WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 14, 1018-1023. Research Article ISSN 2277– 7105

DETERMINATION AND ASSAY OF SOME ANTINEOPLASTIC AGENTS IN PURE FORM AND IN THEIR PHARMACEUTICAL PREPARATIONS USING TITRIMETRIC METHOD

***Parulekar G. T.**

Department of Chemistry, Shri S. H. Kelkar College of Arts, Commerce and Science, Devgad, India.

Article Received on
16 Sep. 2017,
Revised on 06 Oct. 2017,
Accepted on 26 Oct. 2017
DOI: 10.20959/wjpr201714-10035

***Corresponding Author**
Parulekar G. T.
Department of Chemistry, Shri S. H. Kelkar College of Arts, Commerce and Science, Devgad, India.

ABSTRACT

Antineoplastics drugs are the drugs that prevent or inhibit the maturation and proliferation of neoplasms. Antineoplastic agents travel the body and destroy cancer cells. Many of the side effects associated with antineoplastic agents occur because treatment destroys the body's normal cells in addition to cancerous cells. In the present work we have developed a titrimetric method for the determination of some Antineoplastic agents i.e. Capecitabine, Mercaptopurine, Gemcitabine and 5-Fluorouracil in pure form and in their pharmaceutical preparations like Xeloda (Tab.), Purinethol (Tab), Gemzar (Inj) and Adrucil (Inj) by using Nchlorosuccinimide (NCS) reagent in acidic medium. A result shows that the suggested method is accurate, reproducible and precise. It can easily be adopted in an ordinary pharmaceutical laboratory where sophisticated instruments are not available.

KEYWORDS: Antineoplastic agents, titrimetric method, Capecitabine, Mercaptopurine.

INTRODUCTION

Antineoplastic chemotherapy is an important component of small animal practice and is routinely used for selected tumors of horses and cattle. Effective use of antineoplastic chemotherapy depends on an understanding of basic principles of cancer biology, drug actions, toxicities and drug handling safety. In the series of Antineoplastic agents antimetabolites and alkylating agents are well known for exhibiting wide range of pharmacological and biological properties. These agents check the metabolism of neoplasmic cells either by converting or binding the metabolic products of a neoplasmic cells.

Capecitabine (pentyl [1-(3,4-dihydroxy-5-methyl tetrahydrofuron-2-yl)-5-fluoro-2-oxo-1-Hpyrimidin- 4-yl] aminomethanoate) is a pyrimidine antagonists and it acts as prodrug, i.e., enzymatically converted to 5- fluorouracil in tumor tissues. It is a chemotherapeutic agent and is used in the treatment of metastatic breast and colorectal cancers (Zufia *et al.,* 2004, Sylvie *et al.,* 2005, Medikondu *et al.,* 2010). Mercaptopurine is an antimetabolite antineoplastic agent with immunosuppressant properties. It interferes with nucleic acid synthesis by inhibiting purine metabolism and is used, usually in combination with other drugs, in the treatment of or in remission maintenance programs for leukemia. Mercaptopurine is one of a large series of purine analogues which interfere with nucleic acid biosynthesis and has been found active against human leukemias. It is an analogue of the purine bases adenine and hypoxanthine. It is not known exactly which of any one or more of the biochemical effects of mercaptopurine and its metabolites are directly or predominantly responsible for cell death (Wypior *et al.,* 1982). Gemcitabine is a nucleoside analog used as chemotherapy. It is marketed as Gemzar® by Eli Lilly and Company. As with fluorouracil and other analogues of pyrimidines, the drug replaces one of the building blocks of nucleic acids, in this case cytidine, during DNA replication. The process arrests tumor growth, as new nucleosides cannot be attached to the "faulty" nucleoside, resulting in apoptosis (cellular "suicide"). Gemcitabine is used in various carcinomas: non-small cell lung cancer, pancreatic cancer, bladder cancer and breast cancer. It is being investigated for use in oesophageal cancer and is used experimentally in lymphomas and various other tumor types (Mangamma *et al.,* 2012, Annapurna *et al.,* 2013). 5-Fluorouracil (5-FU) is widely used in the treatment of cancer. Over the past 20 years, increased understanding of the mechanism of action of 5-FU has led to the development of strategies that increase its anticancer activity. Despite these advances, drug resistance remains a significant limitation to the clinical use of 5-FU. Emerging technologies, such as DNA microarray profiling, have the potential to identify novel genes that are involved in mediating resistance to 5-FU. Such target genes might prove to be therapeutically valuable as new targets for chemotherapy, or as predictive biomarkers of response to 5-FU-based chemotherapy (Alsarra *et al.,* 2004). Because of the pharmacological importance of these drugs, several methods were reported for quantitative evaluation of these compounds (Indian Pharmacopeia, 2007). Most of the methods employ sophisticated instruments like High pressure thin layer chromatography (HPLC), thin layer chromatography (TLC), I.R. absorption Spectrophotometry and other techniques. Here we report a simple titrimetric method for the assay of the some antineoplastic drugs i.e., Capecitabine, Mercaptopurine, Gemcitabine and 5-Fluorouracil by using NCS reagent in

acidic medium. This reagent has also been used for the oxidation studies of primary and secondary alcohols. Present method may easily be adopted in any pharmaceutical laboratory having no sophisticated instruments.

