Research Article

Drug Testing and Analysis

Received: 25 September 2009 Revised: 14 November 2009 Accepted: 16 November 2009 Published online in Wiley Interscience: 9 February 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.100

Spectroscopic study of structure of diphenhydramine drug and its products obtained via reactions with tetracynoethylene and iodine reagents and applications

M. A. Zayed*[a] and Abeer A. El-Habeeb[b]

The reactions between diphrenhydramine drug (diphen) in the base form and the iodine amphoteric reagent (n-donor and/or σ-acceptor) and tetracyanoethylene as a π-acceptor reagent (TCNE) have been spectrophotometrically studied at different conditions of reactant concentrations, time intervals, temperatures, solvents, and different wavelengths aiming to shed light on the nature of these reactions. Consequently, it is possible to select the proper conditions for spectrophotometric determination of this biologically active drug in its formulations. The reaction mechanism between iodine and diphen involves the formation of diphen-I_2 outer-and inner-sphere complexes, whereas diphen-TCNE reaction involves the formation of charge transfer (CT) complex. The analytical parameters of the suggested spectrphometric procedures have been calculated. The values of the Sandell sensitivity, standard deviation (SD), relative standard deviation (RSD) and recovery % refer to the high sensitivity of these procedures applied in analysis of diphen in its formulations. This research also presents a new diphen-I_2 promising drug derivative that can be used for the same purpose as its parent. Both diphen-drug and diphen-I_2 separated solid product are critically investigated by elemental analyses, FT-IR, ^{1}HNMR, electron ionization mass spectrometry (EI-MS) and thermal analyses (TA). Practical investigation (TA and MS) data of diphen-drug and its reaction product are confirmed by MO calculations. This research gives a clear idea about the possible metabolites and metabolic pathways of diphen and its derivative *in vitro* system that may occur *in vivo* system. The importance of this drug stems from its use as an antihistamine with anticholinergic (drying) and sedative side effects.

Keywords: spectroscopic study; diphenhydramine hydrochloride drug (dimedrol); iodine and tetracyanoethylene reagents; inner- and outer-sphere complexes and CT-complexes; applications

Introduction

Diphenhydramine hydrochloride (dimedrol) has the general formula $C_{17}H_{21}NO.HCl$ of mole mass = 291.8 and structural formula[1] as depicted in Figure 1.

It has the following IUPAC name: 2-benzhydryloxy-N,N-dimethylethanamine hydrochloride.

This compound shows three maximum absorbencies at 253, 258, and 264 nm when examined in alcoholic solution. It was also identified by mass spectrometry,[2] high performance liquid chromatography (HPLC) and thin layer chromatography (TLC).[3–6] Gas chromatography was used for determination of diphen-HCl in tablets.[7,8] Osciollographic argentimetry and its application in the determination of dimedrol and other drugs was performed.[9] Fixed-pH titration[10] was applied for titration of weakly acidic and basic drugs, such as cimetidine, mexilentine HCl, levamisol HCl and diphen-HCl. Diphen-selective membrane sensor was constructed, tested and applied for analysis of its pharmaceutical preparations.[11] Potentiometric titrations with tetra-phenyl-borate using ion-selective membrane electrode[12] was used to determine total amine content in binary mixture of papaverine and dimedrol (diphen-HCl). Some ion-selective electrodes[13] were used to determine dimedrol. The best results were obtained with a membrane containing complexes of dimedrol with molybdophosphoric acid. Precipitation titration of hydrophobic physiologically active amines in the presence of hydrophilic amines were done using ion-selective electrodes.[14] Oxidimetric method[15] was suggested for quantitative determination of dimedrol in drug forms. Different spectrophotometric techniques were suggested and used for analysis and assay of dimedrol in different drug forms.[16–20]

Experimental

Instruments

Spectrophotometric measurements were recorded using Shimadzu 1700 pharma double beam recording spectrophotometer with 1 cm quartz cells. The IR spectra were recorded as KBr disks using a Perkin Elmer FT-IR spectrophotometer M 1430 in the wave number range 400–4000 cm^{-1} in the Micro-analytical Center at Cairo University.

* *Correspondence to: M. A. Zayed, Chemistry Department, Faculty of Science, Cairo University, Giza, A.R. Egypt. E-mail: mazayed429@yahoo.com*

a *Chemistry Department, Faculty of Science, Cairo University, Giza, A.R. Egypt*

b *Chemistry Department, Faculty of Education of Girls, Riyadh, Saudi Arabia*

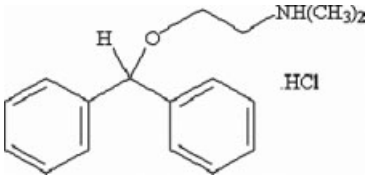


Figure 1. Structural formula of diphenhydramine hydrochloride (dimedrol).

The ^{1}HNMR spectra were measured using an instrument of Model Gemini 2000 (Geneva, Switzerland), and the samples were dissolved in dimethyl sulfoxide (DMSO) and measured in the Central Lab of Chemistry Department, Faculty of Science, Cairo University. Electron ionization mass (EI-MS) spectra were obtained using Shimadzu GC-MS-Qp 1000 EX quadruple mass spectrometer (70 eV); within a scan range of m/z = 50–550.

Thermal analyses (TA) were made using conventional thermal analyzer (Shimadzu system of DTA-50 H and 30 series TG-50). The mass losses of 5 mg sample and heat response of the change of the sample were measured from room temperature up to 600 °C. The heating rate, in an inert argon atmosphere, was 10 °C min^{-1}. These instruments were calibrated using indium metal as a thermal stable material. The reproducibility of the instrument reading was determined by repeating each experiment more than twice.

Procedures

In the case of diphen-iodine, to different aliquots containing (0.1–1.5 ml of 4×10^{-3} M) of working solution of diphen-base (40 μg ml^{-1}), 0.05–1.5 ml of 4×10^{-3} M I_2 solution was added whereas in case of diphen-TCNE method; to 0.04–0.56 ml of working diphen-base solution (2 mg ml^{-1}); 0.3–0.6 ml of 5×10^{-3} M tetracyanoethylene (TCNE) solution was added. The volume of each mixture was completed in 10 ml measuring flask by different solvents in order to select the suitable solvent. The stoichiometry of these reactions was also studied applying molar ratio method (MRM)[21,22] and Job's continuous variation method (CVM).[23]

The absorption spectra of the resulted-complexes in all cases were scanned in the wavelength range 250–800 nm against blank solutions, prepared in the same manner, without drug. The wavelength (λ_{max}) which gave the highest ε value was selected; this wavelength should be far away from that of both drug and reagent.

Validity of Beer's Law

In this study the validity of Beer's Law of diphen-drug reactions with I_2 and TCNE reagents was checked in which 0.3–1.0 ml of 4×10^{-3} M I_2 was added to regularly varied concentrations of diphen-base (3–30 μg ml^{-1}), and 0.4 ml of 5×10^{-3} M TCNE to diphen-base (6–35 μg ml^{-1}) in a 10 ml measuring flask; the volume was completed to the mark with the suitable solvent. The absorbance values were plotted against concentration ranges of the drug to give straight lines pass by origin. These straight lines and/or calibration lines can be used in the microdetermination of diphen-drug in its pharmaceutical products.

The absorbance values of the coloured complexes formed were also measured at different conditions especially λ_{max}, time and temperature against a blank of reaction mixture, prepared in a similar way without drug.

Day-by-day measurements

In daily measurements, the applicability of the proposed tested spectrophotometric procedures and the reproducibly of the results in case of diphen-base-I_2 or diphen-base–TCNE procedure, seven replicates at six or seven different concentrations of diphen-base of 3–30 or 6–35 μg ml^{-1} were carried out in the two procedures respectively. The absorbance values of the samples were measured daily under the optimum conditions for four days and the results obtained were recorded to make statistical calculations of standard deviation (SD), relative standard deviations (RSD), and percent recovery of the results obtained to decide its reproducibly, reliability, accuracy and its precession.

Spectrophotometric microdetermination diphen-drug in its pharmaceutical preparations

Twenty tablets of drug formulation were accurately weighed and the average weight of a tablet was calculated. The tablets were crushed to a fine powder. A portion of the powder equivalent to 100 mg of the drug in its form was dissolved in 100 ml of suitable solvent. In case of liquid formulation, the volume equivalent to 100 mg of drug was also selected and dissolved in the solvent. The resulting solutions were shacked well, filtered through a Whatmann No. 1 filter paper, and washed with the suitable solvent. The filtrate and washings were collected in a 100 ml measuring flask and then diluted to the volume with the same solvent. The microdetermination of each drug was done by application of the same procedure using the previously prepared calibration curves.

Aliquots containing diphen-base of 3–30 or 6–35 μg ml^{-1} extracted from diphen pharmaceutical products were transferred into a 10 ml measuring flask; 1 ml of 4×10^{-3} M I_2 or 0.3 ml of 5×10^{-3} M TCNE was added and the volume was completed to the mark with the suitable solvent. The solution was left to stand for 10 min at room temperature. The absorbance of the mixture was measured at 293 nm in case of I_2-procedure or at 415 nm in TCNE-procedure. The unknown drug concentration was calculated from the corresponding previously prepared calibration curve of each procedure. The interference effect of different excipients, such as like glucose, fructose, and other fillers was tested using ten-fold of interfering gradients.

Preparation of diphen-I_2 solid product

To the drug solution in 10 ml ethanol (96%) iodine solution was dropwisely added until the appearance of a coloured (brownish-yellow) solid product as a precipitate. The precipitate was left for 10 min until completely settled. Iodine solution was added continuously until complete precipitation. The precipitate was left to settle and filtered through small circular Whatmann No.1 filter paper in a suitable Hirsch funnel. The polarity of the product was tested by dissolving it in dichloromethane and reprecipitated by evaporating the solvent in least amount and reprecipitating the compound by dropwisely adding petroleum ether and cooling gradually in refrigerator. The recrystallized compound was then separated by filtration and drying in vacuum desiccators. The yield of the solid product obtained was found to be 75%.

