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Potentiometric Method for the Determination of Lamivudine and Dothiepin Hydrochloride in Pharmaceutical Preparations

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Abstract- A simple and accurate potentiometric titration method for the determination of lamivudine and dothiepin hydrochloride is developed. For this purpose, two N-bromoimides, viz., N-bromosuccinimide (NBS) and N-bromophthalimide (NBP) were used as titrants for determination of the two drugs in acid medium and the end point is determined using a platinum indicator electrode. The method was successfully applied for the determination of two drugs in their pure state or pharmaceutical preparations with mean recovery values of 99.67-100.10 and 99.55-100.33% and relative standard deviations (RSD) were 0.16-0.38 and 0.29-0.58% for lamivudine and dothiepin hydrochloride, respectively. Statistical comparison of the results with official and reported methods showed excellent agreement.

Keywords- Lamivudine, Dothiepin Hydrochloride, N-Bromosuccinimide, N-Bromophthalimide, Potentiometric Titration

1. INTRODUCTION

Lamivudine, 3TC, 2'-deoxy-3'- thiacytidine (Scheme1) is a potent activity against human immunodeficiency (HIV) and hepatitis B viruses (HBV) through inhibition of reversed transcriptase activity. Lamivudine is used in treatment of HBV infections and it has strongly been recommended for the treatment of HIV infections in combination with other antiviral drugs [1]. Lamividine drug and its tablets are described by an official monograph in the B. Pharmacopoeia [2] which describes HPLC procedure for their determinations. Several HPLC methods with UV detection for the determination of 3TC in biological fluids [3-5] and with antiretroviral agents in tablets and plasma have been reported [6-11]. Other methods include capillary electrochromatography [12], voltammetry [13], and spectrophotometry [9, 10, 14-16].

Dothiepin hydrochloride (DOTH), dosulepin hydrochloride (E)–3–(dibenzo [b,e] thiepin –11 (6H)–ylidene)–N,N–dimethylpropan-1-amine hydrochloride (Scheme1) is a tricyclic antidepressant used widely in endogenous depression [1]. Some analytical methods have been reviewed for the determination of dothiepin. The BP [2] specifies non-aqueous titration technique detecting the end point potentiometrically. Other methods includes spectrophotometry [17-20], LC-ESI-MS [21], HPLC [22,23], GC-MS [24,25] ion-selective electrodes [26,27] and spectrofluorimetry [17,20]. The present work aims to introduce new potentiometric titration method for the determination of lamivudine and dothiepin hydrochloride. This method is very simple in application and of low expense in comparison to the previously mentioned techniques and at the same time offer a high degree of accuracy and precision when compared to the official method (non- aqueous titration) [2]. The proposed potentiometric titration method through the oxidation of drug with NBS and NBP yields accurate and reproducible results, and has also been applied to the assay of pharmaceutical preparations.

2. EXPERIMENTAL

2.1. Apparatus

HI 9321 Hanna Microprocessor mV/pH meter with a combined platinum–saturated calomel electrode was used. All titrations were carried out manually; the cell was connected to a water–circulating thermostated at 25±2 °C during the titration.

2.2. Chemicals and reagents

All chemicals and reagents were of analytical–reagent grad and all solutions were prepared with doubly distilled water. Methanol and 1,2- dichloroethane were of HPLC grade (Aldrich) and were used directly as supplied. Pure–grade lamivudine (3TC) and its tablets (Lamidine, 25 mg 3TC / tablet) were provided by Eva pharma, Egypt. Dothiepin

hydrochloride (DOTH) and its capsules (Prothiadin, 50 mg DOTH/ capsule) were obtained by Knoll/Kahira, Cairo, Egypt.

Sulphuric, hydrochloric and acetic acids (BDH,UK) 2 M prepared solution of each was prepared in distilled water. NBS and NBP were obtained from Merck and their solutions (2×10^{-3} M) were prepared fresh daily in distilled water and standardized by the recommended procedure [28].

