Voltammetric Behaviour and Determination of Clonazepam Using a Disposable Screen-Printed Sensor and Its Determination in Serum and Wine

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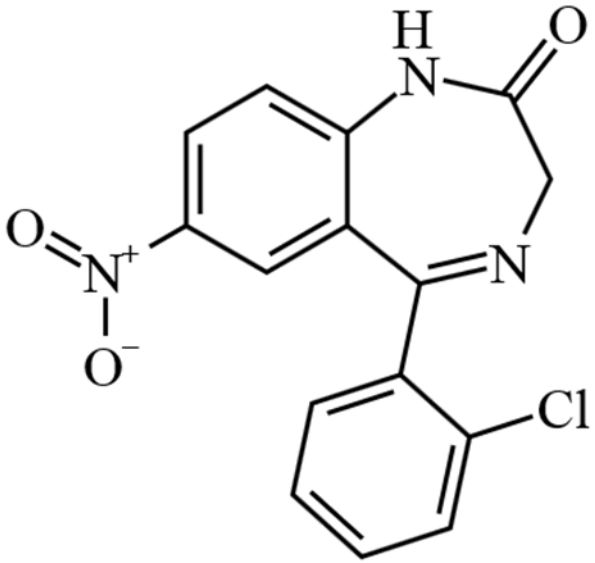
Outline of Talk

- Clonazepam
- Screen-printed Carbon Electrodes
- Cyclic Voltammetry
- Gas chromatography mass spectrometry
- Adsorptive Stripping Voltammetry
- Sample Preparation and Analysis
- Conclusions

Clonazepam

Clonazepam (5-(2-chlorophenyl)-7-nitro-2,3-dihydro-1,4-benzodiazepin-2-one)



Nitro substituted 1,4-benzodiazepine

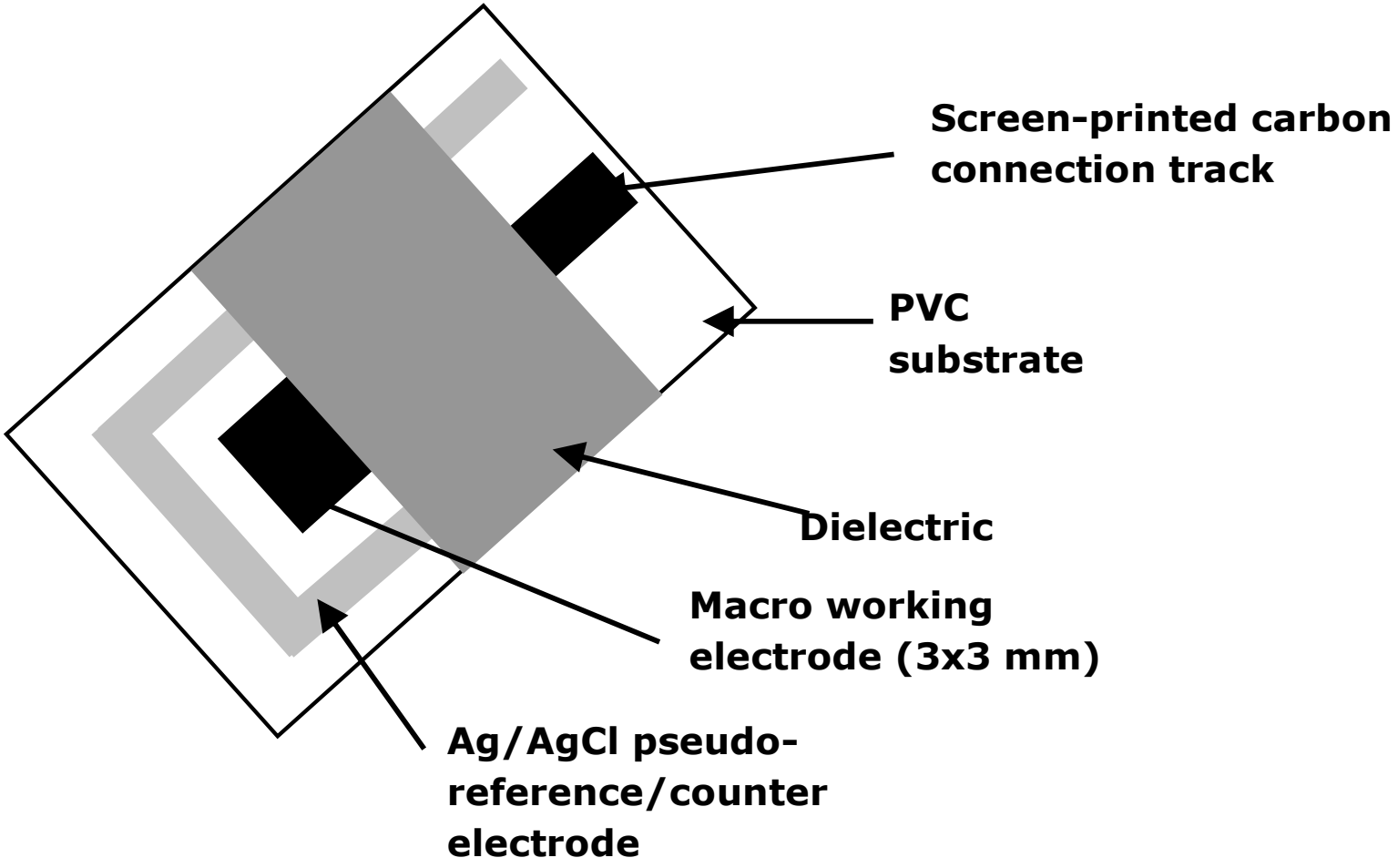
One of the more common benzodiazepines

Prescribed for:

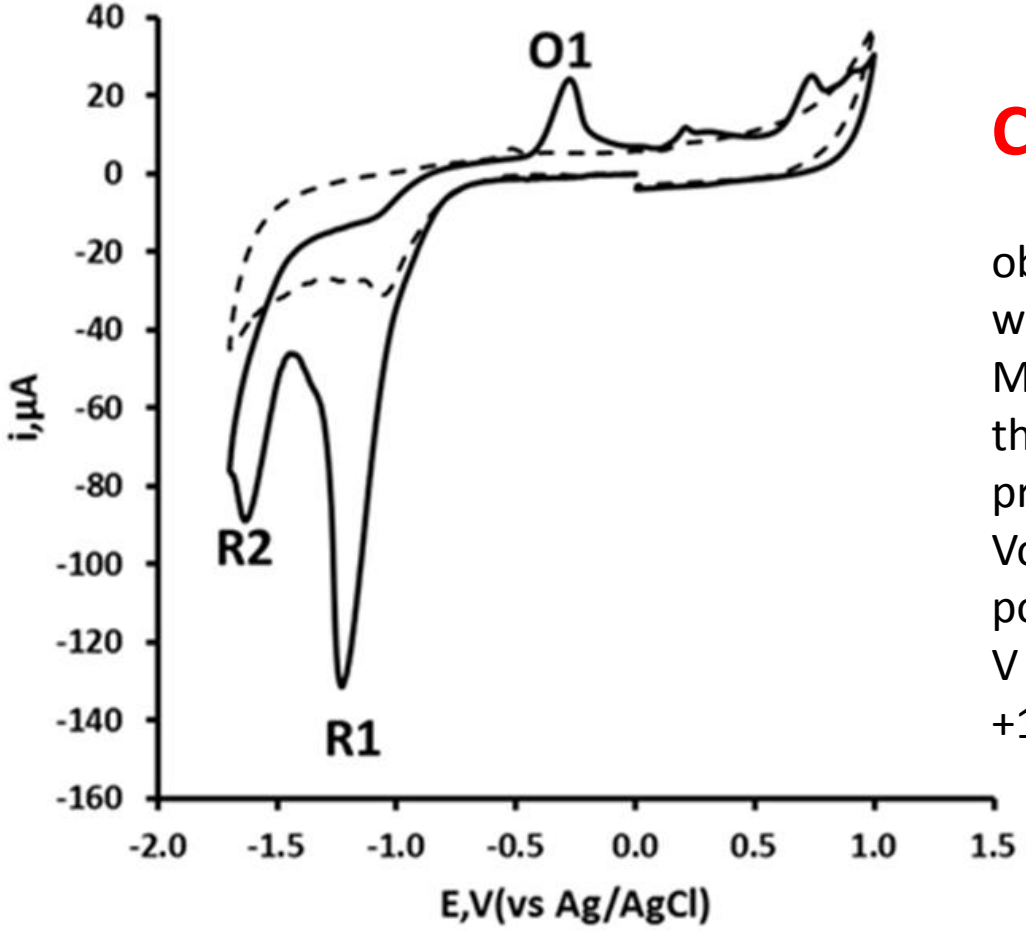
sleep disturbance, depression, anxiety and panic disorders, forms of parasomnia, obsessive–compulsive disorder, seizures, certain types of migraines and epilepsy

Used illegally and has been reported in cases of sexual assault

Screen-printed Carbon Electrode (SPCE)



