Spectrochimica Acta Part A 77 (2010) 324–329

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Spectrophotometric method for the determination, validation, spectroscopic and thermal analysis of diphenhydramine in pharmaceutical preparation

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ARTICLE INFO

Article history:
Received 4 February 2010
Received in revised form 21 May 2010
Accepted 24 May 2010

Keywords:
Diphenhydramine
Charge-transfer complex
2,3-Dichloro-5,6-dicyano-*p*-benzoquinone
Determination
Validation
Spectroscopic techniques
Thermal analysis

ABSTRACT

A sensitive, simple and rapid spectrophotometric method was developed for the determination of diphenhydramine in pharmaceutical preparation. The method was based on the charge-transfer complex of the drug, as *n*-electron donor, with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), as π-acceptor. The formation of this complex was also confirmed by UV–vis, FTIR and ^{1}H NMR spectra techniques and thermal analysis. The proposed method was validated according to the ICH guidelines with respect to linearity, limit of detection, limit of quantification, accuracy, precision, recovery and robustness. The linearity range for concentrations of diphenhydramine was found to be 12.5–150 μg/mL with acceptable correlation coefficients. The detection and quantification limits were found to be 2.09 and 6.27 μg/mL, respectively. The proposed and references methods were applied to the determination of drug in syrup. This preparation were also analyzed with an reference method and statistical comparison by *t*- and *F*-tests revealed that there was no significant difference between the results of the two methods with respect to mean values and standard deviations at the 95% confidence level.

1. Introduction

Diphenhydramine hydrochloride (DPH) 2-(diphenylmethoxy)-*N*,*N*-dimethylethanamine is a chemical mainly used as antihistaminic, antiemetic, sedative, and hypnotic [1] (Fig. 1).

DPH works by blocking the effect of histamine at H_1 receptor sites. This results in effects such as the increase of vascular smooth muscle contraction, thus reducing the redness, hyperthermia and edema that occur during an inflammatory reaction. In addition, by blocking the H_1 receptor on peripheral nociceptors, diphenhydramine decreases their sensitization and consequently reduces itching that is associated with an allergic reaction. This is why diphenhydramine is a popular choice for treatment of the symptoms of allergic rhinitis, hives, motion sickness, and insect bites and stings [2].

Several published methods have been developed for the determination of DPH in pharmaceutical preparations and in biological fluids including: spectrophotometry [3–6], flow injection analysis [7], gas chromatography [8], atomic absorption spectrometry [9], FT-Raman spectroscopy and high performance liquid chromatography [10] and capillary electrophoresis [11–13]. Liquid chromatography/tandem mass spectrometry has been reported for the determination of DPH in dog plasma [14].

The proposed method was based on the charge-transfer reaction between *n*-electron donor and π-acceptor (DDQ) to give a red colored $DDQ^{\bullet -}$ radical anion. DDQ is a strong electron acceptor and has been used for the determination of many electron donors such as chloroquine and pyrimethamine [15], flucloxacillin and dicloxacillin [16], lansoprazole and pantoprazole [17], meloxicam [18], fluoxetine and sertraline [19], ciprofloxacin, enrofloxacin and pefloxacin [20].

In the literature no method has been reported for the determination of DPH by using DDQ. In the present investigation, we report that the development of accurate, reproducible, less time consuming and adequately sensitive spectrophotometric method. The solid complex has been synthesized and structure of the complex has been confirmed by spectral techniques such as UV–vis, FTIR, ^{1}H NMR, and also by thermal analysis.

2. Experimental

2.1. Materials

Pure powder of DPH was obtained from Sigma (St. Louis, MO, USA). Fenotral syrup was obtained from commercial sources in the local pharmacy. The DDQ was purchased from (Fluka, Switzerland). All solvents used were of analytical reagent grade (Merck, Darmstadt, Germany).

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1386-1425/$ – see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.saa.2010.05.031

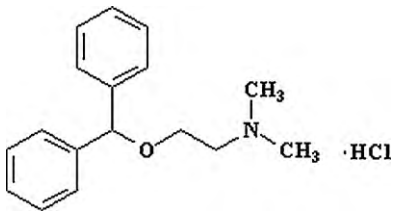


Fig. 1. Molecular structure of diphenhydramine hydrochloride.

2.2. Apparatus

An UV-160A UV–visible spectrophotometer (Shimadzu, Japan) was used for the absorbance measurements.

