Development of spectrophotometric method for analysis of mercaptopurine and dimercaprol for use in quality control and monitoring water pollution

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Abstract

In the present work, spectrophotometric method was developed for analysis of mercaptopurine and dimercaprol to be applied in quality control and monitoring water pollution. These drugs are used for the treatment of cancer and heavy metal poisoning respectively. Both mercaptopurine and dimercaprol react with colourless anhydrous manganese (II) acetate in DMSO to form soluble brown coloured complexes which showed λ_{max} at 350 nm and 360nm during spectrophotometric analysis.

This newly developed method was successfully applied for analysis of commercial drugs and spiked water. The recovery of these drugs from commercial formulations and spiked water samples was in the range between 98.0-99.7% with RSDs in the range 0.56-1.50% that indicated high degree of accuracy and precision of the method.

Keywords: Anhydrous manganese (II) acetate, Mercaptopurine, Dimercaprol, Spectrophotometry, Spiked water.

Introduction

Mercaptopurine and dimercaprol are effective and safe mercapto drugs. Mercaptopurine (MP) is a chemotherapeutic agent used for the treatment of acute myeloid leukaemia, acute lymphoblastic leukaemia, chronic myeloid leukaemiaand various other cancers[11,12,15]. Dimercaprol, commonly known as British Anti-Lewisite (BAL), is used to treat arsenic, gold, copper and mercury poisoning[3,27]. The analysis of these drugs for their active ingredient content is an important part of drug analysis in order to maintain uniform therapeutic standard for all form of medication and to establish safe and practical standards with respect to both dosage and quality. Though these drugs are considered as indispensable and safe, yet they are responsible for polluting the water bodies when enter into water sources by the population after simply flushing out outdated and unused drugs[4].

For the purpose of quality control and monitoring water pollution, there is a dire need to determine these drugs in commercial pharmaceutical formulations and in spiked water samples.Various methods for the analysis of these drugs have been reported using different techniques like spectrophotometry[5,8,22-24,26], fluorimetry[10,14], voltammetry[2,18,25], GC and HPLC[1,6,7,9,13,16,20,21]. Despite the precision and accuracy, all reported methods are time-consuming, expensive, requiring high analytical skill and thus limiting their wide applicability.

However, analysis by spectrophotometry permits low cost, easy operation, good precision and high sensitivity. Methods based on spectrophotometry find wide acceptance due to their simplicity and allowing analysis in less time[19,28]. Therefore, in the present work, an attempt has been made to develop the spectrophotometric method for the determination of mercaptopurine and dimercaprol using anhydrous manganese (II) acetate as chemical sensor.

Material and Methods

Analytical standard mercaptopurine and dimercaprol (Sigma-Aldrich), two commercial formulations viz. MP (Zydus Oncosciences, Ahmedabad, India) and Captomer (Neon laboratories Ltd., Mumbai, India) were each labelled to contain 50 mg of mercaptopurine per tablet. One formulation of dimercaprol viz. BAL injection B.P. (The Boots Company PLC, Nottingham, England) containing 50 mg mL^{-1} of injection was procured and used as received. Dimethyl sulphoxide (DMSO) (Extrapure, Sisco Research Laboratories Pvt. Ltd., Bombay) was dried over calcium oxide and then distilled under reduced pressure[19.] Carbon disulphide (Baker analysed) was used as received.

Preparation of reagents used in analysis: For making the standard solution of anhydrous manganese (II) acetate in DMSO, first the hydrated sample of manganese(II) acetate [$Mg(CH_3COO^-)_2.4H_2O$] was converted into anhydrous form by keeping the sample overnight under acetic anhydride and then white anhydrous compound was filtered and washed with ether, dried and kept in tightly closed bottle. Standard solution of anhydrous manganese (II) acetate in DMSO was prepared by dissolving a little more than calculated amount of the sample in DMSO and standardised against EDTA[28].

