

Determination of thioguanine in pharmaceutical preparations by paper substrate room temperature phosphorimetry

Dong Chuan,^{ab} Yuan Wen,^b Shuang Shaomin^b and Yang Pin^a

^a Institute of Molecular Science, Shanxi University, Taiyuan, 030006 China

^b Department of Chemistry, Shanxi University, Taiyuan, 030006 China.

E-mail: dc@sxu.edu.cn

Received 28th February 2000, Accepted 5th May 2000

Published on the Web 9th June 2000

A room temperature phosphorimetric (RTP) procedure was used for the determination of 6-thioguanine (6-TG). The method is based on paper substrate room temperature phosphorimetry (PS-RTP) using indium sulfate, $\text{In}_2(\text{SO}_4)_3$ as a heavy atom perturber. Various factors affecting the room temperature phosphorescence of 6-TG are discussed. The linear dynamic range for 6-TG is from 3.3 to 334.3 ng per spot with a detection limit of 4.6 ng per spot and a relative standard deviation (RSD) of 2.38%. The recovery of standard 6-TG added to commercial tablets is in the range 96.39–98.44%. The method is simple, rapid and sensitive and can be applied to the analysis of commercial tablets without interference.

Purine compounds are very important in nucleic acid chemistry; for example, adenine and guanine are metabolites of nucleic acids. Medicine containing purine compounds have been used to cure cancer. In addition, at low temperature (77 K) purines have high phosphorescence yields, and detection limits in the picogram range were obtained when purine compounds were adsorbed on solid substrates¹ or in rigid solvents.² However, the requirement for cryogenic conditions has been one of the main limitations for conventional phosphorimetry.³

The great interest in room temperature phosphorimetry (RTP) of purine compounds on solid substrates is mainly a result of the simplicity of the method, the considerable selectivity and the good sensitivity. Gaye and Aaron⁴ carried out a comparative study of the heavy atom effect on the RTP of nine biologically important purines on filter-paper. Thomas and James⁵ and Al-Nosawi *et al.*⁶ demonstrated the enhancement effect of heavy atom perturbers on purine compounds including adenine, guanine and mercapto-substituted purines. Jin *et al.*^{7,8} examined the RTP properties of three purine compounds, *viz.*, purine, 6-mercaptopurine and 6-hydroxypurine, with cadmium acetate as a source of heavy atom perturbation and the effect of pH on the solid substrate (SS)-RTP of purine derivatives. However, there are very few papers dealing with the determination of purine compounds in real samples by RTP. 6-Thioguanine (6-TG) is an important chemotherapeutic drug in the treatment of childhood acute lymphoblastic leukaemia. The determination of 6-TG in capsules by capillary zone electrophoresis⁹ in a 22.2 mM borate buffer (pH = 9.0) with UV detection at 280 nm has been reported. An HPLC method has also been described.¹⁰ In the present investigation, a simple, rapid, sensitive and quantitative method for 6-TG is reported based on RTP using filter-paper as solid substrate and $\text{In}_2(\text{SO}_4)_3$ as heavy atom perturber. Experimental conditions were examined in detail. The method was applied to the analysis of commercial tablets without separation.

Experimental

Apparatus

All RTP spectra and RTP intensity measurements were recorded on an MPF-4 fluorescence spectrophotometer

(Hitachi) equipped with a rotating cylinder phosphoroscope and home-made sample holder for the solid substrate. The spectrometer used a 150 W xenon arc lamp as the excitation light source and an R446F photomultiplier (Hamamatsu) as the detector. A drying box, equipped with a 250 W IR lamp, was used to dry the samples, and the temperature was controlled automatically ($\pm 1^\circ\text{C}$). A 5 μL syringe (Shanghai Medical Laser Instrument Factory) was used for delivery of samples and heavy atom solution.

