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Determination and Assay of Some Antineoplastic Agents in Pure Form and in their Pharmaceutical Preparation

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In the present paper, a simple and convenient titrimetric method is reported for the determination of some antineoplastic agents *i.e.* chloropromazine hydrochloride, promazine hydrochloride, levamisole, fosfestrol, letrozole, and mitomycin in the pure form and in their pharmaceutical preparations *i.e.* relitil-25 (tablet) phenergan (tablet and injection), devormis (tablet), dicaris (tablet), levamole (tablet), vermisole (tablet), honven (tablet), lupride (tablet), progtage (tablet), almito (injection), mitocin (vial), oncicine (tablet), by using N-bromosuccinimide (NBS) reagent in acidic medium. Aliquots containing 1-5 mg of the sample were taken in 100 mL Erlenmeyer flask followed by the addition of 5 mL of (0.02 N) N-bromosuccinimide reagent and 5 mL of (4 N) CH_3COOH. The reaction mixture was shaken thoroughly and allowed to react at room temperature (25-30 °C) for required reaction time (10 min). After the reaction was over 5 mL of 5 % KI was added to it, contents shaken thoroughly and allowed to stand for 1 min. The liberated iodine was titrated against 0.02 N sodium thiosulphate 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 values of 0.02 N sodium thiosulphate for blank and actual experiment. Deviation was found within the error of ± 1 %. The value of standard deviation (SD), relative standard deviation (RSD), coefficient of variation and recovery experiments prove the method to be precise and reproducible.

Keywords: Antineoplastic agents, Titration, Standard deviation, Coefficient of variation.

INTRODUCTION

Antineoplastic agents are antimetabolite and well known for exhibiting a wide range of pharmacological and biological activities. They check the metabolism of neoplasmic cells either by converting or binding the metabolic product of neoplasmic cells. Levamisole, (6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-b]thiazole) is an anthelmintic and immunomedulator. It has been used in humans to treat parasitic worm infections [1] and has been studied in combination with other form of chemotherapy for colon cancer, melanoma and head and neck cancer [2]. Promethazine hydrochloride (10-[(2-dimethyl-amino)propyl] phenothiazine hydrochloride) is long acting antihistaminic, antagonize, the vasodilator and vasoconstrictor action of histamine [3]. Daily dose of promethazine hydrochloride required to maintain an effect on intraderminal injection of histamine in man [3]. Letrozole (4,4'-(1*H*-1,2,4-triazol-1-yl)methylene dibenzonitrile) is an oral non-steroidal aromataze inhibitor for the treatment of hormonally-responsive breast cancer after surgery and also used for the treatment of gynecomastia [4]. Chloropromazine hydrochloride (3-(2-chloro-10*H*-phenothiazine-10-yl)-N,N-dimethylpropane-1-amine) has widely been used in the treatment of schizophrenia and related disorder [5]. Fosfestrol ([4-{4-(4-phasphono-oxyphenyl)hex-3-en-3-yl}phenoxy]phosphonic acid) is a synthetic estrogen used as an antineoplastic agent. Fosfestrol tetrasodium is an inactive synthetic estrogen which is converted in body to an active estrogen diethylstilbestrol and used in the treatment of prostate cancer [6]. Mitomycin ([6-amino-8a-methoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazireno[2',3',3,4]pyrrolo-(1,2a)indol-8-yl] methyl carbamate) is in the family of aziridine containing natural product. It is used to treat upper gastro-intestinal (esophageal carcinoma), anal and breast cancer [7].

Due to the biological and pharmacological importance of these drugs several methods [8-10] were reported for quantitative evaluation of these antineoplastic agents. Most of the methods use sophisticated instruments like HPLC [11], TLC [12], colorimetry [13], infrared spectrophotometry [14] and other techniques. Herein, we reported a simple titrimetric method for assay of some antineoplastic drugs *i.e.* chloropromethazine hydrochloride, promethazine hydrochloride, levamisole, fosfestrol, letrozole, and mitomycin by using N-bromosuccinimide reagent in acidic medium. This reagent [15-19] has been used for allylic or benzylic bromination, oxidation reaction

for primary and secondary alcohol, aromatization reaction and addition to olefinic double bonds. Present method may easily be adopted in any pharmaceutical laboratory having no sophisticated instruments. To establish the authenticity of the method recovery experiments were also done by standard drug addition method.

