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Development and validation of three spectrophotometric methods for determination of pyridostigmine bromide in the presence of its alkaline-induced degradation product

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ABSTRACT

Three simple, accurate and validated spectrophotometric methods were developed for the determination of pyridostigmine bromide (PB) in presence of its alkaline-induced degradation product, 3-hydoxy-*N*-methylpyridinium bromide (3-OH NMP) in powder form and in pharmaceutical formulations. Method A, is first derivative method (^{1}D), which is based on measuring the peak amplitude of the first derivative spectra (^{1}D) of PB at 260 nm. Method B, is first derivative of ratio spectra (^{1}DD) which allows the determination of PB at 267.4 nm using (50 µg/mL) of 3-OH NMP as a suitable devisor. Finally, method C depends on mean centering of ratio spectra (MCR) of PB with different concentrations, which were recorded over 200-400 nm and divided by the spectrum of 20 µg/mL of 3-OH NMP as a devisor. The obtained ratio spectra were mean centered and the concentrations of PB were then determined from the calibration graphs obtained by measuring the amplitudes at 338 nm. The proposed methods were successfully applied for assay of PB both in pure form and in pharmaceutical formulations. The proposed methods were validated in compliance with International Conference on Harmonization (ICH) guidelines. The results obtained by the developed methods were statistically compared to those obtained by the reported HPLC method using F- and student's t-tests showing no significant difference regarding both accuracy and precision.

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1. Introduction

Pyridostigmine bromide, 3-[(dimethylcarbamoyl)oxy]-1-methylpyridinium bromide, [1,2] is parasypathomimetic and reversible choline esterase inhibitor [3], which is used to treat muscle weakness in people with myasthenia gravis [4,5]. Since it is a quaternary amine, it is poorly absorbed in the gut and does not cross the blood-brain barrier, except possibly in stressful conditions [6]. In military medicine, PB is used as a prophylactic agent against intoxication with irreversible organophosphorus AChE inhibitors, such as the nerve agents sarin and soman [6]. The chemical structure, molecular formula and molecular weight are shown in Figure 1. The literature survey reveals several methods for determination of PB including spectrophotometry [7], HPLC [8-11], GC [12-15] and CE [16-18].

PB is determined in the British pharmacopoeia via HPLC method [1]. PB was also assayed in presence of its alkaline induced degradation product via radioisotoptic techniques [19-21] and HPLC methods [22-25]. Since it is very liable to hydrolytic degradation, it is degraded in the alkaline solution into 3-hydroxy-*N*-methylpyridinium bromide as shown in Figure 1 and various analytical techniques; mainly HPLC and GC have been utilized for their determination in biological samples [26].

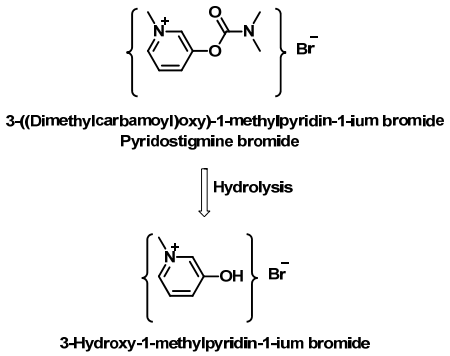

Figure 1. Alkaline hydrolysis of pyridostigmine bromide.

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The novelty of the presented work lies in development and validation of three new spectrophotometric methods for determination of PB in presence of its alkaline degradation product, namely; first derivative, first derivative of ratio spectra and mean centering of ratio spectra. The proposed methods are simple, rapid, don't require preliminary separation steps and can be used easily for the analysis of PB, especially in quality control laboratories.

2. Experimental

2.1. Instrumentations

A double beam UV-visible spectrophotometer (Shimadzu, Japan) model UV-1601 PC with quartz cell of 1 cm path length was connected to IBM compatible computer. The software was UV-PC personal spectroscopy software version 3.7. Data analyses were accomplished with support of PLS-Toolbox 2.0 running under Matlab®, version 7.1.

