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Electroanalytical determination of salbutamol in pharmaceutical formulations using cathodically pretreated boron-doped diamond electrode

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ABSTRACT: This paper examined for the first time the possibilities of the usage of the cathodically pretreated boron-doped diamond (CPT-BDD) electrode for the redox behavior of salbutamol (SAL) using cyclic and adsorptive stripping voltammetry. The cyclic voltammograms showed an irreversible and adsorption-controlled oxidation peak at about +1.0 V in the Britton-Robinson (BR) buffer (pH 9.0) solution. Under the optimized experimental condition, using square-wave adsorptive stripping mode, the compound yielded a well-defined voltammetric response in BR buffer, pH 9.0 at +0.95 V (vs. Ag/AgCl) (after 30 s accumulation at an open circuit condition). A linear calibration graph was obtained in the concentration range of 4.15 to 83 µg mL^{-1} ($1.73x10^{-5}$ -$3.47x10^{-4}$ M). A detection limit of 1.21 µg mL^{-1} ($5.06x10^{-6}$ M) was observed. The suggested method was also applied to the determination of SAL in the drug formulations.

KEYWORDS: Salbutamol; boron-doped diamond electrode; square-wave adsorptive stripping voltammetry; pharmaceutical formulations.

1. INTRODUCTION

The boron-doped diamond (BDD) is a relatively new material used for preparation of solid electrode. Characteristics of BDD electrodes are the widest usable electrochemical potential window among all metal and carbon electrode materials (about 3.5 and 5.0-7.5 V in aqueous and non-aqueous organic solutions, respectively), low and stable background current, a high resistance to deactivation by surface fouling, corrosion stability in highly aggressive media, low adsorption of contaminants, a relative insensitivity to dissolved oxygen, good mechanical robustness [1,2]. Due to that, this electrode, nowadays, plays important role in electroanalytical chemistry and is applied in the different fields. However, it is important to underline that, for many analytes, these properties of BDD electrode are extremely dependent on its electrode surface termination, which can be modified by appropriate electrochemical pretreatment (anodic or cathodic) [3-7] or mechanical treatment [8].

Salbutamol, 2-(*tert*-butylamino)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol (Scheme 1), also known as albuterol, is a β_2-adrenergic bronchodilator widely used for the treatment of bronchial asthma and management of premature labour [9-12]. Salbutamol (SAL) is also applied as a tocolytic agent in humans as well as in veterinary medicine. High doses of SAL may have lipolytic effect and residues of this compound, which are most abundant in liver and meat, can be toxic to humans [13].

Up to now, many methods have been determined for SAL, including spectrophotometry [14-16], high-performance liquid chromatography [17-21] or liquid chromatography [22-25], capillary electrophoresis [26,27], thin layer chromatography [28,29] and immunoassay [30,31]. However, most of these methods need more complicated procedures, costly instrumentation, over analysis time, because they need derivatization or combination with various detection methods when compared to electroanalytical methods. Electroanalytical methods are characterized by low cost, low background current, wide range of potential ranges, good sensitivity, selectivity, precision, accuracy and speed, quick surface renewal, easy fabrication. Several methods

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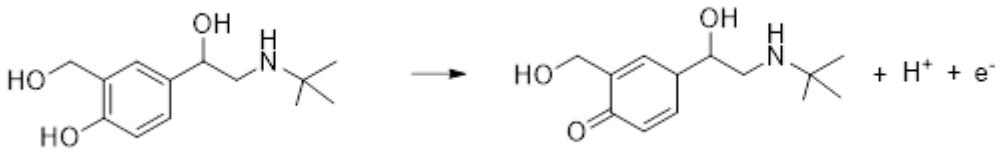
have been investigated for the electrochemical methods of SAL detection such as electrochemical sensors based on molecular imprinting polymers [32], graphene-Au (Gr-Au) nanocomposites [33] poly taurine/zirconia nanoparticles modified electrodes [34], hybrid carbon nanotubes [35], carbon paste electrode modified by iron titanate nanopowders [36], multi-wall carbon nanotubes [37], graphite nanosheet modified electrodes [38], platinum and glassy carbon electrodes [39] and glassy carbon electrode coated with a nanomaterial thin film [40].



