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Note

Liquid chromatography of reserpine and rescinnamine using electrochemical detection

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Reserpine is a *Rauwolfia* alkaloid whose remarkable physiological properties have led to its extensive use in treatment of hypertensive, nervous, and mental disorders. Besides its clinical utility in man, the drug has been used to calm horses participating in various events. A great need thus exists for a highly sensitive and rapid analytical procedure for reserpine. Few papers dealing with high-performance liquid chromatographic (HPLC) measurements of this drug have been published recently [1–5]. Ultraviolet detection following liquid chromatography (LC) yielded a detection limit of 25 ng, which was sufficiently for tablet assays [1, 2]. Lower detection limits can be achieved using fluorescence detection [3]; however, this requires lengthy derivatization reactions. Faster pre-column and post-column reactions for fluorescence detection have also been reported [4, 5]. Electrochemical detection (ED) has not been attempted, despite of its inherent sensitivity and the redox behavior of the drug. This mode of detection has been successfully applied for the detection of numerous electroactive compounds of pharmaceutical significance. The characteristics of a simple and highly sensitive procedure for measuring reserpine and the structurally related drug rescinnamine, based on LC—ED, are reported in the present paper.

EXPERIMENTAL

Apparatus

The LC system [Bioanalytical Systems (BAS), LC-303] consisted of a dual-piston pump (BAS, PM-30A), a Rheodyne Model 7125 injector (20-μl loop), a BAS Biophase ODS 5-μm reversed-phase column (25 cm × 4.6 mm I.D.), and

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an amperometric detector (BAS, LC-3A) equipped with a BAS Model TL-5 thin-layer glassy carbon transducer. The reference electrode was an Ag/AgCl electrode (BAS, Model RE-1). A BAS 3-cm Biophase 5-μm ODS guard cartidge was inserted between the injector and the analytical column. The system was operated at ambient temperature. The flow-rate of the mobile phase was 1.0 ml/min. Cyclic voltammetry was performed in a 10-ml voltammetric cell using a Princeton Applied Research Model 264A voltammetric analyzer.

Materials and procedure

All aqueous solutions were prepared in double-distilled water. Stock solutions of reserpine and rescinnamine (Aldrich) were made up fresh each day by dissolution in acetic acid and ethanol, respectively, and dilution in deionized water. The mobile phase was methanol (LC grade, Fisher)—0.05 *M* KH_2PO_4 in water adjusted to pH 4.5 with phosphoric acid (65:35); a 0.005 *M* concentration of the ion-pairing agent 1-heptanesulfonic acid, sodium salt (Sigma), was maintained. The solvents used in the preparation of the mobile phase were HPLC grade. The urine samples were obtained from healthy volunteers, filtered by passing through a 10—15 μm glass filter, and diluted with the mobile phase solution. Amperometric detection was proceeded by applying the working potential and allowing transient currents to decay. Cyclic voltammetry was performed by scanning the potential between 0.0 and 1.0 V at 50 mV/s.

RESULTS AND DISCUSSION

Cyclic voltammograms for reserpine and rescinnamine recorded over the anodic range of a stationary glassy carbon disk electrode, in the mobile phase used in chromatography, are shown in Fig. 1. Both compounds exhibit a single defined oxidation peak at ca 0.65 V (reserpine) and 0.72 V (rescinnamine).

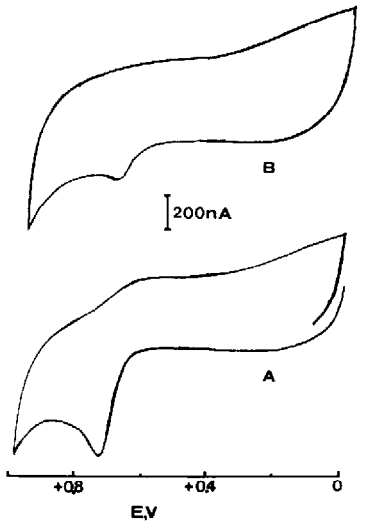

Fig. 1. Cyclic voltammograms of $1 \cdot 10^{-5}$ *M* rescinnamine (A) and reserpine (B) at a glassy carbon electrode. Solution: methanol—0.05 *M* KH_2PO_4, pH 4.5 (65:35), containing 0.005 *M* heptanesulfonic acid. Scan-rate, 50 mV/s.