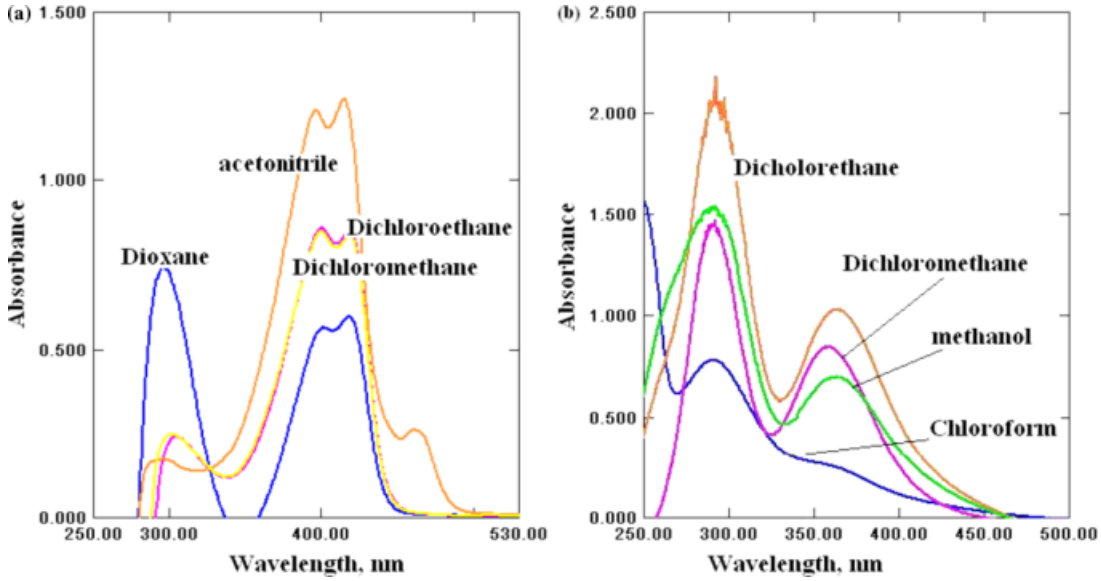


Figure 2. Absorption Spectra of : (a) Diphen-TCNE (b) Diphen-I_2 at different solvents.

Results and Discussion

Spectrophotometric study of reaction between diphen drug and TCEN and iodine reagents in solution

Absorption Spectra, effect of solvent, effect of time, effect of temperature, sequence of addition of reactants and effect of interfering materials

Different solvents were tested as reaction media for interaction between diphen-basic drug and I_2 as a σ- acceptor in the tri-iodide form and the π- acceptor TCNE. 1, 2-dichloroethane was selected as a suitable solvent for diphen-I_2 and acetonitrile for the diphen-TCNA reaction, which may refer to the possible polarity of the reaction product. The absorption spectra of diphen-TCNE CT-complex are shown in Figure 2a. These spectra show two main peaks at 396 nm ($\varepsilon = 1.137 \times 10^4$ L.mole^{-1} cm^{-1}) and at 415 nm ($\varepsilon = 1.147 \times 10^4$ L. mole^{-1}. cm^{-1}) in acetonitrile. The drug and the reagent have no absorption at these wavelength values. Therefore, it is suitable to select either 415 or 396 nm as the suitable maximum wavelength to do further spectrophotometric studies for the formed CT-complex of diphen-base-TCNE in acetonitrile. The absorption spectrum of the reaction product between diphen-basic drug and tri-iodide ion is given in Figure 2B. It shows that both reactants have maximum wavelengths (520 nm for I_2 and 250 nm for basic drug) far from that of the formed products ($\lambda_{max} = 294$ nm). Therefore $\lambda_{max} = 294$ is selected for studying all reaction conditions between the basic drug and I_2, because it has the highest ε value of 3.8×10^4 L.mol^{-1}. cm^{-1}. The sequence of addition study shows that the best one is Drug + Reagent + Solvent (Acetonitrile), $\varepsilon = 1.147 \times 10^4$ L. mole^{-1}. cm^{-1} at 415 nm of diphen-TCNE and $\varepsilon = 0.32 \times 10^4$ L. mole^{-1}. cm^{-1} at 294 nm of diphen-I_2 reaction in dicholorethane.

The effect of time (T in mins), on the formation of the diphen-I_2 and diphen-TCNE products was studied carefully at temperature 25–30 °C and at $\lambda_{max} = 294$ nm and at 415 nm for reactions products respectively. These results show that the maximum absorbance attained at 25 °C ($\varepsilon = 0.32 \times 10^4$ L. mole^{-1}. cm^{-1}) after 7 min, and the colour of the reaction product diphen-I_2 remained unchanged for at least one day. The time also has a pronounce effect at the beginning of the reaction between diphen-base and TCNE reagent up to 10 min at which the absorbance of the formed CT-complex attains the highest ε value of 1.147×10^4 L.mole^{-1} cm^{-1}.

The effect of temperature on the spectra of reaction between diphen and I_2 in 1, 2-dichloroethane medium and diphen-TCNE in acetonitrile was studied at temperature range 10–70 °C and leaving the reaction in thermostat up to 7–10 min. The data obtained show the exponential increase of absorbance with the increase of temperature and the temperature attains maximum effect at 20–30 °C. The fillers are removed during the separation of the basic form of the drug from its hydrochloride formulations as previously explained. Therefore, these fillers have no interfering effects on the spectral measurements.

Stoichiometry of the reaction of diphen drug with I_2 and TCNE reagents and the reactions mechanisms

The stoichiometric ratio of diphen to I_2 or TCNA reagent [R] is obtained by applying both MRM[21,22] and CVM.[23] The data obtained refer to the effective stoichiometric ratio of reactants is [R]/[Drug] = 1:1 (Figure 3).

Spectrophotometric determination of diphen-basic drug using iodine and TCNE reagents

The within-day spectrophotometric measurements of the drug in its pure base-form under selected optimum conditions applying both procedures are given in Tables 1 and 2. Table 3 shows the analytical parameters for microdetermination of diphen-drug by both TCNE and iodine reagents.

Validity of Beer's Law. The variation of the absorbance values of the reaction with the change in the pure drug concentration in the range of 4–40 μg ml^{-1} shows that, Beer's Law is valid over the concentration range 3–30 μg ml^{-1} of diphen drug in case of iodine method and in the range 6–36 μg ml^{-1} in case of TCNE-method, respectively (Table 3). For more accurate results, Ringbom sensitivity for optimum concentration range is applied. The absorbance %

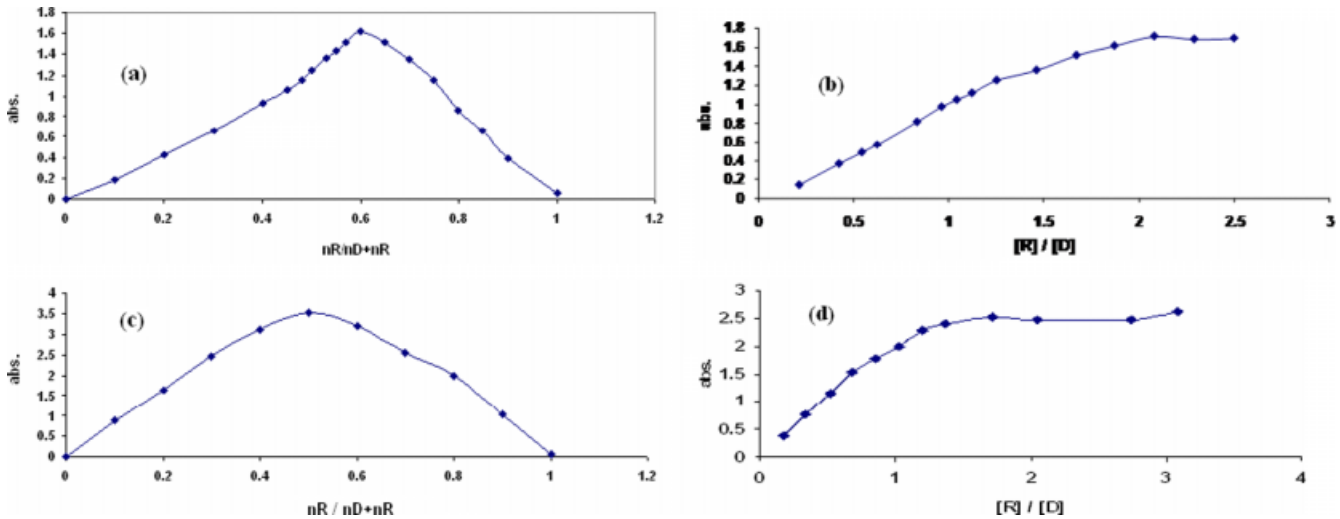


Figure 3. Stoichiometry of the formed compounds : (a) Diphen-TCNE by CVM (b) Diphen-TCNE by MRM (c) Diphen-I_2 by CVM and (d) Diphen-I_2 by MRM.

Table 1. Within-day spectrophotometric micro determination of diphen drug using TCNE reagent under proper selected conditions (t = 10 min, Temp. = 20–30 °C, λ_{max} = 415 nm, [TCNE] = 2.2×10^{-4} M)

Taken µg.ml^{-1}	Found µg.ml^{-1}	Recovery%	S.D.*	R.S.D.* %
6.0	5.92	98.67	0.054	0.91
8.0	8.04	100.5	0.049	0.61
10	9.98	99.8	0.090	0.90
13	13.25	101.92	0.062	0.47
16	15.84	99.00	0.102	0.64
18	18.06	100.33	0.093	0.51
21	21.14	100.67	0.171	0.81
24	23.92	99.67	0.135	0.56
26	26.20	100.77	0.072	0.27
29	29.09	100.31	0.105	0.36
31	31.08	100.26	0.172	0.55
33	33.45	101.36	0.143	0.43
35	34.54	98.68	0.267	0.77

* Seven replicates.