2.2. Materials

2.2.1. Pure standard

Pyridostigmine bromide was kindly supplied by Alexandria Company for Pharmaceuticals & Chemical Industries, Alexandria, Egypt. Its purity was found to be 100.46% according to the established method [1].

2.2.2. Pharmaceutical formulations

Pystonin® sugar coated tablets (Batch No. 4163002) are labeled to contain 60 mg of pyridostigmine bromide, manufactured by Alexandria Company for Pharmaceuticals & Chemical Industries, Alexandria City, Egypt. Mestinon® tablets (Batch No. 80085169) are labeled to contain 60 mg of pyridostigmine bromide, manufactured by Valeant Pharmaceuticals Switzerland GMBH, Birsfelden, Switzerland.

2.2.3. Chemicals and reagents

All chemicals used throughout this work were of analytical grade, and the solvents were of spectroscopic grade (Methanol and hydrochloric acid, Merck, Germany).

2.3. Standard solutions

Working standard solutions of PB and 3-OH NMP (100 µg/mL) were prepared by a suitable dilution of stock solutions (1 mg/mL in methanol) of each component with 0.05 N HCl solutions. All stock standard solutions were freshly prepared on the day of analysis and stored in refrigerator to be used within 24 h.

2.4. Laboratory prepared mixtures

Different mixtures containing different ratios of PB and 3-OH NMP were prepared using their respective working solutions (100 µg/mL).

2.5. Preparation of degradation product

An accurately weighted amount of PB powder equivalent to 0.5 g was dissolved in 15 mL of 0.1 N sodium hydroxide and the solution was left for 30 min. at room temperature. Appropriate volume of the resultant solution was applied on TLC plate where complete degradation was followed up via TLC using methanol-ethyl acetate-triethyl amine-glacial acetic acid (9:1: 0.5:0.05; *v*:*v*:*v*:*v*) as a developing system. After complete degradation, the degradation product was extracted three times each with 50 mL diethyl ether. The extract was then evaporated at room temperature to dryness. The degradation product powder was dried and identified by IR and Mass spectrometry as shown in Figure 2b, and Figure 3b, respectively, and used for preparation of the stock solution of the degradation product. PB was exposed to other stress conditions such as acid hydrolysis, oxidation and photodegradation. Acid hydrolysis was carried out with 0.1 N HCl at room temperature and 80 °C for 3 hrs. Oxidation studies were implemented using 30% hydrogen peroxide solution. Finally, photochemical stability was studied by exposing the drug sample to daylight for 24 hrs. PB showed stability regarding acid hydrolysis, oxidation and photo-stability studies.

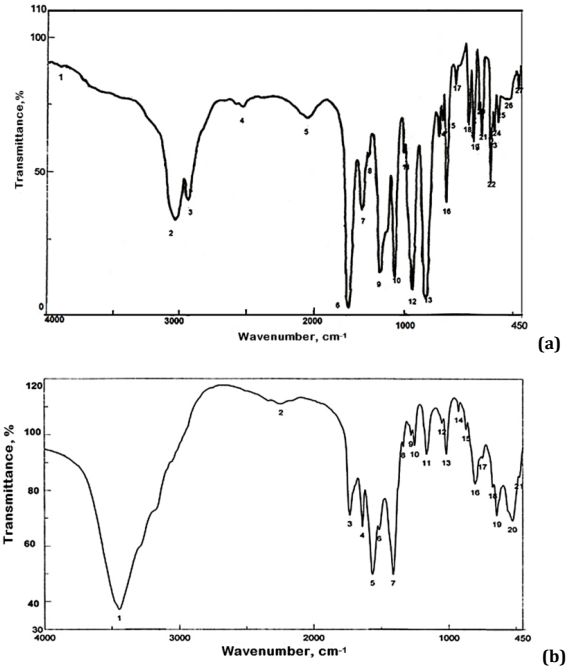

Figure 2. IR spectrum of PB (a) and 3-OH NMP (b).

