

# Potentiometric Determination of Moxifloxacin by ZnO Nanorodes Modified ion Selective Electrode

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## Abstract

Potentiometric determination of moxifloxacin (Moxi) by ion selective electrode based on ZnO nanorods incorporation with HP $\beta$ -CD as sensing ionophore and (KTFPB) potassium tetrakis- (3,5(trifluoromethyl) phenyl borate ion as anionic site (additive) in polyvinyl chloride (PVC) membrane, without inner reference solution was developed. The sensor shows nearly nernstian response over a concentration range ( $5 \times 10^{-8}$  M to  $1 \times 10^{-2}$  M) with a slope of  $21.9 \pm 0.16$  mv decade<sup>-1</sup> of concentration with a limit of detection (LOD) 0.127  $\mu$ M. The electrode exhibits a fast dynamic response of 2 s for a period of 6 months without significant change in its characteristics with excellent stability and sensitivity toward inorganic species. The method is accurate and precise as indicated by the mean recoveries 99.5% with RSD less than 2%. The proposed method was successfully applied for the determination of Moxi in pure form and its pharmaceutical formulations.

**Keywords:** Potentiometric; Ion selective electrode; ZnO nanorode; Moxi

HPLC-fluorescence(HPLC-Fl), capillary electrophoresis(CE), and HPLC-mass spectrometry (HPLC-MS) [4-17].

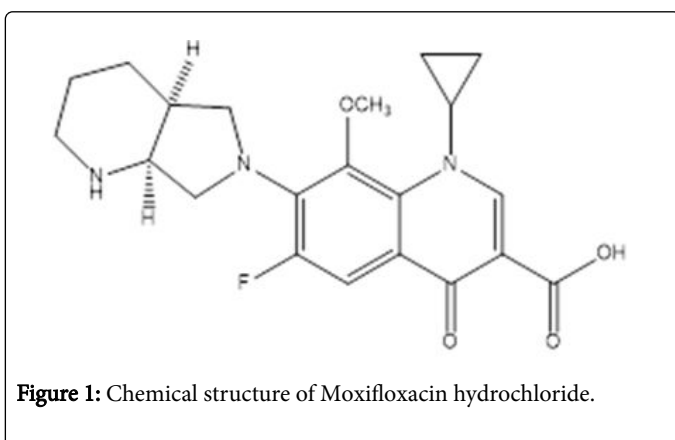
## Introduction

Moxifloxacin (Moxi) chemically is 1-cyclopropyl-7-(2,8-diazobicyclo[4.3.0]nonane)-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid Figure 1, is a synthetic fourth generation fluoroquinolone (fluorinated derivative of the quinolone). Moxi is active against broad spectrum of antibacterial agent, encompassing Gram-negative, Gram-positive bacteria and also antibiotic resistant streptococcus pneumonia [1,2]. The bactericidal action of Moxi results from trapping of enzymes on DNA and lethal release of double-stranded breaks, thereby inhibiting of cell replication [3].

Potentiometric sensors are easy to miniaturize and provides a large dynamic range. In conventional ion selective electrodes, polyvinyl chloride (PVC) is the most commonly used matrix as the selective membrane. The ion-selective membrane exhibits the selectivity with which the sensing material responds to the analyte and an electrochemical equilibrium is reached. The resulting potential difference, formed between the phases, will then be governed by the activity of this specific ion in the two solution phases [17-19].

Different potentiometric methods using ion selective electrodes for determination of Moxi were reported [20-23]. Hefnawy et al. developed PVC membrane sensors for analysis of Moxi. The sensing membranes incorporate ion association complexes of Moxi-cation and sodium tetraphenyl borate (NaTPB), phosphomolybdic acid (PMA) and phosphotungstic acid (PTA) as electroactive materials [21]. The sensors showed good discrimination of Moxi from several inorganic and organic compounds. Elghobashy et al. Constructed Moxi selective electrodes with 2-nitrophenyl octyl ether as a plasticizer in a polymeric matrix of PVC. The Sensors were fabricated using tetrakis (4-chlorophenyl) borate (TpClPB) as an anionic exchanger with and without incorporation of an ionophore [22]. The proposed sensors were successfully applied for the determination of Moxi in bulk powder, pharmaceutical formulation, and biological fluids.

