Regular Article

ANALYTICAL
AND BIOANALYTICAL
CHEMISTRY
RESEARCH

Published by the
Iranian Chemical Society

***Anal. Bioanal. Chem. Res.**, Vol. 11, No. 2, 139-151, April 2024.*

Selective and Low-cost Potentiometric Sensors to Determine Fexofenadine and Rupatadine in Pharmaceuticals Using Sodium Tetraphenyl Boron as an Ion-exchanger

Siddaraju Chikkalingaiah[a] and Rajendraprasad Nagaraju[b,*]

[a]*Department of Chemistry, Maharani's Science College for Women, Mysuru, Karnataka, India*

[b]*PG Department of Chemistry, JSS Research Centre, JSS College of Arts, Commerce, and Science (Research Centre Recognized by the University of Mysore), Ooty Road, Mysuru, Karnataka, India*

(Received 8 October 2023, Accepted 30 December 2023)

In this study, we designed two simple, selective and cost-effective solid-state ion-selective sensors using a polyvinylchloride matrix to detect fexofenadine hydrochloride (FFH) and rupatadine fumarate (RTF) in pharmaceuticals. These sensors utilize the sodium tetraphenyl boron (NaTPB) for ion exchange and β-cyclodextrin for ionophore properties. The FFH sensor shows a linear response within the range of 5×10^{-4} to 2.5×10^{-3} M of FFH at pH levels ranging between 2.5 and 6, exhibiting a Nernstian slope of 56.92 mV decade^{-1}. Similarly, the RTF sensor demonstrates a linear response between 8×10^{-5} and 2.5×10^{-3} M of RTF within the pH range of 2.8-6.4, with a Nernstian slope of 20 mV decade^{-1}. Detection and quantification limits of FFH were found to be 2.5×10^{-4} and 4.5×10^{-4} M, and that of RTF were 2.0×10^{-5} and 6.1×10^{-5} M, respectively. The sensors exhibited excellent selectivity, as indicated by mean percentage recoveries of 100.7 and 99.87 for FFH and RTF, respectively, with a low relative standard deviation (RSD) of less than 2.5%.

Keywords: Fexofenadine, Rupatadine, Sodium tetraphenyl boron, Nernstian slope, Sensors

INTRODUCTION

Fexofenadine hydrochloride (FFH), also identified by its IUPAC name 2-[4-[1-hydroxy-4-[4-[hydroxy (diphenyl) methyl] piperidin-1-yl] butyl] phenyl]-2-methylpropanoic acid (Fig. 1A), is an active carboxylate metabolite originating from the second-generation drug terfenadine [1-3]. FFH effectively alleviates symptoms associated with sneezing, runny nose, sore throat, infectious conjunctivitis, seasonal rhinitis, and idiopathic urticaria. Importantly, it exhibits no adverse effects on electrocardiography [4]. Rupatadine fumarate (RTF), known by its IUPAC name 8-chloro11-{1-[(5-methylpyridin-3-yl) methyl] piperidin-4-ylidene}-6,11-dihydro-5H-benzo [5,6] cyclohepta [1,2-b] pyridine (2E)-but-2-enedioate (Fig. 1B), stands as a second-generation histamine antagonist and a potent inhibitor of platelet-activating factor [5-7].

Both medications are deemed non-sedating when used within therapeutic doses and exhibit limited ability to cross the blood-brain barrier. Their quick absorption and long-lasting effects make them appropriate for once-daily dosing [2,6,8].

The pharmacopeias of both the United States and Europe recommended liquid chromatographic methods for determining FFH [9,10]. However, there is no official monograph for RTF in any pharmacopeia [7]. A review of literature unveiled multiple approaches for quantifying FFH, encompassing techniques like spectrophotometry [3,11-15], thin layer chromatography (TLC) [14], high-performance liquid chromatography (HPLC) [15-17], ultra-high performance liquid chromatography (UHPLC) [18], high-performance thin layer chromatography (HPTLC) [19],

*Corresponding author. E-mail: prasadtnpur@gmail.com

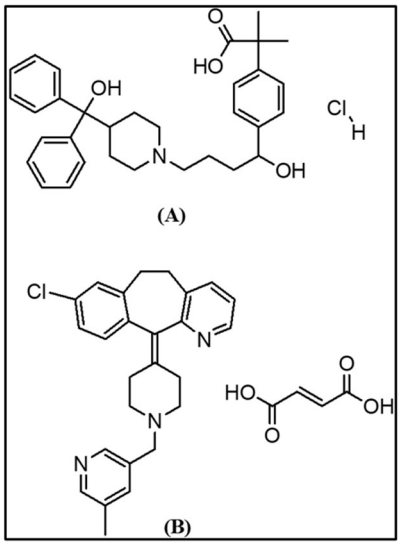

Fig. 1. Chemical structure of (A) FFH and (B) RTF.

liquid chromatography-mass spectrometry (LC-MS/MS) [20], titrimetry [21], capillary electrophoresis [22], voltammetry [23], conductometry [11,24], and potentiometry [25-27]. Regarding RTF, several methods have been reported for its quantification, including spectrophotometry [28,29], spectrofluorometry [30,31], HPLC [32,33], HPTLC [34], LC-MS/MS [35], gas chromatography-mass spectrometry (GC-MS) [36], Ultra performance liquid chromatography (UPLC) [37], voltammetry [38], titrimetry [39], capillary zone electrophoresis [40], and densitometry [41].

Within the array of published methods for FFH and RTF, chromatography, spectrofluorometry, electrophoresis, and voltammetry are consistently associated with drawbacks, such as more reagent consumption or the requirement for sophisticated equipment. Conversely, employing potentiometric sensors for drug analysis has demonstrated simplicity, sensitivity, and selectivity.

This study endeavors to design and validate two solid-state ion-selective sensors supported by a polyvinyl chloride (PVC) matrix. We utilize sodium tetraphenyl boron (NaTPB) as an ion exchanger and β-cyclodextrin (β-CD) as an ionophore to separately quantify FFH and RTF in pharmaceuticals, a novel approach not previously explored using these particular reagents. These methods exhibit high selectivity and offer a more environmentally friendly means of quantifying FFH and RTF in commercial dosage forms.

EXPERIMENTAL

Apparatus

Potential measurements were conducted using a digital potentiometer (PICO, Mumbai, India), while pH measurements were carried out with a pH meter (Elico, Mumbai, India). The measurements utilized an Ag/AgCl reference electrode and an aluminium wire as the conducting material in the indicator electrode.

