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Research Article

A VALIDATED STABILITY-INDICATING RP-HPLC WITH PHOTODIODE ARRAY DETECTOR METHOD FOR THE DETERMINATION OF PIPAZETHATE HYDROCHLORIDE IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, sensitive, rapid and stability indicating reversed phase high-performance liquid chromatographic (RP-HPLC) method with photodiode array (PDA) detection was developed and validated for the determination of pipazethate HCl in bulk drug and dosage forms. Well resolved peaks of target analyte and its degradation products were achieved on a C_{18} reversed phase column, using a simple isocratic mobile phase of water-methanol (40:60, v/v). The flow rate was 1.0 ml/min, the column temperature was 30 ºC and the detection was performed at 230 nm. The method was validated as per International Conference on Harmonization (ICH) guidelines with respect to specificity, linearity, precision, accuracy, limits of detection and quantitation. Pipazethate was subjected to the stress conditions of acidic, basic, oxidative, hydrolytic, thermal and photolytic degradation. The assay was linear over the concentration range of 100-10000 ng/ml and the correlation of coefficient was more than 0.999. Inter and intra-assay precision was less than 4.2%. Inter and intra-assay accuracy was within ± 4.8%.

Keywords: Pipazethate hydrochloride, Stability indicating, HPLC-PDA.

INTRODUCTION

Pipazethate HCl (2-(2-piperidinoethoxy)ethyl 10H-pyrido[3,2-b] [1,4]benzothiadiazine-10-carboxylate hydrochloride is a non narcotic antitussive and cough suppressant drug that acts centrally,[1] it also has some peripheral effects on non-productive cough. The onset of action takes about 10–20 min and the therapeutic action lasts for 4–6 h [1-3]. Spectrophotometric [3-6], conductimetric [7] and potentiometric [8] methods have been reported for determination of pipazethate hydrochloride (P-HCl) with other cough suppressant drugs in pure form and in pharmaceutical preparations. A stability indicating HPLC method has been developed for determination of P-HCl in the presence of its alkaline degradation product; the method has showed linearity over a concentration range of 5-200 µg/ml [9]. P-HCl has been shown to be hydrolyzed upon refluxing in a strong alkaline media; the suggested degradation pathway was through hydrolysis of the ester linkage followed by decarboxylation process to produce a free base molecule as the main alkaline degradation product. The degradation pathway and the structure of the degradation product have been confirmed using IR spectroscopy [9]. Another stability indicating HPLC/HPTLC method has been recently published; authors have studied the kinetic of acidic, alkaline and oxidative degradation processes of P-HCl at different temperatures and investigated the pH rate degradation profile [10]. The HPLC method used CN column and UV detection at 225 nm, and was linear over a concentration range of 0.2-40 and 0.2-20 µg/ml for P-HCl and its main degradation product, respectively [10]. A stability indicating method is a method that will accurately measure the analyte and resolve the analyte from its degradation products [11]. The International Conference on Harmonization (ICH) guidelines requires the establishment of stability indicating methods by conducting of forced degradation studies under a different pH, light, oxidation, dry heat, etc. conditions and separation of the target drug from degradation products [11]. Degradation products may be formed during manufacturing of active ingredients or during storage of the pharmaceutical products. Forced degradation studies provide valuable information about analytical method specificity. Understanding of the possible degradation pathways for target analyte(s) and degradation products that may be generated during either manufacturing or storage can improve the pharmaceutical development process, the products quality and also can avoid many toxicological risks [12]. High-performance liquid chromatography (HPLC) technique has been widely used in stability indicating methods due to high sensitivity, specificity, separation of multiple components and ability to analyze non-volatile, thermally labile and polar compounds [11]. The photodiode array (PDA) detector has many advantages over the conventional UV-Vis spectrophotometer, it has the ability to collect spectral data for many wavelengths simultaneously, while the UV-Vis spectrophotometer has a single channel detector which can collect data for a selected wavelength; degradation products may be unseen at the selected wavelength of the target analyte. The PDA detector also is much faster and gives more reproducible results [13]. P-HCl (Selegon®) is a widely used atitussive compound in the Egyptian market; the increasing utilization of this drug as antitussive especially for children demands the development of a sensitive, simple and stability indicating method that can be used for the routine analysis of P-HCl in raw material and pharmaceutical dosage forms. The aim of this work is to study the stability of P-HCl under a diversity of ICH-recommended forced degradation conditions, to successfully detect and resolve the main degradation products from target analyte and to develop and validate a simple, accurate and highly throughput analytical method for determination of P-HCl in pure form and in pharmaceutical preparations. Degradation products were monitored at different wavelengths to ensure absence of co-elution and the proposed methods was more sensitive than the previous published HPLC methods. Chemical structure of P-HCl is shown in figure [1]

