CHROM. 4351

Determination of degradation kinetics of chlorzoxazone by thin-layer chromatography

In 1955 GARRETT AND CARPER[1] demonstrated the use of chemical kinetics to predict the stability of pharmaceuticals. However, due to the complex nature of most pharmaceuticals and to the complexity of degradation products, in many cases classical analytical methods proved to be too inaccurate and imprecise for such studies. Studies on degradation kinetics of newly developed chlorzoxazone-N-methyl-*d*-glucamine (chlorzoxazone-NMG) in nonaqueous solvents have been problematic. Spectrophotometric analysis was impossible due to the overlap of the absorbance spectra of the degradation products with that of chlorzoxazone (Fig. 1). Due to its low solubility in water-immiscible solvents and to the additional interference of breakdown products, chlorzoxazone could not be separated from its decomposition products by solvent extraction. Although a gas chromatographic method has been reported for the separation and identification of chlorzoxazone[2], it would be unsuitable for this formulation which is heat labile. Thus, TLC, which was initially used pharmaceutically[3] and whose use in this field has been reviewed by COMER AND COMER[4], offers a solution to such problems.

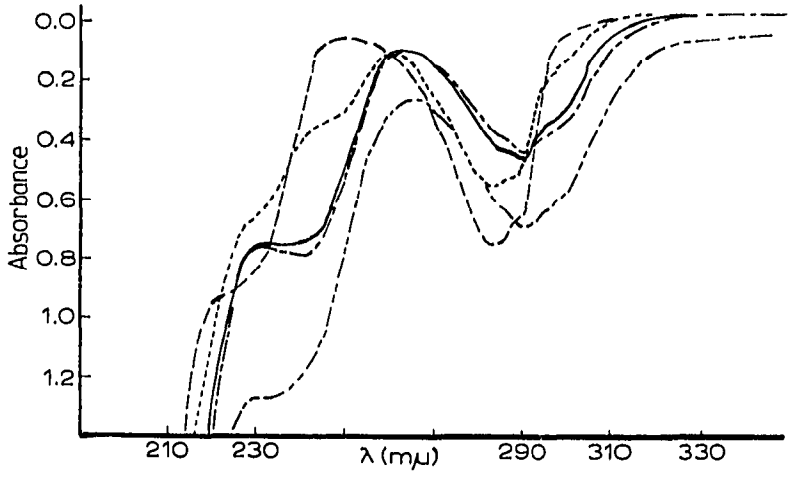


Fig. 1. Spectra of chlorzoxazone and its degradation products. — —, chlorzoxazone and also chlorzoxazone solution in PEG 200 containing sorbitol solution and glycerine (curves superimposed); - - - -, chlorzoxazone-NMG formulation immediately after preparation; ———, chlorzoxazone-NMG formulation after 4 h at 100°; — — — — —, chlorzoxazone-NMG formulation after 6 at 100°; ———————, chlorzoxazone-NMG formulation after 24 h at 100°.

The purpose of this investigation was to develop a simplified and accurate TLC method for the quantitative determination of our heat-labile chlorzoxazone formulations in the presence of decomposition products and to demonstrate the applicability of the method for conducting chemical kinetic studies on the degradation of complex pharmaceutical formulations.

Materials and methods

Chemicals and stability formulations. Chlorzoxazone (trademark Paraflex) was

generously supplied by McNeil Laboratories, Inc. (Fort Washington, Pa.), and N-methyl-*d*-glucamine (NMG) was obtained from K and K Laboratories, Inc. Methanol was spectro grade, while other chemicals and solvents were reagent or U.S.P. grade. Stability data are based on the formulation in Table I; other formulations studied differed in concentrations of NMG, sorbitol solution, glycerine and in the conditions under which they were prepared.

TABLE I

CHLORZOXAZONE-NMG FORMULATION FOR STABILITY STUDIES

Chemical	*Amount*
Chlorzoxazone	5.0 g
N-Methyl-*d*-glucamine	6.0 g
Sorbitol solution (U.S.P.)	5.0 ml
Sodium metabisulphite	0.1 g
Polyethylene glycol 200 q.s.	100.0 ml

Spectra of successively degraded formulations. One-ounce flint glass bottles, each containing about 25 ml of the formulation shown in Table I, were tightly sealed with aluminum foil and placed in an 100° oven. Samples were withdrawn at different time intervals and, after dilution with methanol, were scanned directly using a Perkin-Elmer Model 202 spectrophotometer. The spectra are shown in Fig. 1.