EXPERIMENTAL

N-Bromosuccinimide (NBS) 0.02N (Loba Chemie) was prepared by dissolving accurately weighed 0.356 g of N-bromosuccinimide in 100 mL volumetric flask. Sodium thiosulphate solution (0.02 N) (Merck) was prepared in100 mL distilled water in a volumetric flask. $CuSO_4 \cdot 5H_2O$ (0.02 N) (Qualigens) solution was prepared in distilled water in a 100 mL volumetric flask, potassium iodide 5 % w/v (Qualigens) aqueous solution, 1 % w/v starch solution (Merck) and glacial acetic acid (4 N) (Merck) were also prepared.

Accurately weighed (100 mg) pure sample of chloropromazine hydrochloride, promathazine hydrochloride, levamisole, letrozole, and mitomycin were dissolved in distilled water and fosfestrol was dissolve in ethanol in a 100 mL volumetric flask and solution made up to the mark to give a concentration of 1 mg/mL.

Twenty tablets of pharmaceutical products were crushed to a fine powder and powder equivalent to 100 mg of sample was taken in 100 mL calibrated volumetric flask and dissolved similarly. No residue was noted in any of the sample. As described above, the contents of the injection equivalent to 50 mg of the pure sample were taken to get a concentration of 1 mg/mL.

General procedure: Aliquots containing 1-5 mg of the sample were taken in 100 mL stoppered conical flask and 5 mL of 0.02 N N-bromosuccinimide and 5 mL of 4 N acetic 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 °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 0.02 N sodium thiosulphate solution using starch indicator. A blank experiment was also done under identical condition using all the reagents except sample. The amount of sample was calculated by the difference in titre values of 0.02 N sodium thiosulphate solution used for actual and blank experiments. On the basis of percentage error, the value of SD and coefficient of variation were also calculated (Table-1). The same procedure was applied for the determination of pharmaceutical preparations. Excipients present in pharmaceutical preparations do not interfere.

RESULTS AND DISCUSSION

Taking chloropromazine hydrochloride as a test sample the stoichiometric ratio with the reagent was established. It was observed that three moles of the reagent were needed for complete reaction. Similarly for other compounds also the stoichiometry was determined it was found that the ratio between N-bromosuccinimide reagent and antineoplastic agents varies. *e.g.* levamisole (2:1), promazine hydrochloride (3:1), letrozole (3:1), fosfestrol (2:1) and mitomycin (3:1). The ratio remains constant even under varying reaction conditions *i.e.* change in reaction time, concentration of the reagent, reaction medium, reaction temperature etc. It was observed that a lesser reaction time (< 10 min) gives inaccurate results because of incomplete reaction. Increase in the reaction time (15-20 min) does not change percentage recovery of the sample. As compared to sulphuric acid and nitric acid the use of acetic acid was found as a proper reaction medium. Reaction was also carried out in the absence of acetic acid and it was found that the results were very low because of incomplete dissociation of the reagent. The concentration effect of acetic acid was also studied and 10 mL of 4 N acetic acid was found suitable for accurate results.

N-Bromosuccinimide is the main reactive species with all the compounds, therefore variation in its concentration was essential. While studying various (0.01-1.0 N) concentrations it was found that 0.02 N NBS was sufficient for accurate result. With variable volume of NBS (1-10 mL) it was observed that 5 mL reagent of 0.02 N concentration gives accurate result. Higher concentration (0.03-1.0 N) and volume (6-10 mL) does not improve results. The effect of reaction temperature has also been studied. It was observed that the reaction is very slow at ice cold temperature (0-5 °C). As indicated in Table-1 the best results were obtained at room temperature (25-30 °C). Increase in reaction temperature above (30-100 °C) gives in accurate results because of decomposition of the reagent. On increasing the reaction time (15-30 min) there was no effect on the percentage recovery. Results obtained at standard reaction conditions are reported in Table-1. To verify the precision of the method recovery experiments were done by standard drug addition method (Table-2). 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. Indian pharmacopeia does not describe such type of method for the determination of compounds under study. Only chlorpromazine hydrochloride, promethazine hydrochloride and levamisole have been determined by infrared spectrophotometry and thin layer chromatography in Indian pharmacopeia [20].

Calculation

For each experiment, the amount of sample was calculated by following expression:

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

where, M = molecular weight of the sample, N = normality of sodium thiosulphate solution, B = volume of sodium thiosulphate solution for blank, S = volume of sodium thiosulphate solution for sample, n = stoichiometry of the reaction.