Cyclic Voltammetry



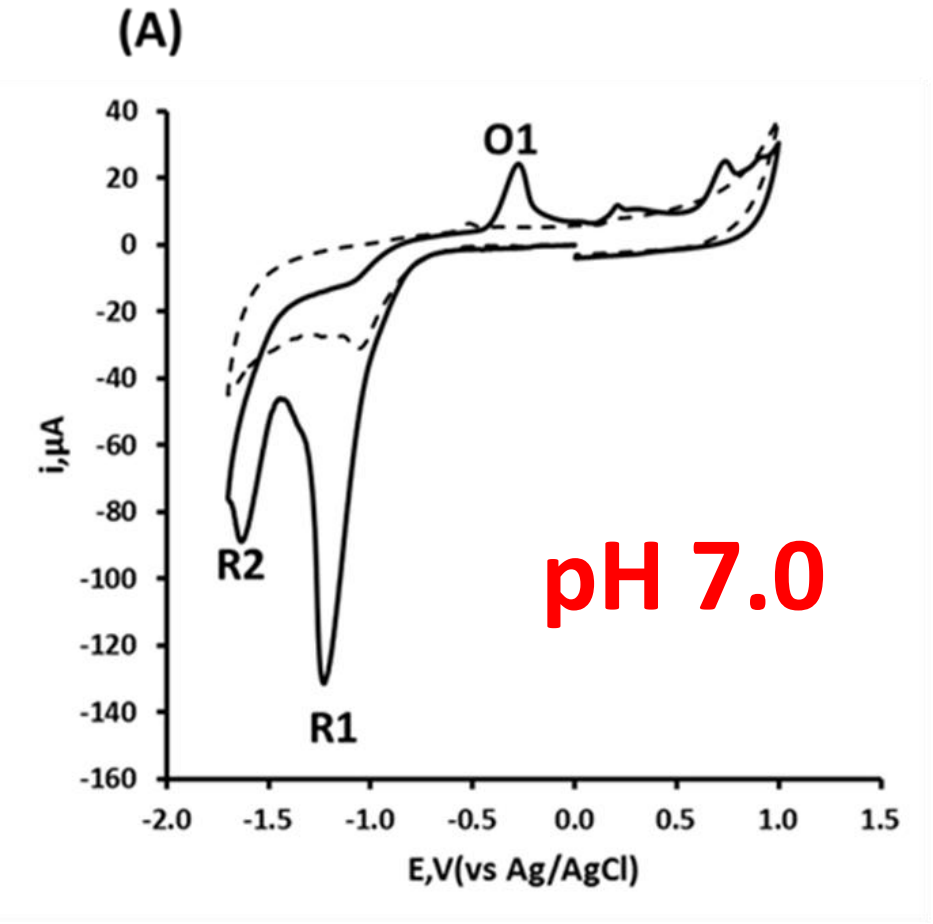
Cyclic Voltammetry

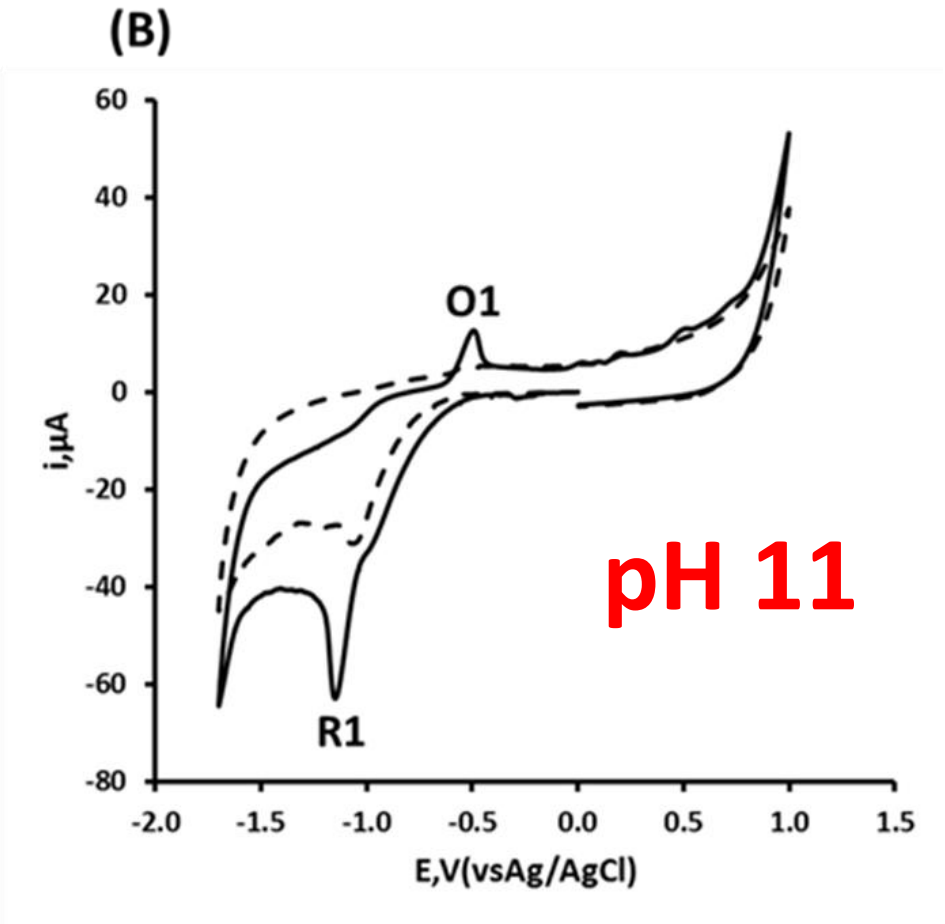
obtained using a scan rate of 50 mV/s with 10 % ethanol, buffered with 0.1 M phosphate at pH 7.0, dashed line in the absence of and solid line in the presence of 1 mM clonazepam. Voltammetric conditions: starting potential 0.0 V, initial switching, -1.7 V and second switching potential +1.0.