ZnO nanowires, nanorods and nanotubes have gained much attraction due to their high surface-to-volume ratio which makes them extremely sensitive to minute surface changes; also they have excellent chemical stability [24]. Moreover, one-dimensional ZnO nanostructures are promising for bio and chemical sensing due to their ease to grow vertically on almost any substrate there high sensitivity, low cost, simplicity and low power consumption [24,25].



**Figure 1:** Chemical structure of Moxifloxacin hydrochloride.

Various analytical methods have been cited in literature for Moxi determinations including spectrophotometry, spectrofluorimetry, atomic absorption spectrometry, conductometry, voltammetry [4-10], high performance liquid chromatography-ultraviolet (HPLC-UV),

In this study potentiometric ZnO nanorods based ion selective electrode without inner reference solution for a simple, sensitive, and rapid determination of Moxi in pharmaceutical formulations is illustrated. Ion selective electrode consisted of PVC, dibutyl phthalate, 2-Hydroxypropyl)- $\beta$ -cyclodextrin (HP $\beta$ -CD) and potassium tetrakis (3,5 (trifluoro methyl)phenyl)borate (KTFPB) as matrix, plasticizer, sensing ionophore and anionic additive, respectively were used.

## Materials and Methods

### Chemicals and reagent

Moxifloxacin hydrochloride was obtained from (98%, Bayer AG, Leverkusen Germany), HP $\beta$ CD (ionophore), tetrakis (3,5(trifluoro methyl)phenyl) borate (KTFPB) (additive), PVC (high molecular weight), dibutyl phthalate (a plasticizer), zinc acetate (ZnAc), Hexamethylenetetramine (HMTA) were purchased from sigma Aldrich (St. Louis, USA), silver wire (0.3 mm diameter), Na<sub>2</sub>HPO<sub>4</sub>, H<sub>3</sub>PO<sub>4</sub>, KOH, acetone, isopropanol, tetrahydrofuran (THF), methanol, (all solvent with HPLC grade), Avelox tablet (Bayer Schering pharma) TRT06R7 labeled to contain 400 mg Moxi per tablet were purchased from local market, Deionized water.

### Instrument and apparatus

pH/mv meter (PHS-3E) (China), Ag/AgCl reference electrode ( $\Omega$  metrohm, Autolab, inner and outer filling by KCl 3M. (Netherlands), sensitive balance, magnetic hot plate, thermometer, oven, SEM (Zeiss Evo LS 10, Germany).

### Seed and growth ZnO nanorods

ZnO nanorods was grown by low temperature aqueous chemical method [25]. A silver wire (0.3 mm) was cut in the length of 5 cm and cleaned by acetone and isopropanol for 2 min in each solution followed by rinsing with deionized water and left to dry at room temperature. The silver wire was immersed three times in a seed

solution prepared by mixing alcoholic solutions of KOH added drop wise to heated, stirred 0.03 M of zinc acetate the resulting solution was kept under stirring for 2 hours at 60°C prior dipping, the wires was left to dry at room temperature. The ZnO was grown by suspending the pre-coated Ag wire in aqueous solutions contains 0.025 M ZnAc with equimolar concentration of HMTA. The beaker was placed in preheated oven at 70°C to 5 hours. The wires were cooled down, washed by deionized water and left to dry overnight. The ZnO nanorods was characterized by SEM (Zeiss Evo LS 10, Germany) Figure 2.

