—Regular Articles—

Kinetic Study on the Degradation of Meclophenoxate Hydrochloride in Alkaline Aqueous Solutions by High Performance Liquid Chromatography

Mohammad Galal El-BARDICY, Hayam Mahmoud LOTFY, Mohammad Abdalla El-SAYED*, and Mohammad Fayez El-TARRAS

Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Kaser El-Aini Street, ET 11562, Cairo, Egypt

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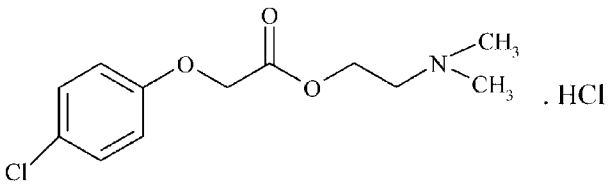
A high performance liquid chromatographic method was developed and validated for determination of meclophenoxate hydrochloride (I) in the presence of its degradation product (p-chlorophenoxy acetic acid) (II). Separation of (I) from (II) was performed using a ZORBAX ODS column with a mobile phase consisting of 0.2% triethylamine in 0.01 M ammonium carbonate: acetonitrile (70 : 30 v/v). The method showed high sensitivity with good linearity over the concentration range of 50 to 400 μg/ml. The method was successfully applied to the analysis of a pharmaceutical formulation containing (I) with excellent recovery. A kinetics investigation of the alkaline hydrolysis of (I) was carried out in sodium hydroxide solutions of 1, 1.5 and 2 N by monitoring the parent compound itself. The reaction order of (I) followed pseudo-first order kinetics. The activation energy could be estimated from the Arrhenius plot and it was found to be 12.331 kcal/mole.

Key words——high performance liquid chromatography; meclophenoxate hydrochloride; p-chlorophenoxy acetic acid; degradation kinetics

INTRODUCTION

Meclophenoxate hydrochloride [CAS number 51-68-3] [(4-chloro phenoxy) acetic acid-2-(dimethyl amino) ethyl ester] is a white powder, soluble in cold water and methanol, sparingly soluble in cold isopropanol and acetone, and practically insoluble in benzene, ether and chloroform.[1)]



It acts as a cerebral stimulant. It is used also as a plant growth regulator.[2)] It has been claimed to aid cellular metabolism in the presence of diminished oxygen concentrations. It has been given mainly for mental changes in the elderly or following strokes and head injury.[2)] Various chromatographic,[3—6)] colorimetric,[7)] radiochemical[8)] and proton magnetic resonance method[9)] have been used for determination of its concentration, which may not be suitable for the evaluation of the stability of meclophenoxate hydrochloride.

The present work aimed to develop a feasible, sensitive and specific analytical procedure for the analysis of meclophenoxate hydrochloride in the presence of its degradation product. Adaptation of the proposed procedure for the analysis of the available dosage form, including expired ones, is also an important task in order to solve problems encountered in quality control. Moreover, kinetic studies and accelerated stability experiments to predict expiry dates of pharmaceutical products necessitate such methods.

EXPERIMENTAL

Samples Meclophenoxate hydrochloride powder was kindly supplied by Minapharm. Its purity was checked in our laboratory according to the reported method of analysis[4)] and was found to be 99.39±1.144.

Lucidril tablets (batch no. 5GE0941 and 010156, expiry March 2004) were purchased from the Egyptian market. Each tablet is claimed to contain 250 mg of meclophenoxate hydrochloride. Lucidril tablets are manufactured by Minapharm Pharmaceutical Company under license to Lipha of France.

Reagents All chemicals used were of analytical grade and de-ionized water was HPLC grade. Sodium

*e-mail: boodi_75@yahoo.com

hydroxide, methanol, chloroform, acetic acid, acetonitrile for HPLC, ammonium carbonate, and triethylamine were obtained from Merck (Germany).

Apparatus Precoated HPTLC plates, silica gel 60 F_{245} 20×20 cm, 0.2 nm thickness, Macheray-Nagel (Germany).