Scheme 1. The proposed mechanism for oxidation of salbutamol

To our knowledge, no study related to the determination of SAL using a BDD electrode has appeared in the literature. In view of this, the present work thus aims to investigate the stripping voltammetry with adsorptive accumulation of SAL at the same electrode surface without any chemical modifications and/or mechanical pretreatment. The practical applicability of proposed method will also be demonstrated on quantification of SAL in the pharmaceutical samples.

2. RESULTS AND DISCUSSION

Prior to the experimental design stage, a preliminary conclusion indicated that BDD electrode without pretreatment was not free from passivating problems, and no suitable electroanalytical responses could be obtained when SAL solutions were analyzed, and thus a way to restore the initial activity of the BDD electrode surface was necessary. Firstly, the electrode was treated by anodically (applying a potential of +1.5 V during 180 s in 0.5 M H_2SO_4). A second procedure consisted in a cathodic one (-1.5 V for 180 s in 0.5 M H_2SO_4). The results shown that the cathodic pretreatment (CPT) of the BDD electrode leads to higher oxidation peak current value than anodic one (data not shown). Thus all subsequent experiments were carried out using a BDD electrode that was cathodically pretreated at −1.5 V for 180 s. This pretreatment was repeated daily before starting the voltammetric measurements. However, it should be noticed that this pretreatment was always preceded by an electrochemical cleaning procedure applying a shorter period (at −1.5 V for 60 s) before each voltammetric experiment in order to guarantee a clean electrode surface.

The oxidation behavior of the compound was first studied by CV without an accumulation step obtained with CPT-BDD electrode. Fig. 1 shows the CV curves of 83 µg mL^{-1} SAL in Britton-Robinson buffer pH 9.0 solution recorded within the potential window from 0.0 and +1.5 V at a scan rate of 100 mV s^{-1}. SAL was found to give one anodic peak at about +1.0 V versus Ag/AgCl, at a BDD electrode. No cathodic peak is observed indicating an irreversible oxidation of SAL. Multi-scan CV recordings revealed that the waves decreased upon the second and subsequent scans at the same BDD electrode, pointing to certain adsorption activity at the electrode surface. The effects of scan rate on the oxidation of SBS at the CPT-BDD electrode were checked by CV in Britton–Robinson buffer, pH 9.0. The oxidation peak shifted slightly towards more positive potentials as the scan rate increased; a typical behavior of irreversible electrochemical reactions [41]. The oxidation peak current (i_p) of SBS increases linearly with the scan rate (v) in the range of 100–600 mV s^{-1}, and can be expressed as following: i_p (µA) = 0.009 v (mV s^{-1}) + 1.504, r = 0.994. Thus, the electrochemical reaction is controlled by the adsorption process.

In addition the linearities of plots of log i_p versus log v are expressed as follows:

$\log i_p$ (µA) = 0.651 $\log v$ (mV s^{-1}) – 1.033 (n = 6, r = 0.987)

The observed responses suggested the possibility of setting up method to determine SAL by AdSV. Among the stripping waveforms, the SW modulation combines good sensitivity with high speed, and reduces problems with poisoning of the electrode surface. As a consequence, further work was dedicated towards studying the influence of nature and acidity of the supporting electrolyte upon the SW response, after performing an accumulation step at CPT-BDD electrode. In Fig. 2A-B, this parameter was established in the pH range 3.0-10.0 of Britton-Robinson buffer by carrying out adsorptive measurements on 41.5 µg mL^{-1} SAL

solution, with an open-circuit mode at 60 s. As can be seen from the Figure 2A and B, on increasing pH > 3.0, peak potential was displaced to less positive values till pH 6.0 then became again almost pH independent (from + 0.997 V at pH 6.0 to +0.969 V at pH 10.0). The relationship between the anodic peak potential and the solution pH value (over a pH range between 3.0 and 6.0) could be fit to the linear regression equation of E_p (V) = -0.064pH + 1.384, with a correlation coefficient of r = 0.996. The slope was found to be -64.0 mV/pH units, which is close to the theoretical value of -59 mV, demonstrating that the number of electron and proton taking part in the electrode reaction is equal. The proposed mechanism for the electrochemical oxidation of SAL is presented in Scheme 1. Furthermore, as can be seen from the Figure 2B, there is two peaks in square wave adsorptive stripping voltammetry (SW-AdSV) for SAL solution. It has been found that the peak at more positive potential (well-defined) is due to the oxidation of SAL whereas the other peak (ill-defined) is due to adsorption of product formed in the well-defined peak reaction and is not related to the oxidation of the well-defined peak product or SAL [36]. The current of the initial peak I_a increased with increasing pH in basic solution, the maximum current (3.16 µA) being obtained at pH 9.0. According to the obtained results, the 0.1 M Britton-Robinson buffer solution at pH 9.0 are the most suitable media for analytical purposes, yielding the high peak current, better peak shape, and also the best background signal, which was chosen for further experiments and development of the methodology.