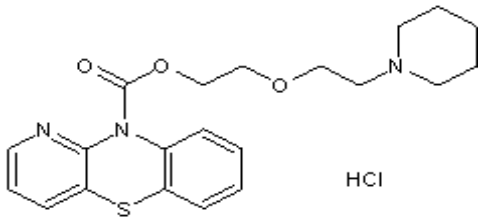

Fig. 1: it shows chemical structure of pipazethate hydrochloride

EXPERIMENTAL

Chemicals and reagents

P-HCl was provided by Egyptian International Pharmaceutical Industries Co. (EPICO) (Cairo, Egypt). Selegon® 20 mg tablets and 40 mg drops were purchased from the local market. Water and methanol (HPLC grade) were purchased from LAB-SCAN, Analytical Sciences (Gliwice, UL, Sowinskiego, Poland). Sodium hydroxide, hydrochloric acid, hydrogen peroxide were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Instrumentations

The HPLC analyses were carried out on a Finnigan Surveyor Plus HPLC system composed of binary pumps, autosampler and Photo diode array detector (PDA) with a Chromquest 5.0 software installed

on a Dell computer (Thermo Electron Corp., Bellefonte, PA, USA). Detection was performed at 230 nm.

Isocratic method for the analysis of P-HCl

The samples were analyzed using a Hypersil Gold (100 mm×4.6mm), 5µm particle size column (Thermo Electron Corp., Bellefonte, PA, USA); the column temperature was maintained at 30ºC .Mobile phase A was water and mobile phase B was methanol; the autosampler utilized methanol as a rinse solution, the total run time was 6.0 min. The elution pumps ran an isocratic flow using 40% A and 60% B at 1.0 ml/min flow rate. The injection volume was 20µl and the loop size was 25 µl.

Preparation of standards and quality controls.

A 100 µg/ml stock solution of P-HCl was prepared in methanol. The solution was prepared by weighing approximately 5.0 mg of P-HCl and quantitatively transferring it into an amber color glass bottle; the solution was diluted to approximately 50 ml using the appropriate solvent volume and stored at approximately 4 ºC. The calibration standards and quality control (QC) samples were prepared by adding the appropriate amounts of the stock solution into a 10 ml volumetric flasks and diluting to volume with water-methanol (40:60, v/v). Nominal concentrations of calibration standards were 100, 300, 500, 1000, 3000, 6000 and 10000 ng/ml of P-HCl. QC samples were prepared at concentrations of 300, 3000 and 6000 ng/ml of P-HCl.

Preparation of tablets and drops solution

Twenty Selegon® tablets, each labeled to contain 20 mg P-HCl were accurately weighed, finally powdered and mixed well. A quantity of powdered tablets equivalent to 5.0 mg of P-HCl were accurately weighted and dissolved in100 ml of methanol (5x20 ml) and filtered into a 100 ml volumetric flask; the volume was completed with methanol to 100 ml, 3000 ng/ml solutions were prepared in water-methanol (40:60, v/v) and 20 µl of the resulting solution was injected into the HPLC system (n=3).

A 125 µl of Selegon® drops solution (labeled to contain 40 mg/ml P-HCl) was aliquoted into a 50 ml volumetric flask already contains approximately 20 ml methanol, the volume was completed with methanol to volume, 3000 ng/ml solutions were prepared in water-methanol (40:60, v/v) and 20 µl of the resulting solution was injected into the HPLC system (n=3).

Forced degradation studies

Pure active pharmaceutical ingredient of P-HCl was stressed under different stress conditions to establish a stability indicating method.

Degradation in solutions

20% methanol was used as a co-solvent in all experiments to avoid any precipitation.