2.3. Preparation of standard drug solutions

Stock solutions (10^{-3} M) were prepared by dissolving the accurate weights of pure solid of 3TC (22.93 mg/100 mL) and DOTH (33.19 mg/100 mL) in distilled water. The solutions were kept in the refrigerator for no more than one week. Dilute solutions whenever required were obtained by appropriate dilution with water.

2.4. General procedure

Volumes (0.5-5.0 mL of 10^{-3} M) containing 0.115-1.146 mg of 3TC and 0.166-1.660 mg of DOTH, 1.0 or 2.0 mL of 2 M H_2SO_4 for 3TC or DOTH, respectively, (when using NBS titrant) and 2.0 mL of 2 M from each HCl and CH_3COOH for 3TC and DOTH, respectively, (when using NBP titrant) were added drop wisely and the volumes were completed to 50 ml with distilled water. The Pt-electrode was immersed in the solution and the titration was carried out potentiometrically using 2×10^{-3} M NBS or NBP solution as titrant from the micro burette (capacity of 5 mL with division of 0.05 mL) and the potential was measured subsequent to each addition of the reagent solution after through stirring for 1-2 min.

A graph of potential values versus the volume of titrant added was constructed and the end point was determined. One milliliter of 2×10^{-3} M NBS or NBP is theoretically equivalent to 0.4585 mg 3TC and 0.6638 mg DOTH.

2.5. Procedure for tablets and capsules

Contents of 10 tablets or capsules containing 3TC or DOTH, respectively, were accurately weighed and a quantity of powder equivalent to prepare 100 mL of 10^{-3} M solution of the drug was transferred to a 100 mL conical flask and extracted with four 20 mL portions of methanol (3TC) or with 1.2-dichloromethane (DOTH). The combined extracts were filtered and evaporated under vacuum and the residue was transferred to a 100 mL volumetric flack and made up to the mark with distilled water. The general procedure was then followed in the concentration ranges already mentioned.

2.6. Procedure for determining the drug-titrant ratio

Two milliliters of 10^{-3} M 3TC or DOTH were transferred to a 50 mL volumetric flask and made up to the mark with distilled water and then followed as directed in general procedure.

3. RESULTS AND DISCUSSION

The potentiometric titration of lamivudine or dothiepin hydrochloride showed a well-defined inflexion on the titration curve, precisely indicating the end point. The potentiometric determination was less subjective and more precise than the use of visual indicators [29-33]. Therefore this method was chosen for the analyses. NBS and NBP have reported to be the most effective oxidizing and brominating agents in acidic medium [29,30]. These reagents have been utilized extensively in the determination of a fast number of compounds, especially those of pharmaceutical interest [31-35]. Recently, some potentiometric titration methods have been used for the determination of some drugs using NBS or NBP [34,35]. The reaction conditions were studied extensively and the molar ratio of the reaction was calculated and the reaction mechanism was also included. The conditions optimized are the choice of medium for a quantitative reaction to proceed towards completion and the amount of reagent added.

3.1. Effect of acids

The effect of acids such as sulphuric, hydrochloric and acetic acids on the quantitative reaction of 3TC and DOTH with NBS or NBP was studied at a suitable ratio [drug]: [reagent] of 1:1. The data indicates that reproducible and stoichiometric results are obtained when using H_2SO_4 in case of NBS reagent and HCl or CH_3COOH when using NBP reagent (Fig. 1 as example) which consistent with those previously published [29,32-36]. The results obtained show sharp inflections lying in the immediate vicinity of the expected end points using 1.0 or 2.0 mL of 2 M H_2SO_4 and 2.0 mL of 2 M HCl or 2 M CH_3COOH for 3TC or DOTH, respectively. The concentrations of these reported acids are maintained constant during the current investigations. H_2SO_4**,** HCl or CH_3COOH acid acts as a H^+ donor and works as supporting electrolyte to keep the ionic strength constant during the titration process. The dilution of acidic drug solution to 50 mL with distilled water gave the best results. Then the dilution of drug solution to 50 mL will be used for all experiment processes.