Reagents and materials

6-TG (chemical correspondent product) was purchased from the Institute of Medicine and Bioproduct Identification (Beijing, China). A stock solution of $5 \times 10^{-3} \text{ mol L}^{-1}$ was prepared by dissolving 6-TG in 1 mL of NaOH solution (0.1 mol L^{-1}), after which the solution was diluted to volume with water in a 50 mL flask. The Britton–Robinson buffer solution consisted of $0.04 \text{ mol L}^{-1} \text{ H}_3\text{PO}_4$, $0.04 \text{ mol L}^{-1} \text{ HOAc}$, and $0.04 \text{ mol L}^{-1} \text{ H}_3\text{BO}_3$. The pH was adjusted by adding different volumes of $0.2 \text{ mol L}^{-1} \text{ NaOH}$ solution to 100 mL of the buffer solution. The working concentration of 6-TG was $1 \times 10^{-4} \text{ mol L}^{-1}$. Water was doubly distilled in a sub-boiling still. $\text{In}_2(\text{SO}_4)_3$ (A.R.) was purchased from Beijing Chemical Industry Factory. All other reagents were of analytical purity. Slow-speed quantitative filter-paper (Hangzhou Xinhua Papermaking Mill, China) was used as the substrate material.

Preparation of sample solutions

Commercial 6-TG tablets were obtained from the Eighth Medical Factory of China (Guangzhou). The tablets were labeled to contain 6-TG at 50 mg per tablet. Twenty tablets were weighed, finely powdered and an amount equivalent to 25 mg of 6-TG was accurately weighed and transferred into a 100 mL calibrated flask. It was then shaken with 10 mL of NaOH solution (0.1 mol L^{-1}) for about 2 min and made up to volume with water. The resulting solution was filtered and the first portion of the filtrate was rejected. The solution was diluted ten times with Britton–Robinson buffer (pH = 12.0) solution before analysis.

Procedures

The slow-speed quantitative filter-paper was cut into 3×1.3 cm strips on which two parallel lines with an interval of 0.2 cm were engraved at the position of the light spot in order to limit the extent of the sample solution. A $3 \mu\text{L}$ volume of $\text{In}_2(\text{SO}_4)_3$ heavy atom solution (1.5 mol L^{-1}) was spotted on the surface of the filter-paper strip. The paper strip was pre-dried at 95°C for 1 min, then $2 \mu\text{L}$ of 6-TG sample solution were spotted at the same position. The paper was then dried again for 3 min. Finally, the substrate was held in position with a solid substrate holder and covered with a quartz glass piece to avoid humidity.¹¹ The holder was placed in the sample compartment, and the RTP intensity was measured or the spectra were recorded. Measurements of the RTP intensity of 6-TG were taken at the corresponding maxima of the excitation (345 nm) and emission (482 nm) wavelengths and the excitation and emission slits were set at 15 nm.

Results and discussion

Phosphorescence spectra of 6-TG

Fig. 1 shows the paper substrate (PS)-RTP spectra of 6-TG in the absence and presence of heavy atom perturbors including NaI and $\text{In}_2(\text{SO}_4)_3$. It can be seen that the excitation and emission spectra of 6-TG exhibit only one peak. The RTP maxima of the excitation and emission wavelengths were 328 and 478 nm, respectively in the absence of a heavy atom. When $\text{In}_2(\text{SO}_4)_3$ was used as the heavy atom, the RTP maxima of the excitation and emission wavelengths were 345 and 482 nm, respectively, whereas with NaI as the heavy atom, the corresponding values were 328 and 478 nm, respectively. It is obvious that the effect of the heavy atom enhanced the phosphorescence emission of 6-TG molecule. It should be noted that NaI only affected the RTP intensity of 6-TG whereas $\text{In}_2(\text{SO}_4)_3$ affected to some extent both the RTP intensity and phosphorescence spectra.

