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Spectrophotometric Determination of Thiols in Pure Substances and Pharmaceutical Preparations

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A spectrophotometric method for determination of mesna (MA), 6-mercaptopurine (MP), 6-thioguanine (TG), and 6-propyl-2-thiouracil (PTU) has been developed. The method was based on the reaction of thiouracils with N,N-dimethyl-p-phenylenediamine (DMPD) in acidic solution in the presence of the Fe^{3+} ion, an oxidising agent. Absorbance of the obtained coloured products was measured at the corresponding optimum wavelengths: 490 nm for MA ($\varepsilon = 1.9 \times 10^3$ L mol^{-1} cm^{-1}), 455 nm for MP ($\varepsilon = 1.9 \times 10^3$ L mol^{-1} cm^{-1}), 460 nm for TG ($\varepsilon = 1.6 \times 10^3$ L mol^{-1} cm^{-1}), and 465 nm for PTU ($\varepsilon = 2.1 \times 10^3$ L mol^{-1} cm^{-1}). Beer's law was obeyed in the following concentration ranges: 8.2×10^{-3}–9.0×10^{-2} g L^{-1} for MA, 6.0×10^{-3}–3.8×10^{-2} g L^{-1} for MP, 4.2×10^{-3}–4.2×10^{-2} g L^{-1} for TG and 4.2×10^{-3}–3.9×10^{-2} g L^{-1} for PTU. The method has been applied for determination of thiols in pharmaceuticals.

Opracowano spektrofotometryczną metodę oznaczania mesny (MA), 6-merkaptopuryny (MP), 6-tioguaniny (TG) i 6-propylo-2-tiouracylu (PTU). Metoda wykorzystuje reakcję tiouracyli z N,N-dimetylo-p-fenylenodiamią w środowisku kwaśnym, w obecności jonów Fe^{3+} jako utleniacza. Absorbancję barwnych produktów reakcji mierzono przy długościach fali $\lambda = 490$ nm dla MA ($\varepsilon = 1.9 \times 10^3$ L mol^{-1} cm^{-1}), $\lambda = 455$ nm dla MP ($\varepsilon = 1.9 \times 10^3$ L mol^{-1} cm^{-1}), $\lambda = 460$ nm dla TG ($\varepsilon = 1.6 \times 10^3$ L mol^{-1} cm^{-1}) i $\lambda = 475$ nm dla PTU ($\varepsilon = 2.1 \times 10^3$ L mol^{-1} cm^{-1}). Prawo Beera jest spełnione w zakresie stężeń 8.2×10^{-3}–9.0×10^{-2} g L^{-1} dla MA, 6.0×10^{-3}–3.8×10^{-2} g L^{-1} dla MP, 4.2×10^{-3}–4.2×10^{-2} g L^{-1} dla TG and 4.2×10^{-3}–3.9×10^{-2} g L^{-1} dla PTU. Opracowaną metodę wykorzystano do oznaczania związków w preparatach farmaceutycznych.

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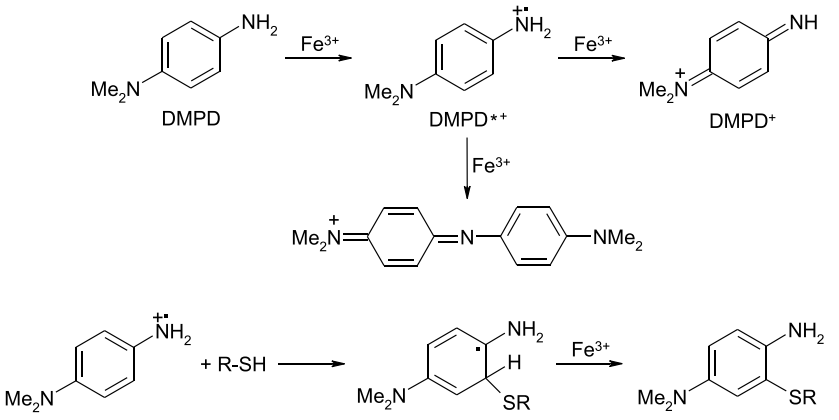
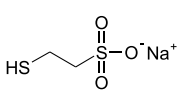
In the presence of an oxidising agent, N,N-dimethyl-p-phenylenediamine (DMPD) reacts with thiols to give orange-red products of a maximum absorbance at 490–510 nm [1]. Previously, this reaction was applied only for determination of a few aliphatic thiols [2, 3]. The reaction for this system proceeds according to Scheme 1 [1].