Liquid chromatograph consists of an ''Agilent'' HPLC instrument, isocratic pump (Model G 1310 A pump, Agilent 1100 series), connected with an ultraviolet detector (Model G1314 A, Agilent 1100 series). The injector was a manual Rheodyne injector (Model 7725I, Rohnert Park, CA, USA) equipped with a 20 μl injector. The instrument was connected to an IBM compatible PC and an HP diskjet 5652 printer.

Mass spectrophotometer, Helwett Packard MS-5988A with electron energy 70 eV for detection of the degradation product.

Chromatographic conditions A ZORBAX ODS column (250 mm×4.5 mm I.D), particle size (5 μm) was used for the analysis. The mobile phase consisted of 0.2% triethylamine in 0.01 M ammonium carbonate: acetonitrile (70 : 30 v/v). The mobile phase was filtered using 0.45 μm membrane filters and degased by ultrasonic vibrations for 30 min. The system was operated at ambient temperature. The flow rate was isocratic at 1.0 ml/min and the detector was operated at 277 nm. The injection volume was 20 μl.

Mass Spectroscopic Conditions Temperature programming from 35℃ to 150℃ (final temperature) at 35℃ min^{-1} and electron energy 70 eV.

Procedures

Preparation of Alkaline Degradation of Meclophenoxate Hydrochloride Weigh 500 mg of meclophenoxate hydrochloride and dissolve in 50 ml of 2 N sodium hydroxide, and then reflux the solution at 100℃ for 25 minutes.

Take 1 ml of the solution, cool at room temperature, and dilute with methanol to test for complete degradation. Spot the degraded solution and standard solution of (I) on HPTLC plates. Place the plates in a chromatographic tank previously saturated for one hour with the mobile phase chloroform: methanol: acetic acid (1 : 1 : 0.1 v/v/v) and then air dry. Visualize the spots under UV light at 254 nm.

Render the medium acidic using concentrated hydrochloric acid to precipitate the degradation product. Filter then recrystalize the degradation product from isopropyl alcohol.

Linearity Into a series of 10 ml volumetric flask, transfer accurately aliquot portions 0.5—4.0 ml at 0.5 ml intervals from the meclophenoxate hydrochloride stock solution (1.0 mg/ml in methanol) and complete to volume with methanol. Inject 20 μl of the previous solutions into the liquid chromatograph using the chromatographic conditions described above. Measure the corresponding peak area and construct a calibration curve representing the relative area under the peak of meclophenoxate hydrochloride to that of the external standard (100 μg/ml), versus the corresponding concentrations of meclophenoxate hydrochloride in μg/ml.

Kinetic Studies

For Studying the Kinetic Order of the Reaction: In a 50 ml volumetric flask, dissolve 500 mg of meclophenoxate hydrochloride in 2 N sodium hydroxide and complete to the mark with the same solvent. Transfer this solution into another clean dry conical flask and reflux in a thermostatically controlled water bath at 80℃ for 25 minutes. Take 1.0 ml samples at 5 minutes intervals, place into 25 ml volumetric flasks half filled with cold methanol, neutralize with 1 ml 2 N hydrochloric acid, and complete the volume with cold methanol. Inject the solutions (initial concentration C_o=400 μg/ml) in the liquid chromatograph using the chromatographic conditions described above. The concentration of (I) was calculated from the regression equation. Plot the log of % of meclophenoxate hydrochloride remaining against time.

For Studying the Effect of Sodium Hydroxide Concentration on the Reaction Rate: Into a series of 50 ml volumetric flasks dissolve 500 mg of meclophenoxate hydrochloride in 1.0, 1.5, and 2 N NaOH and complete to the mark with the same solvent. Transfer these solutions into other clean dry conical flasks, and then reflux in a thermostatically controlled water bath at 80℃ for 25 minutes. Take 1.0 ml samples at 5 minutes intervals and then complete as described in Sec. for Studying the Kinetic Order of the Reaction.

Plot the log of % of meclophenoxate hydrochloride remaining against time for different normalities of NaOH and calculate the rate constant and $t_{1/2}$.