Selection of the filter-paper substrate

RTP emission is the result of an interaction between the analyte molecule and the substrate. Filter-paper is the most common substrate due to its simplicity.¹² Hence, the first decision was to select the type of substrate to act as a support for the 6-TG sample. The RTP spectral properties of 6-TG adsorbed on

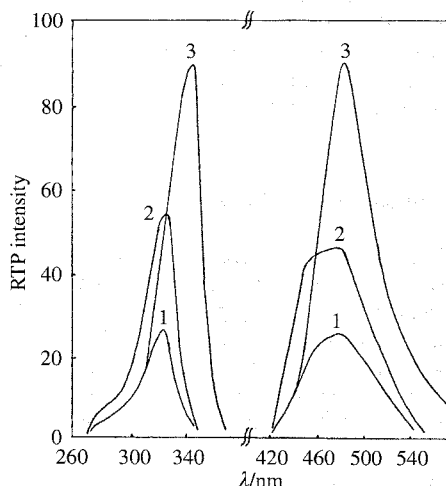


Fig. 1 Phosphorescence spectra of 6-TG ($1 \times 10^{-4} \text{ mol L}^{-1}$) at pH 12. 1, No heavy atom; 2, NaI (1.0 mol L^{-1}) 3, $\text{In}_2(\text{SO}_4)_3$ (1.5 mol L^{-1}).

several types of filter-paper materials were examined in the presence of $\text{In}_2(\text{SO}_4)_3$ and the relative RTP intensities are given in Table 1. The results show that the papers tested can induce RTP of 6-TG. This can be explained by a hydrogen-bonding mechanism.¹³ The main interaction between 6-TG and the hydroxyl groups of the paper was attributed to hydrogen bonding because there are groups such as $-\text{SH}$, $-\text{NH}_2$, $>\text{NH}$ in the 6-TG molecule; the adsorbed state of 6-TG prevented radiationless deactivation of the triplet emission.

It was noticed that Chinese-made filter-papers induced a more intense RTP emission and weaker background emission ($S/N = 12\text{--}17$) than other filter-paper substrates. In addition, the availability of a wide selection of specialty papers with different characteristics and the relatively low cost of these papers are two principal advantages. Among the papers examined, slow-speed quantitative filter-paper is the most favorable substrate because the heavy atom and 6-TG molecules can occupy the interstices between the cellulose fibers. This spatial constraint increases the proximity of the heavy atom and the solute molecules and induces a more efficient heavy atom effect.

Heavy atom effect

The heavy atom effect is a mechanism of considerable analytical importance to the phosphorescence technique.¹⁴ The effect of 25 types of inorganic salts as heavy atom perturbors on the RTP intensities of 6-TG adsorbed on filter-paper was examined. The experiments revealed that most of the inorganic ions did not induce RTP emission of 6-TG. Among the 25 inorganic salts, only $\text{In}_2(\text{SO}_4)_3$ and sodium halides (NaI, NaBr, NaCl) exhibited high phosphorescence enhancement factors (f_{HA}) for 6-TG. When these salts were absent, the RTP signal of 6-TG was observed to decrease markedly. The presence of heavy atom species in the vicinity of a phosphor can enhance its phosphorescence emission by increasing the spin-orbit coupling interaction. $\text{In}_2(\text{SO}_4)_3$, which has a high atomic number, could induce a strong RTP emission of 6-TG because a strong spin-orbit coupling interaction is related to a high atomic number. The influence of heavy atom species is given in Table 2. It was found that no significant change occurred in the shape and position of the maxima of the excitation and emission spectra of 6-TG in the presence of different heavy atoms except for $\text{In}_2(\text{SO}_4)_3$. When $\text{In}_2(\text{SO}_4)_3$ was used as the heavy atom, the excitation and emission maxima of 6-TG were red-shifted 17 and 4 nm, respectively. Fig. 2 shows the relationship between $\text{In}_2(\text{SO}_4)_3$ concentration and RTP intensity of 6-TG. The highest and most stable RTP intensity was obtained when the $\text{In}_2(\text{SO}_4)_3$ concentration was higher than 1.0 mol L^{-1} . The optimum $\text{In}_2(\text{SO}_4)_3$ concentration was selected as 1.5 mol L^{-1} . As for halogen heavy atoms, an investigation of the heavy atom effect indicated the trend of 6-TG RTP enhancement to be $\text{I}^- > \text{Br}^-$

