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Cyclic Voltammetric Behaviour and High-Performance Liquid Chromatography Amperometric Determination of Levamisole

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Abstract: The electrochemical oxidation of levamisole, a glassy carbon electrode, was investigated over the pH range 2.0–10.0. Cyclic voltammetric investigations showed a single oxidation process was recorded, with a peak potential (E_p) shown to be pH-dependent in the range 5.0–8.0; between pH 2.0 and pH 5.0, and above pH 8.0, the E_p was found to be independent of pH, indicating apparent pKa values of 5.0 and 8.0. Peak currents were found to increase with increasing pH values. This voltammetric oxidation process was found to be consistent with a two-electron, two-proton oxidation to the corresponding sulfoxide. Based on these findings, the development of a of method based on the high-performance liquid chromatography separation of levamisole, with electrochemical detection being used for its determination, was explored. The chromatographic conditions required for the separation of levamisole were first investigated and optimized using UV detection. The conditions were identified as a 150 mm × 4.6 mm, 5 µm C_{18} column with a mobile phase consisting of 50% methanol, and 50%, 50 mM, pH 8.0 phosphate buffer. The technique of hydrodynamic voltammetry was applied to optimize the applied potential required for the determination of levamisole, identified as +2.3 V versus a stainless-steel pseudo-reference counter-electrode. Under the optimized conditions, levamisole exhibited a linear response of 1.00–20 mg/L (R^2 = 0.999), with a detection limit of 0.27 mg/L. The possibility of determining levamisole in artificial urine was shown to be possible via simple dilution in the mobile phase. Mean recoveries of 99.7%, and 94.6%, with associated coefficients of variation of 8.2% and 10.2%, respectively, were obtained for 1.25 µg/mL (n = 5) and 2.50 µg/mL (n = 5).

Keywords: levamisole; cyclic voltammetry; hydrodynamic voltammetry; HPLC; amperometry

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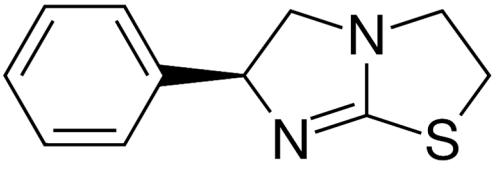
1. Introduction

Levamisole; (6S)-6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-b][1,3]thiazole (i) is commonly used to treat parasitic worm infections in both humans and animals [1], and is listed in the World Health Organisation's List of Essential Medicines as a safe and effective treatment of such infections [2]. Early investigation into the development of levamisole reported improved recovery periods associated with influenza and measles [3]; reports on its use in the treatment on coronavirus (COVID-19) have shown it to possibly aid in recovery [4,5]. Levamisole has been used alongside 5-fluorouracil for the treatment of colorectal cancer, but was withdrawn due to adverse effects [6].



(i)

In the 1970s, reports showed that the administration of tetramisole, a racemate of levamisole, and its isomer, dexamisole, led to an elevation in mood [7], and also showed antidepressant properties [8,9]. Studies on the metabolism of levamisole in horses showed that the drug could be metabolised into pemoline and aminorex. These drugs are both banned in horseracing due to their possible performance-enhancing effects [10]. It is believed that these stimulant-like properties of its metabolite, aminorex [11], and the mode-enhancing properties of levamisole [7–9] have led, at least partly, to its use as an adulterant for cocaine. Levamisole is also popular as an adulterant due to its general availability, as it is used prophylactically for the treatment of animals [12]. It is therefore readily obtainable in large quantities in agrarian-based developing nations where cocaine is predominantly grown and produced. However, the non-regulated use of levamisole via the adulteration of cocaine has been shown to result in serious health issues in humans, such as neutropenia [13], agranulocytosis, and vasculitis [14].

Consequently, there is a need to monitor the use of levamisole in various complex sample matrices, including food, beverages, and biological samples, and in the forensic sciences. The UV absorbance maxima of levamisole occurs at a very low wavelength of around 215 nm [15–17], making its determination by UV-spectroscopy-based approaches difficult due to potential inferences from other sample components, such as albumin, creatinine, urea, and uric acid, which strongly absorb at wavelengths between 200 nm and 220 nm [18].

As far as we are aware, only a small number of reports have been made on the electrochemical behaviour of levamisole. Electrochemical investigations utilising voltammetry have also been shown to be problematic, as there is reportedly a marked interference between levamisole and cocaine [19,20]. Prior to this, early investigations using polarography [21] described the determination of levamisole in animal tissue as part of its racemate mixture, tetramisole hydrochloride. More recently, a potentiometric sensor has been described [22] based on a molecularly imprinted polymer. Cyclic voltammetric behaviour has previously been described on a screen-printed electrode [23] and on a pre-treated, boron-doped, diamond electrode [24]. These studies proposed that the electrochemical oxidation of levamisole proceeds through a two-electron, two-proton, oxidation step, leading to the oxidation of the sulphide group.

The aim of the present investigation was to develop a method based on the high-performance liquid chromatographic (HPLC) separation of levamisole with electrochemical detection (ED) for its determination in urine. We believe that this will overcome the previously reported issues of selectivity recorded by UV-spectrometry-based detection systems and the inference seen when using voltammetry. This present investigation was divided into two main sections. In the first part, we explored the voltammetric behaviour of levamisole on a glassy carbon electrode (GCE). We then optimised the HPLC reverse-phase separation of levamisole, initially employing UV detection and then using electrochemical detection, using the technique of hydrodynamic voltammetry to optimise the applied potential required for the determination of levamisole. The possibility of determining levamisole in artificial urine using the optimised HPLC ED conditions was explored.

2. Materials and Methods

2.1. Chemicals and Reagents

Unless otherwise stated, all chemicals and reagents were purchased from Fisher Scientific Ltd. (Loughborough, UK). Stock solutions of levamisole (Sigma-Aldrich, Dorset, UK) were prepared by dissolving the required mass in deionised water (Purite RO200—Stillplus HP System, Purite, UK) at a concentration of 1.0 mg/mL. Standards for initial studies were made via dilution of the stock solution in phosphate buffer or mobile phase.