Table 2. Within-day spectrophotometric micro determination of diphen drug using iodine reagent under selected proper conditions (λ_{max} = 293 nm, t = 7 min, T = 25–30 °C, [I_2] = 1.4×10^{-4} M)

Taken µg.ml^{-1}	Found µg.ml^{-1}	Recovery%	S.D.*	R.S.D.(%)
4	3.95	98.75	0.054	1.37
8	8.02	100.25	0.091	1.13
12	11.93	99.42	0.106	0.89
16	16.07	100.44	0.136	0.85
20	19.52	97.60	0.151	0.77

* Seven replicates.

is plotted against-log [drug] to obtain the correct concentration limits, found to be 3–8 and 6–26 µg ml^{-1} of diphen using I_2 and TCNE reagents, respectively. The mean recovery values obtained ranged from 98.67 to 100.3% (Table 3). These values indicate

Table 3. Analytical parameters for micro determination of diphen drug by both TCNE and iodine reagents

λ_{max}	TCNE 415 nm	I_2 293 nm
Beers law limit, µg.ml^{-1}	6–36	3–30
Ringbom optimum concentration range, µg.ml^{-1}	6–26	3–8
Limit of detection(µg.ml^{-1})	3.00	2.99
Molar absorptivity, l mol^{-1} cm	89.972×10^2	21.46×10^3
Sandell Sensitivity, µgcm^{-2}	0.0067	0.0055
Regression equation :		
(a)-Slope(specific absorptivity)	0.0382	0.0748
(b)-Intercept	0.0300	0.0017
(c)-Correlation coefficient	0.9956	0.9992
Standard deviation*	0.049–0.267	0.026–0.182
Relative standard deviation %*	0.27–0.91	0.48–1.16
Recovery %	98.67–100.3	98.67–100.3
Calculated t-value** (2.447)	−1.88 to +1.93	−1.23 to +2.33
Calculated F-value** (4.28)	1.02–2.24	1.06–2.86

* seven replicates.
** Theoretical values at 95% confidence limit, F = 4.28, t = 2.447.

the successful application of the proposed spectrophotometric methods for microdetermination of pure drug form. The low values of the calculated standard deviation (SD = 0.049 to 0.267 and 0.026 to 0.182) and relative standard deviation (RSD = 0.29 to 0.91 and 0.84 to 1.16%) for n = 7 indicate the high accuracy and precision of the proposed procedure. This is also supported by the calculated values of Sandell sensitivity (S = 0.0076 and 0.0055 µg cm^{-2}), which indicated the high sensitivity of the applied procedures in spectrophotometric microdetermination of diphen by I_2 and TCNE reagents. The t-test gives t-value of 2.447 and f-test give f-value of 4.28 corresponding to confidence limit of 95%. This indicates the robustness of the applied procedures.

Table 4. Micro determination of diphen in its drug formulations under proper conditions using TCNE reagent in comparison with official method

Taken µg.ml^{-1}	Found µg.ml^{-1} proposed$_1$ method	official$_2$ method	S.D.$_1$*	S.D.$_2$*	F-test**	t-test**
Panadol Night Tablets						
8	7.90	7.96	0.075	0.004	1.36	−1.61
18	18.08	18.03	0.113	0.107	1.12	+0.85
Exylin Syrup						
20	19.69	19.81	0.109	0.138	1.60	−1.81
30	30.51	30.18	0.376	0.251	2.24	+1.93
Ezipect Syrup						
10	10.01	9.98	0.080	0.089	1.24	+0.91
12	11.98	11.95	0.074	0.110	2.20	+0.60
Amydramine Syrup						
14	13.98	14.13	0.148	0.150	1.02	−1.88
26	25.84	25.94	0.160	0.140	1.31	−1.25

* Seven replicates.
** Theoretical values at 95% confidence limit, F = 4.28, t = 2.447.

Table 5. Micro determination of diphen in its drug formulations under proper conditions using iodine reagent in comparison with official method

Taken µg.ml^{-1}	Found µg.ml^{-1} proposed$_1$ method	official$_2$ method	S.D.$_1$*	S.D.$_2$*	F-test**	t-test**
Panadol Night Tablets						
7	7.11	7.01	0.059	0.097	2.76	+2.33
23	22.87	22.79	0.171	0.166	1.06	+0.89
Exylin Syrup						
3	2.99	2.98	0.027	0.045	2.86	+0.51
16	15.98	16.06	0.115	0.129	1.26	−1.23
Ezipect Syrup						
15	14.99	15.04	0.115	0.124	1.16	−0.79
35	34.84	34.53	0.369	0.306	1.45	+1.71
Amydramine Syrup						
12	11.97	11.95	0.073	0.110	2.28	+0.40
25	25.11	24.97	0.106	0.119	1.27	+2.32

* Seven replicates.
** Theoretical values at 95% confidence limit, F = 4.28, t = 2.447.

Application of the proposed spectrophotometric procedures. The tested proposed spectrophotometric procedures are applied for determination of diphen via its reaction with I_2 and TCNE reagents under optimum conditions in Panadol-night tablets and in syrups of alxyline, easybeckt and amideramine pharmaceutical formulation collected from the Saudi Arabian local markets. The results obtained are shown in Tables 4 and 5. These data show that the determined concentration of diphen by the proposed procedures is very near to that obtained by applying the standard procedure[24] as indicated by the confidence and correlation between the data of suggested spectrophotometric procedures using these reagents and official method[24] and applying the f-test and t-test for all results (Tables 4 and 5). These results indicate the reliability, accuracy and precision of the suggested procedures and its success and robustness in analysis of the drug in its dosage form.

Reaction mechanism between I_2 and diphen drug and the identified reaction product

The absorption spectra of diphen-I_2 product in dichloroethane (Figure 2B) refer to two maximum wavelengths at 294 and 360 nm which may be attributed to the formation of two absorbing species[25] of inner and outer sphere complexes[26] leading to the formation of an ion pair as given by the following proposed equations:

$$D + I_2 \longrightarrow D\text{-}I_2 \text{ (outer sphere complex)} \quad (1)$$

$$D\text{-}I_2 \longrightarrow [D\text{-}I]^+I^- \text{ (Inner sphere complex)} \quad (2)$$

$$[D\text{-}I]^+I^- + I_2 \longrightarrow [D\text{-}I]^+I_3^- \text{ (ion-pair)} \quad (3)$$

The formation of I_3^- ions is attributed to the transformation of an outer sphere complex to an inner sphere one liberating iodide ions as a result of reactions between iodine σ-acceptor and diphen-drug (D) as a donor. The reaction product at high concentration of reactants was separated in a solid form by the procedure recommended by Zayed *et al*.[26]

Structure identification of diphen by different physicochemical methods of analyses

Structure identification by thermal analyses (TGA, DTGA and DTA):

The thermogravimetric analysis (TGA) of dimedrol is shown in Figure 4A; differential thermogravimetric (DTGA) is shown in Figure 4B; and differential thermal analysis (DTA) is shown in Figure 4c. The explanation of these results is given in Table 6 and Scheme 1.

The TGA data (Figure 4A) show the first weight loss of 41.9% occurs at 150–280 °C, which may be attributed to the loss of side chain of the formula $OC_2H_4N(CH_3)_2$.HCl of the calculated mass loss = 43% as given by Scheme 1. This weight loss centered at 256.8 °C as given by DTGA (Figure 4B). This first weight loss appeared as an endothermic peak in DTA (Figure 4C) at 160.8 °C and required an energy E_1 = 171.5 J g^{-1}. The second mass loss of practical percent = 54.9% occurs at 280–380 °C followed by another small one appeared as 2.0% in the temperature range 380–600 °C of summation = 56.9%. It appeared as a second endothermic peak in DTA at 241.1 °C, required energy E_2 = 738 J g^{-1} (Table 6 and Figure 4C). This second weightloss may be attributed to the loss of biphenyl-methene of the formula $C_{13}H_{11}$ of calculated weight loss = 57.8%. The DTA (Figure 4C) shows also an exothermic base shift as a final stage; which may be attributed to the increase of heat capacity of the loosed biphenyl radical before going to further thermal degradation.

Structure identification of diphen-HCl (dimedrol) by electron-ionization mass spectrometry (EI-MS)

Of the diverse analytical techniques available today, mass spectrometry (MS) seems to be the most versatile tool.[27] After years of research and development in analytical instrumentation, MS is no longer the expensive and specialized tool it once was, and it is rapidly becoming the detection method of choice in many areas, especially those in which sensitivity and specificity are important.[28] The demands of speed in drug discovery combined with the fast detection capability of MS offer new challenges for

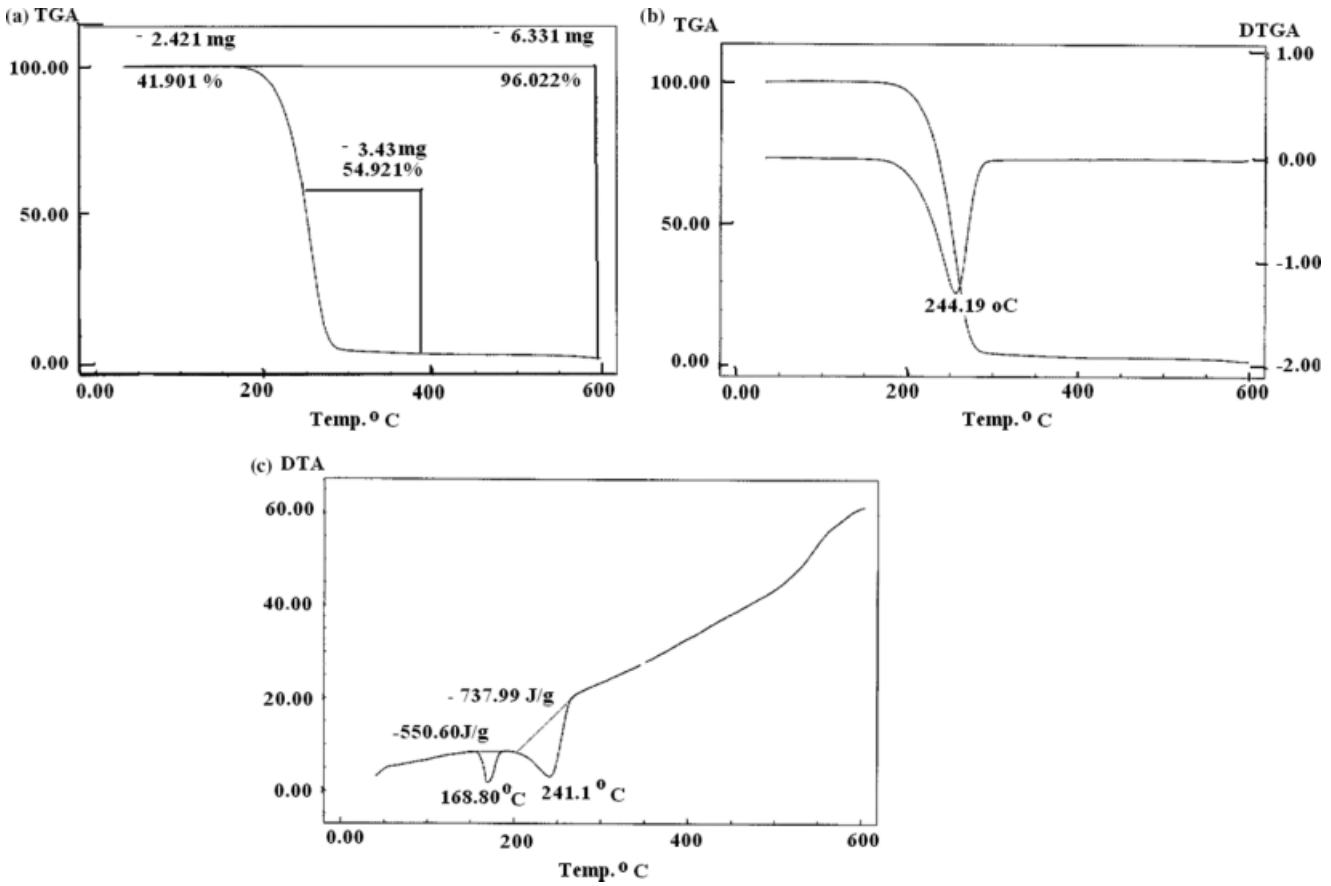
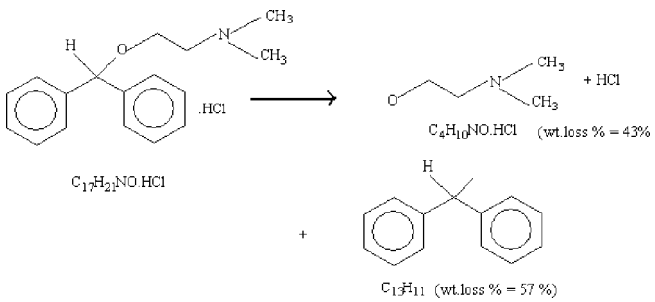


Figure 4. (a) TGA of diphen-HCl, (b) DTGA of diphen-HCl (c) DTA of diphen- HCl.