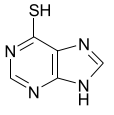


Scheme 1. Mechanism of reaction between DMPD and thiols

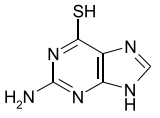
Thiols studied in this paper (see below) are active compounds of drugs used in pharmacotherapy.



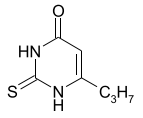
mesna
(MA)



6-mercaptopurine
(MP)



6-thioguanine
(TG)



6-propyl-2-thiouracil
(PTU)

Mesna is a mucolytic agent that breaks disulfide bonds of mucous polypeptide chains [4]. It is also an effective compound in prevention of urinary toxicity associated with high doses of drugs frequently prescribed in the treatment of cancer or as an immunosuppressive agent [5]. 6-Mercaptopurine and 6-thioguanine are important active compounds used in the treatment of leukaemia [6 , 7]. 6-Propyl-2-thiouracil is an active component of thyreostatic drugs [8 , 9].

Several spectrophotometric methods based on colouring reactions of the investigated thiols are known.

Mesna can be determined spectrophotometrically in biological samples applying the Ellman's assay [10] and its modifications [11 , 12]. This method is based on the

reaction of thiols with 5,5'-dithiobis(2-nitrobenzoic acid), in which a coloured 2-nitro-5-thio-benzoic acid anion is produced.

Procedure for determination of 6-mercaptopurine in tablet formulations is based on the measurement of a difference in absorbance between diphenylpicrylhydrazyl solution ($\lambda = 513$ nm) and the same solution after its reaction with thiol [13]. Colouring reactions of 6-mercaptopurine with amminepentacyanoferrate(II) ion ($\lambda = 455$ nm) and nitrosylpentacyano-ferrate(II) ion ($\lambda = 650$ nm) for MP determination in a drug has been also reported [14]. Differential spectrophotometry has been applied for determination of 6-mercaptopurine in tablets [15]. Due to the presence of divalent sulfur atom, induced iodine-azide reaction has been utilised for determination of 6-mercaptopurine [16] and 6-thioguanine [17]. In this method, spectrophotometric measurements at $\lambda = 350$ nm were performed to follow the consumption of iodine.

A decrease in the absorbance intensity of diphenylpicrylhydrazyl ($\lambda = 513$ nm) [13] or oxidized diphenylamine ($\lambda = 580$ nm) [18], caused by reaction with 6-propyl-2--thiouracil, was measured in direct determination of PTU. Colouring reactions of 6-propyl-2-thiouracil with various reagents, such as palladium(II) chloride ($\lambda = 525$ nm) [19], potassium iodate ($\lambda = 465$ nm) [20], 2,6-dichloroquinone-4--chloroimide ($\lambda = 435$ nm) [21], ruthenium(III) ions ($\lambda = 520$ nm) [22], and sodium nitroprusside ($\lambda = 600$ nm) [23] were utilised for PTU determination as well. The kinetic method based on the inhibitory effect of PTU on the Pd(II)-catalyzed reaction of neutral red [24] and crystal violet [25] with hypophosphite ions has been also developed.

In this paper, a spectrophotometric method for determination of mesna, 6-mercaptopurine, 6-thioguanine and 6-propyl-2-thiouracil, based on its reaction with N,N-dimethyl-p-phenylenediamine (DMPD) in the presence of Fe^{3+} ions, has been presented.

EXPERIMENTAL

Apparatus

A double beam UV–VIS spectrophotometer (Cary 100 Bio – Varian, Inc. Palo Alto CA, USA) with 10 mm quartz cells equipped with teflon magnetic stirring bars and teflon lids was used for the absorbance measurements.

