Determination of Sulfamate Residues

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An analytical method for determining residues of ammonium sulfamate in apples and pears is described. The method is based on the sulfamate–nitrous acid reaction, liberating sulfate which is reduced to H_2S and measured spectrophotometrically after treating with zinc, *p*-aminodimethylaniline, and ferric chloride to form methyleneblue. Blanks of 0.1 p.p.m. were obtained on untreated apples and pears and recoveries of about 85% were realized when known quantities of ammonium sulfamate were added to untreated fruit.

A METHOD developed specifically for determining residues of ammonium sulfamate in fruit could have wide applicability for the determination of sulfamate residues. It has a sensitivity of 10 μg. of ammonium sulfamate and shows a blank of 0.1 p.p.m., primarily from reagents, on untreated apple and pear samples. The method is based on the ability to measure microgram quantities of sulfate resulting from the well known reaction of sulfamic acid with sodium nitrite in an acid medium.

$$HSO_3NH_2 + NaNO_2 \xrightarrow{\text{Acid}} NaHSO_4 + H_2O + N_2$$

Before effecting this reaction, it is necessary to separate the sulfamate residues from the fruit tissue by extraction with water, remove interfering colors from this extract with activated carbon, and separate sulfates from the water extract by alumina chromatographic techniques (*6*). The sulfamate is then reacted with sodium nitrite to form sulfate, as shown above, and this sulfate is reduced to hydrogen sulfide using a reducing acid mixture (*1*). The sulfide is distilled into alkaline zinc acetate solution forming zinc sulfide which, in turn, is treated with *p*-aminodimethylaniline and ferric chloride to form methylene blue (*2*). Methylene blue has an absorption maximum at 665 mμ.

Experimental

Reagents. Water (deionized). Barnstead Bantam Demineralizer, Fisher Scientific Co., Pittsburgh, Pa., No. 9-933 or equivalent. All solutions are prepared using deionized water.

Standard Potassium Sulfate Solution (equivalent to 200 μg. per ml. of $NH_4SO_3NH_2$). Dissolve 0.305 gram of reagent grade K_2SO_4 in water, transfer to a 1000-ml. volumetric flask, dilute to volume, and mix well.

Standard Potassium Sulfate Solution (equivalent to 20 μg. per ml. of $NH_4SO_3NH_2$). Pipet 10 ml. of the above solution into a 100-ml. volumetric flask, dilute to volume, and mix thoroughly.

Sodium Hydroxide. 12% aqueous solution.

Hydrochloric Acid. Reagent grade; total S as SO_4. <0.0002%; 1*N*.

Ammonium Hydroxide. Reagent grade; total S as SO_4. <0.0002% 1*N*; 0.1*N*.

Sodium Nitrite. Granular, reagent grade; total S as SO_4. <0.01%.

Zinc Acetate. 1% aqueous solution.

p-Aminodimethylaniline Sulfate. 0.1% in 5.5*M* hydrochloric acid (5 + 6). Eastman Organic Chemical No. 1333 (*N*,*N*-dimethyl-*p*-phenylenediamine sulfate).

Ferric Chloride. 0.023*M* in 1.2*M* hydrochloric acid (1 + 9).

Reducing Acid Mixture. Use fresh, unopened bottles of both hydriodic acid and hypophosphorus acid. Both are somewhat unstable; hydriodic acid is easily oxidized to give free iodine, and hypophosphorus acid, upon standing, will slowly oxidize to orthophosphoric acid. Add 320 ml. of 47% hydriodic acid (Fisher, Certified Reagent A-134), 320 ml. of concentrated hydrochloric acid, and 100 ml. of 30% hypophosphorous acid [J. T. Baker, purified 0178 (50%) diluted to 30%] to a 1000-ml. Erlenmeyer flask. Insert a nitrogen line, and with a gentle stream of nitrogen bubbling through the mixture (40 to 50 ml. per minute), heat to boiling; boil gently for 20 minutes. Cool to room temperature and transfer to an automatic pipettor, similar to Scientific Glass Co. No. J-2130 with a 20-ml. capacity pipet, previously flushed with nitrogen. It is important that the volumes and the concentrations of acids used conform with the specified directions to assure complete reduction efficiency when the reducing acid is applied to sulfate samples at about 100° C. Hypophosphorous acid, if heated too strongly (above 130° C.), will undergo autooxidation and give off phosphine gas. Phosphine gas, if present in the reducing mixture, will distill into the zinc acetate flask and interfere with the methyleneblue formation. Each new lot of acid should be tested by analyzing aliquots of the standard K_2SO_4 solution, as described under Procedure before unknowns are analyzed. If low or erratic results are obtained with the standard K_2SO_4 solution, a new lot of reducing acid mixture should be prepared. The efficiency of the reducing acid mixture should be checked periodically.