Materials and Solutions

Analytical-grade chemicals were exclusively utilized throughout the study. Pure FFH and RTF were sourced from RA Chem Pharma Ltd, India, while Allegra-120 (120 mg FFH/tablet) and Rupamac (10 mg RTF/tablet) were locally procured. Various materials and substances, including Whatman No. 42 filter paper, ion-pair complexing agents such as NaTPB, β-CD as an ionophore, plasticizers like DBP, DOP, nitrophenyl octyl ether (NPOE), dibutyl sebacate (DBS) and dioctyl phthalate (DOP), the matrix substance PVC, and the solvent tetrahydrofuron (THF) were obtained from Merck India Ltd (Mumbai) and utilized in the research. A 1:1 Ethanol and 0.1 M sulfuric acid (H_2SO_4) were prepared by diluting laboratory-grade ethanol and concentrated H_2SO_4 with distilled water to dissolve FFH and RTF, respectively. Additionally, a 1 M potassium chloride (KCl) solution was prepared by dissolving an appropriate amount of solid KCl sourced from Merck, India, in distilled water and used as an electrolyte along with the internal standard solution.

Preparation of Standard FFH and RTF Solutions (5.0 mM)

A precisely measured amount of FFH and RTF was dissolved separately in 1:1 ethanol and 0.1 M H_2SO_4, respectively, to produce standard stock solutions, each with a concentration of 5.0 mM.

Procedure to Obtain FFH-NaTPB and RTF-NaTPB Ion Association Complex

FFH and RTF solutions each of strength 0.005 M were combined with 0.005 M NaTPB in separate 100 ml beakers, at ratios of 1:1 and 1:3 respectively. The mixtures were stirred for 25 min. The resulting FFH^+-TPB^- and $RTF{\cdot}3H^+$-$3TPB^-$ ion associate complexes were collected by filtering the

contents through the Whatman No-42 filter paper and subsequently dried.

Preparation of Membrane Sensors for FFH and RTF

A 20 mg of the dried FFH^+-TPB^- ion-associate complex, along with 100 mg of DOP, 25 mg of β-CD, and 200 mg of PVC was dissolved in 5 mL of THF to create the membrane sensor for FFH analysis. Similarly, for the RTF assay, a membrane was constructed by dissolving 5 mg of the dried RTF·$3H^+$-$3TPB^-$ ion-associate complex, 100 mg of DBP, 100 mg of β-CD, and 250 mg of PVC in 5 ml of THF. These mixtures were promptly spread onto two separate 5 cm diameter Petri dishes and left to dry at room temperature for 24 h. The resulting thin membranes were then fused at one end of two distinct 15 cm glass tubes, allowing them to dry for another 24 h.

The glass tubes stacked with FFH^+-TPB^- and RTF·$3H^+$-$3TPB^-$ membrane sensors were filled with specific solutions: 4 ml of a 1:1 ethanolic FFH (0.005 M) solution mixed with 4 ml of 1 M KCl for FFH analysis, and 4 ml of 0.005M RTF solution combined with 1 ml of 1 M KCl for RTF analysis. A silver electrode was inserted into each tube, and they were sealed individually. These assembled ion-selective electrodes were employed to quantify FFH and RTF, respectively.

Procedure for the FFH/RTF Analysis

Among the two sets of 10 ml volumetric flasks, one set was filled with 0-5 ml of standard FFH (5 mM) solution and another set with 0-5 ml of standard RTF (5 mM) solution. The pH of FFH and RTF solutions were adjusted to the range of 2.5 to 6 and 2.8 to 6.4, respectively. The volume of solution in each flask was then adjusted to the mark with distilled water. The electromotive force (EMF) of each FFH and RTF solutions was then measured using the FFH^+-TPB^- and RTF·$3H^+$-$3TPB^-$ membrane indicator electrodes, respectively, with a shared Ag/AgCl reference electrode.

Calibration curves were generated by plotting EMF against the logarithm of the drug concentration. These curves were employed to determine the respective concentrations of FFH and RTF in the unknown samples. Simultaneously, regression equations were computed using the collected data.

Procedure for Tablet Analysis

Ten Allegra-120 and Rupamac tablets were individually weighed and finely ground into powder. A powder of Allegra-120 tablets equivalent to 67.26 mg of FFH and Rupamac tablets equivalent to 25 mg of RTF were dissolved in 30 ml of 1:1 ethanol and 20 ml of 0.1 M H_2SO_4, respectively. Each solution was vigorously shaken for approximately 20 min, then filtered through Whatman No. 42 filter paper into separate 25 ml volumetric flasks and made up to the mark with distilled water. The standard analytical procedure was subsequently employed to analyze the FFH or RTF content in the respective tablets.

Interference Study

A 5 ml of pure FFH and RTF solutions each of strength 5mM were taken in separate 25 ml standard flasks, 1 ml of interferent solution (1 M) and 10 ml of water were added to each of them and mixed well for about 5 min. The pH of the FFH solution with interferents was adjusted between 2.5 and 6, while the RTF solution with interferents was adjusted to a range of 2.8-6.4. The volume in each flask was adjusted to 25 ml using distilled water, thoroughly mixed, and then subjected to analysis following the standard procedure.

Determination of Selectivity Coefficient ($K_{drug.I}$) of Sensor

Varied aliquots ranging from 1 to 10 ml of 5 mM standard FFH and RTF solutions were taken in different 25 ml volumetric flasks and mixed with a particular interferent of strength 1 M. The pH levels of FFH and RTF solutions with interferents were adjusted to the range of 2.5-6 and 2.8-6.4, respectively. Subsequently, the volume in each flask was adjusted to 25 ml using distilled water and thoroughly mixed. Similarly, a set of solutions containing different interferents was prepared, and the potentials were measured using FFH and RTF sensors. By plotting E_{Cell} against the log[drug], the intersection point was determined. The $K_{drug.I}$ for each interferent was calculated using the formula provided below [25].

$$K_{drug.I} = \frac{[drug]_E}{[I]_E^{Z_{drug}/Z_I}} = \frac{[drug]_I}{[I]_{add}^{Z_{drug}/Z_I}}$$

Where $[drug]_E$ and $[I]_E$ are the strength of FFH/RTF and the interferents, respectively to produce indistinguishable E_{Cell}. The charges on FFH/RTF, and interferents added are

Z_{drug} and Z_I respectively. $[drug]_I$ is the amount of FFH/RTF in the internal solution, and $[I]_{add}$ is the amount (mM) of interferent that has been added or present in the FFH/RTF solution.

RESULTS AND DISCUSSION

NaTPB, a novel ionophore, is employed in potentiometric sensors due to its ease of use and selectivity, particularly for determining FFH and RTF. Within PVC matrix membranes, the drug-NaTPB ion pairs serve as electroactive materials [42]. The simplicity in chemistry, functionality, and response characteristics of NaTPB ion-associates with FFH or RTF makes them highly suitable for constructing sensors to determine these specific substrates. This paper discusses detailed results and performance characteristics of the novel sensors for FFH and RTF using NaTPB, marking a significant advancement in sensor technology.

