Scientific paper

Quantification of the Vasoactive Agent Buflomedil HCl in Pharmaceutical Formulation and Human Serum by Stripping Voltammetry and Liquid Chromatography

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Abstract

Buflomedil HCl, was reduced at the mercury electrode in buffered solutions of various pH values (2–11) via a single 2-electron irreversible step corresponding to reduction of its C=O double bond. Buflomedil HCl has interfacial adsorptive behavior onto the mercury electrode surface and a monolayer surface coverage of 2.37×10^{-10} mol cm^{-2} was estimated. Each adsorbed buflomedil HCl molecule was found to occupy an area of 0.69 nm^2 onto the HMDE surface. Differential-pulse (DP), liner-sweep (LS) and square-wave (SW) adsorptive cathodic stripping voltammetry (AdCSV) methods were described for its determination in the bulk form. The sensitivity of the described electro-analytical methods increases in the direction: DP-AdCSV < LS-AdCSV < SW-AdCSV since their achieved limits of detection were: 2.4×10^{-8}, 1.5×10^{-8} and 1.2×10^{-9} M bulk buflomedil HCl, respectively. Besides, a simple high-performance liquid chromatographic method was also developed for determination of bulk buflomedil HCl with a detection limit of 3.0×10^{-8} M. The described voltammetric and chromatographic methods were successfully applied for determination of buflomedil HCl in its formulations. Besides these methods were applied for determination of buflomedil HCl in spiked human serum (limit of detection varying from 1.5×10^{-9} to 4.5×10^{-8} M buflomedil HCl) without the necessity for pretreatment and/or time-consuming extraction steps prior to the analysis. No significant interferences from excipients or from endogenous human serum substances were obtained. The described SW-AdCS voltammetry method is much more sensitive than the described chromatographic one. However the described chromatographic method is substantially simpler, faster and more sensitive than the previously reported HPLC methods.

Keywords: Buflomedil HCl, Vilatol® and Loftyl ®tablets, Human serum, Determination, Stripping voltammetry, Chromatography.

1. Introduction

Buflomedil HCl (Chart I), 4-(1-pyrrolidinyl)-1-2,4,6-trimethoxyphenyl)-1-butanone hydrochloride, is a vasoactive agent which increase the peripheral and cerebral blood flow in ischaemic tissues of patients with vascular diseases particularly at the microcirculatory level.[1, 2] It is rapidly absorbed from the gastro-intestinal tract, reaching maximal plasma concentration within 1.5–4 h.

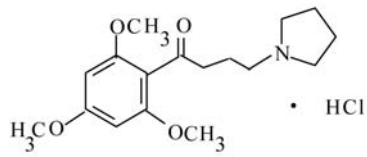

Chart I: Chemical structure of buflomedil HCl

There have been few reports for its determination in formulation and in biological fluids using gas chromatography-mass spectrometry,[3, 4] gas chromatography-ther-

moionic specific detector,[5] high-performance liquid chromatography,[6–8] and reversed-phase ion-pair high-performance liquid chromatography.[9, 10] Gas chromatography is usually limited to the analysis of volatile and thermally stable compounds and methylated derivatization is usually required.[3, 4] However, published HPLC methods that described analysis of buflomedil HCl levels in human serum were not sufficiently sensitive (LOQ = 1.6 ± 0.2 × 10^{-6} M) for therapeutic drug monitoring [7, 9] or used ion-pair reagents in mobile phase [9, 10] which is generally avoided not only because of the added complexity of the mobile phase but also because of baseline drift, irregular peak shapes and width, marked sensitivity of separation to temperature, and slow equilibrium of the column,[11] and/or involve liquid–liquid or solid-liquid extraction prior to analysis [6–9] which is time-consuming and not economically feasible for routine use in pharmacokinetic studies with numerous samples to be analyzed. On the other side, no information is available in literature to date concerning the electroreduction of buflomedil HCl and/or its electroanalytical quantification. Therefore, this work aimed to study the electroreduction of buflomedil HCl at the mercury electrode and to develop adsorptive stripping voltammetric methods for its determination in the bulk form, pharmaceutical formulations and in human serum. Besides, a high performance liquid chromatography – UV detection method was also developed for determination of buflomedil HCl in the same samples. The described voltammetric and chromatographic methods employed a micro volume of plasma (100 μL) and a simple sample preparation without organic solvent extraction prior to the analysis which ensure the applicability and reproducibility of the methods when only small volume of plasma is available. The simple sample preparation also ensures the consistent recovery, the low relative standard deviation and the good linearity which promise the elimination of the internal standard in case of HPLC measurements.

2. Experimental

2. 1. Materials

Bulk buflomedil HCl (Sigma Chemical Co, St. Louis, MO, USA) was used in the present study. Vilatol® tablets; 300 mg/tablet (Global Napi Pharmaceuticals, Egypt) and Loftyl® tablets; 300 mg/tablet (Kahira Pharm. & Chem. Ind. Co., Egypt) were purchased from the local market. Human serum sample of healthy volunteer was stored frozen until assay.