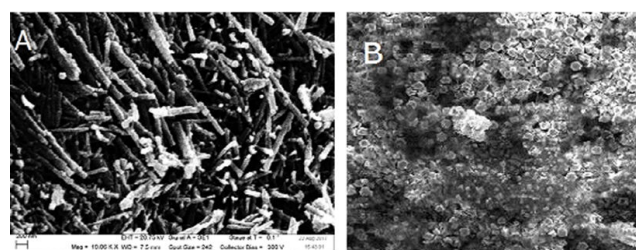


Figure 2: A, B) SEM at different magnifications and view of the ZnO nanorodes grown on Ag wire hydrothermal aqueous chemical method.

### Coating ZnO nanorods with ion selective membrane

ZnO nanorods were coated by ion selective membrane by mixing 33% PVC, 66% DBP plasticizer, 1.2% HP $\beta$ -CD (ionophore), 0.4% KTFPB (ionic additive) in 5 ml THF. The ZnO coated wires was dipped twice into a prepared solution, after each dip the electrode was left to dry at room temperature, then the electrode was conditioned into  $1 \times 10^{-3}$  M of Moxi standard solution for 24 hour prior to use. The membrane was characterized by SEM (Zeiss Evo LS 10, Germany) Figure 3.

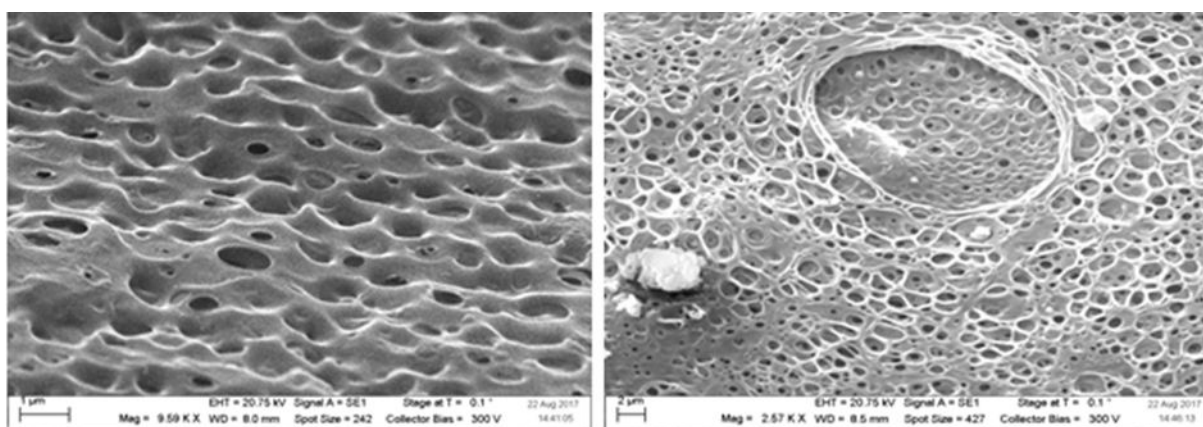


Figure 3: Presents ion selective membrane with KTFPB additive with different magnification.

### Standard drug solutions

Stock standard solutions 0.01 M Moxi.HCl (Mw=437.89 g/mol) was prepared by dissolving accurate weight in deionized water, this solution was kept in the dark at 4°C and it was found that it was stable

for several weeks. Working solutions ranging 0.001-10000  $\mu$ M were prepared by serial dilution of the stock solution by deionized water. The testing series was prepared by adding adequate amount of (0.2 M) phosphate buffer (H<sub>3</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>) [pH=2; 0.2 M] and desired

volume of drug stock solution and the volume completed to mark by deionized water.

### Electrochemical measurements

In a complete potentiometric cell, the Moxi-ZnO-selective electrode was used in conjunction with Ag/AgCl reference electrode (inner and outer filling by KCl 3M). The electrochemical potential between the Moxi-ZnO-selective electrode as cathode and Ag/AgCl reference electrode ( $\Omega$  metrohm, Autolab, inner and outer filling by KCl 3M) as anode was measured with pH/mv meter (PHS-3E).