Table 6. Structure identification of diphen-hydrochloride (dimedrol) drug by thermal analyses

TGA		DTA		
Wt. loss %	Temp. range °C	Peak Temp. °C	Description	E J/g
41.9	150–280	160.8	endothermic	+171.5
54.9	280–380	241.1	endothermic	+738
2.0 Σ Wt.loss% = 98.8	380–600	250–600	Exothermic base shift	

* The main loss occurs at 256.8 °C as given by DTGA.



Scheme 1. Thermal decomposition diphen-HCl (dimedrol).

sample introduction techniques, such as thermal analyses (TA) and different chromatographic techniques.[29] Therefore, it is normal to follow TA decomposition of the diphen by EI-MS fragmentation in comparative way.

Figure 5 shows the EI-MS of diphen-HCl and Scheme 2 shows the suggested pathways of its MS fragmentation. The appearance of the peak at m/z = 256, may be attributed to the main drug molecular ion of mole mass 255 of the general formula $C_{17}H_{21}NO^+$ without HCl molecule. The appearance of the prominent fragment ion of m/z = 58 (RI = 100%) as a base peak, is reasonably account for the loss of the aliphatic part $-CH_2N(CH_3)_2^+$ of the drug molecule of mole mass = 58, as given in suggested path (1). The appearance of the peak at m/z = 77 (RI = 15.5%) path (2) may be attributed to the formation of two fragment ions $C_6H_5^+$ each of mole mass = 77. The appearance of the peak at m/z = 152 (RI = 70%), may be attributed the formation of fragment ion $(C_6H_4-C_6H_4)^+$ as a result of the loss of the aliphatic side chain molecule CH–O–CH_2–CH_2–N

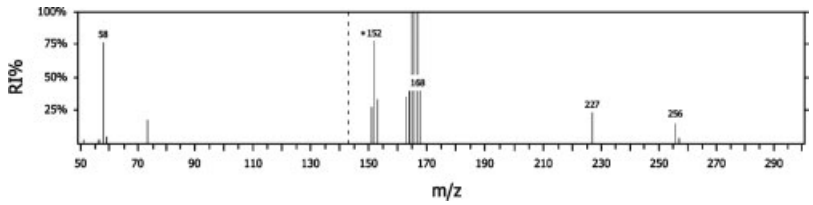
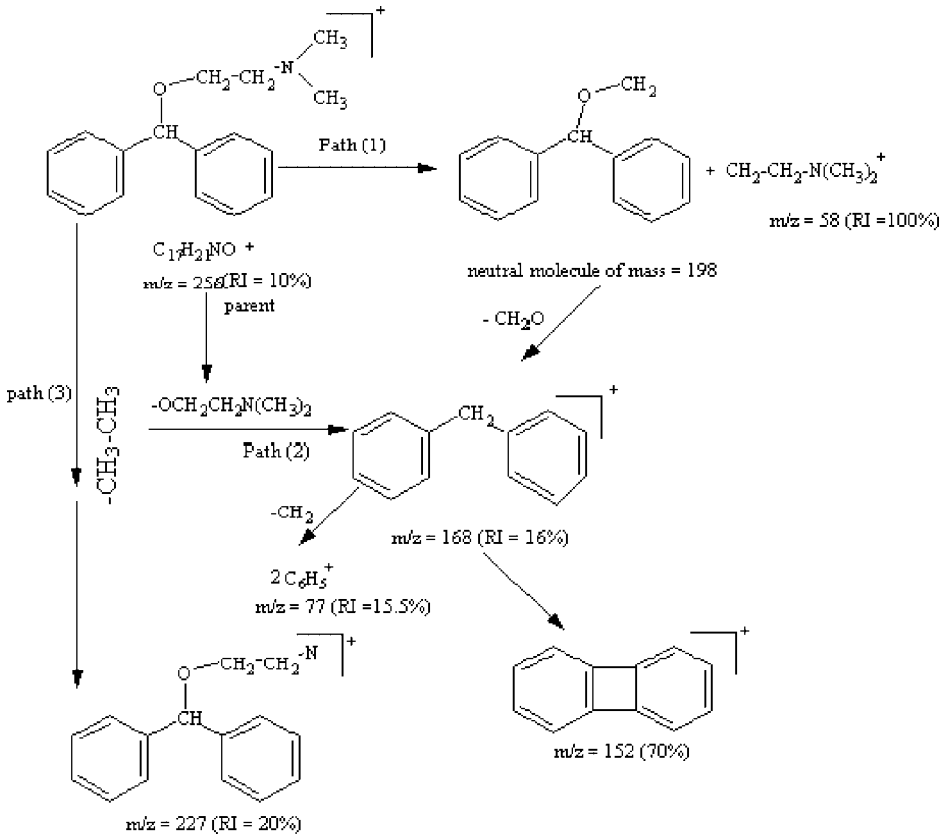


Figure 5. EI-MS of diphen-drug.



Scheme 2. Mass spectral fragmentation pathways of diphen-drug.

$(CH_3)_2$ of mole mass = 101 (path 2). The formation of the fragment ion at m/z = 168 (RI = 16%) may be attributed to the formation of fragment ion $(C_6H_5-CH-C_6H_5)^+$ of mole mass = 168 as a result of the loss of the molecule $-OCH_2-CH_2-N(CH_3)_2$ of aliphatic side chain in the same way as in TA.

The appearance of the peak at m/z = 227 (RI = 20%) may be attributed to the formation of the fragment ion $(C_6H_5)_2CH-O-CH_2-CH_2N^+$ of mole mass = 225 as a result of loss of dimethyl radical as CH_3-CH_3 gas molecule (path 3).

Structure identification of diphen-HCl (dimedrol) by thermal analyses (TA) in comparison with EI-MS and molecular orbital calculations (MOC)

The MOC data of the diphen-HCl involved the calculation of many parameters such as, bond length (in angstrom, A°), bond order, bond strain (in kCal. mol^{-1}), atomic orbital hybridization, charge distribution, heat of formation, electron affinity, and dipole moment of the drug molecule both in neutral form, used in explanation of TA data, and in ionic form, used in explanation of EI-MS data. The calculated data (Tables 7 and 8) were obtained by applying MOC programs and numbering system of the drug molecule (Figure 6).

The charge distribution over atoms in the skeleton of diphen drug neutral molecule is given by Figure 7A and on for the drug ionic form is given by Figure 7B. The MO calculated parameters (Table 7 and Figure 7A) for the neutral diphen molecule can be used to explain the possible rupture bonds during thermal decomposition of the drug molecule. The TGA of diphen-HCl (Figure 4A), shows the first practical weight loss of 41.9% at 150–280 °C is attributed to loss of side chain of $C_4H_{10}NO.HCl$. This is may be explained by MOC data; it occurs as a result of C3–O25 bond rupture. This bond has the MOC parameters as: C3 has a partial charge = +0.151 and SP^3 hybridization that attached to O25 of partial charge = −0.248. The bond has also a length = 1.434°A, bond order = 0.967 and bond strain = −0.071 Kcal $mole^{-1}$. These characters refer to the possibility of rupture of this long bond of high strain during thermal degradation of the drug molecule as a first step (Scheme 1). In EI-MS spectra of the drug (Figure 5) and its fragmentation pathways (Scheme 2), it seems different that, the loss of aliphatic side chain ($C_4H_{10}NO$) occurs in two steps (path 1).

Table 7. The calculated MO-parameters of the diphen drug ionic form

Chemical Sample	atom	Partial Charge	Hybridization	bond	Bond ID	Bond Length (angstrom)	Bond Order	Bond Strain (kcal/mole)
Diphen-ion	C1	−0.063	sp2	C1–C2	1	1.393	1.422	0.058
	C2	−0.116	sp2	C1–C13	2	1.392	1.425	0.014
	C3	0.186	sp3	C5–H22	3	1.097	0.966	0.001
	C4	−0.156	sp2	C1–H23	4	1.098	0.961	0.000
	C5	−0.087	sp2	C2–C3	5	1.510	0.971	0.105
	C6	−0.096	sp2	C2–C10	6	1.398	1.396	0.067
	C7	−0.072	sp2	C3–C4	7	1.507	0.977	0.117
	C8	−0.092	sp2	C4–C5	8	1.396	1.409	0.066
	C9	−0.083	sp2	C4–C9	9	1.397	1.407	0.065
	C10	−0.116	sp2	C5–C6	10	1.391	1.434	0.014
	C11	−0.097	sp2	C3–H24	11	1.122	0.965	0.000
	C12	−0.073	sp2	C3–O25	12	1.445	0.935	0.059
	C13	−0.085	sp2	C6–C7	13	1.391	1.430	0.008
	H14	0.115	s	C6–H14	14	1.096	0.969	0.001
	H15	0.116	s	C7–C8	15	1.392	1.425	0.007
	H16	0.116	s	C7–H15	16	1.096	0.969	0.001
	H17	0.107	s	C8–C9	17	1.390	1.440	0.015
	H18	0.080	s	C8–H16	18	1.096	0.969	0.001
	H19	0.110	s	C9–H17	19	1.096	0.967	0.001
	H20	0.117	s	C10–C11	20	1.388	1.446	0.016
	H21	0.118	s	C10–H18	21	1.100	0.956	0.001
	H22	0.113	s	C11–C12	22	1.392	1.419	0.009
	H23	0.124	s	C11–H19	23	1.095	0.970	0.001
	H24	0.040	s	C12–C13	24	1.390	1.438	0.008
	O25	−0.270	sp3	C12–H20	25	1.096	0.969	0.001
	C26	0.058	sp3	C13–H21	26	1.096	0.968	0.001
	H27	0.078	s	O25–C26	27	1.399	1.021	0.037
	C28	−0.232	sp3	C26–H27	28	1.106	0.969	0.000
	H29	0.096	s	C26–C28	29	1.552	0.924	0.114
	H30	0.123	s	C26–H29	30	1.108	0.967	0.001
	N31	0.525	sp3	C28–H30	31	1.112	0.960	0.002
	H32	0.115	s	C28–N31	32	1.466	0.989	0.117
	C33	−0.203	sp3	C28–H32	33	1.113	0.971	0.000
	C34	−0.234	sp3	N31–C33	34	1.451	1.011	0.046
	H35	0.126	s	N31–C34	35	1.449	1.016	0.052
	H36	0.112	s	C33–H35	36	1.105	0.954	0.000
	H37	0.119	s	C33–H36	37	1.102	0.979	0.000
	H38	0.119	s	C33–H37	38	1.104	0.958	0.000
	H39	0.114	s	C34–H38	39	1.104	0.957	0.000
	H40	0.148	s	C34–H39	40	1.101	0.979	0.000
				C34–H40	41	1.118	0.929	0.000

