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Determination of maleic hydrazide residues in cured tobacco by gas chromatography☆

J. M. Renaud, I. Keller and G. Vuillaume

Research & Development, Philip Morris Europe SA, P.O. Box 11, CH-2003 Neuchâtel (Switzerland)

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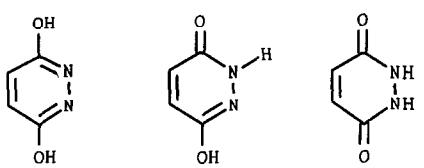
ABSTRACT

A rapid and sensitive method for measuring maleic hydrazide (6-hydroxy-2*H*-pyridazin-3-one) residues in cured tobacco is described. A mixture of free and bound maleic hydrazide is extracted with hydrochloric acid in which maleic hydrazide glycoside is simultaneously hydrolysed. The free maleic hydrazide obtained is methylated using dimethyl sulphate and the derivative is partitioned into chloroform and determined by capillary gas chromatography using a nitrogen–phosphorus detector. The limit of detection of maleic hydrazide is 5 ppm.

INTRODUCTION

Maleic hydrazide (6-hydroxy-2*H*-pyridazin-3-one) (MH) is a systemic plant growth regulator, widely used for tobacco-sucker control. After application to the upper part of the tobacco plant, MH is transcolated within the plant where it inhibits cell division, but not cell extension. Maleic hydrazide can exist in three tautomeric forms [1] (**I–III**) and shows phenolic properties under physiological conditions.



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Correspondence to: Dr. J. M. Renaud, Research & Development, Philip Morris SA, P.O. Box 11, CH-26043 Neuchâtel, Switzerland.

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Towers *et al.* [2] showed that about 15% of the maleic hydrazide in young wheat leaf segments was present as a glycoside. In tobacco, MH is gradually converted into the bound form, mainly during curing but also during storage; conversion rates of up to 50% were observed during curing [3].

Because of growing restriction concerning agricultural chemical residues on tobacco, interest in the determination of MH has arisen. Methods for the determination of total MH on tobacco involve reduction with zinc and hydrolysis with hot alkali to hydrazine [4–9]. The hydrazine is isolated by distillation and determined spectrophotometrically as an azine by addition of *p*-dimethylaminobenzaldehyde. The spectrophotometric procedure was adopted by the International Organization for Standardization (ISO) as a standard method [9]. In addition to the low sample capacity of this method, its major drawback is a lack of specificity and possible interferences [10]. More specific methods utilizing GC have been proposed, but they are restricted to free MH [10,11] and also involve tedious sample preparation steps.

A method is presented here for the routine determination of total, *i.e.*, free and glycosidically bound MH on tobacco. MH glycoside is hydrolysed by

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hydrochloric acid, methylated in aqueous medium with dimethyl sulphate, transferred into an organic solvent and determined by gas chromatography (GC) with nitrogen–phosphorus detection (NPD).

EXPERIMENTAL

Reagents

All solvents were of analytical-reagent grade (Fluka, Buchs, Switzerland). Maleic hydrazide (Riedel-de Haën, Seelze, Germany) and 6-methyluracil (Aldrich, Steinheim, Germany) were of Pestanal grade and 97% purity, respectively. Concentrated hydrochloric acid (32%, $d = 1.16$) and dimethyl sulphate of analytical-reagent grade were obtained from Fluka. An internal standard stock solution was prepared by dissolving 30 mg of 6-methyluracil in 100 ml of water. In order to obtain the extraction solution, 400 ml of concentrated hydrochloric acid and 20 ml of internal standard stock solution were made up to a total volume of 2 l with water. A standard stock solution of MH was prepared by dissolving 40 mg of MH in 100 ml of water. The calibration solution was obtained by dissolving 10 ml of standard MH stock solution and 200 ml of concentrated hydrochloric acid in 1 l of water.

Analytical procedure

Extraction and hydrolysis. A 5-g amount of cut filler or ground tobacco was introduced into a 250-ml flask and extracted with 100 ml of extraction solution for 2 h under reflux. After cooling and filtration, a 1-ml aliquot was transferred into a 5-ml reaction vessel for derivatization.

Derivatization. The 1.0-ml aliquot was made alkaline by addition of exactly 0.4 ml of 10 *M* NaOH; an exact amount of dimethyl sulphate (200 μl) was then added using a disposable syringe and the flask was tightly closed and kept at 75°C for 1 h in a heating module (Pierce, Rockford, IL, USA). At the beginning of the reaction, the flask was shaken regularly until the dimethyl sulphate layer disappeared (usually 7–10 min). After cooling, the dimethyl derivative obtained was extracted with 500 μl of chloroform. Optimum extraction yields were obtained by shaking in a vortex mixer for 3 min and the final phase separation was achieved by centrifugation. An aliquot (200 μl) of the lower organic layer was transferred into a GC vial and analysed by GC–NPD.

