Journal of Chromatography, 507 (1990) 151–156
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1705

High-performance liquid chromatographic method for studies on the photodecomposition kinetics of chlorothiazide

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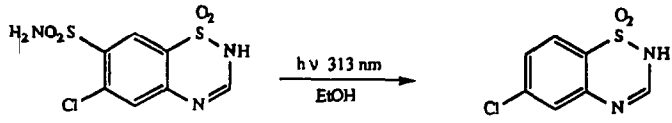
SUMMARY

A simple isocratic high-performance liquid chromatographic method for monitoring the photodecomposition of chlorothiazide in ethanolic solution is described. A 125-mm RP-18 column and an aqueous mobile phase containing 10% methanol and 2% acetic acid were used. The flow-rate was 1.0 ml/min and the UV detector wavelength 265 nm. Samples of 0.2 ml were taken from the reaction vessel and diluted 10-fold. The external standard method was used in the quantitative determinations. The decomposition appeared to proceed according to first-order kinetics. When the solutions were saturated with oxygen, the decomposition was greatly inhibited.

INTRODUCTION

Chlorothiazide (I) was synthesized in 1957 as the first member of the thiazide group[1]. Like the other thiazides, it is used in the treatment of oedema and hypertension[2]. The dose required is larger than that of newer compounds of the group.

The stability of the drug is not a problem in practice, because it is mainly used in tablet form. Hydrolysis of the compound has been studied under various conditions[3–5], but only the structure of the main photolysis product has been elucidated[6]. In ethanolic (ETOH) solution under UV irradiation the free $-SO_2NH_2$ group of the molecule is replaced with a hydrogen atom from the solvent yielding, 6-chloro-2*H*-1,2,4-benzothiadiazine-1,1-dioxide (II).



I II

The aim of this study was to develop a suitable high-performance liquid chromatographic (HPLC) method for the quantitative monitoring of II, as the main photolysis product of I. Because of the very low water solubility of the drug, the photolysis reactions were carried out in ethanolic solution.

0021-9673/90/$03.50

EXPERIMENTAL

Materials

Compound I was kindly supplied by Huhtamäki Oy (Turku, Finland). The isolation of the II was described elsewhere[6]. The identity and purity of the compounds were verified by IR, ^{1}H NMR and electron-impact mass spectrometry and by thin-layer chromatography (TLC). TLC examination of II using chloroform–methanol (4:1) as the eluent did not reveal any UV-absorbing impurities. HPLC-grade methanol (Rathburn) and water and Suprapur (Merck) acetic acid were used in the preparation of the mobile phase. All other chemicals were of analytical-reagent grade.

HPLC instrumentation

The HPLC equipment consisted of an LKB 2150 HPLC pump, LKB 2151 variable-wavelength monitor (at 265 nm), Merck-Hitachi D-2000 chromato-integrator and Rheodyne 7125 injector with a 20-μl loop. A 125 mm × 4 mm I.D. Hibar LiChrospher RP-18 column (particle size 5 μm) was used at a flow-rate of 1.0 ml/min. The mobile phase was methanol–water–acetic acid (10:88:2) (pH 2.7).

Irradiation of solutions of I

The four solutions investigated were in the concentration range 0.5–5 m*M*. Three of them were prepared by diluting of the 5 m*M* solution in a 10-ml volumetric flask to yield 0.5, 2.0 and 3.5 m*M* solutions.

The irradiations were carried out using a high-pressure mercury lamp (Original Hanau TQ 150) immersed in a water-bath (MGW LAUDA WB 20, at 25°C). The 313-nm line from the radiation of the lamp was isolated with a chemical filter consisting of potassium chromate and potassium hydrogenphthalate solutions[6]. The filter solutions in two quartz cuvettes (10 mm I.D.) were placed on a stand on the level of the arc of the lamp, aligned between the lamp and a third quartz cuvette (20 mm I.D.) (Fig. 1). The solution to be irradiated (6.5 ml) in the third cuvette was stirred with a small magnetic bar and another larger whirling bar on the side of the reaction vessel. Radiation from the sides was eliminated with small pieces of black plastic that were also used to support the cuvettes with the help of a press. The irradiation period was 30 min, after which the lamp was switched off and a 0.2-ml sample transferred into a 2-ml volumetric flask and diluted to volume. The 0.5 m*M* solution was analysed as such.

As the light stability of the filter solutions proved to be lower than expected, both of them were replaced with fresh solutions after every irradiation period. The lamp was restarted after 15 min.

When the effect of oxygen saturation was investigated, the gas was bubbled through the solution for 60 min before the irradiation and for 10 min after a sample was taken. All the irradiations were carried out twice.