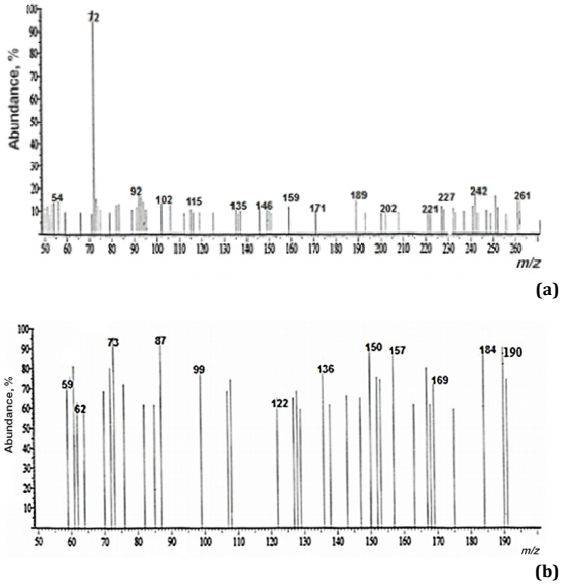

Figure 3. Mass spectrum of PB (a) and 3-OH NMP (b).

2.6. Procedures

2.6.1. Spectral characteristics of PB and PB degradation product

Zero order absorption spectra of 10 µg/mL each of PB and 3-OH NMP were recorded from 200 to 400 nm using 0.05 N HCl as a solvent, Figure 4.

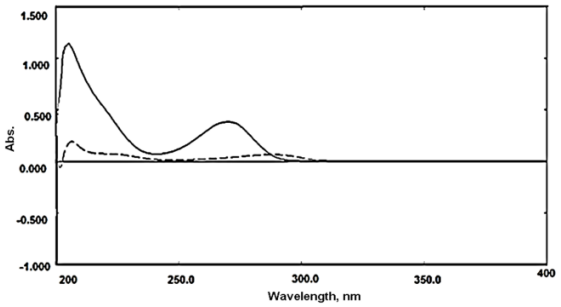

Figure 4. Zero order absorption spectra of 20 µg/mL of PB (__) and 20 µg/mL of 3-OH NMP (---) using 0.05 N HCl.

2.6.2. Linearity and construction of calibration curves

2.6.2.1. First derivative spectrophotometric method (^{1}D)

Accurately measured aliquots equivalent to 100-700 µg of PB were transferred from its working solution (100 µg/mL) into a series of 10 mL volumetric flasks. The volume was completed with 0.05 N HCl to obtain a final concentration range (10-70 µg/mL). ^{1}D curves were recorded at Δλ = 4 nm and scaling factor = 5. The peak amplitudes at 260 nm were recorded then calibration graph was constructed. The regression equation was then computed for the studied drug at the specified wavelength and used for determination of unknown samples containing PB.

2.6.2.2. First derivative ratio spectrophotometric method (^{1}DD)

Accurately measured aliquots equivalent to 100-700 µg of PB were transferred from its working solution (100 µg/mL) into a series of 10 mL volumetric flasks. The volume was completed with 0.05 N HCl to obtain a final concentration range (10-70 µg/mL). ^{1}DD curves were recorded at Δλ = 4 nm and scaling factor = 10. The absorption spectra of these solutions were divided by the absorption spectrum of 50 µg/mL of 3-OH NMP (as a divisor), then the obtained ratio spectra were differentiated with respect to wavelength. The peak amplitudes at 267.4 nm were recorded, then calibration graph was constructed relating the peak amplitudes of ^{1}DD to the corresponding concentrations. The regression equation was then computed for the studied drug at the specified wavelength and used for determination of unknown samples containing PB.

2.6.2.3. Mean centering of ratio spectra spectrophotometric method (MCR)

Accurately measured aliquots equivalent to 100-700 µg of PB were transferred from its working solution (100 µg/mL) into a series of 10 mL volumetric flasks and the volume was completed with 0.05 N HCl to final concentration range (100-700 µg/mL).