Adsorbents and solvents. Several adsorbents (Table II) were examined for selection by a standard procedure. As the stationary phase to be used in further studies, Silica Gel HF_{254} (Brinkmann Instrument, Inc.) was chosen on the basis of easy visualization of chlorzoxazone spots under UV light, stronger binding of the gel to the glass plates and no interference in the analytical procedure.

TABLE II

EVALUATION OF ADSORBENTS FOR THIN-LAYER CHROMATOGRAPHY

Adsorbent	*Binding of adsorbent*	*Detectability of chlorzoxazone*			*Contaminating ions*[a]
		Visible light	*Short wave UV light*	*Long wave UV light*	
Aluminium Oxide G	Poor	No	No	No	Ca^{2+}
Silica Gel G	Poor	No	No	No	Ca^{2+}
Silica Gel H	Good	No	No	No	None
Silica Gel GF_{254}	Good	No	Yes	No	Ca^{2+}
Silica Gel HF_{254}[b]	Good	No	Yes	No	None

[a] Taken from the labels of the containers.
[b] Adsorbent selected for use in TLC.

Because chlorzoxazone is soluble in methanol but is sparingly soluble in chloroform, several mixtures of these solvents (Table III) were examined. The solvent mixture, a 9:1 ratio of chloroform–methanol, which gave an R_F value of 0.58 (Table III) was selected as the mobile phase for further studies. Using a standard TLC method

TABLE III

R_F VALUES OF CHLORZOXAZONE FOR VARIOUS SOLVENT MIXTURES

Ratio chloroform–methanol	*Approximate R_F values*[a]
19:1	0.32
9:1[b]	0.58
4:1	0.87
7:3	1.00
3:2	1.00

[a] Each value is an average of two experiments.
[b] Solvent mixture selected for use in TLC.

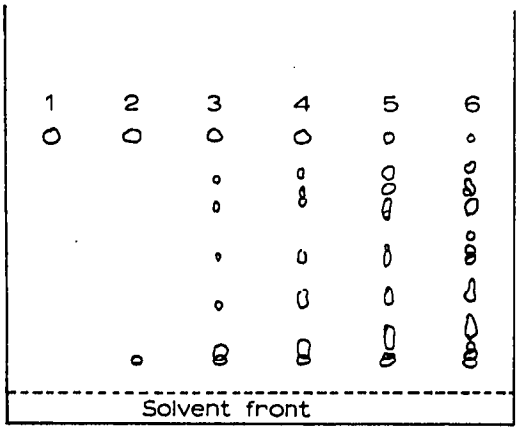
and Silica Gel HF_{254} as the stationary phase, chloroform–methanol (9:1) afforded greater separation of chlorzoxazone from degradation products as well as from other ingredients in the formulation.

Preparation of thin-layer plates. Standard 20 × 20 cm chromatographic plates were coated with a 0.25-mm layer of the adsorbent using a Desaga Model S11 applicator (Brinkmann Instrument, Inc.). The plates were prepared and activated according to instructions from Brinkmann Instrument, Inc. The activated plates were prewashed with the solvent system, dried and stored in a dessicator.

Development of chromatograms. The samples to be assayed were diluted fivefold with methanol. For each formulation, 20 μl of the diluted solutions were applied as four 5-μl spots to the previously prepared chromatograms, and as many as 16 spots (4 formulations) could be run on each chromatogram. These chromatograms were developed in Desaga rectangular trough chambers lined with Whatman No. 3MM filter papers. The chambers were saturated with the solvent vapor by being allowed to stand for about 20 min before use. The solvent front was permitted to rise approx. 14 cm from the bottom edge of the plates. These plates were air dried, and the chlorzoxazone spot was marked under shortwave UV light. Numerous breakdown products were visualized by placing the resolved chromatograms in a saturated iodine chamber for about 15 min (Fig. 2). The size of the areas marked was kept constant for each spot in order to maintain a constant adsorbent blank.

