Development and Validation of an Adsorptive Stripping Voltammetric Method for the Quantification of Vincamine in Its Formulations and Human Serum Using a Nujol-Based Carbon Paste Electrode

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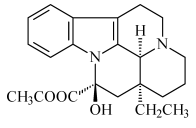
An easy, rapid and selective adsorptive stripping voltammetry (AdSV) method for the determination of vincamine in its formulation and human serum was developed and validated. It was based on the oxidation of the drug onto a Nujol-based carbon paste electrode. The stripping step was carried out by using a square-wave (SW) potential-time voltammetric excitation signal. The optimal experimental variables as well as accumulation parameters were investigated as; frequency f=120 Hz, scan increment ΔE_i=10 mV, pulse-amplitude ΔE_a=25 mV and an accumulation potential E_{acc} of 0.0 V using a Britton–Robinson (B-R) universal buffer of pH 5 as a supporting electrolyte. After validation of the described method, it was applied for determination of vincamine in its formulation and human serum. Mean recovery of 100.41±0.74 (n=5) was achieved for assay of vincamine in Oxybral® capsules. Limits of detection and quantitation of 6.0×10^{-9} M ($2.20\,ng\,ml^{-1}$) and 2×10^{-8} M ($7.33\,ng\,ml^{-1}$) vincamine were achieved in human serum with a mean recovery of 99.5±1.79%, without prior extraction of the drug. No interferences were observed in formulation and/or human serum. Due to high sensitivity and specificity of the developed method, it was successfully applied for evaluating some pharmacokinetic parameters of two healthy volunteers after administration of a single oral Oxybral® capsule.

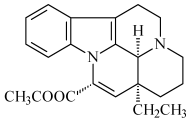
Key words vincamine; square-wave; quantification; stripping voltammetry; carbon paste electrode; Oxybral® capsule

Vincamine, methyl (3α,14β,16α)-14,15-dihydro-14-hydroxy-eburnamienine-14-carboxylate (Chart 1), is one of the main alkaloids of *Vinca minor* plant, a member of the periwinkle family. Vincamine naturally occurs in the D-form and it is a white powder soluble in acid medium but insoluble in alkaline medium.[1] It is claimed to have a selective vaso-regulatory action on cerebral circulation and adopting cerebral blood flow to metabolic needs.[2] Vincamine enhances cerebral metabolism by increasing oxidative decomposition of glucose, increasing energy production leading to an increase in general activity of the human being. Vincamine is quickly absorbed after oral administration, reaching the initial half-life after approximately 2 h, followed by a slower elimination phase. The distribution of vincamine is extensive and it can also pass the blood–brain barrier (BBB).[3] Vincamine is mainly metabolized in the liver and excreted by the kidneys and it may also go through cerebral metabolism.[4] Vinpocetine, ethyl (3α,16α)-eburnamenine-14-carboxylate (Chart 1), is a semi-synthetic derivative of vincamine with the same biological activity.

Various analytical methods have been reported for the quantitation of vincamine in different matrices (*e.g. Vinca* extracts, pharmaceutical formulations, plasma and urine). These methods include spectrophotometry,[5—7] titrimetry,[6] selected ion monitoring,[7] gas chromatography (GC)[9,10] and liquid chromatography (LC).[11] High-performance liquid chromatography (HPLC) with photodiode array detection,[5] UV detection,[12—15] voltammetric detection[16] and mass spectrometric detection[17] was also reported for the assay of vincamine. Most of the previously reported methods require extraction of the drug from its formulations and/or biological fluids prior to the analysis.



Vincamine



Vinpocetine

Chart 1

This paper describes a validated square wave adsorptive anodic stripping voltammetric method for determination of vincamine in its formulation and human serum without the necessity for prior drug extraction.

Experimental

Apparatus All voltammetric measurements were performed with Princeton Applied Research (PAR, Princeton, Oak Ridge, TN, U.S.A.) Potentiostats Models 273 A and 263 A. A voltammetric cell consisting of a C-2 stand with a carbon paste working electrode (BAS Model MF-2010, 3 mm in diameter and 1 mm in depth, Fig. 1), an Ag/AgCl/saturated KCl reference electrode (BAS Model MF-2063) and a platinum wire counter electrode (BAS Model MW-1032) was used. A magnetic stirrer (PAR-305) with a teflon-coated star-shaped magnet was used to provide the convective transport during the preconcentration step. The data were treated through a personal computer connected to the potentiostat and loaded with the 270/250 Research Electrochemistry software version 4.41—copyright© 2000 (PerkinElmer Instruments Inc.).

