Journal of Chromatography, 367 (1986) 405–411
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 841

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET OR ELECTROCHEMICAL DETECTION FOR THE DETERMINATION OF DOPAZINOL IN A PHARMACEUTICAL FORMULATION

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(First received March 26th, 1986; revised manuscript received May 29th, 1986)

SUMMARY

The high-performance liquid chromatographic (HPLC) analysis of dopazinol, an anti-Parkinsonian drug, using either ultraviolet (UV) absorbance detection at 282 nm or oxidative amperometric electrochemical detection at the glassy carbon electrode (+0.92 V *vs.* Ag/AgCl) is described and evaluated. The influence of such factors as mobile phase composition, pH and column temperature on the detection of dopazinol using both detectors is reported. The HPLC analysis of samples resulting from the development and evaluation of a pharmaceutical formulation demonstrated a precision of <2.5% (R.S.D.) in the working ranges of the UV and electrochemical detectors. The limits of detection were 100 ng/ml for the UV detector and 1 ng/ml for the electrochemical detector. The higher sensitivity of the latter makes its use appropriate for the analysis of samples with low concentrations of dopazinol. Studies to compare the UV and electrochemical detectors for the routine analysis of dopazinol in pharmaceutical formulations are presented.

INTRODUCTION

The development of a pharmaceutically efficacious formulation of dopazinol[1], (+) 3,4,4A,5,6,10B-hexahydro-4-propyl-2H-naphth-(1,2B)(1,4)-oxazin-9-ol (Fig. 1), a new highly promising anti-Parkinsonian drug, generated the need for a sensitive, specific analytical method which could be used on a routine basis. Initial analytical investigations examined the utility of high-performance liquid chromatography (HPLC) with ultraviolet (UV) absorbance detection. Recent studies, however, have indicated that dopazinol is an extremely potent drug and that the expected dosage levels may result in samples with concentrations below the working range of an HPLC–UV system.

Amperometric electrochemical detectors for liquid chromatography have been shown to improve detection limits and sensitivity for a wide variety of electrochemically active compounds[2]. Many pharmaceuticals, including sulpha drugs, purines, phenothiazines, phenolic acids, and local anesthetics have been quantitatively deter-

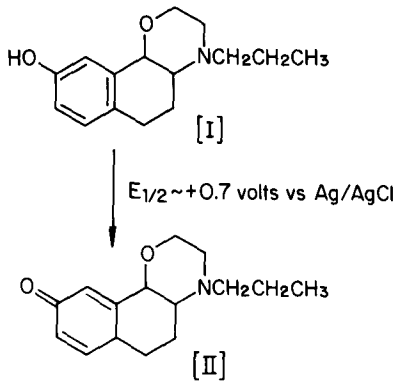

Fig. 1. Postulated electro-oxidation mechanism of dopazinol. I = Dopazinol; II = compound B.

mined using liquid chromatography–electrochemical detection (LC–ED)[3–7]. The phenolic substituent of the dopazinol molecule renders it a suitable candidate for oxidative amperometric ED.

In this paper, an HPLC method for the determination of dopazinol in a pharmaceutical dosage form using either ultraviolet absorbance or oxidative amperometric electrochemical detection is described and evaluated. The influence of such factors as mobile phase composition, pH and column temperature on the HPLC method with these two detectors is reported. Data reported include chromatographic parameters, linearity of detector response and detection limits. The precision and accuracy of the HPLC method with both detectors are also reported.

EXPERIMENTAL

The liquid chromatographic system consisted of a Hewlett-Packard (Avondale, PA, U.S.A.) 1081B isocratic high-performance liquid chromatograph equipped with a Kratos (Ramsey, NJ, U.S.A.) Spectroflow 773 ultraviolet absorbance detector. The UV detector was operated at 282 nm with an attenuation of 0.01 absorbance units full scale (a.u.f.s.). An oxidative amperometric electrochemical liquid chromatographic detector (Model TL-8A, Bioanalytical Systems, West Lafayette, IN, U.S.A.) was operated in series following the UV detector. The electrochemical detector was controlled by a potentiostat (Model LC-2B, also obtained from Bioanalytical Systems) and consisted of a glassy carbon working electrode operated in the direct current mode at +0.92 V *versus* an Ag/AgCl reference electrode with a stainless-steel block auxiliary electrode. A sensitivity range of 500 nA/V full scale was regularly used. The electrochemical detector was enclosed in a Faraday cage which was grounded through the potentiostat ground to prevent stray electromagnetic interference. Each detector was equipped with a Hewlett-Packard 3392A integrator to record all chromatograms. An IBM (IBM Instruments, Danbury, CT, U.S.A.) octyl end-capped HPLC column (150 mm × 4.5 mm I.D., 5 μm particle size) was used. The mobile phase consisted of aqueous 25 m*M* KH_2PO_4, pH 5.4–acetonitrile (55:45, v/v) which was degased with helium prior to use. The injection volume was 20 μl and the