MATERIALS AND METHODS

Chemicals and Solutions Preparation (Dwivedi *et al.*, 1988)

N-chlorosuccinimide (NCS) solution 0.02N (Loba Chemie) 2.6713 g of N-chlorosuccinimide was weighed accurately and dissolved in distilled water in a 100mL volumetric flask.

Sodium thiosulphate solution 0.02N (Merck): 4.9636 g of sodiumthiosulphate was accurately weighed and dissolved in distilled water in a 1000mL volumetric flask.

Copper sulphate solution 0.025N (Qualigens): 0.4994 g of Copper sulphate penta hydrate was weighed accurately and dissolved in distilled water in a 100mL volumetric flask.

Potassium iodide solution 5% w/v (Qualigens): 5 g potassium iodide was accurately weighed and dissolved in distilled water in a 100mL volumetric flask.

Starch solution 1%w/v (Merck): 1 g of starch powder was dissolved in hot distilled water in a beaker with a continuous stirring.

Hydrochloric acid 4N (Merck): 348.64 mL of concentrated hydrochloric acid was diluted with distilled water in 1000 mL volumetric flask and solution was made up to the mark.

Sample solutions

Accurately weighed 50 mg of pure samples were dissolved in minimum amount of hydrochloric acid in 50 mL volumetric flask and then made up to the mark with distilled water to give a concentration of 1 mg/mL. Tablet solutions: Tablets of a particular sample were crushed up to a fine powder and powder equivalent to 50 mg of sample were taken in 50 mL volumetric flask and dissolved similarly. Injection solution: 50 mg of the sample were dissolved in distilled water in 50 mL volumetric flask.

Experimental

Aliquots containing 1-5 mg of the sample were taken in 100 mL stoppered conical flask and 5 mL of 0.02N N-chloro succinimide and 5 mL of 4N hydrochloric acid was added to it. The reaction mixture was shaken well and allowed to react for required reaction time (10 min.) at

room temperature (25-30^{0}C). After the reaction was over 5 mL of 5% potassium iodide solution was added to it, content shaken thoroughly and allowed to stand for a minute. The liberated iodine was titrated against 0.02N sodium thiosulphate solution using starch indicator. A blank experiment was also under identical conditions using all reagents except the sample. The amount of sample was calculated by the difference in titre values of 0.02N sodium thiosulphate solution used for actual and blank experiments. On the basis of percentage error, the value of SD and RSD were also calculated (Table-1). The same procedure was applied for the determination of pharmaceutical preparations.

RESULTS AND DISCUSSION

Table 1: Determination of some Antineoplastic agents in pure form and in their pharmaceutical preparations

Sr. No	Sample	Aliquots taken (mL)	Amount present (mg)	Reaction Time (min.)	Molecularity (n)	Amount obtained by calculation	Error (%)	SD	CV
1	Capecitabine (Pure)	1	0.996	10	2	0.984	1.20	0.0045	0.4573
		3	2.988	10	2	2.975	0.78	0.0089	0.2992
		5	4.980	10	2	4.956	0.48	0.0075	0.1513
	Xeloda (Tab.)	1	0.980	10	2	0.962	1.84	0.0100	1.0395
		3	2.940	10	2	2.927	0.44	0.0132	0.4510
		5	4.900	10	2	4.892	0.16	0.0075	0.1533
2	Mercaptopurine (Pure)	1	0.990	10	3	0.980	1.00	0.0071	0.7245
		3	2.970	10	3	2.936	1.14	0.0062	0.2112
		5	4.950	10	3	4.921	0.58	0.0062	0.1260
	Purinethol (Tab),	1	0.985	10	3	0.977	0.81	0.0075	0.7677
		3	2.955	10	3	2.957	0.06	0.0089	0.2992
		5	4.952	10	3	4.939	0.26	0.0075	0.1513
3	Gemcitabine (Pure)	1	0.995	10	3	0.980	1.51	0.0128	1.3292
		3	2.985	10	3	2.959	0.87	0.0122	0.4123
		5	4.975	10	3	4.957	0.36	0.0137	0.2764
	Gemzar (Inj),	1	0.994	10	4	0.975	1.91	0.0054	0.5538
		3	2.982	10	4	2.968	0.46	0.0093	0.3133
		5	4.970	10	4	4.951	0.38	0.0091	0.1838
4	5-Fluorouracil (Pure)	1	0.984	10	3	0.975	0.91	0.0082	0.8410
		3	2.952	10	3	2.938	0.47	0.0055	0.1872
		5	4.920	10	3	4.911	0.18	0.0033	0.0672
	Adrucil (Inj)	1	0.976	10	3	0.964	1.22	0.0137	1.4391
		3	2.928	10	3	2.907	0.71	0.0121	0.4144
		5	4.880	10	3	4.862	0.36	0.0121	0.2480