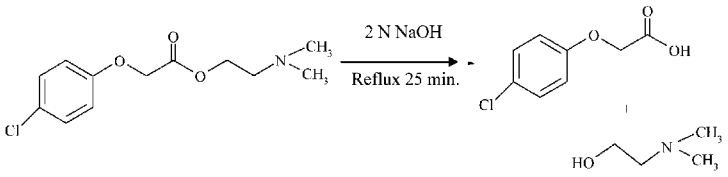
For Studying the Effect of the Temperature on the Reaction Rate: Dissolve three portions each of 500 mg of meclophenoxate hydrochloride in 50 ml volumetric flasks and complete the volume with 1.0, 1.5, and 2 N NaOH respectively. Transfer these solutions into other clean dry conical flasks and then reflux in a

thermostatically controlled water bath at 60℃, 70℃, 80℃ and 90℃ for 25 minutes. Take 1.0 ml samples at 5 minute intervals and then complete as directed under Sec. for Studying the Kinetic Order of the Reaction.

Plot the log of % of meclophenoxate hydrochloride remaining against time at different temperatures. Also plot the Arrhenius plot for the effect of temperature on the rate of hydrolysis.

RESULTS AND DISCUSSION

Degradation of Meclophenoxate Hydrochloride Proposed scheme for preparing the degradation product:



Mass spectroscopy was performed for the degradation product (p-chloro phenoxy acetic acid) and the parent peak was identified at m/z=187, which is the molecular weight of p-chloro phenoxy acetic acid. N, N dimethyl ethanolamine is a volatile compound characterized by a fishy odour which is why TLC cannot detect it. It also has no absorbance at 277 nm so it does not interfere with the proposed method of analysis.

Meclophenoxate and its acid degradation product have the same UV spectrum since the conjugation moiety does not change. Meclophenoxate can be hydrolyzed in aqueous solution[10] but it was stable in water for 6 hrs. This has been confirmed by TLC.

High Performance Liquid Chromatographic Analysis A simple isocratic high-performance liquid chromatographic method is described for the determination of meclophenoxate hydrochloride in the presence of its acid degradation product without prior separation. To optimize the HPLC assay parameters, the type of column and its dimension, mobile phase condition, and choice of wavelength of detection were investigated. Different types of stationary phase C_8 and a ZORBAX ODS column with different dimensions and particles size were used. It was found that the ZORBAX ODS column (250 mm×4.5 mm I.D) with a particle size of 5 μm gave the most suitable resolution. The peak shape improved dramatically with an increase in the percentage of 0.01 M ammonium carbonate to acetonitrile in the mobile phase. At higher ratio of acetonitrile, the retention time was increased with tailing of the drug peak, which was reduced by the addition of triethyl amine. Satisfactory separation was obtained with a mobile phase consisting of 0.2% triethylamine in 0.01 M ammonium carbonate: acetonitrile (70 : 30 v/v) with a retention time of 5.39 ± 0.02 min for meclophenoxate hydrochloride and 2.70 ± 0.04 min for its alkaline degradation product (Fig. 1). System suitability was checked by calculating the capacity factor, tailing factor, column efficiency, and selectivity factor (resolution) (Table 1).[11-14]

Our method offered an advantage compared to the stability indicating HPLC assay performed by Tatsuhara and Tabuchi,[4] whose mobile phase was adjusted to pH 3.1 *i.e.*, acidic. The pH of this method was alkaline (pH 9.0), this allowed the ionization of the acid degradation product which increased its polarity leading to a better resolution.

A linear relationship was obtained between the relative peak area at the selected wavelength (277 nm) and the corresponding concentrations of meclophenoxate hydrochloride in the range of 50—400 μg/ml, by adopting the external standard method for calibration.

The regression equation was computed and found to be:

$$A = 0.0094C + 0.0405 \quad r = 0.9997$$

Where A is the relative peak area of the analyte to that of the external standard (100 μg/ml), C is the concentration of the drug in μg/ml and r is the correlation coefficient.