The recorded absorption spectra of PB from 200-400 nm were divided by the absorption spectrum of 3-OH NMP (20 µg/mL) to obtain the ratio spectra which were then mean centered. These mean centered values of the ratio spectra at 338 nm were recorded and plotted versus the corresponding concentrations. Calibration curve was constructed and regression equation was computed.

2.6.3. Analysis of laboratory prepared mixtures

Mixtures containing different ratios of PB and 3-OH NMP 10-90% were prepared. The volume was adjusted with 0.05 N HCl solution and procedures mentioned under Section 2.6.2 were followed. The concentrations were calculated from the corresponding regression equations.

2.6.4. Application to pharmaceutical formulations (Pystonin® and Mestinon® tablets)

Twenty tablets of each of Pystonin® and Mestinon® were separately weighed, powdered and mixed well. An accurately weighed amount of the powdered tablets equivalent to 100 mg of each pharmaceutical formulation was transferred into two separate 100 mL volumetric flasks and then 75 mL methanol was added to each flask. The prepared solutions were sonicated for 30 min, cooled and completed to volume with methanol to obtain 1000 µg/mL stock solution. The solutions were filtered and diluted with 0.05 N HCl to obtain 100 µg/mL working solutions and the procedures under Section 2.6.2 were followed. When carrying out the standard addition technique, the powdered tablets and pure PB were mixed well together before proceeding in the above mentioned procedures.

3. Results and discussion

PB contains amide group, which is liable to hydrolysis in basic condition, as shown in Figure 1. The first degradation product is aliphatic compound that is very soluble in water and has no absorbance under UV, but the second degradation product is the main degradation product [8], which is precipitated upon hydrolysis with NaOH and was extracted with multiple fractions of ether in our study. The several extractions were collected and evaporated at room temperature and the degradation product (3-OH NMP) powder was identified by IR and mass spectrometry, where the IR spectrum of PB Figure 2a, shows a strong band corresponding to the amide carbonyl group stretching at 1736 cm^{-1} (peak 6) and a band at 2950 cm^{-1} (peak 3) characteristic of C-H stretching of the aliphatic CH_3 in PB. The absence of these two characteristic bands in the IR spectrum of 3-OH NMP in addition to the appearance of single stretching band at 3445 cm^{-1} (peak 1) characteristic of the hydroxyl group is evidence of the cleavage of the carbamate group and complete degradation of the drug at the stated conditions as shown in Figure 2b.

The mass spectra of PB and 3-OH NMP also confirmed their identity as mass molecular ion peaks at *m/z* 260 and 190 corresponding to the intact drug (PB) and its degradation product (3-OH NMP), respectively, as shown in Figure 3a and Figure 3b. Thus the main task of this work was to study the stability of PB according to ICH guidelines [26] for:

i. Stress alkaline hydrolysis: 0.1 N NaOH at room temperature for 30 min.
ii. Stress acid hydrolysis: 0.1 N HCl at 80 °C for 3 h.
iii. Oxidative condition: 30% H_2O_2 for 24 hrs.
iv. Photochemical stability was studied by exposing the drug sample to daylight for 24 hrs.

PB showed stability regarding acid hydrolysis, oxidation and photo-stability studies.

PB acts as parasypathomimetic and reversible choline esterase inhibitor which has been used in treatment of some serious diseases such as myasthenia gravis, hence it is very important to develop some methods which not only are rapid, accurate, precise, but also simple and economic for

determination of the studied drug in its pharmaceutical dosage forms and this was the main task of the developed spectrophotometric methods.

UV-spectrophotometric methods of analysis have advantages of saving time and cost when compared to other methods such as HPTLC and HPLC methods [27,28]. This work concerns with the development of three accurate, precise and rapid spectrophotometric methods namely; first derivative (^{1}D), first derivative of ratio spectra (^{1}DD) and mean centering of ratio spectra (MCR) for analysis of PB in presence of its alkaline induced degradation product.

3.1. Spectrophotometric methods optimization

One of the important steps in development of analytical methods is to optimize the parameters, variables and conditions which will be followed in development and validation to obtain the best results.