Chromatography

The external standard method was used in the quantitative determinations. Unknown concentrations in the samples were calculated from the peak areas of a standard solution, generally 0.5 m*M* with respect to I and II. At the early stage of the work sulphadiazine was tested as an internal standard, but was found to be unsatis-

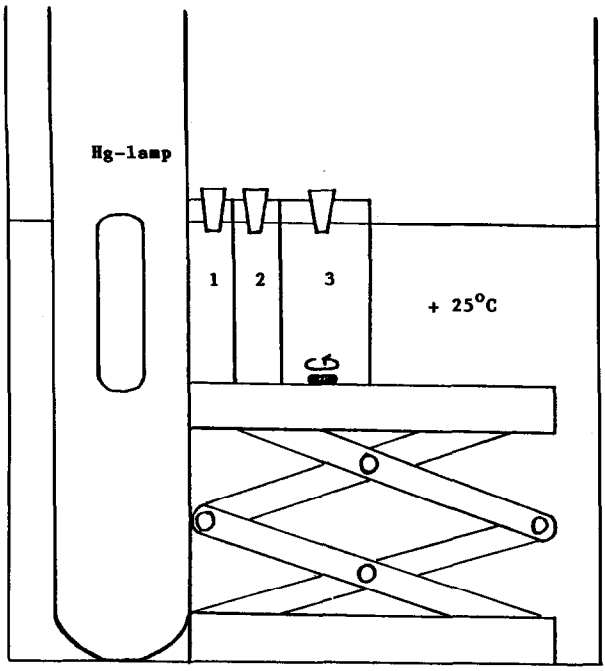


Fig. 1. Schematic representation of the irradiation apparatus used. 1 = Potassium chromate solution; 2 = potassium hydrogenphthalate solution; 3 = reaction vessel.

factory. No other compounds were investigated, as the precision of the peak areas of I and II was good. The injection volume used throughout the study was 3 μl. Before the analysis was started a solution of I was repeatedly injected into the column until the peak area became constant.

The calibration graphs were made with dilutions from 5 mM solutions of I and II over the concentration range 0.1–0.5 mM. The precision of the method was studied by analysing standard solutions of I and II at levels of 0.1 and 0.5 mM six times. In general triplicate injections were made of each sample from the irradiated solutions and an external standard was analysed after every fifth sample.

RESULTS AND DISCUSSION

A typical chromatogram from the irradiated solution is shown in Fig. 2. The retention times for I and II are 3.3 and 21.7 min respectively. The parent compound is well separated from the solvent peaks and from the product. When the flow-rate is 1.0 ml/min, the retention time of the product is relatively long, but it could be shortened by using a higher flow-rate or gradient elution. The calibration graphs were linear over the concentration range studied with correlation coefficients of 0.9995 for I ($n=21$) and 0.9997 for II ($n=21$). The equation corresponding to a typical calibration graph for I is expressed as $y = 1.27 \cdot 10^6 x - (5.3 \cdot 10^3)$ and that for II is $y = 1.43 \cdot 10^6 x - (1.78 \cdot 10^4)$, where x is the concentration of the compound injected (mM) and y is the corresponding peak area (integrator units).

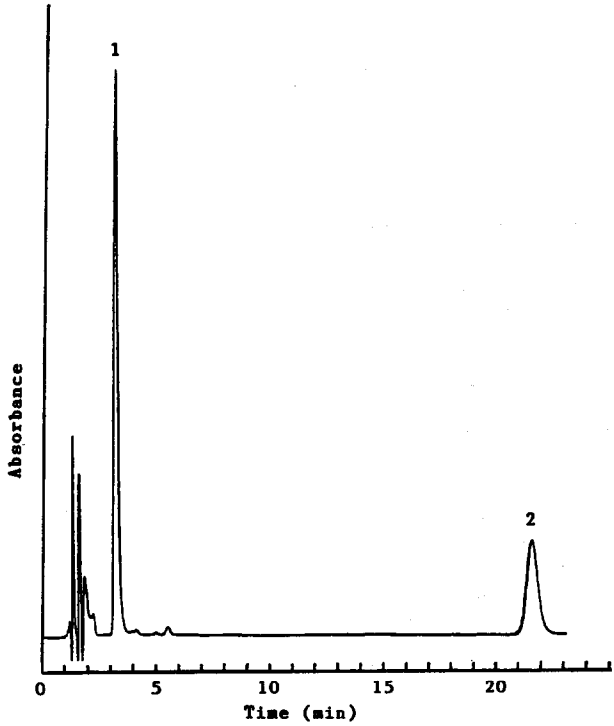


Fig. 2. Typical chromatogram of the irradiated solution. Peak 2 corresponds to II derived from 0.5 m*M* of I (peak 1) after 2 h irradiation exposure.