Analysis of mercaptopurine and dimercaprol: For the analysis of mercaptopurine and dimercaprol, standard curves of these drugs were prepared. Aliquots (0.1-2.0 mL) of the standard solution in DMSO of mercaptopurine (0.001 mol L^{-1}) and dimercaprol (0.0005 mol L^{-1}) were taken in 5 mL measuring flasks and volume made to 2 mL with DMSO. Each solution was also mixed anhydrous manganese (II) acetate (1 ml,~0.01M in DMSO) and the final volume made to 5 mL with DMSO. The absorbance of the resulting brown colour was measured at 350 nm and 360 nm for mercaptopurine and dimercaprol respectively (λmax of

manganese (II) drug complex, fig. 1 and 2). The colour was stable for at least 120 min in each case. The calibration graphs were constructed by plotting the absorbance values against concentration of each drug compound in the usual way and Beer's law is obeyed up to 60 and 65µg ml^{-1} of mercaptopurine and dimercaprol respectively. Optical and calibration characteristics are summarized in table 1. The content of mercaptopurine and dimercaprol present in the commercial formulation and spiked water was determined from the standard curves.

Determination of mercaptopurine and dimercaprol in commercial formulation: Two formulations of mercaptopurine (captomer and 6-MP) each labelled to contain 50 mg of MP per tablet and one formulation of dimercaprol injection B.P. containing 50 mg ml^{-1} of injection were used. A known number of tablets (20 tablets) of each formulation of mercaptopurine were weighed and crushed into powder. Stock solution was prepared by dissolving accurately weighed amount equivalent to 152.16 mg of MP in DMSO and sonicated for 10 min. The solution was filtered and residue was washed 2-3 times with DMSO. The filtrate and washings were diluted to 100 mL with same solvent. The stock solution of dimercaprol from its injection formulation was prepared by dissolving accurate volume of injection to a known volume of DMSO. Suitable aliquots of the extracts were taken for analysis as described for pure compounds. Assay results are given in table 2 and 3.

Determination of mercaptopurine and dimercaprol in spiked water samples: About one liter of tap water was randomly collected from the local area. 10 mL of water sample was transferred into five 25 mL glass tubes and spiked with DMSO solution of mercaptopurine (0.001 mol L^{-1}) in the range 3.0-60.0 µg and of dimercaprol (0.0005 mol L^{-1}) in the range 1.2-14.4 µg. Each solution was then mixed with 5 mL of buffer solution of pH 5, 2 mL cyclohexane and 1 mL of Triton X-100 (1% aqueous solution) and diluted to 25 mL with water.

Table 1
Spectrophotometric determination of mercaptopurine and dimercaprol with anhydrous manganese(II) acetate: optical and calibration characteristics

Characteristics	Mercaptopurine	Dimercaprol
λ_{max} (nm)	350	360
Beer's range (µg mL^{-1})	3.0-60.0	2.4 – 65.0
Stability (min)	120	120
Molar Absorptivity (ε) [L mol^{-1} cm^{-1}]	2.6 x 10^3	1.50 x10^3
Slope	0.0114	0.0265
Intercept	0.1461	-0.0282
Correlation coefficient (r)	0.9864	0.9895