Method Development

A membrane sensor designed for FFH and RTF utilizes the cation exchanger NaTPB and β-CD as an ionophore within the PVC matrix. The plasticizers employed were DOP and DBP for FFH and RTF sensors, respectively. In an aqueous solution, FFH's basic nitrogenous group becomes protonated, forming $FFH^+.Cl^-$. This solution, containing FFH^+ and Cl^-, interacts with NaTPB containing anionic TPB^- in the ratio 1:1, leading to the formation of an ion association complex, FFH^+-TPB^-, following a proposed reaction Scheme 1. Similarly, in an aqueous solution, RTF's three basic nitrogenous groups become protonated, forming $RTF{\cdot}3H^+$. The solution containing $RTF{\cdot}3H^+$ associates with NaTPB, containing anionic TPB^- in a 1:3 ratio, leading to the formation of the ion association complex $RTF{\cdot}3H^+$-$3TPB^-$ according to the suggested reaction scheme 2. Upon placing the respective ion-selective electrodes in the FFH and RTF solutions, an in-situ exchange of ions occurs, influencing the equilibrium partitioning of sample ions at the sample/membrane junction. This interaction at the phase boundary significantly influences the potentiometric response of ion-selective electrodes using polymeric membranes. Consequently, the ion-selective electrode sensor translates changes in FFH and RTF concentration within a solution into an electric potential. The voltage is theoretically linked to the logarithm of the ionic activity [43,44].

The structure of the pure drug, NaTPB, and FFH^+-TPB^- were ascertained by IR spectrometric analysis (Fig. 2). The FT-IR spectrum of FFH showed the characteristic absorption bands at 3362.1 cm^{-1} (−OH stretching), at 2942.7 cm^{-1} (−CH stretching of alkane), at 2648.3 cm^{-1} (−OH of carboxylic acid), at 1712.7 cm^{-1} (C=O stretching of carboxylic acid), at 1472.3 and 1448.1 cm^{-1} (C=C stretching of aromatic ring), at 1252.8 cm^{-1} (C-N stretching of amine), at 1167.57 cm^{-1} (CO stretching of tertiary alcohol) and at 1067.94 cm^{-1} (CO stretching of secondary alcohol) [45,46]. On the other hand, the FT-IR spectrum of NaTPB includes the bands at 3056.4-3002.4 cm^{-1} (C–H stretching), at 1600-1400 cm^{-1} (carbon-carbon stretching vibrations in the aromatic ring) and at around 800 cm^{-1} for B-C characteristics [47]. As observed in

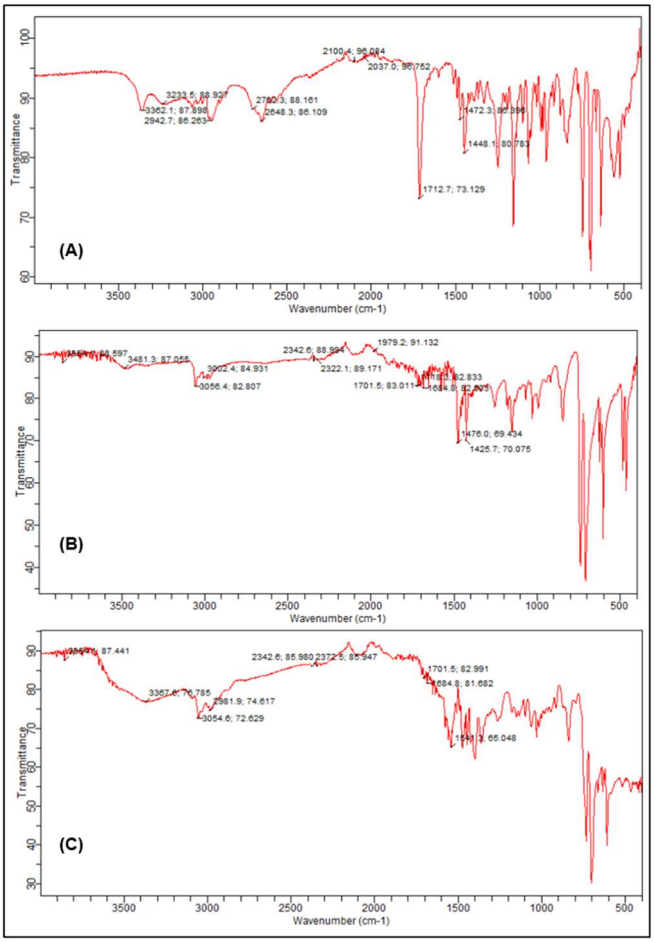

Fig. 2. FTIR spectra of (A) FFH, (B) NaTPB and (C) FFH^+-TPB^- ion-associate.

the FTIR spectrum of FFH^+-TPB^- ion associate, the band at 1712.7 cm^{-1} is missing but can observe one at 1541.3 cm^{-1} revealing the probability of the existence of resonance stabilized carboxylate ion of FFH in the complex [48].

The formation of RTF·$3H^+$-$3TPB^-$ ion-associate was confirmed by recording the IR spectra. The typical spectra for pure RTF, NaTPB and RTF·$3H^+$-$3TPB^-$ ion-associate complex are presented in Fig. 3. The FT-IR spectrum of RTF showed characteristic absorption bands at 3030.3-2898.0 cm^{-1} (C-H stretching of aromatic ring), at 1699.7 cm^{-1} (C=O stretching), at 1436.9 cm^{-1} (C-H splitting of methyl group), 1420.1 cm^{-1} (O-H splitting of carboxylic acid), 1326.9 cm^{-1} (C-N stretching of aromatic amine), at around 1200 cm^{-1} (C-N stretching of an amine group) [49]. The FTIR spectrum of RTF·$3H^+$-$3TPB^-$ ion associate complex has all the bands of combining reagents but the band at 1699.7 cm^{-1} is absent. However, a band observed at 1541.3 cm^{-1} may be due to the existence of resonance stabilized carboxylate ion of RTF in the complex [48].

The systematic representation of the electrochemical cell constructed using the designed membrane sensors for FFH/RTF determination is as shown below:

$Ag\text{-}AgCl_{IR} \| FFH_I$ (1:1 ethanolic drug), KCl (1 M) $| Membrane | [FFH]_{Sample} \| AgCl\text{-}Ag_{SR}$

$Ag\text{-}AgCl_{IR} \| RTF_I$ (0.005 M), KCl (1 M) $| Membrane | [RTF]_{Sample} \| AgCl\text{-}Ag_{SR}$

where '$Ag\text{-}AgCl_{IR}$' and '$Ag\text{-}AgCl_{SR}$' are reference Ag-AgCl electrodes immersed into internal reference FFH_I /RTF_I and sample solution $[FFH]_{Sample}$/$[RTF]_{Sample}$, respectively.
The E_{Cel}l and $[drug]_{Sample}$, are related through the following Nernst equation [50]:

$$E_{Cell} = K + 0.05916\ log[\text{drug}]_{Sample}$$

Where K indicates the potential of the reference electrode, the liquid junction potential, the asymmetry potential, the activity coefficient of FFH, and $[FFH]_I$.