3.2. Effect of NBS and NBP concentrations

For the quantitative determination of drug, the effect of NBS or NBP concentration is examined and 2×10^{-3} M is chosen as an optimum concentration to achieve a constant and highly stable potential reading within 1-2 min of mixing. Concentration less than this led to unstable reading and more time was needed to obtain constant potential values. Raising the temperature does not accelerate the oxidation process and tends to cause inaccurate results and creates difficulty in detecting the end point because of decomposition of NBS and NBP at higher temperature, thus room temperature (25±2 °C) is the most suitable.

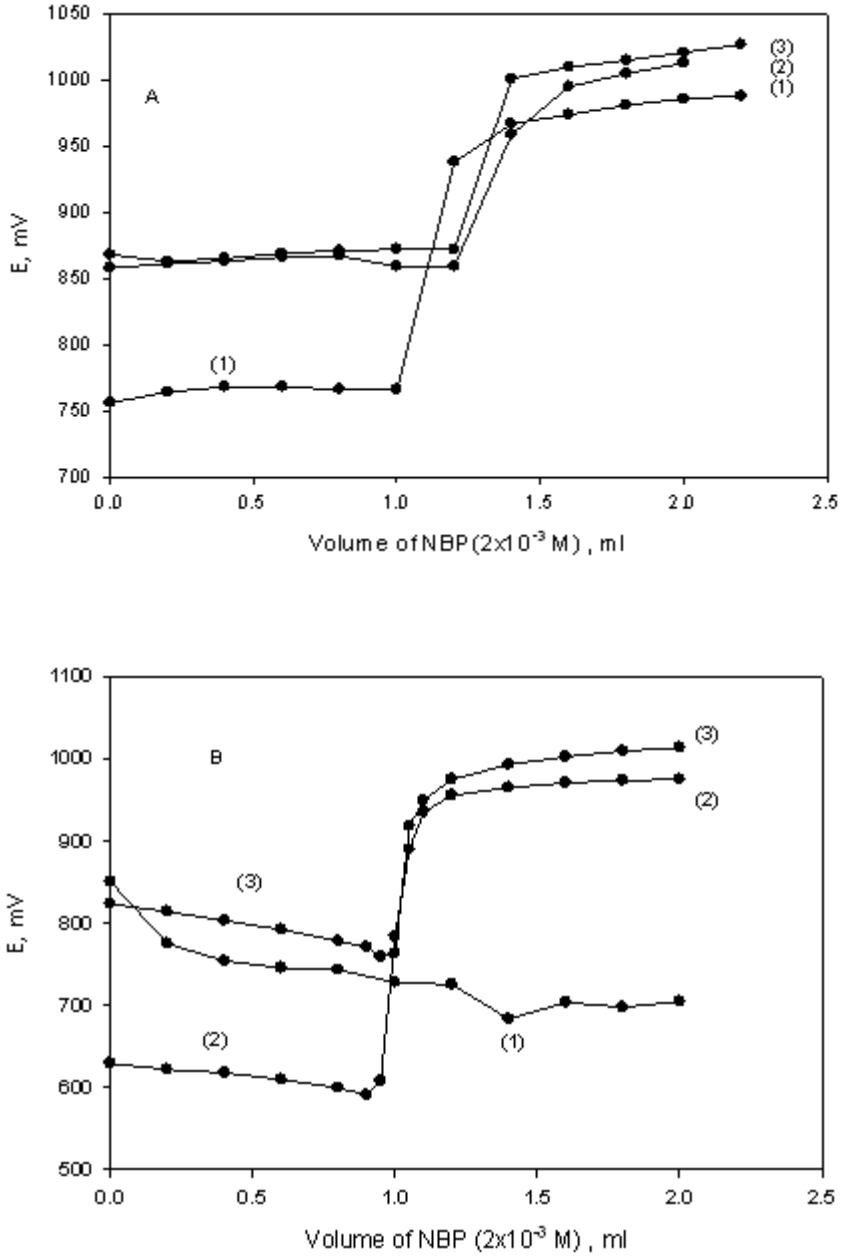
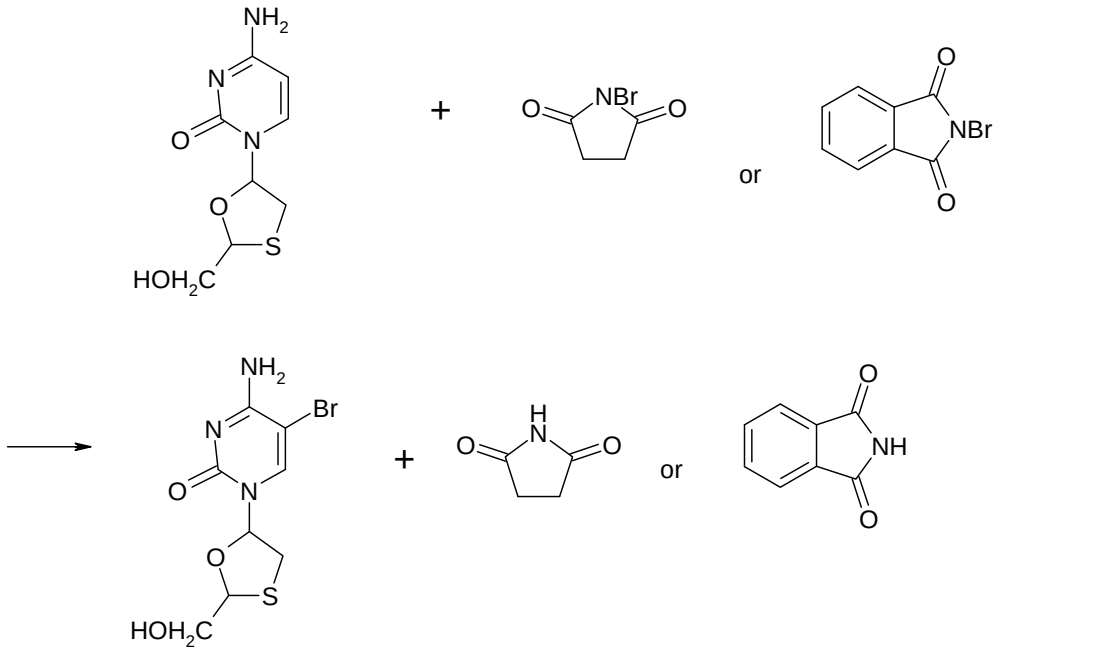