Moxi.TFPB - PVC || Test solution || Ag/AgCl (3M KCl)

The measured potential was plotted against the logarithm of drug concentration. The electrode was washed with deionized water blotted with tissue paper between measurements.

## Results and Discussions

### Optimization conditions

**Effect of pH:** The effect of pH on the potential response of the Moxi-ZnO-ISE was investigated using  $1 \times 10^{-4}$  M solutions in pH range of 2.0-11.0 using  $\text{Na}_2\text{HPO}_4/\text{H}_3\text{PO}_4$  (0.2 M) as a buffer solution. The potential readings corresponding to different pH values were recorded and plotted using the proposed electrode. Increasing in electrode potential was observed in pH range from 2 to 3 and decreased from pH 4 to 11, Figure 4. These result suggested that the inclusion complex of Moxi and HP $\beta$ -CD was suitable in acidic media because Moxi contains secondary amine that capable to bind with protons presents in acidic media resulting positively charged Moxi ion, which therefore can attracted by anionic tetraphenyl borate group present in the additive (KTFPB) and hence facilities the inclusion between Moxi and HP $\beta$ -CD [21,22].

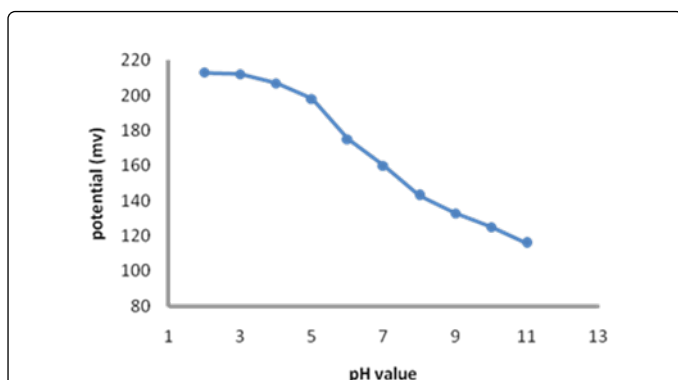


Figure 4: Optimization of pH for Moxi-TFPB- HP $\beta$ -CD, Moxi  $1 \times 10^{-4}$  M, at room temperature, time 2 sec.

**Effect of volume of buffer:** The effect of volume of buffer on the potential response of the Moxi-ZnO-ISE was studied using  $1 \times 10^{-4}$  M

solutions in the range of (0-10) mL using  $\text{Na}_2\text{HPO}_4/\text{H}_3\text{PO}_4$  [pH 2; 0.2 M]. It was found that the potential increased when buffer adding to Moxi solution without buffer and the potential remains constant with adding extra volume of buffer as shown in Figure 5.

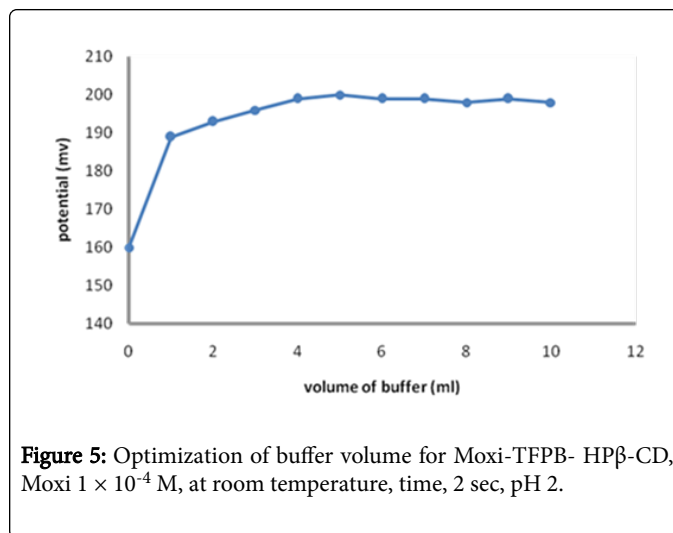


Figure 5: Optimization of buffer volume for Moxi-TFPB- HP $\beta$ -CD, Moxi  $1 \times 10^{-4}$  M, at room temperature, time, 2 sec, pH 2.