2. 2. Solutions and Reagents

(i) A standard solution (1 × 10^{-3} M) of bulk buflomedil HCl was prepared in methanol (Merck), and the desired working solutions (10^{-8}–10^{-4} M) were prepared by appropriate dilution with methanol (for voltammetric measurements) or with the mobile phase: methanol: water: pH 9 borate buffer (80 : 10 : 10, v/v/v) (for HPLC measurements). The buflomedil HCl solutions were stable with time (for at least 7 days at ambient temperature of 25 ± 2 °C and for at least 5 weaks at 4 °C).

(ii) Ten tablets of each of the formulations: Vilatol® (Global Napi Pharmaceuticals, Egypt) and Loftyl® (Kahira Pharm. & Chem. Ind. Co., Egypt) were weighed and the average mass per tablet was determined, and then ground to fine powders. A weighed portion of each of the homogeneous powder equivalent to 1 × 10^{-3} M buflomedil HCl was accurately transferred into a 100-mL volume calibrated flask containing 70 mL methanol (Merck). The content of the flask was sonicated for about 10 min and then filled up with methanol. The solutions were then filtered through a 0.45 μm Milli-pore filter (Gelman, Germany). Convenient concentrations of buflomedil HCl were then obtained by accurate dilutions with methanol (for voltammetric measurements) or mobile phase: methanol: water: pH 9 borate buffer (80 : 10 : 10, v/v/v) (for HPLC measurements).

(iii) Into each of 10 centrifugation tubes (3 ml polypropylene micro-centrifuge tubes) containing a certain concentration of buflomedil HCl, a 100 μL-volume of the human serum was transferred, then mixed well with 1 ml of methanol to denature and precipitate of proteins. The solutions were centrifuged (using an Eppendorf centrifuge 5417C, Hamburg, Germany) for 3 min at 14000 rpm to separate the precipitated proteins. The clear supernatant layers of the solutions were filtered through 0.45 μm Milli-pore filters to produce protein-free human serum samples spiked with various concentrations of buflomedil HCl (10^{-8}–10^{-4} M). No further pre-treatment or extraction steps were required for serum samples prior to the analysis.

(iv) A series of the Britton-Robinson (B-R) universal buffer of pH 2–11,[12] borate buffer of pH 7–11 and phosphate buffer of pH 5–8 [13] were prepared in de-ionized water and used as supporting electrolytes. All the chemicals used were of analytical-reagent grade quality and were used without further purification. A pH-meter (Crison, Barcelona, Spain) was used for the pH measurements. Deionized water was supplied from a Purite-Still Plus de-ionizer connected to an AquaMatic double-distillation water system (Hamilton Laboratory Glass LTD, Kent, UK).

2. 3. Instrumentation

(i) Electrochemical Analyzers Models 263A and 394-PAR (Princeton Applied Research, Oak Ridge, TN, USA) controlled with the software 270/250-PAR were used for the voltammetric measurements. An

electrode assembly (303A-PAR) incorporated with a micro-electrolysis cell and a three-electrode configuration system comprising of a hanging mercury drop electrode (HMDE) as a working electrode (area of HMDE = 0.026 cm^2), an Ag/AgCl/KCl_s reference electrode and a platinum wire auxiliary electrode, was used. A magnetic stirrer (305-PAR) and a stirring bar were used to provide the convective transport during the preconcentration step.

(ii) A liquid chromatographic pump (Bischoff, Switzerland) equipped with a UV- detector (Bischoff Lambda 1000) and a reversed phase column (Prontosil C_{18}, 250 × 4.0 mm, 5 µm) were used. Data acquisition and peak integration was done with the Bischoff McDAcq integrator software v1.5. The injection volume was 20µL with a Rheodyne 7125 injector valve.

(iii) Absorption spectra of buflomedil HCl solutions in methanol was recorded at room temperature within the wavelength range 200–600 nm using a Shimadzu UV–Visible spectrophotometer Model 160A (Kyto, Japan). From the UV spectra of the analyte, the detection wavelength was chosen as 210 nm since at which buflomedil HCl has a much developed absorption band and a good chromatographic response.

2. 4. General Electrochemical Procedure

A known volume of the analyte solution was pipetted into a 10-ml volume calibrated flask and then filled up with borate buffer of pH 7. The solution was introduced into the electrolysis cell, and then deoxygenated with pure nitrogen gas for about 5 min in the first cycle and for 30 s in each successive cycle, while a stream of nitrogen gas was kept over the solution during the measurements. In stripping voltammetric analysis preconcentration of buflomedil HCl onto the surface of HMDE was performed by adsorptive accumulation at –0.6 V (versus Ag/AgCl/KCl_s) for 30 s while stirring the solution at 400 rpm. After equilibrium time of 5 s allowed for the solution to become quiescent, voltammograms were recorded by scanning the potential towards the negative direction using the selected potential-waveform (differential pulse, linear-sweep or square-wave).

2. 5. HPLC Procedure

Buflomedil HCl was quantitated on a C_{18} reversed phase column, using a mobile phase composed of the methanol: water: pH 9 borate buffer (80 : 10 : 10, v/v/v) delivered at a flow rate of 0.8 ml min^{-1} at ambient temperature of 25 ± 2 °C, and with UV detection (wavelength = 210 nm). The mobile phase was sonicated-well before use and the column was equilibrated with the mobile phase flowing through the system before the injection of the standard solution of the analyte. Each standard solution was injected into the chromatographic system (n = 3) and mean values of peak areas (A) were plotted against concentrations (C).