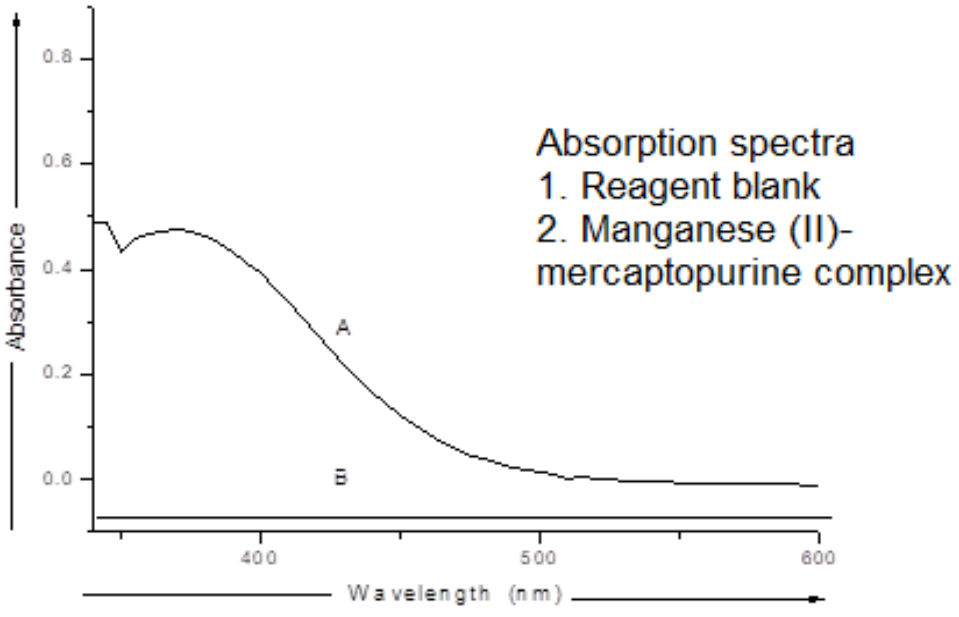


Fig. 1: Absorption spectra of manganese (II) - mercaptopurine complex

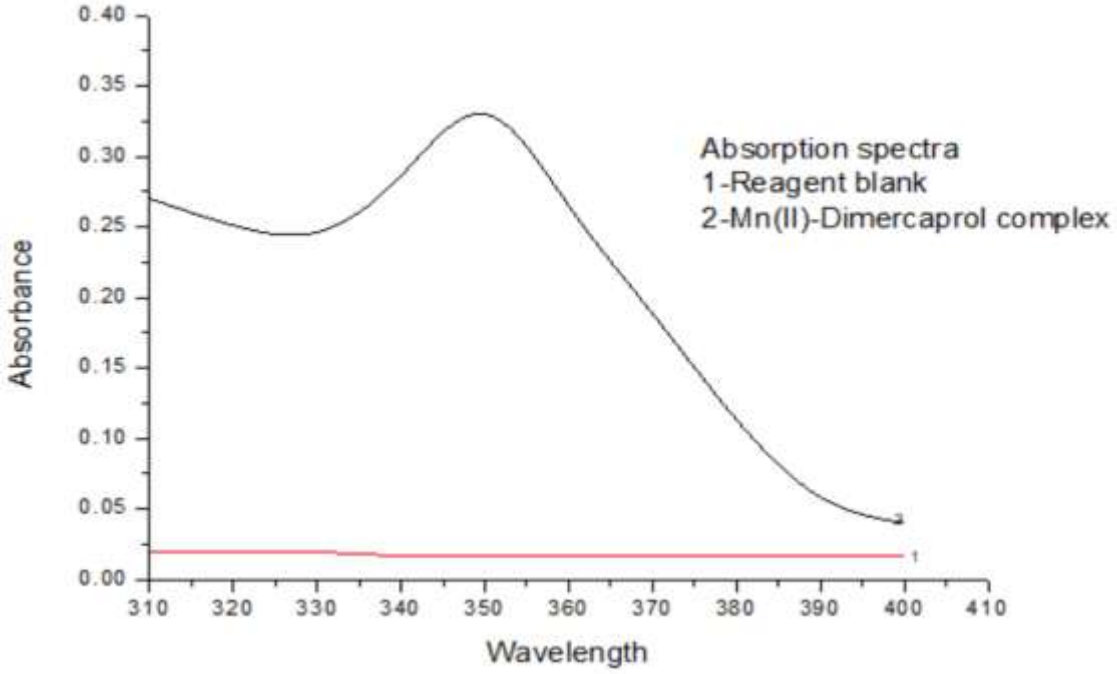


Fig. 2: Absorption spectra of manganese (II) - dimercaprol complex

Table 2
Spectrophotometric determination of mercaptopurine in pharmaceutical dosage forms.

Drug Compound	Formulation	Maker's specification†	Active Ingredient added (µg)	Active Ingredients*	
				Found (µg)	Recovery (%)
Mercaptopurine	Captomer	50 mg per tablet	3.0	2.94±0.045	98.0±1.50
			15.0	14.89±0.193	99.3±1.29
			30.0	29.86±0.255	99.5±0.85
			45.0	44.45±0.298	98.8±0.66
			60.0	59.85±0.334	99.7±0.56
	6-MP	50 mg per tablet	3.0	2.96±0.041	98.7±1.36
			15.0	14.87±0.187	99.1±1.25
			30.0	29.86±0.241	99.5±0.80
			45.0	44.81±0.282	99.6±0.63
			60.0	59.89±0.326	99.8±0.54

*Values are mean of the five determinations with standard deviations (±).
†Makers specificationsare established by independent method[17]

Table 3
Spectrophotometric determination of dimercaprol in pharmaceutical dosage forms.