**Effect of temperature:** The effect of temperature on the potential response of the Moxi-ZnO-ISE was studied using  $1 \times 10^{-4}$  M solutions at the range of temperature (10-80°C) using thermometer presented in Figure 6. It reveals that the potential increased with increasing temperature of drug solution this could be attributed to potentiometric measurements is equilibrium controlled [26], thus increasing solution temperature is resulting faster equilibrium between the electrode surface and Moxi solution.

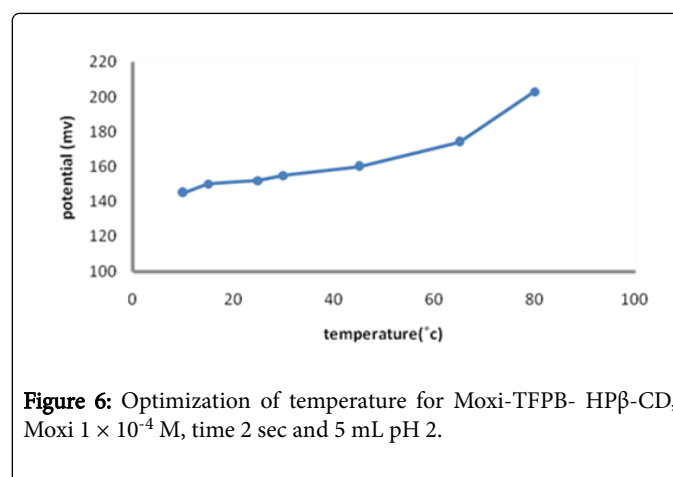
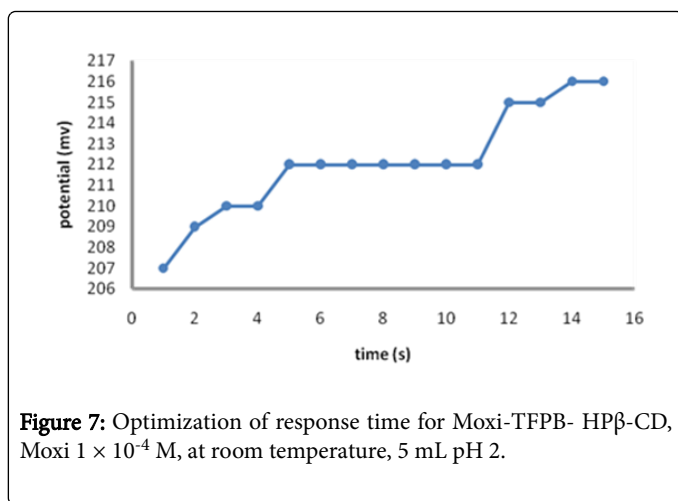


Figure 6: Optimization of temperature for Moxi-TFPB- HP $\beta$ -CD, Moxi  $1 \times 10^{-4}$  M, time 2 sec and 5 mL pH 2.

**Response time:** The response time of potential of the Moxi-ZnO-ISE was studied using  $1 \times 10^{-4}$  M solutions in a period from 0 to 15 second. The potential readings corresponding to time were recorded and plotted using the proposed electrode in Figure 7. The sensor display very fast and stable response within 2 second.



**Figure 7:** Optimization of response time for Moxi-TFPB- HPβ-CD, Moxi  $1 \times 10^{-4}$  M, at room temperature, 5 mL pH 2.