3. Results and Discussion

3. 1. Cyclic Voltammetry Studies

Cyclic voltammograms of 1 × 10^{-4} M buflomedil HCl in the B–R buffer of pH (2–11) containing 10 % (v/v) methanol exhibited a single irreversible cathodic peak, (Fig. 1). The absence of any peak on the reverse scan indicated the irreversible nature of the electrode reaction of buflomedil HCl. The peak potential E_p shifted towards more negative values upon the increase of pH of the medium confirming the involvement of protons in the electrode reaction and that the proton-transfer precedes the electron-transfer process.[14] The irreversible nature of the electrode reaction was also identified from the shift of peak potential E_p to more negative values upon the increase of scan rate v (25–500 mV s^{-1}) at different pH values.[15] Linear plots of peak potential (E_p) versus logarithm of scan rate (v) with slope values of 51–92 mV were obtained at various pH values; from which values of αn_a = 1.16–0.64 and α = 0.58–0.32 were estimated, confirming again the irreversible nature of the electrode reaction of buflomedil HCl at the mercury electrode.[16]

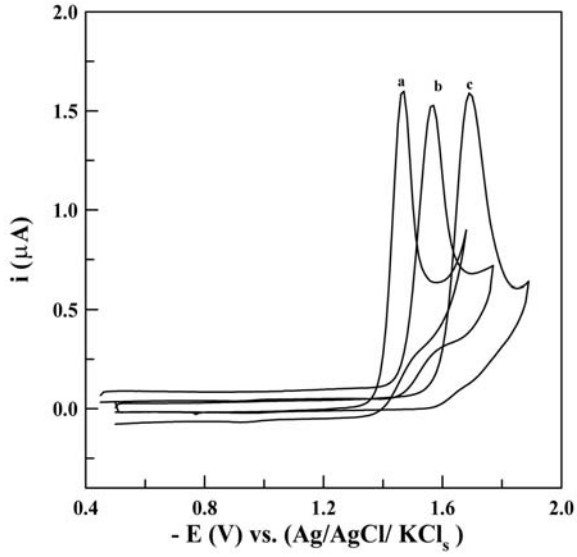

Figure 1. Cyclic voltammograms of 1 × 10^{-4} M buflomedil HCl in the B–R universal buffer of different pH values containing 10 % (v/v) methanol : (a) 5.0 (b) 7.0 and (c) 9.0.

The adsorptive character of the analyte onto the mercury surface was identified by recording cyclic voltammograms of 5 × 10^{-7} M buflomedil HCl at 100 mV s^{-1} in the B–R universal buffer of pH 7 following its preconcentration onto the HMDE by adsorptive accumulation at open circuit conditions (Fig. 2, curve a) and then at E_{acc} = –0.6 V (versus Ag/AgCl/KCl_s) for 30 s (Fig. 2, 1st cycle b

& 2nd cycle c). The peak current magnitude (i_p) was enhanced following preconcentration of the analyte (curve b) indicating its adsorption onto surface of the HMDE. Whereas the repetitive cycle at the same mercury drop (curve c) exhibited a lower peak current magnitude which may be due to desorption of buflomedil HCl from the mercury electrode surface. On the other side, the peak current magnitude increased linearly upon the increase of scan rate ν up to 500 mV s^{-1}. A linear plot of log i_p versus log ν following the regression equation: log i_p = 0.84 log ν – 1.74 (r = 0.996 & n = 5) was obtained with a slope value of 0.84 which is close to the expected theoretical value 1.0 for an ideal reaction of surface species [17] with some diffusion contributions.

The electrode surface coverage (Γ^v mol/cm^2) was calculated using the equation Γ^v = Q/nFA, where Q is the charge consumed by the surface process as estimated by the integration of the area under the peak of the cyclic voltammogram of the analyte,[18] n is the number of electrons consumed in the reduction process (n = 2), F is the Faraday's constant (96,487 C) and A is the electrode surface area (0.026 cm^2). On dividing the number of coulombs transferred (1.189 × 10^{-6} C), by the conversion factor nFA (5017.324 mol C cm^{-2}), a monolayer surface coverage of 2.37 × 10^{-10} mol cm^{-2} was estimated. Each adsorbed buflomedil HCl molecule therefore occupied an area of 0.69 nm^2.

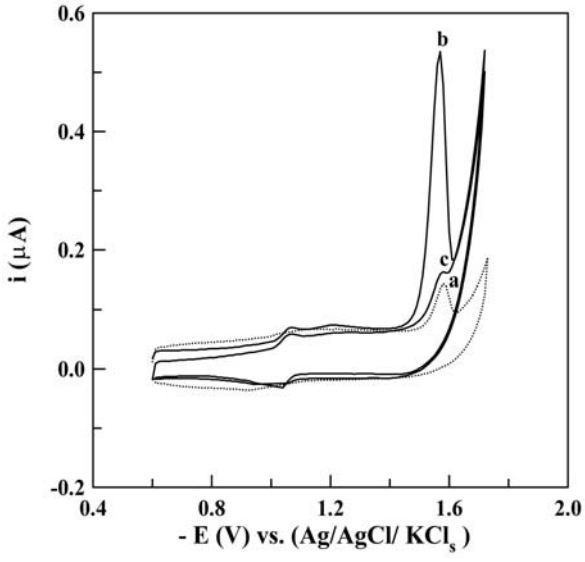

Figure 2. Cyclic voltammograms of 5 × 10^{-7} M buflomedil HCl in the B–R buffer of pH 7 recorded following its preconcentration onto HMDE by adsorptive accumulation under open circuit conditions (a) and then at E_{acc} = –0.6 V for 30 s (1st cycle b and 2nd cycle c); scan rate = 100 mV s^{-1}.