To optimize the ^{1}D, ^{1}DD and mean centering spectrophotometric methods, it was important to test the influence of the following variables:

3.1.1. Solvents

Different solvents were experimented (methanol, ethanol, isopropanol, acetonitrile, purified water, 0.05 N HCl and 0.1 N HCl). It was found that PB is stable in these solvents and 0.05 N HCl was the best solvent regarding both sensitivity and selectivity.

3.1.2. The divisor and its concentration

The effect of divisor concentration on the method selectivity and analytical parameters such as intercept, slope and correlation coefficient of calibration equations was tested. Different concentrations of 3-OH NMP were tested as a divisor (10, 20, 30, 40 and 50 µg/ mL). It was found that 50 µg/mL of 3-OH NMP was the best concentration in case of ^{1}DD method and 20 µg/mL of 3-OH NMP was the best concentration in case of MCR method regarding both selectivity and sensitivity.

3.1.3. Smoothing and scaling factors

Different smoothing factor (Δλ) values were tried such as (2, 4 and 8), where smoothing factor = 4 presented a suitable signal to noise ratio with good resolution in case of both ^{1}D and ^{1}DD. Different scaling factor values were tried such as (10, 5 and 100), where scaling factor = 5 and 10 were the best in case of ^{1}D and ^{1}DD respectively to measure the signal of PB and to decrease the reading error.

3.2. ^{1}D spectrophotometric method

The zero-order absorption spectra of PB and its degradation product 3-OH NMP display sever overlapping Figure 4, which interferes with the direct spectrophotometric determination of PB in presence of its degradate. Derivative spectrophotometry is an analytical technique of great utility for resolving the overlapped spectra. The application of this technique provides selective determination of PB in presence of 3-OH NMP, by measuring the peak amplitude at 260 nm as shown in Figure 5.

The peak amplitude of the first derivative spectrum at λ = 260 nm as shown in Figure 6 was plotted versus the concentration of PB in the range of 10-70 µg/mL. The calibration curve was constructed with mean percentage recovery 99.76±1.526. The following linear regression equation was obtained:

$$Y = 0.0042 \times C + 0.0019 \qquad r = 0.9997 \qquad (1)$$

where Y is the peak amplitude at 260 nm, C is the concentration in µg/mL and r is the correlation coefficient.

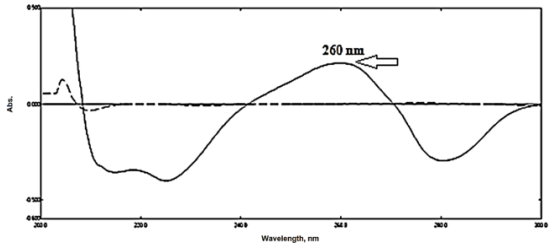

Figure 5. First derivativa spectra of 10 µg/mL of PB (__) and 50 µg/mL of 3-OH NMP (---) using 0.05 N HCl as blank.

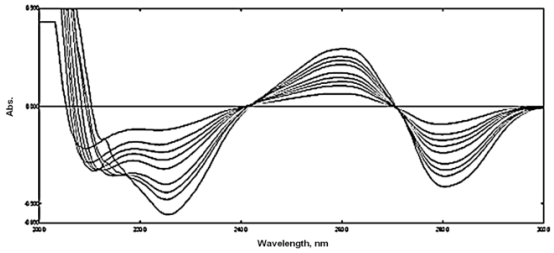

Figure 6. First derivative spectra of PB (____) in the range of 10-70 µg/mL using 0.05 N HCl as blank.

3.3. ^{1}DD spectrophotometric method

First derivative ratio spectrophotometry has the ability to overcome the problem of overlapping spectra. The application of this technique allows selective determination of PB in presence of 3-OH NMP by measuring the peak amplitude at 267.4 nm as shown in Figure 7. The absorption spectrum of PB was divided by the absorption spectrum of 50 µg/mL of 3-OH NMP (as a divisor), and then the obtained ratio spectrum was differentiated with respect to wavelength. The peak amplitude of the first derivative ratio spectrum at λ = 267.4 nm, as shown in Figure 8, was plotted versus the concentration of PB in the range of 10-70 µg/mL.