Dipole moment (Debye)	Electron affinity (eV)	Heat of formation (kcal/mole)	Ionization potential (eV)
14.060	4.394	182.430	12.101

The first step occurs as a result of the C26–C28 bond rupture; followed by the rupture of C3–O25 bond this confirmed by their MOC characters. The MOC characters of C26–C28 are given as C26 has the partial charge = 0.058 and SP^3 hybridization and C28 has the partial charge = −0.232 and SP^3 hybridization, which indicates the low electrostatic attraction characters of the C26–C28 than that of C3–O25 in which C3 has the partial charge = 0.186 of SP^3 hybridization and O25 has a partial charge = −0.270 and SP^3 hybridization. This means that MOC data refer to the rupture of C26–C8 bond before the rupture of the bond C3–O25. This is also confirmed by the comparison of the other MOC characters of the two bonds as C26–C28 has bond length = 1.552°A, bond order = 0.924 and bond strain = 0.114 kCal $mole^{-1}$; whereas C3–O25 has the bond length = 1.445°A, bond order = 0.935 and bond strain = 0.059 kCal $mole^{-1}$. These characters refer to the fact that, C26–C28 is longer, has low bond order and is of higher bond strain than C3–O25. Therefore, the C26–C28 is easily ruptured before the rupture of C3–O25, which actually occurs in path 1 of the EI-MS scheme of the diphen drug. These losses are $CH_2–N(CH_3)_2^+$ fragment ion of m/z = 58 (RI = 100%) as a base peak followed by the loss of CH_2OH leading to the formation of $C_6H_5–CH–C_6H_5$ of m/z = 168. This means that TA degradation

Table 8. The calculated MO-parameters of the diphen-drug neutral form

Chemical sample	atom	Partial charge	Hybridization	bond	Bond ID	Bond length (angstrom)	Bond order	Bond strain (kcal/mole)
Diphen-neutral	C1	−0.090	sp2	C1–C2	1	1.394	1.414	0.073
	C2	−0.84	sp2	C1–C13	2	1.391	1.421	0.017
	C3	0.151	sp3	C5–H22	3	1.097	0.960	0.002
	C4	−0.118	sp2	C1–H23	4	1.097	0.958	0.000
	C5	−0.078	sp2	C2–C3	5	1.513	0.963	0.135
	C6	−0.107	sp2	C2–C10	6	1.398	1.399	0.069
	C7	−0.097	sp2	C3–C4	7	1.513	0.961	0.130
	C8	−0106	sp2	C4–C5	8	1.396	1.405	0.073
	C9	−0.092	sp2	C4–C9	9	1.396	1.405	0.062
	C10	−0.097	sp2	C5–C6	10	1.391	1.426	0.012
	C11	−0105	sp2	C3–H24	11	1.127	0.949	0.000
	C12	−0.099	sp2	C3–O25	12	1.434	0.967	0.071
	C13	−0.104	sp2	C6–C7	13	1.391	1.423	0.008
	H14	0.105	s	C6–H14	14	1.095	0.966	0.001
	H15	0.103	s	C7–C8	15	1.391	1.421	0.008
	H16	0.104	s	C7–H15	16	1.095	0.966	0.001
	H17	0.105	s	C8–C9	17	1.390	1.430	0.013
	H18	0115	s	C8–H16	18	1.095	0.966	0.001
	H19	0.102	s	C9–H17	19	1.096	0.963	0.001
	H20	0.102	s	C10–C11	20	1.389	1.435	0.016
	H21	0.103	s	C10–H18	21	1.102	0.948	0.000
	H22	0.120	s	C11–C12	22	1.391	1.416	0.006
	H23	0.117	s	C11–H19	23	1.095	0.966	0.001
	H24	0.058	s	C12–C13	24	1.390	1.428	0.006
	O25	−0.248	sp3	C12–H20	25	1.095	0.966	0.001
	C26	0.044	sp3	C13–H21	26	1.095	0.966	0.001
	H27	0.077	s	O25–C26	27	1.412	0.985	0.064
	C28	−0.120	sp3	C26–H27	28	1.104	0.964	0.001
	H29	0.039	s	C26–C28	29	1.536	0.969	0.117
	H30	0.060	s	C26–H29	30	1.108	0.970	0.000
	N31	−0.064	sp3	C28–H30	31	1.108	0.976	0.002
	H32	0.052	s	C28–N31	32	1.493	0.983	0.146
	C33	−0.089	sp3	C28–H32	33	1.117	0.961	0.001
	C34	−0.105	sp3	N31–C33	34	1.480	0.994	0.072
	H35	0.046	s	N31–C34	35	1.479	0.995	0.055
	H36	0.021	s	C33–H35	36	1.098	0.983	0.001
	H37	0.048	s	C33–H36	37	1.101	0.977	0.001
	H38	0.021	s	C33–H37	38	1.098	0.984	0.000
	H39	0.049	s	C34–H38	39	1.101	0.976	0.000
	H40	0.065	s	C34–H39	40	1.097	0.983	0.000
				C34–H40	41	1.103	0.969	0.000

Dipole Moment (Debye)	Electron Affinity (eV)	Heat of Formation (kcal/mole)	Ionization Potential (eV)
2.128	−0.177	7.81	9.073

did not feel the stepwise mass losses of the aliphatic side chain of diphen ($C_4H_{10}NO$), which actually detected by the EI-MS. The loss of this side chain in one step in EI-MS is also detected as given by path 2 (Scheme 2), leading to the same fragment ion m/z = 168 in good agreement with TA. In TA Scheme 1, this reminder molecule is completely loosed in second step at 250–600 °C, which actually fragmented to different mass fragment ions as given in path 1 and path 3 in MS. This means also that, TA cannot detect such stepwise fragmentation that actually detected by EI-MS. This mass stepwise fragmentation can be explained by using MOC characters of the ruptured bonds. In path 3 (Scheme 2), the appearance of fragment ion at m/z = 227 (RI = 2%) may be explained by the rupture of the bonds N31–C33 and N31–C34 and consequently the loss of two methyl groups of –N $(CH_3)_2$, of the ionic drug parent, leaving the remainder molecule $(C_6H_5)_2CH–OCH_2CH_2N$ of mole mass = 226. This rupture can be explained by MOC characters of N31–C33 and N31–C34 bonds. The characters of these bonds are: N31 has the partial charge = +0.525 of SP^3 tetrahedral hybridization; C33 has the partial charge = −0.203 of SP^3 hybridization and C34 has the partial charge = −0.234. These values mean the less electrostatic attraction between N31–C33 than between N31–C34. Therefore, the bond N31–C33 is firstly

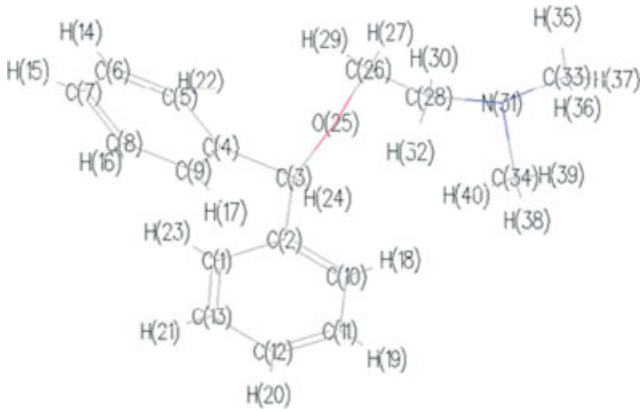


Figure 6. The numbering system of diphen-drug molecule.

ruptured followed by the rupture of N31–C34 to loose two methyl groups as ethane gas. This conclusion is also confirmed by the comparison of the other MOC characters of the two bonds as: N31–C33 bond has a bond length = 1.451°A; bond order = 1.011 and bond strain = 0.046 k Cal mole^{-1}. The bond N31–C34 has, bond length = 1.449°A; bond order = 1.016 and bond strain = 0.052 k Cal mole^{-1}. These values mean that N31–C33 is longer and of lower bond order. Therefore, N31–C31 may rupture at first followed by N31–C34; or maybe both are ruptured at the same time. Thermal analyses does not detect such fragmentation. At the end of path 1 and path 2 which meet together at the step of formation of the fragment ion m/z = 168 (RI = 16%) (Scheme 2); they give two possibilities. The first one is the formation of the fragment ion of m/z = 152 (RI = 70%) as a result of losing CH_2 (H23–C3–H24) radical and the rupture of the two bonds C3–C2 and C3–C4. The MOC characters of the two bonds are: C3 has the partial charge = +0.186 of Sp3 hybridization, C2 has the partial charge = −0.116 of SP^2 hybridization and C4 has the partial charge = −0.156 of SP^2 hybridization. These values of charges on atoms mean higher electrostatic attraction between C3–C4 than between C3–C2 and consequently the possibility of rupture of C3–C2 at first followed by the rupture of the bond C3–C4 to give the molecular ion of m/z = 152. This conclusion can also be confirmed by the comparison of the other MOC parameters of the two bonds as: C3–C2 has a bond length = 1.510°A; bond order = 0.971 and bond strain = 0.105 k Cal mole^{-1}; and C3–C4 has a bond length = 1.507°A, bond order = 0.977 and bond strain = 0.117 k Cal. mole^{-1}. This means that C3–C2 is a longer bond of lower bond order than C3–C4 and consequently ruptures first. The rupture of these bonds leads to the formation of stable molecular ion of m/z = 152. This stability may be attributed to the reconnection of the biphenyl rings (Scheme 2) via rebinding of C2–C4 and C1–C9. The other alternative is the fragmentation of this fragment ion of m/z = 168 into two phenyl radicals each of m/z = 77 (RI = 20%) as a result of ruptured of the bonds C3–C2 and C3–C4 and the loss of CH_2 rad-

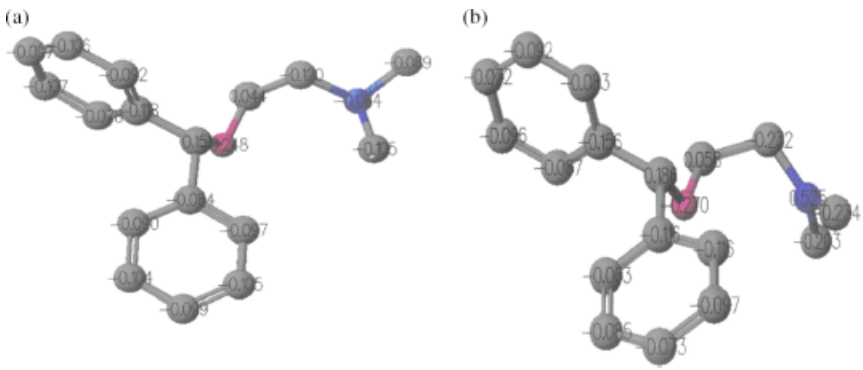


Figure 7. (a). Charge distribution of neutral diphen-drug molecule. (b). Charge distribution of ionic diphen-drug molecule.