A Mettler balance (Toledo-AB104, Greifensee, Switzerland) was used for weighing the solid materials. A pH-meter (Crison, Barcelona, Spain) was used for the pH measurements. An Eppendorf centrifuge (Model 5417 C, Hamburg, Germanny) was used for separation of the precipitated proteins from human serum samples prior to the assay. A micopipetter (Eppendorf—Multipette® plus) was used for transfer of the analyte solutions throughout the present experimental work. The de-ionized water used throughout the present study was supplied from a Purite-Still Plus de-ionizer connected to an AquaMatic bi-distillation water system (Hamilton Laboratory Glass LTD., Kent, U.K.).

Reagents and Solutions. 1. Supporting Electrolytes Britton–Robinson (B-R) universal buffer (pH 2—11), acetate buffer (pH 3.8—6.2), phosphate buffer (pH 2—7.5), potassium chloride (0.1 M) and potassium nitrate (Analytical grade) were prepared in de-ionized water and were used as supporting electrolytes. All the chemicals were of analytical grade and were used without further purifications.

2. Solutions of Bulk Vincamine and Vinpocetine Bulk vincamine was kindly supplied from Glaxo-Smith-Kline (GSK), El Salam City, Cairo,

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Egypt. Bulk vinpocetine was kindly supplied from Medical Union Pharmaceuticals (MUP), Abu-sultan, Ismailia, Egypt. Standard stock solutions of 5×10^{-3} M bulk vincamine and vinpocetine were prepared in methanol (Merck) and stored in dark bottles at 4 °C. The desired solutions (1×10^{-6}—1×10^{-4} M) were prepared by appropriate dilution of the standard stock solutions with methanol.

3. Solutions of Oxybral® Capsules The contents of five Oxybral® capsules (Glaxo-Smith-Kline, El Salam City, Cairo, Egypt) as labeled to containing 30 mg vincamine/capsule were weighed and the average mass of a single capsule was determined, then grounded to a homogeneous fine powder in a mortar. A quantity of the powder equivalent to 30 mg of vincamine was transferred accurately into a 100 ml calibrated flask contains 70 ml methanol (Merck). The content of the flask was sonicated for about 15 min and completed to volume with methanol. The solution was then filtrated through a 0.45 μm milli-pore filter (Gelman, Germany) to separate out the insoluble excipients, rejecting the first portion of the filtrate. The desired concentrations of the drug were obtained by accurate dilution with methanol. The solutions were directly analyzed, according to the general analytical method without extraction of vincamine prior to the analysis.

4. Solutions of Human Serum Serum samples of two healthy volunteers were collected then mixed and stored frozen until assay. Samples of the human serum (each of 1 ml) were fortified with various concentrations (2×10^{-8}—1×10^{-6} M) of vincamine in small tubes. Each of these samples was then completed to 2 ml with methanol to denature and precipitate proteins. After vortexing each of the serum samples for two min., the precipitated proteins were separated by centrifugation for 3 min at 14000 rpm. The clear supernatant layer was filtered through a 0.45 μm milli-pore filter to obtain protein-free human serum samples. Then the analysis was followed up as indicated in the general analytical procedure.

Preparation of the Carbon Paste Electrodes The carbon paste was prepared by thoroughly hand mixing 5 g of graphite powder (Aldrich, Milwaukee, WI, US 1—2 μm) with 1.8 ml of Nujol (Sigma, d=0.84 g/ml) or castor oil (ADWIC, Egypt) in an agate mortar with pestle. The Nujol-based or castor oil-based paste was packed into the well of the electrode and its surface was manually smoothed by polishing on clean paper. The carbon paste electrode (CPE) was then immersed in the supporting electrolyte placed in the electrolysis cell and several sweeps were applied to obtain a low background current. Both Nujol-based CPE (Nj-CPE) and castor oil-based CPE (Ct-CPE) were used to obtain the optimal one.

For electrode surface re-generation after recording each voltammogram, the CPE was transferred into a blank electrolyte solution in the electrolysis cell and series of cyclic scans were continued until a voltammogram corresponding to the residual current was obtained. The electrode was then ready for use in the next measurement.

General Analytical Procedure A 10-ml volume of the B-R universal buffer containing the analyte was introduced into the micro-electrolysis cell and a selected accumulation potential was then applied to the prepared CPE for a selected accumulation time period, while the solution was stirred at 400 rpm. At the end of the accumulation time, the stirring was stopped and a 5 s rest period was allowed for the solution to become quiescent. The voltammogram was then recorded by scanning the potential towards the positive direction using the square-wave potential waveform. Medium exchange method[18] was used during determination of vincamine in the human serum, where the experiment was held after the preconcentration step and the electrode was then transferred into a blank electrolyte solution to proceed the stripping step. This procedure enables to avoid blocking of the electrode surface with low molecular weight proteins which may remain after centrifugation. Moreover, the perfect choice of the optimal preconcentration potential decreases the possibility of adsorption of the remained low molecular weight proteins at the electrode surface.

