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Research Article

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A sensitive and new colorimetric reagent system for the determination of 2-bromo-2-nitropropane 1, 3-diol (bronopol) pesticide in grains and vegetables

Anjum Ansari[1], Smita Joshi[2] and Sulbha Amlathe*[3]

[1]Department of Applied Chemistry, BUIT, Barkatullah University, Bhopal (M.P.), India
[2]Department of Chemistry, Sarojini Naidu Govt. Girls PG (Autonomous) College, Shivaji Nagar, Bhopal (M.P.), India
[3]Department of Applied Chemistry, BUIT, Barkatullah University, Bhopal (M.P.), India

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ABSTRACT

A sensitive spectrophotometric method for the determination of 2-bromo-2-nitropropane 1, 3-diol (bronopol), at mg L^{-1} level is described. The method is based on the reduction of the nitro group of bronopol to amino group with Zinc/HCl which subsequently diazotized with sodium nitrite in acidic medium and coupled with phloroglucinol to formed orange azo dye in alkaline medium. The dye shows maximum absorbance at 425 nm and obeyed Beer's law in the range of 10-50 µg bronopol in 1 mL. The important analytical parameters and optimum reaction conditions have been optimized. The Molar Absorptivity and Sandell's Sensitivity were found to be 4.02×10^3 litres mol^{-1} cm^{-1} (±100) and 0.049 µg $cm^{-2,}$ respectively. The Standard Deviation and the Relative Standard Deviation were found to be ± 0.0073 and 3.63% respectively.The method has been successfully applied to the determination of bronopol in polluted water, grains and vegetables. The method was found to be free from the interference of most of the commonly used pesticide and other common pollutants.

Keywords: Spectrophotometer, Bronopol, Pesticide, Phloroglucinol, Diazotization.

INTRODUCTION

Bronopol is a highly active antimicrobial compound whose chemical formula is 2-bromo-2-nitropropane 1, 3-diol. Bronopol is a white pale yellow crystalline solid with strong odor. Bronopol is readily soluble in non-polar solvents but shows a high affinity for polar organic solvents. Bronopol is used in consumer products as an effective preservative agent, as well as a wide variety of industrial applications as an antimicrobial in cosmetics, external medicaments, shampoos and bath preparations. It is also used as a substitute for formaldehyde in chemical toilets. Bronopol has a broad spectrum of antibacterial activity and is widely used, at concentrations of upto 0.1% (w/v), as a preservative for pharmaceutical and cosmetic products [1-9]. The trade name of bronopol is myacide B10 & myacide AS. Among pesticide it is categorized as bactericide [10]. Bronopol is stable to hydrolysis under normal conditions. However, at warmer temperature and/or higher pHs, rapid hydrolysis may occur. Under these conditions, hydrolysis products include formaldehyde and lesser amounts of other degradates [11, 12]. It causes irreversible eye damage and skin burns. May be fatal if swallowed or inhaled. Harmful if absorbed through the skin [13]. Metabolism studies indicate that Bronopol is primarily excreted in the urine [14, 15]. The use of bronopol in personal care products (cosmetic, toiletries) has declined since the late 1980s due to the recognized potential for nitrosamine formation, hence studied less [16].

Different instrumental methods like HPLC [17, 18], UV-Spectrophotometry [19], liquid chromatography, Reversed-Phase Ion-Pair Chromatography [20], enzymic micro determination [21] etc. are used for its determination [5, 6]. The determination of most of the pesticides containing nitro group are based on reduction of nitro group into amino group and then subsequent diazotization and coupling with suitable reagents to form an azo dye reported [22, 23]. In

the present paper, a new chromogenic reagent has been developed for determination of bronopol. The nitro group of bronopol is reduced with Zn/HCl to form amino bronopol [22], which is subsequently diazotized and coupled with phloroglucinol to form a orange-colored azo dye in alkaline medium. The dye shows an absorbance maxima at 425 nm and obeys Beer's law in the range 10-50 µg in 1mL. The method has been successfully applied to the determination of bronopol in polluted water, grains and vegetables.