The reaction conditions were established after studying the effect of variables such as reaction time, reaction temperature, concentration and volume of N-chlorosuccinimide reagent, concentration and volume of hydrochloric acid and reaction temperature. In the determination of all antineoplastic agents 10min. reaction time was needed to complete the

reaction. A much more reaction time (beyond 10min.) does not improve the results. At lesser reaction time (less than 10min.) the recovery of the sample was low because of the incomplete reaction. It was also established that the prescribed concentration of reagent (0.02N) was suitable for accurate results. An increase in concentration of reagents (0.1-1.0N) does not have any effect on the recovery. A lower concentration (0.03-0.05N) gives inaccurate results because of incomplete reaction. While studying the effect of concentration of hydrochloric acid it was found that recommended concentration (4N) and volume (5 mL) of the acid was suitable for the reaction. While studying the effect of reaction temperature it was noticed that the reaction was complete at room temperature (25-30 0C). On heating the reaction mixture either on water bath or directly on flame gives inaccurate results because of the decomposition of the reagents. If the reaction was carried out at lower temperature (15-0^0C) the speed of the reaction was much more retarded. On increasing reaction time (15-40min.) there was no effect on the percentage recovery.

Results of determinations are reported in table-1, it shows that the suggested method is accurate, reproducible and precise. It can easily be adopted in an ordinary pharmaceutical laboratory where sophisticated instruments are not available. On the basis of molecularity, available literature a possible course of reaction may be suggested for each compound. Since isolation of the final products and their identification has not been possible, it may be proposed that the compounds get converted to corresponding oxidized products. It was found that the stoichiometric ratio of NCS for different drug molecule are different such as capecitabine(2:1), Mercaptopurine (3:1), Gemcitabine (4:1) and 5-Fluorouracil (3:1) in pure form and in their pharmaceutical preparations. Indian Pharmacopoeia does not describe this type of general method for determination of above Antineoplastic drugs. However there are methods for individual compounds e.g. Capecitabine, Mercaptopurine, Gemcitabine and 5-Fluorouracil has been determined by IR absorption Spectrophotometry and Thin Layer Chromatography. Indian Pharmacopoeia-2007 does not describe any method for the determination of capecitabine.

REFERENCES

1. Dwivedi H.C. and I.C. Shukla. Determination of pyrimethamine with N-chlorosuccinimide in acidic medium. Indian Journal of pharmaceutical Sciences, 1988; 50(2): 136-137.
2. Indian Pharmacopeia. The Controller of Publication, Ghaziabad, New Delhi, 2007; 2: 986, 1059.
3. Medikondu Kishore, M. Jayaprakash, T.Vijayabhaskarareddy. Spectro- Photometric Determination of Capecitabine in Pharmaceutical formulations. International Journal of Chemical and Analytical Science. 2010; 1(10): 238-241.
4. Sylvie M. Guichard, Iain Mayer, Duncan I. Jodrell. Simultaneous determination of capecitabine and its metabolites by HPLC and mass spectrometry for preclinical andclinical studies. Journal of Chromatography B, 2005; 826: 232–237.
5. Zufia L., A. Aldaz, J. Giraldez. Simple determination of capecitabine and its metabolites by liquid chromatography with ultraviolet detection in a single injection. Journal of Chromatography B, 2004; 809: 51–58.
6. Wypior, H.J., Schilling, A., Von Meyer, L., Land, W. (1982). A gas chromatographic determination of 6-mercaptopurine in the serum of immunosuppressed transplantation patients. Research in Experimental Medicine (Berl), 181(1): 19-25.
7. Annapurna MM, Kumar BSP, Venkatesh B. Validated stability indicating liquid chromatographic method for the determination of gemcitabine hydrochloride in parenterals. Indo Am J Pharm Res., 2013; 3: 23-36.
8. Mangamma K, Rao DV, Mohan VS, Prasanna AD. Method development and validation of gemcitabine and irinotecan by RPHPLC in pharmaceutical formulation. Int J Chem Anal Sci., 2012; 3: 1500-2.
9. Alsarra I.A. and M.N. Alarifi. Validated liquid chromatographic determination of 5-fluorouracil in human plasma. J. Chromatogr. B Biomed. Appl. 2004; 804: 435–439.