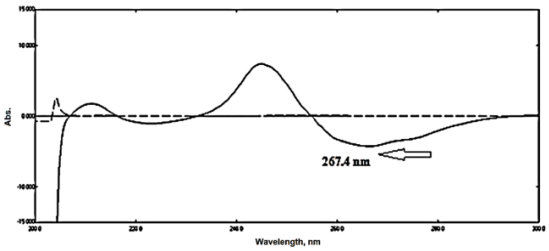

Figure 7. First derivative of ratio spectra of 10µg/mL of PB(__) and 10µg/ml of 3-OH NMP(---) using 50 µg/mL of 3-OH NMP as a devisor and 0.05 N HCl as blank.

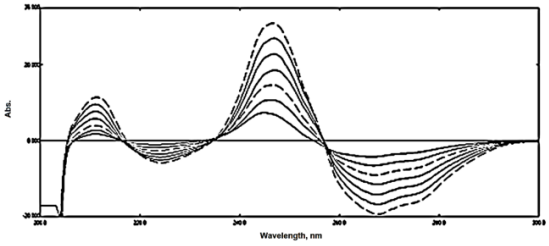

Figure 8. First derivative of ratio spectra of of PB in the range of 10-70 µg/mL using 50 µg/ mL 3-OH NMP as a divisor and 0.05 N HCl as blank.

Table 1. Assay results for the determination of PB in synthetic mixtures with 3-OH NMP prepared to contain 10-90% degradate.

3-OH NMP %	% Recovery	% Recovery	% Recovery
	^{1}D spectrophotometric method	^{1}DD spectrophotometric method	Mean centering method
10%	98.86	98.96	99.86
20%	101.54	101.28	101.57
30%	102.32	99.30	100.19
40%	100.73	101.63	100.88
50%	102.32	98.36	100.63
60%	102.20	101.70	99.05
70%	102.60	99.72	99.85
80%	100.85	99.85	98.90
90%	93.59*	100.36	92.54*
Mean±SD	101.43±1.251	100.13±1.200	100.12±0.903

* Rejected value.

The calibration curve was constructed with mean percentage recovery 99.90±0.762. The following linear regression equation was obtained:

$$Y = 0.0260 \times C + 1.1613 \qquad r = 0.9999 \qquad (2)$$

where Y is the peak amplitude at 267.4 nm, C is the concentration in µg/mL and r is the correlation coefficient.

3.4. Mean centering of ratio spectra spectrophotometric method (MCR)

The developed MCR method depends on the mean centering of the ratio spectra, it gets rid of the derivative steps and therefore signal-to-noise ratio is increased [29]. This method is able to resolve binary and ternary mixtures in complex samples with unknown matrices [30]. The mathematical explanation of this method was discussed and illustrated by Afkhami and Bahram [31-33].

The absorption spectra of PB were recorded in the wavelength range of 200-400 nm and divided by the absorption spectrum of 20 µg/mL of 3-OH NMP to obtain the ratio spectra which were then mean centered. PB was determined by measuring the peak amplitude at 338 nm which corresponds to maximum wavelength as shown in Figure 9. The peak amplitude was plotted versus the corresponding concentration in the range of 10-70 µg/mL. The calibration curve was constructed with mean percentage recovery 99.91%±1.343. The following linear regression equation was obtained:

$$Y = 0.4626 \times C + 0.6773 \qquad r = 0.9998 \qquad (3)$$

where Y is the peak amplitude at 338 nm, C is the concentration in µg/mL and r is the correlation coefficient.