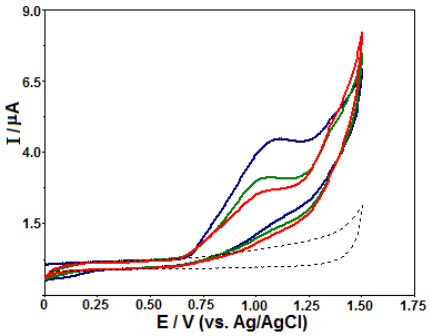


Figure 1. The repetitive cyclic voltammograms of 83 µg mL^{-1} SAL solutions in Britton-Robinson buffer, pH 9.0 for BDD electrode. Scan rate, 100 mV s^{-1}. Dashed lines represent background current.

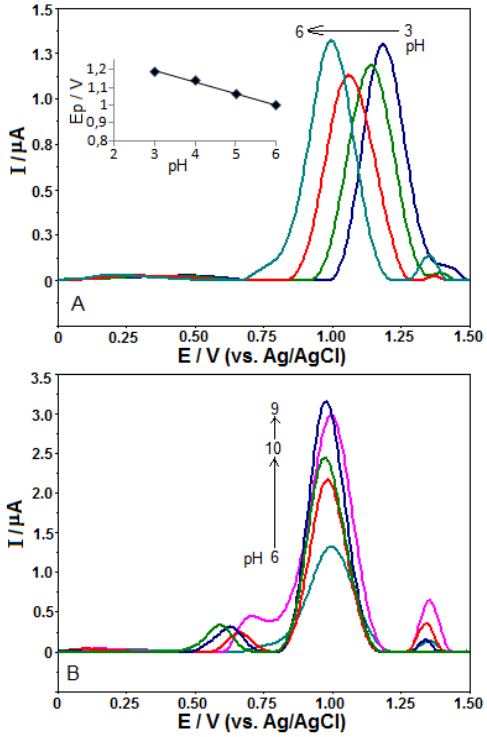


Figure 2. SW stripping voltammograms of 41.5 µg mL^{-1} SAL in Britton-Robinson buffer at CPT-BDD electrode with different pH values (A-3.0-6.0) (B-6.0-10.0). Inset (A): plot of E_p vs. pH. t_{acc}= 60s at open circuit condition; SWV parameters: frequency, 50 Hz; scan increment, 8 mV; pulse amplitude, 30 mV.

Taking into account the pronounced adsorptive characteristics of SAL proved on BDD electrode, the attention was then turned to the effect of accumulation time (t_{acc}) and accumulation potential (E_{acc}) (data not presented) for 41.5 µg mL^{-1} SAL under the optimum experimental conditions. The influence of the t_{acc} upon the analytical signal was examined in the range 0-360 s at open-circuit condition. The current increased linearly with t_{acc} till 30 s beyond which the peak current was almost constant, which indicated that the accumulation of SAL at the electrode surface nearly reached a saturation state. The t_{acc} of 30 s is very short and doubtlessly advantageous for practical use of this electrode. On the other hand, the dependence of the stripping peak current on the E_{acc} was evaluated either at open-circuit condition or over the potential range +0.1 to +0.6 V with an accumulation time of 30 s. The maximum peak current was achieved at the potential of at open-circuit condition. Therefore, the t_{acc} and E_{acc} of 30 s and an open-circuit condition, respectively, were found reasonable, respectively, for the rest of present analytical investigation.