Nitrogen. Cylinder supply, with necessary regulator, pressure reducing valve and needle control valve.

Darco G-60. Activated carbon: Darco Corp., New York.

Alumina. Acid-washed. With stirring, digest reagent grade aluminum oxide (Merck) 71707 at room temperature for 1 hour with 1*N* HCl. Wash with water, allowing the larger particles to settle, and decant off the fines until the residue settles in less than 1 minute for a fall height of 10 cm.

Apparatus. Chromatographic Column, Acid-Washed Alumina. Two columns are required and four are desired for routine use. Insert a plug of glass wool into the lower end of a 50-ml. buret and add a slurry of the acid-washed alumina until a column of about 20 to 25 cm. is formed. Place the tip of the buret through a one-hole rubber stopper, insert into a filtering flask, and apply suction, washing down any particles that may be on the side of the buret with distilled water. Insert a second plug of glass wool at the top of the column and firmly press it into contact with the hard surface of the oxide column. Eliminate sulfate contamination by washing with 50 ml. of 1*N* HCl, followed by 100 ml. of deionized water, 50 ml. of 1*N* NH_4OH, and 50 ml. of 0.1*N* NH_4OH. Continue to wash with an additional 50 ml. of 1*N* NH_4OH and 50 ml. of 0.1*N* NH_4OH, followed with 100 ml. of deionized water. Never allow the column to be left dry. Always fill the buret with deionized water and let stand until ready for use.

Before using the column, wash with an additional 20 ml. of 1*N* NH_4OH and 50 ml. of 0.1*N* NH_4OH, and analyze the total volume of effluent for sulfate by the described procedure. If sulfate is detected, as indicated by a final absorbance reading greater than 0.020, repeat washing the column with the NH_4OH solutions as often as necessary. Secondly, check each column to establish the volume of NH_4OH solutions required to

elute the sulfate. Usually, two washes, each consisting of 20 ml. of 1*N* NH_4OH and 50 ml. of 0.1*N* NH_4OH, are satisfactory. To determine the necessary elution volumes, add 2.5 ml. of the standard K_2SO_4 solution equivalent to 200 μg. per ml. of $NH_4SO_3NH_2$ to 100 ml. of 1*N* HCl and pour this solution through the chromatographic column as described under Procedure. Elute the sulfate as suggested and analyze the ammonium sulfate solution as described, using a 1/5 aliquot.

If necessary, additional NH_4OH may be used to elute completely all sulfate.

Waring Blendor. Equipped with standard size (1000 ml.) glass jar.

Spectrophotometer. Beckman Model B, or its equivalent with a 5-cm. cell.

Centrifuge. International Size 1, Model BE 50, or its equivalent, equipped with either 250- or 500-ml. capacity centrifuge bottles.

Stirring Apparatus. Magnetic.

Reducing Apparatus. See Figure 1.

Procedure

Weigh a 100-gram sample of diced fruit into a Waring Blendor jar, add 125 ml. of distilled water, and blend for 3 to 4 minutes. Transfer, quantitatively, to either a 250- or 500-ml. centrifuge bottle, centrifuge for 10 minutes at 2000 r.p.m. and carefully decant the water extract through glass wool into a 600-ml. beaker. Rinse the blender jar with an additional 125-ml. portion of distilled water, collecting the rinse in the centrifuge bottle. Stopper with a cork plug, shake thoroughly for 2 minutes, and centrifuge as before, combining the water extract with that in the 600-ml. beaker. Repeat the extraction again with another 125-ml. portion of distilled water and combine the extract with those previously collected.

Add 2 grams of Darco to the combined extracts and heat to 60° C. on a hot plate in about 10 minutes. Using a vacuum, filter through Whatman No. 1 filter paper into a flask containing 5 ml. of 12% NaOH solution. Wash the beaker with two 5-ml. portions of distilled water. The direct filtration of the Darco slurry is very slow. Allow overnight for filtration. (The procedure should not be interrupted for any length of time before this point is reached.) Pour the clear filtrate into a 400-ml. beaker using several small portions of distilled water as wash. The pH of the solution should be 11.5 to 12; if not, add additional 12% NaOH solution and carefully concentrate on a hot plate to a volume of about 90 ml. Maintain the pH of the solution above 7 during the concentration step by addition of 12% NaOH if necessary. The concentration should be completed in 1 to 2 hours if the solution is maintained at a boil. An appreciably longer heating time could result in hydrolysis of the $NH_4SO_3NH_2$, giving low results.