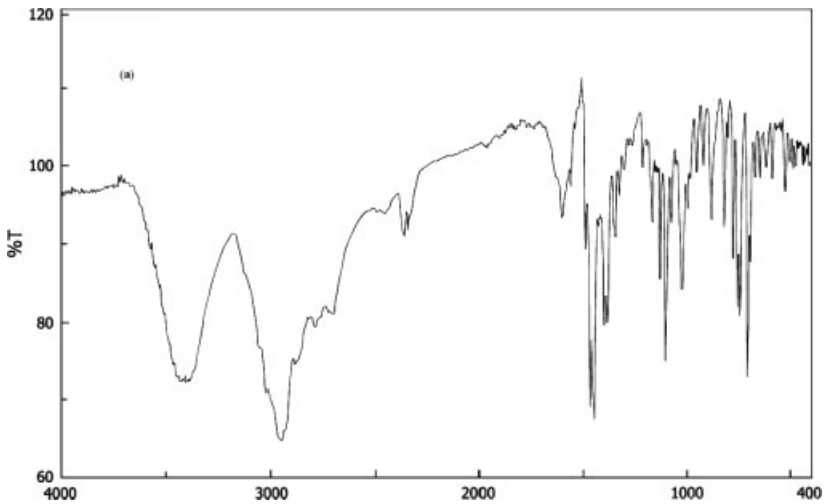


Figure 8. The IR spectra of diphen–I_2 Inner/outer-complex.

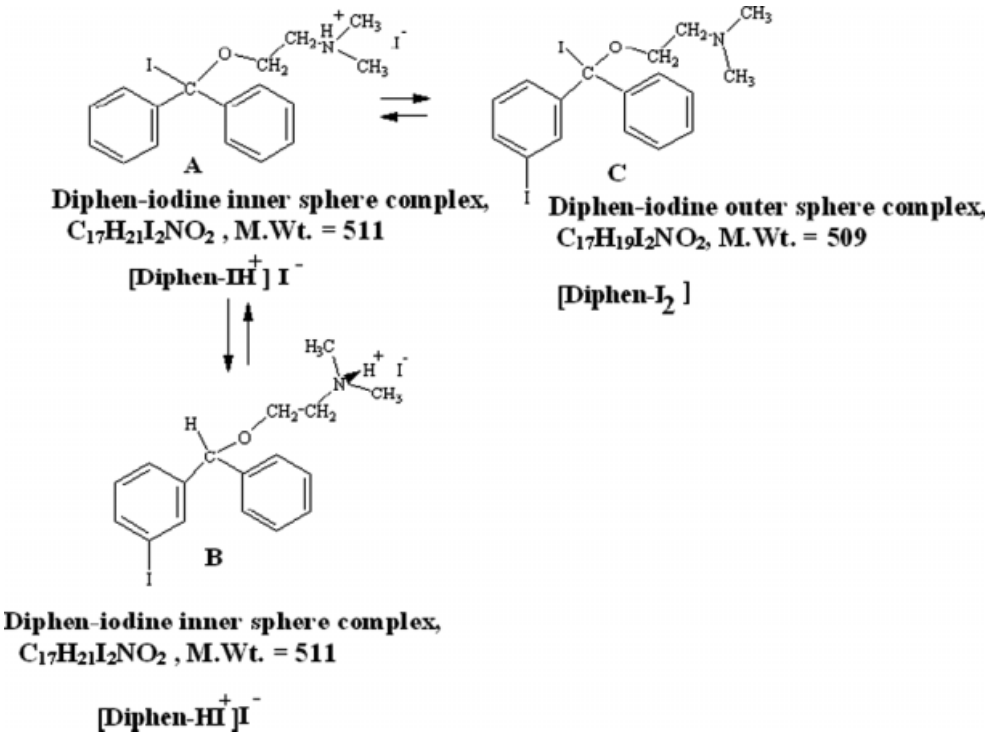


Figure 9. The proposed structural formulae of diphen-I_2 products.

ical followed by recombination of the two phenyl radicals to give the stable molecular ion of m/z = 152 as a final product. Thermal analyses cannot give such detailed fragmentation pathways.

The final conclusion is that the teamwork explanation between TA, EI-MS and MOC gives a highly effective complementary detailed picture of the fragmentation of the drug *in vitro* system which can help to understand the possible metabolites be formed during administration and assimilation of the drug *in vivo* systems.

Structure identification of diphen-I_2 product by different physicochemical methods of analyses

The structure of diphen-iodine solid product has been identified by different physicochemical tools aiming chiefly to shed light on the mechanism of the reaction between diphen and iodine in solution. The methods used for such structure identification of the reaction product obtained are elemental analyses (C, H, and N); IR, ^{1}HNMR, ^{13}CNMR; thermal analyses (TGA, DTGA and DTA), and EI-MS spectrum. The reaction in solution between diphen refers to the formation of compound of 1 : 1 stiochiometric ratio as given by both molar ratio and continuous variation methods. In this reaction the drug acts as donor (D) and iodine reagent acts as an a σ-acceptor to form CT- inner or outer complex, i.e., may be [D-I^-]I^+ or [D]-I_2 product.

Structure identification of the product by elemental analyses:

The elemental analyses of the diphen-I_2 product (1 : 1) are given as the calculated element % (Calcd.%) depending on the proposed general formula and found (F%) as practically obtained. These results are: Calcd. C% = 40.0% (F: C% = 39.47, 39.17%); Calcd. H% = 3.96 % (F: H% = 3.80, 3.87%) and Calcd. N% = 2.74 (F: N% = 2.68, 2.78%). These data refer to the possibility of the general formula of $C_{17}H_{20}I_2NO$ of mole mass = 508. This general formula give mass agree well with that obtained by mass spectrum of this product.

Structure identification of the diphen-I_2 complexes by IR spectra

The IR spectra (as KBr disk) of diphen–I_2 complexes are shown in Figure 8. It shows bands at 420–500 cm^{-1} which may be due to υ_{C-I} different modes of vibrations. The bands at 600–750 cm^{-1} may be due to the different modes of vibrations of υ_{C-N} and υ_{C-O} groups. The bands at 800–1170 cm^{-1} may be attributed to the υ_{C-H} of aliphatic C–H bridge different modes of vibrations, whereas the bands at 1170 to 1750 and 2350–3406 cm^{-1} may be assigned as υ_{C-H} of C–H different modes of the two aromatic rings of the product. The IR spectrum (as a KBr disk) of diphen itself[30] is greatly different from that of the product. The drug parent shows principal peaks at wave numbers: 713,754, 1103, 1017, 1180 and 991 cm^{-1}.

Depending on the results of elemental analyses and IR data, the proposed structural formula of diphen-I_2 product can be given by Figure 9. The more possible structure is that given by either A or B, because this compound is soluble in polar solvents and precipitated by non-polar petroleum ether, and recrystallized from a mixture of polar and non-polar solvents.

Structure identification of diphen-iodine product by ^{1}HNMR and ^{13}CNMR

The above proposed structures of diphen-iodine product (forms A, B and C) are confirmed by practical ^{1}HNMR (Figure 10). These data show that the ^{1}HNMR of parent (Figure 10) is greatly different from that of diphen-I_2 daughter (Figure 11).

The practical ^{1}HNMR of diphen-I_2 product (Figure 11) shows the chemical shift values of different protons of the compounds and

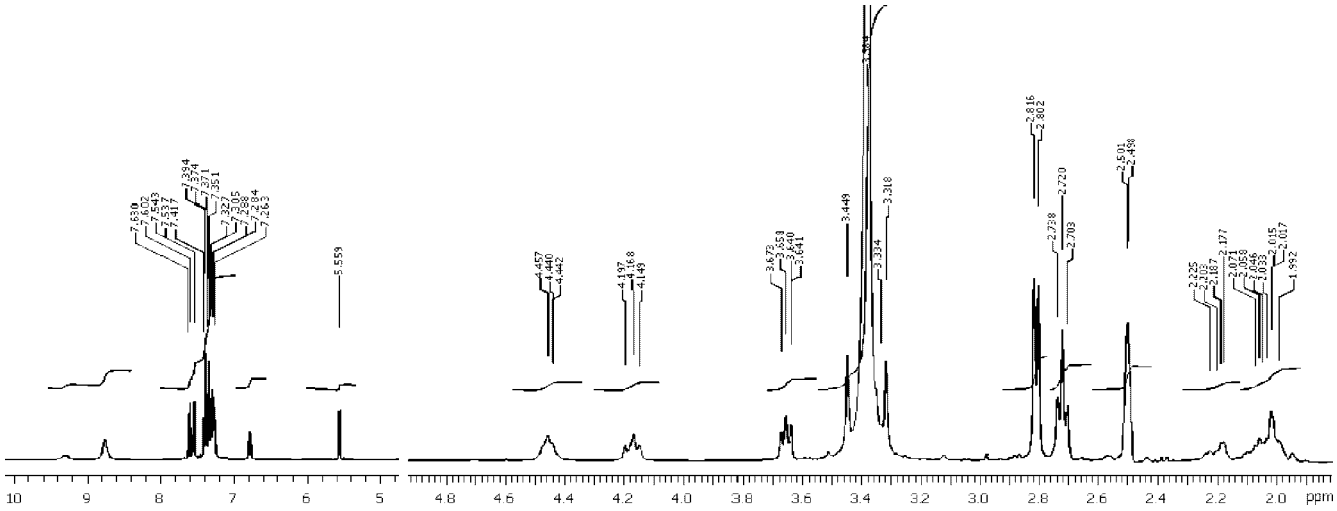


Figure 10. The ^{1}HNMR spectra of diphen-drug.