Reagents and solutions

The following reagents and pharmaceuticals were used: N,N-dimethyl-p-phenylenediamine dihydrochloride and iron(III) nitrate nonahydrate (99% – Sigma–Aldrich, Poland), mesna (98% – Sigma–Aldrich, Poland), 6-mercaptopurine (95% – Sigma–Aldrich, Poland), 6-thioguanine (98% – Sigma–Aldrich, Poland), 6-propyl-2-thiouracil (99% – Sigma–Aldrich, Poland), hydrochloric acid, sulfuric acid, sodium hydroxide

(POCH S.A., Poland), Mistabron 600 mg (PLIVA Kraków, Zakłady Farmaceutyczne S.A., Poland), Mercaptopurinum 50 mg (Zakłady Chemiczno-Farmaceutyczne „Vis" Sp. z. o.o., Poland), Lanvis 40 mg (GlaxoSmithKline, Great Britain), Propycil 50 mg (Solvay Pharmaceuticals GmbH, Germany), Thyrosan 50 mg (SUN-FARM Sp. z. o.o. Poland).

The following solutions were used: 2 g L^{-1} N,N-dimethyl-p-phenylenediamine dichydrochloride in 0.5 (1.0) mol L^{-1} HCl and 2×10^{-2} mol L^{-1} Fe^{3+} in 0.1 mol L^{-1} H_2SO_4.

5×10^{-3} mol L^{-1} solutions of the investigated compounds were freshly prepared. Mesna solution was prepared by dissolving 0.08376 g of the compound in 10 mL of water. Then, 1 mL of this solution was further diluted to 10 mL with water. In order to prepare the solutions of other compounds, 0.08683 g of MP (0.08526 g of TG, 0.11140 g of PTU) was dissolved in 10 mL of 0.2 mol L^{-1} NaOH solution. Then, 1 mL of this solution was diluted to 10 mL with water.

All solutions were prepared using deionised water.

Procedures

2 mL of 2×10^{-3} mol L^{-1} Fe^{3+} and 1 mL of 2 g L^{-1} DMPD solutions in the case of MA and PTU or 2.5 mL of 2×10^{-3} mol L^{-1} Fe^{3+} and 0.5 mL of 2 g L^{-1} DMPD solutions in the case of MP and TG were placed into a 5 mL-in-volume flask, followed by addition of an appropriate volume of the solution of the investigated thiol. The mixture was left to equilibrate for a sufficient time (Tab. 1) and then it was diluted to the mark with water. Finally, the solution was transferred into a spectrophotometic cell and the absorbance was measured at he optimum wavelength (Tab. 1) against blank solution containing all reagents except for thiouracil. A calibration graph was obtained by plotting the measured absorbance *vs* concentration of thiouracil and the corresponding linear regression equation was derived.

Determination of MA in pharmaceuticals

The content of one ampoule was quantitatively introduced into a 100 mL-in-volume calibrated flask containing 10 mL of water. The content was shaken and finally diluted to the mark with water. A 50 µL portion of the drug solution was subjected to the analysis applying general procedure described above. The content of the compound was calculated using the regression equation (Tab. 3).

Determination of MP, TG, and PTU in pharmaceuticals

Ten tablets were accurately weighed and powdered. An accurate average mass of one tablet (0.24542 g – Mercaptopurinum, 0.22950 g – Lanvis 0.19022 g – Propycil, 0.18270 g – Thyrosan) was placed in a 100 mL-in-volume calibrated flask containing 10 mL of 1 mol L^{-1} NaOH solution and 10 mL of water. The flask was placed in an ultrasonic bath and the contents were shaken for 10 min to accelerate dissolution. Finally, the sample was diluted to the mark with water. An appropriate aliquot of the mixture was then transferred to a 2 mL centrifuge tube and centrifuged at 4000 rpm for 2 min. A clear 150 µL-in-volume portion of the drug solution in the tube was subjected to the analysis applying general procedure described above. The content of the compound was calculated using the regression equation given in Table 3.