Solutions for alkali, acidic, oxidative and neutral degradation studies were prepared as follow: A 100 mg of P-HCl was dissolved in 100 ml of methanol-2M NaOH, 2M HCl, 20% H_2O_2, and water (20:80, v/v), respectively, solutions were protected from light and exposed to dry heat (80 ºC) in an oven for 8.0 hours, then further diluted with mobile phase.

Degradation in solid form

For temperature stress studies; a 100 mg of P-HCl powder was protected from light and exposed to dry heat at 80 ºC for 8.0 hours, the powder was dissolved in 100 ml water-methanol (80:20, v/v), then further diluted with mobile phase. For photostability studies; a 100 mg of P-HCl powder was spread on a glass dish in a thin layer (less than 2mm thickness) and exposed to direct day light for 4 hours, the powder was dissolved in 100 ml water-methanol (80:20, v/v), then further diluted with mobile phase.

Validation

The analytical method was validated according to the International Conference on Harmonization (ICH) guidelines [14].

Specificity

Specificity is the ability of the analytical method to discriminate between target analyte and other components that may be present. The specificity of the assay was determined by the complete chromatographic separation of P-HCl peak from its degradation products peaks generated under various stress conditions.

Linearity

Calibration curve test solutions were prepared from pipazethate stock solution at seven concentration levels (100, 300, 500, 1000, 3000, 6000 and 10000 ng/ml) in triplicate. Linearity was established by least squares linear regression analysis of the calibration curve. Peak areas were plotted versus P-HCl concentrations. The lower limit of quantitation (LOQ) was determined as the lowest concentration of the target analyte that was quantified with acceptable precision and accuracy [15]. The LOD was determined as the concentration of analyte that produced a response of three times the background noise [14]

Precision

Precision is the degree of agreement of test results when the analytical method is applied to multiple samples. Intra-day precision (repeatability) was determined by analyzing quality control (QC) samples at three different concentrations (300, 3000 and 6000 ng/ml) for five times within one day. Inter-day (intermediate) precision was determined by analyzing QC samples at concentrations of (300, 3000 and 6000 ng/ml) in triplicates for three consecutive days.

Accuracy

Accuracy is the closeness of test results obtained by the analytical method to the nominal value. Intra-day accuracy was determined by analyzing quality control (QC) samples at concentrations of (300, 3000 and 6000 ng/ml) for five times within one day. Inter-day accuracy was determined by analyzing QC samples at concentrations of (300, 3000 and 6000 ng/ml) in triplicates for three consecutive days.

Ruggedness and Robustness

Robustness is the ability of the analytical method to remain unchanged by small, but deliberate changes in method parameters. To determine the robustness of the proposed method, the experimental conditions were deliberately changed; variation of the mobile phase flow rate by ± 0.1 ml/min, column temperature by ± 3.0 ºC and organic strength of the mobile phase by ± 2.0 % were studied. Ruggedness is the degree of reproducibility of test results under normal operational conditions such as laboratory to laboratory and analyst to analyst. The ruggedness of the method was assessed by comparing the inter-assay results of QC samples that have been conducted by two different analysts on two different days.

Solution stability

The stability of P-HCl in solution was evaluated by storing drug solutions prepared in the mobile phase for 48 hours, at both room temperature and 4ºC, and testing both solutions in triplicates.

RESULTS AND DISCUSSION

Method development

A Hypersil Gold (100 mm×4.6mm), 5µm particle size column maintained at 30 ºC and a simple isocratic method were used for the separation of target analyte and its main degradation products. Hypersil Gold column is a highly pure silica-based C_{18} column with end capping and minimal residual silanols [16]. It is stable over a wide range of pH (1.8-10.6) and offers improved peak shape with no tailing for neutral and basic analytes in comparison to other C_{18} columns. [17] The end capping increases the column capacity to resolve related components; the column shows excellent symmetrical peak shape and high sensitivity for wide range of analytes using either isocratic or gradient mobile phase at ambient temperature and 30°C [17]. Different chromatographic conditions were optimized to obtain an acceptable chromatographic resolution

between target analyte and its main degradation products, different percentages of methanol (50-85%) were applied; 60% methanol was found to be the optimum organic percentage, increasing the percentage of methanol (e.g. 85%) showed early eluted analyte peak at 2.2 min and poor chromatographic separation. Other chromatographic conditions such as flow rate and column temperature were optimized. Symmetrical peak shape (Figure 2) was obtained under the proposed chromatographic conditions; the tailing factor was 1.06 for P-HCl peak, other system suitability parameters; capacity factor (k´), selectivity (α) and resolution (Rs) are summarized in Table 1.