flow-rate was 0.8 ml/min. Column temperature was 50°C. In order to decrease analysis time, an IBM octyl endcapped mini-column (50 mm × 4.5 mm I.D., 5 μm particle size) was used for initial characterization of the electrochemical detector.

Voltammetric scans were performed using a Bioanalytical Systems Model CV-1A cyclic voltammetry system with a glassy carbon working electrode, an Ag/AgCl reference electrode and a platinum wire auxiliary electrode. The supporting electrolyte for all cyclic voltammogram was the HPLC mobile phase noted above. A scan rate of 250 mV/s was used.

Dopazinol and its deshydroxy analogue (compound B) were obtained from the reference standard collections in-house. All solvents were HPLC grade.

Stock solutions (0.5 mg/ml) were prepared by dissolving approximately 25 mg of dopazinol or compound B in 50 ml of tetrahydrofuran (THF). Standard solutions of the desired concentrations were prepared by diluting the stock solution with THF. Stock solutions of dopazinol in THF stored at 5°C and protected from light were found to be stable for at least 5 days.

Chromatographic parameters with the exception of column efficiency were calculated based on the U.S.P. guidelines[8]. Column efficiency (N) was computed from

$$N = 5.54[t_R/W_{0.5}]^2$$

where t_R is the compound retention time and $W_{0.5}$ is the peak width at half peak height.

Dopazinol was extracted from the formulation matrix by shaking an aliquot of the formulation in a sealed flask for approximately 1 h in 25 ml of THF. An aliquot of the resulting solution was centrifuged at approximately 15 000 rpm (15 600 g) for 1 min and a 20-μl aliquot of the clear supernate was injected directly for HPLC analysis.

RESULTS AND DISCUSSION

HPLC with ultraviolet absorbance detection of dopazinol was performed at 282 nm, the wavelength of maximum absorbance for the drug in the HPLC mobile phase. The mobile phase conditions chosen were based on studies to optimize chromatographic behavior and analysis time as a function of mobile phase composition and pH. The capacity factor (k') of dopazinol was found to increase with decreasing amounts of organic modifier in the mobile phase, to increase with increasing mobile phase pH and to remain unchanged with varying buffer salt concentrations from 10 mM to 25 mM. Using the HPLC conditions specified, HPLC–UV for dopazinol was found to be linear over the concentration range from 0.5 μg/ml to 8.8 μg/ml and to have a detection limit (2σ) of $\approx$ 100 ng/ml. Accuracy, as determined by a recovery study from spiked placebo pharmaceutical formulations was found to average 101.4% recovered ± 0.8% (R.S.D.) as shown in Table I. No significant absorbance was contributed by the formulation excipients. HPLC–UV precision as measured by the R.S.D. of the assay value of 12 similar dopazinol solutions at 5 μg/ml was 0.53%.

HPLC–ED parameters were based upon the electrochemical behavior of dopazinol in the mobile phase. Cyclic voltammetric investigations of dopazinol showed an oxidative wave at the glassy carbon electrode at $E_{1/2} \approx +0.7$ V *vs.* Ag/AgCl (Fig.

TABLE I

ACCURACY OF DOPAZINOL METHOD

Concentration (μg/ml)	*Area*		*Recovery (%)*	
	UV	*ED*	*UV*	*ED*
2.92	28 865.5	280 315	102.7	101.9
2.92	28 381.0	281 990	101.0	102.5
4.38	21 791.5	214 060	102.3	99.3
4.38	21 506.5	215 160	100.9	99.8
5.84	28 895.0	282 780	101.0	100.8
5.84	29 234.0	283 935	102.1	101.2
7.30	36 268.0	348 530	100.7	100.5
7.30	36 410.5	346 665	101.0	100.0
7.76	78 645.5	866 130	101.7	100.0
7.76	86 507.0	841 810	100.4	97.2
Average			101.4	100.3
R.S.D.			0.8	1.5