3. 2. Stripping Voltammetry Studies

Based on the adsorption behavior of buflomedil HCl onto the HMDE, stripping voltammetric methods were optimized for its trace determination applying different potential-waveforms:

3. 2. 1. Differential-Pulse Stripping Voltammetry Method

Differential-pulse adsorptive cathodic stripping (DP-AdCS) voltammograms of 1 × 10^{-6} M bulk buflomedil HCl in the B-R universal buffer (pH 2–11) , phosphate buffer (5–8) and in the borate buffer (pH 7–11) following its preconcentration onto the HMDE by adsorptive accumulation at –0.5 V for 30 s exhibited a single peak over the entire pH range. A better enhanced peak current magnitude was achieved in borate buffer of pH 7. The optimum instrumental conditions for achieving a better developed DP-AdCS voltammetric peak were identified by studying the effect of each of accumulation potential E_{acc} (–0.2 to –0.7 V), accumulation time t_{acc} (0–120 s), scan rate ν (2–10 mVs^{-1}) and pulse-height a (10–50 mV) on the peak current magnitude (i_p) of 1 × 10^{-6} M bulk buflomedil HCl in borate buffer of pH 7. The results indicated that the optimal operational conditions which generated a well-shaped better enhanced peak current were E_{acc} = –0.6 V (versus Ag/AgCl/KCl_s), t_{acc} = 80 s, ν = 10 mV s^{-1} and a = 25 mV.

DP-AdCS voltammograms of various concentrations of buflomedil HCl were recorded under the optimized operational conditions and a linear variation of the peak current magnitude (i_p) with concentration (C) was obtained within the concentration range 8.0 × 10^{-8} to 5.0 × 10^{-6} M; the corresponding regression equation was: i_p *(μA) = 0.13 C (μM) ± 0.15*, (r = 0.997, n = 11). A limit of detection (*LOD*) of 2.4 × 10^{-8} M and a limit of quantitation (*LOQ*) of 8.0 × 10^{-8} M bulk buflomedil HCl were achieved by means of the optimized DP-AdCS voltammetric method using the expression *k.S.D/b* , [19] where k = 3 for *LOD* and 10 for *LOQ*, *S.D* is the standard deviation of the calibration curve and b its slope.

3. 2. 2. Linear-Sweep Stripping Voltammetry Method

In order to optimize the operational conditions for assay of bulk buflomedil HCl by LS-AdCS voltammetry, effect of each of scan rate v (20–150 mV s^{-1}) and preconcentration potential E_{acc} (–0.1 to –1.0 V) on its peak current magnitude of 5 × 10^{-7} M in the borate buffer of pH 7 was studied following its preconcentration onto the HMDE by adsorptive accumulation for 30 s. A better enhanced peak current magnitude (i_p) was achieved at a scan rate (ν) of 100 mV s^{-1} following preconcentration of buflomedil HCl onto the HMDE by adsorption accumulation at –0.6 V (versus Ag/AgCl/KCl_s).

On the other side, the effect of preconcentration time $t_{acc.}$ on LS-AdCS voltammetric peak current magnitude of various concentrations of bulk buflomedil HC-

l (1×10^{-6}, 5×10^{-7}, 1×10^{-7} and 5×10^{-8} M) in the borate buffer of pH 7 at 100 mV s^{-1} following preconcentration by adsorptive accumulation at –0.6 V was also investigated. For analysis of 1×10^{-6}, 5×10^{-7}, 1×10^{-7} and 5×10^{-8} M buflomedil HCl the response was linear up to 20, 40, 70 and 80 s, respectively, then leveled off which may be due to complete coverage of the mercury electrode surface with the analyte species. This means that the lower the concentration of the analyte, the longer of the accumulation duration is. Thus, preconcentration time of choice will be dictated by the sensitivity needed. Accordingly, the optimum operational conditions of the described LS-AdCS voltammetric method can be summarized as: E_{acc} = –0.6 V, $t_{acc.}$, ≤ 80 s, and v = 100 mV s^{-1} using the borate buffer of pH 7 as a supporting electrolyte.

LS-AdCS voltammograms of various concentrations of buflomedil HCl were recorded under the optimized operational conditions. A linear calibration graph over the concentration range of 5×10^{-8} to 1×10^{-6} M bulk buflomedil HCl was obtained; the corresponding regression equation was: $i_p(\mu A) = 0.428$ C (μM) + 0.019 (r = 0.996 and n = 7). Limits of detection (LOD) and quantitation (LOQ) of 1.5×10^{-8} M and 5×10^{-8} M bulk buflomedil HCl, respectively, were achieved, by means of the described LS-AdCS voltammetric method.[19]

