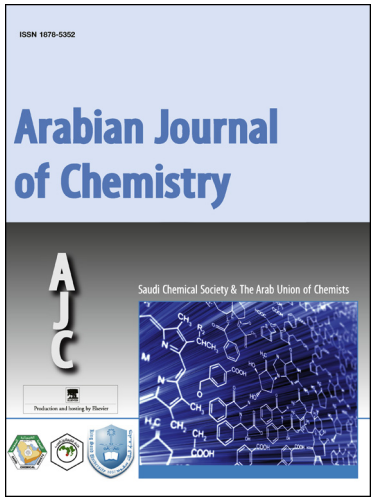

HPLC-DAD stability indicating determination of pentoxyverine citrate. Application to degradation kinetics and assay of syrup dosage form

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HPLC-DAD stability indicating determination of pentoxyverine citrate. Application to degradation kinetics and assay of syrup dosage form

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Abstract

The study describes the development, validation and application of a simple and reliable HPLC-DAD procedure for the stability-indicating determination of the cough suppressant drug pentoxyverine citrate (PTV). The method involved the use of Waters Symmetry C8 (3.9×150 mm, 5 μm) column with gradient elution of the mobile phase composed of 0.025 M phosphoric acid and acetonitrile. The gradient elution started with 10%

(by volume) acetonitrile, ramped up linearly to 60% in 10 min then it was kept constant till the end of the run. The mobile phase was pumped at a flow rate of 1 mL/min. The multiple wavelength detector was set at 210 nm, and quantification of the analyte was based on measuring its peak area. Retention time for PTV was about 7.03 min. Reliability and analytical performance of the proposed HPLC method was validated with respect to linearity, range, precision, accuracy, specificity, robustness, detection and quantification limits. Calibration curve of PTV was linear in the range 10–150 μg/mL with correlation coefficient >0.9998. The drug was subjected to forced-degradation conditions of hydrolysis, oxidation and dry heat. The proposed method proved to be specific and stability-indicating by resolution of the drug from its forced-degradation products. The validated HPLC method was applied to the analysis of PTV in syrup form where it was successfully resolved from the pharmaceutical additives and quantified with recoveries not less than 97.7 %. Moreover, the proposed method was utilized to investigate the kinetics of acidic and basic hydrolysis of PTV, and to derive its pH-rate of degradation profile in Britton–Robinson buffer within the pH range 2–12. Finally, the proposed method made use of DAD as a tool for peak identity and purity confirmation.

KEYWORDS

Pentoxyverine citrate; HPLC-DAD; Stability-indicating determination; Forced degradation; Kinetics; Syrup dosage form.

1. Introduction

Pentoxyverine citrate (PTV; also known as carbetapentane citrate) (Fig. 1), chemically known as 2-[2-(diethylamino)ethoxy]ethyl 1-phenylcyclopentanecarboxylate dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate (The British Pharmacopoeia, 2010), is a centrally acting cough suppressant used for non-productive cough (Sweetman et al., 2009). The monograph of PTV in the British Pharmacopoeia (BP) describes a potentiometric non-aqueous titration procedure for analysis of the bulk powder (The British Pharmacopoeia, 2010), however no official methods can be found for assay of the drug in dosage forms.

The quantification of PTV in pharmaceutical preparations and/or biological samples was addressed in various reports. Analytical methods in these reports involved the use of colorimetry (Lin et al., 1984; Weclawska et al., 1987), derivative spectrophotometry (Lin et al., 1992; Wen, 2000), flow-injection chemiluminescence (Zhang et al., 2009) and different sensors and electrodes based on ion-pair complexes (Hopkala et al., 1994; Yin, 2004). Also, the scientific literature showed the use of separation techniques such as capillary electrophoresis with electrochemiluminescence detection (Liu et al., 2005), HPLC-UV detection (Gad-Kariem et al., 1997; Jiang et al., 2008) and LC-MS for determination of PTV in human plasma (Yu et al., 2009; Wen et al., 2010). Recently, HPLC-DAD was applied for the determination of syrups containing PTV in combination with other drugs (Dönmez et al., 2011). Finally, characterization of PTV and its metabolites in urine was carried out using GC-MS (Westphal et al., 2012). To the best of our knowledge, no reports have been published yet dealing with the forced degradation of PTV or its stability indicating assay in a complex pharmaceutical dosage form such as the syrup form.

The aim of the present study is the development, validation, and application of a simple, reliable and specific HPLC-DAD method for the analysis of PTV in syrup dosage form. The method was thoroughly tested for its specificity and stability-indicating properties

by resolution of the drug from its forced hydrolytic, oxidative, and dry heat degradation products. The developed HPLC method was also applied to study the kinetics of acidic and basic hydrolysis of PTV. Pseudo-first order rate constants and half-lives were calculated in each case. In addition, Arrhenius plot was used to predict the shelf stability of PTV in acidic solutions at room temperature, and pH-rate profile of PTV degradation in Britton–Robinson buffer solutions was investigated.

2. Experimental

2.1. Instrumentation

The HPLC-DAD system consisted of Agilent 1200 series (Agilent Technologies, Santa Clara, CA, USA) (quaternary pump, vacuum degasser and diode array and multiple wavelength detector G1315 C/D and G1365 C/D) connected to a computer loaded with Agilent ChemStation Software. A Rheodyne manual injector with 20 µL loop was used. The column used was Waters Symmetry C8 (3.9 × 150 mm, 5 µm particle size).

2.2. Materials

Pentoxyverine citrate was kindly supplied by Borg Pharmaceutical Industries Co., Alexandria, Egypt. HPLC-grade acetonitrile (Scharlau Chemie S.A., Sentmenat, Spain), HPLC-grade methanol (Fisher Scientific UK Limited, Loughborough, Leicestershire, UK), analytical grade of ortho-phosphoric acid, hydrochloric acid, sodium hydroxide, 50% hydrogen peroxide, glacial acetic acid, boric acid and high purity distilled water were used. The pharmaceutical preparation assayed in the study is Cabella® syrup (Borg Pharmaceutical Industries Co., Alexandria, Egypt, BN 034015) labeled to contain 21.3 mg pentoxyverine citrate in each 10 mL syrup.

2.3. General procedure

A gradient mobile phase system consisting of (A) 0.025 M phosphoric acid and (B) acetonitrile was used. The separation was achieved with a linear gradient program as follows: 10% v/v B at zero time; from 0 to 10 min, ramp up to 60% v/v B; from 10 to 15 min, holding 60% v/v B. After 15 min, the gradient program was returned to the initial conditions and the analytical column was reconditioned for 3 min. The flow rate was 1.0 mL/min. The injection volume was 20 µL. The eluant was monitored by the diode array detector from 190 to 400 nm, and chromatograms were extracted at 210 nm. All determinations were performed at 25ºC.

PTV stock solution (1000 µg/mL) was prepared in HPLC-grade methanol. The working solutions were prepared by dilution of the stock solutions with distilled water to reach the concentration range 10–150 µg/mL PTV respectively. Triplicate injections were made for each concentration and chromatographed under the previously described LC conditions. The peak areas were plotted against the corresponding concentrations to construct the calibration graphs.

2.4. Assay of syrup dosage form

Accurate volumes of Cabella® syrup were diluted with distilled water to obtain final concentrations within the analytical range of PTV (10–150 µg/mL) then treated as under General Procedure. Recovery values were calculated from similarly treated standard solutions. For standard addition assay, sample solutions were spiked with aliquots of PTV standard solution to obtain total concentrations within the previously specified range then treated as under General procedure. Recovered concentrations were calculated by comparing the analyte response with the increment response attained after addition of the standard.

2.5. Forced degradation and stability-indicating study

Forced degradation studies were carried out on PTV standard as well as its commercial syrup dosage form according to the following conditions:

(a) Acidic and basic conditions: PTV solution was treated with 1 mL of 1 M HCl or 1 M NaOH. The acidic degradation solution was placed in a water bath at 100°C for 1 hr, while the solution in NaOH was kept at room temperature for 1 hr. After the specified time, all solutions were neutralized by adjusting the pH to 7.0 and then diluted with distilled water to reach a final concentration of 75 µg/mL of PTV. The same procedure was applied to an aliquot of the syrup dosage form.