### Electrode composition

The electrode shows linear nernstian response over a wide range of concentration 0.05-10000  $\mu$ M, stable, sensitive and very fast response. This attributed to electrode compositions. The ZnO nanorods increased the surface area for distribution of the membrane compared if it directly attached to silver wire, thus increased the sensitivity of the electrode and decreases the response time. HPβ-CD is used as sensing ionophore, the most important property of CDs is their ability to form supramolecular inclusion complexes with many appropriately sized organic ions and molecules in aqueous, non-aqueous and mixed media [27,28]. The driving forces for the complexation are non-covalent, including vanderWaals forces and directed hydrogen bonding. Water molecules in CD cavity are displaced by more hydrophobic guest molecules present in the solution to attain a non-polar/non-polar association and decrease of CD ring strain resulting in a more stable lower energy state [29]. On constructing an ISE, the amount of the sensing ionophore in the electrode matrix should be sufficient to obtain reasonable complexation at the electrode surface that is responsible for the electrode potential [30,31].

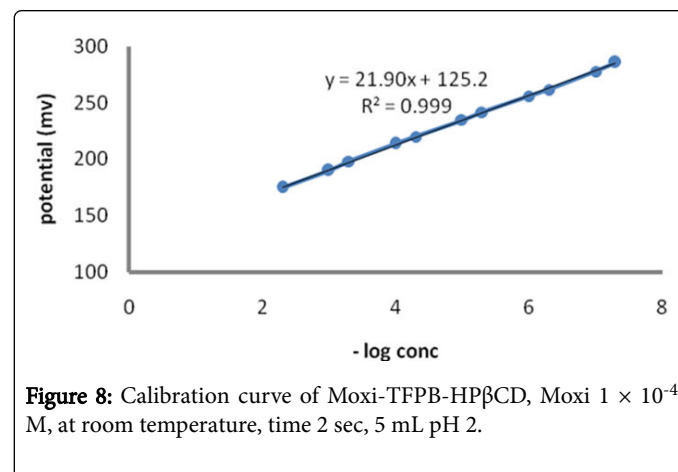
The function of KTFPB as lipophilic ionic additives is to promote the interfacial ion exchange kinetics and decrease the electrode resistance through enhancing the ionic mobility in the electrode matrix. The response of ISEs containing ionic sites can be distinguished whether the incorporated ionophore acts as an electrically charged or uncharged carrier [32,33].

### Statistical data

The analytical methods were validated with respect to linearity, limit of detection (LOD), limit of quantification (LOQ) and precision according to ICH [34].

**Calibration curve and statistical data for Moxi:** The measuring range of a potentiometric sensor was the linear part of the calibration curve as shown in Figure 8. The critical response of the sensor was determined and the results were summarized in Table 1. LOD and LOQ were determined using the formula  $LOD$  or  $LOQ = K \cdot SD_a / b$ , where  $K = 3.3$  for LOD and 10 for LOQ,  $SD_a$  is the standard deviation of the intercept, and  $b$  is the slope. The values of LOD and LOQ were found to be 0.127 and 0.3836  $\mu$ M respectively. The sensor show nearly nernstian response over the concentration range 0.05-10000  $\mu$ M for Moxi standard solution. Calibration curve slope for electrode were 21.9

mV decade<sup>-1</sup>. The electrode exhibited a fast dynamic response of 2 s for a period for more than 6 months without significant change in the electrodes parameters.



**Figure 8:** Calibration curve of Moxi-TFPB-HPβCD, Moxi  $1 \times 10^{-4}$  M, at room temperature, time 2 sec, 5 mL pH 2.

Parameter	Value
Slope, mv decade-1	21.9 ± 0.16
Intercept, mv decade-1	125.24 ± 0.84
Linear Correlation coefficient ( R2 )	0.9995
Linear range, $\mu$ M	0.05-10000 $\mu$ M
LOD, $\mu$ M	0.127
LOQ, $\mu$ M	0.3836
Response time, second	>5 s
Life time, month	6 month
*PDL, $\mu$ m	0.001 $\mu$ M
*PDL= practical detection limit	

**Table 1:** Parameter for Moxi potentiometric method.

**Accuracy and precision of the potentiometric method:** The accuracy and precision of the proposed method was determined at three concentration levels of Moxi by apply three replicate samples of each concentration. The standard deviations for the results did not exceed 2% are listed in Table 2, indicating high reproducibility of the results and precision of the method. This good level of precision was suitable for quality control analysis of Moxi and in the pharmaceutical formulations.