Removal and elution of spots. A simple scraping and eluting unit was obtained by modifying an assembly described by MOTTHER AND POTTERAT[5]. The glass portion of a medicinal dropper (10 cm long) was used for this purpose. The collars were filed off, and one side of this open end was flattened in a flame to give a smooth scraping surface. The narrow end of the dropper, firmly packed with glass wool, was attached to a vacuum line. Each area was scraped off the plate and completely drawn into this assembly by the vacuum. The assembly was supported above a volumetric flask. The chlorzoxazone was eluted with 8–9 ml of methanol without any transfer of the adsorbent, and the volume made up to 10 ml with methanol. These solutions were filtered through teflon filter discs having a 5-μ pore diameter (Millipore LSWP01300). The absorbances of these solutions were recorded at 238 mμ using a Beckman-DU spectrophotometer. The readings were corrected with the adsorbent blank (methanol)[6], and the concentrations of the drugs were determined from the calibration curve.

Preparation of the calibration curve. Solutions of chlorzoxazone were prepared



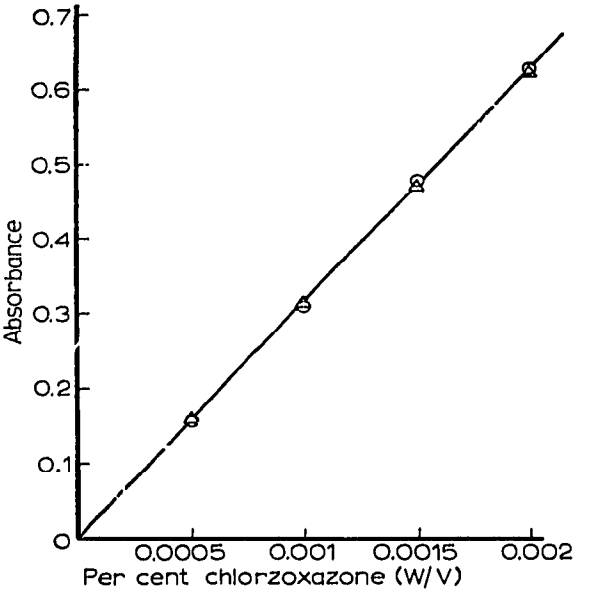


Fig. 2. Thin-layer chromatogram showing separation of chlorzoxazone from its solutions and from its degradation products (each position represents 5 μl of 1.0% chlorzoxazone after dilution with methanol). 1 = chlorzoxazone; 2 = chlorzoxazone solution in PEG 200 containing sorbitol solution and glycerine; 3 = chlorzoxazone-NMG formulation immediately after preparation; 4 = chlorzoxazone-NMG formulation after 4 h at 100°; 5 = chlorzoxazone-NMG formulation after 6 h at 100°; 6 = chlorzoxazone-NMG formulation after 24 h at 100°.

Fig. 3. Calibration curves for chlorzoxazone. O—O, after TLC-elution; △—△, after direct dilution.

in polyethylene glycol 200 (PEG 200) containing 2.5% each of sorbitol solution, propylene glycol and glycerine. These solutions were diluted with methanol to give different concentrations of chlorzoxazone. Each solution was resolved using the TLC method previously described, and after filtration the absorbance was measured. The readings were used to prepare a calibration curve.

A similar calibration curve was prepared by direct dilution of chlorzoxazone with methanol using absorbance readings at the same wavelength. Both these curves are shown in Fig. 3.

Reproducibility and recovery by TLC. Chlorzoxazone solution (5 w/v %) was prepared in PEG 200 containing a 5 % (v/v) sorbitol solution. The solution was assayed by the TLC–elution method and by a direct method in which the test solution was diluted with an appropriate amount of methanol and absorbances were read at 283 mμ. The results are shown in Table IV.

TABLE IV

COMPARISON OF THE TLC METHOD WITH THE DIRECT DILUTION METHOD

Method	*Replications (No.)*	*Actual value (mg/ml)*	*Mean assay value (mg/ml)*	*Standard deviation (mg/ml)*
Direct dilution	12	50.0	50.00	±0.15
TLC	12	50.0	49.77	±0.33

Accelerated stability studies. Chlorzoxazone-NMG formulations were placed in 40, 50 and 60° ovens. The samples were withdrawn at predetermined time intervals and assayed by the TLC–elution method. Data are shown graphically in Fig. 4. An Arrhenius plot, constructed for the rate constants calculated from Fig. 4, is shown in Fig. 5.