Table 1: Shows System Suitability Parameters

Deg. Medium	Deg. Product	Retention time (min)	Capacity factor (k´)	Selectivity (α)	Resolution (r_s)
Alkaline	DGB-1	1.5	0.29	10.52	3.8
	DGB-2	2.6	1.24	2.46	3.7
	DGB-3	3.5	2.01	1.52	2.0
	DGB-4	6.1	4.25	1.43	2.0
Acidic	DGA-1	1.5	0.29	10.25	4.9
	DGA-2	2.0	1.00	3.05	3.8
Oxidative	DGO-1	1.5	0.29	10.25	4.9
	DGO-2	1.9	0.64	4.76	4.7
	DGO-3	3.0	1.59	1.92	2.1
Neutral	DGN-1	5.9	4.09	1.44	1.6
	P-HCl	4.7	3.05	-	-

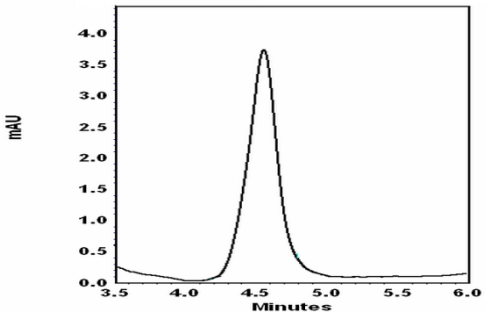

Fig 2: shows hplc chromatogram of low qc sample (300 ng/ml) p-hcl at 230 nm using a hypersil gold c_{18} column and water _ methanol (40:60, v/v) isocratic mobile phase.

The main advantage of the photodiode array (PDA) detection is the ability to collect multiwavelength spectral information from one injection [13]. The absorbance ratio at different wavelengths can be applied to confirm absence of any co-elution of different components in a signal chromatogram. P-HCl showed main absorption peaks at 230, 251 and 276 nm. Analyte peak were monitored using the three different wavelengths; under the proposed chromatographic conditions 230 nm was found to be the optimum wavelength for quantitation at which the highest detector response was obtained.

Degradation behavior of pipathezate hydrochloride

Using of a 20% methanol as a co-solvent was found to be very convenient to avoid precipitation and also to avoid many time consuming steps such as filtration, washing, drying and reconstitution of degradation products; other degradation products may be formed during multiple preparation steps of the main degradation products and lead to misjudging, as the main degradation products and their stability are unknown in most cases. On the other hand, separation and identification of the degradation products was out of scope of this work; monitoring degradation products were carried out to measure the degree of method selectivity. All stressed samples tested in solid and solution form remained colorless with no any precipitation. Degradation products were monitored at 230, 251 and 276 nm to compare different elution profiles of degradation products at multiple wavelengths and to ensure absence of co-elution.

Alkaline conditions

It was observed that P-HCl showed alkaline degradation upon heating in methanol-2.0 M NaOH (20:80, v/v) for 8 hours at 80 °C. The main alkaline degradation product (DGB-1) peak was eluted at approximately 1.5 min, other small peaks (DGB-2, 3, 4) were eluted at 2.6, 3.5 and 6.1 minutes, respectively. (Figure3). Similar elution profiles with different peak intensities were observed at 251 and 276 nm.