TGA–DSC (thermogravimetric analysis–differential scanning calorimetry) curves were obtained with a TA SDT Q 600 thermal analyzer apparatus using flowing nitrogen 100 mL min^{-1}, temperature range 25–750 and 25–1200 °C, at heating rate of 20 °C min^{-1}.

The FTIR spectra were recorded with ATR technique on a PerkinElmer Spectrum One Bv 5.0 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian UNITY INOVA 500 MHz spectrometer. TMS was used as internal standard in ^{1}H NMR.

2.3. Solutions

10 mg mL^{-1} of DPH stock solution was prepared in water. DDQ solution was prepared 2.0 mg mL^{-1} in acetonitrile. The DPH stock and DDQ solutions were stored at 4 °C.

2.3.1. Preparation of diphenhydramine base solution

A 1 mL standard stock solution of DPH was transferred into the 12 mL each glass tube. Then, 1 mL of concentrated ammonia solution was added and shaken for 1 min. The alkaline aqueous layer was extracted with three portions of chloroform each of 3 mL. The chloroform phase was filtered through anhydrous sodium sulfate, into a 10 mL calibrated flask and the volume. It was made up to 10 mL by adding chloroform (1.0 mg mL^{-1}, calculated as diphenhydramine base (DP)). From this solution, 5.0 mL was transferred into 10 mL calibrated flasks and evaporated to dryness in a water bath at 45 °C under a stream of nitrogen. Then, the residue completely dissolved in acetonitrile using an ultrasonic bath and then completed to the mark with acetonitrile (0.5 mg mL^{-1}, calculated as free base).

2.4. Procedures

2.4.1. General procedure

Transfer aliquots of 0.25–3.0 mL of diphenhydramine base solution (0.5 mg mL^{-1}) into a series of 10 mL calibrated flasks. Bring the volume to 3.0 mL with acetonitrile, added 1.0 mL DDQ reagent. The reaction mixture was mixed and the volume was completed to 10 mL with acetonitrile. The immediately formed dark red color was measured at λ_{max} = 460 nm against a blank prepared in the same manner except for an addition of drug and the calibration graph was obtained.

2.4.2. Analysis of dosage forms

4 mL of fenotral syrup was transferred into stoppered glass tubes. The solution was alkalized with 1.0 mL of concentrated ammonia and then extracted with chloroform as indicated in Section 2.3.1. After preparation of diphenhydramine base solution, proceed as described in Section 2.4.1.

2.5. Determination of molar ratio

Applying the continuous variation method [21] for the reaction of DDQ reagent with DP and refer to the stoichiometry of the drug donor (D) to the reagent acceptor (A) of ratio 1:1 (D:A).

2.6. Method validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures [22,23].

2.6.1. Linearity

The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The calibration curves were constructed by plotting concentration versus absorbance, using linear regression analysis.

2.6.2. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where S is the slope of the calibration curve and σ is the standard deviation of intercept of regression equation.

2.6.3. Precision and accuracy

DP samples (12.5, 75.0 and 150 μg/mL) in five replicates were analyzed on the same day to determine the intra-day precision and accuracy, and on each of five separate days to determine inter-day precision and accuracy. Precision was expressed as the relative standard deviation (RSD %). Accuracy was expressed as the mean relative error (RME %).

2.6.4. Recovery

The % recovery of the added pure drug was calculated as, % recovery = $[(C_t - C_s)/C_a] \times 100$, where C_t is the total drug concentration measured after standard addition, C_s the drug concentration in the formulation sample and C_a is the drug concentration added to formulation.

2.6.5. Robustness

The robustness of the spectrophotometric method was determined by analysis of samples under a variety of conditions such as small changes in the reagent concentration and reaction time.