(b) Oxidation with H_2O_2: PTV solution was treated with 0.5 mL of hydrogen peroxide 5%. The solution was placed in a water bath at 80°C for 1 hr. After the specified time, the solution was diluted with distilled water to reach a final concentration of 75 µg/mL PTV. An aliquot of the syrup dosage form was similarly treated.

(d) Dry heat degradation: An amount of PTV powder (50 mg) was kept in an oven at 90°C for 12 hrs. After the specified time, the powder was dissolved in methanol, and aliquots of this methanolic stock were diluted with distilled water to reach a final concentration of 75 µg/mL PTV.

After the previous treatments, solutions were filtered with a 0.45 µm filtration disk prior to injection to the column.

2.6. Kinetics study

2.6.1. Acidic hydrolysis

Accurate volumes of PTV stock solution giving final concentration of 75 µg/mL were transferred into a series of glass tubes and mixed with 1-mL volumes of 1 M HCl. The tubes were heated at different temperatures (70°, 80° and 100°C). At the specified time intervals, the contents of the tubes were neutralized to pH 7.0 using predetermined volumes of 1 M NaOH.

2.6.2. Basic hydrolysis

Basic hydrolysis of PTV was carried out at room temperature. Aliquots of PTV stock solution giving final concentration of 75 µg/mL were separately mixed with 1-mL volumes of

(0.1, 0.5 and 1.0 M) NaOH solutions. In each case, the solutions were left at room temperature for different time intervals. The contents of each flask were then separately neutralized with predetermined volumes of 1 M HCl solution.

2.6.3. pH-rate profile

Accurate volumes of PTV stock solution giving final concentration of 75 µg/mL were transferred into a series of glass tubes and mixed with 2-mL volumes of Britton–Robinson buffer solutions. The pH values used for measurement of the pH-rate profile of the degradation of PTV are within pH 2–12 in one unit pH intervals. The tubes were placed in a water bath at 100°C for different time intervals, and then the contents of each tube were neutralized using 1M NaOH or 1M HCl.

For all the above kinetic study solutions, the neutralized solutions were then quantitatively transferred to a series of 10 mL volumetric flasks and appropriately diluted to volume with water. The solutions thus prepared were analyzed as described under general procedure. The concentration of the remaining PTV was calculated at each temperature and time interval. Data were further processed and degradation kinetics constants were calculated.

3. Results and discussion

3.1. Optimization of chromatographic conditions

A gradient liquid chromatographic method coupled with diode array detection was developed to provide a suitable procedure for the routine quality control analysis of PTV in its complex syrup dosage form. The most important aspect in LC method development is the achievement of sufficient resolution of the target drug from all other compounds present in the sample with acceptable peak symmetry in a reasonable analysis time. To achieve this goal, several experiments were carried out in order to optimize both the stationary and mobile phases. For optimization of the stationary phase, several reversed phase columns (Zorbax SB-

C8 (4.6 × 250 mm), Zorbax SB-C18 (4.6 × 250 mm), Zorbax Eclipse XDB-C18 (4.6 × 150 mm), Waters Symmetry C18 (3.9 × 150 mm) and Waters Symmetry C8 (3.9 × 150 mm)) were tested. The best clear separation between the eluting peaks, sharper symmetric PTV peak and relatively shorter retention time were attained by using the Waters Symmetry C8 column; hence, it became the column of choice for this study. Other columns especially those of 250 mm length resulted in longer retention times for the eluting peaks and broader PTV peak.

The mobile phase composition was evaluated using various proportions of 0.025 M phosphoric acid solution and acetonitrile. Gradient elution was found necessary in order to ensure complete separation between PTV and all other substances in the analyzed samples (forced degradation products or inactive constituents of the syrup dosage form) in a reasonable run time and acceptable peak shape. The best chosen gradient program started with 10% (by volume) acetonitrile ramped up linearly to 60% in 10 min then kept at this percentage afterwards. Flow rate was kept constant at 1.0 mL/min allover the run, and temperature was adjusted at 25ºC. It is noteworthy to mention that the applied gradient program produced stable baseline without any drift or deformation.

Quantification was achieved using diode array detection based on peak area measurement. PTV is considered a weak UV absorbing compound. It exhibits considerable absorbance only in the short UV region (below 230 nm); consequently, 210 nm was found suitable to record all chromatograms in this study. The above described chromatographic conditions showed symmetric PTV peaks with good system suitability parameters. Fig. 2A shows a typical chromatogram for standard PTV. The drug elutes at retention time 7.03 ± 0.056 min with retention factor (k′) = 3.18. The column performance (apparent efficiency) can be expressed by the number of theoretical plates (N) for the analyte peak which equals about 63780.

3.2. Validation of the proposed method

3.2.1. Linearity and concentration range

The linearity of the proposed HPLC procedure was evaluated by analyzing a series of different concentrations of PTV (n = 7) (Fig. 3). The linear regression equation was generated by least squares treatment of the calibration data. Under the optimized conditions described above, the measured peak areas were found to be proportional to concentrations of the analyte. Table 1 presents the performance data and statistical parameters including linear regression equation, concentration range, correlation coefficient, standard deviations of the intercept (S_a), slope (S_b) and standard deviations of residuals ($S_{y/x}$). Regression analysis shows good linearity as indicated from the correlation coefficient value (0.9998). In addition, deviation around the slope can be further evaluated by calculation of the RSD% of the slope (S_b%) which was found to be less than 1.0 %. The analysis of variance test for the regression line reveals that, for equal degrees of freedom, an increase in the variance ratio (F values) means an increase in the mean of squares due to regression and a decrease in the mean of squares due to residuals. The greater the mean of squares due to regression, the steeper is the regression line. The smaller the mean of squares due to residuals, the less is the scatter of experimental points around the regression line. Consequently, regression lines with high F values (low significance F) are much better than those with lower ones (Table 1).

3.2.2. Detection and quantification limits

According to the pharmacopoeial recommendations (The United States Pharmacopeia, 2011), limit of detection (LOD) and quantification (LOQ) were determined, at signal to noise ratio of 3:1 and 10:1, respectively (Table 1). The LOQ value (7.6 µg/mL) was verified by its nearness to the lower concentration of the working range.

3.2.3. Precision and accuracy

The within-day (intra-day) precision and accuracy for the proposed method were studied at three concentration levels of PTV using three replicate determinations for each concentration within one day. Similarly, the between-day (inter-day) precision and accuracy were tested by analyzing the same three concentrations using three replicate determinations repeated on three days. Recoveries were calculated using the corresponding regression equations and they were satisfactory. The percentage relative standard deviation (RSD %) and percentage relative error (E_r %) were less than 1.5 % proving the high repeatability, intermediate precision and accuracy of the developed method for the estimation of PTV in bulk form (Table 2).

3.2.4. Specificity

Specificity is defined as the ability to access unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components (The United States Pharmacopeia, 2011), and this will be demonstrated in details in the following sections of this study.

3.2.5. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters (The United States Pharmacopeia, 2011). Robustness was examined by evaluating the influence of small variations in different conditions such as source of acetonitrile (Scharlau Chemie S.A., Spain or SDS, France or Labscan, Poland), working wavelength (± 2 nm), flow rate (± 0.05 mL/min) and column temperature (± 2ºC). These variations did not have any significant effect on the measured responses or the chromatographic resolution. RSD% for the measured peak areas using these variations did not exceed 3%.

3.2.6. Stability of solutions

The stability of standard working solutions as well as sample solutions in the diluting solvent (distilled water) was examined and no chromatographic changes were observed within 24 hours at room temperature. Also, the stock solutions prepared in HPLC-grade methanol were stable for at least one week when stored refrigerated at 4ºC. Retention time and peak area of PTV remained unchanged and no degradation could be observed during these periods.

