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DETERMINATION OF SOME ANTINEOPLASTIC AGENTS IN THE PURE FORM AND IN THEIR PHARMACEUTICAL PREPARATION WITH N-BROMOSUCCINIMIDE REAGENT

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ABSTRACT

A simple, quick and convenient method has been developed for the determination of some antineoplastic drugs like cyclophosphamide, chlorambucil, melphalan, busulphan, methotrexate, mercaptopurine and 5-fluorouracil in pure form and in their pharmaceutical preparation with the use of N-bromosuccinimide reagent. The value of percentage error, coefficient of variation (CV) and standard deviation (SD) prove the method to be precise and reproducible. To establish authenticity of the method, recovery experiments were also carried out by standard drug addition method.

KEYWORDS

Antineoplastic agents, Pharmaceuticals, N-Bromosuccinimide and Titration.

INTRODUCTION

In the series of antineoplastic drugs and alkylating agents are also used. These compounds alkylate the substance with which they react by joining it through a covalent bond. Any antitumor agent whose activity is explainable by such a mechanism is called alkylating agents. According to Zhuorong Li, Jiye Han and Yongying[1] alkylating agents are nitrogen mustards alkyl sulfonates, ethyl enimines, nitrosoureas, triazenes etc. Alkylating agents are used in chemotherapy of neoplastic diseases. Biological evaluation correlate that derivative of melphalan moiety prevent carcinogenesis for multiple myeloma and arnidine. Analogues of chlorambucil are potent against the growth of human breast cancer MCF-7 cells. Antineoplastic agents are known to exhibit a wide range of pharmacological properties and biological activities such as anticancer, antitumor, antioxidant, antinociceptive, antiinflammatory and immunomodulating activities. Because of great medicinal value of these compounds their estimation has widely been studied [1-10]. Most of the above methods

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involve shophisticated instruments and complicated techniques. In the present paper we describe a simple, quick and convenient method for the determination of above antineoplastic drugs with N-bromosuccinimide reagent.

EXPERIMENTAL

Reagent and solutions:

N-bromosuccinimide (0.02M) solution:

356 mg of NBS (CDH) was accurately weighed and dissolved in minimum volume of warm water and the solution was made up to the mark with distilled water in 100ml volumetric flask. It was standardised iodometrically against standardised sodium thiosulphate (0.02N) solution.

Sodium thiosulphate (0.02N) solution:

4.9636g of sodium thiosulphate (B.D.H.) was weighed accurately and dissolved in minimum amount of distilled water in a 1000ml volumetric flask. The solution was made up to the mark with distilled water. It was standardised by titrating with primary standard copper sulphate (0.025N) solution using potassium iodide (10%) and starch solution (1%) as indicator.

Copper sulphate (0.02N) solution:

0.4994g of copper sulphate (G.R.) was weighed accurately and dissolved in minimum amount of distilled water in a 100ml volumetric flask containing small amount of dilute sulphuric acid to check the hydrolysis of copper sulphate. The solution was made up to the mark with distilled water.

Potassium Iodide (Baker analysed reagent)

10% w/v aqueous solution was prepared in distilled water.

Starch solution:

1% w/v aqueous solution of starch was prepared in distilled water.

5N sulphuric acid solution:

5N v/v aqueous solution of sulphuric acid was prepared by diluting concentrated acid with distilled water.

Samples solutions:

Accurately weighed 100mg of pure samples of the compounds were dissolved in distilled water in a 100ml volumetric flask and solution made up to the mark to give concentration of 1mg/ml.

Tablets solution:

Twenty tablets of pharmaceutical products were crushed to a fine powder and the powder equivalent to 100mg of sample was taken in 100ml calibrated volumetric flask and dissolved in same way as described for the pure sample.

Injections solution:

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Solution of injection of above compounds having an amount equivalent to 100mg of the pure sample were taken and dissolved in distilled water in 100 ml volumetric flask to get a concentration of 1mg/ml.