3. Results and discussion

3.1. Absorption spectra and mechanism of the reaction

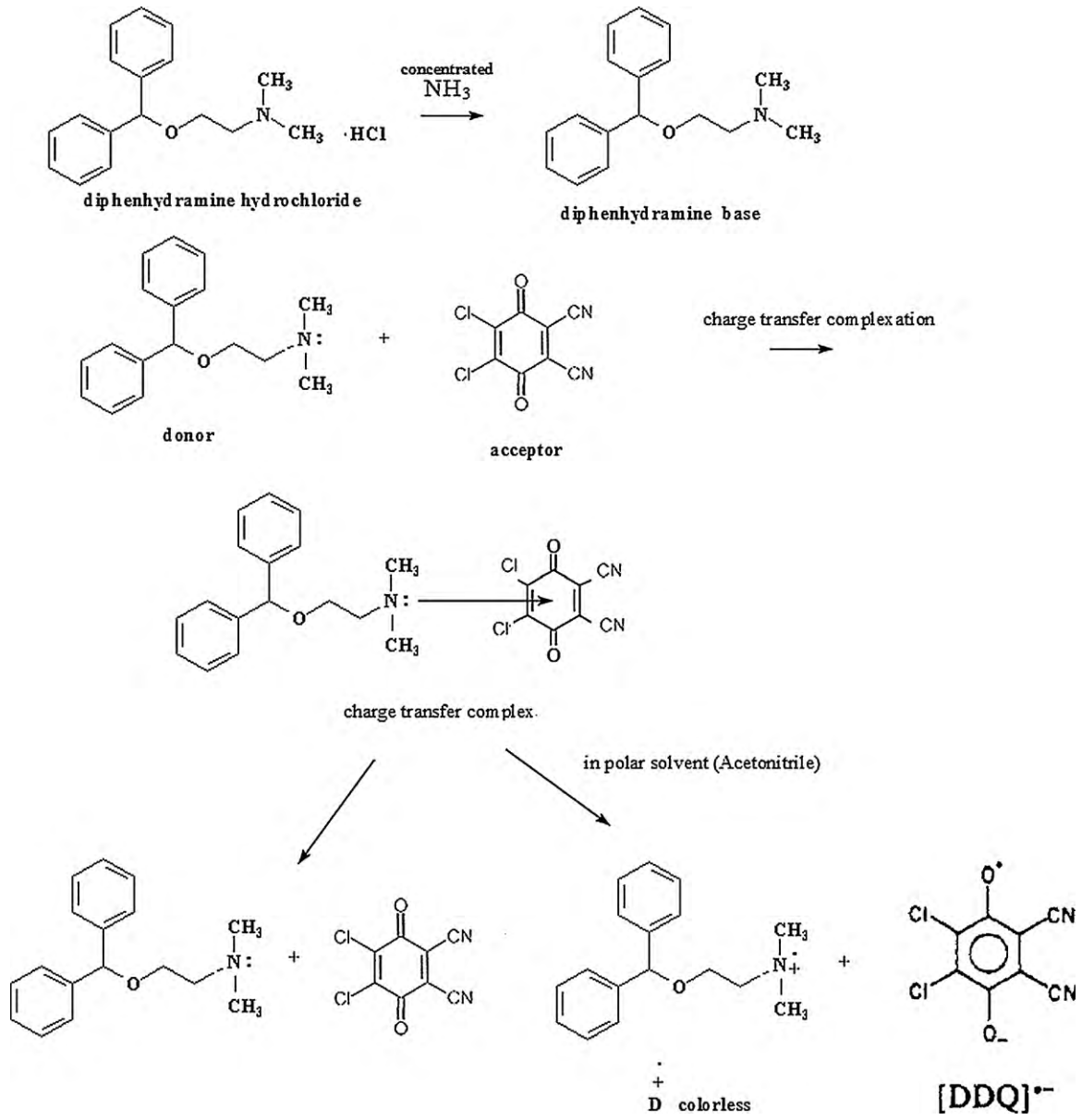
The method is based on the formation of intensely red colored $DDQ^{\bullet-}$ radical anion by interaction of the drug with DDQ in acetonitrile at ambient temperature. As can be represented in the following scheme, the radical anion results from dissociation of the original charge-transfer complex (CT-complex) formed by interaction of the drug (n-electron donor, D) with DDQ (π-acceptor, A) (Scheme 1).

Interaction of DDQ with DP formed a red color product in acetonitrile with absorption maxima at 460, 541 and 586.5 nm (Fig. 2). Measurements were carried out at 460 nm, at which higher sensitivity was achieved.

3.2. Optimization of reaction conditions

3.2.1. Effect of reagent concentration

The influence of the concentration of DDQ was examined by addition of different volumes of 0.2% reagent in the range of



Scheme 1.

