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CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHODS IN DETERMINATION OF KINETICS OF 6–MERCAPTOPURINE DISULFIDE DISTRIBUTION

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Abstract: 6–Mercaptopurine disulfide is an intermadiate in the auto–oxidation reaction of 6–mercaptopurine. To determine its concentration in the presence of 6–mercaptopurine and other products of its decomposition, a HPLC method was proposed. Time changes in 6–mercaptopurine disulfide concentration ware assessed by UV spectrometry. Absorbance measurements at λ = 289 nm and 323 nm allowed to follow variations in the concentrations of 6–mercaptopurine disulfide and 6–mercaptopurine, respectively.

Keywords: 6–mercaptopurine disulfide, determination, HPLC and UV methods.

6–Mercaptopurine (6–MP) is a cytostatic from the group of antimetabolites of purine bases. The direction of transformations of this compound is determined by the sulfuryl group present in the 6–mercaptopurine molecule. 6–MP easily undergoes oxidation reaction to 6–mercaptopurine disulfide which, as a result of the disproportionation reaction, decomposes into 6–mercaptopurine and purine–6–sulfinic acid. The latter react wiht oxygen and, in a basic medium, forms hypoxantine or purine–6–sulfonic acid (1–2).

The aim of the paper is to test the usefulness of HPLC and UV methods for monitoring of changes in the concentrations of 6–mercaptopurine disulfide and 6–mercaptopurine.

EXPERIMENTAL

Chemicals

6–Mercaptopurine hydrate, s. 118F0075, Sigma Chemical Co., USA; theophylline, Aldrich–Chemie, Germany.

All other reagents used were analytical–grade reagents (pure for analysis).

Apparatus

A spectrophotometer M–40, Carl Zeiss (Jena, Germany), a high–performance liquid chromatograph, an L–6000A (Hitachi) pump, an L3 3UV detector (Pye Unicam), a PM 8351 recorder (Philips), a feeder with a loop feeding 20 µl (Berkley, California), UV 10 VEB thermostat, Prüfgerate–Werk, Medingen, Germany, and a N–517 pH–meter (Mera–Elwro, Wrocław) equipped with a complex GK 2401 C electrode, Radiometer (Denmark).

Synthesis of 6–mercaptopurine disulfide

6–Mercaptopurine disulfide (6–MP disulfide) was obtained as a result of oxidation of 6–mercaptopurine (6–MP) by a water solution of iodine (3). 6–MP, 1.0 g (0.0066 mol), was dissolved in 600 cm^3 hot phosphate buffer, pH 7.6. A water solution of iodine, 6 cm^3, (0.0032 mol), was added within 5 minutes with constant stirring. The resulting precipitate was filtered off, washed with water until the components of the buffer disappeared and recrystallized from 85% ethanol.

Kintic measurements

Decoposition of 6–mercaptopurine disulfide was analyzed at 308 K in a (1:1) methanol – 0.1 $mol \cdot dm^{-3}$ hydrochloric acid and 0.1 $mol \cdot dm^{-3}$ sodium hydoxide, The ionic strenght of the solutions was adjusted to 0.5 $mol \cdot dm^{-3}$ with a solution of sodium chloride (4 $mol \cdot dm^{-3}$). The solutions of suitable pH and ionic strength of 0.5 $mol \cdot dm^{-3}$ were thermostated in calibrated flask till 308 K; at which a sample of 6–MP disulfide was added. At certain time intervals, the solution of 6–MP disulfide was sampled at amounts of 2.0 cm^3, immediately neutralized and cooled.

INVESTIGATIONS, RESULTS AND DISCUSSION

The degree of decomposition of 6–MP disulfide and formation of 6–MP were followed spectrophotometrically by following changes in the UV spectra of the solutions at λ_{max} 289 nm and 323 nm (Figure 1), and the HPLC method (Figure 2). In the latter method the following parameters were used, *viz.*, stationary phase: silica gel chemically bound

to octadecylsilane RP–18, dp = 5 μm; mobile phase: methanol – potassium dihydrophosphate (0.5 mol·dm^{-3}) – acetic acid (1.05 kg·dm^{-3}) – water (300:40:5) ad 1000; detection wavelenght, 289 nm; mobole phase flow rate, 1.5 cm^3·min^{-1}; internal standard, theophylline at $4.27.10^{-4}$ mol·dm^{-3}.