EXPERIMENTAL SECTION

2.1 Apparatus: A carl-zeiss specol with 1-cm matched cells was used for all spectral measurements. A UV-Visible Spectrophotometer 2201 and systronics spectrophotometer 166 with matched 1-cm cells were used for all spectral measurements. All glassware's were calibrated.

2.2 Raegents: All chemicals used were of AnalaR grade. Demineralized or distilled water were used throughout the experiment.

Standard Bronopol Solution
A stock solution of 1mg/ml of bronopol was prepared in distilled water. A working standard of 100 µg /ml solution was prepared fresh daily by the appropriate dilution of the stock.

Zinc Dust
Finely divided zinc of AR grade has been used.

Hydrochloric Acid
2 M hydrochloric acid was prepared by appropriate dilution of Concentrated HCl.

Sodium Nitrite Solution
0.5% aqueous solution is prepared by pre oven dried sodium nitrite A. R. grade reagent.

Sulphamic Acid
3% solution in water was prepared by dissolving 3 gm sulphamic acid in 100 ml distilled water.

EDTA
0.1 M solution in water was prepared by dissolving 3.7 gm EDTA in 100 ml distilled water.

Phloroglucinol
0.3 % aqueous solution was prepared by dissolving 300 mg phloroglucinol in 100 ml distilled water.

Sodium Hydroxide
5 M solution was prepared by dissolving 20 gm sodium hydroxide in 100 ml distilled water and standardized by standard procedure [24].

PROCEDURE
The procedure involves following two steps:

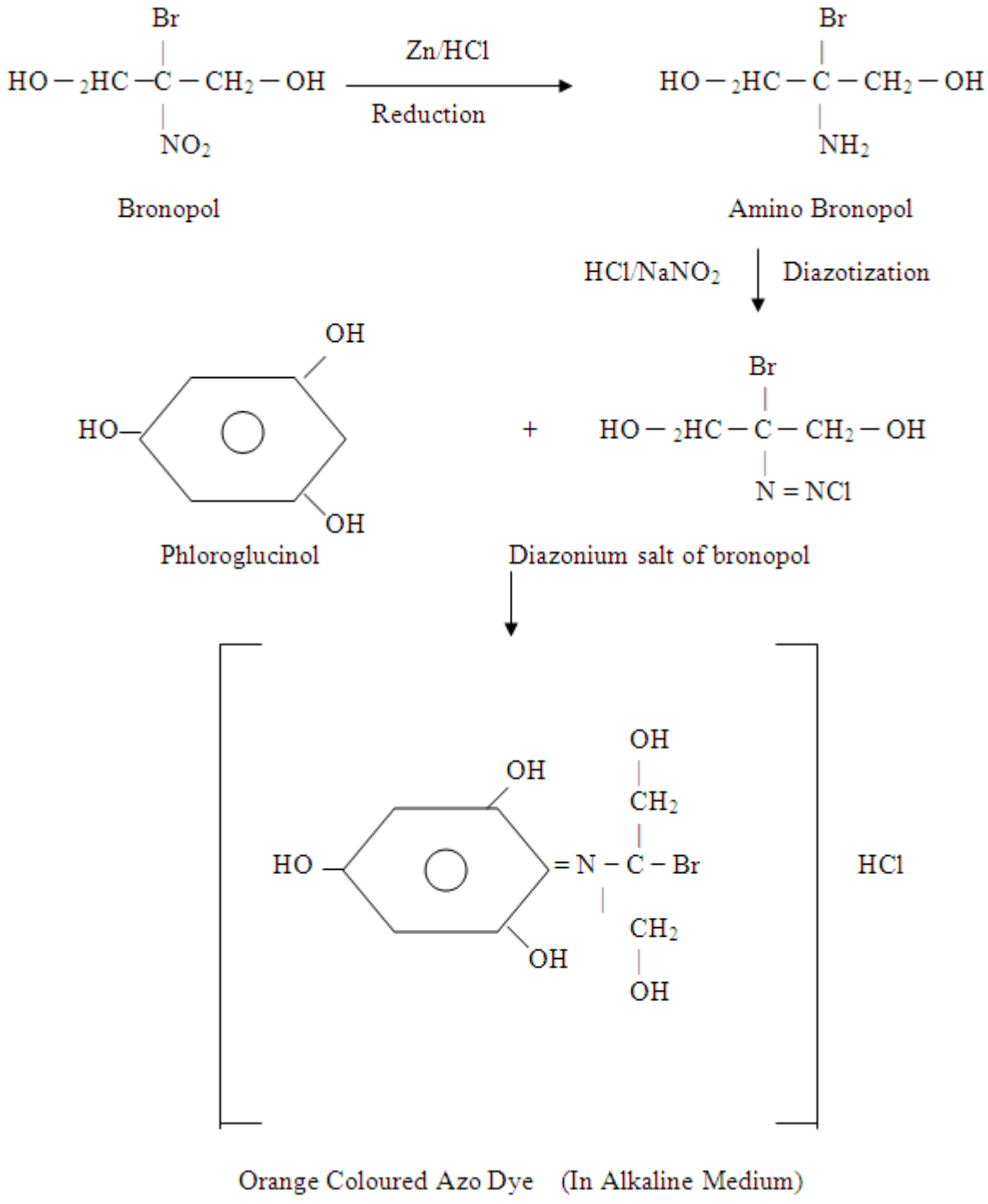
STEP-1 REDUCTION OF BRONOPOL
An aliquot of standard solution containing 10 mg of bronopol was taken in a test tube. To this 0.6 g zinc dust followed by 12 mL of 5M hydrochloric acid were added. The contents were vigorously boiled for 2 minutes and then boiled gently for another 10 minute. Then the mixture was cooled and filtered into a 100 mL volumetric flask. The washings of test tube were added to the volumetric flask, volume is made upto the mark with distilled water.

