Novel Redox Behaviour of the 1,4-Benzodiazepine Lorazepam and Its Analytical Application

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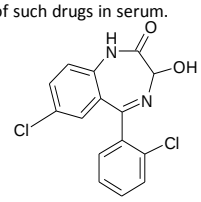
Introduction

Lorazepam, 7-chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2H-1,4-benzodiazepin-2-one, (I) is one of the most commonly administered and abused members of the 1,4-bezodiazepine class of drugs. Previous electrochemical investigations have focused on its cathodic behaviour showing two pH dependent reduction processes resulting from the $2e^-$, $2H^+$ reductions of the 4,5-azomethine and the 3-hydroxyl groups [1,2]. However, to our knowledge there have been no reports on anodic redox behaviour of this molecule. In this present study we have identified several previously unreported oxidation processes (figure 1, O1, O2 and O3). The effects of both pH and scan direction on these were studied and mechanisms given to explain these observations. The possibility of determining lorazepam by liquid chromatography dual electrode detection in the redox mode (LC-DED) was then explored. The results showed this to be a promising approach for the determination of such drugs in serum.



(I)

Experimental

Cyclic voltammetry (CV) was performed with a Pstat10 potentiostat interfaced to a PC for data acquisition via the General Purpose Electrochemical System Software Package (GPES) version 3.4 (Autolab, Windsor Scientific Limited, Slough Berkshire UK). The voltammetric cell (Metrohm, Switzerland) contained a glass coated platinum wire auxiliary electrode, a saturated calomel electrode (SCE) (Russell, Fife, UK) and a 6 mm diameter glassy carbon electrode (GCE) as the working electrode. Cyclic voltammograms were obtained utilising a supporting electrolyte consisting of 50 % acetone, 50 % 100 mM phosphate buffer (v/v). Degassing was achieved by purging with oxygen free nitrogen (BOC, Guildford, UK) for 5 minutes to eliminate oxygen reduction waves. A starting potential of 0.0 V was used, with an initial switching potential of -2.0 V and a second switching potential of +2.0 V, with a final potential of 0.0 V. LC-DED investigation were undertaken as described previously [3,4]. Liquid chromatographic conditions: 50 % organic modifier/50 % 100 mM phosphate buffer pH 2.1; 0.8 ml/min C_{18} 250 x 4.6 mm, 5 µm. Generator = -2.45 V, detector = +1.0 V (vs. Ag/AgCl).

Results and Discussion

Typical cyclic voltammograms for lorazepam obtained in 0.1 M phosphate buffer containing 50 % acetone are shown in figure 1. Similar cathodic behaviour is seen if the scan is first implemented in the negative (figure 1a) or positive (figure 1b) direction, with a single reduction peak (R1) being obtained. A smaller reduction wave is also observable at -0.6 V which we believe is a result of the reduction of oxygen substituted species such as aldehydes or mesityl oxide present as impurities in the acetone [5,6]. Figure 1c shows the anodic section of the voltammogram of lorazepam obtained at pH 4. If the scan is first implemented in the negative direction (not shown), then on the return anodic section two oxidation peaks are obtained (O1 and O3). However, without this negative scan, peak O1 is absent but O3 is present together with a new peak, O2. This indicates that O1 results from the oxidation of a compound formed during the reduction of lorazepam on the initial negative going scan.

Interestingly, at pH values above 4, (figure 1d) the three oxidation peaks are still observable. Again, O1 only occurred if the cyclic voltammogram had been implemented in the negative direction first. However, O2 is now present with or without prior reduction. We believe these observed differences in the cyclic voltammetric behaviour of lorazepam must result from two different mechanisms that occur above and below pH 4.

Previous investigations [1,2] at Hg electrodes have shown that at low pH values lorazepam is reduced in a $4e^-$, $4H^+$ process resulting from the simultaneous $2e^-$, $2H^+$ reduction of the 4,5 azomethine bond and the $2e^-$, $2H^+$ reduction of the 3-OH group. This possibility may be deduced from the i_p versus pH plot shown in figure 1e; clearly the magnitude of the current for R1 decreases by 50 % when the pH is changed from pH 2 to pH 6. This is illustrated in scheme I. Figure 3f shows that protons are also involved in the electrodes reactions.