Fig. 1. Effect of acids (1.0 mL of 2 M) on the potentiometric titration of lamivudine (A), and dothiepin (B) (2.0 mL of 10^{-3} M) with NBP (2×10^{-3} M), (1) HCl, (2) CH_3COOH, (3) H_2SO_4, total volume=50 mL

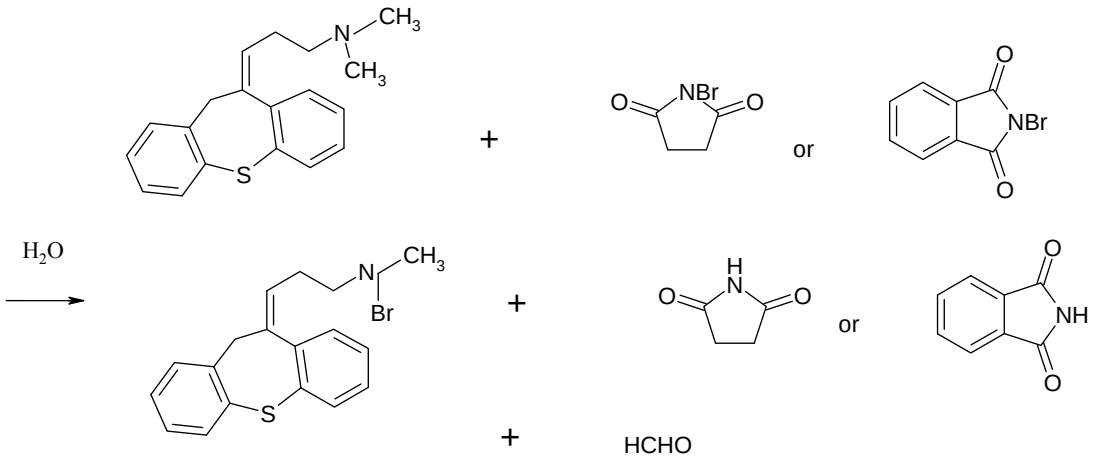
3.3. Molar ratio

As shown in Figs. 2&3, it is confirmed that 1.0 mole of NBS or NBP was required for complete bromination of each 1.0 mole of 3TC or DOTH, as illustrated in Scheme1.

Lamivudine



Dothiepin



Scheme 1. Bromination reactions of lamivudine and dothiepin with NBS or NBP

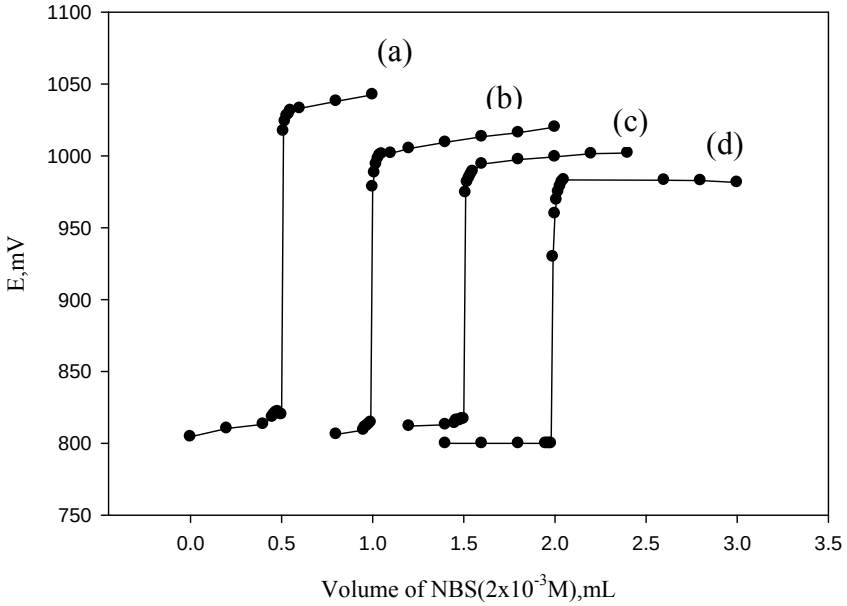


Fig. 2. Typical titration curves for potentiometric determination of end point of the reaction between NBS (2×10^{-3} M) and lamivudine using Pt electrode.(a) 1.0 mL of 10^{-3} M, (b) 2.0 mL of 10^{-3} M, (c) 3.0 mL of 10^{-3} M, (d) 4.0 mL of 10^{-3} M lamivudine, 1.0 ml of 2 M H_2SO_4 in the total volume=50 mL