3. 2. 3. Square-Wave Adsorptive Stripping Voltammetry Method

Effect of preconcentration potential ($E_{acc.}$) on the SW-AdCS voltammetric peak current magnitude of 5×10^{-7} M buflomedil HCl in the borate buffer of pH 7 was also examined over the potential range of –0.1 to –1.1 V following its preconcentration onto the HMDE by adsorptive accumulation for 30 s. The peak current magnitude was better enhanced over the potential range (–0.5 to –0.7) V. At lower and higher potentials the peak current decreased, so a preconcentration potential of –0.6 V (vs. $Ag/AgCl/KCl_s$) was chosen for further studies. The influence of square-wave pulse parameters namely: frequency f (10–90 Hz), scan increment ΔE_s (2–12 mV) and pulse-amplitude a (10–50 mV) on the SW-AdCS voltammetric peak current magnitude of 5×10^{-7} M buflomedil HCl in the borate buffer of pH 7 following its preconcentration onto the HMDE by adsorptive accumulation at –0.6 V for 30 s was examined. A better developed and symmetrical peak was obtained at the following pulse parameters: f = 80 Hz, ΔE_s = 10 mV and, a = 25 mV. Also effect of varying the preconcentration time ($t_{acc.}$) on the peak current magnitude of various concentrations of bulk buflomedil HCl (1×10^{-6}, 5×10^{-7}, 1×10^{-7} and 5×10^{-8} M) in the borate buffer of pH 7 was evaluated. As shown in Figure (3), for analysis of 1×10^{-6}, 5×10^{-7}, 1×10^{-7} and 1×10^{-8} M buflomedil HCl the response was linear up to 20, 40, 70 and 80 s, respectively. This means that the lower the concentration of the analyte, the longer of the accumulation duration is. Thus, preconcentration time of choice will be dictated by the sensitivity needed. Accordingly, the optimum operational conditions of the described SW-AdCS voltammetric method can be summarized as: E_{acc} = –0.6 V, $t_{acc.}$, ≤ 80 s, f = 80 Hz, ΔE_s = 10 mV and a = 25 mV using the borate buffer of pH 7 as a supporting electrolyte. For the optimized adsorptive stripping voltammetric methods, influence of the rest time (5–10 s) and area of the HMDE (0.01 to 0.026 cm^2) were also considered and a time period of 5 s and a HMDE area of 0.026 cm^2 were chosen in the present study applying the different potential-waveforms.

Using the described SW-AdCSV method for assay of standard solutions of various concentrations of buflomedil HCl, a linear calibration curve was obtained over the concentration range 4×10^{-9} to 4×10^{-7} M; the corresponding regression equation was: $i_p(\mu A) = 4.52\ C\ (\mu M) + 0.35$ (r = 0.999 and n = 12). Limits of detection (*LOD*) and quantitation (*LOQ*) of 1.2×10^{-9} and 4.0×10^{-9} M of bulk buflomedil HCL, respectively, were achieved [19] by means of the described SW-AdCSV method.

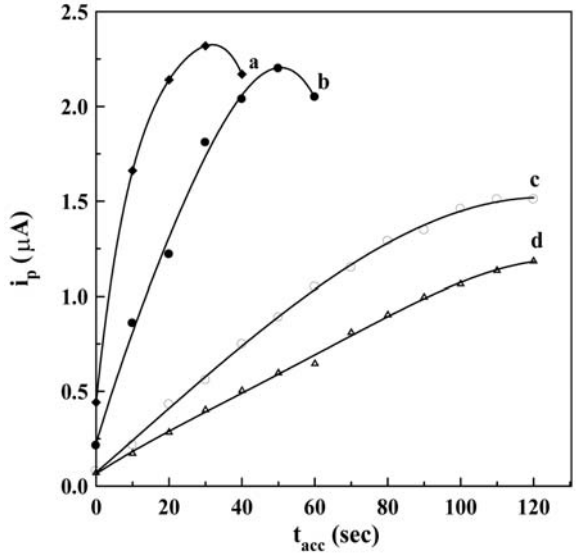

Figure 3. Effect of accumulation duration (t_{acc}) on SW-AdCSV peak current of :(a) 1×10^{-6}, (b) 5×10^{-7}, (c) 1×10^{-7} and (d) 5×10^{-8} M buflomedil HCl in the borate buffer of pH 7; E_{acc} = –0.6 V, a = 25 mV, f = 80 Hz, *and* ΔE_s = 10 mV .

3. 3. Chromatography Study

Several studies were carried out to optimize the experimental conditions of a high performance liquid chromatographic method for determination of bulk buflomedil HCl. These include detection wavelength, and nature, proportion and flow rate of the mobile phase. The absorption spectrum of 1×10^{-5} M buflomedil HCl methanolic solution exhibits two absorption bands at 210 and 283 nm. The absorption band at 210 nm was better developed