Optimization of Parameters

Membrane composition. Initially, a sequence of trials with different quantities of materials such as ion exchangers, ionophore, plasticizer, and matrix substance were executed to yield optimal membranes. The functionality of these membranes for FFH/RTF sensing was assessed through potentiometry. The FFH^+-TPB^- membrane sensor, developed using 20 mg of ion-associate, 100 mg of DOP, 25 mg of β-CD, and 200 mg of PVC, demonstrated highly reliable results. Similarly, the RTF·$3H^+$-$3TPB^-$ membrane sensor, comprising 5 mg of ion-associate, 100 mg of DBP, 100 mg of β-CD, and 250 mg of PVC, yielded highly dependable outcomes. Additionally, attempts to establish calibration lines with varied material quantities at concentrations differing from those specified above did not exhibit acceptable Nernstian behavior. The dissolution of materials was found suitable in 5 ml THF. The volume of THF larger than 5 ml did not significantly alter the outcomes.

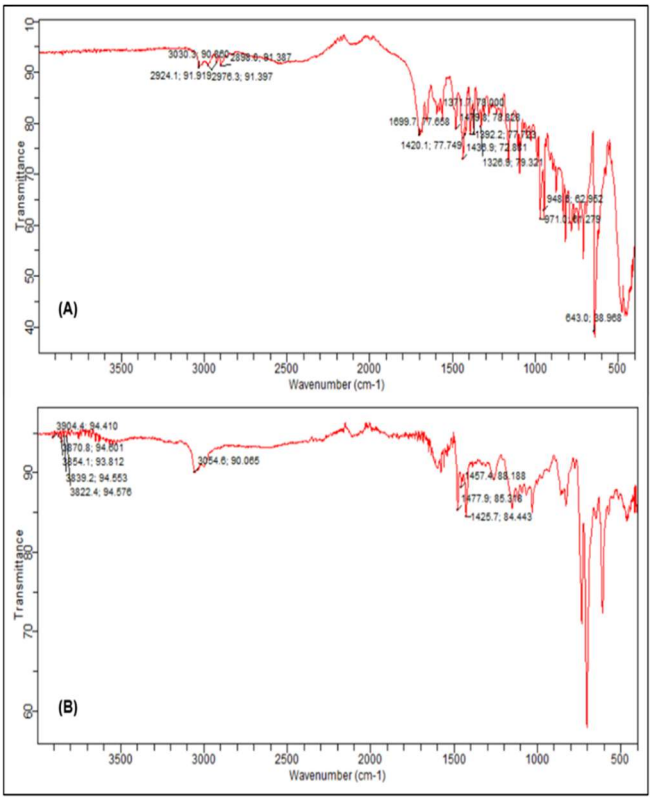

Fig. 3. IR Spectra of (A) Pure RTF (B) RTF·$3H^+$-$3TPB^-$ ion-associate.

Choice of Plasticizer

The membrane was developed using different plasticizers such as nitrophenyl octyl ether (NPOE), DOP, DBP, and dibutyl sebacate (DBS). The membranes composed of

100 mg of DOP and 100 mg of DBP exhibited consistent potential responses and Nernstian behavior for FFH and RTF assays, respectively. Details regarding the performance of sensors composed of different plasticizers in different amounts are summarized in Table 1.

The Concentration of FFH in the Internal Reference Solution

An FFH/RTF and KCl solutions of different concentrations were used as internal standards for creating a calibration plot of E_{cell} against log[drug]. Quantification of FFH using an internal standard solution containing 4 ml of 5.0 mM FFH and 4 ml of 1 M KCl solution, RTF quantification using 4 ml of 5.0 mM RTF with 1 ml of 1 M KCl solution yielded outstanding results, meeting the predicted Nernstian response criteria of the sensor (Fig. 4).

Table 1. Performance of Sensors Composed of Different Plasticizers in Different Amounts

Plasticizer	Amounts (mg)	FFH-NaTPB sensor		RTF-NaTPB sensor	
		Slope* ± SD	Confidence limit (CL) at 95%	Slope* ± SD	Confidence limit (CL) at 95%
DBP	50.0	37.23 ± 0.52	0.64	31.23 ± 1.54	1.91
	75.0	41.00 ± 0.89	1.10	25.62 ± 1.25	1.55
	100.0	46.11 ± 1.23	1.52	20.00 ± 0.87	1.10
	125.0	49.32 ± 0.89	1.10	20.89 ± 1.03	1.28
	150.0	51.36 ± 1.03	1.28	21.00 ± 0.98	1.21
DBS	50.0	45.60 ± 1.11	1.37	41.23 ± 1.46	1.81
	75.0	46.23 ± 0.97	1.20	40.25 ± 1.25	1.55
	100.0	49.28 ± 0.88	1.09	38.24 ± 0.84	1.04
	125.0	50.23 ± 0.68	0.84	35.56 ± 1.22	1.51
	150.0	51.22 ± 1.56	1.93	33.45 ± 0.68	0.84
DOP	50.0	51.68 ± 1.26	1.56	39.57 ± 0.88	1.09
	75.0	54.29 ± 1.88	2.33	40.01 ± 1.03	1.27
	100.0	56.92 ± 1.45	1.80	40.89 ± 1.06	1.31
	125.0	56.21 ± 0.87	1.08	43.22 ± 1.88	2.33
	150.0	56.00 ± 1.00	1.24	44.16 ± 1.57	1.94
NPOE	50.0	51.26 ± 0.85	1.05	38.22 ± 1.09	1.35
	75.0	50.22 ± 0.54	0.67	41.00 ± 1.11	1.37
	100.0	49.65 ± 0.99	1.23	40.65 ± 0.79	0.98
	125.0	50.00 ± 0.77	0.95	40.55 ± 0.62	0.77
	150.0	51.55 ± 1.23	1.52	39.99 ± 0.64	0.79

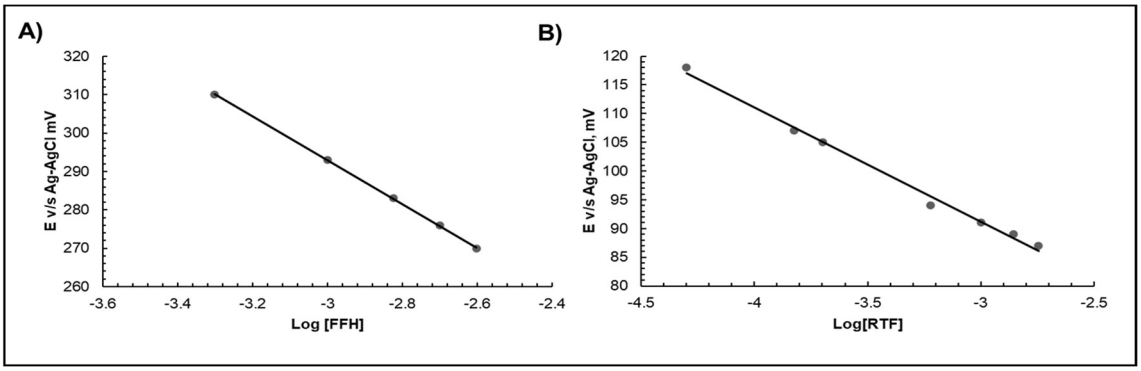

Fig. 4. Calibration curves while using (A) FFH Sensor and (B) RTF Sensor.