To study the reproducibility, accuracy and precision of the described method for determination of vincamine in bulk form and pharmaceutical formulation, recovery experiments were carried out by means of both the calibration curve and standard addition methods. All the data were obtained at room temperature.

Pharmacokinetic Studies Pharmacokinetics of vincamine was studied in plasma of two healthy male volunteers following a single oral Oxybral® capsule labeled to contain 30 mg vincamine. Subjects were caffeine and alcohol free for at least 12 h before the administration of the drug and all over the counter medications were avoided for 24 h prior to the study. The two volunteers gave their written informed consent prior to anticipating in the study (at Ramadan Specialized Hospital, Tanta City, Egypt). Blood samples (1 ml each) were collected before initiation and at 0.5, 1.0, 1.5, 2, 3, 4, 8 and 12 h after the oral administration. The blood samples were centrifuged immediately at 3000 rpm for 15 min and then the plasma fractions were rapidly separated and stored in coded polypropylene tubes at −20 °C until the assay. Following separation of proteins by methanol, the plasma samples were analyzed using the described square-wave stripping voltammetric procedure.

Results and Discussion

Choice of the Working Electrode The anodic cyclic voltammograms for the oxidation of vincamine in Britton–Robinson buffer were recorded at both Nujol-based and castor oil-based carbon paste electrodes (Fig. 1). It is clear that the characteristics of the voltammetric response strongly depends on the composition of the electrode.[19] At Nj-CPE, the peak potential shifted about 40 mV more positive than that of Ct-CPE with almost double peak current intensity. Therefore, Nj-CPE was used as a working electrode in this analytical study.

Electrochemical Oxidation of Vincamine at Nj-CPE Cyclic voltammetry was applied as a diagnostic tool to get information about electrochemical oxidation of vincamine onto a Nj-CPE in the B-R universal buffer of pH (2—11). In the forward scan, a single well-defined anodic peak was observed over the whole pH range, while no peaks were observed in the cathodic scan; pointing to the irreversible nature of the oxidation process. The peak current intensity recorded without preconcentration (t_{acc}=0 s) was practically pH-independent (Fig. 2, a), while after preconcentration of the drug for 180 s, the peak current intensity was much enhanced at pH values 5—6 (Fig. 2, b). This behavior suggested a higher rate of adsorption of vincamine within this pH range. The peak potential shifted to less positive values on the increase of pH of the medium (Fig. 2, c) denoting that protons are involved in the electrode reaction process and that the proton-transfer reaction precedes the electrode process.[20] The E_p *vs.* pH plot consists of two straight portions within pH ranges 2—5 and 5—11 (Fig. 2, c) with slope values of 46 and 22 mV/pH unit, respectively.

Figure 3 showed two cycles (a, b) for 5×10^{-6} M vincamine solution in a B-R universal buffer of pH 5 at scan rate of 0.10 V s^{-1} following preconcentration of the drug by adsorptive accumulation onto the Nj-CPE at 0.0 V (*vs.* Ag/AgCl/KCl$_s$). The peak current decreases with succeeding potential scans suggesting adsorptive behavior of vincamine onto the

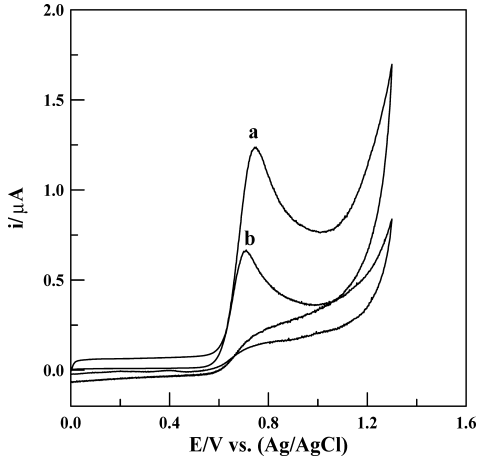

Fig. 1. Cyclic Voltammograms of 5×10^{-6} M Vincamine in a B-R Universal Buffer of pH 5 Following Its Preconcentration onto (a) Nj-CPE and (b) Ct-CPE (E_{acc}=0.0 V, t_{acc}=180 s and Scan Rate=0.10 V s^{-1})