For testing authenticity of the recommended procedure standard deviation (SD) and coefficient of variation (CV) were also calculated. At least nine determinations were carried out and the results noted. The proposed method was further justified by recovery experiments through standard drug addition method. A known amount of the pure compound was taken and to this, varying amounts of pharmaceutical product of the same compounds were added. The total amount of the sample was found by the following expression:

TABLE-1
MILLIGRAM DETERMINATION OF SOME ANTINEOPLASTIC AGENTS IN PURE FORM AND IN THEIR PHARMACEUTICAL PREPARATIONS WITH (0.02 N) NBS REAGENT IN ACIDIC MEDIUM

Sample	Aliquots taken (mL)	Amount present* (mg)	Reaction time (min)	Molecularity (n)	Amount obtained by calculation**	Error (%)	SD	CV
Chloropromzine hydrochloride (Pure)	1	0.998	10	2	0.989	-0.90	0.0039	0.3943
	3	2.995	10	2	2.974	-0.70	0.0026	0.0874
	5	4.994	10	2	4.964	-0.50	0.0017	0.0342
Relitil-25 (Tablet)	1	0.962	10	2	0.953	-0.93	0.0040	0.4197
	3	2.884	10	2	2.862	-0.76	0.0031	0.1083
	5	4.810	10	2	4.784	-0.54	0.0027	0.0564
Promazine hydrochloride (Pure)	1	0.964	10	3	0.951	-1.65	0.0034	0.3574
	3	2.887	10	3	2.859	-0.96	0.0028	0.0979
	5	4.815	10	3	4.789	-0.53	0.0024	0.0501
Phenergan (Tablet)	1	0.965	10	3	0.956	-0.93	0.0029	0.3033
	3	2.900	10	3	2.872	-0.96	0.0023	0.0800
	5	4.837	10	3	4.809	-0.57	0.0018	0.0374
Phenergan (Injection)	1	0.962	10	3	0.948	-1.45	0.0021	0.2215
	3	2.908	10	3	2.892	-0.55	0.0017	0.0588
	5	4.800	10	3	4.789	-0.22	0.0021	0.0438
Levamisole (Pure)	1	0.989	10	2	0.968	-1.12	0.0025	0.2582
	3	2.945	10	2	2.908	-1.25	0.0022	0.0756
	5	4.898	10	2	4.872	-0.53	0.0019	0.0390
Devormis (Tablet)	1	0.982	10	2	0.972	-1.01	0.0041	0.4218
	3	2.945	10	2	2.916	-0.98	0.0036	0.1234
	5	4.902	10	2	4.870	-0.65	0.0031	0.0637
Dicaris (Tablet)	1	0.974	10	2	0.964	-1.02	0.0028	0.2905
	3	2.920	10	2	2.896	-0.82	0.0024	0.2890
	5	4.864	10	2	4.830	-0.69	0.0019	0.0393
Levamole (Tablet)	1	0.978	10	2	0.965	-1.32	0.0032	0.3316
	3	2.932	10	2	2.914	-0.61	0.0028	0.0960
	5	4.894	10	2	4.868	-0.53	0.0021	0.0431
Vermisole (Tablet)	1	0.973	10	2	0.962	-1.13	0.0025	0.2599
	3	2.917	10	2	2.895	-0.75	0.0021	0.0725
	5	4.862	10	2	4.837	-0.51	0.0015	0.0310
Fosfestrol (Pure)	1	0.969	10	2	0.959	-1.03	0.0020	0.2038
	3	2.908	10	2	2.885	0.79	0.0016	0.0554
	5	4.846	10	2	4.818	-0.57	0.0010	0.0207
Honven (Tablet)	1	0.954	10	2	0.944	-1.05	0.0018	0.1907
	3	2.863	10	2	2.841	-0.76	0.0017	0.0634
	5	4.771	10	2	4.748	-0.48	0.0031	0.0358
Letrozole (Pure)	1	0.964	10	3	0.934	-1.05	0.0022	0.3854
	3	2.832	10	3	2.811	-0.74	0.0029	0.0961
	5	4.720	10	3	4.695	-0.53	0.0030	0.0660
Lupride (Tablet)	1	0.884	10	3	0.878	-1.01	0.0016	0.1822
	3	2.661	10	3	2.693	-0.83	0.0012	0.0455
	5	4.435	10	3	4.412	-0.52	0.0014	0.0317
Progtage (Tablet)	1	0.923	10	3	0.913	-1.02	0.0022	0.2410
	3	2.769	10	3	2.749	-0.72	0.0024	0.0873
	5	4.615	10	3	4.591	-0.52	0.0030	0.0653
Mitomycin (Pure)	1	0.991	10	3	0.981	-1.00	0.0076	0.7447
	3	2.973	10	3	2.951	-0.73	0.0051	0.1728
	5	4.955	10	3	4.932	-0.46	0.0035	0.0710
Almito (Injection)	1	0.936	10	3	0.926	-0.99	0.0042	0.4536
	3	2.808	10	3	2.784	-0.86	0.0038	0.1365
	5	4.680	10	3	4.651	-0.62	0.0026	0.0559
Mitocin (Vial)	1	0.964	10	3	0.952	-1.24	0.0021	0.2206
	3	2.892	10	3	2.868	-0.83	0.0078	0.2720
	5	4.820	10	3	4.802	-0.37	0.0096	0.1999
Oncicine (Tablet)	1	0.986	10	3	0.972	-1.32	0.0067	0.6893
	3	2.946	10	3	2.916	-0.71	0.0062	0.2111
	5	4.930	10	3	4.910	-0.41	0.0081	0.1650