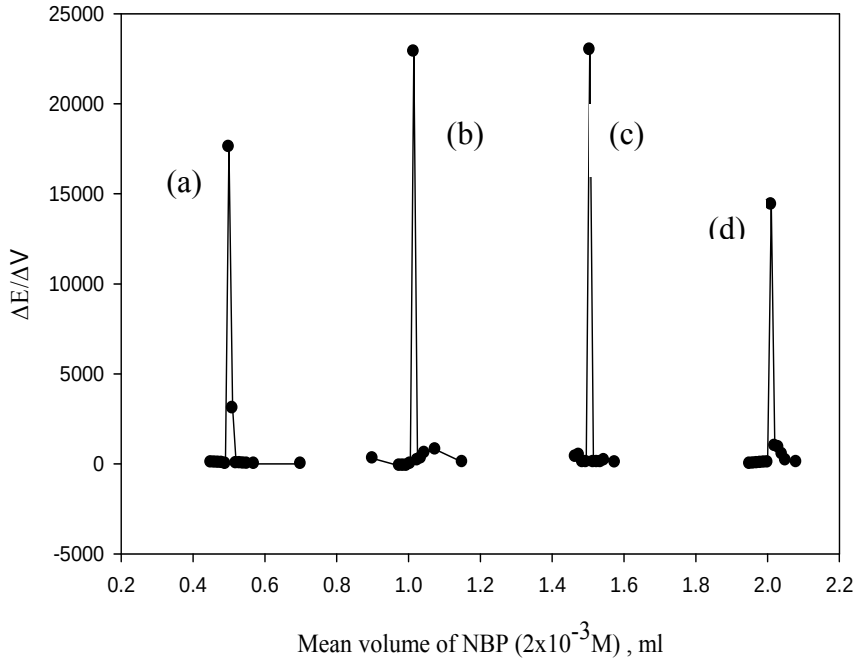


Fig. 3. Differential curves of potentiometric titration of dothiepin with NBP (2×10^{-3}M), (a) 1.0 mL of 10^{-3} M , (b) 2.0 mL of 10^{-3} M , (c) 3.0 mL of 10^{-3} M, (d) 4.0 mL of 10^{-3} M dothiepin, 2.0 mL of 2 M CH_3COOH in the total volume=50 mL

3.4. Reaction mechanism

N-Bromosuccinimide (NBS) is found to react quantitatively with lamivudine and dothiepin in sulphuric acid medium, whereas hydrochloric and acetic acids are more suitable with N-bromophthalimide (NBP) for lamivudine and dothiepin, respectively. It is reported to mention here that bromination of some drugs as sulphonamides are expected to occur only in acidic medium [30]. The bromination of lamivudine with NBS or NBP in H_2SO_4 or HCl medium, respectively, can postulate in Scheme 1. This indicates that the cytosinic-NH_2 group in the para position, which activates the ring for electrophillic substitution. Then the bromination of cytosinic-NH_2 results in the formation of bromo derivative in the ortho position to the cytosinic-NH_2 group.

Table 1. The potentiometric determination of lamivudine and dothiepin in pure solutions using NBS and NBP

Taken (mg)	**NBS**			**NBP**		
	Found[a] (mg)	Recovery (%)	RSD (%)	Found[a] (mg)	Recovery (%)	RSD (%)
Lamivudine						
0.115	0.116	100.87	1.58	0.114	99.13	0.84
0.229	0.228	99.56	1.35	0.228	99.56	0.76
0.459	0.461	100.44	1.23	0.459	100.00	0.57
0.688	0.690	100.29	0.83	0.691	100.44	0.62
0.917	0.914	99.67	0.95	0.915	99.78	0.72
1.146	1.144	99.82	0.78	1.147	100.09	0.69
Mean	-	100.11	1.12	-	99.83	0.70
Dothiepin						
0.166	0.168	101.20	1.02	0.165	99.40	0.54
0.332	0.333	100.30	0.60	0.331	99.70	0.60
0.664	0.663	99.85	0.67	0.668	100.60	0.10
0.996	0.996	100.00	0.50	0.998	100.20	0.19
1.328	1.330	100.15	0.53	1.340	100.90	0.75
1.660	1.659	99.94	0.74	1.658	99.88	0.41
Mean	-	100.24	0.68	-	100.11	0.43

[a] Average of five determinations

In case of dothiepin compound, the reaction with NBS or NBP can be explained that the tertiary amino group in the side-chain of drug is present in the form of a dialkylamino substituted group, owing to its basicity; the nitrogen atom is the site of the reaction with organic brominating agents. Tertiary amines are reported to react with brominating agents to

form a secondary amine with the separation of one of the alkyl group in the form of an aldehyde [37]. The reaction mechanism was proposed and given in Scheme 1.The actual brominating species here is the bromium ion (Br^+) produced by hydrolytic fission [38]. Also the N-Br bond in NBS or NBP is very polar owing to the fact that the nitrogen atom is attached to either two C=O groups. In the bromination reaction there is no possibility of any of the bromine escaping during the titrations. NBS or NBP reacts readily and quantitatively with aqueous solutions of lamivudine and dothiepin, being itself reduced to succinimide or phthalimide, respectively, (Scheme 1).