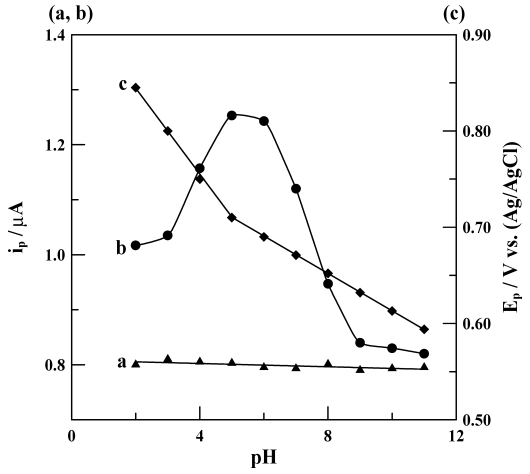

Fig. 2. Influence of pH of the B-R Universal Buffer on the Cyclic Voltammetric Response of 5×10^{-6} M Vincamine; (a) Peak Current (i_p) without Preconcentration, (b) Following Preconcentration onto Nj-CPE by Adsorptive Accumulation at 0.0 V for 180 s and (c) on the Peak Potential (E_p)

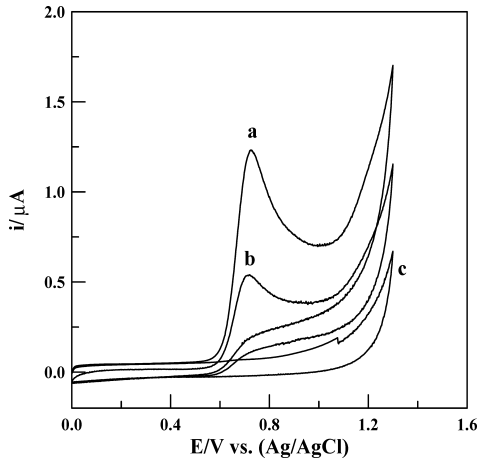

Fig. 3. Cyclic Voltammograms of 5×10^{-6} M Vincamine (a) First Cycle, (b) Second Cycle and (c) 5×10^{-6} M Vinpocetine in a B-R Universal Buffer of pH 5 Recorded at Scan Rate of 0.10 V s^{-1}, Following Preconcentration onto Nj-CPE by Adsorptive Accumulation at E_{acc}=0.0 V for 180 s

Nj-CPE.

Effect of scan rate (ν) on the peak potential (E_p) and peak current (i_p) was examined from 25 to 300 mV s^{-1}. The peak potential shifted linearly to more positive values with the increase of scan rate confirming the irreversible nature of the oxidation process of vincamine at the Nj-CPE.[21] Value of αn_a, product of transfer coefficient α and number of electrons n_a transferred in the rate-determining step, was determined from the linear correlation obtained between the peak potential E_p and the logarithm of the scan rate ν according to the equation of the totally irreversible electrode reaction[22]:

$$E_p=(2.303RT/\alpha n_aF)\log(RTk_f^\circ/\alpha n_aF)-(2.303RT/\alpha n_aF)\log\nu$$

A value of αn_a=0.82 was obtained. Accordingly, the number of electrons, n_a, transferred in the rate-determining step should equal two (n_a=2) and hence, the transfer coefficient α should be 0.41. A linear Randles–Seveik plot (i_p *vs.* ν) was obtained (correlation coefficient r=0.996) confirming that adsorption of vincamine onto the Nj-CPE is the means of mass transport.[23]

Oxidative response of vincamine onto the Nj-CPE may be attributed to the oxidation of hydroxyl group of the drug moiety *via* transfer of two electrons. This was confirmed by recording cyclic voltammogram for vinpocetine, a derivative of vincamine with the same formula but without hydroxyl group (Chart 1). No peak was observed neither in anodic nor cathodic direction (Fig. 2, c) indicating that the hydroxyl group was the unique electroactive center in the vincamine molecule.

Square-Wave Voltammetric Studies. 1. Optimization of an Analytical Method Square-wave voltammograms of 5×10^{-7} M vincamine following its preconcentration by adsorptive accumulation onto the Nj-CPE for 120 s showed a single well-defined irreversible anodic peak in B-R universal buffer over the pH range 2—11. As previously mentioned, vincamine has an adsorptive behavior onto the Nj-CPE and the adsorption process reaches a maximum value at B-R buffer solution of pH 5. Various buffers such as acetate (pH 3.8—6.2) and phosphate (2.5—7.2) buffers, and also some salt solutions (0.1 M) such as potassium chloride and potassium nitrate solutions were tested as supporting electrolytes. A B-R universal buffer of pH 5 was the best compromise with respect to much enhanced peak current intensity and sharper response. Therefore it was used as a supporting electrolyte in the rest of the present analytical work.