2.2. Artificial Urine

Artificial urine was prepared following the method described by Khan et al. [25] by adding 0.1 g of potassium chloride, 4.0 g of sodium chloride, 0.57 g of anhydrous di-sodium

hydrogen orthophosphate, and 0.1 g of potassium dihydrogen orthophosphate to a 0.5 L volumetric flask. This was then made up to the mark with deionized water, following adjustment to approximately pH 6 with sodium hydroxide or hydrochloric acid. The solution was used immediately after preparation.

2.3. Cyclic Voltammetry

Cyclic voltammetric investigations were performed using a voltammetric cell comprising of a 3 mm diameter glassy carbon working electrode (GCE), an Ag/AgCl reference electrode in a 3.0 M KCl solution, and a platinum wire counter-electrode. Before use, the GCE was manually polished on a polishing mat modified with an aqueous slurry of 5 μm aluminium oxide. The GCE was then rinsed with deionised water and dried with a tissue. The electrodes were connected to a CompactStat potentiostat (Ivium, Eindhoven, The Netherlands), connected to a computer with an electrochemical system software package, Ivium software Windows 10 version, for data acquisition and control. Cyclic voltammetric investigations were then recorded in 0.1 M phosphate buffer, and then in the same solution containing 2.0 mM levamisole. A starting and end potential of 0.0 V vs. Ag/AgCl was used, with a switching potential of +2.5 V vs. Ag/AgCl.

2.4. High-Performance Liquid Chromatography

High-performance liquid chromatographic studies were undertaken using an Agilent 1200 infinity II HPLC system with a 150 mm × 4.6 mm Hypersil Gold C18, 5 μm column, thermostatically controlled at 30 °C, connected to a 7125-valve manual injector fitted with a 20 μL sample loop (Rheodyne, Cotati, CA, USA). Standards and sample extracts were determined using a mobile phase of 50% methanol (Fischer, Far UV, HPLC grade) 50% pH 8 50 mM phosphate buffer, at a flow rate of 1.0 mL/min.

2.5. Electrochemical Detection

The thin film amperometric detector cell was similar to that which we have previously described [26]. This consisted of an upper Kel-F block containing a 3 mm diameter GCE working electrode, which was bolted to a stainless-steel block acting as the pseudo-reference/counter-electrode. The upper Kel-F-containing GCE section and lower stainless pseudo-reference/counter-electrode were separated by Teflon gasket (BAS, Congleton, Cheshire, UK). The inlet for this amperometric thin layer cell was connected to the outlet of the Agilent 1100 UV detector via a suitable PEEK connector and tubing. The potential of cell was set at +2.3 V vs. the pseudo-reference/counter stainless-steel electrode (SS) and controlled by an Ivium CompactStat potentiostat (Ivium Technologies, Eindhoven, The Netherlands) interfaced with a PC, to ensure instrument control and data acquisition.

2.6. Hydrodynamic Voltammetry

Fixed volumes of a standard solution were injected and the applied potential of the amperometric thin layer cell varied over the range +1.2 V and +2.5 V (vs. SS) in 0.1 V steps. The recorded peak current was then plotted against the applied potential and the resulting hydrodynamic voltammogram was used to identify the optimum potential based the potential of the plateau of the hydrodynamic wave.

2.7. Analytical Application

Separate artificial urine samples, prepared following the method described in Section 2.2, were fortified with sufficient levamisole to concentrations of 1.25 μg/mL and 2.50 μg/mL, respectively, and diluted 1:1 in mobile phase. The resulting solutions were then introduced to the HPLC system, and the concentration of levamisole were determined using the optimised amperometric conditions.

2.8. Limit of Detection and Limit of Quantification

The limit detection and limit of quantification were calculated based on the assumption that the electrochemical response, y, is linearly related to the standard concentration, x, over a limited concentration range. This can be expressed using the following Equation (1):

$$y = mx + c \quad (1)$$

Therefore, the limit detection and limit of quantification can be expressed as follows:

$$\text{Limit detection} = 3\ \frac{Sa}{m} \quad (2)$$

$$\text{Limit of quantification} = 10\ \frac{Sa}{m} \quad (3)$$

where Sa is the standard deviation of the response and m is the slope of the calibration curve. The standard deviation of the response, y, was then estimated from the standard deviation of the y-residuals from the calculated regression line.

3. Results

3.1. Cyclic Voltammetric Behaviour of Levamisole

Figure 1a shows typical cyclic voltammograms, recorded in the absence and presence of 2.0 mM levamisole using a supporting electrolyte of 0.1 M pH 7.0 phosphate buffer. In the presence of levamisole, the voltammogram shows an oxidation peak at 1.3 V (Ag/AgCl). This was presumed to be the result of the two-electron, two-proton oxidation of the sulphur group. Although not a highly sensitive technique, cyclic voltammetry can provide important qualitative information about the redox processes of the analyte and its products [27]. Figure 1b shows the cyclic voltammetric behaviour of levamisole from pH 2 to pH 10. At a low pH, the electrochemical oxidation of levamisole is proposed to proceeds through a two-electron, two-proton oxidation, leading to the oxidation of the sulphur group [23,24]. However, no reports have been made on the voltammetric behaviour of levamisole under higher pH conditions. As we were interested in developing a reverse-phase HPLC method for levamisole, we focused our initial cyclic voltammetric studies on the range pH 2–10, where most reverse-phase columns are stable. In our present investigation, the cyclic voltammetric behaviour of levamisole was investigated for the pH range 2–10. No measurable peak could be obtained in our cyclic voltammetric investigations at pH 2. Plots of peak potential (E_p) vs. pH showed a 30 mV/pH unit slope between pH values 4 and 8 (Figure 2). At pH 8 and above, E_p values were found not to change with increasing pH. This is consistent with the pKa value of 7 reported for levamisole [28,29]. Above the pH value of 8, the oxidation of levamisole becomes independent of pH. It is believed that this could result from the hydrolysis of the thiazole ring, followed by electrochemical oxidation [30]. It should be noted that reports also cite pKa values of 8 [31,32] and 9.5 [20,33] for levamisole, which may help to explain why there was a shift in the peak potentials from pH 8 to 10 after the pKa value of 7.0.