STEP-2 DIAZOTIZATION & COUPLING OF REDUCED BRONOPOL
In 1 mL of above solution 0.5 mL of sodium nitrite solution and 1mL 2M hydrochloric acid were added and left for 10 minute with occasional shaking for the complete diazotization. The excess of sodium nitrite was removed by the addition of 1 mL of sulphamic acid. The metal ions were masked with 1 mL of 0.1 M EDTA solution. The resulting dye is coupled with 0.5 mL of phloroglucinol and kept for 10 minute. The solution was then made alkaline with addition of 1 mL of 5 M NaOH solution and again left for 10 minute for complete color reaction. The absorbance of this dye was measured at 425 nm against a reagent blank.

RESULTS AND DISCUSSION

4.1 Spectral Characteristics

The orange dye shows maximum absorption at 425 nm. The absorbance spectra of the dye are shown in **Fig. 1**. The reagent blank is colourless and shows negligible absorption at this range.



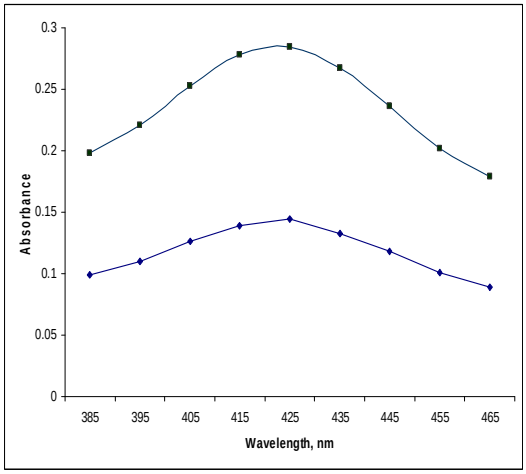


Fig. 1: Absorption Spectra of the Dye and Reagent Blank
A. Concentration of Bronopol = 200 µg/10 ml.
B. Concentration of Bronopol = 100 µg/10 ml.
C. Reagent Blank

4.2 THE COLOUR REACTION:

Bronopol first reduced into Amino Bronopol. Then amino bronopol was diazotized with sodium nitrite in presence of hydrochloric acid to form an additional compound, which is subsequently coupled with pholoroglucinol to form an orange coloured azo dye in alkaline medium.