3.5. Potentiometric titration of drug

NBS or NBP is found to react quantitatively with lamivudine and dothiepin in sulphuric acid medium in case of NBS and hydrochloric acid or acetic acid medium in case of lamivudine or dothiepin, respectively, with NBP. Lamivudine and dothiepin are directly titrated potentiometrically with 2×10^{-3} M NBS or NBP as a titrant.

The titration curves of drug show one well-defined S-shaped stoichiometric end point using a Pt-electrode as an indicator electrode. The titration data is given in Fig 2. As representative curves for NBS, which represent the plot of E *vs*. volume (mL) of titrant for the determination of drug. The first differential curves which represent the plot of $\Delta E / \Delta V$ *vs*. mean volume of titrant, are characterized by sharp inflections lying in the immediate vicinity of the expected end points (Figs. 3.as example for NBP) these sharp inflections permit the accurate of the end point. The results in Figs. 2&3 indicate that the two reagents (NBS & NBP) are equal for accurate and sensitive methods for the determination of the two drugs.

3.6. Analytical results

The results of the drug determination presented in Table 1 showed that good recoveries and low standard deviations were obtained. The optimum concentration range for determination of lamivudine is 0.115-1.146 mg with mean recovery values 100.11 and 99.83% and coefficient of variations 1.12 and 0.70% using NBS and NBP, respectively. The concentration range for the determination of dothiepin is 0.166-1.660 mg with mean recovery values 100.24 and 100.11% and coefficient of variations 0.68 and 0.43% using NBS and NBP, respectively.

In order to establish whether the proposed method exhibits any fixed or proportional bias, a simple linear regression [39] of observed drug concentration against the theoretical values was illustrated. The calculated t-values are 0.11-0.94, which are lower than the tabulated value at 95% confidence level and five degrees of freedom (2.571). Thus there are no systematic differences between the determined and the true concentration over a wide range (0.115-1.146 mg for lamivudine and 0.166-1.660 mg for dothiepin). The results of different statistical treatments of the data are shown in Table 2.

Table 2. Linear regression analysis of data obtained from determination of lamivudine and dothiepin using NBS and NBP

Drug	Reagent	Slope of the regression line [a]	Intercept of the regression line [a]	Correlation coefficient (r)	RSD (%)	t-test (2.571)[b]
Lamivudine	NBS	0.9979	0.0009	1.0000	0.20	0.12
	NBP	1.0009	-0.0005	0.9999	0.22	0.11
Dothiepin	NBS	0.9995	0.0008	0.9999	2.00	0.28
	NBP	1.0020	0.0003	1.0000	0.60	0.94

[a] Observed vs. theoretical

[b] Tabulated 95 % confidence limit at 5-degrees of freedom

3.7. Determination of the active components in pharmaceuticals

In order to evaluate the applicability of the potentiometric method to pharmaceuticals, the determinations of lamivudine and dothiepin were carried out on Lamidine tablets and Prothiadin capsules, respectively, under the same conditions as employed for the pure drugs. The fact that the shapes of the potentiometric titration curves of pure drugs and their corresponding pharmaceuticals are nearly the same proves that the excipients which might be present in the pharmaceutical preparations had no effect of the titration curves.