In the pH range between 4 and 8, a slope of the 30 mV/pH unit was recorded. The Nernst equation (Equation (1)) predicts that such a slope is the result of an oxidation process involving a one-proton, two-electron oxidation.

$$E_p = E^{0'} - \frac{2.3\ RTp}{zF}\ \text{pH} \quad (4)$$

where E_p, is the peak potential, $E^{0'}$ is the formal oxidation potential, R is the universal gas constant, T is the temperature (kelvins), F is the Faraday constant, p is the number

of hydrogen ions, and z is the number of electrons. If the universal constant values are substituted into Equation (4), we can obtain Equation (5).

$$E_p = -0.059 \frac{p}{z} \text{ pH} \quad (5)$$

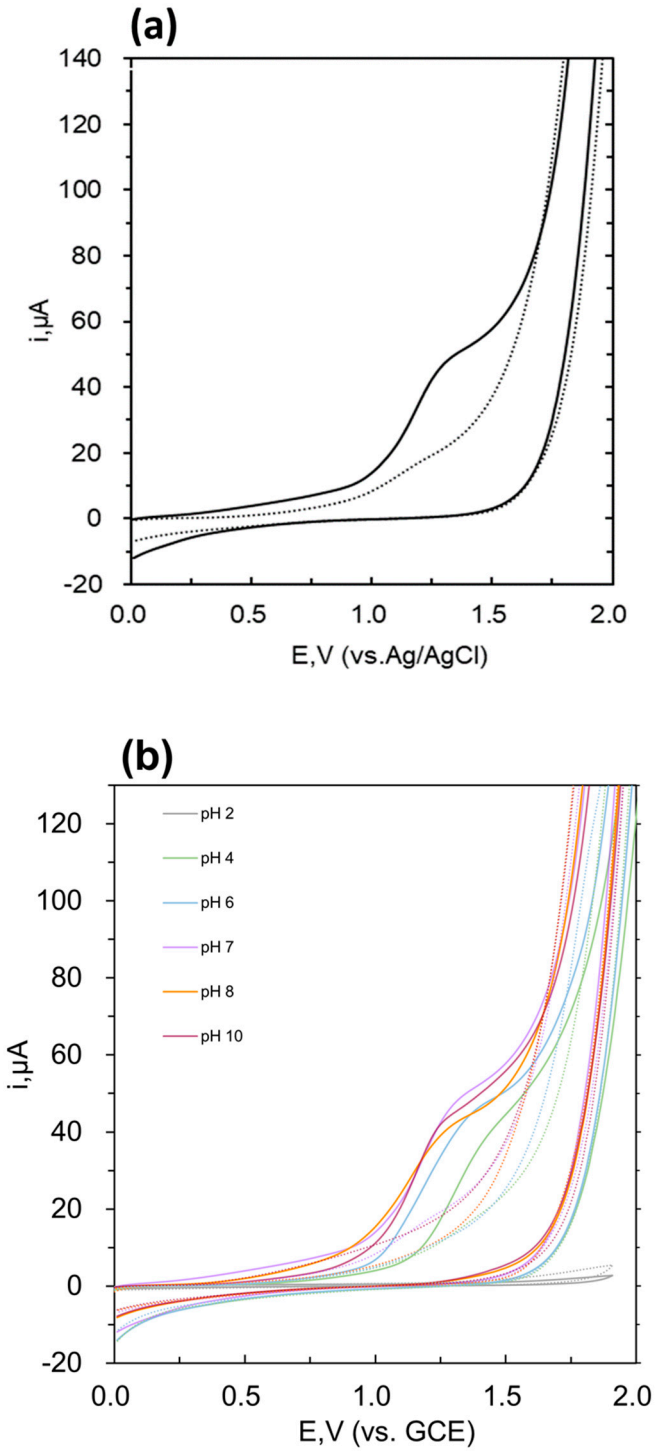


Figure 1. (**a**) Cyclic voltammograms obtained in the presence (solid line) and absence (dotted line) of 2 mM levamisole in 100 mM pH 7 phosphate buffer; (**b**) cyclic voltammograms obtained at pH 2, pH 4, pH 6, pH 7, pH 8, and pH 10 for 2 mM levamisole. Scan rate 50 mV/s; starting and end potential 0.0 V; switching potential +2.5 V. Only the applied potential section from 0.0 V to +2.0 V is shown.

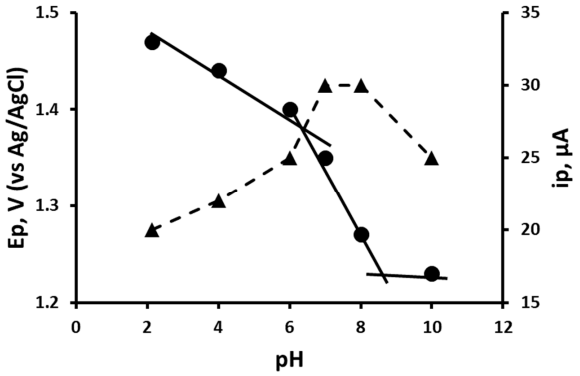
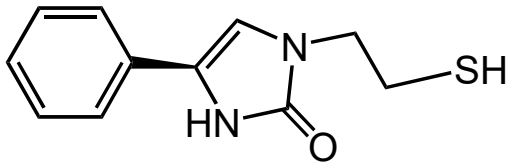


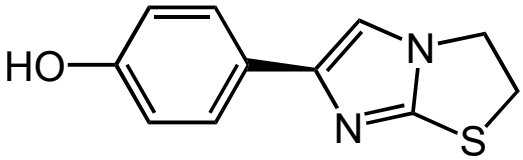
Figure 2. The effect of phosphate buffer pH on the resulting peak potential (E_p, ●) and peak current (I_p, ▲) of a 2.0 mM levamisole in 0.1 M phosphate buffer solution. Voltammetric conditions as shown in Figure 1. Levamisole concentration 2 mM.