RESULTS AND DISCUSSIONS

In preliminary experiments the optimum conditions for the reaction were investigated; they included: molar ratio of DMPD and Fe^{3+} ions, concentration of HCl solution used for dissolution of DMPD, optimum reaction time, and analytical wavelength. The established values are given in Table 1.

Table 1. Optimum conditions for determination of the studied compounds

Compound	c HCl, mol L^{-1}	Molar ratio Fe^{3+}:DMPD, mol:mol	λ, nm	ε, L mol^{-1} cm^{-1}	t, min	Range of determination, mg mL^{-1}
MA	0.5	4.0:1.0	490	1.9×10^3	15	8.2×10^{-3}–9.0×10^{-2}
MP	0.5	10.0:1.0	455	1.9×10^3	30	6.0×10^{-3}–3.8×10^{-2}
TG	1.0	10.0:1.0	460	1.6×10^3	25	4.2×10^{-3}–4.2×10^{-2}
PTU	0.5	4.0:1.0	465	2.1×10^3	30	4.2×10^{-3}–3.9×10^{-2}

Different concentrations of the studied compounds were determined under optimal conditions and the corresponding determination ranges were found. The results of determinations are presented in Table 2.

Table 2. Results of determination of the studied compounds; n = 6

Compound	Taken		Found $\bar{x} \pm t_{0.95} \cdot \frac{s}{\sqrt{n}}$		RSD, %
	μmol	mg	μmol	mg	
MA	0.2500	0.0410	0.2504 – 0.0033	0.0411 – 0.0005	1.30
	0.5000	0.0821	0.5095 – 0.0010	0.0836 – 0.0002	0.20
	1.000	0.1642	1.018 – 0.003	0.1671 – 0.0004	0.30
	1.500	0.2463	1.458 – 0.001	0.2392 – 0.0001	0.06
	2.000	0.3283	2.038 – 0.002	0.3345 – 0.0003	0.10
	2.750	0.4515	2.797 – 0.001	0.4591 – 0.0002	0.04

(Continuation on the next page)

Table 2. (Continuation)

Compound	Taken		Found $\bar{x} \pm t_{0.95} \cdot \frac{s}{\sqrt{n}}$		RSD, %
	µmol	mg	µmol	mg	
MP	0.2000	0.0304	0.2121 – 0.0011	0.0323 – 0.0002	0.50
	0.2500	0.0380	0.2642 – 0.0020	0.0402 – 0.0003	0.70
	0.5000	0.0761	0.5074 – 0.0018	0.0772 – 0.0003	0.30
	1.000	0.1522	1.002 – 0.002	0.1524 – 0.0003	0.20
	1.250	0.1902	1.240 – 0.001	0.1886 – 0.0001	0.10
TG	0.1250	0.0209	0.1335 – 0.0010	0.0233 – 0.0002	0.70
	0.2500	0.0418	0.27314 – 0.0046	0.0467 – 0.0008	1.60
	0.5000	0.0836	0.4979 – 0.0023	0.0843 – 0.0004	0.40
	1.000	0.1672	0.9948 – 0.0030	0.1673 – 0.0005	0.30
	1.250	0.2090	1.238 – 0.010	0.2079 – 0.0017	0.80
PTU	0.1250	0.0213	0.1245 – 0.0010	0.0212 – 0.0002	0.80
	0.2500	0.0426	0.2624 – 0.0008	0.0447 – 0.0001	0.30
	0.5000	0.0851	0.5093 – 0.000	0.0867 – 0.0001	0.10
	1.000	0.1702	1.012 – 0.002	0.1724 – 0.0003	0.20
	1.250	0.1915	1.110 – 0.014	0.1891 – 0.0002	0.10

The proposed method was successfully applied to the determination of some thiols in pharmaceutical formulations without the need of any sophisticated procedure. It was accurate, relatively fast, and free from the interference from excipients. The method gave accurate results with recovery values confirming its applicability to the determination of thiols in pharmaceuticals (Tab. 3). The contents of active compounds were found using the equations given in Table 3.

