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A NEW TITRIMETRIC METHOD FOR THE ASSAY OF SOME ANTINEOPLASTIC AGENTS

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ABSTRACT

In the present work we have developed a new method for the determination of some Antineoplastic agents i.e. capecitabine, cytarabine, etoposide, doxorubicin hydrochloride and methotrexate in pure form and in their pharmaceutical preparations like xabine (Tab.), xeloda (Tab.), cytabine (Inj.), cytaraside (Vial), etosid (Tab.), peltasol (Inj.), zodox (Vial), oncodox (Inj.), biotrexate (Inj.), and imutrex (Inj.) by using N-chlorosuccinimide (NCS) reagent in acidic medium. This reagent in acidic medium produces chloronium ion (Cl^+) which is highly reactive. It may react with allylic hydrogen or hydroxyl group of the substances and forming respective oxidation products. Aliquots containing 1-5 mg of sample were taken in 100 mL erlenmeyer flask followed by the addition of 5mL of 0.02N N-chlorosuccinimide reagent (NCS) and 5mL of 4N hydrochloric acid. The reaction mixture was shaken thoroughly and allowed to react at room temperature (25-30^{0}C) for required reaction time (10min.). After the reaction was over 5mL of 5% KI solution was added to it, contents shaken thoroughly and allowed to stand for a minute. The liberated iodine was titrated against 0.02N sodiumthiosulphate solution using starch indicator. A blank experiment was also run under identical conditions using all the reagents except sample. Results were calculated by the difference in the titre value of 0.02N sodiumthiosulphate for blank and actual experiment. Maximum deviation was found within the error of ± 1%. The value of standard deviation (SD), relative standard deviation (RSD) and recovery experiments prove to be precise and reproducible.

KEYWORDS: Antineoplastic agents, titrimetric method, standard deviation (SD), relative standard deviation (RSD), recovery experiment,

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INTRODUCTION

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-H-pyrimidin-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. Cytarabine or Cytosine arabinoside is 1-β-arabinofuranosylcytosine. It is also known as Ara-C, arabinofuranosylcytidine and is used in the treatment of cancers of white blood cells such as acute myeloid leukemia (AML) and non-Hodgkin lymphoma. Doxorubicin hydrochloride known as (8S,10S)-10-{[(2R,4S,5S,6S)-4-amino-5-hydroxy-6- methyloxan-2-yl]oxy}-6,8,11-trihydroxy-8-(2-hydroxyacetyl)-1-methoxy-5,7,8,9,10,12-hexahydrotetracene-5,12-dionehydrochloride is an antineoplastic antibiotic obtained from streptomyces peucetius. Etoposide [4'-demethyepipodophyllotoxin-9-[4,6,-0-ethylidine-β-D glucopyranoside] inhibits DNA synthesis by forming a complex with topoisomerase-II and DNA. This complex induces breaks in double strand DNA and prevents repair by topoisomerase-II binding. It acts primarily in the G-2 and S-phases of the cell cycle. Methotrexate (MTX) (2S)-2-[(2,4-diaminopteridin-6-yl)methyl] (methyl)-amino}phenyl) formamido] pentanedioic acid is an antimetabolites and antifolate drug mainly used in the treatment of autoimmune diseases, ecotopic pregnancy, and for the induction of medical abortions. It acts by inhibiting the metabolism of folic acid.

Because of the pharmacological importance of these drugs, several methods were reported for quantitative evaluation of these compounds. Most of the methods employ sophisticated instruments like High pressure thin layer chromatography[1-4] (HPLC), thin layer chromatography (TLC), I.R. absorption Spectrophotometry[5-8], and other techniques[9-12]. Here we report a simple titrimetric method for the assay of the some antineoplastic drugs i.e., capecitabine, cytarabine, doxorubicin hydrochloride, etoposide and methotrexate by using NCS reagent in acidic medium. This reagent has also been used for the oxidation studies of primary and secondary alcohols[13-14]. Our laboratory has been engaged in developing titrimetric methods with different reagents[15-16]. Present method may easily be adopted in any pharmaceutical laboratory having no sophisticated instruments. To establish authenticity of the method recovery experiments were also done by standard drug addition method.