Table 1 RTP intensities and signal-to-noise ratios (S/N) of 6-TG adsorbed on several papers

Substrate ^a	Net RTP intensity ^b	S/N
Paper(quant. M)	77	16
Paper(quant.F)	65	14
Paper(quant. S)	80	17
Paper(qual.)	55	12
Paper(W. No. 40)	79	13
Paper(W. No. 41)	77	7
Paper(S & S)	92	5

^a Paper source: Chinese-made quantitative middle (M), fast (F), slow (S) speed and qualitative filter-papers, UK-made Whatman No. 40 and No. 41 filter-papers, and German-made Schleicher & Schull No. 589 filter-paper.
^b 6-TG ($1 \times 10^{-4} \text{ mol L}^{-1}$), $\text{In}_2(\text{SO}_4)_3$ (1.5 mol L^{-1}), Britton-Robinson buffer (pH = 12).

> Cl⁻, with the heavier ions inducing the greater RTP enhancement.

Effect of pH

In general, purine derivatives containing one or two -NH₂ groups produce intense RTP in neutral or weakly alkaline solutions. However, purine and its derivatives with a -SH substituent produce intense RTP in a strongly alkaline solution. There is one -NH₂ and one -SH group in the 6-TG molecule and the molecule may be negatively charged in a strongly alkaline medium.⁸ Hence, 6-TG should produce intense RTP in a strongly alkaline solution. The effect of pH on 6-TG RTP is shown in Fig. 3. RTP was quenched at pH ≤ 10 or pH ≥ 13. The highest RTP intensity was obtained at pH 11.0–12.5, the appropriate pH for determination being 12.0. 6-TG can produce a more intense RTP emission due to the strong electron-donating action of the -SH and -NH₂ groups in alkaline medium, but the RTP intensity of 6-TG is quenched in acidic medium, because the -SH and -NH₂ groups are protonated and have a strong electron-attracting ability.

Table 2 Enhancement factors (f_{HA}) of the PS-RTP of 6-TG (1×10^{-4} mol L⁻¹) with different heavy atoms

Heavy atom ^a	$\lambda_{\text{ex}}/\lambda_{\text{em}}^b$	I_p	f_{HA}^c
None	328/478	18.1	1.0
In ₂ (SO ₄) ₃ (1.5)	345/482	92.0	5.08
NaI (1.0)	328/478	40.7	2.25
NaBr (1.0)	328/478	40.1	2.21
NaCl (1.0)	328/478	32.9	1.82
Tl ₂ SO ₄ (0.1)	—	9.5	0.52
LaCl ₃ (0.5)	—	9.8	0.54
YCl ₃ (0.1)	—	5.7	0.31
Pb(Ac) ₂ (1.0)	—	4.8	0.27
Cd(Ac) ₂ (1.0)	—	9.6	0.53
AgAc (0.05)	—	0.7	0.04
HgCl ₂ (0.2)	—	0.4	0.02

^a The concentrations (mol L⁻¹) of heavy atoms are given in parentheses. Ac = CH₃COO⁻. ^b $\lambda_{\text{ex}}/\lambda_{\text{em}}$ is the position of the maxima of the excitation and emission spectra of 6-TG. — = No heavy atom enhancement effect. ^c $f_{\text{HA}} = I_p/I_{p_0}$, where I_p is the net intensity with heavy atom, and I_{p_0} is the net intensity without the heavy atom.

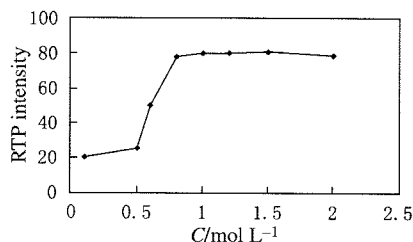


Fig. 2 Effect of In₂(SO₄)₃ concentration on RTP of 6-TG. 6-TG (1×10^{-4} mol L⁻¹), Britton–Robinson buffer (pH = 12).

