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Spectrophotometric Determination of Halogen-Containing Organic Pharmaceuticals Using 1,5-Bis(6-methyl-4-pyrimidyl)carbazone. IV

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A spectrophotometric method for the determination of chlorine, bromine or iodine ion by using 1,5-bis(6-methyl-4-pyrimidyl)carbazone has been established. The ranges of determination were 0.355—3.55 ppm of Cl^-, 0.799—7.99 ppm of Br^- and 1.27—12.7 ppm of I^-. The proposed method could be applied to the analyses of various halogen-containing organic compounds, including sulfur-containing compounds. Eleven commercial pharmaceutical preparations gave precision (R.S.D.) values in the range of 0.2% to 5.6% (replicate analysis), when they were processed by the oxygen flask combustion method.

Keywords——spectrophotometric determination; silver complex; oxygen flask combustion; halogen-containing compound; 1,5-bis(6-methyl-4-pyrimidyl)carbazone; drug assay

In the previous paper,[1] a new spectrophotometric method was reported for the determination of small amounts of chlorine ion by using 1,5-bis(6-methyl-4-pyrimidyl)carbazone (MPC). The method (MPC method) is based on the reduction of the absorbance of silver–MPC complex at 530 nm after the reaction of chlorine ion with the complex to form AgCl in 1 M ethanolic acetic acid solution in the presence of an appropriate surfactant.

As the MPC method is principally based on the reactions between halogen and silver ions, the method could be applicable for the determination of bromine- or iodine-containing substances as well. However, there are some practical problems to be solved for this purpose. Firstly, sulfate ion produced by the combustion of sulfur compounds interfered with the colorimetric reaction. Secondly, the addition of a large amount of hydrazine hydrate to the absorbing liquid was found to be undesirable for the measurement of absorbance. The purpose of this study was to overcome these problems and to apply the modified MPC method to the determination of various pharmaceutical preparations such as powder, tablets and capsules which contain bromine, iodine or chlorine.[2]

Experimental

Reagents——The colorimetric reagent MPC was prepared as described by Ichiba *et al.*[1] Pharmaceutical materials and preparations were purchased commerically, or kindly supplied by the companies mentioned in the acknowledgments. All other chemicals were of the purest grade commercially available. All aqueous solutions were prepared in distilled and deionized water.

Stock solutions of halogen ions (X^-: Cl^-, Br^-, I^-) were prepared by dissolving NaCl, KBr or KI in water at 0.125 M. One-tenth of a percent of an MPC solution in dimethylsulfoxide, 1 M ethanolic acetic acid solution and other reagents were prepared according to the previous paper.[1]

Apparatus——Absorption spectra and absorbance measurements were made with a Shimadzu model UV-240 spectrophotometer using 10 mm cells.

Procedure——The sample was weighed out accurately so that it contained about 5×10^{-5} mol of halogen ion. The weighed samples were then processed by the oxygen flask combustion method to form the inorganic halides. The absorbing liquid used was 5 ml of H_2O_2 ($1 \rightarrow 5$) for chlorine- or bromine-containing compounds, but in the case of iodine-containing compounds, it was a mixture of 1 ml of 0.1 M $NH_2NH_2 \cdot H_2O$ and 5 ml of water. In the case of sulfur compounds, 1 ml of 0.25 M barium acetate was added after the combustion process, and the resulting $BaSO_4$ was filtered off after making up the volume to 25.0 ml with water.

Standard solutions of X^- and a blank solution were prepared by the same procedure as described in the previous paper.[1] The absorbance was measured at the wavelength of maximum absorbance at around 530 nm, after the reaction of Ag–MPC with X^- to form AgX in 1 M ethanolic acetic acid solution. The amounts of halogen-containing compounds in milligrams were calculated by using the following equation:

$$\frac{(A_o - A_s)}{(A_o - A_t)} \times 6.25 \times 10^{-2} \times \frac{M_r}{N}$$

where,

A_s, A_t, A_o: absorbances of sample, standard, or blank solution, respectively,

M_r: molecular weight of sample,

N: number of halogen atoms per molecule of sample.

Results and Discussion

As indicated in the previous paper,[1] sulfate ion interfered with the colorimetric reaction due to precipitation of Ag^+ as Ag_2SO_4 in a concentration-dependent manner. Therefore, $SO_4{}^{2-}$-containing sample solution was treated with barium acetate to remove $SO_4{}^{2-}$ as $BaSO_4$ by filtration with 0.45 μm membrane filter. When this treatment was applied to sulfur-containing halogen compounds such as chlorpropamide, furosemide, trichlormethiazide, the recoveries were improved to 96.80—101.3% (R.S.D. = 0.8—2.0%).