General Procedure:

Aliquots containing 1-5mg of the samples were taken in 100ml stoppered conical flask followed by the addition of 5ml of 0.02N, N-bromosuccinimide solution. Contents were shaken properly and 5ml of glacial acetic acid was added to it. The reaction mixture was shaken thoroughly and allowed to react at room temperature (25-30°C) for required reaction time. After the reaction was over 5ml of 10% KI solution was added to it and contents shaken thoroughly and allowed to stand for a minute. The liberated iodine was titrated with 0.02 N Sodium thiosulphate solution using starch as indicator. A blank experiment was also run under identical condition using all the reagents except the sample. The amount of the sample was calculated by the following expression. On the basis of percentage error and the value of SD and CV were calculated (Table-1). In the case of pharmaceutical preparations the same procedure was applied. It was observed that the excipients present in pharmaceuticals do not interfere.

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Table -1

Determination of some antineoplastic drugs in pure form and in their pharmaceutical preparations with 0.02 N N-bromosuccinimide reagent in acidic medium.

S. No.	Sample	Aliquots Taken (mL)	Amount Present* (mg)	Reaction Time (min.)	Molecu-larity	Amount obtained by Calculation** (mg)	Error (%)	SD	CV
1.	Cyclophosphamide (pure)	5.00	4.99	5	2	4.920	-1.40	0.0012	0.0243
(a)	Cyclophophamide (tab)	5.00	4.99	5	2	4.920	-1.40	0.0012	0.0243
(b)	Cyclophosphamide (inj)	5.00	4.99	5	2	4.940	-1.00	0.0066	0.1336
(c)	Cycloxan (Tab)	5.00	4.99	5	2	4.930	-1.20	0.0091	0.1845
(d)	Endo-Astra (Tab)	5.00	4.99	5	2	4.920	-1.40	0.0012	0.0243
2.	Chlorambucil (Pure)	5.00	4.99	5	2	4.960	-0.60	0.0053	0.1068
(a)	Leukeram (Inj.)	5.00	4.99	5	2	4.980	-0.20	0.0088	0.1767
3.	melphalan (Pure)	5.00	4.99	5	2	4.920	-1.40	0.0012	0.0243
(a)	Alkeran (Inj)	5.00	4.99	5	2	4.950	-0.80	0.0093	0.1878
4.	Busulphan (Pure)	5.00	4.99	5	2	4.930	-1.20	0.0091	0.1845
(a)	myleram (tab)	5.00	4.99	5	2	4.930	-1.20	0.0093	0.1886
(b)	Myram (tab)	5.00	4.99	5	2	4.920	-1.40	0.0013	0.0264
5.	Methotrexate (Pure)	5.00	5.00	5	4	4.978	-0.45	0.0084	0.0168

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(a)	Biotrexate (Inj)	5.00	4.74	5	4	4. 718	-0.46	0.0081	0.0171
(b)	Metrorex (inj.)	5.00	4.85	5	4	4.827	-0.47	0.0011	0.0229
(c)	Neotrexate (Tab)	5.00	4.50	5	4	4.478	-0.49	0.0013	0.0293
(d)	Zexate (Tab)	5.00	4.50	5	4	4.473	-0.59	0.0022	0.0049
6.	Mercaptopurine (Pure)	5.00	5.00	5	2	4.952	-0.96	0.0029	0.0058
(a)	Puri-nethol (Tab)	5.00	4.88	5	2	4.879	-0.02	0.0022	0.0448
(b)	Mercepthol (Tab)	5.00	4.88	5	2	4.854	-0.54	0.0013	0.0268
(c)	Zypurin (Tab)	5.00	4.93	5	2	4.921	-0.18	0.0016	0.0315
(d)	Empurine (Tab)	5.00	4.97	5	2	4.963	-0.14	0.0010	0.0205
7.	5-fluorouracil (Pure)	5.00	5.00	5	1	4.984	-0.32	0.041	0.0082
(a)	Fivoflu (Inj)	5.00	5.00	5	1	4.994	-0.12	0.018	0.0364
(b)	Fluracil (Inj)	5.00	5.00	5	1	4.977	-0.47	0.0011	0.0221
(c)	Fludin (Inj)	5.00	5.00	5	1	4.984	-0.32	0.0092	0.0184
(d)	Tevafluoro (Tab)	5.00	4.75	5	1	4.728	-0.46	0.0081	0.0171