Table 3. Results of determination of 6-propyl-2-thiouracil in pharmaceuticals; n = 6

Drug	Regression equation x, mg	Declared content, mg	Found, mg $\bar{x} - t_{0.95} \cdot \frac{s}{\sqrt{n}}$	RSD, %
Mistabron (mesna)	$y = 2.2779x + 0.0044$ $R^2 = 0.9990$	600	600.7 – 1.7	0.27
Mercaptopurinum (6-mercaptopurine)	$y = 2.5135x + 0.0125$ $R^2 = 0.9986$	50	50.00 – 0.39	0.74
Lanvis (6-thioguanine)	$y = 1.928x + 0.0119$ $R^2 = 0.9981$	40	40.77 – 0.28	0.67
Propycil (6-propyl-2-thiouracyl)	$y = 2.4824x + 0.0078$ $R^2 = 0.9992$	50	49.50 – 0.28	0.55
Thyrosan (6-propyl-2-thiouracyl)		50	50.70 – 0.26	0.49

It was found that the reaction rate and absorbance value depend on the acidity of the reaction solution and the molar ratio of DMPD to Fe^{3+} ions. The reaction slowed down and the absorbance of the product decreased with the increasing acidity of the reaction mixture. The same behaviour was observed in case of the decreasing amount of Fe^{3+} ions in relation to the amount of DMPD.

In 0.5 and 1.0 mol L^{-1} HCl solutions and in the presence of more than 2.5 moles of DMPD (in relation to 1.0 mole of the oxidiser) the reaction with thiol did not proceed. This was caused probably by fast dimerization of $DMPD^{*+}$ cation radical (Scheme 1). Reaction occurred neither in 2 mol L^{-1} HCl solution when the amount of DMPD was higher than 4.5 mol, nor in 5 mol L^{-1} HCl solution when the amount of DMPD was higher than 1.0 mol (in relation to 1.0 mol of Fe^{3+} ions). Probably the colourless $DMPD^{+}$ cation, which does not react with thiols, was being formed faster than $DMPD^{*+}$ cation radical, which can react with thiol (Scheme 1).

In the studied reaction, all investigated thiols gave orange-coloured products except for mesna, which gave a pink product. The colours of the products were stable for *ca* 30 min. As it is shown in Table 1, the analytical wavelengths are different than those reported in the literature [1]. Only mesna absorbed at the literature wavelength. This difference is probably caused by the presence of a heterocyclic system in the molecular structures of thiopurines and thiouracil. The absorption spectra obtained under the optimum experimental conditions against blank solution are shown in Figure 1.

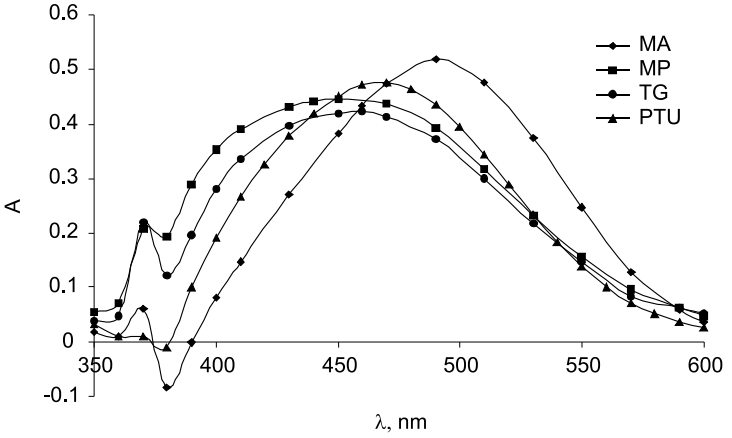


Figure 1. Absorption spectra of thiol compounds determined under optimum conditions ($c = 2.5 \times 10^{-4}$ mol L^{-1})

The comparison of the established determination ranges for pure substances (Tab. 1) with the ranges reported in the literature shows the advantage of the iodine-azide method for MP ($1–5 \times 10^{-5}$ mg mL^{-1}) [16] and TG (5×10^{-7}–4×10^{-5} mg mL^{-1}) [17] and of the kinetic method for PTU (1.7×10^{-6}–2.48×10^{-4} mg mL^{-1}) [24, 25]. Other literature methods allow one to determine the same or higher concentrations of pure thiols, as in case of PTU (20–80 mg mL^{-1}) [22].