4.3 Effect of Varying Reaction Conditions

1) Effect of time

It was found that minimum 10 minutes are required for reduction. The reduced bronopol required at least 10 minute for diazotization and 10 minute for complete coupling reaction with 0.3 % phloroglucinol. An increase in time decreases its absorbance values.

2) Effect of temperature

The most suitable range of temperature for reduction, complete diazotization and coupling was found to be 20-40 ^{0}C. At higher temperature colour system violates Beer's law and at low temperature the dye shows lower absorbance value **[Fig. 2]**.

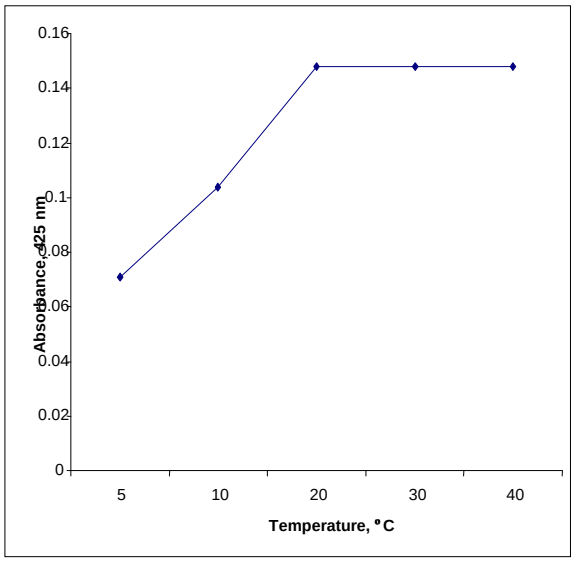


Fig. 2: Effect of Temperature on the Final Absorbance Concentration of Bronopol = 100 µg/10 ml

3) Effect of molarity

At the time of colour development strong alkaline medium was required. Hence 1 ml of 5M NaOH was used. Whereas final volume contains 3 M NaOH.

4) Effect of reagent concentration

For complete reduction, 6 g zinc and 12 ml of 5 M hydrochloric acid were required. 0.5 ml of 0.5% sodium nitrite was found to be sufficient for diazotization. An increase in amount, it shows lower absorbance. Acidity during diazotization was maintained with 2 M HCl **[Fig. 3]**.

The effect of amount of phloroglucinol on coupling reaction was also studied and 0.5 ml of 0.3% aqueous phologlucinol was found to be the best for coupling. An increase in concentration and / or volume of phloroglucinol shows lower absorbance **[Fig. 4]**.

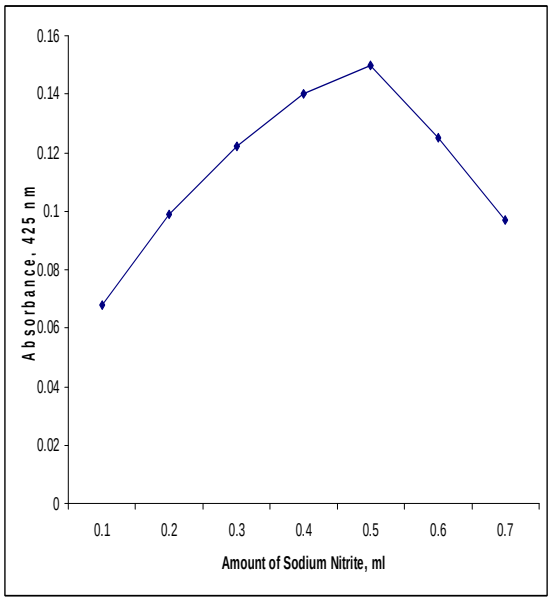


Fig. 3 Effect of Amount of Nitrite on the Final Absorbance
Concentration of Bronopol = 100 µg/10 ml