In the above conditions, linear relations were obtained in the UV method over the ranges of 6–MP disulfide and 6–MP concentrations of (0.35 to 5.32). 10^{-4} mol·dm^{-3} and (0.43 to 5.22)·10^{-5} mol·dm^{-3}, respectively

$A_{D6-MP} = f(c_{D6-MP})$ $y = (21650 \pm 702) \cdot x$ $r = 0.9989$
$A_{6-MP} = f(c_{D6-MP})$ $y = (19142 \pm 392) \cdot x$ $r = 0.9996$

and in the HPLC method over the following concentration ranges: (0.1356 to 1.0848) · 10^{-5} mol·dm^{-3} D6–MP and (0.226 to 3.626) · 10^{-4} mol·dm^{-3} 6–MP;

$h_{D6-MP}/h_{IS} = f(c_{D6-MP})$ $y = (13339 \pm 934) \cdot x$ $r = 0.9972$
$h_{D6-MP}/h_{IS} = f(c_{6-MP})$ $y = (9681 \pm 587) \cdot x$ $r = 0.9979$

where A_{D6-MP} and A_{6-MP} are the absorbances of 6–MP disulfide and 6–MP and h_{D6-MP}, h_{6-MP}, h_{IS} are the heights of the peaks attributed to 6–MP disulfide, 6–MP and internal standard, respectively. The values of direction coefficients were userd for the calculation of concentrations of 6–MP disulfide and 6–MP in the kinetic studies.

