

CORESTA RECOMMENDED METHOD N° 4

DETERMINATION OF MALEIC HYDRAZIDE RESIDUES IN TOBACCO

(September 1976)

1. PRINCIPLE

Tobacco is boiled with sodium hydroxide solution to remove volatile basic interfering compounds. Zinc granules are then added, and the nascent hydrogen produced, reduces the maleic hydrazide to succinic hydrazide which is then hydrolysed to liberate hydrazine. The hydrazine is distilled off and collected in dilute sulphuric acid, and finally reacted with *p*-dimethylamino-benzaldehyde to form a yellow compound which can be measured spectrophotometrically.

2. BASIC METHOD

2.1 Chemicals

- (1) *p*-dimethylaminobenzaldehyde (*p*-DAB) - 2 % w/v in *N* sulphuric acid. Stored in the dark, and in a refrigerator, this solution is stable for one month.
- (2) Granulated zinc - 30 mesh.
It has been found that the grade of zinc used is of prime importance. It is recommended that the zinc to be used is checked by comparing the colour produced by standard solutions of hydrazine sulphate and *p*-DAB, with that from maleic hydrazide after reduction and distillation.
- (3) Maleic hydrazide standard solution, 10 µg/ml.
Dissolve 0.010 g maleic hydrazide in 100 ml 0.1 *N* sodium hydroxide solution and dilute to 1 litre with distilled water. This solution is stable.
- (4) Ferrous chloride. Ensure that the ferrous chloride is interference free by testing with *p*-DAB.
- (5) Sodium hydroxide solution - 50 % w/v in distilled water.
- (6) Sulphuric acid - *N* solution.
- (7) Anti-foam agent such as paraffin wax or refined vegetable oil.
- (8) High boiling point oil for thermometer well.
- (9) Anti-bumping granules.
- (10) Hydrochloric acid 10 % v/v for cleaning.

2.2 Apparatus

- (1) Distillation apparatus - see fig. 1. The distillation flask has a capacity of 500 ml, double wall thickness, and has a thermometer well.
The distillation flask is connected to the steam generator via a three-way tap. The third way is used to pass steam, through a condenser, to waste, when not required *i.e.* when changing the distillation flask.
- (2) Spectrophotometer with 10 mm cells.
- (3) Measuring cylinder - 100 ml.
- (4) Volumetric flask - 25 ml.
- (5) Pipettes - 2 ml.

Safety note :

It is advisable to use a safety screen in front of the apparatus during the distillation.

3. PROCEDURE

Transfer 1 ± 0.01 g ground tobacco to the distillation flask. Add 50 ml of 50 % sodium hydroxide solution, a small piece of paraffin wax or 1 ml refined vegetable oil, and a few anti-bumping granules. Add 1 ml high boiling point oil to the thermometer well and insert thermometer. Heat the flask carefully, swirling frequently, until the temperature reaches $165\text{ }^{\circ}\text{C}$; this should take about 10-15 minutes. Remove the flask from the heat, and allow to cool for 5 minutes. Remove the thermometer and wipe the socket joint clean and dry. Add 0.5 ± 0.05 g ferrous chloride and 15 ± 0.5 g zinc granules. Replace the thermometer and immediately connect the flask to the distillation apparatus (fig. 1) using a thin film of high vacuum silicone grease on the socket joint. Place the 100 ml measuring cylinder, containing 10 ml *N* sulphuric acid, so that the condenser delivery tube is below the surface of the acid. Ensure a rapid flow of cooling water to the condenser.

