Asian Journal of Chemistry Vol. 20, No. 7 (2008), 5573-5580

Study of the Formation of Artifacts Following Dichloromethane Reaction with Some Nitrogenous Drugs

ALI MOHAMMADI*†, MOHSEN AMINI‡, MORTEZA PIRALI HAMEDANI‡, HOSSEIN HEIDARI TORKABADI and RODERICK B. WALKER§
Department of Drug and Food Control, School of Pharmacy
Medical Sciences/University of Tehran, Tehran, Iran
Fax: (98)(21)6461178; Tel: (98)(21)6959090; E-mail: alimohammadi@tums.ac.ir

In this work, the quaternization reaction of some nitrogenous drugs in dichloromethane under stress condition and room temperature at different times are studied. Under these conditions, drug-chloromethochloride adducts or artifacts were found to be formed for clozapine, ofloxacin and olanzapine. The structures of the resultant adducts were elucidated using ^{1}H NMR spectroscopy. In addition, the amount of intact drug was determined using in-house validated HPLC methods with UV detection.

Key Words: Adducts, Dichloromethane, Clozapine, Ofloxacin, Olanzapine, Chloromethochloride.

INTRODUCTION

The interaction of amines and some alkaloids with halogenated hydrocarbon solvents is well known[1,2]. These solvents and in particular dichloromethane are often used in many areas of research and development processes of various pharmaceutical compounds including synthesis, identification, purification, extraction, assay and metabolic studies. Information regarding interactions which will have an effect on the results of the relevant processes under investigation will improve quality control and therefore, enhance quality assurance purposes. In this work, the quaternization reactions of some drugs (Fig. 1)[3,4] in dichloro-methane under reflux and room temperature conditions at different times are reported. The structures of adducts were elucidated using ^{1}H NMR spectroscopy. In addition, the amount of intact drug was determined using in-house validated HPLC methods with UV detection.

†Medical Nanotechnology Research Centre, Medical Sciences/University of Tehran, Tehran, P.O. Box 14155-6451, Iran.

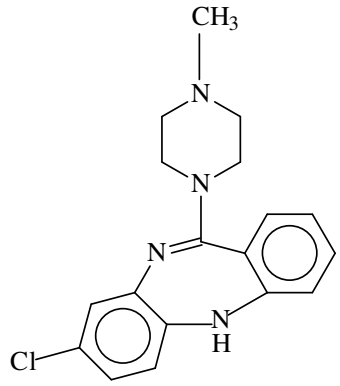
‡Department of medicinal chemistry, School of Pharmacy, Medical Sciences/University of Tehran, Tehran, Iran.

§Department of Pharmaceutics, Faculty of Pharmacy, Rhodes University, Grahamstown, South Africa; E-mail: r.b.walker@ru.ac.za

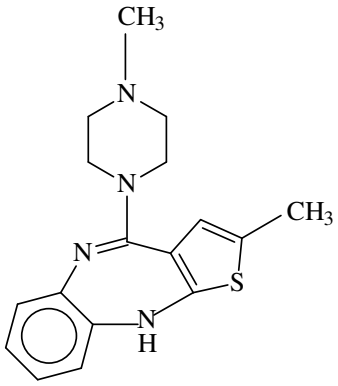
EXPERIMENTAL

Working standard powdered drugs were investigated (Fig. 1) and supplied by the Food and Drug Quality Control Laboratory of the Ministry of Health and Medical Education, Tehran, Iran. The drugs were used without further purification. Acetonitrile, methanol, dichloromethane, sodium hydroxide, hydrochloric acid, phosphoric acid and sodium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany). All chemicals were at least of analytical grade and were used as received.

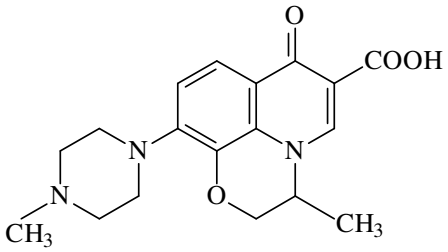
Purified HPLC grade water was obtained by reverse osmosis and filtration through a Milli-Q® system (Millipore, Milford, MA, USA).