Some of the methods described in the literature do not require long reaction time; however in some cases the reaction time in the presented method is comparable to [14] or even shorter [13, 20] than in the literature procedures.

To the best of our knowledge, the presented method is the first spectrophotometric procedure for determination of mesna and 6-thioguanine in pharmaceuticals.

In conclusion, the proposed method for determination of thiols is accurate, inexpensive, simple, and gives reproducible results. Moreover, it requires the use of only commonly available reagents. An additional advantage of the developed method is the use of reagents, which keep their quality for several weeks if refrigerated.

REFERENCES

1. Kubáň V., Dasgupta P.K. and Marx J.N., *Anal. Chem.(Warsaw)*, **64**, 36 (1992).
2. Wei Lei and Dasgupta P.K., *Anal. Chim. Acta.*, **226**, 165 (1989).
3. Yakimova V.P., *Zh. Anal. Chim.*, **46**, 1357 (1991).
4. Semczuk B., Gołabek W., Kłos A., Horoch A., Siwiec H. and, Kupisz K., *Otolaryngologia Pol.* XXXIV, 6 (1980).

5. El-Yazigi A., Yusuf A. and Al-Rawithi S., *Ther. Drug Monit.,* **17**, 153 (1995).
6. Burchenal J.H., Murphy M.L. and Ellison R.R., *Blood*, **8**, 965 (1953).
7. Murphy M.L., Tan C.T., Ellison R.R., Karnofsky D.A. and Burchenal J.H., *Proc. Am. Assoc. Cancer Res.*, **2,** 36 (1955).
8. Palumbo A. and d'Ischia M., *Biochem. Biophys. Res. Commun.*, **282**, 793 (2001).
9. Elias A.N., *Med. Hypotheses*, **62**, 431 (2004).
10. Ellman G.L., *Arch. Biochem. Biophys.*, **82**, 70 (1959).
11. Stekar J., *Aerztl. Lab.*, **28**, 187 (1982); CA 97: 65875m.
12. Brock N., Hilgard P., Pohl K., Ormstad K. and Orrenius S., *J. Cancer Res. Clin. Oncol.*, **108**, 87 (1984).
13. Berg B.H., *Acta Pharm Suecica*, **8**, 453 (1971).
14. Jaksevac-Miksa M., Hankonyi V. and Karas-Gasparec V., *Acta Pharm. Jugosl.*, **29**, 139 (1979).
15. Taneja D.K. and Jain N.K., *Indian Drugs*, **33**, 403 (1996).
16. Chrzanowska M. and Sloderbach A., *Chem. Anal. (Warsaw)*, **42**, 381 (1997).
17. Ciesielski W., *Chem. Anal. (Warsaw)*, **36**, 613 (1991).
18. Emara K.M., Refaat I.H., Abdel-Wadood H.M. and Hassan M.G., *Bull Pharm. Sci. Assit. Univ.*, **28**, 225 (2005); CA 144:398516.
19. Stankovic B., Jovanovic T., Music S. and Koricanac M. Z., *Farmaco*, **51**, 679 (1996).
20. Bruno S., *Boll. Chim. Farm.*, **102**, 478 (1963); CA 59:15123.
21. Ratliff C.R., Gillialand P.F. and Hall F.F., *Clin. Chem.*, **18**, 1373 (1972).
22. Renhardt F. and Hoppe I., *Seyler's Z. Physiol. Chem.*, **293**, 268 (1953); CA 49:135976.
23. Mohamed E., Tawakkol M.S. and Aboul-Enein H.Y., *Chem. Biomed. Environ. Instrum.*, **11**, 241 (1981).
24. Barzegar M., Rahmani A., Jabbari A. and Mousavi M.F., *Pharmazie*, **58**, 114 (2003).
25. Jabbari A., Barzegar M. and Mohammmadi M., *Ind. J. Chem.*, **44A**, 1215 (2005).

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