CALCULATION

$$mg\ of\ sample = \frac{M \times N(B - S)}{2n}$$

Where, M = Molecular weight of the sample
N = Normality of sodium thiosulphate solution
B = Volume of sodium thiosulphate for blank
S = Volume of sodium thiosulphate for sample
n = No. of moles of N-bromosuccinimide consumed per mole of sample

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Tab = Tablet
Inj = Injection
* For each sample nine estimations were done
** Average of nine determinations

RESULTS AND DISCUSSION:

The reaction conditions were established after studying the effect of variables such as reaction time, concentration of reagent, reaction temperature, volume of the reagent and glacial acetic acid. Variation in the reaction time was found to influence the speed of the reaction to an appreciable extent.

In the determination of cyclophasphamide, chlorambucil, melphalan, busulphan, methotrexate, 6-mercaptopurine and 5- fluorouracil the reaction was completed within 5 minutes. At a lesser reaction time (less than five minutes) the recovery of the sample is low because of the incomplete reaction. A much more reaction time (beyond 5 minutes) does not improve the results. It was also established that the prescribed concentration of the reagent (0.02N) was suitable for accurate results. An increase in the concentration of the reagent (0.025-0.5N) does not have any effects on the percentage recovery. A lower concentration (0.01-0.015N) gives inaccurate results because of incomplete reaction. In was also noticed that the reactivity of reagent was slow without the presence of ionizing medium. Since mineral acids like HNO_3 and H_2SO_4 may decompose the reagent glacial acetic acid was used. While studying the effect of variation in volume of glacial acetic acid it was found 5 ml of acid is suitable for best results. The best recovery of sample was obtained at room temperature (25-30°C). On heating the reaction mixture on direct flame, on a boiling water bath, inaccurate results were obtained. It may be due to decomposition of the reagent. If the reaction is carried out at lower temperature (0-5°C) the speed was much more retarted. Even increasing reaction time the results were not satisfactory, at this temperature. Results reported in table-1 shows that the suggested method is accurate reproducible and precise. It can easily be adopted in an ordinary pharmaceutical laboratory. On the basis of molecularity and available literature, a possible course of reaction may be suggested for each antineoplastic drugs. It may be proposed that the compounds gets oxidised to corresponding oxidised products.

POSSIBLE COURSE OF REACTION:

On the basis of the nature of the reagent and the structure of the compound the following reaction may be proposed for different compounds e consumes two moles of the reagent and gets brominated at active positions giving rise to dibromo derivative.

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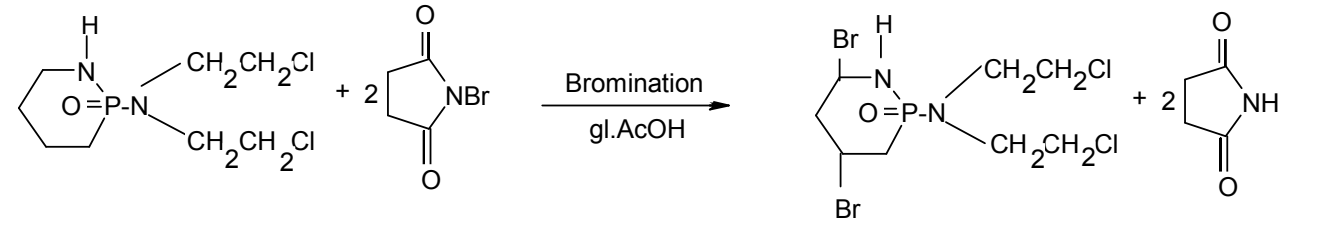
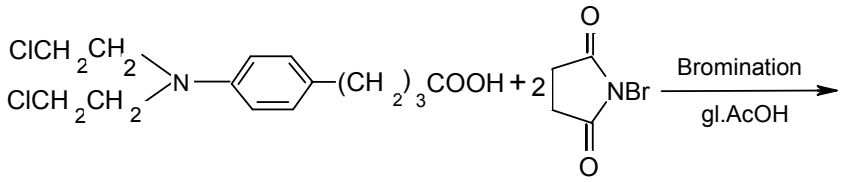
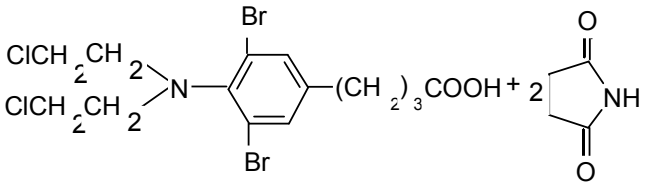