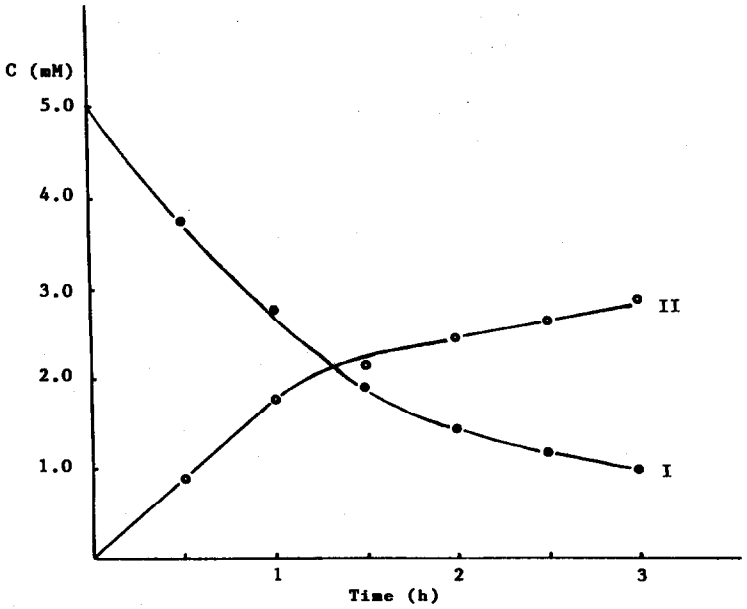


Fig. 3. Time courses for I and II in the photodegradation of a 5 m*M* solution of I.

TABLE I

FIRST-ORDER RATE CONSTANTS FOR THE PHOTODECOMPOSITION OF VARIOUS CONCENTRATIONS OF I IN ETHANOLIC SOLUTION

Concentration (m*M*)	0.5	2.0	3.5	5.0
k_{obs} (min^{-1})	$1.4 \cdot 10^{-2}$	$1.2 \cdot 10^{-2}$	$1.1 \cdot 10^{-2}$	$9.8 \cdot 10^{-3}$

The precision of the peak area expressed as the relative standard deviation was 0.5% ($n=6$) for I and 1.0% ($n=6$) for II at 5 m*M* and 2.1% ($n=6$) for I and 2.4% ($n=6$) for II at 0.1 m*M*. An example of product analysis is shown in Fig. 3. The disappearance of I followed first-order kinetics over the concentration range studied, which is normally the case in photochemical decomposition reactions[7]. The rate constants for the degradation were determined from the slopes of linear plots of the logarithm of residual I against time and are presented in Table I. The decomposition rate was found to be inversely proportional to the initial concentration of the parent compound with a correlation coefficient of -0.998. This can be explained by a greater inner filter effect in concentrated solutions.

The oxygen saturation of the solutions had a similar inhibiting effect on the decomposition of I as for hydrochlorothiazide[8] because of the ability of oxygen gas to quench excited states (Fig. 4).

By using the irradiation method presented it is possible to carry out photolysis reactions under exact and almost monochromatic conditions. As only one solution is

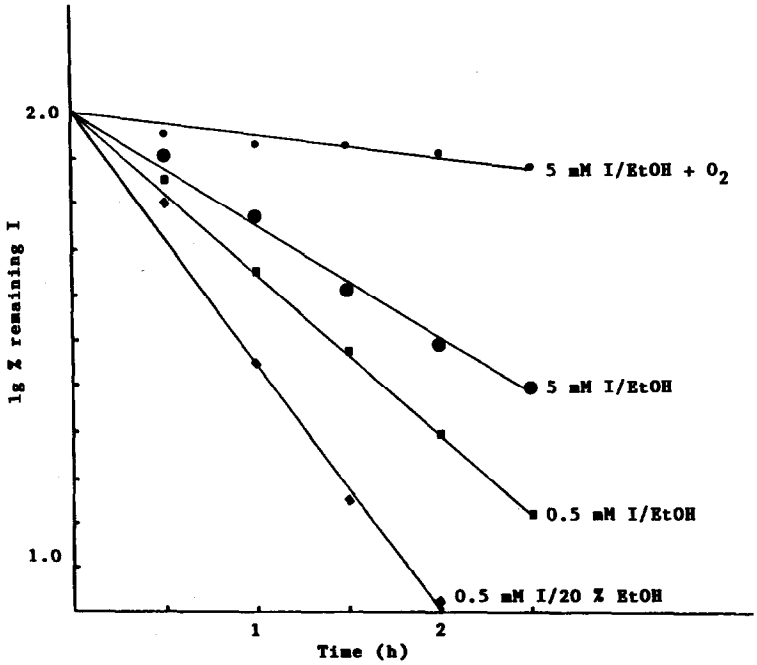


Fig. 4. Effect of concentration, oxygen saturation and solvent composition of the decomposition rate. EtOH = Ethanol.

irradiated at a time, the lamp used should have a relatively constant intensity. The HPLC procedure described is simple and precise and can be modified and used in similar investigations of the photochemical reactions of the thiazides.

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