Drug Compound	Formulation	Maker's specification†	Active ingredient added (µg)	Active ingredient*	
				Found (µg)	Recovery (%)
Dimercaprol	B.A.L injection B.P.	50 mg per mL			
			2.4	2.37±0.033	98.9±1.37
			7.2	7.15±0.069	99.3±0.96
			14.4	14.30±0.088	99.3±1.16
			28.8	28.66±0.204	99.5±0.71
			57.6	57.37±0.472	99.6±0.82

*Values are mean of the five determinations with standard deviations (±).
†Makers specification are established by independent method[17]

The content of each flask was transferred into 25 mL centrifuging tubes and kept in the thermostatic bath at 55°C for 10 min. Separation of the phases was done by centrifugation at 3500 rpm for 10 min. The aqueous phase was easily removed by pipette. The surfactant-rich phase was dissolved in 2 mL DMSO and mixed with 1 mL of anhydrous manganese(II) acetate solution (in DMSO) and the final volume was made to 5 mL with DMSO. The absorbance of the brown colour was measured at 350 and 360 nm respectively against a reagent blank prepared under similar conditions. The results are summarized in table 4.

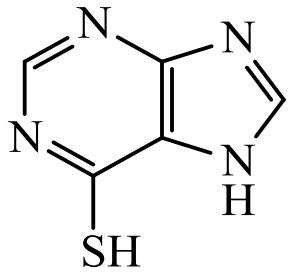
Results and Discussion

Manganese (II) acetate forms coloured complexes with water insoluble organosulphur compounds including mercaptans but its original light pink colour deepens in organic solvents and consequently restricts its use in organic solvents. In order to extend the advantages of this reagent in organic analysis, in the present work, anhydrous manganese (II) acetate was stabilized by treating manganese (II) acetatetetrahydrate with acetic anhydride.

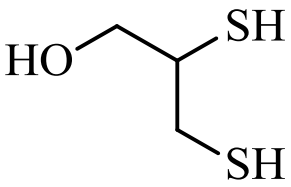
The resulting reagent form was quite stable and formed stable and colourless solution in DMSO and in other organic solvents. This reagent was found suitable for the spectrophotometric determination of mercaptopurine and dimercaprol drugs.

The proposed method was based on the formation of brown complexes with the mercapto group of the drug compounds. Mercaptopurine with one mercapto function reacts with the reagent in 4:1 molar ratio, dimercaprol with two functions reacts in 2:1 molar ratio forming an intense brown complex in each case.The above stoichiometry has also been established by photometric titrations of the drug compounds at respective wavelengths. An inverted L-shaped titration curve with intersections at 4:1 and 2:1 molar ratios with mercaptopurine and dimercaprol was obtained (Fig. 3).

The reagent forms colourless solution in DMSO which was stable and reacts with mercaptopurine and dimercaprol to form corresponding drug manganese (II) soluble brown complexes. The analysis was accomplished by measuring the colour at 350 nm and 360nm respectively. Optimizations of various experimental parameters, stoichiometry and proposed mechanism of colour reaction were also studied in developing the method for its application to the analysis of these drugs in pharmaceutical formulation and water samples. Structures of mercaptopurine (I) and dimercaprol (II) and chemical reaction (III) are shown in scheme 1.



(I)



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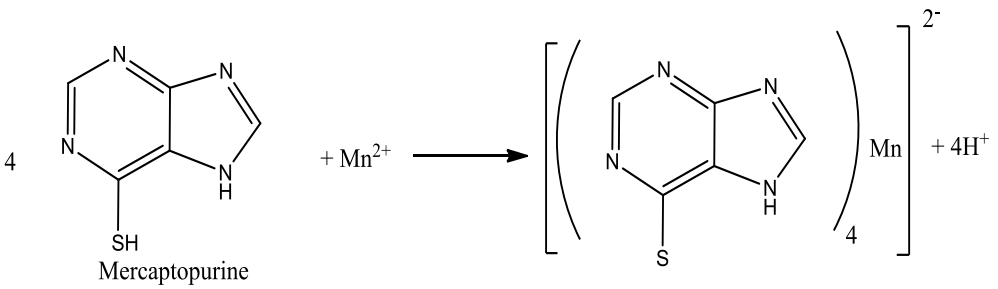

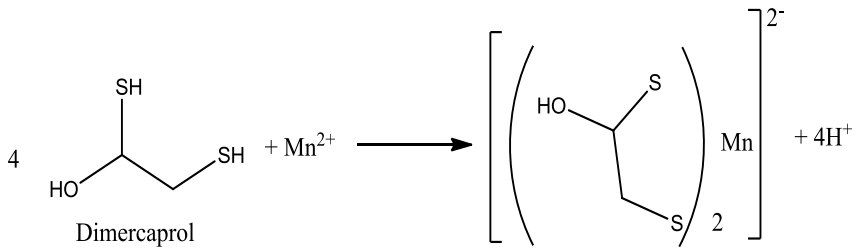

(III)