Method Validation The selectivity and specific-

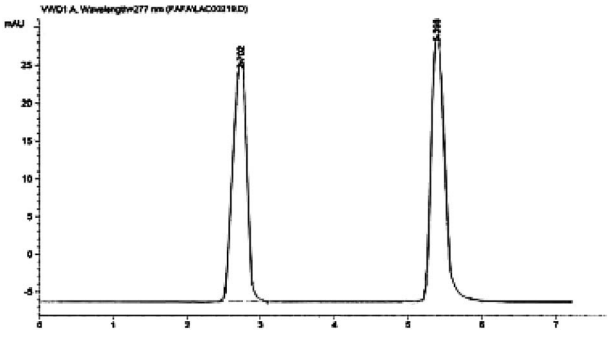


Fig. 1. Liquid Chromatographic Separation of Meclophenoxate

HCl (100 μg ml^{-1}) and its degradation product (100 μg ml^{-1}) using the chromatographic conditions described in the text.

Table 1. Statistical Analysis of the Parameters Required for System Suitability Test of HPLC Method

Parameter	Obtained value		Reference value
	Meclophenoxate HCl	Degradation product	
Resolution (R)	3.676		R>0.8
T (tailing factor)	1.05	1.0	T=1 for a typical symmetric peak
α (relative retention)	1.99		>1
k' (column capacity)	4.398	1.702	1—10 acceptable
N (column efficiency)	728.4	268.16	Increases with efficiency of the separation
HETP	0.0343	0.093	The smaller the value, the higher the column efficiency

Table 2. Determination of Meclophenoxate Hydrochloride in Laboratory Prepared Mixtures by the Proposed Method

Concentration (μg.ml^{-1})		Percentage %		HPLC method
Meclophenoxate HCl	Degradation product	Meclophenoxate	Degradation product	Recovery % Meclophenoxate HCl
350	50	87.5	12.5	99.12
300	100	75	25	101.56
250	150	62.5	37.5	98.75
200	200	50	50	102.03
150	250	37.5	62.5	98.51
100	300	25	75	100.47
50	350	12.5	87.5	99.07
Mean				99.94
S.D.				1.319

Table 3. Determination of Meclophenoxate Hydrochloride in Lucidril Tablets by the Proposed Method

Lucidril tablets claimed to contain 250 mg Batch number	HPLC method Recovery % ± S.D.*	Reported method** Recovery % ± S.D.*
5GE0941	100.87 ± 1.197	100.29 ± 1.411
010156 (expiry 3/04)	83.91 ± 1.002	84.12 ± 1.373

* Average of three determinations. ** Chromatographic method.

ity of the proposed method was proved by the analysis of laboratory prepared mixtures containing different ratios of the drug and its degradation product, and it was found to be valid until the content of degradation product was 87.5% (Table 2).

To ascertain the accuracy of the proposed procedure, it was successfully applied for the determination of meclophenoxate hydrochloride in Lucidril tablets (Table 3) and its validity was assessed by applying the standard addition technique. The small relative standard deviations indicate that the method is accurate (Table 4).

The results obtained for the analysis of meclophenoxate hydrochloride in the pure powdered form were statistically compared with those from a previously reported method.[4] A significant difference was not observed[17] (Table 5).

The precision of the suggested method was also expressed in terms of relative standard deviation of the inter-day and intra-day analysis. The method was checked for its robustness by minor changes in assay conditions and it proved to be robust. Changes in instruments or personnel did not alter the results, which indicate the ruggedness of the proposed method. The obtained assay parameters and a validation sheet are presented in Table 6.

Table 4. Application of Standard Addition for the Determination of Meclophenoxate Hydrochloride by the Proposed Method

Batch number	Standard added (mg)	HPLC method	
	Meclophenoxate HCl	Found of added (mg)	Recovery % of added
5GE0941	100.00	98.96	98.96
	150.00	152.00	101.33
	200.00	202.42	101.21
Mean±S.D.			100.53±1.277
010156	100.00	98.50	98.50
(Expiry 3/04)	150.00	151.60	101.06
	200.00	198.70	99.35
Mean±S.D.			99.63±1.303

Table 5. Statistical Comparison for the Results Obtained by the Proposed Method and the Reported Method for the Analysis of Meclophenoxate Hydrochloride in Pure Powder Form

	HPLC method	Reported method*
	Meclophenoxate HCl	Meclophenoxate HCl
Mean	99.94	99.39
S.D.	1.148	1.144
Variance	1.317	1.308
n	8	6
F test	1.006 (4.88)	
Student's *t* test	0.888 (2.179)	

The figures in parenthesis are the corresponding tabulated values at $p=0.05$.[17] * Chromatographic method.