Since the square-wave response markedly depends on the parameters of the excitement signal, voltammograms of 5×10^{-7} M vincamine in a B-R buffer of pH 5 following preconcentration onto the Nj-CPE for 120 s were recorded at the various instrumental conditions (frequency f=20—120 Hz, scan increment ΔE_i=2—10 mV and pulse-amplitude ΔE_a=25—75 mV). Although the square-wave adsorptive anodic stripping (SW-AdAS) voltammetric peak current intensity of vincamine was linearly increased upon the increase of each of these parameters, a sharp peak with much enhanced current intensity was obtained under the following instrumental conditions: frequency f=120 Hz, scan increment ΔE_i=10 mV and pulse-amplitude ΔE_a=25 mV using a B-R universal buffer of pH 5 as a supporting electrolyte.

Effect of varying accumulation potential (E_{acc}=−0.20 to +0.30 V) on the SW-AdAS voltammograms of 5×10^{-7} M vincamine recorded under the optimized instrumental parameters following its preconcentration onto the Nj-CPE for 120 s in a B-R universal buffer of pH 5 was also evaluated. A much enhanced peak current was achieved at 0.0 V (Fig. 4). Hence, an accumulation potential (E_{acc}) of 0.0 V (*vs.* Ag/AgCl/KCl_s) was used throughout the present study. On the other side, SW-AdAS voltammograms of 2×10^{-7} and 5×10^{-7} M vincamine were recorded under the optimal instrumental parameters at increased preconcentration time (E_{acc}= 0.0 V). As shown in Fig. 5, the magnitude of the peak current depended linearly on both the analyte concentration and the accumulation time. Apparently, lower concentration of the analyte requires longer preconcentration time. This meant that, the choice of preconcentration time was dictated by the sensitivity required. In the present analytical method, preconcentration times of 90, 180 and 300 s were applied (Table 1).

2. Method Validation Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical ap-

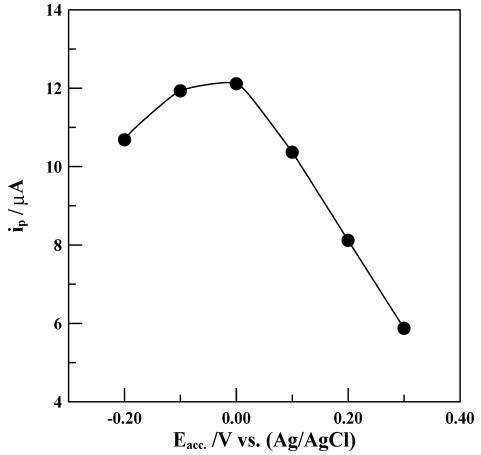

Fig. 4. Effect of Accumulation Potential (E_{acc}) on the SW-AdAS Voltammetric Peak Current (i_p) of 5×10^{-7} M Vincamine in a B-R Universal Buffer of pH 5, Following Preconcentration onto Nj-CPE by Adsorptive Accumulation at $E_{acc}=0.0$ V for 120 s

Instrumental parameters; frequency $f=120$ Hz, scan increment $\Delta E_i=10$ mV and pulse-amplitude $\Delta E_a=25$ mV.

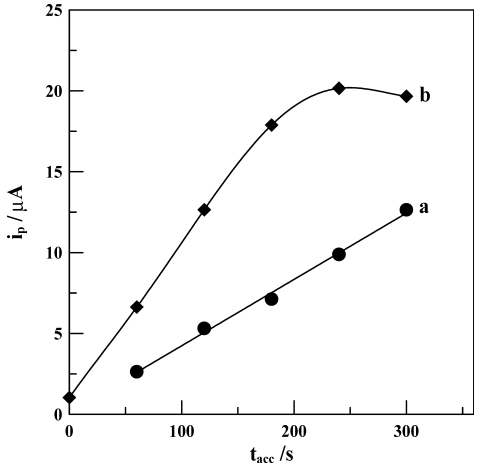

Fig. 5. Effect of the Accumulation Time (t_{acc}) on the SW-AdAS Voltammetric Peak Current of (a) 2×10^{-7} and (b) 5×10^{-7} M Vincamine in a B-R Buffer of pH 5 Following Preconcentration onto Nj-CPE by Adsorptive Accumulation at 0.0 V

Other instrumental parameters are as those given in Fig. 4.

plications. The elements required for method validation are: linearity range, limits of detection and quantitation, accuracy, precision, selectivity, robustness and intermediate precision.[24)]

Linearity: As shown in Table 1, linear relations of peak current (i_p) with concentrations of vincamine were obtained referred to different accumulation times onto the Nj-CPE by the described SW-AdAS voltammetric method. A good linearity is evident from values of the correlation coefficients $r=0.994$—0.999 (Table 1), thus confirmed validity of the described SW-AdAS method for determination of vincamine.