Turn the 3-way tap to admit steam to the distillation flask, and distil at a rapid rate, (distillation temperature $200 \pm 10\text{ }^{\circ}\text{C}$), to collect at least 100 ml distillate in 20 minutes. Remove the cylinder, rinsing the condenser delivery tube with a few ml of distilled water. Filter the distillate through a medium porosity sintered glass filter funnel into a 250 ml beaker, washing out the cylinder and funnel with about 5 ml distilled water, and adding the washings to the beaker. Add a few anti-bumping granules and evaporate carefully on a hot plate to about 6 ml (DO NOT EVAPORATE BELOW 6 ml). Alternatively, concentrate in a rotary evaporator. Cool the concentrated distillate and quantitatively transfer to a 25 ml graduated flask. Add 2 ml *p*-DAB, and dilute to volume with distilled water. Stopper the flask, mix well, and allow to stand for 15-30 minutes. Measure the absorbance of the solution at 425, 455 and 485 nm using a 10 mm cell and 2 ml *p*-DAB diluted to 25 ml as reference. Calculate the corrected absorbance using the following formula.

$$A_{\text{corr}} = A_{455} - \frac{A_{425} + A_{485}}{2}$$

From a standardisation curve read off the maleic hydrazide concentration.

Preparation of standardisation curve

Clean 500 ml distillation flasks, transfer 1 ± 0.01 g untreated tobacco, add 50 ml 50 % sodium hydroxide and 0, 1, 2, 5 or 10 ml standard solution, *i.e.* equivalent to 0, 10, 20, 50, 100 μg maleic hydrazide. Predigest, distil, and measure the absorbance as given in (3). Plot A for each standard against μg maleic hydrazide, to obtain the standardisation curve. If desired, a simple factor from curve slope may be derived.

Cleaning of distillation flask

After distillation, remove the hot flask from the apparatus using heat resistant gloves, and wearing safety glasses. Remove the thermometer and seal the well with a small cork. Rinse the steam inlet tube free of sodium hydroxide. Pour the contents of the distillation flask into an iron sieve in the sink to trap the zinc granules. Rinse the flask three times with water and twice hydrochloric acid (10 % v/v) to remove the encrusted caustic and zinc granules. Fill the flask with hydrochloric acid (10 %) to remain until next use. Rinse three times with water before re-use. NB : it is essential to remove all residual zinc as it would cause premature decomposition of maleic hydrazide in the predigest stage of the next determination.

The distillation flasks must be regularly inspected as they have a very limited life due to the action of the concentrated sodium hydroxide solution.

Note 1

Proteins present in tobacco can cause excessive foaming. Decomposition of the proteins by an acid pre-digestion stage can reduce foaming.

Some tobaccos, especially those containing a high proportion of bases, contain natural interfering substances which produce a reddish colour with *p*-DAB : these can be removed by a carbon clean up.

Both the acid pre-digestion and carbon clean-up may be included in the procedure if necessary.

Acid pre-digestion

The acid pre-digestion precedes the alkaline pre-digestion.

50 ml 3 *N* hydrochloric acid is added to the 1 g tobacco sample, together with a small piece of paraffin wax to control foaming. The flask is heated on a hot plate to boiling point and boiled at a moderate rate until the volume has been reduced to 20-25 ml. Wash down the walls with distilled water to give a total volume of about 50 ml. Reduce the volume again to 20-25 ml and cool. (Note : the partially pre-digested tobacco may be left overnight at this stage. However, once sodium hydroxide has been added, the method must be completed as quickly as possible).

Slowly add 50 ml 70 % w/v sodium hydroxide solution, swirling to mix. Then proceed with distillation as before.

Carbon clean-up

The carbon clean-up stage is introduced immediately after the distillation stage. The distillate (100 ml) is shaken vigorously with 2 g activated carbon (NORIT "A") for 1 minute. Filter using a Buchner funnel and then concentrate to 6 ml develop the colour using *p*-DAB as before.

Note 2

Some people prefer to carry out the distillation stage in a current of nitrogen in place of steam. If nitrogen is used, it is essential to ensure that sufficient water is present in the distillation flask to prevent the concentration of sodium hydroxide reaching an unacceptably high level.

Repeatability

Joint experiments carried out by the CORESTA Pesticide Sub-Group indicate that the repeatability of this method is $\pm 5\%$.