Consequently, a plot of E_p values against pH for a reaction involving an equal number of hydrogen ions and electrons would give a slope equal to 0.059 V/pH unit. Reactions involving a greater number of electrons than hydrogen ions would give slopes that are fractions of the 0.059 V/pH unit. In this present investigation, plots of E_p in the range pH 4–8 gave a slope of 0.030 V/pH, indicating a voltammetric oxidation involving one proton and two electrons.

Studies on the chemical oxidation of levamisole have shown that (ii) is the main product that is formed [34]. The biological oxidation of levamisole has also led to a number of different products, including (ii) and its phenolic derivative (iii) [35], and the drugs aminorex and pemoline [10].



(ii)



(iii)

Masui et al. [36] have studied the voltammetric oxidation of a number of different amines. They postulated that oxidation proceeds via one-proton, two-electron oxidation to produce the corresponding aldehyde and primary amine. This matches the predicted behaviour that can be seen in this present study. Figure 3 presents the overall mechanism we propose for electrochemical oxidation, reported here for levamisole. Amjad et al. [37] studied the voltammetric behaviour of a number of compounds that are structurally related to levamisole, showing the voltammetric one-proton, two-electron oxidation of 2-amino-5-nitrothiazole. The protonated form of levamisole (iv), predominating at low pH values (pKa = 7), can be oxidised via two-electron, one-proton oxidation (vi). This initial oxidation reaction results in the formation of free radicals (v); which could lead to the possible formation of a dimer, as is commonly reported in a number of studies undertaken on related compounds [37–40].

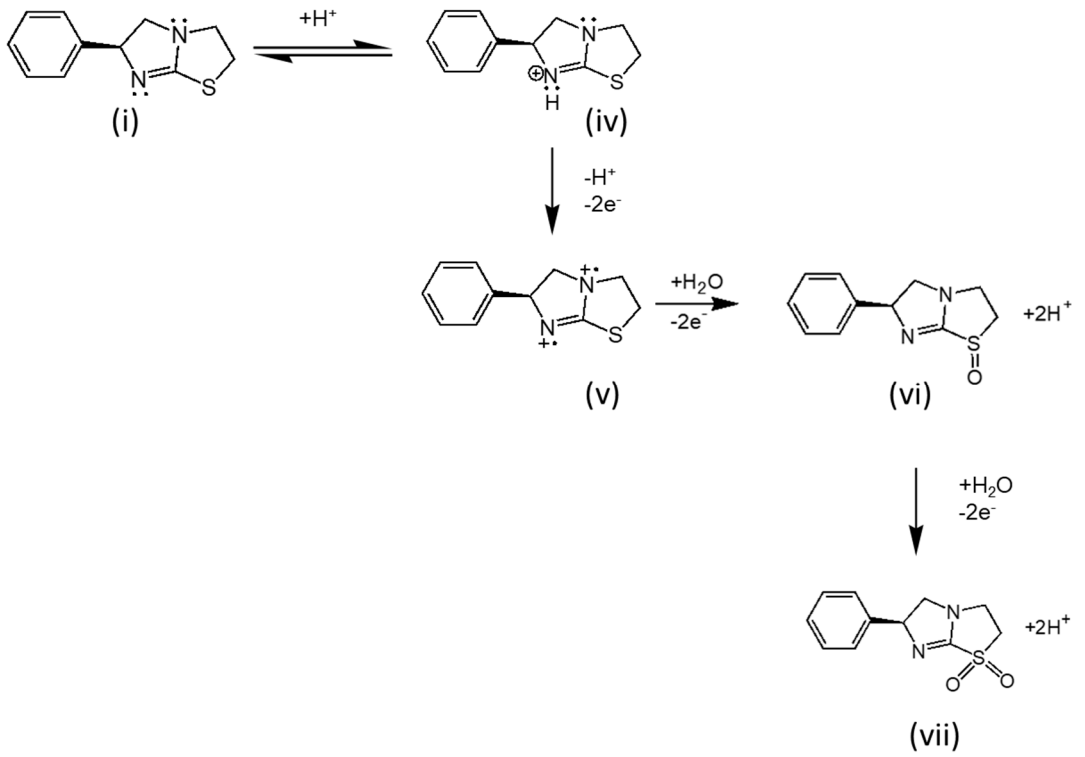


Figure 3. Electrochemical oxidation of levamisole.

3.2. Effect of pH on the Liquid Chromatographic Separation of Levamisole

Studies were first undertaken with a mobile phase comprising 50% methanol and 50% 0.1 M phosphate buffer over the pH range 2–8. The pH of the mobile phase was found to have a marked effect on the retention time of levamisole, and the relationship of capacity factor (k′) for levamisole with the pH of the mobile phase is shown in Figure 4. At pH values below 4, levamisole was found to be unretained, with a k′ below 0.25. This was considered to result from levamisole being in its polar, ionised form (pKa 7). Increasing the mobile phase pH resulted in an increase in retention times, with k′ values of 1.5 being recorded above mobile phase pH values of 6. As the maximum peak currents were recorded at pH 8, subsequent investigations were undertaken with a mobile phase of 50%, methanol 50%, 100 mM, pH 8 phosphate buffer.