Electrode Conditioning Time

The FFH and RTF sensors underwent conditioning by immersing them in standard FFH and RTF solutions, respectively, for varying durations. Based on the potential values response to standing time (as depicted in Fig. 5), the dynamic surface necessitates approximately four hours of activation at 25 °C for proper utilization.

Effect of pH

The impact of pH on the E_{cell} was investigated by measuring the potential of an FFH/RTF solution in the pH range of 0.5 to 8. The required pH was maintained either by adding diluted NH_3 or 1 M NaOAc. The consistency in potential was established in the pH range 2.5 to 6 for FFH solution and between pH 2.8 and 6.4 for RTF solution (Fig. 6). The deviation from the Nernstian response in the pH below and above these ranges was probably due to the lesser availability of FFH^+/$RTF.3H^+$ for in-situ exchange of ions. Consequently, an ion-selective electrode sensor transforms variation in the concentration of FFH^+/$RTF.3H^+$ in a solution into an electric potential [43,44]. The resulting slopes of the calibration curves due to different pH under study were summarized in Table 2.

Response Time

The developed and conditioned FFH/RTF sensors were tested for their response time, affirming their ability to sense FFH/RTF solutions in under 5 s. The outcomes of the study of response time are made available in Fig. 7.

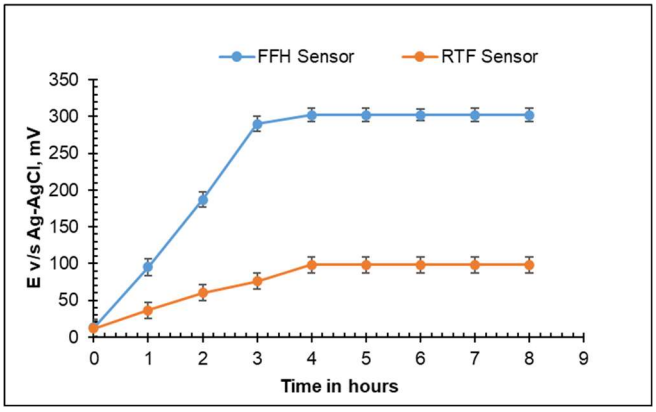

Fig. 5. Effect of sensor's contact time to produce stable potential readings in FFH Sensor (1.0 mM FFH) and RTF Sensor (1.5 mM RTF).

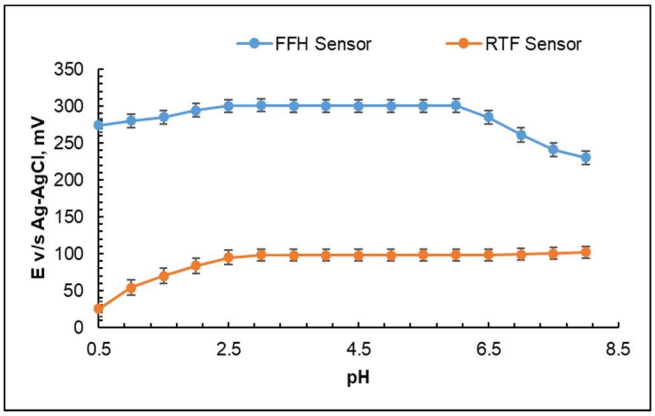

Fig. 6. Effect of pH on the potentials of FFH (1.0 mM) and RTF (1.5 mM) solutions measured using FFH and RTF sensors.

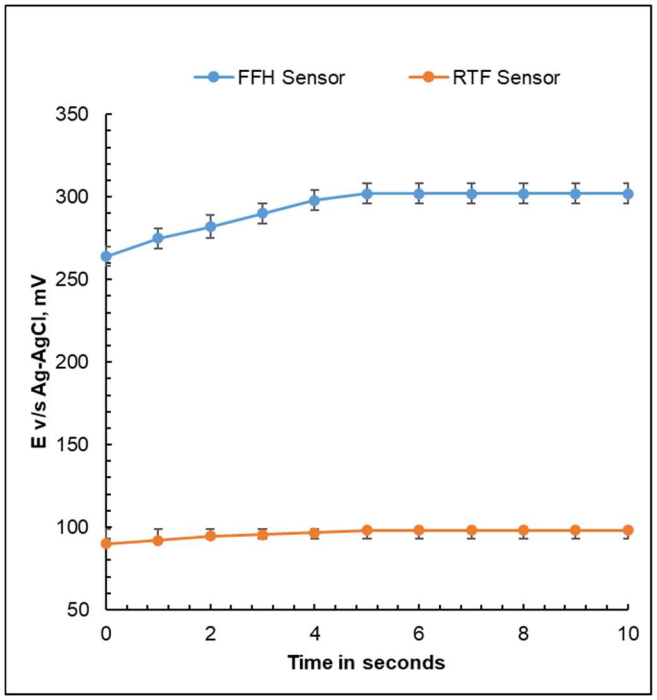

Fig. 7. Effect of response time on the potentials of FFH (1.0 mM) and RTF (1.5 mM) solutions measured using FFH and RTF sensors.

Lifetime of the Sensor

The developed FFH^+-TPB^- and $RTF{\cdot}3H^+$-$3TPB^-$ sensors exhibited excellent performance, maintaining a consistent mean Nernstian slope of 56.92 and 20 mV $decade^{-1}$ for measuring FFH and RTF, respectively, over 60 days of

Table 2. Results of Evaluation of the Eeffect of pH on the Behavior Proposed FFH-NaTPB and RTF-NaTPB Sensors

pH	Slope of the Calibration plot* ± SD (mV decade^{-1})	
	FFH-NaTPB Sensor	RTF-NaTPB Sensor
0.5	51.99 ± 0.80	05.09 ± 0.53
1.0	53.13 ± 0.62	11.00 ± 0.70
1.5	54.07 ± 0.69	14.26 ± 0.90
2.0	55.78 ± 0.61	17.11 ± 0.91
2.5	56.92 ± 0.72	19.35 ± 0.85
2.8	56.92 ± 0.72	19.94 ± 0.86
3.0	57.13 ± 0.91	20.00 ± 0.77
3.5	56.92 ± 0.71	19.96 ± 0.68
4.0	56.92 ± 0.69	19.96 ± 0.66
4.5	56.92 ± 0.68	19.96 ± 0.65
5.0	56.92 ± 0.67	19.96 ± 0.59
5.5	56.92 ± 0.91	20.00 ± 0.54
6.0	57.11 ± 0.86	20.00 ± 0.61
6.4	55.97 ± 0.90	20.02 ± 0.68
6.5	54.07 ± 0.80	20.06 ± 0.70
7.0	49.52 ± 0.83	20.18 ± 0.83
7.5	45.73 ± 0.79	20.37 ± 0.88
8.0	43.64 ± 0.88	20.77 ± 0.87

*Mean value of five determinations.