2, curve C). The voltammetric activity is postulated to be due to the electro-oxidation of the phenolic hydroxy sustituent of dopazinol to the quinone (Fig. 1). A similar reaction mechanism has been reported for a number of substituted phenols[9]. Confirmation of this assignment is based upon the lack of electro-activity of Compound B, an analogue of dopazinol not containing the phenolic hydroxy substituent (Fig. 2, curve B). The lack of a reductive wave in the cyclic voltammogram indicates that the oxidation of dopazinol is irreversible. Solution pH over the range of 2 to 8 was shown to be directly related to $E_{1/2}$ for dopazinol oxidation with lower pH values

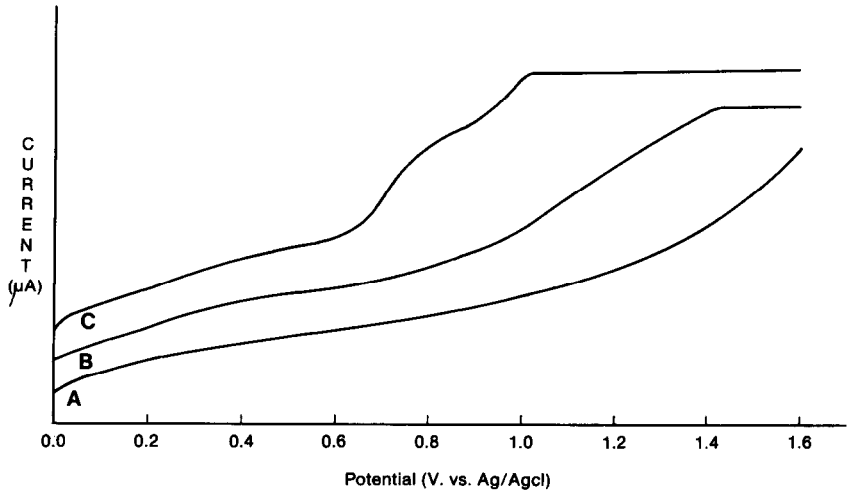

Fig. 2. d.c. voltammograms obtained using a glassy carbon electrode and 0.025 *M* KH_2PO_4–acetonitrile (55:45) supporting electrolyte. (A) Supporting electrolyte; (B) $9.20 \cdot 10^{-3}$ *M* compound B; (C) $8.08 \cdot 10^{-3}$ *M* dopazinol. Scan rate = 250 mV/s, sensitivity = 5 μA/V. Note: Increasing current is indicated along the *y*-axis in the direction away from the origin.

corresponding to lower $E_{1/2}$ values. High sensitivity determinations of dopazinol in solutions of pH > 7 (where the $E_{1/2}$ exceeded 1 V *vs.* Ag/AgCl) were not possible due to unacceptably low signal/noise ratios.

The current–voltage curve shown for dopazinol in the mobile phase solvent system (Fig. 2) demonstrates that a maximum current for the oxidation of the analyte is obtained at potentials > ≈ +0.85 V *vs.* Ag/AgCl. However, when operating at potentials > +1.0 V high background currents due to the oxidation of trace impurities in the mobile phase were noted. As a result, an applied potential of +0.92 V *versus* the Ag/AgCl reference electrode was chosen as a compromise between maximizing the current response and minimizing the background current.

Mobile phase modifications were studied to optimize the response of the EC detector. Decreasing the amount of organic solvent in the mobile phase resulted in increased signal from the electrochemical detector. The concentration of the phosphate buffer in the mobile phase in the range of 10 m*M* to 25 m*M* did not affect the current generated; however, an increase in mobile phase pH from pH 3 to pH 6 caused the response from the electrochemical detector to increase. The mobile phase