$R\text{-}NO_2 + 4e^- + 4H^+ \rightarrow R\text{-}N(H)OH + H_2O$ **(R1)**

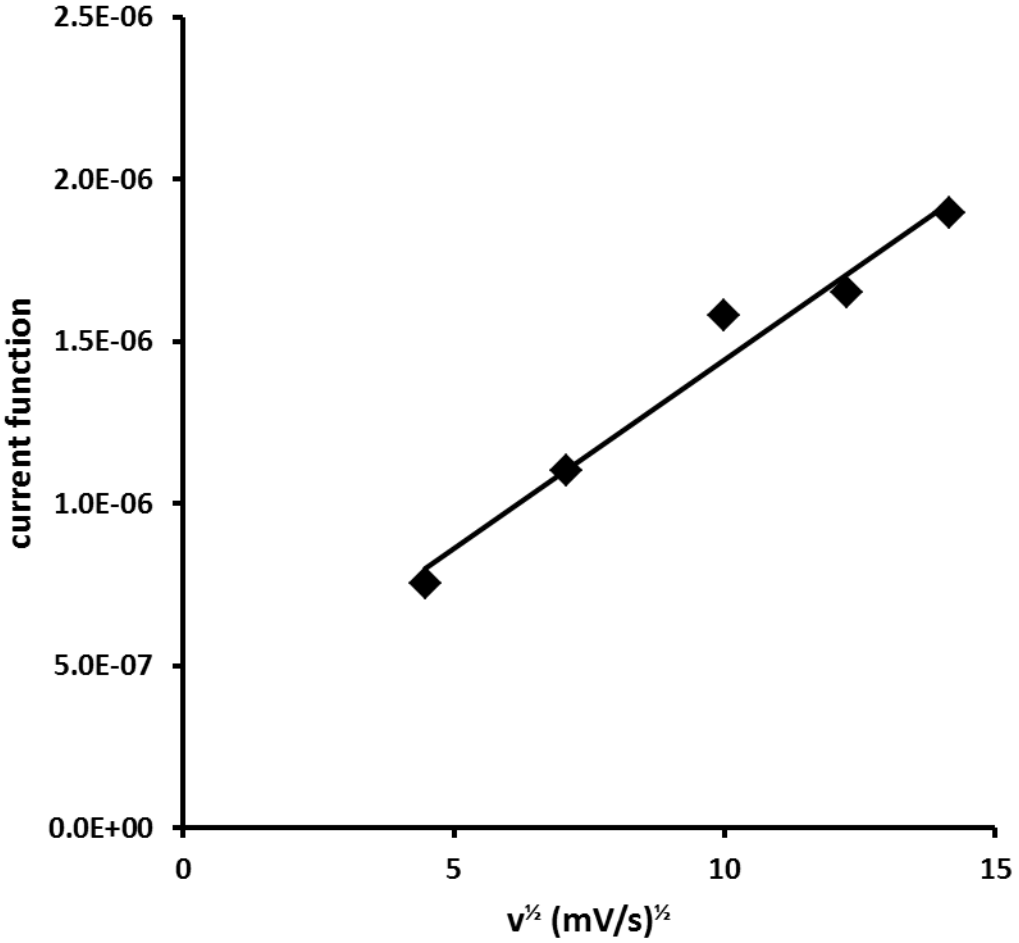
$R\text{-}C{=}N\text{-}R' + 2e^- + 2H^+ \rightarrow R\text{-}C(H)\text{-}N(H)\text{-}R'$ **(R2)**

$R\text{-}N(H)OH \rightarrow R\text{-}N{=}O + 2e^- + 2H^+$ **(01)**





Cyclic voltammograms obtained using a scan rate of 50 mV/s with 10 % ethanol, 0.1 M phosphate at (A) pH 7.0 and (B) pH 11, dashed line in the absence of and solid line in the presence of 1 mM clonazepam. Voltammetric conditions: starting potential 0.0 V, initial switching, -1.7 V and second switching potential +1.0 V.