Table 3. The Selectivity Coefficients of the Sensors for Various Interferents

Interferent	Selectivity coefficient, $K_{drug,I}$*	
	FFH	RTF
Ag^+	0.095	0.111
NH_4^+	0.023	0.085
Na^+	0.015	0.091
K^+	0.112	0.023
H^+	0.123	0.013
Ca^{2+}	0.220	0.112
Co^{2+}	0.123	0.095
Zn^{2+}	0.090	0.086
Glycine	0.160	0.123
Urea	0.163	0.086
Uric acid	0.058	0.212
Glucose	0.061	0.125
Oxalate	0.026	0.226
Formic acid	0.022	0.165
Citric acid	0.122	0.121
Tartaric acid	0.221	0.097
Benzoic acid	0.233	0.023
Salicylic acid	0.121	0.054
Phthalic acid	0.111	0.026
Boric acid	0.099	0.126

*Average of 5 determinations.

standard use. However, variations in the measured potential were noted after this 60-day period.

Evaluation of Selectivity Coefficients

The FFH/RTF solution, previously examined, was intentionally mixed with various interferent solutions (1 M), as outlined in the investigation [42,51]. The resulting selectivity coefficient values, all below 1 (as in Table 3) indicated the absence of interference from the added substances.

Method Validation

The suggested methods underwent validation in compliance with current IUPAC regulations [42,52] and ICH guidelines [51] for assessing linearity, accuracy, precision, recovery studies, sensitivity, robustness, and ruggedness.

Linearity of a Calibration Curve, Regression Data, and Performance Characteristics

The measured EMF demonstrates a linear correlation with the concentrations of drug solutions within specific ranges: 5×10^{-4} to 2.5×10^{-3} M for FFH (Fig. 4a) and 8×10^{-5} to 2.5×10^{-3} for RTF (Fig. 4b). The Nernstian behavior is evident through the slopes of 56.92 mV decade^{-1} for FFH and 20 mV decade^{-1} for RTF. These values further demonstrated that the stoichiometry of the reaction between

FFH and NATPB is 1:1 and that of RTF and NaTPB is 1:3. The equations derived from curve-fitting regression data are as follows:

$Y = 56.92 X + 338$ for FFH and $Y = 20 X + 117$ for RTF

The determination of the Limit of Detection (LOD) followed IUPAC Guidelines [42,52], computed from the intersection of the extrapolated linear segments of the calibration curve with the x-axis. Additional performance characteristic values for the FFH and RTF membrane sensors are detailed in Table 4 below.

Accuracy and Precision

In assessing intra-day variations, researchers examined three different concentrations of FFH and RTF solutions, each replicated seven times. For inter-day variations, three different concentrations of FFH and RTF solutions were studied, with five replicas for each. The % RSD and % RE reported in Table 5 signify the accuracy and precision achieved by the proposed scientific method.

Robustness and Ruggedness

The operational temperature was intentionally increased by 2 °C during the analysis of FFH solutions at strength 1.0, 1.5, and 2.0 mM. RTF solution at strength 0.32, 0.96, and 1.60 mM were also analyzed under a similar set of conditions. Across temperatures of 23, 25, and 27 °C, the % RSD calculated remained under 3%, highlighting the robustness of the proposed methods.

Table 4. Sensor's Performance Features and Regression Data

Parameter	FFH-NaTPB sensor	RTF-NaTPB sensor
Linear range, M	5×10^{-4} to 2.5×10^{-3}	8×10^{-5} to 2.5×10^{-3}
Limit of detection (LOD), M	2.5×10^{-4}	2.0×10^{-5}
Limit of quantification (LOQ), M)	4.5×10^{-4}	6.1×10^{-5}
Slope (m), mV decade^{-1}	56.92	20.00
Intercept (b), mV	338	117
Correlation coefficient (R)	0.9997	0.9986
R^2	0.9994	0.9972
pH (Optimum)	2.5-6	2.8-6.4
Lifetime, days	> 2 months	

Table 5. Results Indicating the Precision and Accuracy of the Proposed Sensors

Sensor	Drug taken (M)	Intra-day variations			Inter-day variations		
		Drug found* (M)	%RSD	%RE	Drug found$ (M)	%RSD	%RE
FFH-NaTPB	1.0	1.03	2.11	3.0	1.02	1.68	2.0
	1.5	1.49	1.65	0.67	1.52	2.66	1.33
	2.0	2.02	1.11	1.0	2.03	2.99	1.5
RTF-NaTPB	0.32	0.316	3.12	1.25	0.317	2.98	0.94
	0.96	0.975	2.56	1.56	0.985	3.26	2.60
	1.60	1.589	3.10	0.69	1.603	3.33	0.19

Drug: FFH (for FFH-NaTPB sensor) or RTF (for RTF-NaTPB sensor). *Mean value of seven measurements; $Mean value of five measurements.

Three analysts assessed the FFH and RTF solutions mentioned earlier, utilizing three potentiometers to monitor instrumental and inter-person differences, respectively. The calculated % RSD, reported as less than 3.16% in Table 6, further confirmed the reliable performance of the sensors.

Application to Tablet Analysis

The validated membrane sensors were utilized to assess five replicates of tablet extracts containing FFH at strengths of 1.0, 1.5, and 2.0 mM as well as RTF at strengths of 0.32, 0.96, and 1.60 mM. The analysis determined the quantity of FFH and RTF in the respective tablets, their % recovery, and %RSD. These findings aligned well with the reference methods results for FFH [3] and RTF [28]. The experimental t- and F-test values were lower than the tabulated values at the 95% confidence level indicating that, both the procedures are accurate and précised. Additionally, the average percentage recovery of FFH and RTF close to 100% with a standard deviation below 2% (as shown in Table 7) strongly supports the absence of any discernible difference between the suggested and reference methods.

Table 6. Results of Robustness and Ruggedness of the Proposed Sensors

Sensor	Concentration of drug (M)	%RSD values for varied parameters		
		Robustness	Ruggedness	
		(Varying T by 2 ^{O}C)	Inter-analysts	Inter-potentiometric
FFH-NaTPB	1.0	2.56	1.12	2.10
	1.50	2.23	1.65	2.00
	2.00	2.85	1.33	2.32
RTF-NaTPB	0.32	1.86	1.19	2.98
	0.96	2.43	2.64	3.16
	1.60	2.22	2.03	2.87

Drug: FFH (for FFH-NaTPB sensor) or RTF (for RTF-NaTPB sensor).