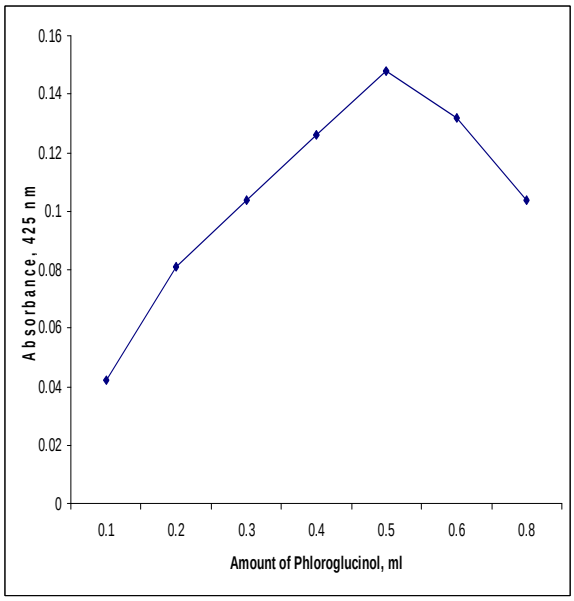


Fig. 4 Effect of Amount of Phloroglucinol on the Final Absorbance
Concentration of Bronopol = 100 µg/10 ml

1 ml of 5 M NaOH was sufficient after coupling reagent. An increase in volume of NaOH showed lower absorbance values or causing turbidity in the solution **[Fig. 5]**.

4.4 Beer's Law, Molar Absorptivity, Sandell's Sensitivity, and Reproducibility

Beer's law was obeyed in the range of 100 to 500 µg bronopol in 10ml of the final volume **[Fig. 6]**. The Molar Absorptivity and Sandell's Sensitivity were found to be 4.02×10^3 liters/mol/cm (±100) and 0.049 µg $cm^{-2,}$ respectively.

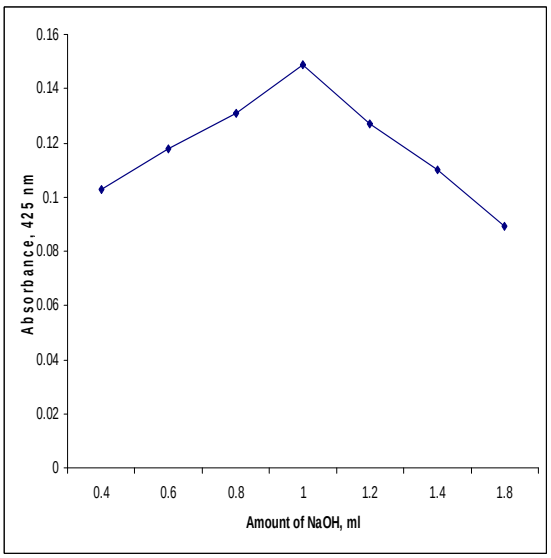


Fig. 5 Effect of Amount of NaOH
on the Final Absorbance
Concentration of Bronopol = 100 µg/10 ml

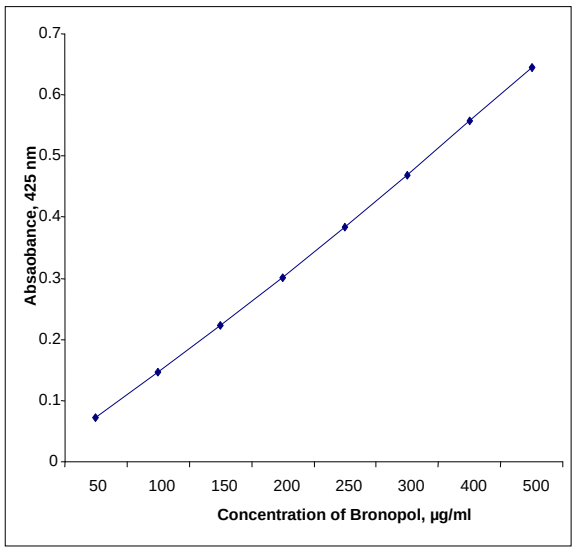


Fig. 6 Calibration Curve for Spectrophotometric
Determination of Bronopol with Phloroglucinol

The reproducibility of the method was checked by analyzing 100 µg/ 10 ml of bronopol for a period of 7 days [**Table I**]. The Standard Deviation and the Relative Standard Deviation were found to be ± 0.0073 and 3.63% respective.