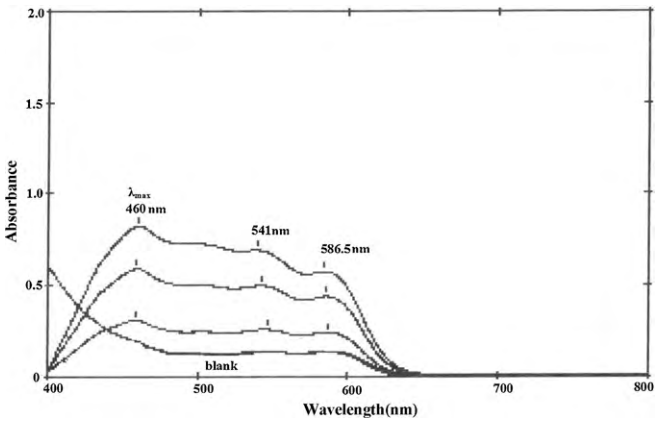


Fig. 2. Absorption spectrum of CT-complex (different concentrations).

0.25–3.0 mL. The maximum absorbance was obtained when 1.0 mL DDQ solution was utilized.

3.2.2. *Effect of solvent*

The formation of charge-transfer complexes where a reaction occurs between π-acceptors and *n*-electron donors can be influenced by the polarity of the solvent. Absorbance spectral characteristics of DP with DDQ in different solvents were examined. Double distilled water, methanol, ethanol, acetonitrile and chloroform were tested for this investigation. Experimental results indicated that acetonitrile gave the maximum absorbance and stable among the studied solvents.

3.2.3. *Effect of time and temperature*

The optimum reaction time was determined by monitoring the color development at room temperature (25 ± 5 °C). Complete color intensity was attained 2 min.

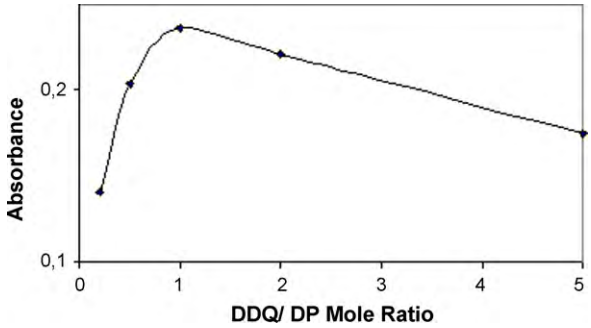


Fig. 3. Mole ratio of DDQ to DP.

3.2.4. Stoichiometry

Job's method of continuous variation was used for determining the molar ratio of DP to each of the analytical reagent employed in the charge-transfer reactions. The ratio was 1:1 in all cases (Fig. 3).

3.3. Method validation

3.3.1. Linearity, detection and quantification limits

Under the specified optimum reaction conditions, the calibration curves for DP with the reagent employed in the present work were constructed. The regression equations for the results were derived using the least-squares method. In all cases, Beer's law plots ($n = 3$) were linear with very small intercepts and good correlation coefficients in the general concentration range of 12.5–150 μg/mL. The LOD and LOQ were found to be 2.09 and 6.27, respectively (Table 1).

3.3.2. Precision and accuracy

The intra-day and inter-day relative standard deviation (RSD) values obtained by the proposed method were found to be within 0.29–0.10%. Accuracy of the methods expressed as relative mean error (RME) was below −4.6%.

3.3.3. Recovery

Recovery studies were carried out by the standard addition method. In this study, different concentrations of pure drug (50, 100 and 150 μg/mL) were added to a known preanalyzed formulation sample and the total concentration was determined using the proposed method ($n = 5$). The mean recovery was found to be 97.67% (Table 2).

3.3.4. Robustness

Robustness of the procedures was assessed by evaluating the influence of small variation of experimental variables: concentrations of acceptor reagent, and reaction time, on the analytical performance of the method. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time. The small variations in any of the variables did not significantly affect the results; recovery percentages were 97.72. This provided an indication for the reliability of the proposed method during routine work.

Table 1
Optical characteristics, statistical data of the regression equations and validation parameters for DP.

Parameters	Value[a]
Molar absorptivity (L/mol cm)	1.2×10^3
Sandell's sensitivity (μg cm^{-2}/0.001 A)	2.11×10^{-2}
Regression analysis	
Slope	0.0045
Intercept	0.0239
Regression coefficient (r)	0.9997
Linearity (μg/mL)	12.5–150
LOD (μg/mL)	2.09
LOQ (μg/mL)	6.27

[a] Values are mean of three determinations.

Table 2
Results of standard addition method ($n = 5$).