Concentration	Taken (-log c)	Found (-log c)	Recovery % ± RSD*
$5 \times 10^{-4}$ M	3.3	3.46	104.8 ± 0.97
$1 \times 10^{-5}$ M	5	5.08	101.6 ± 0.67
$1 \times 10^{-7}$ M	7	6.98	99.7 ± 0.49
*values are mean of three determinations			

**Table 2:** Precision of the potentiometric method for Moxi determination.



Parameter		Recovery% ± RSD*
Standard conditions		99.7 ± 0.85
pH	1.5	99.3 ± 0.52
	2.5	98.9 ± 0.50
Temperature (°C)	30	98.5 ± 0.89
	40	96.4 ± 0.99
Volume of buffer (mL)	2	99.1 ± 0.25
	8	99.3 ± 0.42
Reaction time (sec)	2	97.4 ± 0.47
	10	99.0 ± 0.39
* values are mean of 3 determinations		

**Table 3:** Robustness of potentiometric method for Moxi determination.

Sample content M	Standard added M	p C	Found log c	Recovery (% ± RSD)*
1 × 10 <sup>-4</sup>	1 × 10 <sup>-4</sup>	3.7	3.61	97.3 ± 0.28
1 × 10 <sup>-4</sup>	2 × 10 <sup>-4</sup>	3.5	3.41	97.16 ± 0.50
1 × 10 <sup>-4</sup>	3 × 10 <sup>-4</sup>	3.4	3.26	95.88 ± 0.29
1 × 10 <sup>-4</sup>	4 × 10 <sup>-4</sup>	3.3	3.14	95.2 ± 0.52
1 × 10 <sup>-4</sup>	5 × 10 <sup>-4</sup>	3.2	3.06	95.6 ± 0.30

**Table 4:** Recovery of the potentiometric method of Moxi.

**The sensitivity:** The sensitivity was tested by adding some inorganic salts and diluted acids and bases, it was found that the electrode shows excellent sensitivity toward testing species with RSD less than 2%.

**Reproducibility:** The electrode response shows excellent repeatability during analysis and very stable response with intraday RSD did not exceeded 2%, and interday analysis with RSD less than 5%.

## Conclusion

It can be concluded that Moxi-ZnO-ISE offers a viable technique for the direct determination of Moxi in pharmaceutical preparations. The sensor allows simple, rapid, and reproducible determination over a wide linear range of concentration with the same sensitivity without the need of complex sample manipulations. The sensor exhibits a good selectivity towards the drug in the presence of various pharmaceutical recipients, long life time and time- labor saving.

The procedure avoid the usual pretreatment steps necessary for Moxi assays and presents some general advantages over common chromatographic and spectroscopic procedure, it makes use of less sophisticated equipments (there for being easier to operate and providing lower cost of analysis ) and surpasses color and turbidity problems associated with suspensions and colloids.

Sensor accomplished LOD and LOQ of 0.127 μM, 0.3836 μM, respectively with a fast response time of less than 5 seconds.

**Robustness of potentiometric method for Moxi:** Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed, whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variables did not significantly affect the procedures, recovery values were shown in Table 3.

**Analysis of pharmaceutical formulations:** A proposed method was applied to the pharmaceutical formulations and indicates the high accuracy of the proposed method for determination of Moxi. The proposed method has advantage of being virtually free from interferences by excipients. The percentage was 99.5 ± 0.84.

**Recovery study of the potentiometric method:** To a fixed amount of the drug in the dosage form and pure drug (the standard) were added at five different levels and the total was found by the proposed method each test was performed in triplicate. Table 4 revealing good accuracy and no interference from excipients. Recovery was calculated as the amount found / amount taken × 100. Values are mean ± R.S.D. for three determinations.

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