The SW response markedly depends on the parameters of the excitement signal. In order to obtain the maximum development of the SW-AdSV peak current, the various instrumental conditions (square-wave frequency, 25 Hz ≤ f ≤ 125 Hz; pulse amplitude, 20mV ≤ a ≤ 70 mV; and scan increment, 6mV ≤ ΔE_s≤ 16mV) were studied for 41.5 µg mL^{-1} SAL in selected electrolytes following pre-concentration for 30 s under at an open-circuit condition. The peak currents increased with the f due to the increase in the effective scan rate but the peak shape and baseline were distorted at f values higher than 50 Hz.This was attributed to the greater contribution of the capacitive current at higher frequencies. The voltammetric responses for SAL determination as a function of variation in a demonstrated that peak current values increased upon increase of this parameter. However, the best peak morphology and sharper one was obtained at 60 mV. In addition, at higher values of 12 mV, an increase in ΔE_s resulted in a decrease in peak current. To account for the results, in subsequent experiments, values of f = 50 Hz, a = 60mV, and ΔE_s = 12 mV were adopted.

The previously optimized SWV parameters were employed to record the analytical curve for SAL in 0.1 M BR buffer (pH 9.0) using the CPT-BDD electrode. The proposed method offered well-defined concentration dependence. Fig. 3 displays stripping voltammograms obtained by successive additions of SAL over the 4.15 to 83 µg mL^{-1} (1.73x10^{-5} -3.47x10^{-4} M) concentration range. The peak current at a potential of +0.95 V increased proportionally with the SAL concentration (Fig. 3, inset) to yield a highly linear calibration plot, I_p (µA) = 0.104 C (µg mL^{-1})+1.069 (r: 0.999, n: 8), where I_p is the adsorptive stripping peak current, C the SAL concentration, r the correlation coefficient, and n the number of experiments.

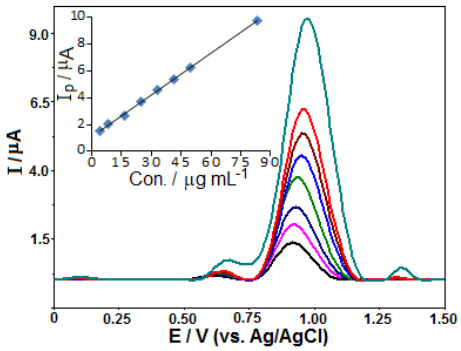


Figure 3. SW stripping voltammograms in Britton-Robinson buffer, pH 9.0 with containing different concentration of SAL, 4.15, 8.3, 16.6, 24.9, 33.2, 41.5, 49.8 and 83.0 µg mL^{-1}. Inset depicts a corresponding calibration plot for the quantitation of SAL. Electrode= CPT-BDD; t_{acc}= 30s at open-circuit condition; SWV parameters: frequency, 50 Hz; scan increment, 12 mV; pulse amplitude, 60 mV.

From the data obtained by the analytical curves, the detection (LOD) and quantification (LOQ) limits were calculated using the formulas 3 s/m and 10 s/m, respectively, where s is the standard deviation of the peak current (three runs) of the lowest concentration of the related linearity range, and m the slope of the calibration plot. LOD and LOQ were found to be 1.21 µg mL^{-1} (5.06x10^{-6} mol L^{-1}) and 4.03 µg mL^{-1} (1.69x10^{-5} M), respectively.

The precision of the CPT-BDD electrode has been evaluated by repetitive determinations of at a concentration level of 4.15 µg mL^{-1} for SAL at pH 9.0. The results of seven replicate measurements showed a relative standard deviation (R.S.D.) of 4.84% indicating that the results are repeatable. Further, inter-day precision was examined by measuring the current response of the BDD electrode for three consecutive days

for the same 4.15 µg mL^{-1} concentration of SAL and the R.S.D. was found to be 7.08%. The experimental results indicated that the BDD electrode possessed a good sensitivity and precision for determination of SAL.

