mixed complexes when several drugs are present. Experiments are in progress to develop a method that would produce only one chromatographic peak per secondary amino drug in a mixture.

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