3.3. Analysis of syrup dosage form

The optimized HPLC-DAD procedure was applied for the assay of PTV in the pharmaceutical formulation available in the local market (Cabella® syrup). Accurate volumes of the syrup were diluted with distilled water to reach the concentration range of PTV (10–150 µg/mL) then solutions were injected to the column. A representative chromatogram obtained from the syrup sample solution is shown in Fig. 4A. PTV eluted at its specific retention time, and the inactive ingredients of the formulation appeared as well-resolved peaks at retention times 5.37 min (saccharin sodium), 7.43 min (methyl paraben) and 10.10 min (propyl paraben). Resolution (R_s) is a measure of the degree of separation between adjacent peaks. A resolution value of 1.5 is usually regarded as sufficient for the baseline separation of closely eluted peaks (Kazakevich et al., 2007). Resolution (R_s) was calculated between PTV and its nearest peak at 7.43 min, and it was found 4.41. Additionally, resolution was found 9.84 between PTV and the preceding peak at 5.37 min. The diode-array detector enables peak purity verification of the analyte where no signs of co-elution from any of the inactive components were detected. Recoveries were calculated using both external standard and standard addition methods. The assay results revealed satisfactory accuracy and precision as indicated from % recovery, SD and RSD% values (Table 3). The resolution and quantification of PTV in its complex syrup dosage form without any interference from the

inactive ingredients can be considered a clear evidence for the specificity of the proposed method.

A reference HPLC method was adopted for the determination of PTV in its commercial product (Dönmez et al., 2011). Recovery data obtained from the developed HPLC method were statistically compared with those of the reference method using the Student's t- and the variance ratio F-tests. In both tests, the calculated values did not exceed the theoretical ones at the 95% confidence level which indicated that there were no significant differences between the recoveries obtained from both methods (Table 3). It is evident from these results that the proposed method is applicable to the analysis of PTV in its syrup commercial formulation with minimum sample preparation and satisfactory level of accuracy and precision.

3.4. Forced-degradation and stability-indicating aspects

Forced degradation studies help in identification of the likely degradation products of a drug substance, which can in turn help in establishing the degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating power of the proposed analytical procedure (Klick et al., 2005). Also, forced degradation studies are useful when the degraded products or impurities are unknown or not available (Klick et al., 2005). Forced degradation experiments were carried out on PTV standard solutions in order to produce the possible relevant degradation products and test their chromatographic behavior using the developed method. Hydrolytic, using strong acidic (1 M HCl) and strong basic (1 M NaOH) media, oxidative (5% H_2O_2) and dry heat degradation experiments were conducted, and the resulting chromatograms were compared with that obtained from standard untreated solution of the drug (Fig. 2A).

No signs of degradation of PTV could be observed in 1 M HCl medium at room temperature for 24 hrs; however, about 37% decrease in peak area as well as the appearance

of a degradation product peak at 11.03 min were clearly noticed after heating with 1 M HCl at 100°C for 1 hr (Fig. 2B). On the other hand, PTV was susceptible to degradation by strong alkali at room temperature. Treatment of PTV with 1 M NaOH and keeping the solution at room temperature for 1 hr resulted in about 26% degradation and the appearance of the same previously mentioned degradation peak at 11.03 min (Fig. 2C). Being as ester, PTV is expected to hydrolyze in both acidic and basic media with the formation of the hydrolysis product (1-phenylcyclopentanecarboxylic acid) which appears as a common degradation peak at 11.03 min. Another extra peak at 14.54 min appears only in the chromatograms of basic degradation. This peak shows an absorption spectrum which is very similar to that of the intact drug. This suggests that the drug chromophore was not altered, and this impurity is probably produced from a reaction in the side chain. Further investigation revealed that this extra peak appears only when the solution contains methanol (the solvent used for preparation of PTV stock), while aqueous PTV solutions does not produce this extra peak. This can be explained by the *in-situ* formation of the methyl ester of the hydrolysis product (1-phenylcyclopentanecarboxylic acid). This esterification reaction can occur as an artifact reaction when acid/base hydrolysis reactions are performed using an alcohol co-solvent system such as methanol (Baertschi et al., 2005). This side reaction does not occur in acid degradation solutions, simply because acid hydrolysis is initiated only at high temperature (100°C) where methanol evaporates and does not remain in the solution.

PTV was found stable to oxidative degradation at room temperature after 24 hrs, however, about 7% decrease in peak area as well as the appearance of a degradation product peak at 7.35 min were obviously detected after heating with 5% H_2O_2 at 80°C for 1 hr (Fig. 2D). Resolution between the peaks of PTV and the oxidative degradation product at 7.35 min was found 2.95. Hydrogen peroxide reacts with tertiary amines to form tertiary amine oxides (Baertschi et al., 2005). The degradation peak at 7.35 min shows an absorption spectrum

which is identical to that of the intact drug. This implies that the drug chromophore is not involved in the reaction with H_2O_2, and the new peak is most likely for a side chain oxidation product (e.g. N-oxide of the tertiary nitrogen of PTV). Dry heat forced degradation of PTV revealed slight degradation of the drug after heating at 90°C for 12 hrs. After the specified time, solutions were prepared from the powder, and main peak appeared at its specific retention time with area about 90% of that of standard of the same concentration (i.e. about 10% degradation), additionally, two small extra peaks were observed at 2.83 and 14.07 min (Fig. 2E).

Similar forced degradation experiments were carried out on aliquots of the syrup dosage form. As mentioned earlier, a hydrolysis product peak at 11.03 min could be seen in the chromatograms of the acid and base degradations (Fig. 4B and 4C). Also, obvious changes were noticed for the inactive ingredients peaks especially in acidic medium. These changes included decrease in the peak areas and the emergence of several new degradation peaks at retention times: 2.98, 3.05, 3.65, 4.07 and 4.57 min (Fig. 4B and 4C). Oxidative degradation of the syrup produced similar observations to those obtained with the standard PTV (lower peak area for the drug and the evolution a well resolved degradation product peak at retention time 7.30 min), besides, no signs of change could be seen for the inactive ingredients peaks at 5.44, 7.43 and 10.11 min. Fig. 4D shows the chromatogram of the dosage form solution after treatment with 5 % H_2O_2 / 80°C for 1 hr.

It is noteworthy to mention that peak purity test results obtained from the diode-array detector (DAD) confirm that PTV peak was homogenous and pure in all the analyzed samples subjected to forced degradation conditions.

3.5. Degradation kinetics

The kinetics of acidic hydrolysis of PTV was investigated in 1M HCl at different temperatures (70^o, 80^o and 100^oC) while basic hydrolysis was studied in NaOH solutions of

different strengths (0.1, 0.5 and 1.0 M) at room temperature. Since the decomposition rate of PTV at lower strengths of HCl and/or at room temperature was too slow to obtain reliable kinetic data, solutions of 1 M HCl at elevated temperatures were used to investigate the acidic hydrolysis kinetics. However, significant basic hydrolysis of PTV could be observed at a faster rate compared with that of acidic hydrolysis. Thus milder conditions of NaOH strength at room temperature were applied for basic hydrolysis kinetic study.