No corresponding peak is observed in the reverse, cathodic scan. According to Adams [6], the electrode reaction of methoxy-substituted indole alkaloids (including reserpine-like compounds) involves the introduction of a hydroxyl group at the indole-5 position. The above cyclic voltammetric data indicate that amperometric detection might provide a useful approach for monitoring these drugs in chromatographic effluents. Stirring the solution for different time periods prior to the voltammetric scan resulted in larger anodic peaks, indicating strong accumulation of the drugs; this behavior can be used as a basis for a sensitive adsorptive stripping procedure for reserpine and rescinnamine [7]. Such interaction with the electrode surface caused no serious filming problem in the amperometric measurements reported in this study. While reserpine also undergoes reduction at high cathodic potentials [8], oxidative LC—ED is preferred over reductive one, because difficulties associated with deaeration of the mobile phase (and sample) and large noise levels are eliminated.

In order to determine the optimum potential required for the amperometric assay of reserpine, a hydrodynamic voltammogram was generated by repetitive 100-ng injections of the drug while changing the operating potential (Fig. 2). A well defined wave is observed, with maximum (mass transport limited) signal at potentials higher than +0.9 V. For rescinnamine, the maximum signal was observed at potentials higher than +1.0 V (not shown). These hydrodynamic voltammetric response is in general agreement with the cyclic voltammetric data described above. The optimum operating potentials were determined to be +0.9 V (reserpine) and +1.0 V (rescinnamine).

Mobile phase pH plays a significant role in terms of both the electrochemical response and the chromatography of reserpine (Fig. 3). Changes of the pH from 3.5 to 6.0 result in a similar retention time (ca. 9 min); an increase in retention time, up to 25 min, is observed upon further increase of the pH to 7.5. Maximum electrochemical sensitivity occurs at pH 4.5; a gradual decrease in peak current is observed by increasing the pH over the 4.5—7.5 range. A mobile phase pH of 4.5 was used throughout the study.

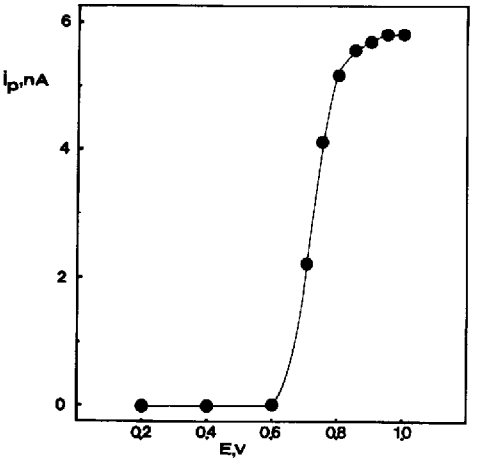

Fig. 2. Hydrodynamic voltammogram for injections of 100 ng reserpine. Flow-rate, 1.0 ml/min; mobile phase, as solution described in Fig. 1.

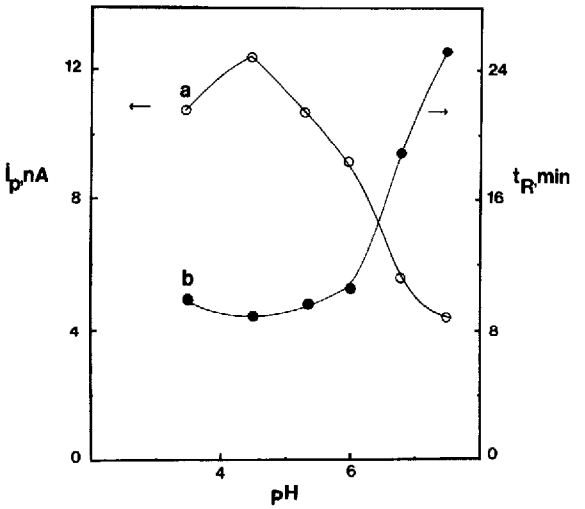

Fig. 3. Dependences of the peak current (a) and retention time (b) on the mobile phase pH. Injections of 150 ng reserpine; operating potential , +0.90 V. Flow-rate and mobile phase as in Fig. 2.

The sensitivity and linearity of the response were evaluated using eight successive injections of reserpine solutions of increasing amount (5—40 ng). Well defined peaks were obtained at the nanogram level. The peak response increased linearly with increasing amounts of the drug; the slope of the calibration plot corresponded to a sensitivity of 73.6 nA/μg (correlation coefficient, 0.999; intercept, 0.2 nA). Similarly, a calibration curve for rescinnamine was linear over the range 10—50 ng injected (slope, 53.1 nA/μg; intercept, 0.3 nA; correlation coefficient, 0.999; not shown).