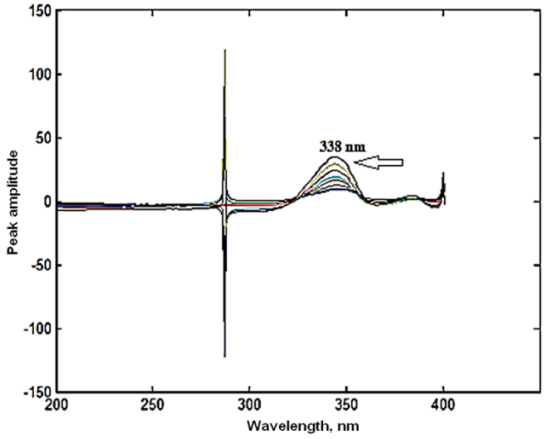

Figure 9. The mean centered ratio absorption spectra of PB in the range of 10-70 µg/mL using 0.05 N HCl as blank.

3.5. Stability-indication and selectivity

The assessment of the stability-indicating efficiency and selectivity of the proposed methods was proved by application of these methods to laboratory prepared mixtures containing different ratios of PB and 3-OH NMP. Results given in Table 1 indicate that three proposed methods are valid for determination of intact PB in presence of its alkaline-induced degradation product. Compared to the derivative method, MCR eliminates the derivative step and so the signal-to-noise ratio is enhanced.

As the suggested methods could effectively determine the drug in presence of its degradate (3-OH NMP), they can be employed as stability indicating ones.

3.6. Application of the proposed methods to the pharmaceutical formulation

The suggested methods were successfully applied for the assay of PB in its pharmaceutical formulations (Pystonin® and Mestinon® tablets), showing good percentage recoveries. The validity of the developed methods was further assessed by applying the standard addition technique and confirmed the accuracy of the methods as shown in Table 2.

3.7. Methods' validation

The international conference on harmonization (ICH) guidelines [34] for analytical method validation has been followed throughout methods' validation.

3.7.1. Linearity

Under optimum experimental conditions, PB was determined in triplicates in the range of 10-70 µg/mL for ^{1}D, ^{1}DD and MCR, Table 3.

3.7.2. Accuracy

The accuracy of the proposed methods was checked by determination of different blind samples of PB. The accuracy was ascertained also by application of the standard addition technique and through analysis of market pharmaceutical preparation by the suggested methods. The good percentage recoveries of pure drug samples provide good accuracy of the proposed methods, Table 2.

3.8. Precision

3.8.1. Repeatability

Three different concentrations of PB (20, 30 and 40 µg/mL) for ^{1}D method, (10, 60, 70 µg/mL) for ^{1}DD method and (10, 20, 30 µg/mL) for MCR method were determined in triplicates in the same day to estimate intraday variation.

Table 2. Determination of pyridostigmine bromide in pharmaceutical formulations by the proposed methods and application of standard addition technique

Method	Pharmaceutical formulation	Taken (µg/mL)	Found % ± SD	Pure added (µg /mL)	Recovery%
^{1}D method	Pystonin® tablets Batch. No (4163002)	20	101.46±1.004	10	99.76
				20	101.59
				30	101.38
				Mean±SD	100.91±1.003
	Mestinon® tablets Batch. no (80085169)	20	96.83±1.578	10	100.00
				20	98.05
				50	99.61
				Mean±SD	99.22±1.032
^{1}DD method	Pystonin® tablets Batch. No (4163002)	20	96.48±1.315	10	100.69
				40	99.09
				50	101.53
				Mean±SD	100.44±1.238
	Mestinon® tablets Batch. no (80085169)	20	94.99±0.995	8	102.07
				10	99.13
				12	98.91
				Mean±SD	100.04±1.764
Mean centring method	Pystonin® tablets Batch. No (4163002)	20	98.84±1.229	15	99.72
				20	97.64
				25	100.05
				Mean±SD	99.14±1.302
	Mestinon® tablets Batch. no (80085169)	20	96.25±1.233	10	98.51
				15	101.88
				25	101.32
				Mean±SD	100.57±1.808

Table 3. Regression and analytical validation parameters of the proposed methods for determination of PB in presence of 3-OH NMP.