Cyclophosphamide N-bromosuccinimide Dibromocyclophosphamide Succinimide

Similarly chlorambucil gives corresponding brominated product by consuming two moles of the reagent.



Chlorambusil N-bromosuccinimide



Dibromochlorambusil Succinimide

Malphalan has activated benzene ring at ortho position to substituted amino group therefore these positions are brominated and give dibromo derivative.

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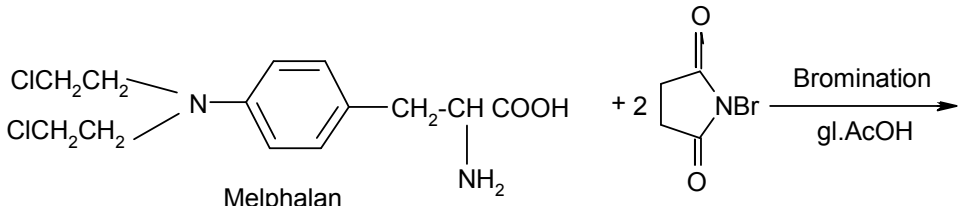
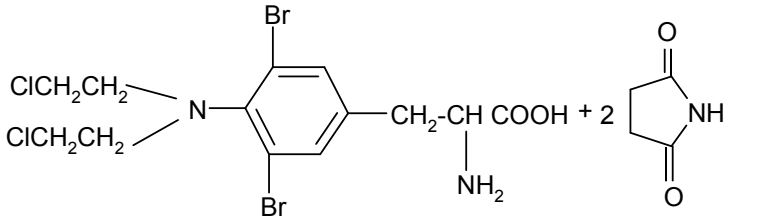
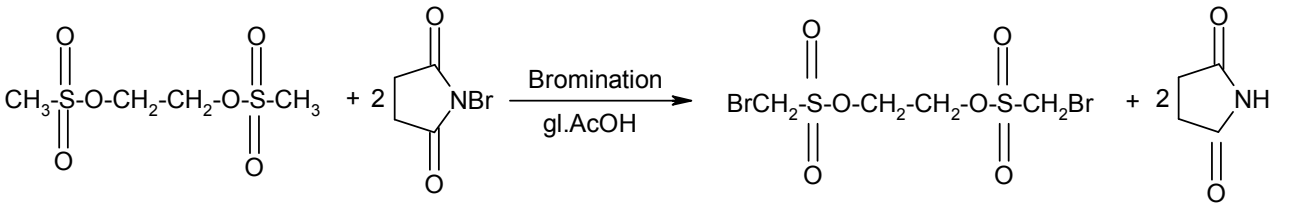


Melphalan N-bromosuccinimide



Dibromomelphalan Succinimide

In case of Busulphan following reaction may be proposed.



Busulphan N-bromosuccinimide Dibromobusulphan Succinimide

Methotrexate consumes 4 moles of NBS and gives tetrabromomethotrexate. The following reaction may be proposed.