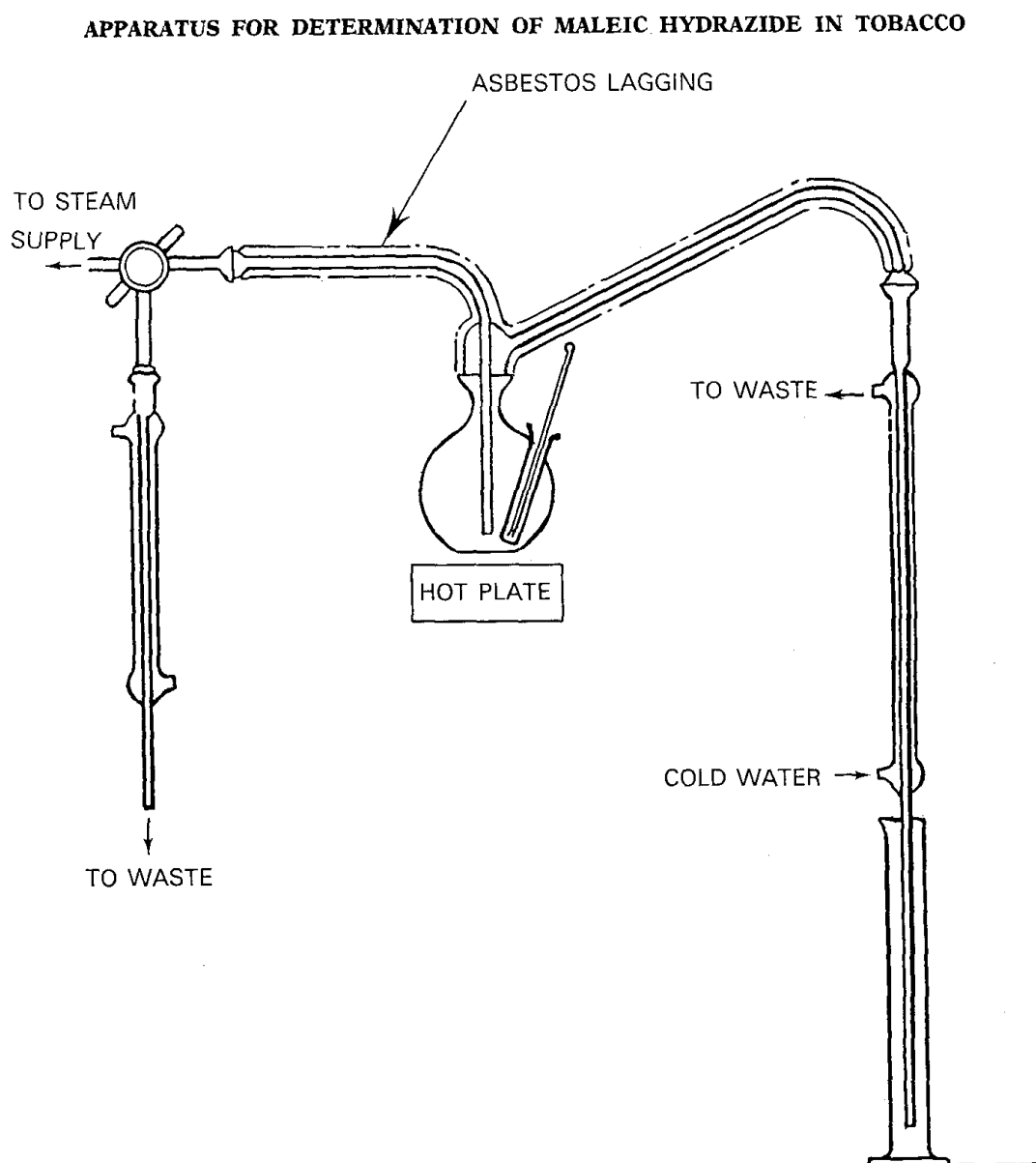


Figure 1