In the process of the conversion of organic iodine compounds into inorganic ones by an oxygen flask combustion method, it is generally recommended that hydrazine hydrate should be added to the absorbing liquid to reduce iodine molecule or other oxidized iodine species into I^-. Therefore, the effect of its concentration on the coloration was examined (Table I). The presence of a reducing agent in a quantity of more than one equivalent with respect to iodine was essential, but it was found that the larger the amount of hydrazine, the larger the variation of the absorbance. The most effective concentration of hydrazine hydrate was found to be 1×10^{-4} M. Iodine-containing compounds such as amidotrizoic acid, iopanoic acid and triiodophenol were determined in the presence of 1×10^{-4} M hydrazine hydrate, and the results were satisfactory in terms of reproducibility (R.S.D. = 0.5—1.1%).

The calibration curves for the determination of bromine and iodine ions were obtained by the standard procedure using KBr or KI stock solutions. They conformed to Beer's law up to 8×10^{-5} M bromine and iodine ions. The relative standard deviations were 0.2—1.7% ($n = 5$) at each measured point and the correlation coefficients (r) of the regression equations for bromine and iodine were 0.997 and 0.998, respectively.

The results for the determination of the parent drugs in pharmaceutical preparations (powder, tablets or capsules) are summarized in Table II. Although some deviations from the labeled amounts were observed, they did not exceed the respective tolerance range.

TABLE I. Effect of Hydrazine Hydrate[a]

$NH_2NH_2 \cdot H_2O$ ($\times 10^{-4}$ M) taken	Absorbance ± S.D. ($n = 5$)	R.S.D. (%)
0.5	0.478 ± 0.007	1.4
1.5	0.482 ± 0.005	1.0
5.0	0.488 ± 0.007	1.4
10.0	0.502 ± 0.010	2.0

[a] 0.5—10 × 10^{-4} M $NH_2NH_2 \cdot H_2O$ was added to a reagent mixture consisting of 1×10^{-4} M Ag–MPC and 6×10^{-5} M I^- in 1 M ethanolic acetic acid solution, and the absorbance was measured at 530 nm. S.D., standard deviation; R.S.D., relative standard deviation.

TABLE II. Application of the MPC Method for the Determination of Pharmaceutical Preparations

Drug	Labeled amount	Halogen	Found ± S.D.	R.S.D. (%)
Furosemide tab.	40 mg	Cl[a]	42.56 ± 0.82 mg	1.94 ($n=5$)
Hydrochlorothiazide tab.	25 mg	Cl[a]	24.29 ± 0.50 mg	2.04 ($n=5$)
Clomifene citrate tab.	50 mg	Cl	47.38 ± 1.83 mg	3.87 ($n=5$)
Indometacin tab.	25 mg	Cl	26.18 ± 1.46 mg	5.59 ($n=5$)
Chlordiazepoxide powder	10%	Cl	10.10 ± 0.02%	0.20 ($n=5$)
Trichlormethiazide tab.	2 mg	Cl[a]	2.12 ± 0.06 mg	2.86 ($n=5$)
Chlormezanone tab.	100 mg	Cl[a]	102.10 ± 1.55 mg	1.52 ($n=5$)
Clofibrate cap.	250 mg	Cl	262.00 ± 3.14 mg	1.20 ($n=3$)
Clorpropamide tab.	250 mg	Cl[a]	248.50 ± 2.63 mg	1.06 ($n=3$)
Meglumine amidotrizoate inj.	49.32%	I	50.01 ± 0.30%	0.60 ($n=6$)
Sodium iopodate cap.	500 mg	I	468.20 ± 17.27 mg	3.69 ($n=6$)

a) Sulfur-containing halogen compounds, which were treated with barium acetate and subjected to the MPC method. S.D., standard deviation; R.S.D., relative standard deviation; tab., tablets; cap., capsules; inj., injection.

It is concluded from the above results that the MPC method is particularly useful for the determination of halogen-containing parent drugs in pharmaceutical preparations provided that: i) the ingredients and their approximate proportions are known; ii) they contain no halogen-containing substances other than the parent drugs.

In the case of halogenide (chloride, bromide or iodide), which also reacts with Ag–MPC, the content of inorganic halogens should be quantitated prior to the oxygen flask combustion process.

The MPC method presented here is a clean one as compared with conventional spectrophotometric methods using toxic mercuric compounds,[3] and is also a simple one, as the oxygen flask combustion process significantly simplified the pretreatment of samples to remove diluents such as starch and lactose, and other ingredients.

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References and Notes

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