Table 7. Results of Analysis of FFH and RTF Tablets Using Proposed Sensor and Statistical CP Omparison with Results of the Official/Reference Methods

Sensor	Tablets analysed	Drug/Tablet (In mg)	Found*	
			%Label claim ± SD	
			Reference method	Proposed method using Drug-NaTPB sensor
FFH-NaTPB	Allegra-120	120	97.74 ± 1.15	98.67 ± 1.56 t = 1.07 F = 1.84
RTF-NaTPB	Rupamac	10	99.12 ± 0.84	98.59±1.12 t = 0.85 F = 1.78

*Mean value of 5 determinations. (Tabulated t-value at the 95 % confidence level and for four degrees of freedom is 2.77). (Tabulated F-value at the 95 % confidence level and for four degrees of freedom is 6.39).

Recovery Study

Respective FFH and RTF solutions at 50%, 100%, and 150% strength were introduced into pre-analysed tablet extracts. After adjusting the pH to the optimal range, the potential of these resultant solutions was measured. The newly introduced sensors showcased their accuracy through an average recovery of 100.7% for FFH and 99.87% for RTF, accompanied by a %RSD value lower than 2.5% (as indicated in Table 8).

Comparison Study

The current study was compared with previously published electroanalytical methods, particularly potentiometric approaches employing a membrane sensor for FFH. Abbas *et al.* developed a membrane sensor using the reineckate (REN) exchanger and DOP as a plasticizer within a PVC matrix [25]. A report was also found with three ion-selective membranes utilizing the molybdophosphoric acid (MPA) exchanger with different plasticizers - DBP, NPOE, and tributyl phthalate (TBP) [26]. Additionally, Rajendraprasad et al. engineered a sensor using Alizarin Red S (ARS), β-CD, and NPOE as the ion exchanger, ionophore, and plasticizer, respectively, utilizing PVC matrix [27]. Table 9 documents the potentiometric attributes: detection limit, selectivity, pH influence, linear range, and lifespan of the FFH^{+}-TPB^{-} sensor, comparing them with the reported values. Similarly, Table 10 summarizes the performance character of the $RTF{\cdot}3H^{+}$-$3TPB^{-}$ sensor and compares them with those of Devnani *et al*.'s voltammetric method [38]. This comparative analysis reveals that the superior choice of proposed potentiometric sensors for FFH and RTF is due to applicability over a wider pH range, rapidity in response, need for low-cost instruments, better selectivity, and applicability for assaying dosage forms. Moreover, the proposed sensors perform exceedingly well for more than 60 days.

Table 8. Results of Accuracy Assessment in Recovery Study by Standard-addition Procedure

Sensor	Drug from tablet extract (M)	Pure Drug added (M)	Total Drug found (M)	% Drug recovered*	%RSD
FFH-NaTPB	1.0	0.5	1.497	99.52	1.23
	1.0	1.0	2.006	100.56	2.11
	1.0	1.5	2.520	102.02	2.22
RTF-NaTPB	0.96	0.48	1.43	97.52	2.08
	0.96	0.96	1.93	100.8	1.89
	0.96	1.44	2.42	101.3	2.22

Drug: FFH (for FFH-NaTPB sensor) or RTF (for RTF-NaTPB sensor). *Mean value of five measurements.

Table 9. Comparison of Performance Characteristics of Different FFH Ion Selective Electrodes

Performance characteristics	FFH-REN-DOP [25]	FFH-MPA-DBP [26]	FFH-MPA-NPOE [26]	FFH-MPA-TBP [26]	FFH-ARS-NPOE [27]	FFH-NaTPB-DOP (proposed work)
Linear range (M)	2.5×10^{-6}-1.0×10^{-2}	8.0×10^{-6}-1.0×10^{-1}	1.31×10^{-5}-1.0×10^{-2}	2.5×10^{-5}-1.0×10^{-1}	2.5×10^{-6}-1.25×10^{-3}	5×10^{-4}-2.5×10^{-3}
LOD (M)	1.3×10^{-6}	5.6×10^{-6}	3.5×10^{-6}	3.9×10^{-6}	3.5×10^{-7}	2.5×10^{-4}
Slope (mV decade^{-1})	62.3	57.01	56.70	14.30	56.18	56.92
pH range	2.0-4.5	2.0-4.5	2.5-4.0	2.0-4.0	2.0-5.5	2.5-6
Life time (days)	-	41	36	13	62	60

Table 10. Comparison of Performance Characteristics of Proposed RTF Sensor with Existing Voltametric Method

Performance characteristics	Voltametric method [38]	RTF-NaTPB-DBP (Proposed work)
Linear range	400-1400 ng ml^{-1}	8×10^{-5} to 2.5×10^{-3} M
LOD	56.78 ng ml^{-1}	2.0×10^{-5} M
Slope (mV $decade^{-1}$)	0.0043 ng ml^{-1}	20.00 mV $decade^{-1}$
pH range	6.5	2.8-6.4
Life time (days)	-	60

CONCLUSIONS

The inherent advantages of the two ion-selective potentiometric sensors designed for FFH and RTF assays lie in their simplicity, use of less dangerous and user-friendly chemicals, and the absence of the necessity for sophisticated equipment or highly specialized operators. These sensors exhibited exceptional Nernstian slopes of 56.92 and 20 mV $decade^{-1}$ for FFH and RTF, respectively, demonstrating selectivity within the ranges of 5×10^{-4} to 2.5×10^{-3} M for FFH and 8×10^{-5} to 2.5×10^{-3} M for RTF. The Limit of Detection (LOD) values validated the effectiveness of these methods for directly quantifying FFH and RTF in pharmaceuticals, ensuring high precision with an average recovery of 100.7% for FFH and 99.87% for RTF. Consequently, recommending the routine use of potentiometric assays employing the FFH^{+}-TPB^{-} and $RTF{\cdot}3H^{+}$-$3TPB^{-}$ sensors for quality control analysis in pharmaceutical preparations and therapeutic administration laboratories seems advisable.

ACKNOWLEDGMENT

The authors are very grateful to the JSS Mahavidyapeetha and JSS College of Arts, Commerce and Science, for having extended all the required amenities to carry out this work.