Limits of Detection and Quantitation: Limits of detection (LOD), defined as 3-times the baseline noise, and limits of quantitation (LOQ), defined as the lowest concentration assessed with acceptable accuracy and precision, were evaluated following various preconcentration times (Table 1). The achieved LOD and LOQ values following preconcentration of the drug onto the Nj-CPE for 300 s were 3.0×10^{-9} (1.10 ng ml^{-1}) and 1×10^{-8} M (3.67 ng ml^{-1}), respectively. The achieved LOD revealed the high sensitivity of the described SW-AdAS voltammetric method over most of the previously reported ones for determination of vincamine; (10 ng ml^{-1}),[8,15)] (5 ng ml^{-1}),[13,14,16)] (3 ng ml^{-1}),[9)] (0.25 mg ml^{-1})[10)] and (0.05 mg ml^{-1}).[11)]

Table 1. Characteristics of the Calibration Curves of the SW-AdAS Voltammetric Determination of Vincamine under the Optimal Procedural Conditions

Acc. duration (s)	Linearity range (M)	Least square equation[a)]		Corr. coeff. (r)	LOD (M)
		Intercept (μA)	Slope (μA/μM)		
90	2×10^{-7}—8×10^{-6}	0.066	19.40	0.994	6.0×10^{-8}
180	5×10^{-8}—5×10^{-7}	0.091	41.07	0.997	1.5×10^{-8}
300	1×10^{-8}—4×10^{-7}	0.121	63.53	0.999	3.0×10^{-9}

a) Average of three determinations.

Table 2. Influence of Minor Changes in the Optimal Procedural Conditions on Assay of 5×10^{-7} M Vincamine, Following Its Preconcentration onto a Nj-CPE; $f=120$ Hz, $\Delta E_i=10$ mV and $\Delta E_a=25$ mV

Variable	Conditions	%R±S.D. ($n=5$)
Robustness		
pH of the medium[a)]		
4.5	$E_{acc}=0.0$ V	98.78±0.55
5.0		99.53±0.76
5.5		100.34±0.88
Accumulation potential (E_{acc})[a)]		
−0.05 V	pH=5.0	98.26±1.05
0.0 V		99.53±0.76
+0.05 V		98.61±1.22
Intermediate precision		
Potentiostat 273A-PAR (Lab. 1)	pH=5.0	99.53±0.76
Potentiostat 263A-PAR (Lab. 2)	$E_{acc}=0.0$ V	102.74±1.58
Day (1)		99.53±0.76
Day (2)		98.93±0.70
Day (3)		100.26±1.35

a) Potentiostat 273A-PAR.

Accuracy: The accuracy of the described SW-AdAS voltammetric method was evaluated as recovery of a known concentration (5×10^{-7} M) of vincamine following preconcentration onto the Nj-CPE for 120 s. The mean percentage recovery obtained by the calibration curve method was 99.53% (Table 2).

Precision: The high sensitivity of an analytical method is usually accompanied by very good precision. The analytical performance of the described SW-AdAS voltammetric method was evaluated, as relative standard deviation, for five replicate measurements of 5×10^{-7} M vincamine solution following preconcentration for 120 s. The achieved relative standard deviation of 0.76% confirmed the very good precision of the described method (Table 2).

Stability: The stability of vincamine in a B-R buffer solution of pH 5 was evaluated under the optimal operational conditions by monitoring the changes in the anodic peak height of 5×10^{-7} M vincamine following its preconcentration for 120 s over a period of 3 h. The electroanalytical signal showed no difference in either acidic or alkaline media over this time period which confirmed stability of vincamine

over the studied pH range. Moreover under storage conditions for more than a month, neither the stock solutions of vincamine nor the serum calibration samples showed any degradation.

Robustness: The robustness[24] of the described SW-AdAS voltammetric method for assay of vincamine was examined by minor changes in assay conditions (pH 5±0.5 and accumulation potential E_{acc} 0.0±0.05 V). As shown in Table 2, changes in instruments or personnel did not alter the results, which indicate the ruggedness of the described method.

Intermediate Precision: The intermediate precision[24] of the described SW-AdAS voltammetric method was examined by applying it to assay of vincamine using two PAR-Potentiostats, 273A (Lab. 1) and 263A (Lab. 2), at different elapsed time. The results obtained due to Lab.-to-Lab. and even day-to-day were found reproducible (Table 2), since there was no significant difference in the recovery and/or standard deviation values.