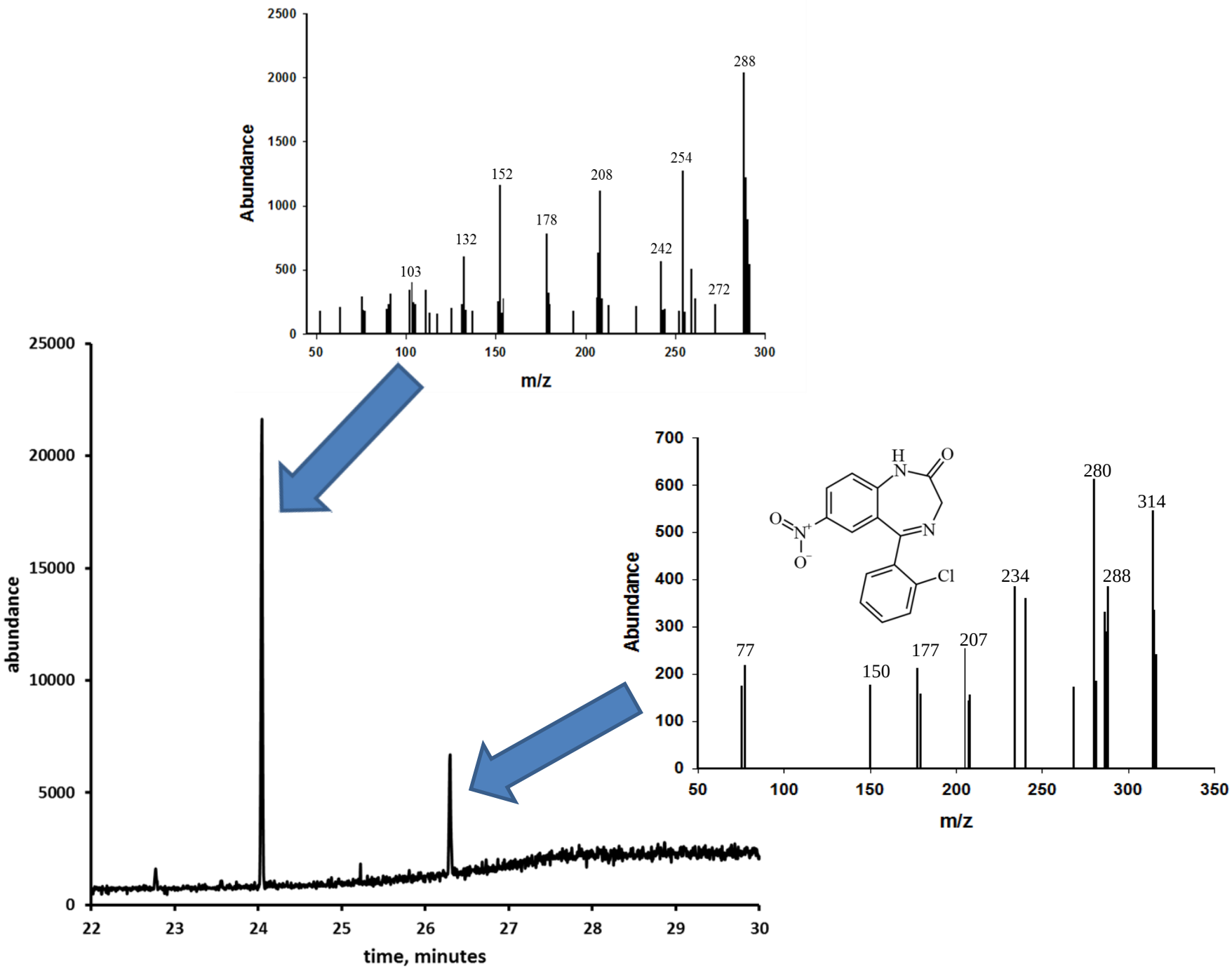


ip ≠ v½

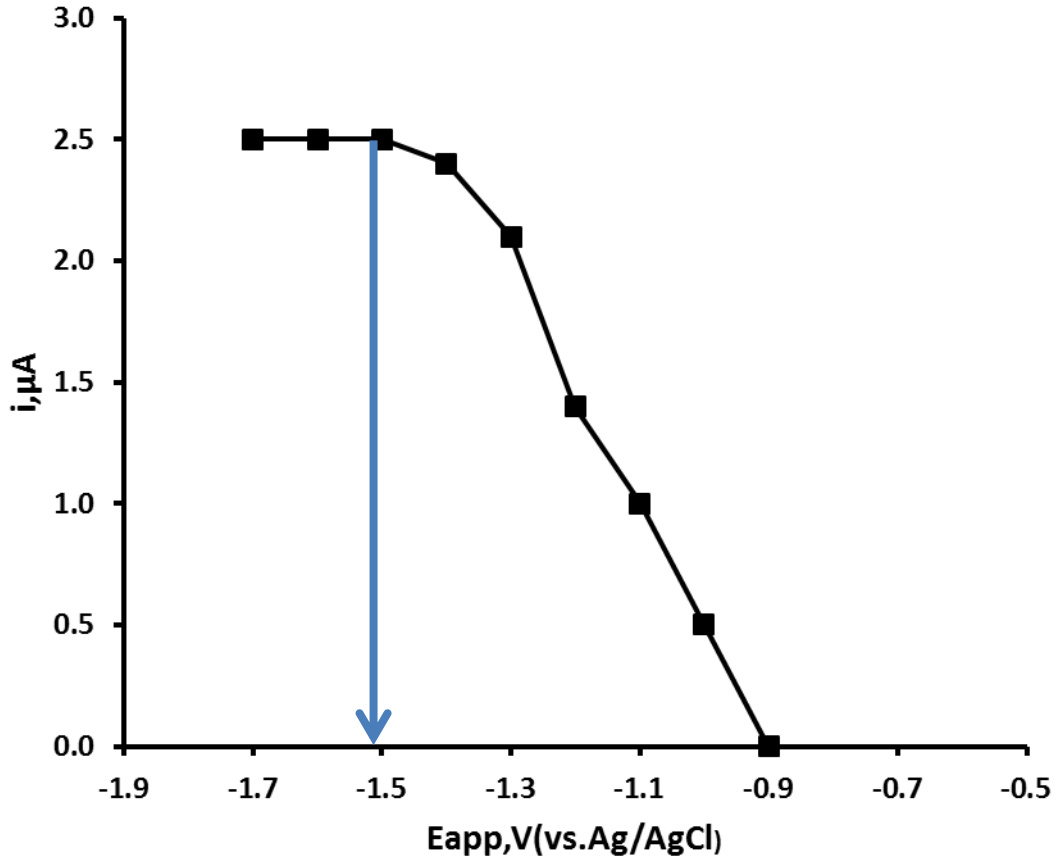
Positive slope. Indicative of reactant adsorption

Current function plot for pH 11 O1 peak.