Clozapine (pKa = 3.7, 7.6)



Olanzapine (pKa = 5.0, 7.4)



Ofloxacin (pKa = 7.6)

Fig. 1. Structural formulae of the studied drugs

^{1}H NMR spectra were recorded using a Bruker AC 80 MHz spectrometer with DMSO (Merck, Spectrophotometric grade) as the solvent. The HPLC system used for the determination of intact drug and consisted of a Waters® 600 controller solvent delivery module, a Waters® 717 plus autosampler, a solvent degasser, a variable wavelength detector (Waters Chromatography Division, Milford, MA, USA). A Millenium® Chromatographic Data System was coupled to the detector *via* a SAT/IN Module (Waters Chromatography

Division, Milford, MA, USA) and was used to record and evaluate the data collected during chromatographic analysis. Chromatographic separations were performed as follows:

Clozapine: Column: μBondapak C_{18}, 5 μm, 300 mm × 3.9 mm i.d., Mobile phase: methanol-KH_2PO_4 (0.01 M) buffer pH 7 (70: 30 v/v), Flow rate: 1 mL/min, UV detection wavelength: 254 nm, Column temperature: ambient, Injection volume: 20 μL.

Olanzapine: Column: μBondapak C_{18}, 5 μm, 300 mm × 3.9 mm i.d., Mobile phase: methanol-water (pH adjusted to 3 using dilute phosphoric acid (60:40 v/v), Flow rate: 1 mL/min, UV detection wavelength: 254 nm, Column temperature: 65 °C, Injection volume: 20 μL.

Ofloxacin: Column: Hypersil-CN column, 5 μm, 150 × 4.6 mm i.d., Mobile phase: methanol-water (pH adjusted to 3 using with dilute phosphoric acid (50:50 v/v), Flow rate: 1 mL/min, UV detection wavelength: 280 nm, Column temperature: ambient, Injection volume: 20 μL.

The mobile phases were filtered through a 0.45 μm Chrom Tech Nylon-66 filter prior to use.

Preparation of stock and standard solutions: Stock solutions of drug standards were prepared separately in methanol. Aliquots of the standard stock solutions were transferred separately using A-grade bulb pipettes into 10 mL volumetric flasks and the solutions were made up to volume with the relevant mobile phase to yield final concentration ranges of 1-40, 1-20 and 1-20 μg/mL for clozapine, olanzapine and ofloxacin, respectively. The stock solutions were freshly prepared on each day of analysis.

Synthesis of drug-chloromethochloride adducts: A suitable amount of each drug was accurately weighed and dissolved separately in dichloromethane (1 mg/mL). The resulting solution was refluxed at 70 °C for 72 h. The reaction solution was allowed to cool at room temperature and the resultant precipitate (adduct) was harvested by filtration. The precipitate was washed with dichloromethane and then dried under gentle steam of nitrogen whilst protecting the material from light using aluminium foil. The dried material was stored at 4 °C until required for analysis.

Investigation of formation of adduct under room temperature: In order to determine whether the adducts were formed at room temperature a suitable amount of each drug powder was accurately weighed, transferred into a volumetric flask and dissolved in dichloromethane (1 mg/mL). The volumetric flasks were sonicated for 10 min to effect complete dissolution and the solutions were then made up to volume with dichloromethane (1 mg/mL). The final solutions were protected from light using aluminium foil and left to stand in the laboratory. A 1 mL aliquot of the dichloromethane solution was sampled at 0, 0.25, 0.50, 1, 2, 4, 6 and 24 h during storing in the laboratory. The dichloromethane solution was then dried under a gentle

steam of nitrogen and any residue dissolved in the mobile phase into a 10 mL volumetric flask. In order to quantitate the amount of intact drug remaining a 20 μL aliquot of the reconstituted solution was injected onto the HPLC system using the relevant mobile phase.

RESULTS AND DISCUSSION

As mentioned previously all dichloromethane solutions were refluxed for 72 h. Under these stress conditions drug - chloromethochloride adducts (Fig. 2.) were formed for clozapine, ofloxacin and olanzapine. The structure of the relevant adduct was elucidated using ^{1}H NMR spectroscopy and the certificate of ^{1}H NMR spectrums of clozapine, ofloxacin, olanzapine and their dichloromethane adducts were assinged as follows:

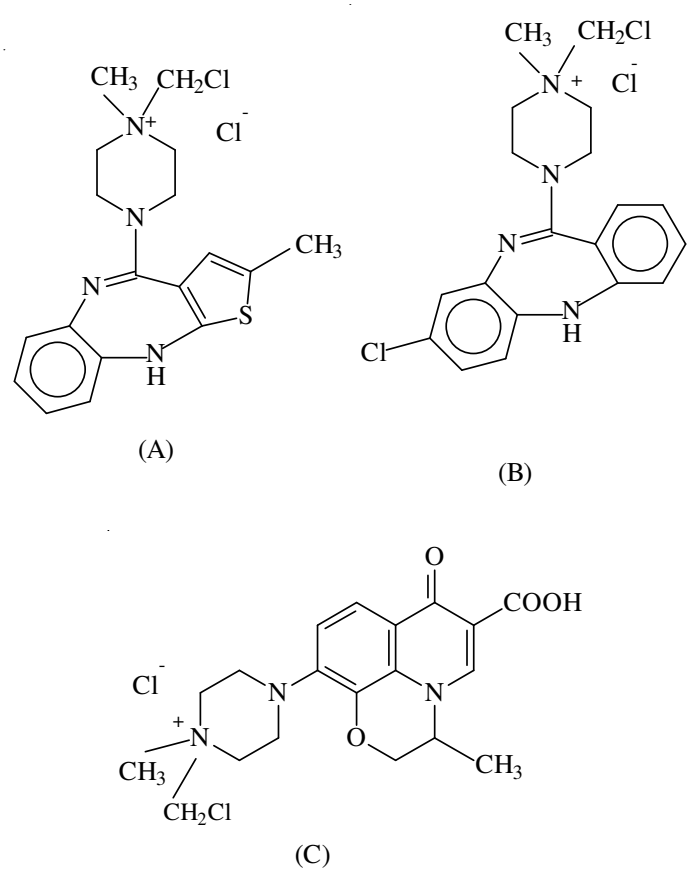


Fig. 2. Structures of drug-chloromethochloride adducts: (A) olanzapine chloromethochloride; (B) clozapine chloromethochloride; (C) ofloxacin chloromethochloride

Clozapine: ^{1}H NMR(DMSO-d_6) δ: 2.20 (S, 3H, N-CH_3), 2.37-2.50 (m, 4H, $H_{3,5}$-piprazine), 3.20-3.35 (m, 4H, $H_{2,6}$-piprazine), 6.65 (S, 3H, aromatic), 6.90-7.33 (m, 4H, aromatic), 7.5 (bS, 1H, NH).

Clozapine adduct: ^{1}H NMR (D_2O) δ: 3.63 (S, 3H, NCH_3), 3.90-4.20 (m, 8H, $H_{2,3,5,6}$-piprazine), 5.61 (S, 2H, -CH_2Cl), 6.90-7.33 (m, 7H, aromatic).

Ofloxacin: ^{1}H NMR (DMSO-d_6) δ: 1.41 (d, J = 6.4 Hz, 3H, CH_3), 2.23 (S, 3H, NCH_3), 2.30 (m, 1H, CH), 2.40 (m, 4H, $H_{3,5}$-piprazine), 3.20-3.40 (m, 4H, $H_{2,6}$), 4.35-4.55 (m,CH_2-benzoxazine), 7.5 (d, J = 12H_2, 1H, 8H-aromatic), 8.9 (S, 1H, HS-dihydropyridine), 11.09 (bS, 1H, aCOOH).

Ofloxacin adduct: ^{1}H NMR (DMSO-d_6) δ: 1.45 (d, J = 6.4 Hz, 3H, CH_3), 2.66 (m, 1H, CH), 3.10-3.60 (m, 8H, $H_{2,3,5,6}$-piprazine), 3.70 (S, 3H, NCH_3), 4.40-4.55 (m, CH_2-benzoxazine), 4.7-5.0 (m, 1H, H_3-benzoxazine), 5.66 (S, 2H, CH_2Cl), 7.40-7.70 (m, 1H, H_8-aromatic), 8.9 (S, 1H, HS-dihydropyridine), 11.16 (bS, 1H, COOH).

Olanzapine: ^{1}H NMR (DMSO-d_6) δ: 2.20 (S, 3H, Me-thiazole), 2.27 (S, 3H, N-CH_3), 2.30-2.55 (m, 4H, $H_{3,5}$-piprazine), 3.25-3.38 (m, 4H, $H_{2,6}$-piprazine), 6.33 (S, 1H, H-thiazole), 6.5-6.80 (m, 4H, aromatic), 7.57 (S, 1H, NH).

Olanzapine adduct: ^{1}H NMR (DMSO-d_6) δ: 2.20 (S, 3H, methiazole), 3.30 (S, 3H, NCH_3), 3.40-3.75 (m, 8H, $H_{2,3,5,6}$-piprazine), 5.57 (S, 2H, -CH_2Cl), 6.30 (S, 1H, H-thiazole), 6.50-6.80 (m, 4H, aromatic), 7.70 (S, 1H, NH).