EXPERIMENTAL

REAGENTS AND SOLUTIONS

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. Sodiumthiosulphate solution 0.02N [Merck]: 4.9636 g of sodiumthiosulphate was accurately weighed and dissolved in distilled water in a 1000mL volumetric flask. Coppersulphate solution 0.025N [Qualigens]: 0.4994 g of Coppersulphate pentahydrate 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.

General Procedure

Aliquots containing 1-5 mg of the sample were taken in 100 mL stoppered conical flask and 5 mL of 0.02N N-chlorosuccinimide 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 sodiumthiosulphate 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 sodiumthiosulphate 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. Excipients present in pharmaceutical preparations do not interfere.

Calculation

The amount of sample in each experiment was calculated by following expression-

Weight (mg) of sample = $\frac{M \times N(B-S)}{n}$

Where, M= molecular weight of sample, N= normality of N-chlorosuccinimide reagent solution, B= volume of sodiumthiosulphate solution used for blank experiments, S= volume of sodiumthiosulphate solution used for sample experiments, n= no. of moles of N-chlorosuccinimide consumed per mole of sample. On the basis of the results obtained, SD and RSD were calculated. Recovery experiments were carried out (Table2-6) to show the authenticity of the method by following expression-

% Recovery = $\frac{N(\sum XY)-(\sum X)(\sum Y)}{N(\sum X^2)-(\sum X)^2} \times 100$

Where, N= total No. of observations, X= amount of drug added, Y= amount of drug obtained by calculation.

RESULTS AND DISCUSSION

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

S. No.	Sample	Aliquots taken (mL)	Amount present* (mg)	Reaction time (min.)	Molecu--larity (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
A	Xabine (Tab.)	1	0.982	10	2	0.966	1.02	0.0070	0.7277
		3	2.946	10	2	2.931	0.50	0.0115	0.3923
		5	4.910	10	2	4.900	0.20	0.0169	0.3449
B	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.	Cytarabine (Pure)	1	0.998	10	3	0.989	0.90	0.0046	0.4651
		3	2.994	10	3	2.978	0.53	0.0065	0.2183
		5	4.990	10	3	4.982	0.16	0.0040	0.0803
A	Cytabine (Inj.)	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
B	Cytaraside (Vial)	1	0.978	10	3	0.961	1.73	0.0040	0.4162
		3	2.934	10	3	2.922	0.40	0.0038	0.1300
		5	4.890	10	3	4.875	0.30	0.0077	0.1579
3.	Etoposide (Pure)	1	0.988	10	2	0.971	1.72	0.0095	0.9824
		3	2.964	10	2	2.943	0.70	0.0013	0.0442
		5	4.940	10	2	4.918	0.44	0.0091	0.1850
A	Etosid (Tab.)	1	0.981	10	2	0.952	0.98	0.0120	1.2448
		3	2.943	10	2	2.920	0.78	0.0125	0.4210
		5	4.905	10	2	4.878	0.55	0.0142	0.2921
B	Peltasol (Inj.)	1	0.976	10	2	0.964	1.22	0.0137	1.4391
		3	2.928	10	2	2.907	0.71	0.0121	0.4144
		5	4.880	10	2	4.862	0.36	0.0121	0.2480
4	Doxorubicin hydrochloride (Pure)	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
A	Zodox (Vial)	1	0.974	10	4	0.957	1.74	0.0054	0.5643
		3	2.922	10	4	2.915	0.23	0.0091	0.3122
		5	4.870	10	4	4.860	0.20	0.0061	0.1255
B	Oncodox (Inj.)	1	0.976	10	4	0.959	1.76	0.0094	0.9802
		3	2.928	10	4	2.914	0.47	0.0065	0.2231
		5	4.880	10	4	4.860	0.55	0.0065	0.1339
5	Methotrexate (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
A	Biotrexate (Inj.)	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
B	Imutrex (Inj.)	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

Tab. = Tablet, Inj. = Injection *In each sample nine determinations were done,
*****Average of nine determinations.***

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 ^{0}C). 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 ^{0}C) the speed of the reaction was much more retarded. On increasing reaction time (15-40min.) there was no effect on the percentage recovery.