Table I: Reproducibility of the Method
Concentration of Bronopol = 140 µg/ 10ml

No. of Days	Absorbance* at 425 nm
1	0.205
2	0.195
3	0.210
4	0.190
5	0.201
6	0.205
7	0.201
	Mean = 0.201
	Standard Deviation = ± 0.0073
	Relative Standard Deviation = 3.63%

** Mean of three repetitive analyses*

4.5 Effect of Foreign Species

The effect of various interfering co pollutants and pesticide were studied to assess the validity of the method. Known amounts of co pollutants and pesticide were added to the solution containing 100 µg bronopol per 10 ml prior to analysis and then solution was analyzed by the proposed method. The tolerance limits of various interfering species in alkaline medium were improved after masking with EDTA. Commonly found pollutants such as pyridine, hydrazine, acetone and pesticide (methyl parathion) did not interfere with this method. The tolerance limits for various interfering species are shown in **Table II**.

Table II; Effect of Foreign Species

Foreign Species	Tolerance limit in µg mL^{-1}
Acetone, hydrazine, pyridine	1000
Phenol	2000
P-nitroaniline	2500
Aniline	5000
Benzidine	20 000
Cu^{2+} , Mg^{2+}	1000
SO_4^{2-} , Cl^-	1000
K^+	1, 00,000
I^-	1, 00,000
Ammonia	1000
NO^{2-}	1000
$Cr_2O_7^-$	1000
Pesticide (methyl parathion)	1000
Carbaryl, Zineb	1000

Note. Concentration of bronopol was 100 µg 10mL-1
Amount that may vary by ±2%.
Masked with 1mL of 0.1 M EDTA.

5. APPLICATIONS

The method has been applied for the determination of bronopol in cotton, citrus, tomato, chili, ginger, vegetables, flowers, grapes, rice and agricultural wastewater. Since the samples analyzed were found to be free from bronopol, synthetic samples were prepared by crushing of the tomato, chili, ginger, rice etc. and mixing of known amount of bronopol and then analyzed by the proposed method and recovery were checked.

5.1 Determination of bronopol in waste water from agriculture field

Water samples (collected from the run-off water from agricultural field where bronopol was sprayed as a bactericide) were filtered and the filtered samples were analyzed for bronopol by the proposed method. The results obtained are in good agreement [**Table III**].

5.2 Determination of bronopol in grains and vegetables

Different samples of rice, ginger, tomato and chili were collected from the market. These samples were blended in a mixer. To these samples, known amounts of bronopol was added and kept for some time. The blended pulp was washed with 2x5 mL of water. These extracted samples were then filtered and 1mL of each sample (extracted) was analyzed by the proposed method [**Table III**].

Table III: Recovery of Bronopol from Waste Water and Spray Residues*

Sample	Bronopol (µg) Added	Found	% Recovery
Wastewater	50	44.20	88.40
	100	95.40	95.40
	200	192.50	96.25
Rice	50	46.10	92.20
	100	96.30	96.30
	200	194.80	97.40
Tomato	50	45.20	90.40
	100	93.80	93.80
	200	193.70	96.85
Ginger	50	41.00	82.00
	100	85.00	85.00
	200	168.00	84.00
Chili	50	46.90	93.80
	100	98.30	98.30
	200	196.98	98.49

**Mean of three replicate analyses*

CONCLUSION

It can be concluded that Bronopol, which is used as bactericide, can be precisely determined to 100 µg $10mL^{-1}$ using the proposed method. The method is simple, rapid and sensitive. Stability of the color formed, reproducibility, and noninterference of common ions make the method more versatile to determine quantities of bronopol pesticide in plant materials and agriculture wastewater.

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