^{1}H NMR analysis of the adducts indicated that the CH_2Cl signal occurred at 5.61, 5.66 and 5.57 ppm for clozapine, ofloxacin and olanzapine, respectively, while the relevant N-CH_3 signal occurred at a lower chemical shift for the parent compound.

The in-house analytical method used for the determination of each drug was validated with respect to parameters such as linearity, limit of quantitation (LOQ), limit of detection (LOD), precision and accuracy[5-8].

Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 1-40, 1-20 and 1-20 μg/mL for clozapine, olanzapine and ofloxacin, respectively. Peak areas of the drugs were plotted *versus* their respective concentrations and linear regression analysis performed on the resultant curves. Correlation coefficients were found to be more than 0.998 for all drugs with % RSD values ranging from 0.30-3.50 % across the concentration ranges studied. Typically, the regression equations were: y = 58.8x + 7.03 (R = 0.999), y = 93.96x + 37.9 (R = 0.999) and y = 59.74x + 7.88 (R = 0.998) for clozapine, olanzapine and ofloxacin, respectively.

The intra- and inter-day variability or precision data are summarized in Table-1 and were assessed by using standard solutions prepared to produce solutions of three different concentrations of each drug. Repeatability or intra-day precision was investigated by injecting nine replicate samples of each of the samples at three different concentrations. Inter-day precision was assessed by injecting the same three samples over three consecutive days.

TABLE-1
INTRA- AND INTER-ASSAY PRECISION DATA (n = 9)

Actual concentration	Measured concentration (μg/mL), RSD (%)	
Clozapine (μg/mL)	Intra-day	Inter-day
1	1.03, 1.50	1.025, 3.06
10	9.92, 2.05	10.31, 1.45
40	39.90, 0.45	40.80, 0.75
Ofloxacin (μg/mL)	Intra-day	Inter-day
1	1.02, 3.10	1.01, 2.85
5	5.15, 2.79	4.94, 1.64
20	20.15, 1.75	20.09, 2.10
Olanzapine (μg/mL)	Intra-day	Inter-day
1	0.99, 2.30	1.00, 2.50
10	9.85, 2.53	10.28, 3.501
20	19.95, 1.55	20.25, 0.88

Data expressed as mean for measured concentration values.

Accuracy was determined by interpolation of replicate (n = 6) peak areas of three accuracy standards of different concentration, from a calibration curve that had been prepared as previously described. In each case, the percent relevant error and accuracy (Table-2) was calculated and found to be less than 3.0 % for each of the compounds under investigation.

Typical chromatograms obtained following the analysis of standard drug solutions in mobile phase and drug solutions following treatment with dichloromethane at room temperature are depicted in Fig. 3. Following storage at room temperature and in dichloromethane for 15 min, 100 % of clozapine had been quaternized with methylene chloride. Consequently the peak for clozapine was not seen in the HPLC chromatogram following analysis (Fig. 3B). In contrast only 60 % of the ofloxacin quaternized with dichloromethane under the same conditions (Fig. 3D). Complete quaternization of the ofloxacin by methylene chloride occurred following exposure to dichloromethane for 4 h. Drug-chloromethochloride adducts are insoluble in dichloromethane. Therefore, produced adducts did not appear on chromatograms. Although there is no direct correlation between the structure of

TABLE-2
ACCURACY DATA (n = 6)

Clozapine concentration (μg/mL)	Interpolated concentration	RSD (%)	RE (%)
1	0.980 ± 0.026	2.75	2.05
10	10.125 ± 0.140	1.35	1.23
40	40.350 ± 0.180	0.45	0.88
Ofloxacin concentration (μg/mL)	**Interpolated concentration**	**RSD (%)**	**RE (%)**
1	1.028 ± 0.032	3.20	2.81
5	4.920 ± 0.100	2.03	1.62
20	20.250 ± 0.220	1.07	1.26
Olanzapine concentration (μg/mL)	**Interpolated concentration**	**RSD (%)**	**RE (%)**
1	1.024 ± 0.015	1.45	2.42
10	9.750 ± 0.100	1.02	2.48
20	20.110 ± 0.130	0.65	0.56

Data expressed as mean ± SD for interpolated concentration values and mean data shown for % RE.