3. Interference Studies Voltammetric techniques have found widespread use in drug analysis, since the voltammetric methods usually involve a simple dilution step and most of the ingredients used do not interfere in the subsequent determination.[25] The selectivity of the method for the assay of vincamine was examined in the presence of the common inactive ingredients usually present in its formulations (*e.g.* starch, gelatin, lactose, talc and magnesium stearate). The ingredients are electro-inactive and its adsorption onto the Nj-CPE was very limited under the optimum operational conditions. A mean recovery for 5×10^{-8} M vincamine in the presence of ingredients was found to equal 98.23±1.16% (n=5), which showed no significant interference from ingredients. Accordingly, the described stripping voltammetric method can be considered selective.

4. Analytical Application Analysis of Vincamine in Oxybral® Capsules: The described SW-AdAS voltammetric method was successfully applied for the determination of vincamine in its pharmaceutical formulations (Oxybral® capsules of 30 mg vincamine/capsule). The obtained results (Table 3) were statistically compared with those obtained by the compendial spectroscopic method.[26] Since the calculated value of F does not exceed the theoretical value (Table 3), there was no significant difference between the described and reported methods with respect to reproducibility.[27] Also, no significant difference was noticed between the two methods regarding accuracy and precision as revealed by t-value,[27] Table 3. The accuracy of the described SW-AdAS voltammetric method was also judged by applying the standard addition method,[28] Table 4. This means that the described voltammetric method should be applicable to the analysis of this and other similar formulation products containing vincamine.

Assay of Vincamine in Spiked Human Serum: The described SW-AdAS voltammetric method was also successfully applied for assay of vincamine in spiked human serum without the necessity for its prior extraction. Since results showed that vincamine is strongly adsorbed onto the Nj-CPE, medium exchange method was used following accumulation of the drug and before recording each voltammogram to minimize interferences resulting from macro solution constituents and to increase the efficiency of the stripping step. No interfering peaks have been observed in blank serum.

Table 3. Statistical Comparison for the Results Obtained by the Proposed SW-AdAS Voltammetric and the Compendial Spectroscopic[26] Methods for the Analysis of Vincamine in Oxybral® Capsules, 30 mg

Parameter	Proposed SW voltammetric method	Compendial spectroscopic method
Mean (%)	100.41	99.31
S.D (%)	0.74	1.04
n	5	5
F-value	1.975	
Student's t-test	1.927	

Theoretical F-value=6.39 and t-test=2.31 at 95% confidence limit for $n_1=n_2=5$.

Table 4. Results of Application of Standard Addition to the Determination of Vincamine in Oxybral® Capsules, 30 mg, by the Proposed Voltammetric Method

Standard added (μg ml^{-1})	Recovery (%)	Mean±S.D.
20	99.42	
40	100.70	99.61±1.00
60	98.72	

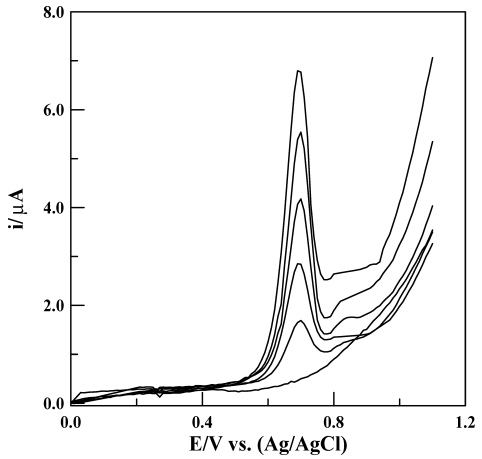

Fig. 6. SW-AdAS Voltammograms for Successive Additions of Vincamine Spiked in Human Serum Sample, Recorded in a B-R Buffer of pH 5 Following Preconcentration onto Nj-CPE by Adsorptive Accumulation at 0.0 V for 300 s

Each addition affected a 2×10^{-8} M vincamine and the dotted line represents the blank serum solution. Other instrumental parameters are as those given in Fig. 4.

Variation of the peak current *versus* concentration of vincamine was linear within the range of $2\times10^{-8}-5\times10^{-7}$ M (Fig. 6) followed the regression equation; i_p (μA)=55.15 C (μM)−0.235 (r=0.995 and n=10). Reproducibility of the described method in spiked human serum was evaluated for 10 replicate measurements of 5×10^{-8} M (1.83 ng ml^{-1}) vincamine and found to equal 99.5±1.79% (Table 5). The achieved LOD and LOQ were 6.0×10^{-9} M (2.20 ng ml^{-1}) and 2×10^{-8} M (7.33 ng ml^{-1}), respectively.