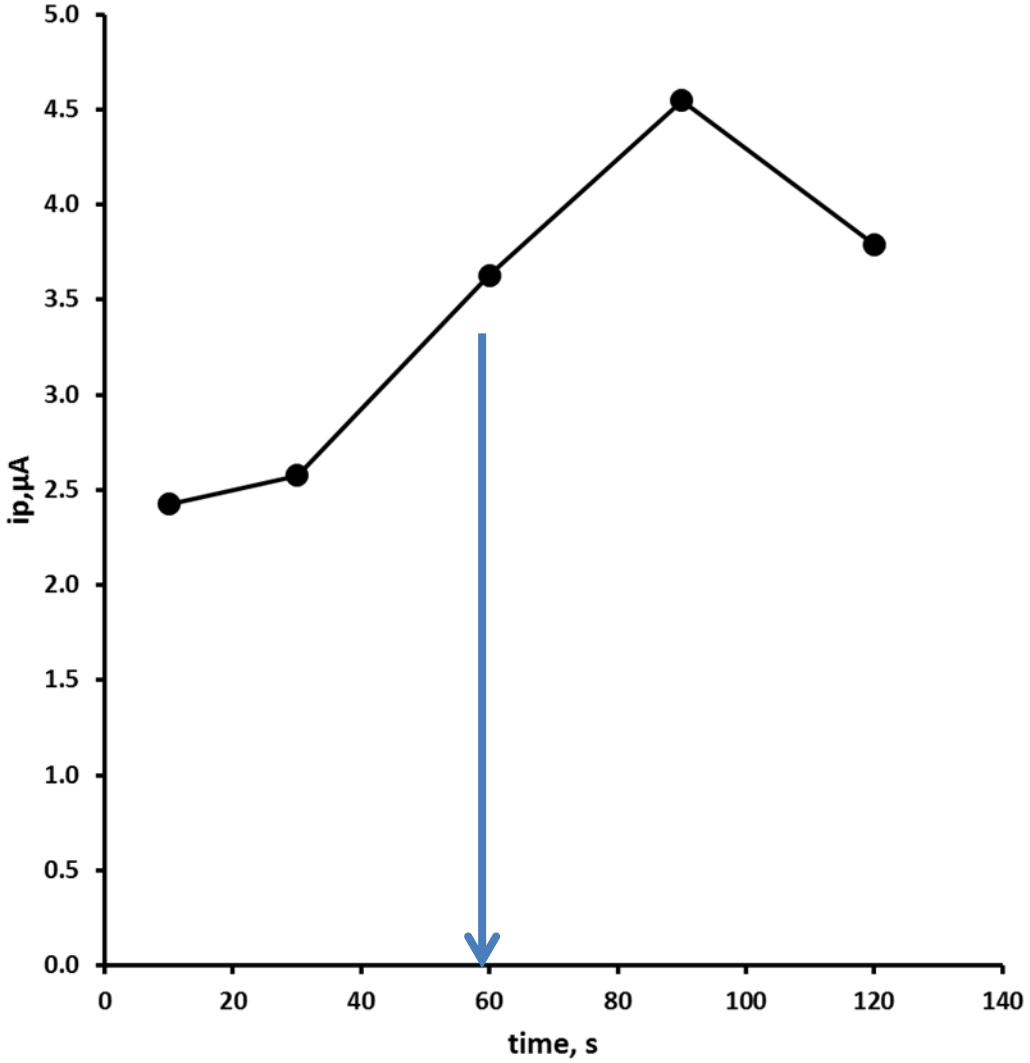
Gas chromatography mass spectrometry


Abundance
m/z
288
254
152
208
178
132
242
103
272
abundance
time, minutes
Abundance
m/z
280
314
288
234
207
177
77
150
Cl
H
N
O
N
O
O

Adsorptive Stripping Voltammetry



Effect of accumulation potential on 0.1 mM clonazepam O1 oxidation peak obtained by linear sweep voltammetry at pH 11. A new SPCE was used for each determination.



Effect of accumulation time on 0.1 mM clonazepam O1 oxidation peak obtained by linear sweep voltammetry at pH 11. A new SPCE was used for each determination.

Differential Pulse Adsorptive Stripping Voltammetry Performance Characteristics

- The calibration plot was linear over the range 2.05 – 8.00 µg/mL (R^2 = 0.984), with a sensitivity of 21.8 nA/µg/mL

- The theoretical detection limit, defined as three times the mean baseline noise was calculated to be 1.96 µg/mL.

- A coefficient of variation of 7.4 % was obtained for a 7.1 mg/L solution of clonazepam.