3.5.1. Acidic hydrolysis

A regular decrease in the concentration of PTV with increasing time intervals was observed especially at 100°C. At the selected temperatures (70, 80 and 100°C), the degradation process followed pseudo-first order kinetics (Fig. 5). From the slopes of the straight lines, it was possible to calculate the corresponding degradation rate constants and half lives ($t_{1/2}$) at each temperature, based on the equation (Maher et al., 2012):

$$\text{Log } C = \log C_0 - Kt/2.303 \qquad (1)$$

where the slope of the line = $-K/2.303$, C and C_0 are the drug concentrations measured at a given time t and at zero time, respectively and K is the rate constant. These values are gathered in Table 4. Additionally, log K values were then plotted against the reciprocals of the absolute temperature (1/T) to construct the Arrhenius plot (Maher et al., 2012; Florence et al., 2006). Arrhenius equation was found to be:

$$\log K = 13.80 - (5286.84/T) \qquad (2)$$

Arrhenius plot is presented in Fig. 6. The K_{25} is used as a measure of the stability of the drug at room temperature. The log K_{25} value can be obtained by extrapolation on the Arrhenius plot as illustrated in Fig. 6, however, for more accurate result, it was calculated from equation (2) where the log K_{25} = –3.9411, and therefore K_{25} had the value of 1.145×10^{-4} hr^{-1}. Accordingly, it was found that half life-time ($t_{1/2}$) of PTV in 1 M HCl at room temperature is

about 252 days (Table 4). It is clear from these results that PTV can be considered stable at room temperature even in strong acidic medium.

3.5.2. Basic hydrolysis

For each NaOH strength (0.1, 0.5 and 1.0 M), log concentration of intact PTV remaining was plotted against time (Fig. 7) and it was found that the degradation followed pseudo first order kinetics. In each case, pseudo first order rate constants were calculated from the slopes of the corresponding plot, equation (1). The corresponding ($t_{1/2}$) values were also calculated and presented in Table 4.

3.5.3. pH-rate profile study

The effect of different pH values on the degradation of PTV was studied using Britton-Robinson buffer of pH values ranging from 2–12 at 100°C for different time intervals. The apparent first order degradation rate constants were calculated at each pH and plotted versus the pH values resulting in pH-rate profile curve (Fig. 8). Obviously, PTV hydrolyses in alkaline pH values faster than in acidic pH solutions. The lowest degradation rate constant is within the pH range 4–6 at which the drug solution is most stable.

4. Conclusion

This study described a simple, selective and reliable HPLC-DAD procedure for the assay of pentoxyverine citrate in its complex pharmaceutical dosage form (syrup). Simplicity was illustrated by the minimum requirement of organic solvents since water was used as a solvent for preparation of working and sample solutions. This suggests that the proposed method is cost-effective and environment-friendly. To our present knowledge, no attempts have been made yet to develop a comprehensive stability indicating assay procedure for pentoxyverine in bulk form and in pharmaceutical formulations by any analytical methodology. A significant advantage in the study is the separation of the analyte from

dosage form excipients as well as from several degradation peaks obtained by the hydrolytic, oxidative and dry heat forced-degradation experiments. The chemical structures of the hydrolytic and oxidative degradation peaks were proposed based on their absorption spectra obtained from DAD. Reliability was guaranteed by testing the various validation parameters of the method and the successful application to syrup dosage form. The described method is superior to the previously reported HPLC-DAD method for the assay of pentoxyverine in syrup (Dönmez et al., 2011). The proposed method made use of DAD as a tool for peak identity and purity confirmation; however, it can be adapted to conventional HPLC-UV detection which is the most popular in quality control laboratories. Finally, the proposed method was successfully applied to study the kinetics of acidic and basic degradation processes of pentoxyverine and to derive the pH-rate profile curve for the drug.

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Table 1. Analytical parameters for the determination of PTV using the proposed HPLC-DAD method.

Parameter	PTV
Wavelength (nm)	210
Linearity range (μg/mL)	10 – 150
Intercept (a)	-2.30
S_a [a]	12.42
Slope (b)	20.72
S_b [b]	0.16
RSD% of the slope	0.77
Correlation coefficient (r)	0.99986
$S_{y/x}$ [c]	18.66
F [d]	17721
Significance F	4.54×10^{-10}
LOD [e] (μg/mL)	2.28
LOQ [f] (μg/mL)	7.60

[a] Standard deviation of the intercept
[b] Standard deviation of the slope

[c] Standard deviation of residuals
[d] F equals the mean of squares due to regression divided by the mean of squares about regression (due to residuals)
[e] Limit of detection
[f] Limit of quantification

Table 2. Precision and accuracy for the determination of PTV using the proposed HPLC-DAD method.

	Nominal value (µg/mL)	Found ± SD[a] (µg/mL)	RSD(%)[b]	E_r(%)[c]
Within-day	10	9.91 ± 0.08	0.81	-0.90
	40	40.16 ± 0.28	0.70	0.40
	100	100.44 ± 0.83	0.83	0.44
Between-day	10	9.93 ± 0.12	1.21	-0.70
	40	40.38 ± 0.41	1.02	0.95
	100	101.22 ± 1.28	1.27	1.22

[a] Mean ± standard deviation for three determinations.
[b] % Relative standard deviation.
[c] % Relative error.

Table 3. Analysis of PTV in its pharmaceutical preparation (Cabella® syrup) by the proposed HPLC-DAD method and the reference method.

	External standard	Reference method [c]	Standard addition
%Recovery ± SD[a]	99.16 ± 0.87	98.72 ± 0.96	99.49 ± 1.06
RSD%[b]	0.88	0.97	1.07
t	0.75		
F	1.23		

[a] Mean ± standard deviation for five determinations.
[b] % Relative standard deviation.
[c] Dönmez et al., 2011.
Theoretical values for t and F at P = 0.05 are 2.31 and 6.39, respectively.

Table 4. Pseudo first-order rate constants (K) and half lives ($t_{½}$) for the acidic and basic hydrolysis of PTV.

Acidic hydrolysis using 1 M HCl		
Temperature (^{o}C)	**K (hr^{-1})**	**$t_{½}$ (hr)**
100	0.4314	1.61
80	0.0624	11.11
70	0.0253	27.39
25	1.145×10^{-4}	6052.40 (252 days)
Basic hydrolysis at room temp		
NaOH strength (M)	**K (hr^{-1})**	**$t_{½}$ (hr)**
1.0	0.2724	2.54
0.5	0.1272	5.45
0.1	0.0330	21.00