The ability to detect minute levels of reserpine and rescinnamine depends on the signal-to-noise characteristics of the detector. Injections of 5-ng solutions of the drugs were used to estimate the minimum detectable quantities

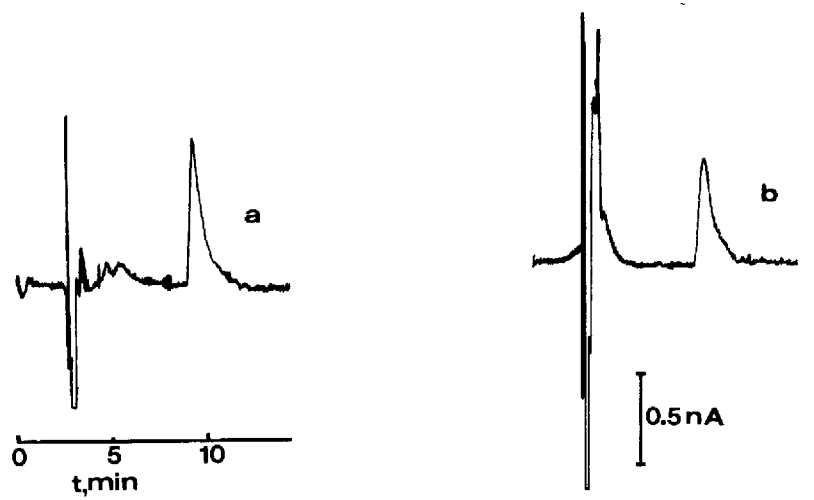

Fig. 4. Chromatograms for injections of 5 ng rescinnamine (a) and reserpine (b). Conditions as in Fig. 3, except for the operating potential (a) of +1.0 V.

(Fig. 4). Based on a signal-to-noise ratio of 3, detection limits would be 0.8 ng (rescinnamine) and 0.9 ng (reserpine).

The reproducibility was assessed from a series of eight successive injections of a 100-ng reserpine solution (conditions as in Fig. 3). The mean peak current found was 8.34 nA, with a range of 8.16—8.52 nA and a relative standard deviation of 1.5%. The reproducible data, obtained over a 3-h period, indicate the absence of electrode "poisoning" (due to adsorption of reserpine).

To test the specificity of the procedure, a range of electroactive drugs that may be co-administrated with reserpine was screened. As a result of differences in the retention behavior the 50-ng reserpine peak was not affected by the presence of 50 ng of the cardiovascular drugs propranolol, pindolol and nicardipine, the antidepressant despiramine, or the antibiotic tetracycline (conditions as in Fig. 3). In contrast, because of the similarity of their redox and retention behaviours, rescinnamine would appear as a shoulder on the reserpine peak. Such interference is common also to fluorescence monitoring of reserpine [4].

The suitability of the described method for direct measurement in body fluids is illustrated in Fig. 5. A urine sample containing reserpine (10 μg/ml) was diluted 1:25 (8 ng injected) and used without any preliminary treatment to yield the chromatogram shown in Fig. 5b. The reserpine peak (retention time, 11 min) is not affected by other naturally occurring electroactive sample constituents that are eluted earlier. The absence of interferences obtained in untreated urine samples is attributed to the selectivity of electrochemical detection. Direct proportionality between the peak height and the reserpine level is indicated from the standard additions plot (shown also in Fig. 5). Quantitation is thus feasible by comparing the peak currents of reserpine from unknown samples with those obtained from blank urine to which known amounts of reserpine were added. Urine samples collected from different volunteers yielded response characteristics similar to those shown in Fig. 5. Samples containing lower levels of the drug, or other body fluids, may be assayed by employing a simple clean-up procedure instead of the dilution step.

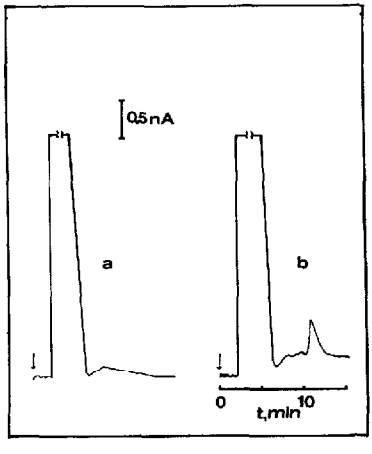

Fig. 5. Chromatograms obtained before (a) and after (b) spiking a urine sample with reserpine at 10 μg/ml and diluting 1:25 with the mobile phase. Conditions as in Fig. 3.

An internal standard would be needed, in routine clinical applications, to overcome variation in recovery.

In conclusion, the LC—ED approach described here offers a sensitive and rapid method for trace measurement of reserpine and rescinnamine. Because of the incomplete resolution of reserpine and rescinnamine the method can measure only one drug in the absence of the other, using external standard addition. The limit of detection is 27-fold lower than that of UV absorbance detection. The use electrochemical detection is suitable for analysis performed in complex biological matrices without the need for elaborate treatment procedures.

ACKNOWLEDGEMENTS

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