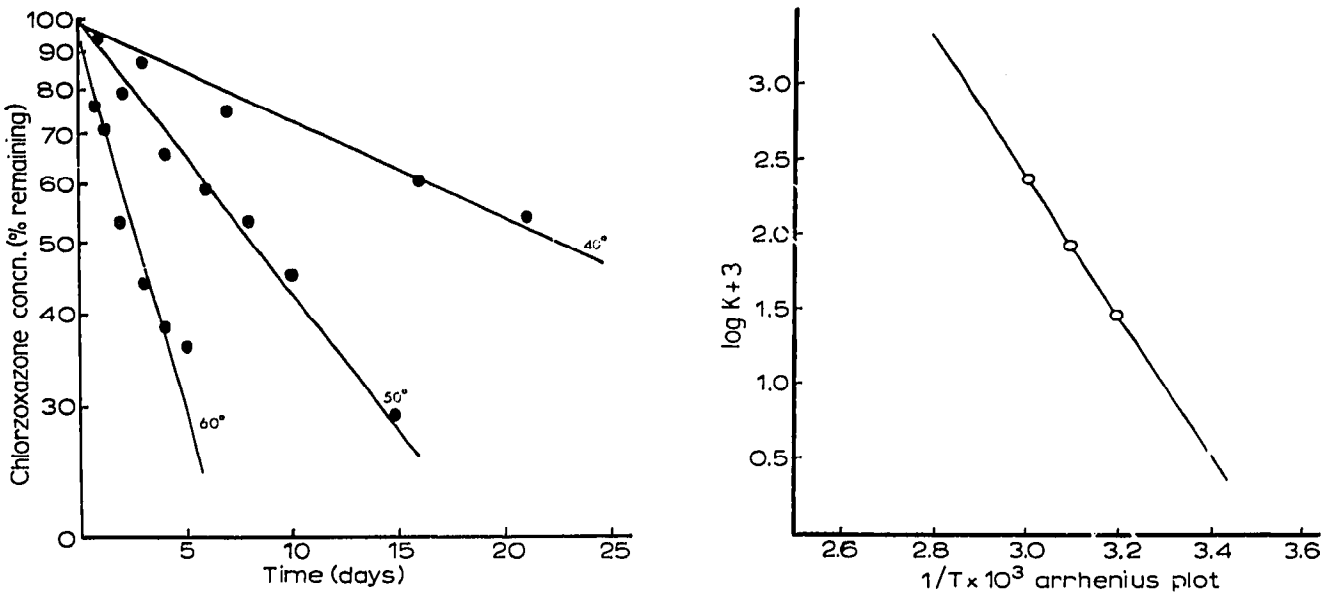


Fig. 4. Apparent first-order plots for degradation of chlorzoxazone-NMG formulation.

Fig. 5. Arrhenius plot for chlorzoxazone-NMG formulation.

Results and discussion

Two factors, *i.e.* the resolution of the active ingredient in the formulation and a satisfactory procedure for the quantitative analysis of this ingredient, were essential for the successful utilization of TLC in the study on degradation kinetics of complex pharmaceuticals.

Fig. 2 shows the resolution of chlorzoxazone and of its formulations at different levels of degradation. It is apparent from this figure that the spots of other products in the formulation neither overlapped nor interfered with the chlorzoxazone spots.

Excellent recovery was made possible by the simple collecting unit which could scrape, remove and elute the chlorzoxazone spots in the same unit without transfer. Almost complete recovery (99.5 %) of chlorzoxazone showed the accuracy of this TLC–elution method (Table IV). The small loss (0.5 %) was well within the limits of experimental error. The accuracy of the method was also apparent from the superimposed calibration curves (Fig. 3) based on direct dilution of chlorzoxazone and its recovery after being chromatographed. The low value of the standard deviation (99.5 $\pm$ 0.66, see Table IV) also indicated the high precision of this method.

The TLC–elution method was used successfully for studying the degradation kinetics of chlorzoxazone-NMG formulations. Fig. 4 shows good first-order degradation plots of the formulation under study. Rate constants of degradation calculated from Fig. 4 gave a good Arrhenius plot (Fig. 5). Using this plot it was possible to

predict the stability of the formulation at any desired temperature. The predicted values were well within the limits of experimental error. Using this TLC–elution method, the stability of other chlorzoxazone-NMG formulations could also be predicted with similar accuracy and precision.

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