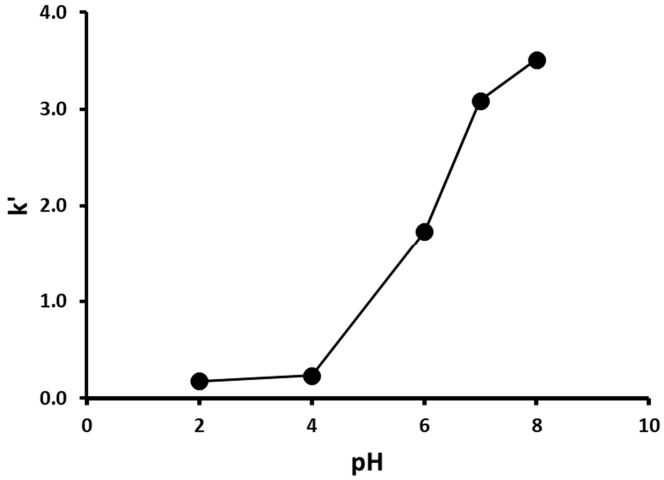


Figure 4. Effect of mobile phase pH on the capacity factor (k′) of levamisole. Each point is the mean of two separate runs.

3.3. Hydrodynamic Voltammetry

We next explored the possibility of utilising the electrochemical oxidation of levamisole following its HPLC separation using the optimised chromatographic conditions identified above. The HDV obtained over the potential range +1.2–+2.6 V (vs. SS) for levamisole is shown in Figure 5. The HDV exhibits two waves for the oxidation of levamisole, unlike the single oxidation peak recorded previously by cyclic voltammetry (Figure 1). It is believed that the second oxidation peak also occurred via cyclic voltammetry, but its presence was masked by the background current resulting from the oxidation of the water that was present as part of the supporting electrolyte.

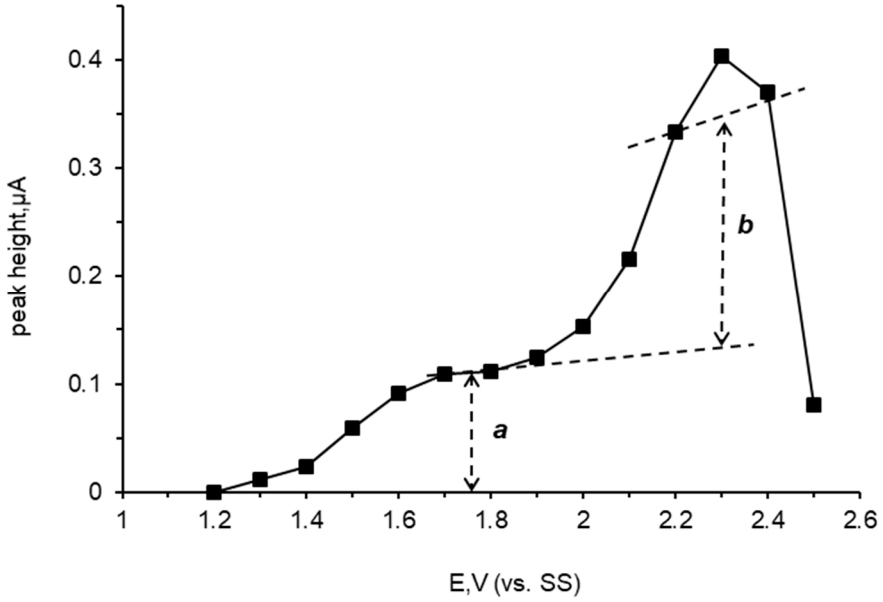


Figure 5. Hydrodynamic voltammogram obtained at flow rate of 1.0 mL/min for a 20 μL injection volume of 10 mg/L levamisole in 50% methanol, 50 mM, pH 8.0 phosphate buffer. Each point is the mean of two separate runs. (a) difference between 0 current and the current at first plateau; (b) distance between the current at first and second plateau. Applied potential (E_{app}) = +1.20–+2.50 V (vs. SS).

We believe that the first hydrodynamic voltammetric wave (a) results from the same two electron oxidation process that are recorded in the cyclic voltammetric investigations presented above. The second hydrodynamic voltammetric wave (b) is double the current magnitude and is consequently believed to result from a further four-electron oxidation.

Previous reports [23,24] have postulated that the voltammetric oxidation of levamisole progresses via the two-electron, two-proton oxidation of the thiazole sulphur to produce sulfoxide (vi).

It is possible that this group can be oxidised further, via the loss of two more electrons to the corresponding sulfone (vii). The overall reaction that forms (viii) would result in a four-electron oxidation step. This would give the same relative current ratios seen in our HDV study (Figure 5) if a two-electron oxidation, as predicted by our cyclic voltammetric investigation, is correct.

The optimum oxidation potentials for the determination of levamisole were considered to be either +1.8 V or +2.3 V (vs. SS) based on the two wave maxima that were recorded. Both applied potentials offer possible advantages, with the higher potential offering the possibility of greater sensitivity, and the lower offering improved selectivity. Consequently, additional investigations were made into the relative analytical advantages of using either potential of +1.8 V and +2.3 V (vs. SS).

3.4. Calibration Plot, Limit of Detection, and Precision

Figure 6 shows the chromatograms obtained for a series of standard solutions of levamisole, over the range 1.0–20 mg/L, in mobile phase, determined using the optimized HPLC-ED conditions. A linear response was recorded over the entire investigated range. The limits of detection, and quantification, based on signal-to-noise ratios of 3 and 10, respectively, were found to be 0.27 mg/L and 0.72 mg/L.

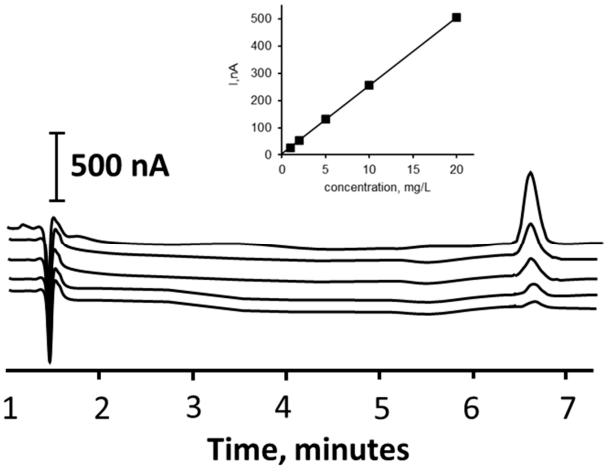


Figure 6. Chromatograms obtained for injections of 1.0, 2.0, 5.0, 10, and 20 mg/L levamisole. Inset: calibration curve for standard levamisole solutions. Applied potential E = +2.30 V (vs. SS); mobile phase: 50 mM pH 8.0 phosphate buffer solution, methanol (50:50 *v*/*v*) at a flowrate of 1.0 mL/min; and an injection volume of 20 µL.