Table 3 summarizes the results obtained for each drug in the corresponding pharmaceuticals, expressed as percentages of the nomial contents. The recoveries agree well enough with the nomial contents and the RSD values are less than 1.0%. Thus, the precision of the method is very satisfactory for the determination of lamivudine and dothiepin in corresponding pharmaceutical dosage forms. The results obtained were compared statistically by Student's t-test (for accuracy) and Variance ratio F-test (for precision) with the official method (BP) [2] for lamivudine and reference method (DDQ) [19] for dothiepin, at 95% confidence level with eight and four degrees of freedom for t-and F-tests, respectively, as shown in Table 3.

Table 3. The potentiometric titration for determination of lamivudine and dothiepin in some pharmaceutical preparations

Pharmaceutical	Drug	Reagent	Found ±SD (%)[a]	
			Potentiometric method	Compared Method
Lamidine tablets [c]	Lamivudine	NBS	99.68±0.38 t=0.67 F=1.80	99.87±0.51[2] (2.306) [b] (6.39) [b]
		NBP	99.76±0.46 t=0.36 F=1.23	
Prothiadin capsules [d]	Dothiepin	NBS	99.73±0.62 t=0.47 F=2.65	99.48±1.01[19] (2.306) [b] (6.39) [b]
		NBP	99.69±0.51 t=0.42 F=3.92	

[a] Mean and standard deviation for five determinations

[b] The tabulated values of t- and F-tests at 95% confidence limit

[c] Lamidine tablets containing 50 mg of lamivudine per tablet, Eva Pharma, Egypt

[d] Prothiadin capsules containing 25 mg of dothiepin hydrochloride per capsule, Al Kahira Co., Egypt, under licence of Knoll AG., Ludwigshafen, Germany

The results showed that the t-and F-values were less than the critical values, indicating that there was no significant difference between the proposed and official or reported method [2,19].The results clearly demonstrate the utility of the proposed potentiometric titrimetry for analysis of each drug in pharmaceuticals.

The recovery studies of standard additions to commercial pharmaceuticals were also carried out to provide further support for the validity of the method. The related data are given in Table 4. The mean percentage recoveries and their relative standard deviations were found to be 99.55-100.33 and 0.16-0.58%, respectively. These results also confirm the precision and the validity of the potentiometric method. As a result of this study, the proposed method can be successfully applied to the determination of lamivudine and dothiepin and the analysis of some of their pharmaceutical preparations.

Table 4. Recovery studies of standard additions to some pharmaceutical preparations

Pharmaceutical	Drug	Reagent	Taken (mg)	Added (mg)	Found[a] (mg)	Recovery± RSD%
Lamidine tablets [b]	Lamivudine	NBS	0.4	0.2	0.598	99.67±0.16
			0.4	0.4	0.799	99.87±0.38
			0.4	0.6	1.001	100.10±0.27
		NBP	0.4	0.2	0.599	99.83±0.26
			0.4	0.4	0.798	99.75±0.37
			0.4	0.6	0.998	99.80±0.34
Prothiadin capsules [c]	Dothiepin	NBS	0.6	0.3	0.898	99.78±0.41
			0.6	0.6	1.201	100.10±0.33
			0.6	0.9	1.499	99.93±0.29
		NBP	0.6	0.3	0.896	99.55±0.36
			0.6	0.6	1.204	100.33±0.52
			0.6	0.9	1.495	99.67±0.58

[a] Average of five determinations

[b] Lamidine tablets containing 50 mg lamivudine per tablet, Eva Pharma, Egypt

[c] Prothiaden capsules containing 25 mg dothiepin hydrochloride per capsule, Al Kahira Co., Egypt, under licence of Knoll AG., Ludwigshafen, Germany

4. CONCLUSION

The simplicity, accuracy and precision of the proposed potentiometric titrimetry indicate that this method is quite suitable for routine quality control analysis of pharmaceutical preparations. The extraction of drug from tablets or capsules with methanol or 1,2-dichloroethane, respectively, to remove any excipients could be interfered. In addition, the interacting reagents i.e. NBS and NBP are easily available and less expensive**.**

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