Scheme 1: Structures of mercaptopurine and dimercaprol are shown in scheme 1 and chemical reaction

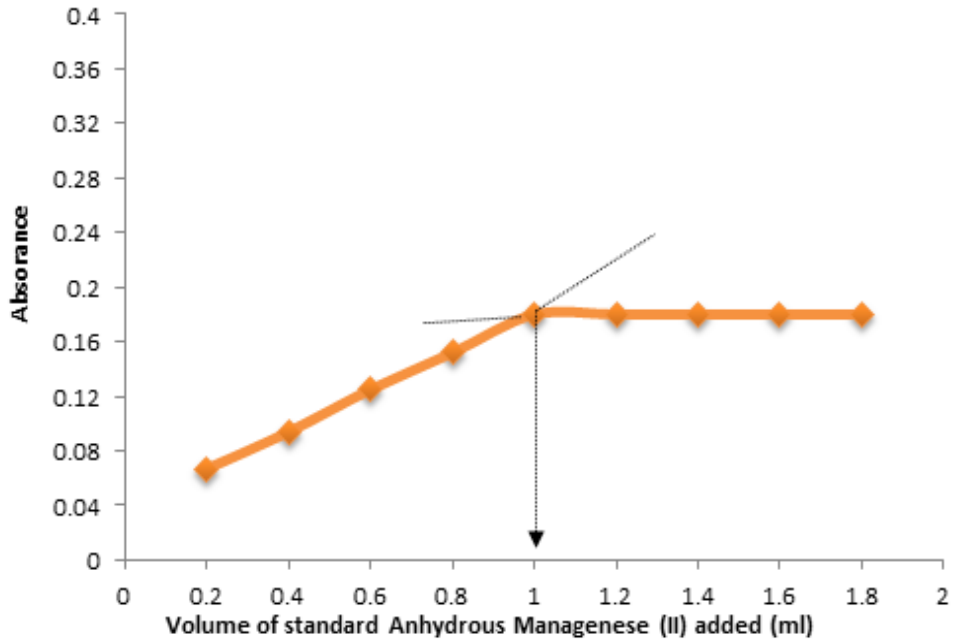

Fig. 3: Typical photometric titration curve of mercaptopurine and dimercaprol with anhydrous manganese (II) acetate

Table 4
Spectrophotometric determination of mercaptopurine and dimercaprol in spiked water samples.

Drug Compound	Active Ingredient added (µg)	Active Ingredient*	
		Found (µg)	Recovery (%)
Mercaptopurine	3.0	2.65±0.042	88.3±1.40
	15.0	13.56±0.209	90.4±1.39
	30.0	27.62±0.324	92.1±1.08
	45.0	42.51±0.348	94.5±0.77
	60.0	57.32±0.378	95.5±0.63
Dimercaprol	2.4	2.10±0.038	87.5±1.58
	7.2	6.63±0.096	92.2±1.33
	14.4	6.71±0.142	93.2±0.99
	28.8	13.42±0.228	94.8±0.79
	57.6	5.67±0.374	94.9±0.65

*Values are mean of the five determinations with standard deviations (±)

The colour which developed instantaneously on mixing thereagents was stable for 2 hours.

The Beer's law was obeyed up to 60 and 65µg ml^{-1} of mercaptopurine and dimercaprol solution respectively. The proposed method shows good precision and accuracy with maximum relative standard deviation (RSD) of 1.50% in the determination of mercaptopurine and dimercaprol in the range of 3.0-60.0 µg and 2.6-65.0 µg. The method has successfully been applied to the analysis of each drug in their commercial dosages forms and validated for their determination in spiked water samples. In the formulation analysis, the recoveries were in the range 98.0–99.7% of the nominal contents with RSD's in the range 0.56 - 1.50 % (Table 2 and 3).

Mercaptans in various biological and pharmaceutical preparations have been determined by numerous methods involving a variety of procedures and techniques. Broadly, the methods of determining mercapto group depend on one of the following fundamental procedures i.e. oxidation of mercapto group to disulphides, mercaptide formation and alkylation. These methods have, however, been described to lack specificity and doubts have frequently been expressed over the stoichiometry of oxidation. Slow oxidation, less solubility of drug compounds in water and interference from

other sulphur containing groups are some of the disadvantages of these methods. To overcome these problems, non-aqueous spectrophotometric method has been developed for the analysis of mercaptans. The method has successfully been applied to the analysis of some commercial drugs viz. dimercaprol and mercaptopurine.