and at which buflomedil HCl had a good chromatographic response, therefore it was chosen for detection of the analyte throughout this study. Several binary or ternary eluents with different ratios were tested as mobile phases such as: [acetonitrile : water], [methanol : water] [acetonitrile : water : phosphate buffer] and [methanol : water : phosphate buffer], [acetonitrile : water : borate buffer], [methanol : water : borate buffer]. The mobile phase comprised of methanol : water : pH 9 borate buffer (80 : 10 : 10, v/v/v) with a flow rate of 0.8 ml min^{-1} was chosen. It provides a single well-separated peak for buflomedil HCl from the solvent fronts and has the best peak resolution, retention time and area counts with the least band tailing (retention time for buflomedil HCl was approximately 5.5 min and the total analysis time for each chromatographic run was 7.5 min). Representative HPLC chromatograms of 1×10^{-5} M bulk buflomedil HCl in different mobile phases are shown in Figure (4). The quantitative analysis of buflomedil HCl by the described HPLC method showed a linear peak area (*A*) with concentration (*C*) over the range of 10^{-7}–10^{-4} M (Fig. 4; inset); the corresponding regression equation was: *A (V * s) = 0.0448 C (μM) + 0.055* (r = 0.998, n = 9). Limit of detection (*LOD*) of 3×10^{-8} M and limit of quantitation (*LOQ*) of 1×10^{-7} M bulk buflomedil HCl were estimated using the expression *3 S.D./ b*.[19] The results indicated the reliability of the described stripping voltammetry and chromatographic methods for the assay of bulk buflomedil HCl, however the described SW-AdCS voltammetry method is much more sensitive.

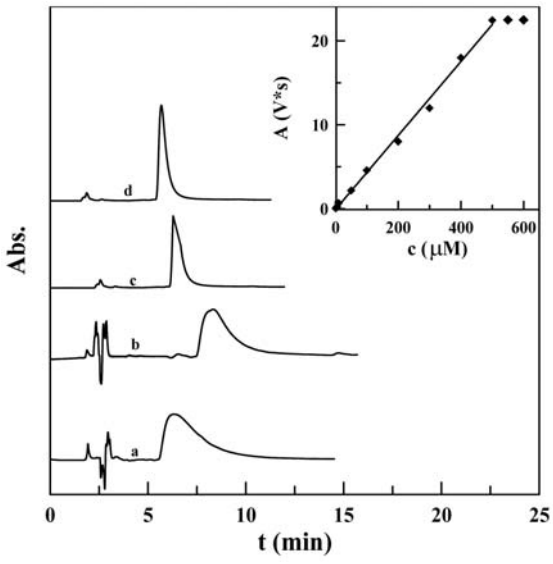

Figure 4. Representative HPLC chromatograms of 1×10^{-5} M buflomedil HCl at 210 nm in different mobile phases: (a) methanol : water (90 : 10, v/v), (b) methanol : water (80 : 20, v/v), (c) methanol : water : pH 9 borate buffer (80 : 17 : 3, v/v/v) and (d) methanol : water : pH 9 borate buffer (80 : 10 : 10, v/v/v). Inset: Plot of peak area (*A*) vs. concentration (*C*) of bulk buflomedil HCl**;** Mobile phase: methanol : water : pH 9 borate buffer (80 : 10 : 10, v/v/v) and flow rate = 0.8 ml min^{-1} at 25 ± 2 °C.

3. 4. Methods Validation

3. 4. 1. Selectivity

An attractive feature of an analytical method is its relative freedom from interference of the foreign species. The selectivity [20] of the described stripping voltammetric methods was identified through possible interferences from excipients usually present in the pharmaceutical formulations (such as: lactose, starch, gelatin, talc and magnesium stearate). This was carried out by analysis of 1×10^{-7} M bulk buflomedil HCl solution in the absence and presence of these common excipients, following preconcentration of buflomedil HCl onto the HMDE by adsorptive accumulation at –0.6 V for 60 s in both cases. Statistically, no significant differences in the mean percentage recoveries (%R) and relative standard deviations (RSD%) were obtained by the described DP, LS and SW-AdCS voltammetric methods in the absence of excipients (98.7 ± 2.4, 99.7 ± 1.3, and 100.0 ± 1.4%) and in their presence (98.4 ± 2.2, 98.4 ± 1.3, and 98.6 ± 1.2 %), respectively. Similar studies were carried out by the described chromatographic method and no significant differences in the mean percentage recovery and relative standard deviation in the absence (98.9 ± 1.1) and presence (98.0 ± 1.3) of excipiants were noticed. The results suggested the selectivity of the described stripping voltammetric and chromatographic methods for assay of bulk buflomedil HCl without interferences from excipients.

3. 4. 2. Intra-Day and Inter-Day Precision:

The precision and accuracy of the described DP-AdCS, LS-AdCS, SW-AdCS voltammetric and chromatographic methods were evaluated through intra-day and inter-day assays.[20] The mean percentage recoveries (%R) and relative standard deviations (RSD%) shown in Table 1, indicated the high precision and accuracy of the described methods for assay of bulk buflomedil HCl.

3. 4. 3. Robustness and Inter-Laboratory Precision

In regard to assay robustness[20] of the described DP-AdCS, LS-AdCS and SW-AdCS voltammetric methods, influence of small variation of some of the most important operational conditions including pH (7 to 8), preconcentration potential (–0.5 to –0.7 V) and preconcentration time (75 to 85 s), on recovery and standard deviation of 1×10^{-7} M bulk buflomedil HCl was studied. The obtained mean percentage recoveries and relative standard deviations (99.6 ± 0.4 to 97.4 ± 0.2) were insignificantly affected within the studied range of variation of the operational conditions, and consequently the described stripping voltammetry methods were reliable for assay of bulk buflomedil HCl and they could be considered robust.