Cool in an ice bath, add 10 ml. of concentrated HCl, measured by graduate, and

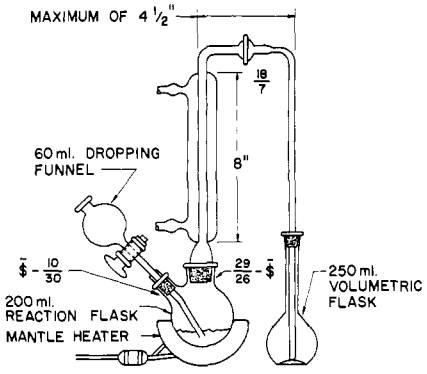

Figure 1. Sulfate reducing apparatus

2 teaspoonsfuls of acid-washed (wet) Al_2O_3, and stir continuously on a magnetic stirrer for 1 hour. Allow to settle and filter with vacuum through No. 40 filter paper. Wash the Al_2O_3 remaining in the beaker by resuspending in 5 ml. of distilled water, allowing to settle, and decanting the supernatant onto the filter. Repeat this washing operation a second time. The HCl acid concentration must remain between 2 and 5% for complete adsorption of SO_4 on Al_2O_3, and, therefore, the wash volumes should not exceed those prescribed.

Remove the final traces of sulfate using the prepared alumina column. Drain the water from the column, wash with 20 ml. of 1*N* HCl, and elute the sample through the column. Wash the column with 20 ml. of 1*N* HCl, combine with the sample eluate and transfer to a 400-ml. beaker using a minimum amount of distilled water as wash. (As all undesirable SO_4 has now been removed, this is a convenient stopping point.) Before reusing this column, it must be regenerated as described below. This column should be reserved for sulfate clean-up.

To regenerate the alumina column, continue to wash with 50 ml. of 1*N* HCl, followed in order with 100 ml. of deionized water, 20 ml. of 1*N* NH_4OH, and 50 ml. of 0.1*N* NH_4OH. Repeat the NH_4OH wash using 20 ml. of 1*N* solution and 50 ml. of 0.1*N* solution. Complete the wash with 100 ml. of deionized water. The column is now ready for reuse. No change in the adsorption power of the column has been observed after 100 analyses (*6*).

Add 0.1 gram of reagent grade $NaNO_2$ to the solution in the 400-ml. beaker and place on a magnetic stirrer for 30 minutes, thus allowing sufficient time for the nitrous acid to react.

Pass this solution, now containing sulfate resulting from the nitrous acid–sulfamate reaction, through a second alumina column, prepared as described under Apparatus, drained, and washed with 20 ml. of 1*N* HCl. Wash the beaker and stirring bar with 50 ml. of 1*N* HCl and add to the column, followed by 100 ml. of deionized water. Discard the eluate.

Elute the sulfate from the column using the necessary volumes of NH_4OH solutions as determined previously. Measure the volume of the combined ammonium sulfate solution and transfer 1/5 the volume to a 200-ml. round-bottomed flask equipped with a 29/26 joint and 10/30 side arm. Ammonium sulfamate in the range of 0.5 to 8 p.p.m. may be determined using the above aliquot. If maximum sensitivity is desired, transfer the entire ammonium sulfate solution to the reaction flask. Add 3 to 4 glass beads and concentrate to about 5 ml. using an electric heating mantle. Continue to concentrate to a volume of less than 0.5 ml. using a steam bath.

Assemble the reducing apparatus as illustrated (Figure 1) with the sulfate solution to be analyzed already in the reaction flask and the receiving flask containing 130 ml. of 1% zinc acetate solution plus 5 ml. of 12% sodium hydroxide solution. With the cooling water running through the condenser, add 20 ml. of the reducing acid mixture to the reaction flask via the dropping funnel. Immediately insert the nitrogen line allowing a constant flow of nitrogen to bubble gently (40 to 50 ml. per minute) through the system. Heat the contents of the flask to boiling (5 to 6 minutes) and continue to boil under reflux for exactly 5 minutes. Continue the nitrogen flow for an additional 5 to 6 minutes before disconnecting the equipment.

Without delay, remove the dropping tube from the receiving flask, washing the end of the tube with small amounts of water, and add, by pipet, 25 ml. of 0.1% *p*-aminodimethylaniline solution; swirl to dissolve the solids. Add, by pipet, 5 ml. of 0.023*M* ferric chloride solution, mix, and allow to stand for 10 minutes. Dilute to the mark with water, mix well, and allow to stand for at least 20 minutes. (The solution must be kept out of direct sunlight and standing time should not exceed 2 hours.) Prepare a reagent blank solution by adding to a 250-ml. volumetric flask 130 ml. of 1% zinc acetate, 5 ml. of 12% sodium hydroxide, 25 ml. of 0.1% *p*-aminodimethylaniline solution; swirl to dissolve the solids, and then add 5 ml. of 0.023*M* ferric chloride solution. Mix and continue as with the sample solutions.