A special attribute of the method is that it has been tested on a good number of mercaptans before applying to the listed drugs, thus the method would give the users a confidence in extending to more and more drugs/formulations containing mercaptans. The immediate development of the colour with colourless reagent, stability of the colour and reagent solution, non-extraction of coloured product, established stoichiometry of the colour reaction and above all, simplicity and rapidity of the procedure are other features of the proposed method. Hence, the present spectrophotometric method developed for the analysis of mercaptopurine and dimercaprol could be applied in quality control and in monitoring water pollution.

In general, drugs are considered indispensable and safe but when water bodies enter through excretion and/or when people flush out the outdated, then unused drugs are found to pollute the water resources. Therefore, to protect the natural resources form contamination, it is always advisable to follow the drug take back options as they are best way for safely disposal of unused or expired medicines.

Conclusion

A simple and rapid spectrophotometric method for determination of mercaptopurine and dimercaprol in pharmaceutical formulations has been developed. The method was based on the reaction of the mercapto group of the drug with anhydrous manganese (II)-acetate in DMSO to form brownish manganese (II) drug complex showing λ_{max} at 350 nm and 360 nm respectively. The proposed method showed good precision and accuracy with maximum relative standard deviation (*RSD*) of 1.05%. The high recoveries of drugs from their commercial pharmaceutical formulations and spiked water samples in the range 98.0-99.7% with RSDs in the range 0.56-1.50% indicated good accuracy and precision of the proposed method.

The immediate development of the colour with colourless reagent, stability of the colour and reagent solution, non-extraction of coloured product, established stoichiometry of the colour reaction and above all, simplicity and rapidity of the procedure are other attractive features of the proposed method. Overall, the present spectrophotometric method developed for the analysis of mercaptopurine and dimercaprol could be applied in quality control and monitoring water pollution.

References

1. Abdelsayed M.E., Maksoud A.S., Sidhom I., Gad Z.M. and Hanafi S.R., HPLC Determination of the Levels of 6-Mercaptopurine Metabolites Suitable for the Clinical Risk Assessment of its Toxicity among Egyptian Children with Acute Lymphocytic Leukemia, *Journal of Analytical and Bioanalytical Techniques,* **8(2),** 1-11 **(2017)**

2. Barek J., Berka A., Dempírová L. and Zima J., Polarographic and voltammetric determination of 6-mercaptopurine and 6-thioguanine, *Collect Czech. Chem. Commun*., **51,** 2466-2472 **(1986)**

3. Bastrup-Madsen P., Dimercaprol in acute lead poisoning, *The Lancet*, **256,** 171-172 **(1950)**

4. Bottoni P., Caroli S. and Caracciolo A.B., Pharmaceuticals as priority water contaminants, *Toxicological and Environmental Chemistry*, **92(3),** 549–565 **(2010)**

5. Besada A., Tadros N.B. and Gawargious Y.A., Copper (II)-neocuproine as colour reagent for some biologically active thiols: Spectrophotometric determination of cysteine, penicillamine, glutathione and 6-mercaptopurine, *Mikrochimica Acta*, **99(1-2),** 143–146 **(1989)**

6. Byers C.E., Holloway E.R., Korte W.D., Smith J.R., Clarkson E.D., Platoff G.E. and Capacio B.R., Gas Chromatographic-Mass Spectrometric Determination of British Anti-Lewisite in Plasma, *Journal of Analytical Toxicology,* **28(5),** 384–389 **(2004)**

7. Bruunshuus I. and Schmiegelow K., Analysis of 6-mercaptopurine, 6-thioguanine nucleotides and 6-thiourie acid in biological fluids by high-performance liquid chromatography, *Scandinavian Journal of Clinical and Laboratory Investigation*, **49(8),** 779–784 **(1989)**

8. Chwatko G., Spectrophotometric Method for the determination of total thiols in human urine, *Annals of Clinical Laboratory Science,* **43(4),** 424-428 **(2013)**

9. Ding T.L. and Benet L.Z., Determination of 6-mercaptopurine and azathioprine in plasma by high-performance liquid chromatography, *Journal of Chromatography B: Biomedical Sciences and Applications,* **163(3),** 281–288 **(1979)**