3.5. Studies of Possible Interferences

Common drugs reported as cocaine adulterants were investigated using the optimised HPLC-ED method that we developed (Figure 7). To provide the equivalent of a ten-fold concentration ratio compared to that of levamisole, 10 mg/L of paracetamol, aspirin, caffeine, benzocaine, phenacetin, and lidocaine were investigated as possible interferences. Caffeine did not show a response under the optimised conditions. All of the investigated compounds were found to have electrochemical oxidative responses, but were chromatographically separated from levamisole.

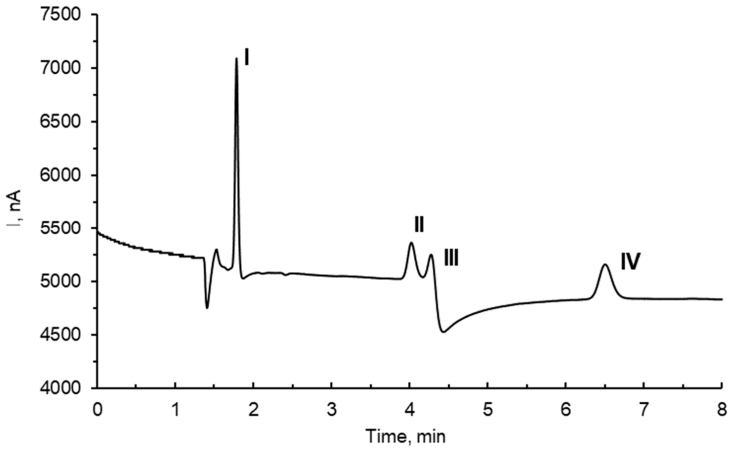


Figure 7. Chromatogram obtained by HPLC-ECD for 2 ppm mixed standard solutions. Applied potential = +2.30 V (vs. SS). Well-defined peaks obtained within the retention times of 0–8 min: (I) paracetamol at 1.76 min; (II) phenacetin at 4.02 min; (III) benzocaine at 4.28 min; (IV) levamisole at 6.55 min.

4. Analytical Application

To assess the performance of our optimized-HPLC ED approach, five replicate determinations on artificial urine, fortified with 1.25 mg/L and 2.5 mg/L levamisole, were undertaken. Aliquots of the samples were placed in artificial urine (Section 2.2). Quantification was achieved by external calibration with a mean recovery of 99.6% (%CV = 8.2%. HorRat = 0.53 [41], n = 5), and a mean recovery of 94.6% (%CV = 10.8%, HorRat = 0.78 [41], n = 5) was obtained for 1.25 mg/L and 2.50 mg/L, respectively. The data summarized in Table 1 suggest that similarly good performance characteristics could be achieved in real human urine samples, as we showed previously for the determination of p-nitrophenol, using a similar approach [42].

Table 1. Recovery and precision data for levamisole obtained on artificial urine samples.

Sample	Native, mg/L	Added mg/L	%Rec
1	ND	1.25	100.3
2	ND	1.25	100.3
3	ND	1.25	87.5
4	ND	1.25	97.1
5	ND	1.25	113
		Mean	99.6
		SD	8.14
		%CV	8.2
		HorRat	0.53
6	ND	2.33	93.0
7	ND	2.13	85.1
8	ND	2.17	86.7
9	ND	2.84	113.7
10	ND	2.37	94.6
		Mean	94.6
		SD	10.2
		%CV	10.8
		HorRat	0.78

5. Conclusions

An assay utilising HPLC with amperometric detection was successfully developed for the determination of levamisole in artificial urine using a C_{18} reverse-phase column with a mobile phase of pH 8, phosphate buffer, and methanol. This approach offers advantages over UV, as its absorbance maxima is at very low wavelength of around 215 nm [15–17], making its determination by spectroscopy difficult due to the inferences from other sample components. Direct electrochemical investigations utilising voltammetry, have also been shown to be problematic, as there is reportedly a marked interference between levamisole and cocaine [19,20]. The utilisation of a chromatographic separation step, as was developed in this report, can overcome these issues. Cyclic voltammetric studies showed a $2e^-$, $2H^+$ oxidation, postulated to be the oxidation of the thiazole sulphur of levamisole, which produces sulfoxide. Hydrodynamic voltammetric studies showed the same oxidation process, as well as a further, previously unreported oxidation process, which is believed to result from the oxidation of this species via the loss of two more electrons to the corresponding sulfone.