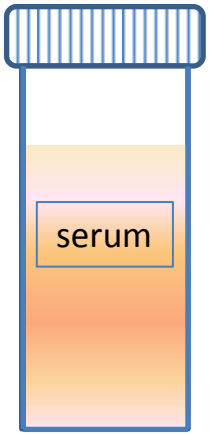
Evaluation Using Wine and Serum

Sample Preparation

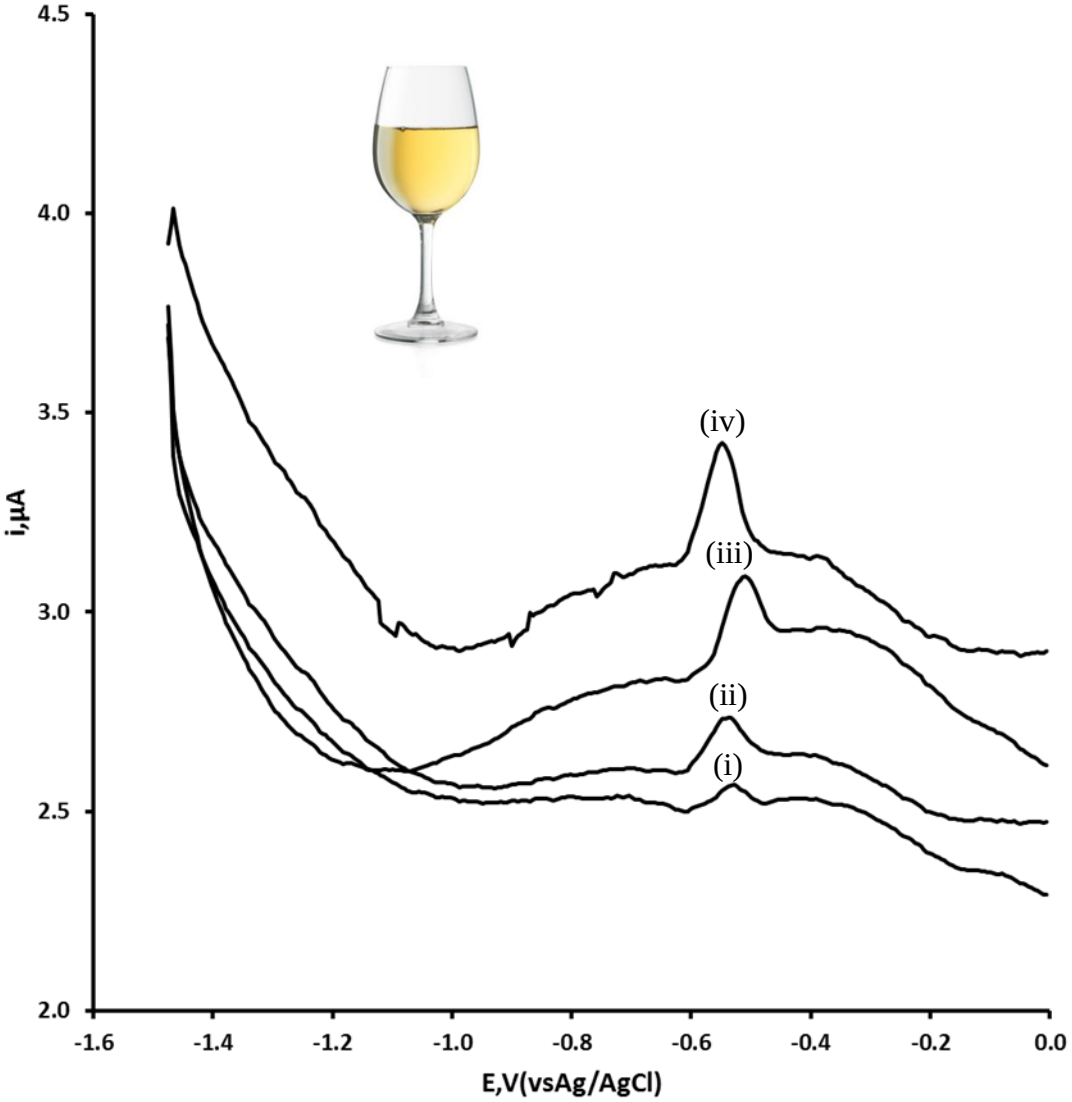
Wine samples

- A 25 mL aliquot of wine was treated with 1.9 g of trisodium phosphate to produce a concentration of 0.2 M of this salt.
- A 5 mL aliquot of this mixture was diluted with sufficient water and ethanol to give a 0.1 M phosphate/10 % ethanol solution.
- A 100 µL aliquot of this solution was examined directly on the strip using the optimised DPAdSV conditions.
- The concentration of clonazepam was determined using the method of multiple standard additions.