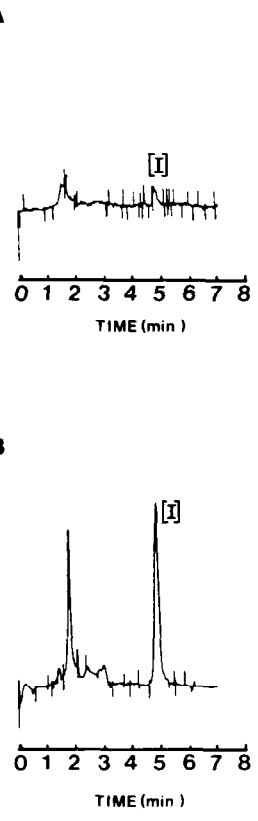

Fig. 3. Simultaneously obtained chromatograms of 100.4 ng/ml dopazinol (≈ 2 ng injected). I = dopazinol. (A) UV detector: wavelength = 282 nm; attenuation = $2 \cdot 10^{-5}$ a.u.f.s. (B) electrochemical detector: glassy carbon working electrode; Ag/AgCl ref. electrode; stainless-steel aux. electrode; potential = +0.92 V; attenuation = 0.8 nA.f.s.

conditions which produced optimum chromatography were found to be at or near optimum for ED response as well.

Current ranges on the electrochemical detector of 500 nA full scale (nA.f.s.), 200 nA.f.s. and 100 nA.f.s. were found to be easily usable for routine analysis. Detector sensitivity ranges < 100 nA.f.s. resulted in decreased signal-to-noise ratios due to significantly increased baseline noise.

Studies were performed to evaluate the linearity and detection limit of the electrochemical detector as compared to the UV detector. The electrochemical detector also demonstrated linearity over a concentration range of 0.5 μg/ml to 8.8 μg/ml. However, at these relatively high concentrations the UV detector is the detector of choice due to the precision problems to be addressed later. The chromatograms in Fig. 3 illustrates that the electrochemical detector is capable of easily detecting dopazinol at the 100 ng/ml (UV detection limit) concentration level. The detector maintains linear response at concentration levels well below this concentration. For example, linear regression analysis of the LC–ED results for duplicate injections over the range of 50.2 ng/ml to 502 ng/ml dopazinol yielded a correlation coefficient (r) = 0.9998. The detection limit of the electrochemical detector (2σ) was found to be 1 ng/ml (20 pg injected). These results clearly demonstrate the superiority of ED over UV detection for measuring low concentrations of dopazinol.

In order to validate the use of the HPLC–ED method for the determination of dopazinol, studies similar to those done for the HPLC–UV system concerning injection precision, assay precision and accuracy were performed. Relatively high injection concentrations (5 μg/ml dopazinol) caused imprecision problems for the electrochemical detector (R.S.D. = 4.20%, n = 12). This imprecision is most likely attributable to a "coating" phenomenon which occurs on the electrode surface when high concentrations are oxidized. Experiments at lower concentrations demonstrated improved precision of ED (Table II). It was concluded that at lower concentrations ($\leqslant$ 500 ng/ml), the electrode is "conditioned" after a few injections and the coating phenomenon becomes insignificant. Since ED will only be used to analyze samples containing low levels of dopazinol, coating is not a critical problem.

To determine accuracy, a recovery study from spiked placebo pharmaceutical formulations was also done using ED. The response of the electrochemical detector to extracts of placebo formulation spiked with standard solutions of dopazinol was compared to the response for standard solutions of dopazinol. An average recovery of 100.3% ± 1.5% (R.S.D.) was obtained for the electrochemical detector (Table I). The extracts of blank placebo formulations showed no interferences.

TABLE II

PRECISION OF DOPAZINOL METHOD

Concentration (ng/ml)	*R.S.D. (%)*		*Number of samples (N)*
	UV	*ED*	
5000	0.53	4.20	12
500	1.10	1.10	7
250	1.70	0.54	11
100	4.60	2.30	13

A comparative study of the chromatographic parameters resulting from UV and electrochemical detectors in series demonstrated that the HPLC peaks were essentially the same for both detectors (UV: $k' = 1.99$, $N = 4719$, $T_{5\%} = 2.3$; ED: $k' = 1.72$, $N = 3433$, $T_{5\%} = 2.3$).

CONCLUSION

The data obtained established the reliability of both the ultraviolet and the oxidative amperometric electrochemical detector for the determination of dopazinol in pharmaceutical formulations with ease, accuracy, precision and speed. The inclusion of the electrochemical detector as an optional detector for the HPLC method for the determination of dopazinol in samples with concentrations $\leqslant 500$ ng/ml greatly increases the versatility of the method. Both methods have been shown to be accurate, precise and specific for dopazinol in the sample matrices tested. Using the oxidative amperometric detector described here, samples containing nanograms per milliliter of dopazinol can be routinely assayed.

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