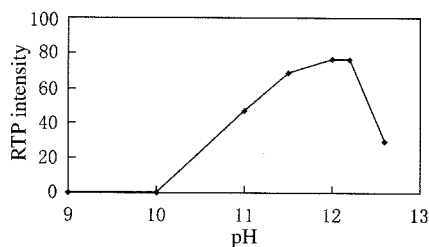


Fig. 3 Effect of pH on RTP of 6-TG. 6-TG (1×10^{-4} mol L⁻¹), In₂(SO₄)₃ (1.5 mol L⁻¹), pH was adjusted with Britton–Robinson buffer.

Effect of drying temperature, pre-drying time and drying time

Extensive research has shown a decrease in RTP for samples exposed to moisture in the ambient atmosphere.^{15–17} On the one hand, the effect of moisture was considered to disrupt hydrogen bonding on the paper surface, which has the additional detrimental effect of allowing the transport of oxygen to the vicinity of the phosphor atom. On the other hand, water molecules can compete with the analyte for bonding sites on the paper support, thus decreasing the analyte–support adsorption process and increasing the probability of collisional and vibrational quenching. These two processes decrease the number of analyte–support hydrogen bonds, which are necessary to maintain a rigid microenvironment.

The sample drying conditions, including drying temperature, pre-drying time and drying time, were examined in detail and the following results were obtained. (1) The highest RTP intensities occur between 90 and 100 °C. (2) Pre-drying time is the time required to dry the wet substrate on which the reagent solution, In₂(SO₄)₃ has been spotted. The appropriate pre-drying time is 1.0 min. (3) The RTP intensity of increases 6-TG with the drying time, the highest and most stable RTP signal being obtained after 3.0 min.

Analytical characteristics

Slow-speed quantitative filter-paper using In₂(SO₄)₃ as heavy atom salt is the most preferable system for PS-RTP. On the basis of the procedures, a calibration graph of log *P* vs. log *C* of 6-TG was obtained and its regression equation was log *P* = 6.506 + 1.083 log *C*, where *P* is the relative RTP intensity and *C* is the concentration of 6-TG (in mol L⁻¹). The slope (1.083), intercept (6.506) and correlation coefficient (0.998) were the average values of six determinations. Their RSD values were 0.0023, 0.0007 and 0.0014%, respectively. The linear dynamic range of the regression equation was from 3.3 to 334.3 ng per spot. The RSD value of the method was 2.38% and the limit of detection was 4.6 ng per spot, corresponding to three times the standard deviation of the blank (*n* = 11).

Analytical application

Twenty tablets of 6-TG were finely powdered. A portion of the powder containing approximately 25 mg of 6-TG was accurately weighed and transferred into a 100 mL calibrated flask. Then, five 6-TG standards (0, 10, 25, 50, 100 mg) were transferred into 100 mL calibrated flasks containing the sample and shaken with 10 mL of NaOH solution (0.1 mol L⁻¹) for about 2 min before being made up to volume with doubly distilled water. The resulting solutions were filtered and the first portion of the filtrate was rejected. The five filtrates were diluted ten times with Britton–Robinson buffer (pH = 12.0) solution before their RTP intensities were measured to determine the recoveries.

The proposed method was successfully applied to the analysis of commercial dosage forms of 6-TG. The results, shown in Table 3, were obtained by using the standard additions method and compared with those obtained by the Pharmacopeia method,¹⁸ which involves measurement of the UV absorption of 6-TG solution at 348 nm. The recovery studies were carried out after adding known amounts of 6-TG to the pre-analyzed formulation of commercial 6-TG tablets. The recovery studies were performed with four different concentration levels of 6-TG and the average recovery was 97.42%. All the recoveries were found to be close to 100%. The high percentage recoveries

Table 3 Recovery of standard 6-TG added to commercial tablets^a

Added/mg	Found/mg		Recovery $\pm s$ (%) ^c		RSD (%)	
	Proposed method	Reported method ^b	Proposed method	Reported method ^b	Proposed method	Reported method ^b
10	9.78	10.27	97.80 \pm 3.86	102.70 \pm 1.92	3.95	1.87
25	24.61	25.32	98.44 \pm 3.15	101.28 \pm 0.81	3.20	0.80
50	48.53	50.41	97.06 \pm 1.88	100.82 \pm 0.74	1.94	0.73
100	96.39	99.62	96.39 \pm 2.32	99.62 \pm 1.27	2.41	1.27