Author Contributions: Conceptualization, K.C.H.; methodology, K.C.H.; software, K.C.H. and T.Y.J.C.; validation, K.C.H. and T.Y.J.C.; formal analysis, K.C.H. and T.Y.J.C.; investigation, T.Y.J.C.; resources, K.C.H.; data curation, T.Y.J.C.; writing—original draft preparation, K.C.H. and T.Y.J.C.; writing—review and editing, K.C.H.; supervision, K.C.H.; project administration, K.C.H.; funding acquisition, K.C.H. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Keiser, J.; Utzinger, J. Efficacy of current drugs against soil-transmitted helminth infections: Systematic review and meta-analysis. *JAMA* **2008**, *299*, 1937–1948. [CrossRef]
2. World Health Organization. *Model List of Essential Medicines*, 21st ed.; World Health Organization: Geneva, Switzerland, 2019.
3. Janssen, P.A.J. The Levamisole Story. In *Progress in Drug Research/Fortschritte der Arzneimittelforschung/Progrés des Recherches Pharmaceutiques*; Jucker, E., Ed.; Birkhäuser: Basel, Switzerland, 1976; Volume 20. [CrossRef]
4. Firozabad, A.R.; Meybodi, Z.A.; Mousavinasab, S.R.; Sahebnasagh, A.; Jelodar, M.G.; Karimzadeh, I.; Habtemariam, S.; Saghafi, F. Efficacy and safety of Levamisole treatment in clinical presentations of non-hospitalized patients with COVID-19: A double-blind, randomized, controlled trial. *BMC Infect. Dis.* **2021**, *21*, 297. [CrossRef]
5. Keim, C.K.; Schwartz, R.A.; Kapila, R. Levamisole-induced and COVID-19-induced retiform purpura: Two overlapping, emerging clinical syndromes. *Arch. Dermatol. Res.* **2023**, *315*, 265–273. [CrossRef]
6. Lee, K.C.; Ladizinski, B.; Federman, D.G. Complications associated with use of levamisole-contaminated cocaine: An emerging public health challenge. *Mayo Clin. Proc.* **2012**, *87*, 581–586. [CrossRef] [PubMed]
7. Vanhoutte, P.M.; Nueten, J.M.V.; Verbeuren, T.J.; Lapuron, P.M. Differential effects of the isomers of tetnamisole on adrenergic neunotransmission in cutaneous veins of dog. *J. Pharmacol. Exp. Ther.* **1977**, *200*, 127–140.
8. Brugmans, J.; Van Lommel, R.; Baro, F. Clinical aspects of tetramisole, an anthelmintic with antidepressant activity. *Psychopharmacology* **1972**, *26*, 85.
9. Bobon, J.; Bobon, D.P.; Bourdouxhe, S.; Pinchard, A. A pilot open trial of dexamisole (R 12.563) in retarded depression. *J. Int. Med. Res.* **1974**, *2*, 171–174. [CrossRef]
10. Gutierrez, J.; Eisenberg, R.L.; Koval, N.J.; Armstrong, E.R.; Tharappel, J.; Hughes, C.G.; Tobin, T. Pemoline and Tetramisole 'Positives' in English racehorses following Levamisole administration. *Ir. Vet. J.* **2010**, *63*, 498–500. [CrossRef]
11. Hofmaier, T.; Luf, A.; Seddik, A.; Stockner, T.; Holy, M.; Freissmuth, M.; Ecker, G.F.; Schmid, R.; Sitte, H.H.; Kudlacek, O. Aminorex, a metabolite of the cocaine adulterant levamisole, exerts amphetamine like actions at monoamine transporters. *Neurochem. Int.* **2014**, *73*, 32–41. [CrossRef] [PubMed]
12. Waller, P.J. From discovery to development: Current industry perspectives for the development of novel methods of helminth control in livestock. *Vet. Parasitol.* **2006**, *139*, 1–14. [CrossRef]
13. Knowles, L.; Buxton, J.A.; Skuridina, N.; Achebe, I.; Legatt, D.; Fan, S.; Zhu, N.Y.; Talbot, J. Levamisole Tainted Cocaine Causing Severe Neutropenia in Alberta and British Columbia. *Harm Reduct. J.* **2009**, *6*, 30. [CrossRef] [PubMed]
14. Larocque, A.; Hoffman, R.S. Levamisole in cocaine: Unexpected news from an old acquaintance. *Clin. Toxicol.* **2012**, *50*, 231–241. [CrossRef] [PubMed]
15. Misquith, J.; Dias, A. Validated Ultra Violet Spectroscopic Methods for The Determination of Levamisole Hydrochloride. *Inter. J. Pharm. Tech Res.* **2012**, *4*, 1631–1637.
16. Syed, S.M.; Farooqui, Z.S.; Gawale, K.; Hamde, D. A Validated UV Spectroscopic Method for determination of Levamisole HCl. *Int. J. Pharm. Res. Appl.* **2020**, *5*, 97–101.
17. Sowjanya, S.; Devadasu, C. Development of RP-HPLC Method for the Simultaneous Quantitation of Levamisole and Albendazole: Application to Assay Validation. *Int. J. Anal. Chem.* **2018**, *2018*, 5746305. [CrossRef]
18. Lin, T.-J.; Yen, K.-T.; Chen, C.-F.; Yan, S.-T.; Su, K.-W.; Chiang, Y.-L. Label-Free Uric Acid Estimation of Spot Urine Using Portable Device Based on UV Spectrophotometry. *Sensors* **2022**, *22*, 3009. [CrossRef]
19. de Jong, M.; Sleegers, N.; Kim, J.; Van Durme, F.; Samyn, N.; Wang, J.; DeWael, K. Electrochemical fingerprint of street samples for fast on-site screening of cocaine in seized drug powders. *Chem. Sci.* **2016**, *7*, 2364–2370. [CrossRef]
20. Rocha, R.G.; Stefano, J.S.; Arantes, I.V.S.; Ribeiro, M.M.A.C.; Santana, M.H.P.; Richter, E.M.; Munoz, R.A.A. Simple Strategy for Selective Determination of Levamisole in Seized Cocaine and Pharmaceutical Samples Using Disposable Screen-printed Electrodes. *Electroanalysis* **2019**, *31*, 153–159. [CrossRef]
21. Holbrook, A.; Scales, B. Polarographic determination of tetramisole hydrochloride in extracts of animal tissue. *Anal. Biochem.* **1967**, *18*, 46–53. [CrossRef]
22. Sadeghi, S.; Fathi, F.; Abbasifar, J. Potentiometric sensing of levamisole hydrochloride based on molecularly imprinted polymer. *Sens. Actuators B Chem.* **2007**, *122*, 158–164. [CrossRef]