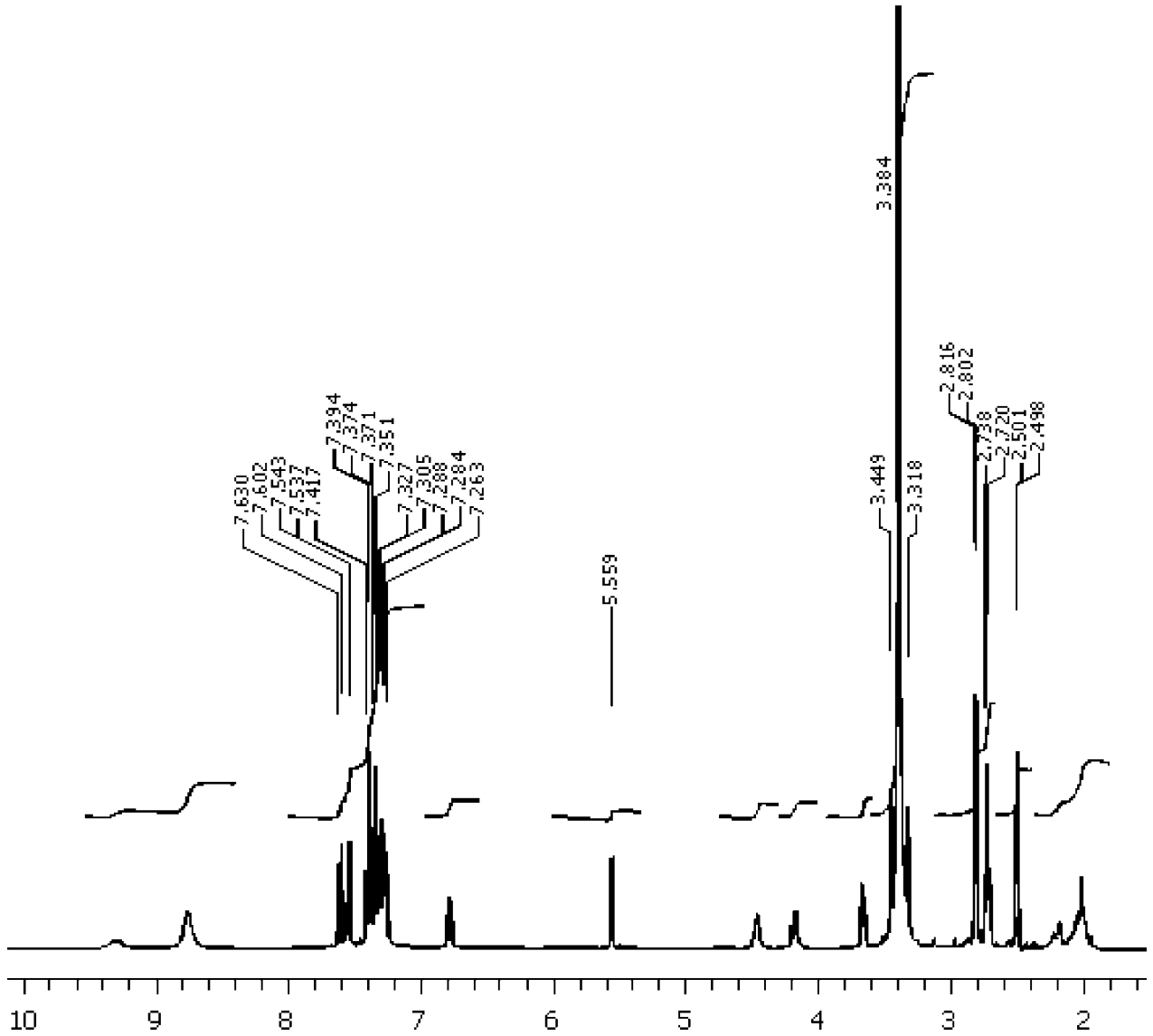


Figure 11. The ^{1}HNMR of diphen-I_2 product.

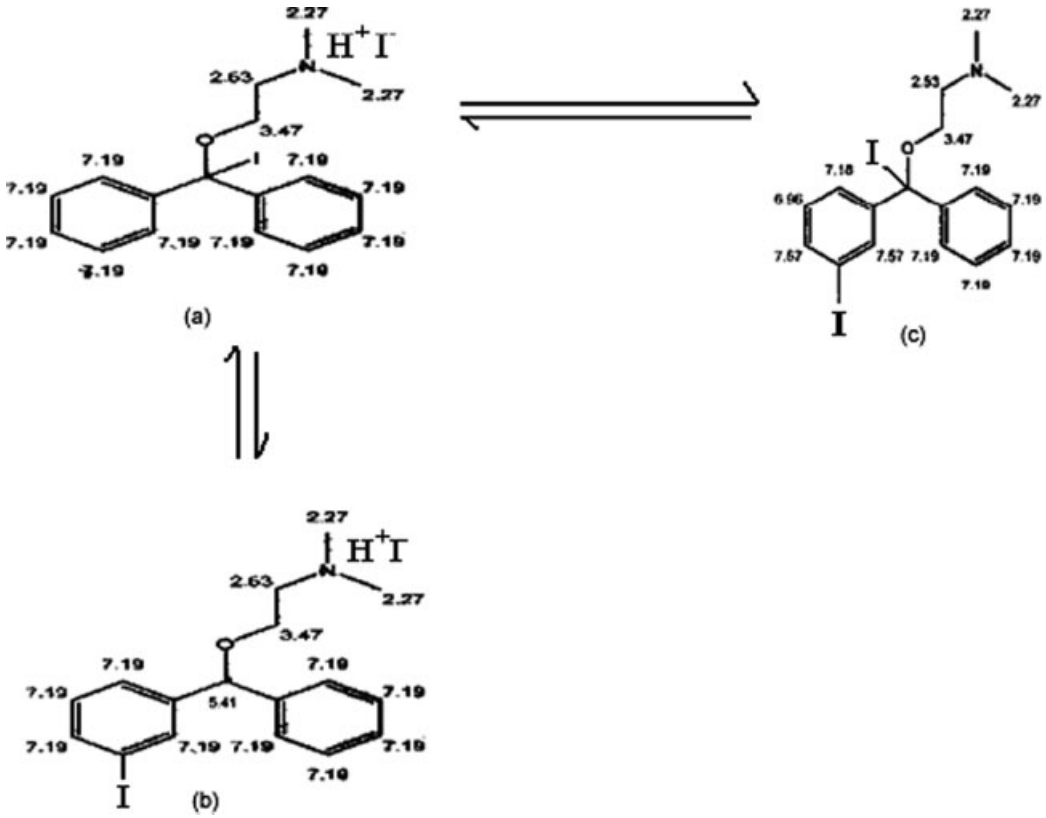


Figure 12. Theoretical ^{1}HNMR of the form B or C of diphen-I_2 Inner/or outer-complex.

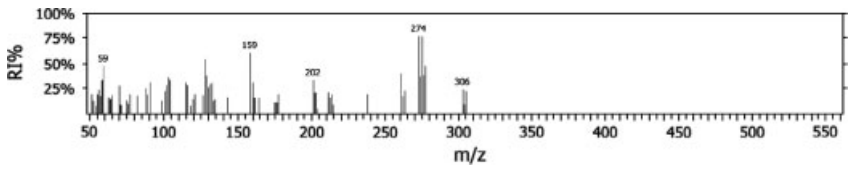


Figure 13. EI-MS of diphen-I_2 compound.

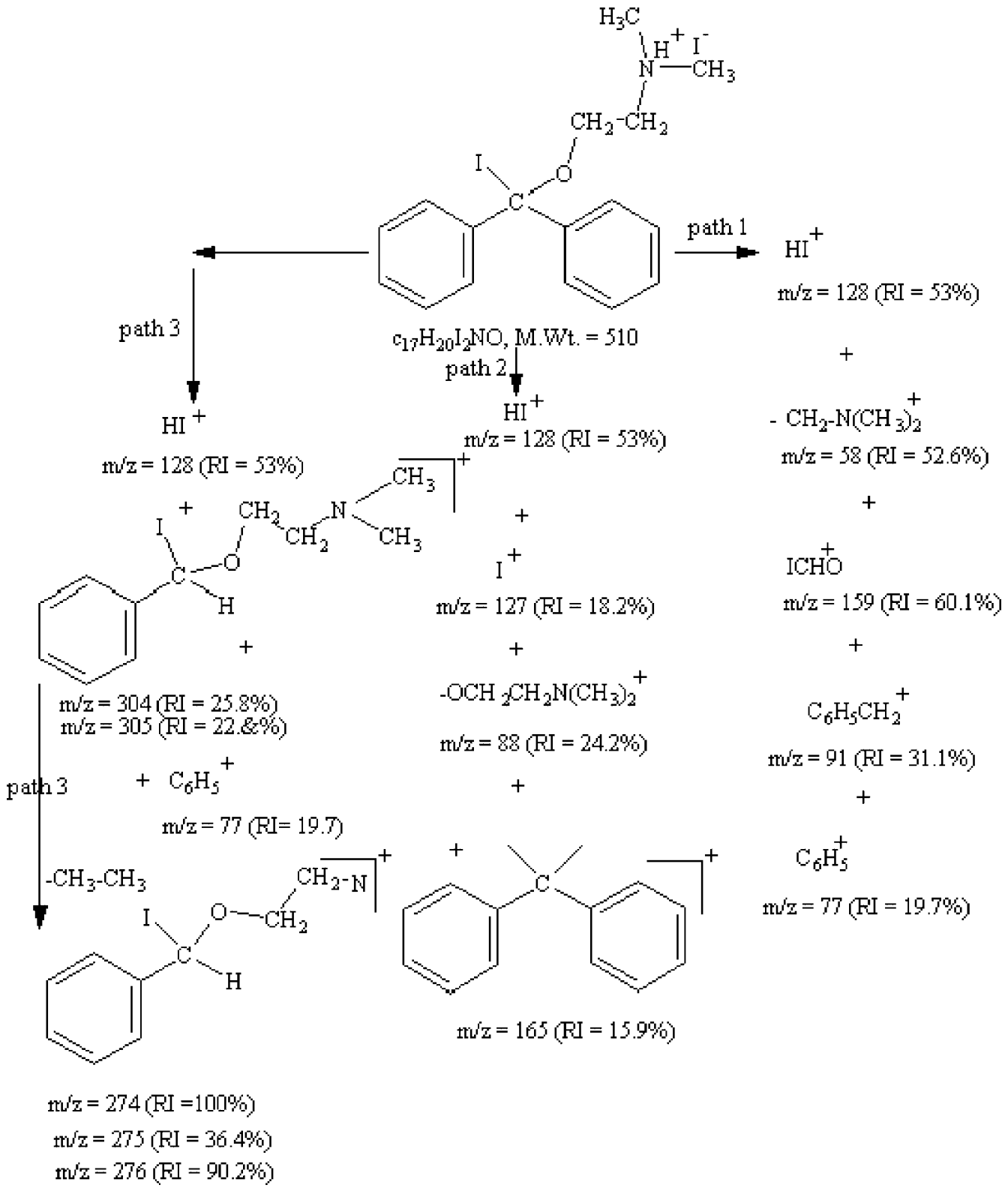
their mutual interactions. The protons of aliphatic side chain of O–CH_2–CH_2–N $(CH_3)_2$ appeared at σ = 2.498–2.816 ppm for the first two protons and at 3.318–3.449 ppm for the second nearby protons respectively (Figure 11). Some of their mutual interactions appeared at σ = 2.816–2.498 ppm and at σ = 4.149–4.457 ppm respectively. The six protons of two methyl groups attached to nitrogen atom in this side chain show their chemical shifts of σ = 1.992–2.225 ppm and their possible mutual interactions at σ = 2.216–2.816 ppm. The ten protons of the two benzene rings have their chemical shifts of σ = 7.263–7.630 ppm and their possible mutual interactions of σ = 6.768–6.793 ppm and 8.758 ppm.