^a Declared amount is 50 mg per tablet. ^b See ref. 18. ^c s = Standard deviation of six determinations.

indicate that there is no interference from ingredients and excipients of the commercial 6-TG tablets. This is a further indication of the accuracy of the proposed method. Georget *et al.*⁹ applied capillary zone electrophoresis to the determination of 6-TG in capsules with a recovery of 101.7% and an RSD of 0.75%. However, capillary zone electrophoresis requires expensive instrumentation. The proposed method offers the advantages of accuracy, precision, and speed as well as less consumption of reagents. In addition, the method is suitable for the routine analysis of drugs in their dosage forms and in drug control laboratories.

Conclusion

PS-RTP was employed for the determination of 6-TG by using $\text{In}_2(\text{SO}_4)_3$ as a heavy atom perturber. The method has the advantages of simplicity, rapidity, high sensitivity and selectivity. The method was applied to the determination of 6-TG in pharmaceutical preparations with satisfactory results.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (980003) and Shanxi Province Youth Foundation of China (981006).

References

- 1 R. J. Mayer, J. M. Holman and A. C. Bridges, *J. Chromatogr.*, 1974, **39**, 393.
- 2 J. J. Aaron and J. D. Winefordner, *Anal. Chem.*, 1972, **44**, 2127.
- 3 T. Vo-Dinh, *Room Temperature Phosphorimetry for Chemical Analysis*, Wiley, New York, 1984.
- 4 M. D. Gaye and J. J. Aaron, *Anal. Chim. Acta*, 1988, **205**, 237.
- 5 K. A. Thomas and D. S. James, *J. Agric. Food Chem.*, 1980, **31**, 388.
- 6 A. I. Al-Nosawi, J. N. Miller and J. W. Bridges, *Analyst*, 1980, **105**, 488.
- 7 W. J. Jin, H. R. Zhou, X. Yang and C. S. Liu, *Appl. Spectrosc.*, 1995, **49**(3), 156.
- 8 W. J. Jin, R. H. Zhu, X. H. Shang, C. Dong, W. Y. Zhang and C. S. Liu, *Spectrochim. Acta, Part A*, 1997, **53**, 1735.
- 9 S. Georget, J. Vigneron, I. May, A. Perrin, M. A. Hoffman and M. Hoffman, *J. Clin. Pharm. Ther.*, 1999, **24**(4), 273.
- 10 C. W. Keuzenkamp-Jansen, R. A. De Abreu, J. P. Bokkerink and J. M. Trijbels, *J. Chromatogr. B*, 1995, **672**, 53.
- 11 C. Dong, C. S. Liu and K. C. Feng, *Fenxi Huaxue*, 1993, **21**(7), 755.
- 12 R. J. Hurtubise, *Phosphorimetry: Theory, Instrumentation and Application*, VCH, New York, 1990.
- 13 H. Z. Xie, C. Dong, W. J. Jin, Y. S. Wei, C. S. Liu, S. S. Zhang and B. L. Zhou, *Anal. Chim. Acta*, 1996, **319**, 239.
- 14 M. Zander, *Phosphorimetry*, Academic Press, New York, 1968.
- 15 E. M. Schulman and C. Walling, *J. Phys. Chem.*, 1973, **77**, 902.
- 16 S. L. Wellons, R. A. Paynter and J. D. Winefordner, *Spectrochim. Acta, Part A*, 1974, **30**, 2133.
- 17 T. Vo-Dinh, E. Lue-Yen and J. D. Winefordner, *Anal. Chem.*, 1976, **48**, 1186.
- 18 Pharmacopoeia of People's Republic of China, ed. C. Yueli, Chemical Industry Press, Beijing, 1990, pp. 682-684.