Finally, the selectivity of the proposed method was evaluated. Various possible interferents were investigated for their effects on the determination of SAL. The tolerance limit was defined as the maximum concentration of the potentially interfering substance that caused an error less than ± 5.0% in the determination of the compound. To evaluate the interferences of these foreign species NaCl, KNO_3, $Mg(NO_3)_2$, lactose, cornstarch, talc, uric acid and ractopamine, systematic studies were performed. It was found that this method has good selectivity to the determination of SAL. At about one hundred-fold concentrations of NaCl, KNO_3 and $Mg(NO_3)_2$, 20-fold concentration of cornstarch, talc, lactose or uric acid and 10-fold concentration of ractopamine did not interfere with the current responses of 4.15 µg mL^{-1} SAL. It is known that determination of SAL is important in the presence of ractopamine. Hence, the development electroanalytical method has been tested in the presence of ractopamine. Fig. 4 displays stripping voltammograms obtained by successive additions of SAL over the 4.15 to 83 µg mL^{-1} concentration range while holding the concentration of ractopamine constant at 10 µg mL^{-1}. The peak current at a potential of +0.95 V increased proportionally with the SAL concentration (Fig. 4, inset) to yield a highly linear calibration plot, I_p (µA) = 0.097 C (µg mL^{-1})+0.251 (*r*: 0.997, *n:* 7). The results showed that the development method has been used to determine SAL in the presence of ractopamine.

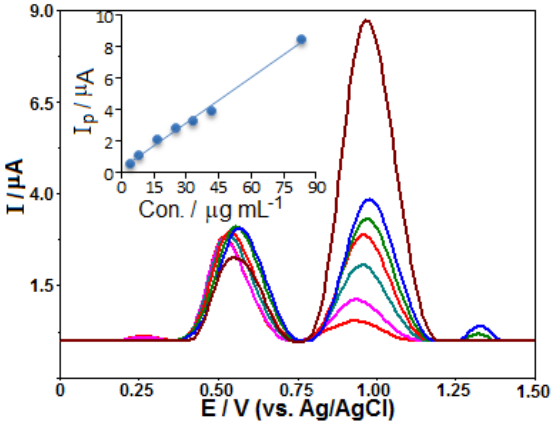


Figure 4. SW stripping voltammograms in Britton-Robinson buffer, pH 9.0 with 10 µg mL^{-1} ractopamine containing different concentration of SAL, 4.15, 8.3, 16.6, 24.9, 33.2, 41.5 and 83.0 µg mL^{-1}. Inset depicts a corresponding calibration plot for the quantitation of SAL. Other operating conditions as indicated in Fig.3.

In order to assess the feasibility of the analytical system from the practical point of view, the above-described electroanalytical methodology was applied for SAL determination in the commercially available product. The analyzed solutions were prepared as it was described above (in Section 4.4), after simply dissolving samples in selected medium and diluting the resulting solution to a target concentration within the linear range. The diluted real samples were almost similar to aqueous sample in behavior. Quantification for the sample was performed by means of the calibration curve method from the related regression equation. Taking into account the successive dilutions of the sample, SAL content was calculated to be 3.92 mg per tablet (RSD of 4.85%), which approximates the label value of 4.0 mg per tablet declared by producer. The results achieved by the CPT-BDD electrode are in good agreement with the labelled SAL content in the sample, thus indicating the feasibility of the method for SAL determination in pharmaceutical formulation. To check the validity of the proposed method, the spike/recovery experiments were performed. The recovery studies were carried out by adding the appropriate volume of standard SAL solutions prepared in supporting electrolyte to the previously determined SAL content of the tablet sample. Recovery of SAL was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure SAL. In Table 1, the results of the analysis of spiked samples of the tablet are shown. The recoveries of the compound in the tablet samples were examined at least three times. It was found that SAL amount can be quantitatively recovered by the proposed method, being thus a guarantee of the accuracy of the voltammetric determination of SAL in the pharmaceutical formulations.

Table 1 Results of the recovery analysis of SAL in the sample of tablet

SAL added (μg mL^{-1})	*Level determined[a] (μg mL^{-1})*	*Recovery (%) ± RSD (%)*
4.15	3.81	91.8 ± 5.08
8.3	7.82	94.2 ± 4.33
16.6	15.52	93.5 ± 3.89

[a]Values reported are the average of three independent analysis of each spiked sample

3. CONCLUSION

BDD electrode as relatively novel electrode material (without any electrode surface modification) was used for the first time in combination with SW-AdSV to elaborate a simple analytical method for the determination of SAL. The proposed method, after optimization of SW-AdSV parameters was successful applied for the SAL quantification in the pharmaceutical formulation samples with simplicity, satisfactory results and recoveries.