Table 6. Assay Parameters and Method Validation for Meclophenoxate Hydrochloride

Parameter	HPLC method
	Meclophenoxate HCl
Range (μg ml^{-1})	50—400
Slope	0.0094
Intercept	0.0405
Mean	99.94
S.D.	1.148
Variance	1.317
RSD%	1.148
Correl. Coef. (r)	0.9997
*RSD%[a)]	1.110, 1.236
*RSD%[b)]	1.428, 1.418
**LOQ	15 μg ml^{-1}

* RSD%[a)] and RSD%[b)] are the intra-day, and inter-day ($n=5$) relative standard deviation of concentrations (200 and 300 μg ml^{-1}) for meclophenoxate HCl. ** LOQ: limit of quantification.

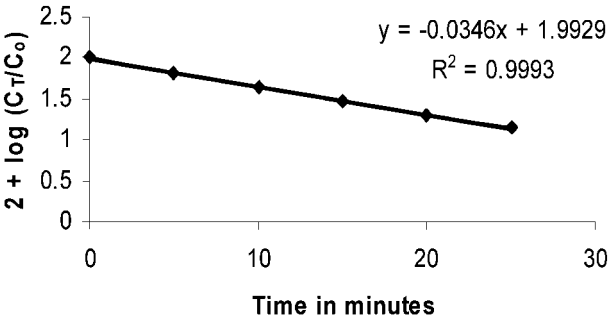


Fig. 2. First Order Plot of the Hydrolysis of Meclophenoxate Hydrochloride (1000 mg%) with 2 N NaOH at 80℃

Kinetics of the Degradation A linear relationship was obtained by plotting the log concentrations of the remaining (I) against time (Fig. 2). Since the hydrolysis was performed in a large excess of sodium hydroxide (2.0 M), it follows a pseudo-first order reaction rate[15] which is the term used when two reactants are involved in the reaction but one of them is in such a large excess (NaOH) that any change in its concentration is negligible compared with the change in concentration of the other reactant (drug).

Different parameters that affect the rate of the reaction were studied. The effect of temperature was studied by conducting the reaction at different temperatures using different concentrations of the alkaline solution (Figs. 3, 4 and 5). At each temperature the rate constant and $t_{1/2}$ were calculated and then the log of the rate constant was plotted against the reciprocal of the temperature in Kelvin units (Arrhenius plot (Fig. 6)) to demonstrate the effect of temperature on the rate constant. It was concluded that as the temperature increased the rate of hydrolysis increased with a decrease in the $t_{1/2}$ (Table 7). Also, the energy of activation was determined by calculating the rate constant from the following equation.[16]

$$\log \frac{K_2}{K_1} = \frac{E_a}{2.303R}\left(\frac{T_2 - T_1}{T_1 T_2}\right)$$

Where "E_a" is the activation energy, "T_1" and "T_2" are the two temperatures degrees in Kelvin, "R" is the gas constant, and "K_1" and "K_2" are the rate constants at the two temperatures used.

The calculated "E_a" was found to be 51.148 kilo joule mol^{-1} which was a comparatively low value for esters, suggesting the instability of (I) in alkaline medium.