Concentration of drug in formulations (μg/mL)	Concentration of pure drug added (μg/mL)	Total concentration of drug found (μg/mL)	% analytical recovery (±SD)[a]
25	25	49.23	$96.92 \pm 1.2 \times 10^{-2}$
25	75	97.03	$96.04 \pm 1.0 \times 10^{-2}$
25	125	150.06	$100.04 \pm 1.5 \times 10^{-2}$

[a] Values are mean of five determinations

Table 3
Determination of DP in pharmaceutical preparations by the proposed and Ref. [3] method.

Drug		Proposed method	Reference method[c]
Fenotral syrups (12.5 mg/5 mL)	% recovery ± SD[a]	$98.48 \pm 3.1 \times 10^{-1}$	$98.12 \pm 3.9 \times 10^{-1}$
	t[b]		1.56
	F[b]		1.58

[a] Values are mean of six determinations.
[b] The tabulated values of t and F at 95% confidence limit are 2.23 and 5.05, respectively.
[c] Ref. [3].

3.4. Application

Commercially available DP in syrup was subjected to analysis both by the proposed method and by a reference method [3]. Statistical comparison of the results by the Student's t-test and the variance-ratio F-test at the 95% confidence level, revealed there was no significant difference between the accuracy and precision of the two methods (Table 3).

3.5. Spectroscopic studies on the solid CT-complex

3.5.1. Infrared absorption spectra

Infrared spectra of electron donor DP, acceptor DDQ and its CT-complex [(DP) (DDQ)] are shown in Fig. 4. In the spectra of the CT-complex main bands of both the donor and the acceptor are observed. From the data given in Table 4, decreases and increases in the vibration frequencies of a particular band have been used as evidence for a particular site of a CT interaction. For example, ν (C=C), ν (C–H) and ν (C–O) stretching vibration bands of the free base DP show some changes in both band intensities and wave number values upon complexation. In the CT-complex, the DDQ gains electrons from the donors and it changes to the ben-

Table 4
FTIR spectral data of DP, DDQ and CT-complex.

Assignment (cm^{-1})	DP	DDQ	DP–DDQ
ν (C≡N)	–	2233	2205, 2113
ν (C=C)	1492	1551	1573
ν (C=O)	–	1669	–
ν (C–H_{alkyl})	2956	–	2956, 2870
ν (C–H_{aryl})	3032	–	3028
ν (C–O)	1108	–	1103

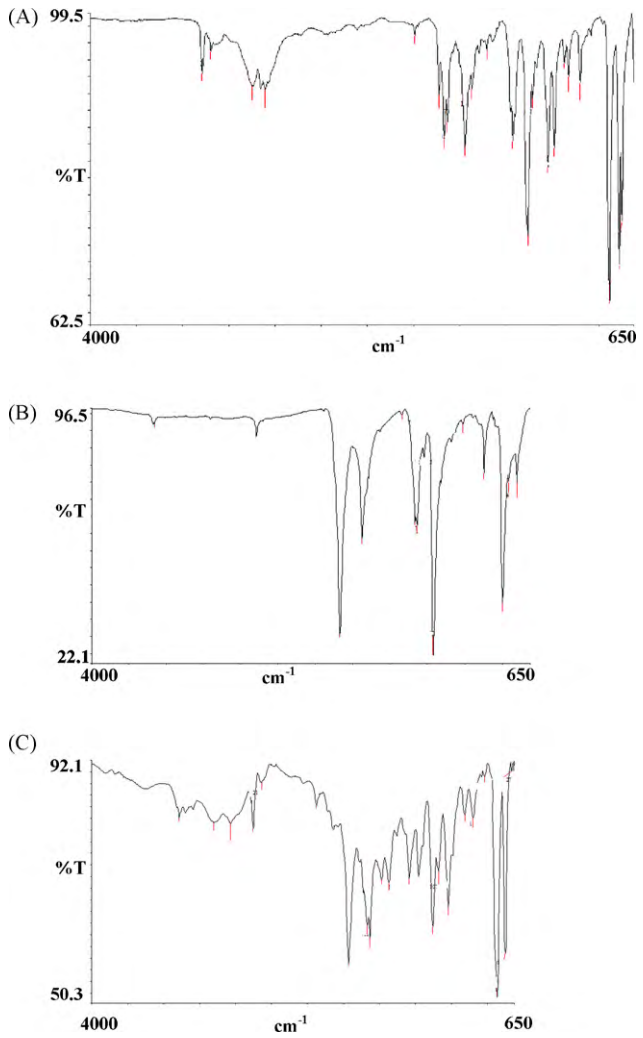


Fig. 4. FTIR spectra of (A) DP, (B) DDQ and (C) CT-complex.