4. MATERIALS AND METHODS

4.1. Chemicals

Salbutamol hemisulfate (SBS) salt standard was purchased from Sigma. SBS stock solution (1 mg mL^{-1}) was prepared in water. This stock solution is correspond to 0.83 mg mL^{-1} SAL solution. Tablet dosage form containing the active compound was procured from commercial local pharmacies. Other reagents used were of analytical grade (Merck or Sigma), and their solutions were prepared with deionised water (uric acid was prepared 0.1 M NaOH) further purified via a Milli-Q system (Millipore, resistivity ≥ 18.2 MΩ cm). Britton–Robinson (BR) buffer solution (pH 3-10) was prepared in usual way by mixing of 0.1 M of all necessary components (acetic acid, boric acid and orthophosphoric acid), with pH adjusted with sodium hydroxide (1.0 M).

4.2. Apparatus

The cyclic (CV) and square-wave (SWV) voltammetric experiments at a boron-doped diamond (BDD) electrode were performed using a μAutolab type III electrochemical analyzer controlled with the GPES 4.9 software (EcoChemie, The Netherlands). All SW voltammograms were smoothed using a Savicky and Golay algorithm and baseline-corrected by the moving average method (peak width of 0.01 V), using the GPES software. A three-electrode cell system was used: a BDD working electrode (Windsor Scientific Ltd.; diameter of 3mm, the boron doping level of 1000 ppm), a Pt-wire auxiliary electrode, and an Ag/AgCl (3 M NaCl) (Model RE-1, BAS, USA) reference electrode to which all electrode potentials hereinafter are referred. The pretreatment of BDD electrode was firstly polarized in a 0.5 M H_2SO_4 by applying -1.5 V during 180 s; thus, the BDD surface was made predominantly hydrogen-terminated. Afterwards, the electrode was pretreated for 60 s under the same experimental conditions. In this study, the first cathodic surface pretreatment was daily performed before starting the experimental work. The other step in the procedure was applied before each voltammetric experiment. The pretreatment procedure was carried out in an independent electrochemical cell.

4.3. Adsorptive stripping voltammetric procedure

The general procedure for stripping voltammetric analysis of SAL was as follows: The three-electrode system was immersed in a voltammetric cell containing required aliquot of the SAL working solutions and supporting electrolyte at a desired pH. A selected accumulation potential was then applied to a BDD electrode surface for a selected pre-concentration period, while the solution was stirred at 500 rpm. At the end of the accumulation period, the stirring was stopped and a 5 s rest period was allowed for the solution to become quiescent. Then, the voltammogram was recorded by scanning the potential toward to positive direction between 0.0 to +1.5 V using SW waveform.

The best instrumental parameters for SWV which was used for investigating the determination of SAL were as follows: frequency, 50 Hz; pulse amplitude, 60 mV; scan increment, 12 mV. Successive measurements were carried out by repeating the above assay protocol on the working electrode. All measurements were performed in triplicate at laboratory temperature.

4.4. Sample preparation

Ventolin® (GlaxoSmithKline) tablets labeled as containing 4 mg SAL were used for the present analytical applications. Ten tablets were weighed and the average mass per tablet was determined. The tablets were carefully grounded to a fine powder in a mortar with a pistil. An adequate amount of the resulting powder was weighed and transferred into a 25-mL calibrated dark flask, which was completed to the volume with BR buffer pH 9.0. The content of the flask was sonicated for about 30 min. to complete dissolution. The desired concentrations of SAL were obtained by taking suitable aliquots from the upper clear layer of the mixture and diluting with 0.1 M BR buffer (pH 9.0) solution. An aliquot volume of these solutions was transferred to the voltammetric cell containing the same solution, and analyzed in the day of preparation according to the procedure developed for the pure electrolyte using the calibration curve method from the related regression equation.

Author contributions: Conception - Y.Y; Design - Y.Y.; Supervision - Y.Y.; Resources - Y.Y., Z.Ş.; Materials - Y.Y., Z.Ş.; Data Collection and/or Processing - Y.Y., P.T.P.; Analysis and/or Interpretation - Y.Y., P.T.P.; Literature Search - H.S.A., A.A.A.; Writing manuscript - Y.Y., P.T.P.; Critical Reviews - P.T.P, H.S.A., A.A.A., Y.Y., Z.Ş.; Other - Z.Ş

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