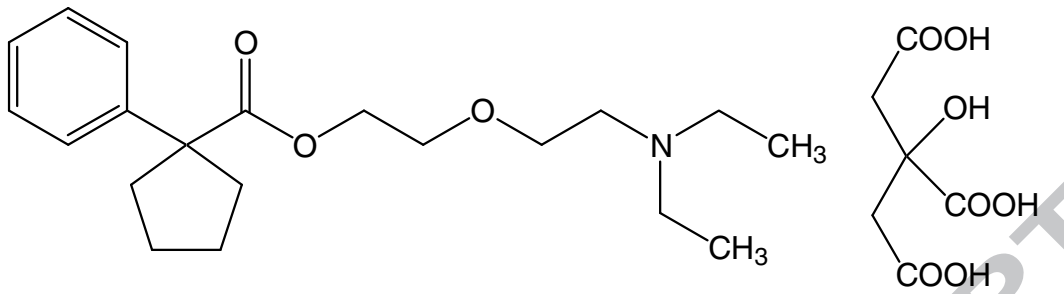
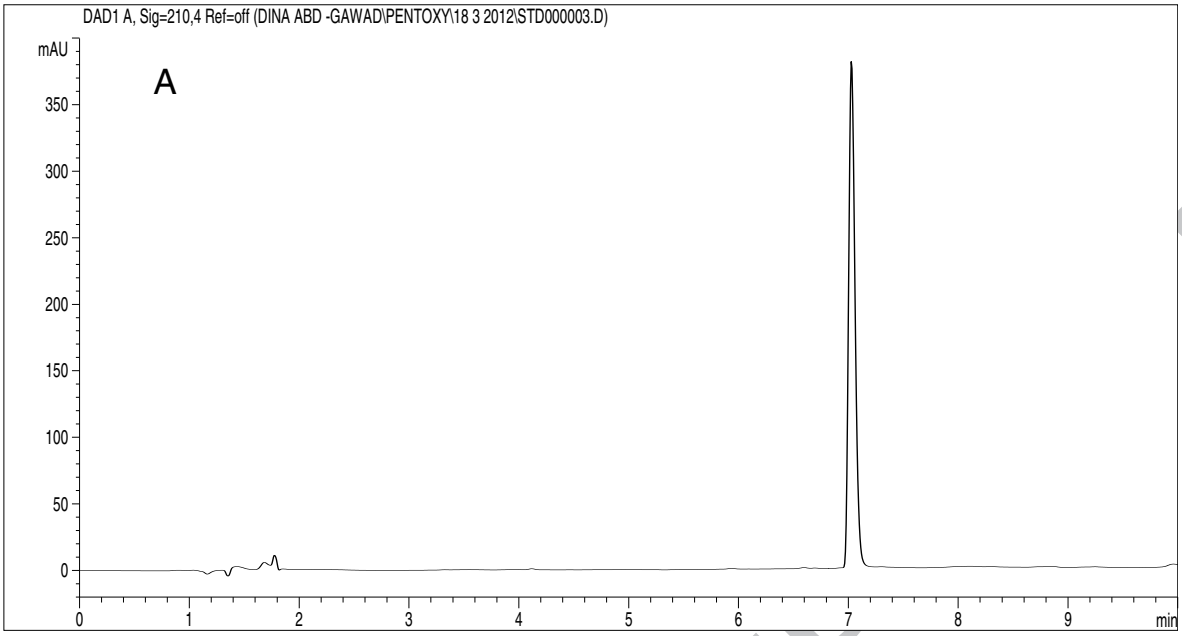
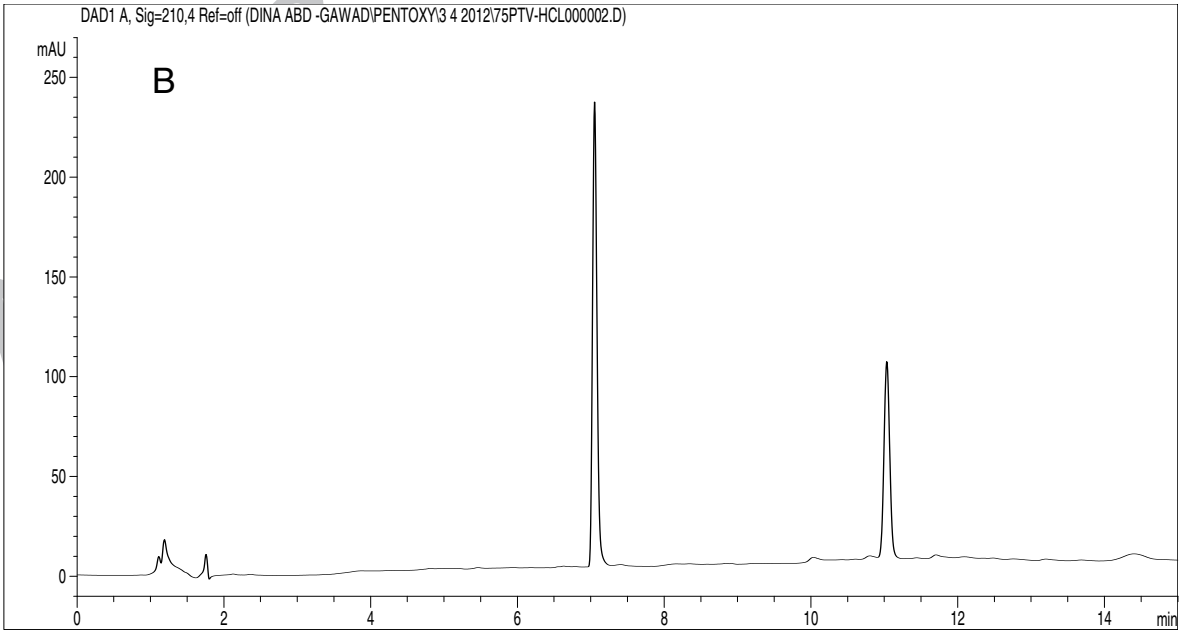
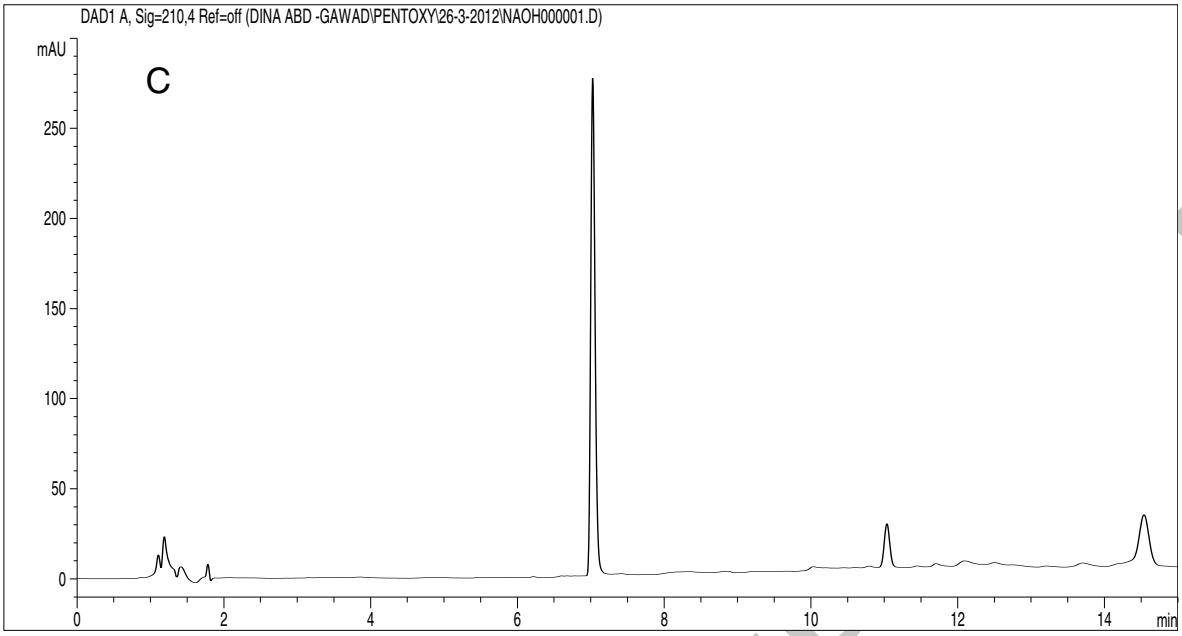
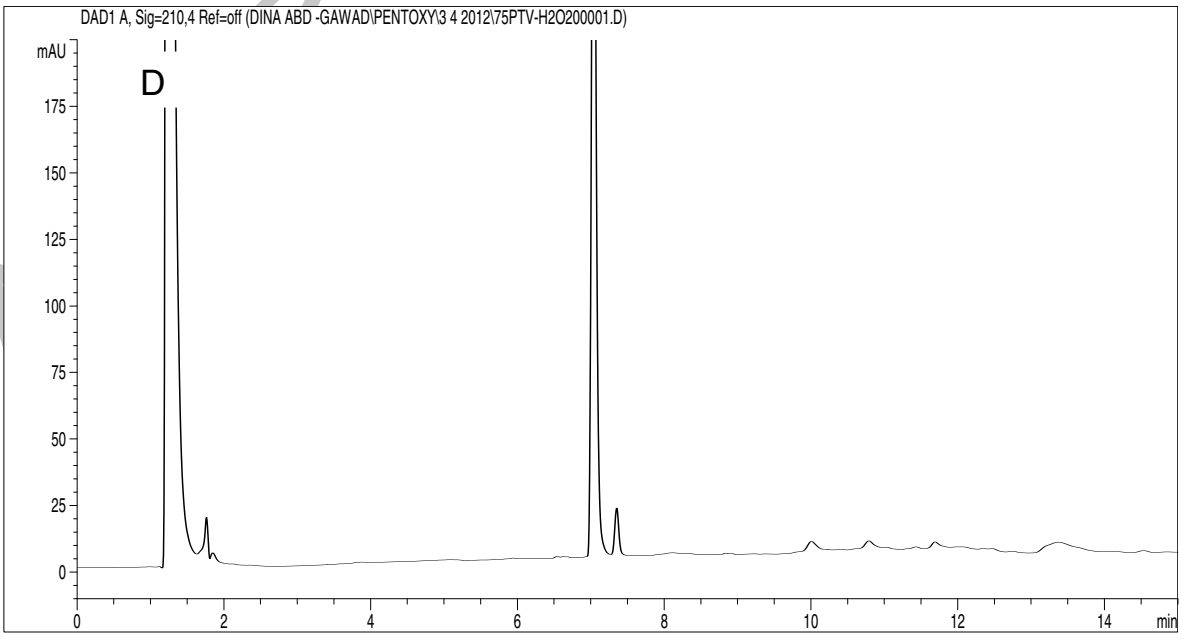


Figure 1. Chemical structure of pentoxyverine citrate (PTV).