23. Stefano, J.S.; Dias, A.C.; Arantes, I.V.S.; Costa, B.M.C.; Silva, L.A.J.; Richter, E.M.; Banks, C.E.; Munoz, R.A.A. Batch-injection Amperometric Analysis on Screen-printed Electrodes: Analytical System for High-throughput Determination of Pharmaceutical Molecules. *Electroanalysis* **2019**, *31*, 518–526. [CrossRef]
24. Lourencao, B.C.; Medeiros, R.A.; Thomasi, S.S.; Ferreira, A.G.; Rocha-Filho, R.C.; Fatibello-Filho, O. Amperometric flow-injection determination of the anthelmintic drugs ivermectin and levamisole using electrochemically pretreated boron-doped diamond electrodes. *Sens. Actuators B Chem.* **2016**, *222*, 181–189. [CrossRef]
25. Khan, L.; Read, H.; Ritchie, S.; Proft, T. Artificial Urine for Teaching Urinalysis Concepts and Diagnosis of Urinary Tract Infection in the Medical Microbiology Laboratory. *J. Microbiol. Biol. Educ.* **2017**, *18*. [CrossRef]
26. Peng, X.; Giltrow, D.; Bowdler, P.; Honeychurch, K.C. Liquid Chromatography Electrochemical Determination of Nicotine in Third-Hand Smoke. *Electroanalysis* **2017**, *29*, 374–379. [CrossRef]
27. Monteiro, M.C.; Winiarski, J.P.; Santana, E.R.; Szpoganicz, B.; Vieira, I.C. Ratiometric Electrochemical Sensor for Butralin Determination Using a Quinazoline-Engineered Prussian Blue Analogue. *Materials* **2023**, *16*, 1024. [CrossRef]
28. Tyrpenou, A.E.; Xylouri-Frangiadaki, E.M. Determination of Levamisole in Sheep Muscle Tissue by High-Performance Liquid Chromatography and Photo Diode Array Detection. *Chromatographia* **2006**, *63*, 321–326. [CrossRef]
29. Robertson, S.J.; Martin, R.J. The Activation of Nicotinic Acetylcholine Receptors in the Nematode Parasite Ascaris suum by the Application of Levamisole to the Cytoplasmic Surface of Muscle Membrane. *Pestic. Sci.* **1993**, *37*, 293–299. [CrossRef]
30. Wahyuni, W.T.; Putra, B.R.; Marken, F. Voltammetric detection of vitamin B1 (thiamine) in neutral solution at a glassy carbon electrode via in situ pH modulation. *Analyst* **2020**, *145*, 1903–1909. [CrossRef] [PubMed]
31. El-Kholy, H.; Kemppainen, B.; Ravis, W.; Hoerr, F. Pharmacokinetics of levamisole in broiler breeder chickens. *J. Vet. Pharmacol. Ther.* **2006**, *29*, 49–53. [CrossRef] [PubMed]
32. Robertson, A.P.; Bjorn, H.E.; Martin, R.J. Resistance to levamisole resolved at the single-channel level. *FASEB J.* **1999**, *13*, 749–760. [CrossRef] [PubMed]
33. Zubenia, N.; Kormosh, Z.; Semenyshyn, D.; Kochubei, V.; Korolchuk, S.; Savchuk, T. Design and application of a levamisole-selective membrane sensor. *Anal. Bioanal. Electrochem.* **2016**, *8*, 466–477.
34. Dickinson, N.A.; Hudson, H.E.; Taylor, P.J. Levamisole: Its Stability in Aqueous Solutions at Elevated Temperatures. *Analyst* **1971**, *96*, 235–243. [CrossRef]
35. Wolford, A.; McDonald, T.S.; Eng, H.; Hansel, S.; Chen, Y.; Bauman, J.; Sharma, R.; Kalgutkar, A.S. Immune-mediated agranulocytosis caused by the cocaine adulterant levamisole: A case for reactive metabolite(s) involvement. *Drug Metab. Dispos.* **2012**, *40*, 1067–1075. [CrossRef]
36. Masui, M.; Sayo, H.; Tsuda, Y. Anodic oxidation of amines. Part I. Cyclic voltammetry of aliphatic amines at a stationary glassy-carbon electrode. *J. Chem. Soc. B* **1968**, *1968*, 973–976. [CrossRef]
37. Amjad, U.; Lashin, A.; Abbasi, R.; Rana, U.A.; Al-Arifi, N.; Khan, G.S.; Ali, S.; Kraatz, H.-B.; Shaha, A. pH and Temperature Responsive Electrooxidation of Thiazole Derivatives, and Preliminary Screening of Their Antioxidant Activity. *J. Electrochem. Soc.* **2016**, *163*, H350–H358. [CrossRef]
38. Jordan, P.H.; Hart, J.P. Voltammetric behaviour of morphine at a glassy carbon electrode and its determination in human serum by liquid chromatography with electrochemical detection under basic conditions. *Analyst* **1991**, *116*, 991–996. [CrossRef]
39. Garrido, J.M.P.J.; Delerue-Matos, C.; Borges, F.; Macedo, T.R.A.; Oliveira-Brett, A.M. Voltammetric Oxidation of Drugs of Abuse III. Heroin and Metabolites. *Electroanalysis* **2004**, *16*, 1497–1502. [CrossRef]
40. Teófilo, K.R.; Arantes, L.; Marinho, P.A.; Macedo, A.; Pimentel, D.M.; Rocha, D.P.; de Oliveira, A.C.; Richter, E.; Muñoz, R.; dos Santos, W.T. Electrochemical detection of 3,4-methylenedioxymethamphetamine (ecstasy) using a boron-doped diamond electrode with differential pulse voltammetry: Simple and fast screening method for application in forensic analysis. *Microchem. J.* **2020**, *157*, 105088. [CrossRef]
41. Horwitz, W.; Albert, R. The Horwitz Ratio (HorRat): A Useful Index of Method Performance with Respect to Precision. *J. AOAC Int.* **2006**, *89*, 1095–1109. [CrossRef] [PubMed]
42. Honeychurch, K.C.; Hart, J.P. Voltammetric Behavior of p-Nitrophenol and Its Trace Determination in Human Urine by Liquid Chromatography with a Dual Reductive Mode Electrochemical Detection System. *Electroanalysis* **2007**, *19*, 2176–2184. [CrossRef]