Table 1: Precision and accuracy of analysis of 5×10^{-7} M bulk buflomedil HCl by the described stripping voltammetric methods and 1×10^{-5} M by the described chromatographic one (n = 5)

Method	Intra-day			Inter-day		
	% R	Accuracy RE %	Precision RSD %	% R	Accuracy RE %	Precision RSD %
DP-AdCSV	97.8	–2.2	0.6	97.2	–2.8	1.2
LS-AdCSV	98.7	–1.3	2.3	97.0	–1.7	1.7
SW-AdCSV	99.6	–0.8	1.0	98.6	–1.4	1.9
HPLC	99.8	–0.3	0.6	98.4	–1.6	1.7

Also, the robustness of measurements by means of the described chromatographic method was evaluated by intentional minor modifications in the composition of the constituents of mobile phase (± 2%) and rate of its flow (± 0.02). Practically, insignificant effect was observed in peak area or retention time confirming the robustness of analysis by the described chromatographic method.

On the other side, the inter-laboratory precision of measurements using the described voltammetric methods was examined by assay of 1×10^{-7} M buflomedil HCl using two PAR- Potentiostats- Models 263A, Lab. (1) and 394, Lab. (2) under the same operational conditions at different elapsed times by two different analysts. The mean percentage recoveries obtained due to Lab. 1 (98.6 ± 1.3) to Lab. 2 (99.4 ± 1.1) and even day to day (99.1 ± 0.3 to 98.5 ± 0.7) were found reproducible, since there is no significant difference between the recoveries or relative standard deviations.

3. 5. Applications

3. 5. 1. Analysis of Vilatol® and Loftyl® Tablets

The described DP-AdCS, LS-AdCS and SW-AdCS voltammetric and chromatographic (HPLC) methods were successfully applied for analysis of buflomedil HCl in vilatol® (300 mg/tablet) and Loftyl® tablets (300 mg/tablet) using the calibration curve and standard addition methods,[21] without the necessity for its extraction prior to the analysis. The satisfactory obtained voltammetric and HPLC results (Table 2) were statistically compared with those obtained by a reported gas chromatographic (GC) method.[3] The calculated *F* -values did not exceed the theoretical one (Table 2), which means that there is no significant difference between the described and reported methods with respect to reproducibility.[22] Also, no significant difference was noticed between the methods regarding accuracy and precision as revealed by *t* –test,[22] Table (2). This is an indication of the non-interference from excipients in the analysis of buflomedil HCl in their vilatol® and Loftyl® dosage forms by means of the described voltammetric and HPLC methods and consequently each of them can provide a method for the quality control of buflomedil HCl-containing pharmaceutical preparations.

3. 5. 2. Analysis of Spiked Human Serum

Quantitative assay of various concentrations of buflomedil HCl spiked in human serum at levels comparable to or even less than that found in human plasma after drug

Table 2: Analysis of Buflomedil HCl in each of (a) vilatol ® and (b) Loftyl® tablets (300 mg/tablet) by the described stripping voltammetric (5×10^{-7} M) and HPLC (5×10^{-5} M) methods (n = 4) and a reported GC method. [3]

Method	(% R ± S.D)		(Calculated)	
	Calibration curve method	Standard addition method	*F-value*	*t-test*
DP-AdCSV	(a) 98.5 ± 0.5	97.5 ± 1.7	1.6	0.9
	(b) 97.1 ± 1.5	98.2 ± 2.4	1.3	0.4
LS-AdCSV	(a) 99.3 ± 0.3	99.7 ± 0.7	1.8	2.0
	(b) 98.3 ± 1.5	97.9 ± 1.4	1.3	0.6
SW-AdCSV	(a) 99.7 ± 0.7	100.3 ± 0.6	3.1	2.2
	(b) 98.6 ± 1.6	97.8 ± 1.2	1.1	0.9
HPLC	(a) 98.3 ± 0.8	98.5 ± 0.9	4.0	1.1
	(b) 97.8 ± 1.5	97.5 ± 2.2	1.3	0.2
Reported GC	(a) 98.8 ± 0.4	97.8 ± 1.5		
	(b) 97.6 ± 1.7	98.2 ± 2.4		

Theoretical *F-value* = 6.6 and *t-test* = 2.45 at 95% confidence limit for $n_1 = 4$ and $n_2 = 4$.

administration [5, 23–26] was carried out by the described DP-AdCS, LS-AdCS and SW-AdCS voltammetric (t_{acc} = 80 s) and chromatographic methods without the necessity for sample pretreatments or time-consuming extraction steps prior to the analysis. Representative SW-AdCS voltammograms of blank human serum and of various concentrations of buflomedil HCl spiked human serum without any interfering voltammetric peaks from endogenous human serum constituents are shown in Figure 5. Similarly, HPLC chromatograms of human serum spiked with various concentrations of buflomedil HCl showed a satisfactory separation of peak of buflomedil HCl (retention time = 5.8 min) from the overlapped peaks of endogenous components in human serum fronts (retention time = 1.8–2.4 min), i.e there was no any interference from endogenous human serum constituents.

Linear dependence of peak current magnitude (i_p) (e.g., Fig. 5, Inset) or peak area (A) on concentration of buflomedil HCl spiked in human serum were obtained over the ranges shown in Table (3). Linearity ranges, corresponding regression equations, limits of detection (*LOD*) and limits of quantitation (*LOQ*) of buflomedil HCl spiked in human serum achieved by means of the described DP-AdCS, LS-AdCS, SW-AdCS voltammetric and chromatographic methods are also reported in Table (3).