Pharmacokinetic Studies: Vincamine is almost completely metabolized and main metabolites are vincamine conjugates (sulphates and glucuronides).[29] Since hydroxyl group is the unique electroactive center in vincamine molecule, these metabolites should be electro-inactive. Therefore, the proposed SW-AdAS voltammetric method might allow a selective determination of vincamine in biological fluids without interferences from its metabolites. This advantage was proved

Table 5. Reproducibility of the Described SW-AdAS Voltammetric Method for Determination of Vincamine in Spiked Human Serum

Concentration (M)		Recovery (%)	Mean±S.D.
Added	Found		
5×10^{-8}	4.86×10^{-8}	97.2	99.5±1.79
	4.88×10^{-8}	97.6	
	5.03×10^{-8}	100.6	
	4.95×10^{-8}	99.0	
	5.08×10^{-8}	101.6	
	4.91×10^{-8}	98.2	
	5.11×10^{-8}	102.2	
	5.05×10^{-8}	101.0	
	4.89×10^{-8}	97.8	
	4.99×10^{-8}	99.8	

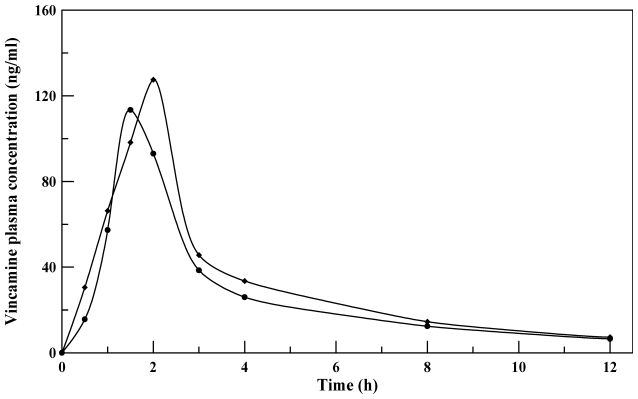

Fig. 7. Mean Plasma Concentration–Time Profiles for Two Male Subjects Following an Oral Administration of a Single Oxybral® Capsule, 30 mg

by studying pharmacokinetics of vincamine in plasma of two healthy male volunteers following the administration of a single oral Oxybral® capsule −30 mg. The obtained plasma concentration–time profiles of the two subjects are shown in Fig. 7. The following parameters were assessed for the period of 0—12 h: area under the plasma concentration–time curves from time zero to the last measurable sample time ($AUC_{0—12}$) and to infinity ($AUC_{0—\infty}$); maximum plasma concentration (C_{max}); time of the maximum concentration (t_{max}); elimination constant (K_{el}) and elimination half-life time ($t_{1/2}$). C_{max} and t_{max} were obtained directly from the concentration–time curve. The area under concentration–time curve from time zero (predose) to time of last quantifiable concentration ($AUC_{0—12}$) was calculated using the linear trapezoidal method. The terminal rate constant (K_{el}) was calculated by applying a log–linear regression analysis to at least the last three time points. The terminal half-life time ($t_{1/2}$) was calculated as [$\ln 2/K_{el}$]. Area under the plasma concentration–time curves from time zero to infinity ($AUC_{0—\infty}$) was calculated as [$AUC_{0—12}+(C_{12}/K_{el})$]. Some pharmacokinetic parameters obtained for the two volunteers were listed in Table 6.

Conclusion

The electrochemical oxidation of vincamine onto a Nj-CPE was identified in buffered solutions and a validated SW-AdAS voltammetric method was described for its determination. The described method is simple, sensitive, selective and no need for sample pre-treatment steps. Therefore, it can be used for analysis of vincamine either in bulk powder, pharmaceutical dosage forms or human serum. Moreover, the described method is low-cost and can be applied in laboratories lacking sophisticated instruments such as HPLC, GC-MS or LC-MS.

Table 6. Pharmacokinetic Parameters Estimated for Two Male Volunteers Following an Oral Administration of a Single Oxybral® Capsule, 30 mg

Parameter/unit	Estimated values[a)]	
	Subject (1)	Subject (2)
C_{max} (ng ml^{-1})	113.42	127.50
t_{max} (h)	1.5	2.0
$AUC_{0—12}$ (ng h ml^{-1})	329.11	395.08
$AUC_{0—\infty}$ (ng h ml^{-1})	367.35	428.26
K_{el} (h^{-1})	0.17	0.22
$t_{1/2}$ (h)	4.08	3.15

a) Average of two measurements.

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