In an acid medium, 6–MP disulfide decomposes

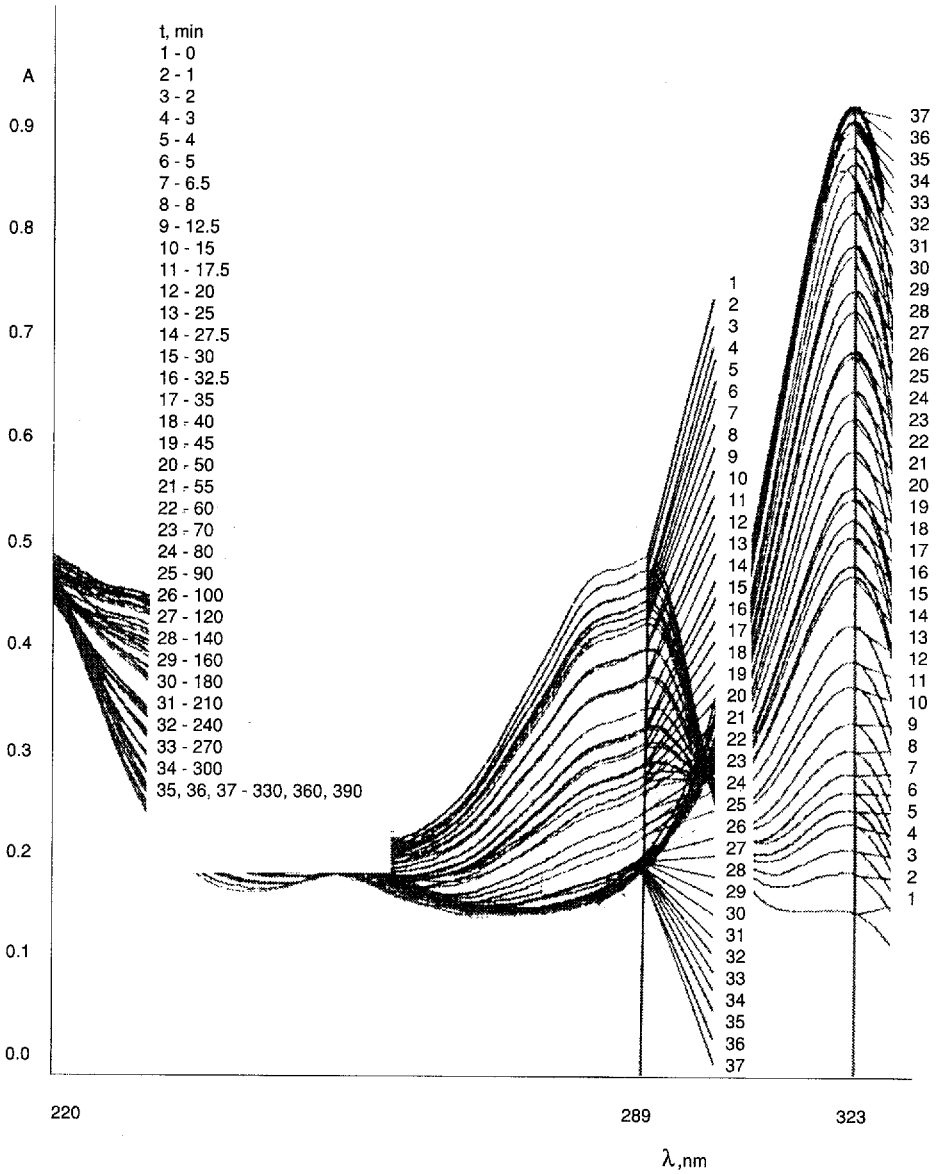

Figure 1. UV spectral changes for the decomposition of 6–mercaptopurine disulfide (289 nm) and formation of 6–mercaptopurine (323 nm) in methanol solution of 1:1 ratio of hydrochloric acid (0.1 mol · dm^{-3}) at 308 K.

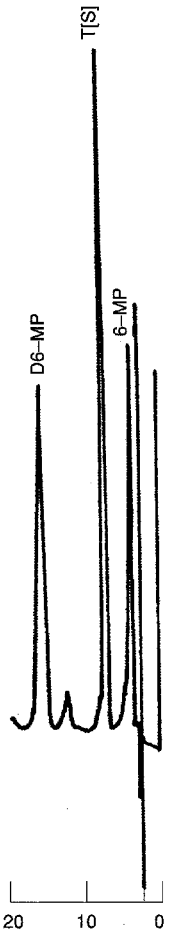

Figure 2. Chromatogram of 6–mercaptopurine disulfide, 6–mercaptopurine and toephylline (internal standard). Column: LiChrosorb RP–18,5 µm (25 cm x 4 mm). The mobile phase: methanol – potassium dihydrophosphate (0.5 mol · dm^{-3}) – acetic acid (1.05 kg · dm^{-3}) water (300:40:5) ad 1000; the mobile phase flow rate 1.5 cm^3 · min^{-1}; the detection wavelength 289 nm, the sensitivity of detector 0.16 AUFS. Retention times in minutes.

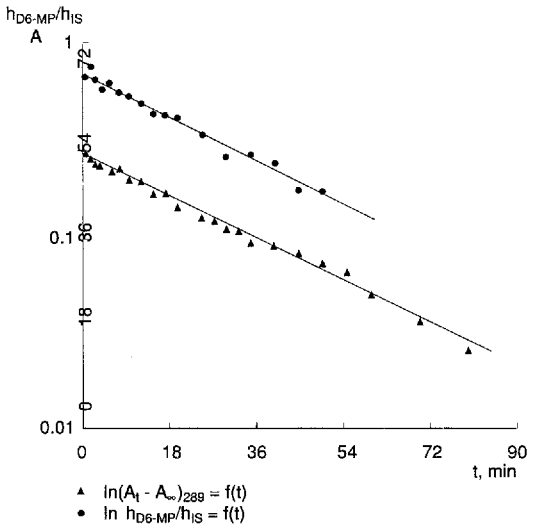

Figure 3. The plots $\ln(A_t - A_\infty)_{289} = f(t)$ and $\ln(h_{D6-MP}/h_{IS}) = f(t)$ for the decomposition of 6–mercaptopurine disulfide in methanol solution of 1:1 ratio or hydrochloric acid (0.1 mol · dm^{-3}) at 308 K.

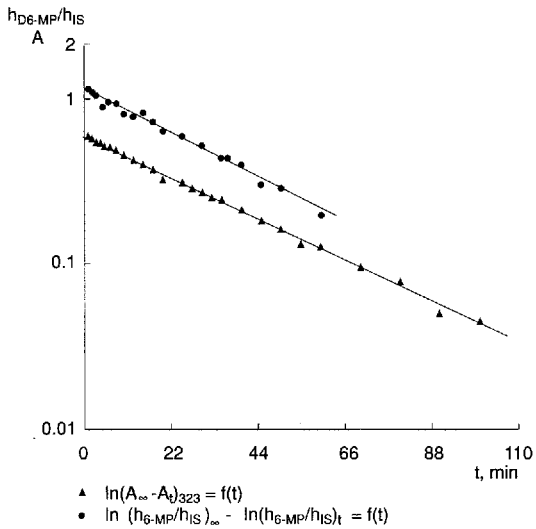

Figure 4. The plots $\ln(A_\infty - A_t)_{323} = f(t)$ and $\ln(h_{6-MP}/h_{IS})_\infty - \ln(h_{6-MP}/h_{IS})_t = f(t)$ for the formation of 6–mercapotopurine in methanol solution of 1:1 ratio of hydrochloric acid (0.1 mol · dm^{-3}) at 308 K.