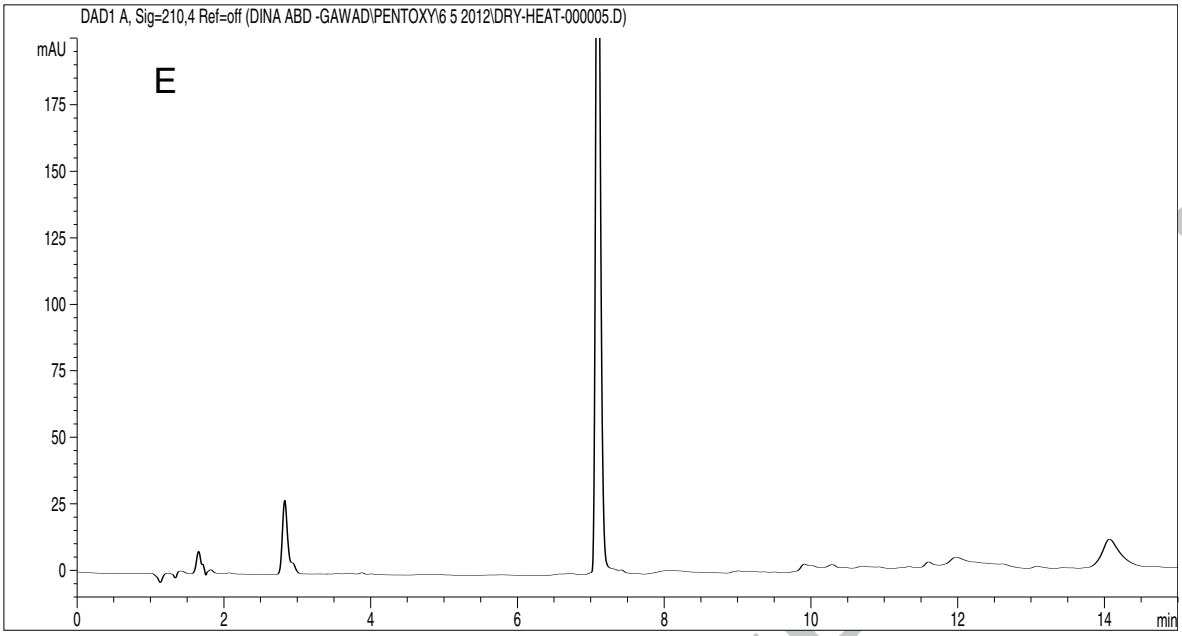


Figure 2. HPLC chromatogram of 20 µL injection of 75 µg/mL standard PTV solution (A). Chromatograms of solutions of 75 µg/mL PTV after exposure to acid degradation (1 M HCl / 100°C for 1 hr) (B), base degradation (1 M NaOH / room temp for 1 hr) (C), oxidative degradation (5 % H_2O_2 / 80°C for 1 hr) (D) and dry heat degradation (90°C for 12 hr) (E).

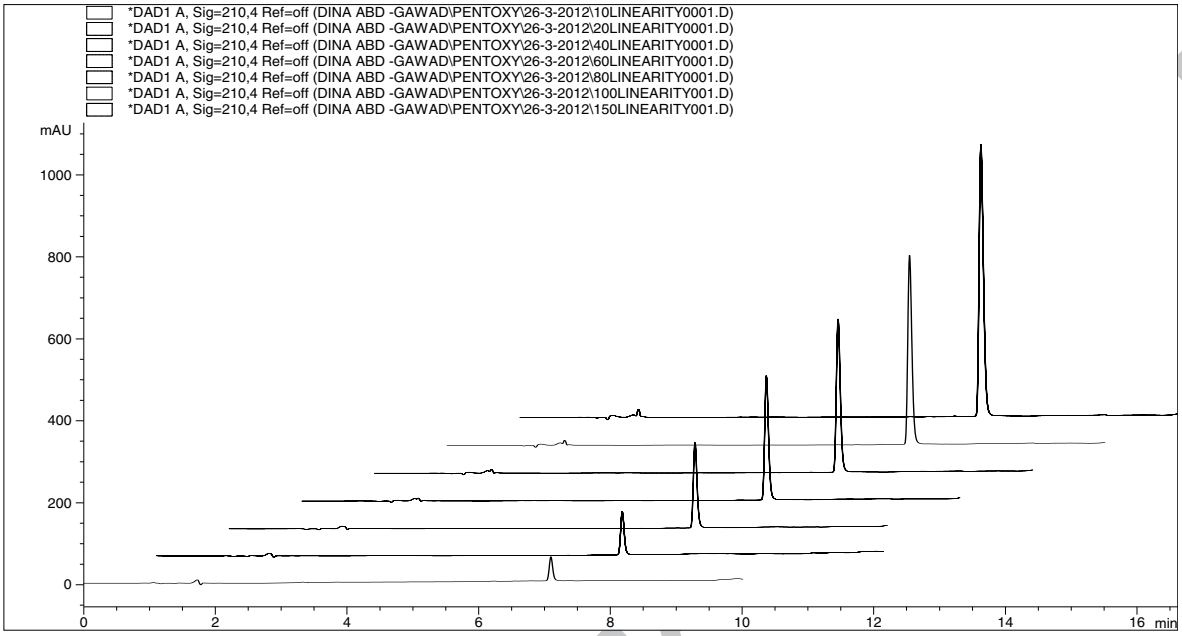
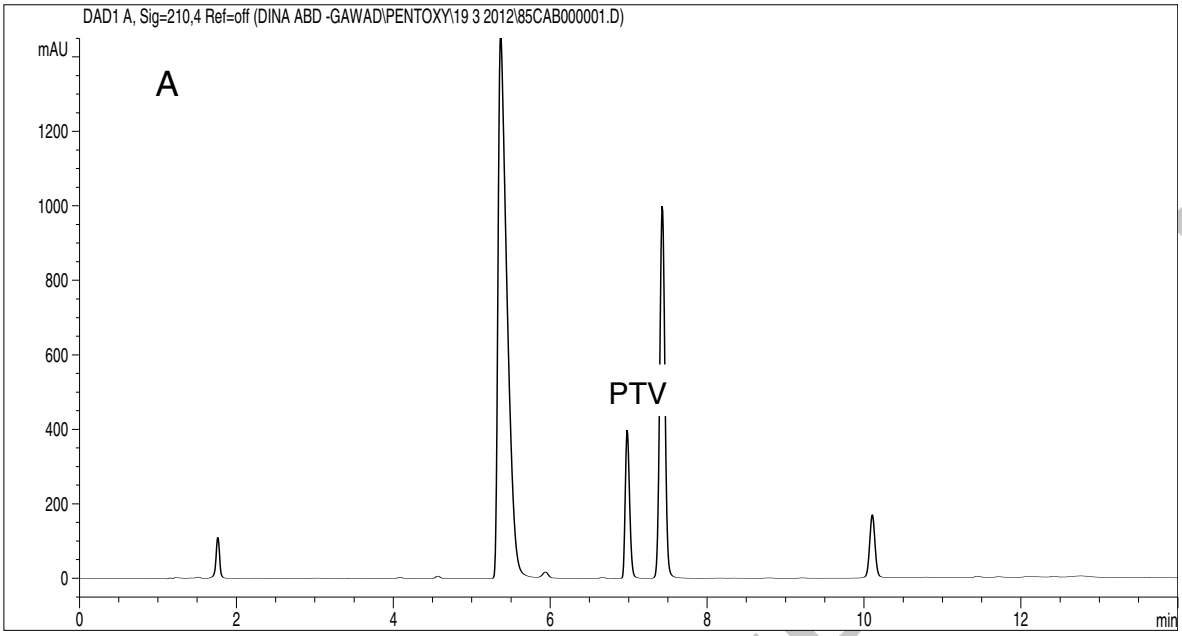
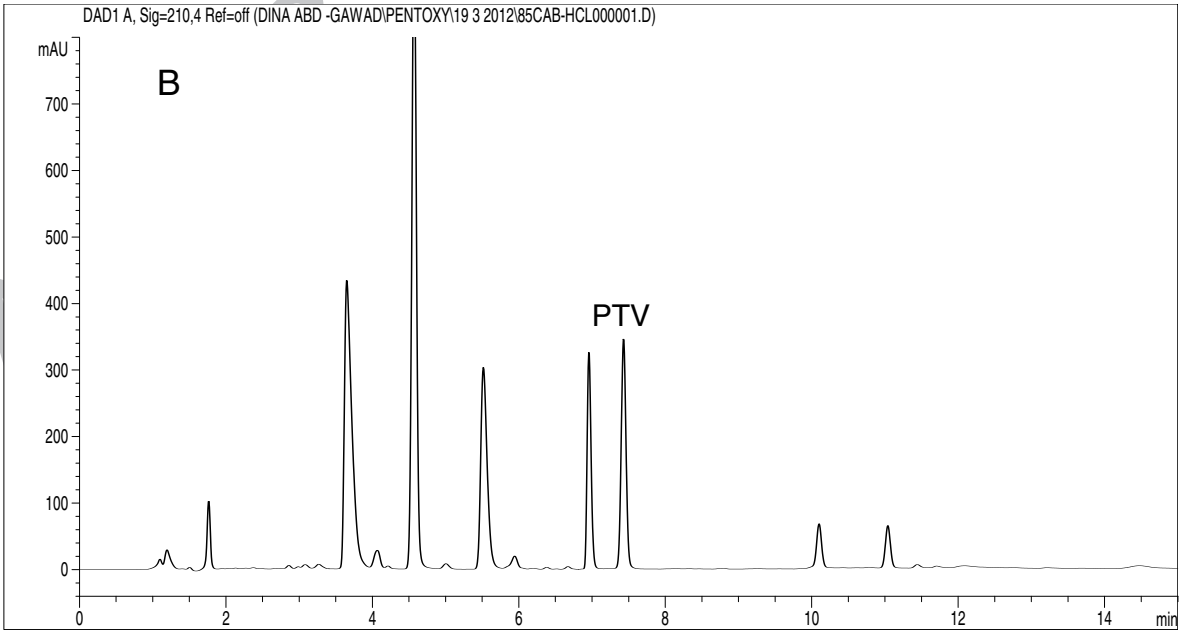