*In each sample nine determinations were done; **Average of nine determinations.

TABLE-2
RECOVERY STUDIES BY STANDARD DRUG ADDITION METHOD

Name of drugs	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 (%)
Chloropromazine hydrochloride	1	3	0.998	0.692	1.942	0.953	0.918	0.925	99.07
	2	3	0.998	1.924	2.895	1.906	3.667	3.702	
	3	3	0.998	2.886	3.848	2.859	8.251	8.329	
	4	3	0.998	3.848	4.801	3.812	14.669	14.807	
		ΣN = 12		ΣX = 9.620		ΣY = 9.526	ΣXY = 27.501	ΣX^2 = 27.793	
Promathazine Hydrochloride	1	3	0.967	0.965	1.907	0.956	0.923	0.931	98.16
	2	3	0.967	1.930	2.863	1.912	3.690	3.725	
	3	3	0.967	2.895	3.819	2.869	8.120	8.381	
	4	3	0.967	3.860	4.775	3.824	14.761	14.900	
		ΣN = 12		ΣX = 9.650		ΣY = 9.560	ΣXY = 27.494	ΣX^2 = 27.937	
Levamisole	1	3	0.989	0.982	1.940	0.968	0.951	0.964	98.58
	2	3	0.989	1.964	2.908	1.936	3.802	3.857	
	3	3	0.989	2.946	3.876	2.904	8.555	8.679	
	4	3	0.989	3.928	4.844	3.972	15.209	15.429	
		ΣN = 12		ΣX = 9.820		ΣY = 9.680	ΣXY = 28.517	ΣX^2 = 28.929	
Fosfestrol	1	3	0.969	0.954	1.898	0.944	0.901	0.910	98.95
	2	3	0.969	1.908	2.842	1.888	3.602	3.641	
	3	3	0.969	2.862	3.786	2.832	8.105	8.191	
	4	3	0.969	3.816	4.730	3.776	14.409	14.562	
		ΣN = 12		ΣX = 9.540		ΣY = 9.440	ΣXY = 27.017	ΣX^2 = 27.304	
Letrozole	1	3	0.984	0.923	1.847	0.913	0.843	0.852	98.92
	2	3	0.984	1.846	2.760	1.826	3.371	3.408	
	3	3	0.984	2.769	3.673	2.739	7.584	7.667	
	4	3	0.984	3.692	4.586	3.652	13.483	13.631	
		ΣN = 12		ΣX = 9.230		ΣY = 9.130	ΣXY = 25.281	ΣX^2 = 25.558	
Mitomycin	1	3	0.991	0.986	1.953	0.972	0.958	0.972	98.82
	2	3	0.991	1.964	2.925	1.944	3.818	3.857	
	3	3	0.991	2.946	3.897	2.916	8.591	8.679	
	4	3	0.991	3.944	4.869	3.888	15.334	15.555	
		ΣN = 12		ΣX = 9.860		ΣY = 9.720	ΣXY = 28.701	ΣX^2 = 29.063	

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

where, N = total number of observations, X = amount of drug added, Y = amount of drug obtained by calculation.

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