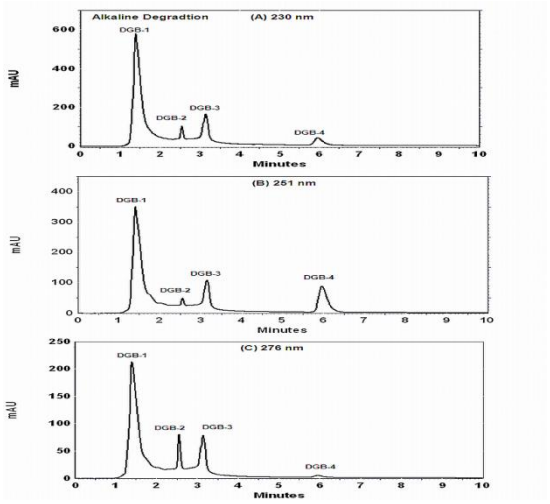

Fig 3: Shows Hplc Chromatogram Of Alkaline Degradation Products At (A) 230 Nm, (B) 251 Nm And (C) 276 Nm

Acidic conditions

Acidic degradation of P-HCl was also observed upon heating in methanol-2.0 M HCl (20:80, v/v) for 8 hours at 80 °C. The acidic degradation products (DGA) showed the main peak at 1.5 min and another smaller peak at 2.0 min (Figure 4). Both peaks were observed at 251 and 276 nm but with different ratios.

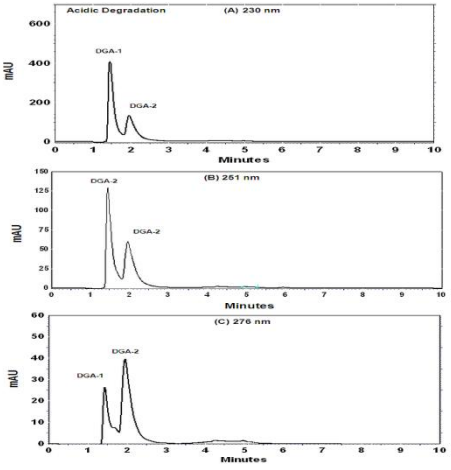

Fig 4:Shows Hplc Chromatograms Of Acidic Degradation Products At (A) 230 Nm, (B) 251 Nm And (C) 276 Nm

Oxidative conditions

Pipazethat hydrochloride was found to be liable also to oxidative degradation; heating in methanol-20% H_2O_2 (20:80, v/v) for 8 hours at 80 °C resulted in strong peak (DGH-1) at 1.5 min and two small peaks at 1.9 and 3.0 min (Figure 5). Similar elution profiles with different peak intensities were observed at 251 and 276 nm.

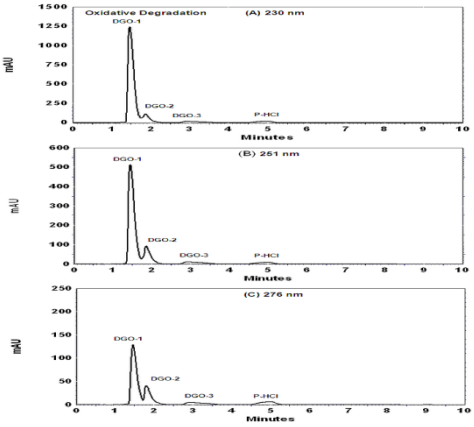

Fig 5: shows hplc chromatograms of oxidatives degradation products at (a) 230 nm, (b) 251 nm and (c) 276 nm

Neutral conditions

P-HCl was found to be more stable under neutral conditions. A small (*negligible*) peak was observed at 5.9 min (less than 1.5 % of the P-HCl peak area) upon heating in methanol-water (20:80, v/v) for 8 hours at 80 °C. However this peak showed higher abundance at 251 nm (approximately 11% of the P-HCl peak area) and was absent at 276 nm (Figure 6)

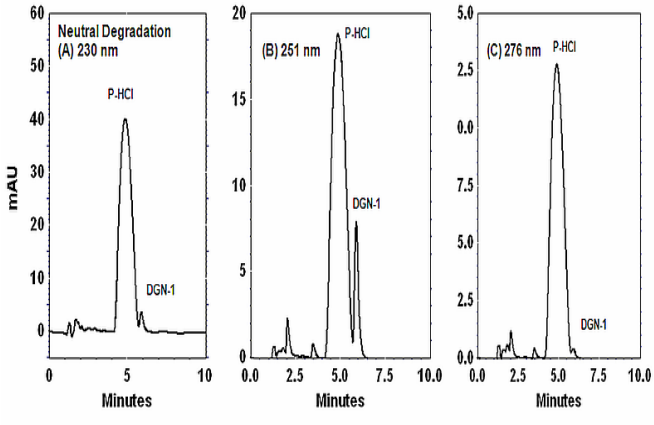

Fig 6: Hplc Chromatograms Of Neutral Degradation Products At (A) 230 Nm, (B) 251 Nm And (C) 276 Nm

Temperature stress and photo-stability studies

P-HCl was found to be stable under the proposed heat conditions. No degradation product peaks were observed on exposure of drug powder to direct day light for 4 hours.