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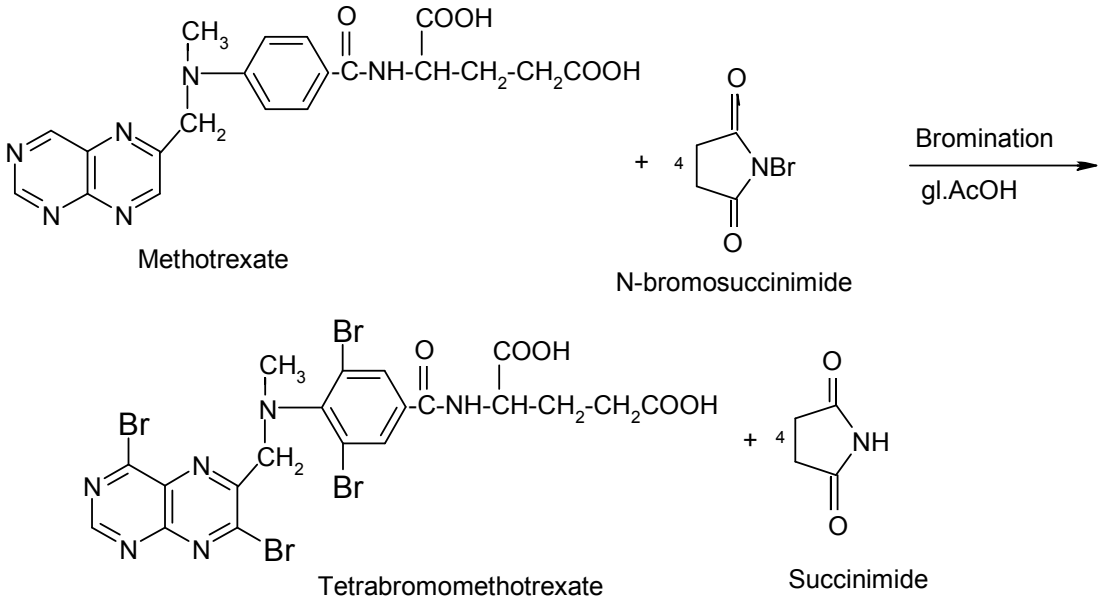
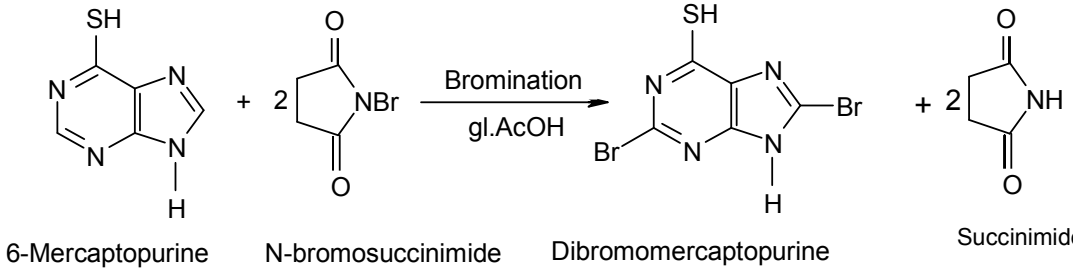


5-mercaptopurines also gives corresponding brominated products by consuming 2 moles of N-bromosuccinimide.



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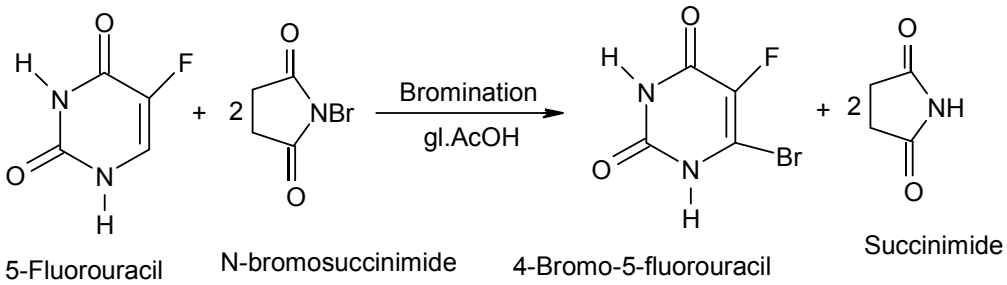

5-Fluorouracil N-bromosuccinimide 4-Bromo-5-fluorouracil Succinimide

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