Serum Samples

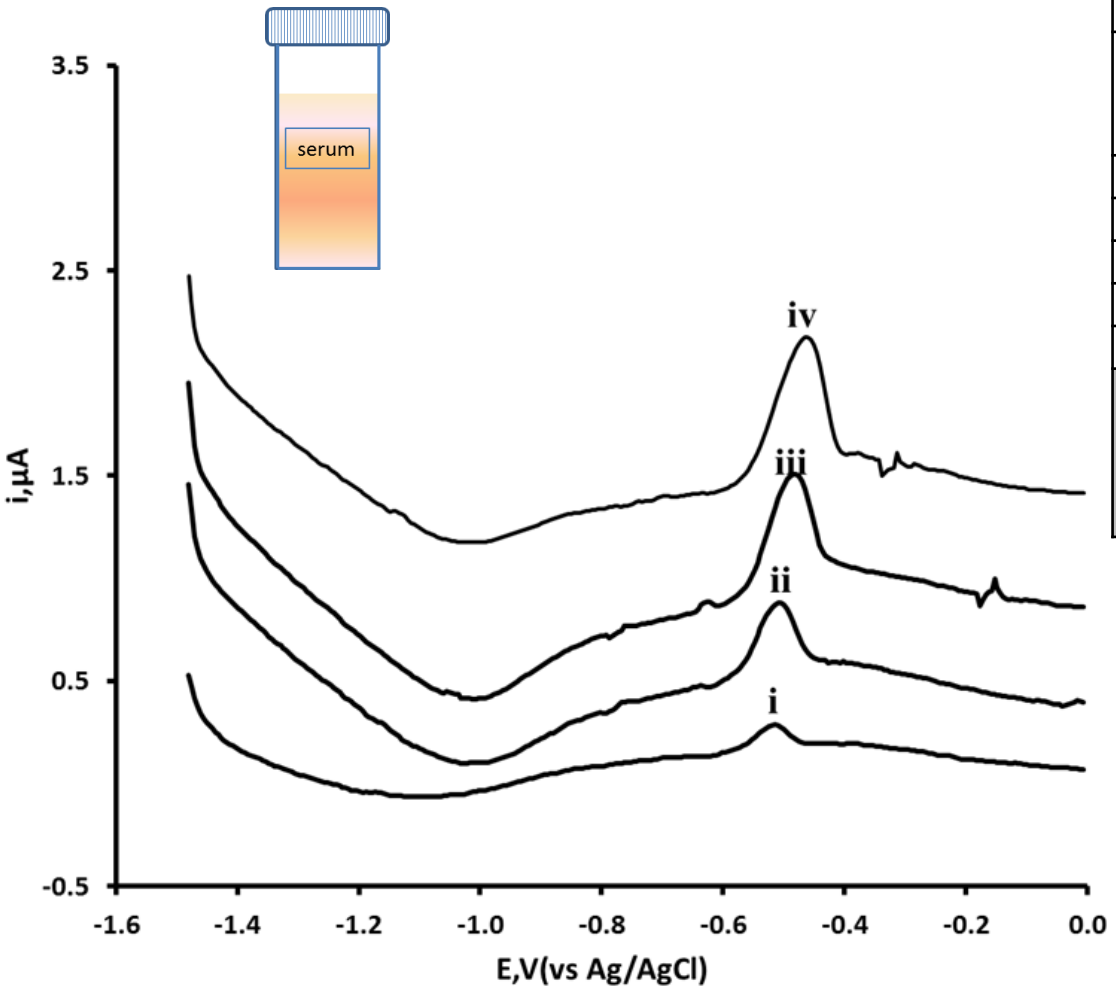


- Serum samples were extracted with ethyl acetate
- Blown down to dryness under nitrogen
- reconstituted in the optimised supporting electrolyte.
- These were then measured in the same manner as described for the white wine samples



White Wine			
Original concentration (µg/mL)	Fortified Concentration (µg/mL)	Detected Concentration (µg/mL)	Recovery (%)
ND	3.16	2.44	77.4
ND	3.16	2.67	84.6
ND	3.16	2.62	82.9
ND	3.16	2.66	84.4
ND	3.16	2.45	77.7
ND	3.16	2.87	90.9
ND	3.16	2.66	84.4
		Mean	**83.2**
		Standard Deviation	4.62
		Coefficient of Variation	5.6

Adsorptive stripping voltammetry of white wine fortified with 20 µM clonazepam standard addition of (i) 0 µM (ii) 10 µM, (iii) 20 µM and (iv) 30 µM. Conditions: -1.5 V for 60 s, 100 µL sample volume. Differential pulse voltammetric conditions as described in Section 2.3. A new SPCE was used for each determination.



Serum			
	Fortified Concentration (µg/mL)	Detected Concentration (µg/mL)	Recovery (%)
ND	12.63	11.38	90.1
ND	12.63	9.69	76.7
ND	12.63	9.17	72.6
ND	12.63	13.50	106.9
ND	12.63	11.97	94.8
		Mean	**88.2**
		Standard Deviation	**13.9**
		Coefficient of Variation	**15.8**

Adsorptive stripping voltammetry of serum fortified with 12.63 µg/mL clonazepam. Standard addition of (i) 0 µg/mL (ii) 3.16 µg/mL, (iii) 6.31 µg/mL and (iv) 9.47 µg/mL. Conditions: -1.5 V for 60 s, 100 µL sample volume. A new SPCE was used for each determination.

Conclusions

- A simple and rapid method for the determination of clonazepam in wine and serum samples by DPAdSV in conjunction with SPCE sensors has been successfully developed.
- Using an applied potential of -1.5 V for 60 s, concentrations as low as 2.0 mg/L could be readily be determined in white wine and serum.
- A theoretical detection limit of 1.96 μg/mL was found.
- The proposed method gives reliable results using the standard addition method.
- In future studies we will investigate the possibility of using this technique to determine other drugs and nitro compounds.

Acknowledgements

- The authors would like to thank the HEFCE for funding. They are grateful to Gwent Electronic Materials Ltd for supplying the screen-printed sensors.

Honeychurch, K. C., Brooks, J. and Hart, J. P. (2016) Development of a voltammetric assay, using screen-printed electrodes, for clonazepam and its application to beverage and serum samples, *Talanta*, 147, 510-515.