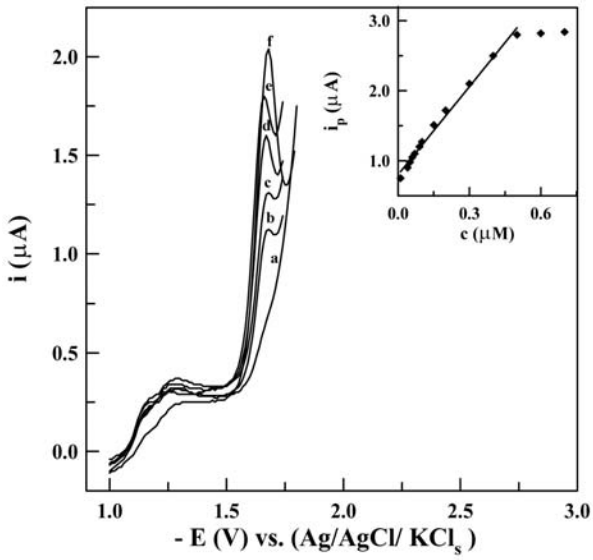

Figure 5. Representative SW-AdCS voltammograms in the borate buffer of pH 7 for different concentrations of buflomedil HCl spiked in human serum: (a) blank human serum; (b) 1×10^{-8}; (c) 5×10^{-8}; (d) 9×10^{-8}; (e) 1.3×10^{-7}; and (f) 2×10^{-7} M buflomedil HCl (t_{acc} = 80 s, E_{acc} = –0.6V, f = 80 Hz, ΔE_s = 10 mV and a = 25 mV). Inset : Plot of peak current (i_p) vs. concentration (C) of buflomedil HCl spiked in human serum.

The obtained mean recoveries and standard deviations of 98.2 ± 0.5 (DP-AdCSV), 98.9 ± 0.7 (LS-AdCSV), 99.5 ± 0.9 (SW-AdCSV), and 98.3 ± 0.8 (HPLC) indicated good accuracy and precision of analysis of buflomedil HCl in spiked human serum by the described voltammetric and chromatographic methods. The results indicated that the described voltammetric and chromatographic methods are sensitive enough for assay of buflomedil HCl in spiked human serum since they offer limits of quantitation (LOQ) of 5×10^{-9} to 1.5×10^{-7} M buflomedil HCl (Table 3). The typical plasma concentrations reported in literature for healthy volunteers each administrated a single oral dose of 248–450 mg buflomedil HCl were in the range 1.6×10^{-7}–1.5×10^{-5} M. [5, 23–26] Therefore, the described analytical methods are considered efficient enough for the assay of buflomedil HCl at different therapeutic dose levels for pharmacokinetic studies as well as therapeutic drug monitoring. On other hand, the described SW-AdCS voltammetry method is much more sensitive than the described chromatographic one. However the described chromatographic method is substantially simpler, faster and more sensitive than the previously reported HPLC methods. [6–10]

4. Conclusion

The electroreduction of buflomedil HCl at the mercury electrode was studied and discussed. Based on the adsorptive character of buflomedil HCl at the mercury surface, three validated stripping voltammetric methods were described for its analysis in the bulk form, formulations and human serum without the necessity for extraction prior to the analysis. Besides a new, specific and sensitive HPLC-UV method was described for determination of buflomedil HCl in formulations and human serum wit-

Table 3: Characteristics of the calibration curves of Buflomedil HCl in spiking human serum by means of the described stripping votammetric and HPLC methods (n = 4).

Method	Linearity range (M)	Regression equation	LOD (M)	LOQ (M)
DP-AdCSV	1×10^{-7} – 4×10^{-6}	i_p (μA) = 0.09 C (μM) + 0.12	3.0×10^{-8}	1×10^{-7}
LS-AdCSV	7×10^{-8} – 3×10^{-6}	i_p (μA) = 0.29 C (μM) + 0.02	2.1×10^{-8}	7×10^{-8}
SW-AdCSV	5×10^{-9} –5×10^{-7}	i_p (μA) = 4.20 C (μM) + 0.056	1.5×10^{-9}	5×10^{-9}
HPLC	1.5×10^{-7}–1×10^{-4}	A (V*s) = 0.038 C (μM) + 0.25	4.5×10^{-8}	1.5×10^{-7}

hout solvent extraction prior to the analysis. The described stripping voltammetric methods showed clear advantages over the described and reported chromatographic methods such as short time of analysis, much sensitive and required cheaper reagents and equipments. The described methods could be recommended for use in quality control and clinical laboratories.

6. References

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Povzetek

Poleg tega smo razvili tekočinsko kromatografsko metodo za določanje buflomedil HCl z mejo določitve $3{,}0 \times 10^{-8}$ M. Opisani metodi smo uporabili za določanje analita v farmacevtskih pripravkih in v človeškem serumu, z različnimi mejami detekcije, med $1{,}5 \times 10^{-9}$ M in $4{,}5 \times 10^{-8}$ M buflomedil HCl, brez predpriprave vzorca ali ekstrakcije. Interferenc s strani ekscipientov nismo opazili. Medtem ko ima voltametrična metoda nižjo mejo detekcije, je kromatografska metoda enostavnejpa, hitrejša in bolj občutljiva kot prej opisane HPLC metode.