Figure 3. 3-dimentional plot of HPLC chromatograms of serial concentrations of standard PTV: 10, 20, 40, 60, 80, 100 and 150 µg/mL.





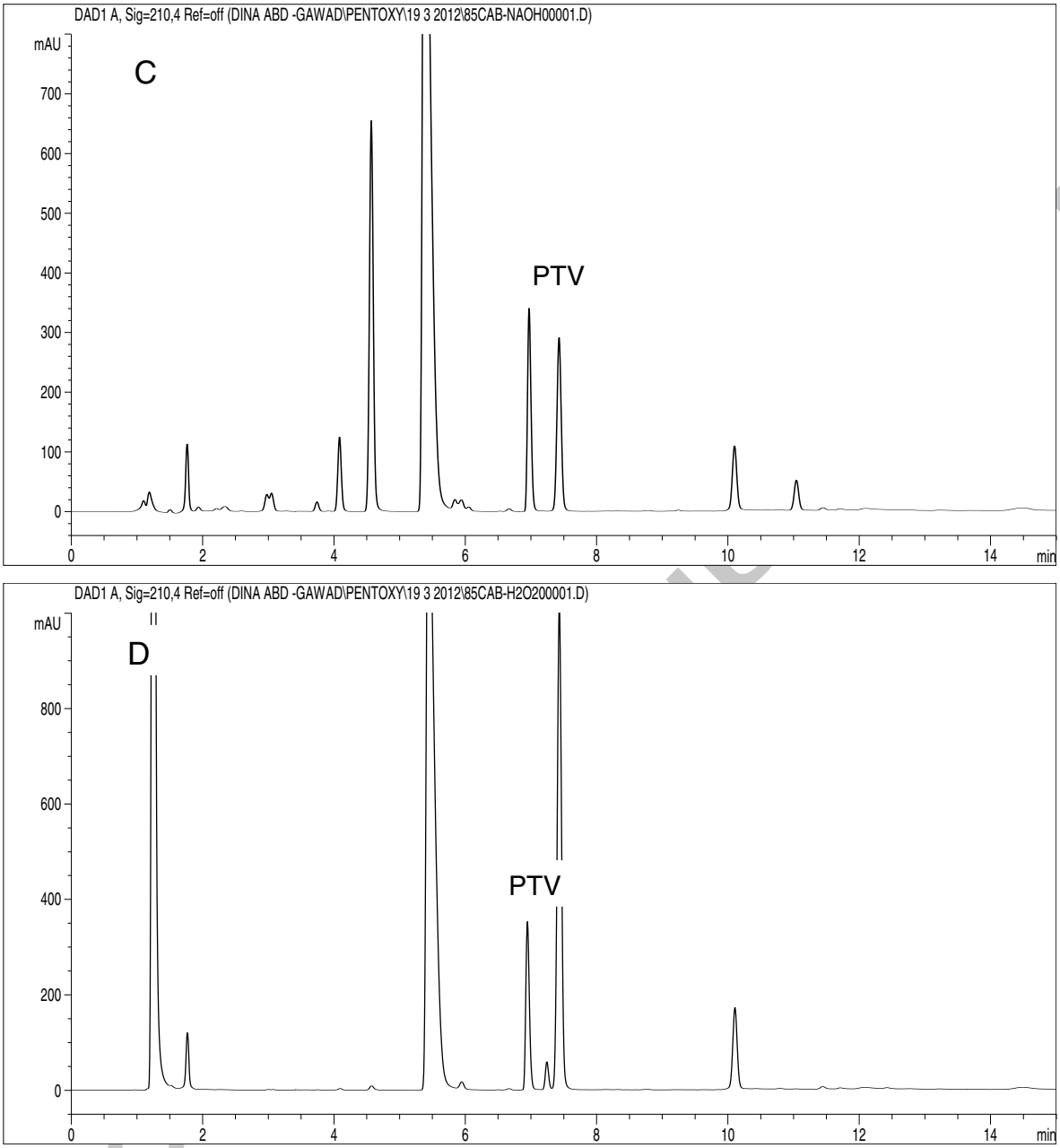


Figure 4. HPLC chromatogram of 20 µL injection of a solution containing 85.2 µg/mL PTV obtained from Cabella® syrup (A). Chromatograms of solutions of 85.2 µg/mL PTV obtained from Cabella® syrup after exposure to acid degradation (1 M HCl / 100°C for 1 hr) (B), base degradation (1 M NaOH / room temp for 1 hr) (C) and oxidative degradation (5 % H_2O_2 / 80°C for 1 hr) (D).