Determine the absorbance of the sample solution at 665 mμ, using 5-cm. cells and the reagent blank solution in the reference cell. The amount of ammonium sulfamate in the sample is then determined from a calibration curve covering ammonium sulfamate concentrations over the range of 0 to 160 μg. The calibration curve is prepared using aliquots of a standard potassium sulfate solution, calculated as equivalent amounts of ammonium sulfamate, that have been subjected to the reducing procedure described above. Aliquots representing 20, 60, 100, and 160 μg. of $NH_4SO_3NH_2$ are suggested for calibration purposes.

Table I. Apparent Ammonium Sulfamate Level in Blanks of Untreated Fruit

Variety	Sample Weight Analyzed, Grams	Apparent Blank, μg.	Apparent Blank, P.p.m.
	Apples		
Rome	100	12	0.12
Rome	100	10	0.10
Rome	100	14	0.14
Rome	100	9	0.09
Rome	100	11	0.11
Rome	100	10	0.10
Stayman	100	15	0.15
Stayman	100	10	0.10
Stayman	100	11	0.11
Stayman	100	12	0.12
Stayman	100	10	0.10
Red Delicious	100	10	0.10
	Av.	11	0.11
	Pears		
D'Anjou	100	14	0.14
D'Anjou	100	11	0.11
	Av.	13	0.13

Table II. Recovery of Known Amounts of Ammonium Sulfamate Added to Untreated Fruit Samples

Sample Weight, Grams	$NH_4SO_3NH_2$ Added, μg.	$NH_4SO_3NH_2$[a] Found, μg.	Recovery, %	Level, P.P.M.
		Apples		
100	10	9	90	0.1
100	20	18	90	0.2
100	30	27	90	0.3
100	40	32	80	0.4
100	50	44	88	0.5
100	60	55	92	0.6
100	100	92	92	1.0
100	100	84	84	1.0
100	200	169	85	2.0
100	300	249	83	3.0
100	400	354	89	4.0
100	500	404	81	5.0
100	600	539	90	6.0
		Av.	87	
		Av. dev.	3.6	
		Pears		
100	20	16	80	0.2
100	40	33	83	0.4
100	60	49	82	0.6
100	100	87	87	1.0
		Av.	83	
		Av. dev.	2.0	

[a] Corrected for average blank.

The volume of an aliquot must be reduced to less than 0.5 ml., as with the sample, before reduction.

Discussion

Ammonium sulfamate hydrolyzes relatively easily in acid medium but is more stable in alkaline systems. During those steps in the procedure in which the fruit extract must be acidified (while in contact with the alumina), it is necessary to proceed without undue delay. This is no longer a problem once the sulfamate has been converted to sulfate. It is also necessary to proceed immediately with the color development procedure at the conclusion of the reduction step and after complete absorption of the liberated hydrogen sulfide. Sulfide is readily oxidized to sulfite on contact with atmospheric oxygen.

To obtain low and consistent blanks it is necessary to use very pure reagent and deionized water as specified; pass the fruit extract through an alumina column to remove final traces of sulfate prior to the conversion of sulfamate to sulfate.

Several reducing acid mixtures were considered: $HI-H_3PO_2-HCl$ (*1*, *3*, *5*,); $SnCl_2-H_3PO_4$ (*4*); and titanium–H_3PO_4 (*7*). The former was chosen because of its simplicity in preparation and handling. It is very important, however, that the mixture be prepared as directed under Reagents if optimum reducing conditions are to exist. It is equally important that the volume of the sulfate solution be reduced to 0.5 ml. or less, prior to addition of the reducing acid solution. The times and nitrogen flow rate suggested during the reduction steps are also critical.

Results

Blanks of 0.1 p.p.m. as ammonium sulfamate, inclusive of equipment and reagents, were obtained on several varieties of apples and one of pears (Table I). Such sulfur-containing pesticides as parathion, captan, thiram, and Guthion do not interfere. Recovery of ammonium sulfamate over the range of 0.1 to 6 p.p.m., when added to untreated apples, averaged 87%. Recoveries obtained on pears over the range of 0.2 to 1 p.p.m. averaged 83% (Table II). Equally satisfactory results should be obtained on a wide variety of materials.

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