In the estimated ^{1}HNMR data of the first proposed form of the compound (A) and the estimated data of the other alternative proposed forms (B or C) of the CT-compound are shown in Figure 12. The comparison between the practical and theoretical HNMR data can help to select the more possible proposed form (A, B or C) representing the CT-complex of diphen-I_2. In the estimated ^{1}HNMR of the form (A), the ten benzene rings protons have chemical shifts σ = 7.19–6.26 ppm; the chemical shifts of the two protons of O–CH_2–appeared as σ = 1.37–2.53 ppm and of CH_2–N appeared as σ = 2.04–3.47 ppm. The estimated chemical shifts of six protons of two methyl groups attached to N of the aliphatic side chain are of σ = 1.42–2.27 ppm. These obtained chemical shift values agree well with those practically obtained by the form (A), i.e., may be this is the most suitable proposed structural formula of the diphen – I_2 inner and outer sphere complexes. This conclusion is confirmed by the inspection of theoretical estimated chemical shifts of protons of the proposed structure (form B or C) in which iodine atom substituted in one of the two benzene rings. These data show that, the protons of the aliphatic side chain O–CH_2CH_2–N $(CH_3)_2$ and the two benzene rings appeared of σ = 2.27, 2.53, 3.47, 5.41, 7.17, 7.18, 7.19, 7.57, and 7.96. Therefore diphen-I_2 daughter can be represented by form B as inner sphere form and C as an outer sphere complex.

Structure identification by electron-ionization mass (EI-MS) spectra

Figure 13 shows the EI-MS spectra of the diphen – I_2 product. It seems greatly different from that of the parent drug (Figure 5).

This spectrum shows many fragment ions at m/z = 56 (RI = 23.5%), 58 (RI = 32.6%), 59 (RI = 47%), 77 (RI = 19.7%), 127 (RI = 18.2%), 128 (RI = 53%), 159 (RI = 60.6%),161 (RI = 30.3%), 274 (RI = 100%), 276 (RI = 90.2%), 305 (RI = 25.8%)



Scheme 3. EI-MS fragmentation pathways of diphen-I_2 compound.

and 306 (RI = 22.7%). These fragment ions are explained by Scheme (3). The appearance of the peak at m/z = 128 (RI = 53%), is attributed to the formation of HI^+ fragment ion from the product (forms A or B) as given by the first step of all proposed pathways (Scheme 3). In path 1, the second step explained the appearance of the peak at m/z = 58 (RI – 52.6%); which may be attributed to the formation of the fragment ion $-CH_2-N(CH_3)_2^+$ of the mole mass = 58 as a result of first decomposition of the aliphatic side chain of the product. This step is followed by the formation of the fragment

ion ICH_2O^+ (mole mass = 157) which appeared at m/z = 159 (RI = 60.1%). The third step involved the formation of the fragment ion $C_6H_5CH_2^+$ (mole mass = 91); which appeared at m/z = 91 (RI = 31.1%). The final step of this pathway is the appearance of the fragment ion at m/z = 77 (RI = 19.7%) due to the fragment ion $C_6H_5^+$.

In path 2, the appearance both peaks at m/z = 128 (RI = 53%) and m/z = 127 (RI = 18.2) are attributed to both fragment ions HI^+ and I^+ respectively as a result of their elimination from the entity of the diphen-I_2 complexes. It followed by the appearance of the fragment ion $-OCH_2CH_2N(NCH_3)_2^+$ at m/z = 88 (RI = 24.2%) as a result of decomposition of the side chain of the CT-complex. Finally the appearance of the fragment ion $(C_6H_5)_2C^+$ of m/z = 165 (RI = 15.9%). In path 3, both fragment ions HI^+ of m/z = 128 (RI = 53%) and $C_6H_5^+$ of m/z = 77 (RI = 19.7%) are attributed to their elimination from the moiety of the parent Ct-complex leaving the more predominant fragment ion $C_6H_5-CHI-O-CH_2-CH_2-N(CH_3)_2^+$ of m/z = 304 (RI = 25.8) or of m/z = 305 (RI = 22.7%). The last fragment ion loosed CH_3-CH_3 gas molecule obtained by the decomposition of the two methyl groups; leaving the most predominant fragment ion $C_6H_5-CHI-CH_2CH_2-N^+$ of m/z = 274 (RI = 100) which appeared as a base peak together with other alternation fragment ions of m/z = 275 (RI = 36.4) and of m/z = 276 (RI = 90.2).

References

[1] D. Barlocco, G. Cignarella, F. Montesano, A. Leonardi, M. Mella, L. Toma, *J. Med. Chem.* **1999**, *42*, 173.
[2] G. R. Tonn, A. Mutlib, F. S. Abott, D. W. Rurak, J. E. Axelson, *Biol. Mass. Spectrom.* **1993**, *22*, 633.
[3] D. Digregorio, J. Sherma, *J. Liq. Chromatogr. Related Technol.* **1999**, *22*, 1599.
[4] E. E. Muller, *J. Sherma, Liq. Chromatogr. Related Technol.* **1999**, *22*, 153.
[5] X. A. Gu, B. Fryirs, and E. Mather, *J. Chromatogr. B: Biomed. Appl.* **1998**, *719*, 135.
[6] L. Ye, J. T. Stewart, *J. Liq. Chromatogr. Rel. Technol.* **1996**, *19*, 711.
[7] Y. Xing, *Yaowu-Fenxi-Zazhi* **1991**, *11*, 186.
[8] Y. Zhang, C. Yang, *Zhongguo.yaoxue.zazhi* **1991**, *26*, 163.
[9] R. Ragonese, M. Mlholland, J. Kalman, *J. Chromatogr.* **2000**, *870*, 45.
[10] G. Xu, *Fenxi-Shiyanshi* **1997**, *16*, 17.
[11] A. Erdem, M. Ozasoz, L. Kirilmaz, C. L. Kilinc, T. Dalbarti, *Electrolysis (NY)* **1997**, *9*, 932.
[12] V. V. Egorov, V.A. Repin, *Zh. Anal. Kihm.* **1995**, *50*, 463.
[13] I. P. Gorelov, T. V. Tolokonova, *Zh. Anal. Kihm.* **1995**, *50*, 298.
[14] V. V. Egorov, V. A. Repin, *Zh. Anal. Kihm.* **1994**, *49*, 745.
[15] M. E. Blazheevskii, V. K. Zinchuk, *Farm.Zh. (Kiev)* **1988**, *3*, 43.
[16] A. Sachan, P. Trivedi, *Indian Drugs* **1999**, *36*, 735.
[17] S. Kalil, *J. Pharm. Biomed. Anal.* **1999**, *21*, 697.
[18] K. N. Wu, C. Fang, B. Tian, *Yaowu.Fenxi. Zazhi.* **1999**, *19*, 337.
[19] J. Guan, M. Xu, S. Cao, *Zhongguo Yiyuan. Yaoxue. Zazhi.* **1993**, *13*, 120.
[20] C. Nerin, J. Cacho, A. Garnica, *J. Pharm. Biomed. Anal.* **1993**, *11*, 411.
[21] J. H. Yoe, A. L. Jones, *Indust. Eng. Chem. Analyst. Ed.* **1944**, *16*, 111.
[22] A. E. Harvey, D. L. Manning, *J. Am. Chem. Soc.* **1950**, *72*, 4488.
[23] W. C. Vosburge, G. R. Cooper, *J. Am. Chem. Soc.* **1941**, *63*, 437.
[24] British pharmacopoeia **2000**, General Medical Council (Great Britain), The Stationery Office, p. 2208; 011322320X, R921.561/X3, 1, 200.00.
[25] W. Kemp, *Organic Spectroscopy*, 2nd edn, Macmillan Education Ltd: Hong Kong, **1982**.
[26] M. A. Zayed, S. M. Khalil, H.M. El-Qudaby, *Spectrochim. Acta A.* **2005**, *62*, 461.
[27] M.A. Zayed, M. F. Hawash, M.A. Fahmey, Abeer A. El-Habeeb, *Spectrochim. Acta Part A.* **2007**, *67*, 522.
[28] F. A. Nour El-Dien, M. A. Zayed, G. G. Mohammed, Reham G. El-Nahas, *J. Biomed. Biotechnol.* **2005**, *PII*, 1.
[29] E. H. Kerns, R. A. Rourick, K. J. Volk, M. S. Lee, *J. Chromatogr. Biomed. Appl.* **1997**, *698*, 133.
[30] Abeer A. El-Habeeb, *Ph.D. Thesis* **2007**, Comparative analytical study using integrated analytical chemistry techniques in analysis of some psychiatric and cold-antihistaminic drugs. Faculty of Education of Girls, Riyadh Saudi Arabia.