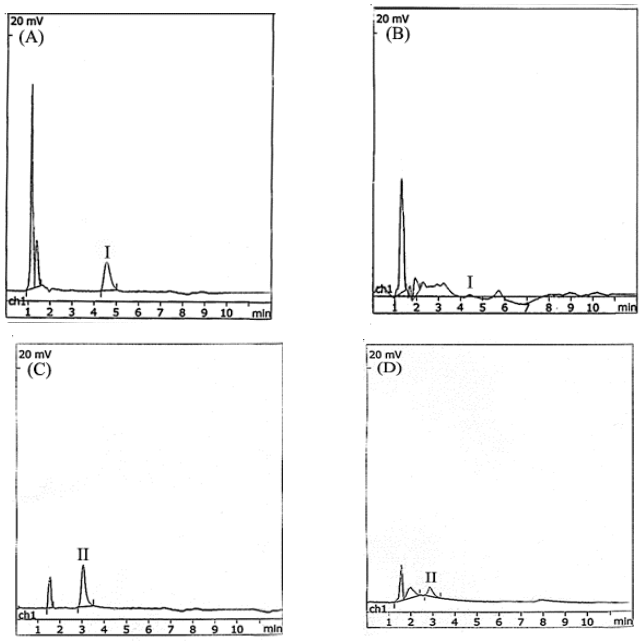


Fig. 3. Typical HPLC chromatograms of: (A) clozapine standard solution in mobile phase (1 μg/mL); (B) clozapine solution in dichloromethane following storage for 15 min at room temperature (observed concentration: not detected) (C) ofloxacin standard solution in mobile phase (1 μg/mL); (D) ofloxacin solution in dichloromethane following storage for 15 min at room temperature (observed concentration: 0.38 μg/mL) showing clozapine (I) and ofloxacin (II)

compounds and their interaction with dichloromethane, it is notable that their basicity and unfavourable steric parameters at the amine nitrogen are important structural features of the amino functional group and which define the nucleophilic nature of this reaction. Such interactions will have an effect on the results of drug analysis and their interpretation in particular when the drugs to be analyzed are present in low concentrations or amounts as is often the case in metabolic studies. A previously reported HPLC method for the quantitation of ofloxacin[9] revealed a low recovery of *ca.* 80 % which may be a consequence of quaternization effects following extraction with dichloromethane. It is therefore, important that a thorough examination of the impact of this organic solvent on drug stability be ascertained prior to its use in analytical procedures.

Conclusion

In this paper, the possibility of the interaction of some nitrogenous drugs with dichloromethane under stress and room temperature conditions have been reported. Under these conditions, drug-chloromethochloride adducts were formed for clozapine, ofloxacin and olanzapine. This reaction is an unwanted consequence of the use of dichloromethane and may generate interference in drug samples analyzed following extraction with this commonly used solution. It is therefore important that a thorough investigation in the use of this organic solvent be undertaken out prior to its use in quality control studies.

ACKNOWLEDGEMENT

The authors would like to acknowledge financial assistance from Medical Sciences/University of Tehran, Tehran, Iran.

REFERENCES

1. A.H. Beckett and H.M. Ali, *J. Chromatogr. B*, **177**, 255 (1979).
2. A.H. Gainza, R.I. Konyeaso and R.A. Asenjo, *J. Pharm. Sci.*, **83**, 226 (1994).
3. A.C. Moffat, M.D. Osselten and B. Widdop, Clarke's Analysis of Drugs and Poisons in Pharmaceuticals, Body Fluids and Postmortem Material, Pharmaceutical Press, London, edn. 3 (2004).
4. M.J. O′Neil, A. Smith, P.E. Heckelman and S. Budavari, The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals, Merck & Co. Inc., White House Station, New Jersey, edn. 13 (2001).
5. J.C. Miller and J.N. Miller, Statistics for Analytical Chemistry, Chichester, Ellis Horwood (1984).
6. A. Mohammadi, I. Haririan, N. Rezanour, L. Ghiasi and R.B. Walker, *J. Chromatogr. A*, **1116**, 153 (2006).
7. A. Mohammadi, N. Rezanour, M.A. Dogaheh, F.G. Bidkorbeh, M. Hashem and R.B. Walker, *J. Chromatogr. B*, **846**, 215 (2007).
8. United States Pharmacopeia 28-NF 23, United States Pharmacopeial Convention, Rockville, MD, p. 2748 (2005).
9. D. Fabre, F. Bressolle, J.M. Kinowski, O. Bouvet, F. Paganin and M. Galtier, *J. Pharm. Biomed. Anal.*, **12**, 1463 (1994).

(*Received*: 19 October 2007; *Accepted*: 15 May 2008) AJC-6579