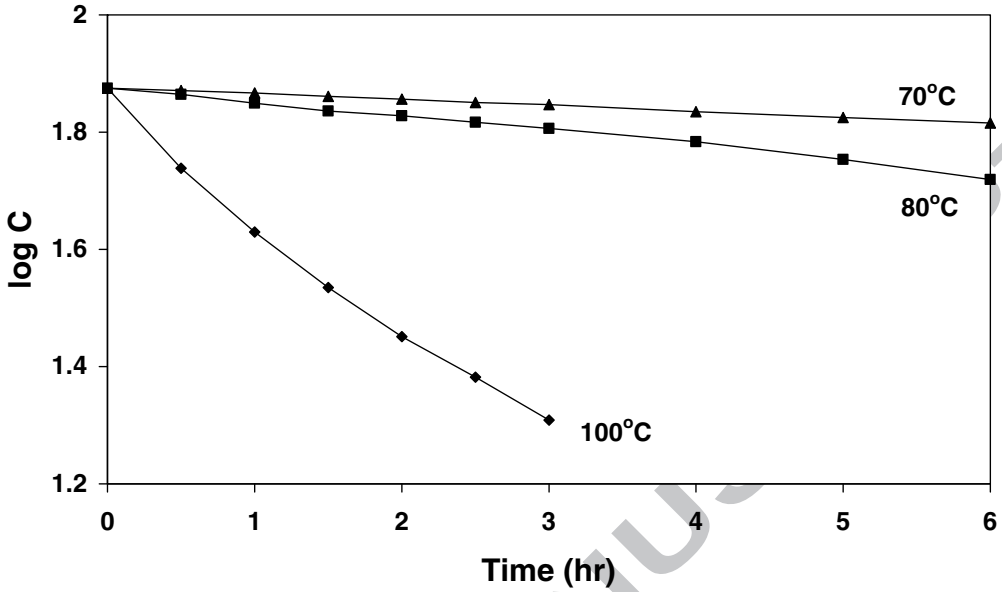


Figure 5. Kinetic study of the acid-induced degradation of PTV at different temperatures.

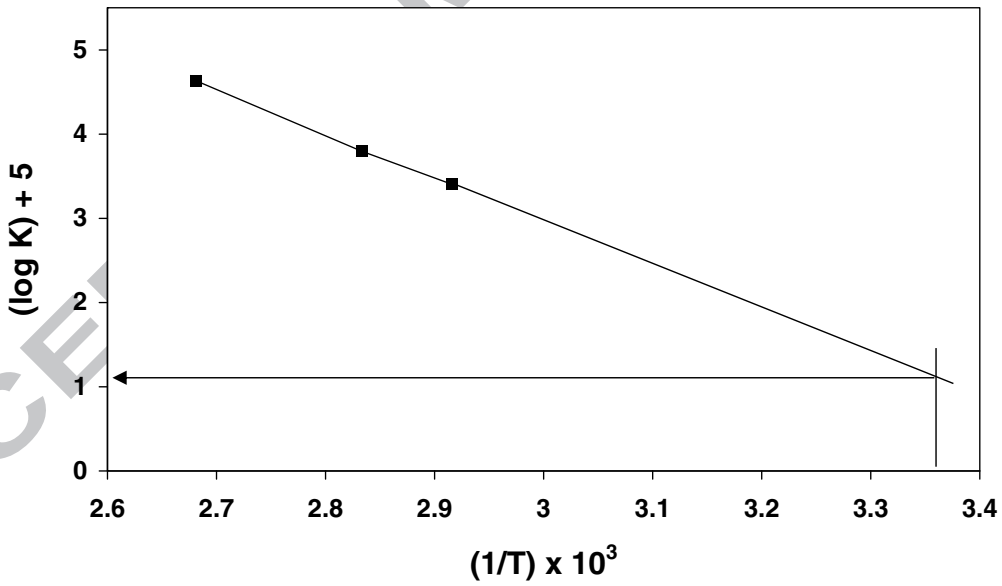


Figure 6. Arrhenius plot for the acid-induced degradation of PTV.

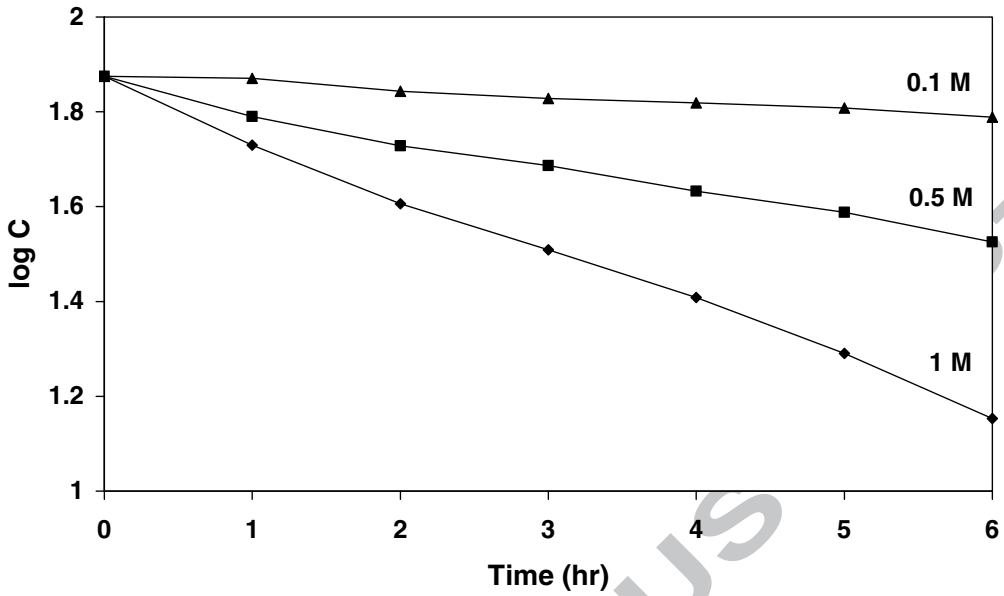


Figure 7. Kinetic study of the base-catalyzed hydrolysis of PTV at different sodium hydroxide strengths.

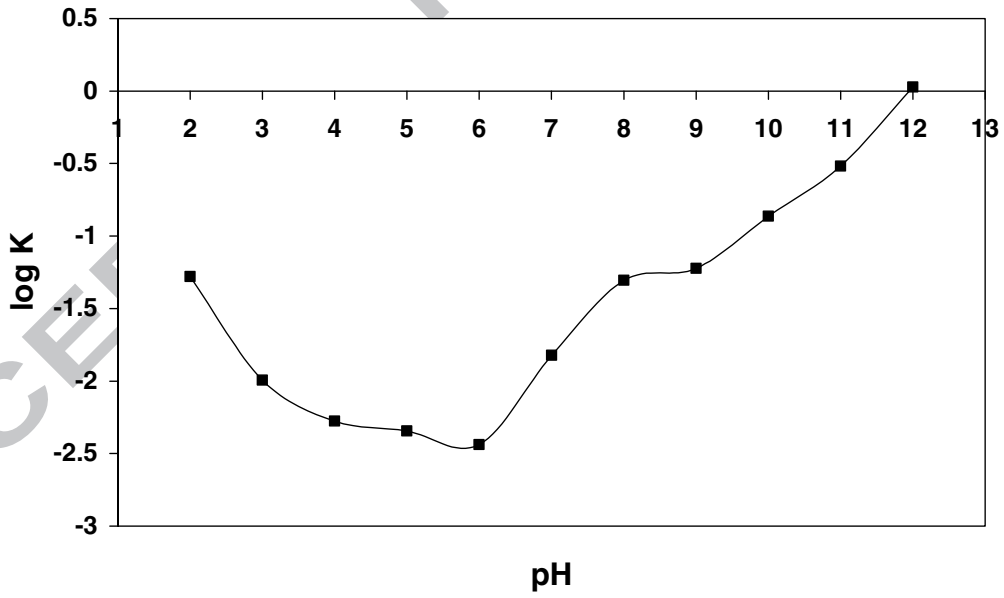


Figure 8. pH-Rate profile curve of PTV using Britton Robinson buffer at 100°C.