Another factor that affects the rate of the reaction is the alkaline strength of NaOH, thus different nor-

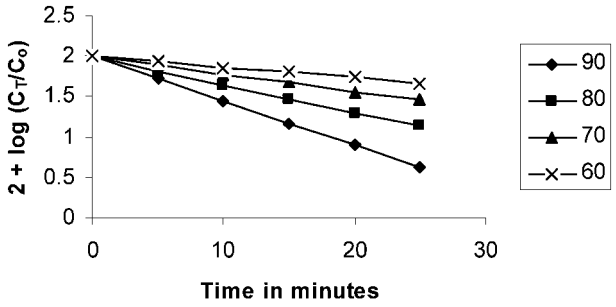


Fig. 3. First Order Plot of the Hydrolysis of Meclophenoxate Hydrochloride (1000 mg%) with 2 N NaOH at Different Temperatures

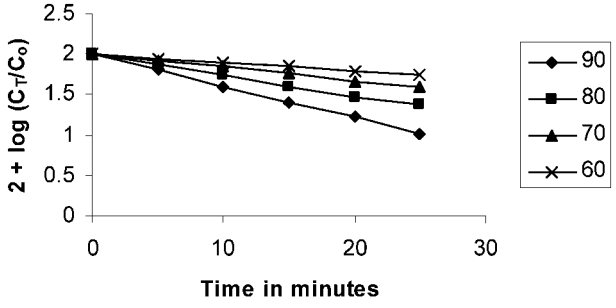


Fig. 4. First Order Plot of the Hydrolysis of Meclophenoxate Hydrochloride (1000 mg%) with 1.5 N NaOH at Different Temperatures

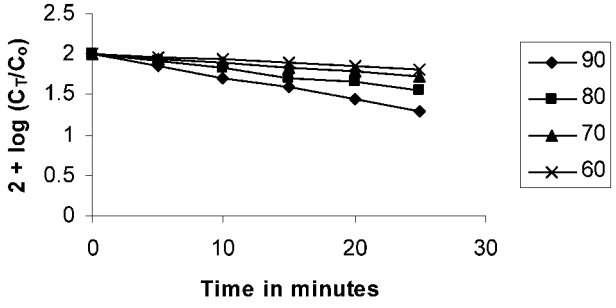


Fig. 5. First Order Plot of the Hydrolysis of Meclophenoxate Hydrochloride (1000 mg%) with 1.0 N NaOH at Different Temperatures

malities of NaOH solutions were used to study the hydrolysis reaction. The rate of hydrolysis increased with an increasing NaOH concentration, although the effect was minor compared to the effect of temperature (Figs. 3, 4 and 5) and (Table 7).

In conclusion, the alkaline hydrolysis of meclophenoxate hydrochloride was found to follow a pseudo first order reaction rate. Also the reaction rate increases with increase in the temperature and the strength of the alkaline solution.

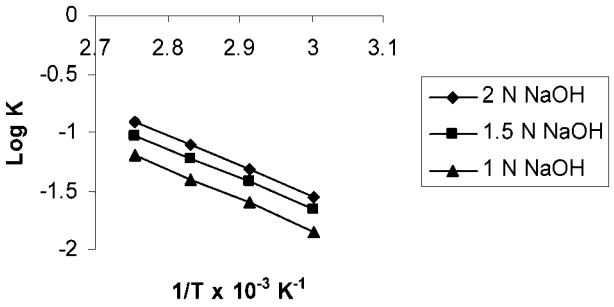


Fig. 6. Arrhenius Plot for the Hydrolysis of Meclophenoxate Hydrochloride (1000 mg%) with 1.0, 1.5 and 2.0 N NaOH

Table 7. Kinetic Data of Meclophenoxate Hydrochloride Hydrolysis

Normality of NaOH	Temperature	K in min^{-1}	$t_{1/2}$ in min
2.0 N NaOH	90℃	0.125	5.54
	80℃	0.079	8.77
	70℃	0.049	14.14
	60℃	0.028	24.75
1.5 N NaOH	90℃	0.093	7.45
	80℃	0.059	11.74
	70℃	0.038	18.23
	60℃	0.022	31.50
1.0 N NaOH	90℃	0.063	11.00
	80℃	0.040	17.32
	70℃	0.025	27.72
	60℃	0.014	49.50

CONCLUSION

The proposed HPLC method provides a simple, sensitive, and selective method suitable for the quality control analysis of meclophenoxate hydrochloride either in the pure powdered form or available pharmaceutical dosage forms.

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