Table 2
Recovery studies of capecitabine by standard drug addition method.

S. No.	Number of observation (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg) Y	XY	X^2	Recovery (%)
1	3	0.996	0.982	1.950	0.966	0.949	0.964	99.35
2	3	0.996	1.964	2.916	1.932	3.794	3.857	
3	3	0.996	2.846	3.882	2.898	8.248	8.100	
4	3	0.996	3.928	4.848	3.864	15.178	15.429	
	∑N=12		∑X= 9.720		∑Y= 9.660	∑XY= 28.169	$\sum X^2$= 28.350	

Table 3
Recovery studies of cytarabine by standard drug addition method.

S. No.	Number of observation (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg) Y	XY	X^2	Recovery (%)
1	3	0.998	0.984	1.964	0.975	0.959	0.968	99.08
2	3	0.998	1.968	2.939	1.950	3.838	3.873	
3	3	0.998	2.952	3.914	2.925	8.635	8.714	
4	3	0.998	3.936	4.889	3.900	15.350	15.492	
	∑N=12		∑X= 9.838		∑Y= 9.750	∑XY= 28.782	$\sum X^2$= 29.047	

Table 4
Recovery studies of etoposide by standard drug addition method.

S. No.	Number of observation (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg) Y	XY	X^2	Recovery (%)
1	3	0.988	0.981	1.923	0.952	0.934	0.962	98.51
2	3	0.988	1.962	2.875	1.904	3.736	3.849	
3	3	0.988	2.943	3.827	2.856	8.405	8.661	
4	3	0.988	3.924	4.779	3.808	14.943	15.398	
	∑N=12		∑X= 9.810		∑Y= 9.520	∑XY= 28.018	$\sum X^2$= 28.870	

Table 5
Recovery studies of doxorubicin hydrochloride by standard drug addition method.

S. No.	Number of observation (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg) Y	XY	X^2	Recovery (%)
1	3	0.984	0.974	1.932	0.957	0.932	0.949	93.15
2	3	0.984	1.948	2.889	1.914	3.728	3.795	
3	3	0.984	2.922	3.846	2.271	6.636	8.538	
4	3	0.984	3.924	4.803	3.828	14.914	15.179	
	∑N=12		∑X= 9.740		∑Y= 8.970	∑XY= 26.210	$\sum X^2$= 28.561	

Table 6
Recovery studies of methotrexate by standard drug addition method.

S. No.	Number of observation (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg) Y	XY	X^2	Recovery (%)
1	3	0.995	0.990	1.960	0.980	0.970	0.980	98.98
2	3	0.995	1.980	2.940	1.960	3.881	3.920	
3	3	0.995	2.970	3.920	2.940	8.732	8.821	
4	3	0.995	3.960	4.900	3.920	15.523	15.682	
	∑N=12		∑X= 9.900		∑Y= 9.800	∑XY= 29.106	$\sum X^2$= 29.403	

Results of determinations are reported in table-1. To verify the precision of the method recovery experiments were done by standard drug addition method (Table2-7). 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), cytarabine (3:1), etoposide (2:1), doxorubicin hydrochloride (4:1), and methotrexate (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. cytarabine, etoposide, doxorubicin hydrochloride and methotrexate has been determined by IR absorption Spectrophotometry and Thin Layer Chromatography. Indian Pharmacopoeia-2008 does not describe any method for the determination of capecitabine.

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