Different elution profiles of acidic, basic, oxidative and neutral degradation products and different peak intensities suggested that different degradation products may be formed under different stress conditions and with different extent. Target analyte was resolved from all generated degradation products; the chromatographic resolution R_s between target analyte and different degradation products peaks ranged from (1.6-4.9). Monitoring degradation products at different wavelengths confirmed the same elution profiles but with different peak intensities and was found to be a good tool to confirm absence/presence of other low abundant peaks as shown in the neutral stress conditions.

Method validation

Specificity

The results of the stress studies designated a high degree of specificity. All degradation product peaks were chromatographically resolved from the analyte peak as shown in figures (3-6).

Linearity

The linear calibration curve for the assay method was obtained over the concentration range of (100–10000 ng/ml); the correlation coefficient was more than 0.999 (n=3), excellent correlation between peak area and analyte concentrations was obtained. The mean ± SD, the percent relative standard deviations of slopes, intercepts and the correlation coefficient of the calibration curves, LOD and LOQ are summarized in table 2.

Table 2 :Shows Lod, Loq And Regression Equation Data

Parameters	P-HCL
LOD (ng/ml)	35.0
LOQ (ng/ml)	100
Regression equation:	
Linearity range (ng/ml)	100-10000
Slope ± SD (n=3)	187.41±4.60
%RSD of slope	2.45
Intercept ± SD (n=3)	-5488.23 ± 397.50
%RSD of intercept	7.24
Correlation coefficient ± SD (n=3)	0.9995 ± 0.0004
%RSD	0.04

Precision

The inter and intra-assay precision (measured as percent relative standard deviation; %RSD) were less than 3.7 and 4.2 %, respectively.

Accuracy

The inter and intra-assay accuracy (measured as percent difference from nominal; %DFN) were within ± 4.8 and 4.3%, respectively. Inter and intra-assay precision and accuracy were summarized in table 3

Table 3 :Shows Intra And Inter_Assay Precision And Accuracy Of Qc Samples

	QC samples Conc. (ng/ml)		
	300	3000	6000
Intra-assay (n=5)			
Mean	300.12	2952.00	6253.13
%RSD	2.46	4.11	0.83
%DFN	0.04	-1.60	4.22
Inter-assay (n=11)			
Mean	298.36	2925.79	6285.67
%RSD	1.90	3.61	0.78
%DFN	-0.55	-2.47	4.76

Robustness and ruggedness

Changing of the mobile phase flow rate by ± 0.1 ml/min, column temperature by ± 3.0 ºC and organic strength of the mobile phase by ± 2.0 % did not result in significant effects on the chromatographic resolution of the proposed method. The inter-assay precision and accuracy of two sets of QC samples prepared by two different analysts and analyzed in two different days were within 3.4% and ± 4.3%, respectively.

Solution stability

The results from stability experiments showed that P-HCl was stable in mobile phase for up to 48 hours at both room temperature and 4ºC.

Dosage forms analysis

The validated method was applied for the determination of P-HCl in commercially available Selegon® tablets and drops; the recoveries

were 101.42 ± 1.56 % and 100.11 ± 3.48, respectively. The proposed method was found to be suitable for the analysis of P-HCl without interference from the excipients.

CONCLUSIONS

A simple, rapid and stability-indicating RP-HPLC method has been developed and validated for the routine analysis of P-HCl in bulk form and dosage forms. The results of stress testing carried out according to the ICH guidelines shows that the proposed method is selective and stability-indicating. The method is capable of separating target analyte from its degradation products. Monitoring degradation products at multiple wavelengths was found to be a good analytical tool for confirmation and comparison of degradation product elution profiles and peak intensities at different wavelengths.

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