to 6–MP, what was evidenced by measurements of UV spectra and retention times of the appropriate reference substances.

Decomposition of 6–MP disulfide in a methanol solution of (1:1) hydrochloric acid is a pseudo–first order reaction described by the equations:

$$\ln(A-A_\infty)_{289} = \ln(A_o-A_\infty)_{289} - k_{obs} \cdot t$$

or

$$\ln(h_i/h_{IS}) = \ln(h_o/h_{IS}) - k_{obs} \cdot t$$

where A, A_o are the 6–MP disulfide absorbances at time t and t = 0 ; A_∞ absorbance at infinite time, $t \rightarrow \infty$, h_i, h_o are the heights of the 6–MP disulfide peak at time t and t=0, h_{IS} is the height of the peak due to the internal standard; k_{obs} – the observed rate constant of the pseudo–first order reaction of 6–MP disulfide decmposition.

The plots of $\ln(A_t - A_\infty)_{289} = f(t)$ and $\ln(h_{D6-MP}/h_{IS}) = f(t)$ are linear for these reactions (Figure 3), and their slope is equal to the rate constant of the reaction. The reaction rate constants obtained for 6–MP disulfide decomposition in a (1:1) methanol solution of

hydrochloric acid (0.1 mol · dm^{-3}) at 308 K, by the HPLC and UV metthods are as follows:

HPLC: $k_{obs} = (4.36 \pm 0.58) \cdot 10^{-4}$, s^{-1}

UV: $k_{obs} = (4.42 \pm 0.30) \cdot 10^{-4}$, s^{-1}

The rate constants of 6–MP formation were determined in the same conditions as those of 6–MP disulfide decomposition and were calculated from the semilogarithmic relationships (Figure 4):

$$\ln (A_\infty - A_t)_{323} = f(t)$$

and

$$\ln(h_{6\text{–}MP}/h_{IS})_\infty - \ln(h_{6\text{–}MP}/h_{IS})_t = f(t)$$

The rate constsnts of 6–MP formation in a 1:1 methanol solution of hydrochloric acid (0.1 mol · dm^{-3}) at 308 K are:

HPLC: $k_{obs} = (4.53 \pm 0.22) \cdot 10^{-4}$, s^{-1}
UV: $k_{obs} = (4.42 \pm 0.08) \cdot 10^{-4}$, s^{-1}

The concentration of 6–MP from t_o to t_∞ varies from c_o to c_∞. At $t \rightarrow \infty$, the molar concentration c_∞ (mol · dm^{-3}) of 6–MP is $2c_o$ (mol · dm^{-3}) of that of 6–MP disulfide. The difference plot $\ln(c_\infty - c_o)_{6\text{–}MP} = f(t)$ is recilinear and its slope is equal to that of the plot $\ln(c)_{D6\text{–}MP} = f(t)$.

A statistical analysis of the rate constants of 6–MP disulfide decomposition and 6–MP formation was made. No statistically significant differences were found between either the rate constants of 6–MP disulfide decomposition or 6–MP formation as determined by the UV and HPLC methods.

In 0.1 mol/dm^3 methanol solution of sodium hydroxide (1:1) at 308 K, 6–MP disulfide decomposes to yield 6–MP in statu nascendi and the rate of the process cannot be established.

REFERENCES

1. Undenberg W.J.M, Lake O.A., Wilting J.: European Conference on Analytical Chemistry EUROANALYSIS V, Cracow, Poland, August 26–31 (1984).
2. Pawełczyk E., Zając M., Majewski W., Majewska B.: Acta Polon. Pharm. – Drug Research 45, 259 (1988).
3. Doerr I.L., Wempen I., Clarke D.A., Fox J.J.: J. Org. Chem. 26, 3401 (1961).

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