zenoid form having the unshared electrons on the oxygen atoms. In the FTIR spectrum, the lack of the ν (C=O) absorption provides further evidence the presence of $[DDQ]^{\bullet-}$ radical anion for this complex. The ν (C≡N) and ν (C=C) stretching vibration bands for the free DDQ occur at 2233 and 1551 cm^{-1}, respectively, and at 2205–2113 and 1573 cm^{-1} upon complexation. The split of the ν (C≡N) into two bands in the case of CT-complex could be related to such as symmetry and dipole moment changes in the complexed DDQ compared with the free molecule. All these changes could be related to both electronic and symmetry changes upon complexation.

3.5.2. ^1H NMR spectra

^1H NMR spectra of the donor (DP) and the CT-complex are measured in $CDCl_3$ and are shown in Fig. 5A and B (Table 5). The spectrum of CT-complex was compared with the donor. Methyl

Table 5
^1H NMR for DP, DDQ and CT-complex.

^1H NMR (δ, ppm)	DP	DP–DDQ
$-CH_3$	2.2 (s)	2.9 (s)
$-CH_2$	2.5 (t)	3.3 (t)
$-O-CH_2$	3.5 (t)	3.7 (t)
–CH	5.3 (s)	5.3 (s)
$-CH_{aryl}$	7.15–7.30 (m)	7.16–7.26 (m)

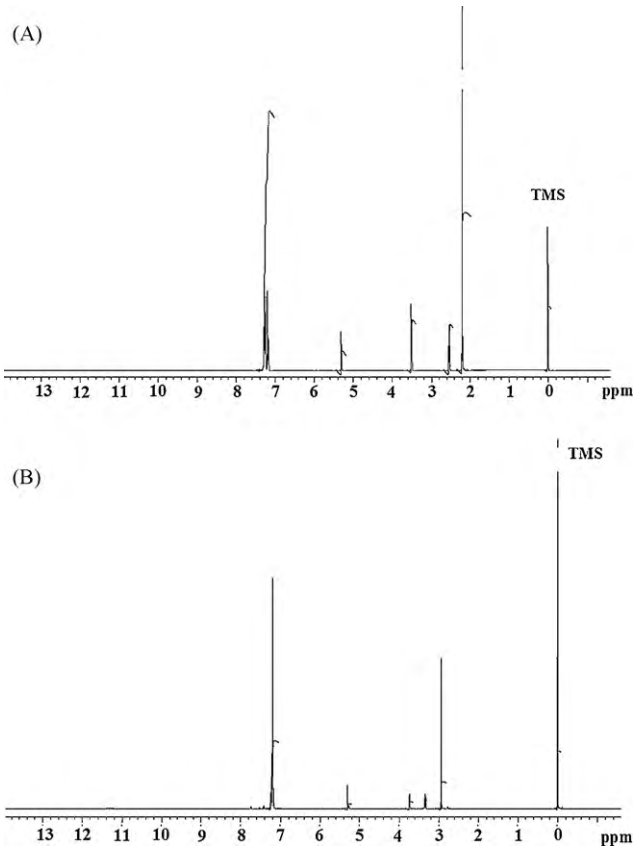


Fig. 5. ^1H NMR spectra of (A) DP and (B) CT-complex in $CDCl_3$.

protons ($-CH_3$) at nitrogen atom gave one sharp singlet NMR signal at 2.2 ppm while methylene protons ($-CH_2-$) gave a triplet at 2.5 and 3.5 ppm. Moreover, methine proton (–CH–) gave a singlet signal at 5.3 ppm. In the CT-complex, while signals of methyl and methylene protons shifted to 2.9, 3.3 and 3.7 ppm, respectively, signal of methine proton did not change. All the observed proton peaks in the spectra of the donor also exist in the CT-complex spectra with small shift to higher ppm values. These changes in the δ (ppm) values of the donor (DP) and CT-complex support the charge migration from the donor (base) toward the acceptor (acid).