Parameter	^{1}D spectrophotometric method	^{1}DD spectrophotometric method	Mean centering method
Calibration range (µg/mL)	10-70	10-70	10-70
Slope	0.0042	0.2602	0.4626
Intercept	0.0014	1.1613	0.6773
Correlation coefficient (r)	0.9997	0.9999	0.9998
Accuracy, %	99.76±1.526	99.90±0.762	99.91±1.343
Specificity and Selectivity	101.43±1.251	100.13±1.200	100.12±0.903
Precision			
Repeatability (RSD%) *	1.354	0.918	0.400
Intermediadte Precision (RSD%) *	1.733	1.299	1.480
LOD ** (µg/mL)	2.406	2.872	2.748
LOQ ** (µg/mL)	7.314	9.479	9.069

* RSD% and RSD%; the intra- and inter-day relative standard deviation of concentrations (20,30 and 40 µg/ mL) for ^{1}D method, (10, 60 and 70 µg/mL) for ^{1}DD method and (10, 20 and 30 µg/mL) for MCR method.
** Limit of detection and quantitation are determined via calculations for ^{1} D, ^{1}DD and MCR methods (LOD = 3.3 × SD/slope, LOQ = 10 × SD/slope) [26].

Table 4. Statistical analysis of the three proposed methods (^{1}D spectrophotometric method, ^{1}DD spectrophotometric method and Mean centering method) and the reported HPLC method for determination of PB in presence of its alkaline-induced degradation product.

Parameters	^{1}D method	^{1}DD method	Mean centering method	Reported HPLC method [1]
Mean	99.85	99.90	99.91	100.46
SD	1.635	0.929	1.343	0.718
Variance	2.673	0.863	1.804	0.515
n	7	7	7	7
Student's *t*-test (2.45) *	0.384	0.238	0.366	-
F- test (4.28) *	3.049	1.676	3.503	-

* The values between parenthesis are corresponding to the theoretical values of *t* and *F* (p = 0.05).

Good percentage recoveries and acceptable relative standard deviation (RSD%) values were obtained confirming the repeatability of the three methods Table 3.

3.8.2. Intermediate precision

The previous procedures were repeated on the same concentrations on different five days to determine the intermediate precision. Good results and acceptable relative standard deviation (RSD%) are shown in Table 3.

3.9. Specificity

Specificity of the method was tested by how accurately and specifically the analyte of interest is determined in presence of other components (impurities, degradates or excipients) [34]. It was achieved by analysis of different synthetic laboratory prepared mixtures containing different ratios of PB spiked with appropriate levels of 3-OH NMP; demonstrating that the assay results are unaffected by the presence of 3-OH NMP and confirming the specificity of the proposed methods as shown in Table 1.

3.10. Limits of detection and quantitation (LOD and LOQ) [34]

The approach based on the standard deviation of the response and the slope was used for determining the detection and quantitation limits, Table 3.

$$LOD = 3.3 \times SD / slope \quad (4)$$

$$LOQ = 10 \times SD / slope \quad (5)$$

Acceptable detection and quantitation limits are shown in Table 3.

The statistical comparison of the results obtained by the proposed methods and those obtained by the reported method on the pure PB as shown in Table 4 indicating that there is no significant difference between the proposed methods and the reported one with respect to accuracy and precision.

4. Conclusion

The presented work provides simple, accurate and selective analytical methods for determination of PB in presence of its degradation product which is separated upon alkaline hydrolysis of PB both in bulk powder and in pharmaceutical formulations. The developed methods are found to be rapid, simple, accurate, reproducible, selective and easy to apply. The suggested methods also allowed the quantitative determination of PB and its degradate without prior separation**.** Compared to the derivative method, MCR eliminates the derivative steps.

Application of the proposed methods to assay of PB in laboratory-prepared mixtures and pharmaceutical formulations shows that neither the alkaline-induced degradation product nor the additives interfere with the determination of PB; indicating that the proposed methods could be applied as stability indicating methods for the determination of pure PB and in the presence of its alkaline-induced degradation product either in bulk powder or in pharmaceutical formulations.

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