10. Finkel J.M., A fluorometric method for the estimation of 6-mercaptopurine in serum, *Analytical Biochemistry,* **21(3),** 362–371 **(1967)**

11. Flora S.J.S. and Pachauri V., Chelation in Metal Intoxication, *International Journal of Environmental Research and Public Health*, **7(7),** 2745–2788 **(2010)**

12. Gaynon P.S., Mercaptopurine in childhood acute lymphoblastic leukaemia, *The Lancet Oncology*, **18(4),** 425–426 **(2017)**

13. Guetens G., De Boeck G., Wood M., Maes R.A., Eggermont A.A., Highley M. and Tjaden, Hyphenated techniques in anticancer drug monitoring, *Journal of Chromatography A*, **976(1-2),** 229–238 **(2002)**

14. Hu Y.J., Liu Y., Jiang W., Zhao R.M. and Qu S.S., Fluorometric investigation of the interaction of bovine serum albumin with surfactants and 6-mercaptopurine, *Journal of Photochemistry and Photobiology B: Biology*, **80(3),** 235–242 **(2005)**

15. Iesavand H., Rahmati M., Afzali D. and Modiri S., Investigation on absorption and release of mercaptopurine anticancer drug from modified polylacticacid as polymer carrier by molecular dynamic simulation, *Materials Science and Engineering: C*, **105,** 110010 **(2019)**

16. Ika M. andalusia R., Supandi S. and Harahap Y., Simultaneous analytical method development of 6-mercaptopurine and 6-methylmercaptopurine in dried blood spot using ultra performance liquid chromatography tandem mass spectrometry, *International Journal of applied Pharmaceutics*, **9**, 168-171 **(2017)**

17. Indian Pharmacopoeia, **6(2),** 1229, 1654 **(2010)**

18. Keyvanfard M., Khosravi V., Karimi-Maleh H., Alizad K. and Rezaei B., Voltammetric determination of 6-mercaptopurine using a multiwall carbon nanotubes paste electrode in the presence of isoprenaline as a mediator, *Journal of Molecular Liquids*, **177,** 182–189 **(2013)**

19. Perrin D.D., Armarego W.L. and Perrin R.D., Purification of Laboratory chemical, Pergamon Press Ltd., 196 **(1960)**

20. Perrett D. and Rudge S.R., The determination of thiols and related compounds using high-performance liquid chromatography, *Journal of Pharmaceutical and Biomedical Analysis*, **3(1),** 3–27 **(1985)**

21. Sabourian R., Mirjalili S.Z., Namini N., Chavoshy F., Hajimahmoudi M. and Safavi M., HPLC methods for quantifying anticancer drugs in human samples: A systematic review, *Analytical Biochemistry*, **610,** 113891 **(2020)**

22. Sharma D.K., Sambra B.S., Verma N. and Verma B.C., Titration of Mercaptans and Some Mercaptan-Based Drugs with Iodine Monobromide in Non-Aqueous Dimethyl Sulfoxide, *Collection of Czechoslovak Chemical Communications*, **62(1),** 42–46 **(1997)**

23. Skowron M. and Ciesielski W., Spectrophotometric determination of methimazole, D-penicillamine, captopril and disulfiram in pure form and drug formulations, *Journal of Analytical Chemistry*, **66(8),** 714–719 **(2011)**

24. Tu C.Q. and Wen X.R., Spectrophotometric Determination of 6-Mercaptopurine in Pharmaceutical Sample Using Fe (III)-Potassium Ferricyanide System, *Advanced Materials Research*, **881-883,** 479–483 **(2014)**

25. Verma B.C., Verma N., Singh J. and Sharma D.K., Pulse polarographic determination of British Anti Lewisite (BAL) injection and analysis of some sulpha drugs, *Journal of Scientific and Industrial Research*, **58,** 869-871 **(1999)**

26. Verma B.C., Jamwal V.S., Sambra B.S. and Sharma D.K., New Colorimetric and Photometric Titration Procedures For The Determination Of Dimercaprol In Injections, *Indian J. Pharm. Sci.*, **57(6),** 249-251 **(1995)**

27. Vilensky J.A. and Redman K., British anti-lewisite (dimercaprol): An amazing history, *Annals of Emergency Medicine*, **41(3),** 378–383 **(2003)**

28. Vogel A.I., Quantitative Inorganic, Analysis, 3rd Ed., Elbs and Longman, London, 34 **(1975)**.

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