3.5.3. Thermal analysis measurements

Thermogravimetric (TGA) and differential scanning calorimetric (DSC) analysis were carried out in order to confirm the decompositions and structures of the formed solid CT-complex. TGA and DSC curves of the DP, DDQ and CT-complex are shown in Fig. 6. The DP donor shows one degradation step at 190–330 °C corresponds to the loss of DP. The found weight loss of this step is 92%. The DDQ acceptor has one degradation step at 170–290 °C corresponding to the loss of DDQ. The found weight loss of this step is 95%. The thermal decomposition of the complex in inert atmosphere proceeds approximately with three main degradation steps. The first stage and second stage of degradation occur within a temperature range of 25–160 and 160–380 °C. The found weight loss associated with these stages of decomposition is 26.2% and 52.0% (78.2%) may be attributed to loss of the DDQ and diphenyl group of donor molecules, which is in good agreement with the calculated value of 79.0%. The third decomposition temperature at 380–1200 °C is related to the decomposition of the $C_6H_{11}NO$ group of the donor. The weight loss of this step is 20.1% in agreement with the calculated value of 20.9%.

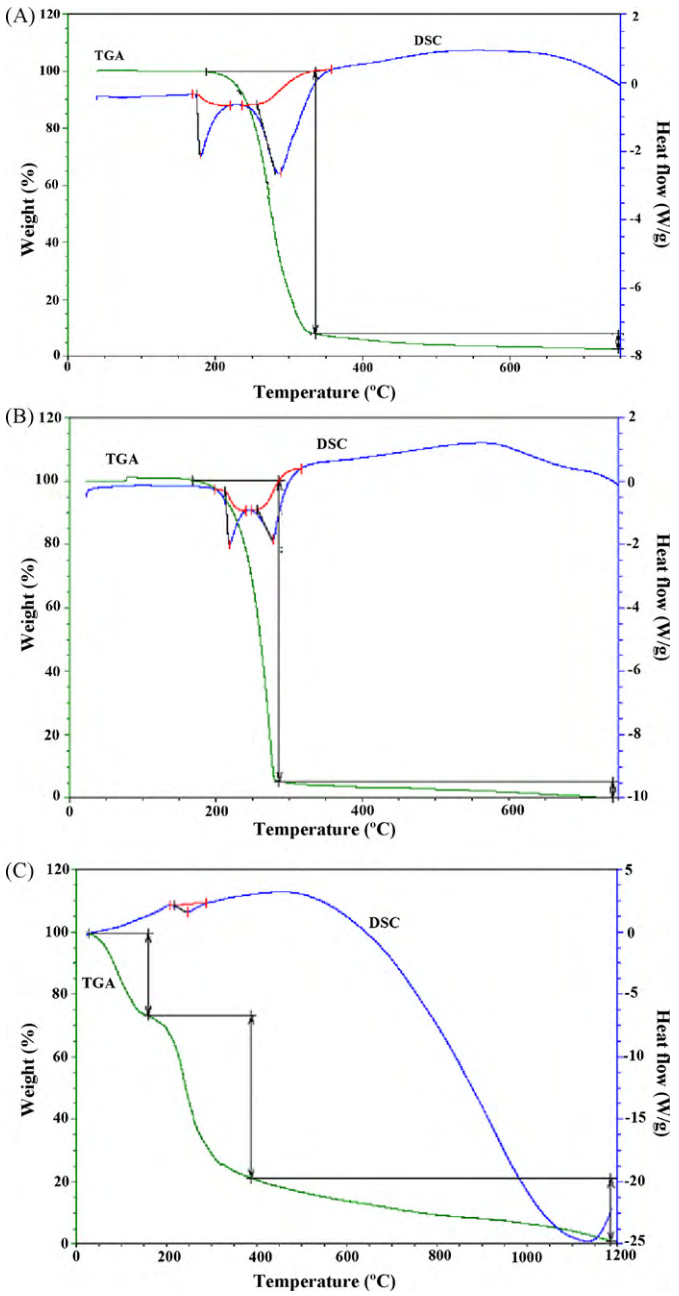


Fig. 6. TGA and DSC diagrams of (A) DP, (B) DDQ, and (C) CT-complex.

4. Conclusion

The proposed method was simple, rapid, accurate, precise and inexpensive and can be used for routine analysis of DP in pharmaceutical preparation. The proposed method for DP determination has many advantages over other analytical methods due to its sensitivity, simplicity, rapidity and low cost [8–14]. It does not require any extraction, heating, buffer or any other agent. Unlike the gas chromatographic and HPLC procedures, the instrument is simple and is not of high cost. The proposed method is suitable for routine quality control.

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