REFERENCES

[1] D.A. Handley, A. Magnetti, A.J. Higgins, Expert. Opin. Invest Drug. 7 (1998) 1045.
[2] K. Simpson, B. Jarvis, Drugs. 59 (2000) 301.
[3] A.R. Breier, M. Steppe, E.E.S. Schapoval, Anal. Lett. 40 (2007) 2329.
[4] D.I. Bernstein, W.F. Schoenwetter, R.A. Nathan, W. Storms, R. Ahlbrandt, J. Mason, Ann. Allergy Asthma Immunol. 79 (1997) 443.
[5] M. Merlos, M. Giral, D. Balsa, R. Ferrando, M. Queralt, A. Puigdemont, J. García-Rafanell, J. Forn, J. Pharmacol. Exp. Ther. 280 (1997) 114.
[6] J. Mullol, J. Bousquet, C. Bachert, W.G. Canonica, A. Gimenez-Arnau, M.L. Kowalski, E. Martí-Guadaño, M. Maurer, C. Picado, G. Scadding, P. Van Cauwenberge, Allergy. 63 (2008) 5.
[7] E. Vuurman, E. Theunissen, A. van Oers, C. van Leeuwen, J. Jolles, Hum. Psychopharmacol. 22 (2007) 289.
[8] The United States Pharmacopoeia: USP 24: the National Formulary: NF 19, USP Convention, Rockville, Md, USA, 2000.
[9] European Pharmacopoeia 6.0. 2280 (2008) 1888.
[10] H.K. Trivedi, M.C. Patel, Sci. Pharm. 80 (2012) 889.
[11] S. Ashour, M. Khateeb, R. Mahrouesh, Pharm. Anal. Acta. S2 (2013) 003.
[12] M.S. Raghu, K. Basavaiah, K.N. Prashanth, K.B. Vinay, ISRN Spectroscopy. 2012 (2012) 1.
[13] R.V. Rele, J. Chem. Pharm. Res. 8 (2016) 350.
[14] S. Mahmood, Z. Ahmad, M. Arshad, J. Chem. 1 (2018) 01.
[15] G.R. Barabde, S.R. Ambadekar, A. Nishad, J. Res. Pharm. Sci. 2 (2016) 01.
[16] N. Malothu, T. Paladugu, P. Katamaneni, Int. J. Pharm. Biol. Sci. 8 (2018) 619.
[17] E. Akbel, I. Bulduk, European J Sci Tech. 32 (2012) 1048.
[18] G. Rajitha, A.G. Sushmita, Int. J. Pharm. Sci. Nanotech. 13 (2020) 5069.
[19] N. Tamilselvi, K. Sruthi, R. Arivukkarasu, P. Vanathi,

D. Visakh, Research J. Pharm. and Tech. 9 (2016) 469.
[20] M.L Stanton, M.S. Joy, R.F. Frye, J Chromatogr B. 878 (2010) 497.
[21] M.S. Raghu, C.S. Shantharam, K. Yogesh Kumar, J Anal Pharm Res. 7 (2018) 1.
[22] P. Mikus, I. Valásková, E. Havránek, Drug Dev. Ind. Pharm. 31 (2005) 795.
[23] A. Golcu, B. Dogan, S.A. Ozkan, Anal. Lett. 38 (2005) 1913.
[24] S. Ashour, M. Khateeb, Canadian Chem. Trans. 1 (2013) 292.
[25] M.N. Abbas, A.A.A. Fattah, E. Zahran, Anal. Sci. 20 (2004) 1137.
[26] A.M. Abass, O.S. Hassan, H.T. AL Rikabi, A. Ahmed, Egypt. J. Chem. 64 (2021) 6293.
[27] N. Rajendraprasad, C. Siddaraju, Anal. Bioanal. Electrochem. 14 (2022) 934.
[28] R.V. Rele, S.S. Patil, Der. Pharma. Chemica. 4 (2012) 2278.
[29] P.G. Patel, V.M. Vaghela, S.G. Rathi, N.B. Rajgor, V.H. Bhaskar, J. Young Pharmacists. 1 (2009) 354.
[30] A. Almahri, M.A. Abdel-Lateef, E. Samir, S.M. Derayea, M.A. El Hamd, Luminescence. 36 (2021) 651.
[31] G. Rana, M.I. El-Awady, M.M. Tolba, F. Ibrahim, R Soc. Open Sci. 8 (2021) 2111962.
[32] L.C. Rupali, M.P. Mahajan, S.D. Swant, Der. Pharma. Chemica. 4 (2012) 1047.
[33] V.K. Redasani, A.R. Kothawade, S.J. Surana, J. Anal. Chem. 69 (2014) 384.
[34] M.T. Patil, A.D. Ankalgi, J. Curr. Pharma. Res. 3 (2013) 791.
[35] C. Sun, Q. Li, L. Pan, B. Liu, P. Gu, J. Zhang, L. Ding, C. Wu. J. Pharm. Biomed. Anal. 111 (2015) 163.
[36] M. Katselou, S. Athanaselis, P. Nikolaou, S. Qammaz, A. Dona, C. Spiliopoulou, I. Papoutsis, Biomed. Chromatogr. 32 (2018) e4260.
[37] N. Kumar, D. Sangeetha, P. Sunil Reddy, Curr. Pharmaceut. Analysis. 9 (2013) 61.
[38] H. Devnani, P. Singh, S. Saxena, S.P. Satsangee, J. Int. J. Pharmaceut. Sci. Res. 5 (2014) 4792.
[39] R.V. Rele, S.A. Mahimkar, S.A. Sawant, Anal. Chem. Indian J. 8 (2009) 561.
[40] S.L. Dalmora, C. dos Santos Butzge, F.T. Machado, M.E. Walter, M.E. de Ávila Dalmora, R.B. Souto, Talanta. 94 (2012) 1.
[41] K. Czerwinska, E. Wyszomirska, E. Kublin, E. Malanowic, A.P. Mazurek, Acta Poloniae. Pharmaceutica. 73 (2016) 1467.
[42] Y. Umezawa, P. Bühlmann, K. Umezawa, K. Tohda, S. Amemiyaiupac. Pure. Appl. Chem. 72 (2007) 1851.
[43] N. Rajendraprasad. Anal. Bioanal. Electrochem. 13 (2021) 33.
[44] E. Bakker, P. Bühlmann, E, Pretsch, Talanta 63 (2004) 3.
[45] B. Singh, G. Saini, M. Vyas, S. Verma, S. Thakur, Future J. Pharm. Sci. 5 (2019) 1.
[46] J.H. Ahn, J. Kim, N.U. Rehman, H.J. Kim, M.J. Ahn. H.J. Chung, Pharmaceutics. 12 (2020) 547.
[47] H.F. Shurvell, J.A. Fanira, Canadian J. Chem. 46 (1968) 2081.
[48] C. Brian, Smith, Spectroscopy. 33 (2018) 20.
[49] L.C. Henríquez1, G.M. Redondo, R.V. Zúñiga, G.C. Berrocal, J. Drug Deliv. Ther. 8 (2018) 42.
[50] D. Harvey, McGraw-Hill Companies (ed). Textbook of Modern Analytical Chemistry (DePauw University). **(**2000) 475.
[51] ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology, CPMP/ICH/381/95.
[52] P.B. Richard, L. Erno, IUPAC, Recommendations for nomenclature of ion-selective electrodes. Pure & Appl. Chem. 66 (1994) 2527.