LC-DED investigations were undertaken to exploit this behaviour. Figure 2 shows the resulting chromatograms obtained for two serum sample extracts. Clearly, in the presence of lorazepam a well-defined chromatographic peak is seen at retention time of 8.5 minutes corresponding to lorazepam. The response was found to be linear with concentration over the range 32.1 ng to 4.0 µg, with a detection limit of 15 ng on column. The percentage recovery for a serum fortified at 16 µg/ml lorazepam was found to be 77.9 %, with a corresponding %CV of 5.7 %.

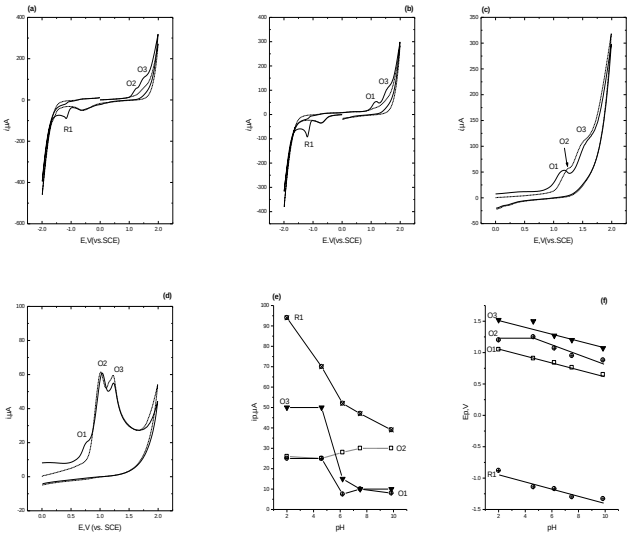
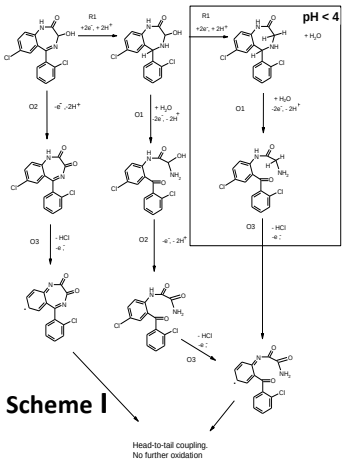


Figure 1. Cyclic voltammograms obtained in the presence (solid line) and absence (dotted line) of 1 mM lorazepam in 50 % 0.2 M pH 4 phosphate buffer-50 % acetone. Scan rate 50 mV/s, starting and end potential 0.0 V. (a) initial switching potential +2.0 V, second switching potential -2.0 V. (b) initial switching potential -2.0 V, second switching potential +2.0 V. (c) anodic section, voltammetric conditions as figure 3a (d) anodic section, pH 8, other voltammetric conditions as figure 3a (blanks omitted for clarity) (e) i_p versus pH and (f) Ep versus pH.



Scheme I

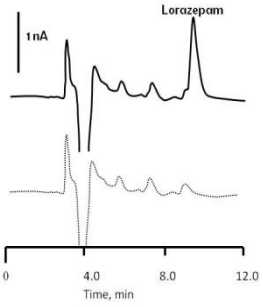


Figure 2. Typical chromatograms obtained in the redox mode for bovine serum extract. Solid line, fortified with lorazepam (16.0 mg/l), dotted line unadulterated.

Conclusions

- This is first report on the electrochemical anodic redox behaviour of lorazepam
- The underlying mechanism for the redox peaks observed by cyclic voltammetry has been investigated.
- The number and nature of the peaks was found to dependant on both pH and scan direction.
- We have utilised this behaviour for the determination of lorazepam in biological fluids by LC-DED.

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References

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