Calibration. A 1-ml volume of calibration solution was introduced into a 5-ml reaction vessel and derivatized in parallel with the tobacco extract. Three replicates were required.

Instrumentation. A Carlo Erba Model 5160 gas chromatograph with split injector and a nitrogen–phosphorus detector was used, equipped with a 30 m × 0.25 mm I.D. fused-silica column coated with a 0.25-μm layer of DB-17 (J&W, Rancho Cordova, CA, USA). A Spectra-Physics DP-700 integrator was used for data processing. The injector and detector temperatures were set at 250 and 270°C, respectively. The column temperature was initially 140°C for 3 min, then programmed from 140 to 200°C at 5°C/min and from 200 to 250°C at 20°C/min; the final temperature was held for 15 min. The column head pressure was set at 120 kPa of 99.999% helium. A 30 ml/min flow of 99.99% nitrogen was used as make-up gas. A 1-μl volume of sample was injected with a splitting ratio of 1:30. Quantification was performed by the internal standard technique using 6-methyluracil as internal standard.

GC–MS apparatus and conditions. A Hewlett-Packard Model 5988 GC–MS system was used. The chromatographic conditions were identical with those mentioned above. The spectra were recorded in the electron impact mode with the following conditions: interface temperature, 250°C; source temperature, 190°C; and ionization energy, 70 eV. Ions were detected at m/z 140 (92%, $M^{+\cdot}$), 112 (36%), 82 (29%), 80 (47%) and 69 (100%).

Nuclear magnetic resonance. ^{1}H NMR spectra were recorded in deuterated chloroform at 200 MHz on a Bruker WP 200 spectrometer. Tetramethylsilane was taken as the reference for the calculation of chemical shifts. The ^{1}H NMR chemical shifts were 3.6 (s, 3H), 3.8 (s, 3H) and 6.9 ppm (s, 2H).

RESULTS

The chromatogram of an extract of flue-cured tobacco is shown in Fig. 1. The retention times of methylated MH and 6-methyluracil are 5.32 and 14.29 min, respectively. A recovery of 95% was determined on tobacco samples fortified with 80 ppm of MH. The precision of the method was determined at two concentration levels by analysing two

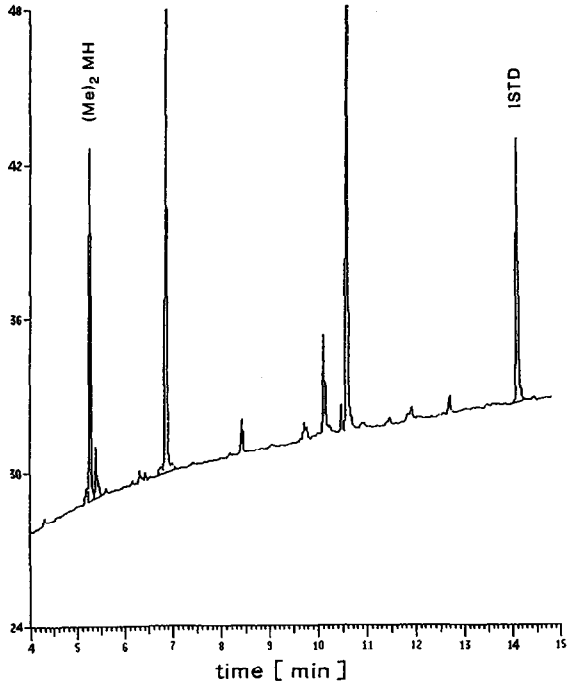

Fig. 1. Gas chromatogram of a Virginia tobacco extract containing 209 ppm of MH.

different tobacco samples containing 70 and 150 ppm of MH. At both levels the relative standard deviation based on eleven replicates was 3%. The detection limit, corresponding to a signal-to-noise ratio of 3, was 5 ppm.

The linearity of MH detection was examined for standard solutions and for fortified tobacco extracts between 10 and 550 ppm (Figs. 2 and 3). The amounts detected by the GC method increased linearly in the range considered for both the standard solutions and the tobacco extracts with a good correlation (r^2 = 0.999 and 0.997, respectively).

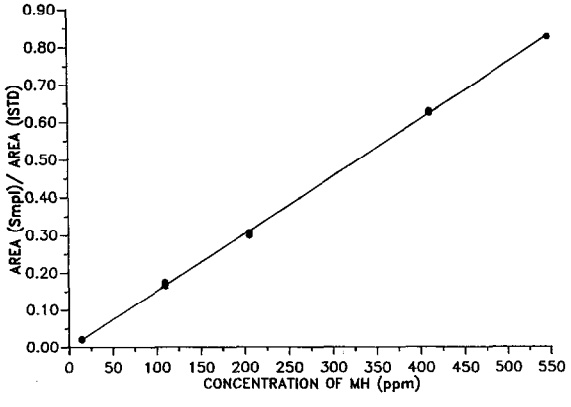

Fig. 2. Linearity of MH detection in standard solutions.

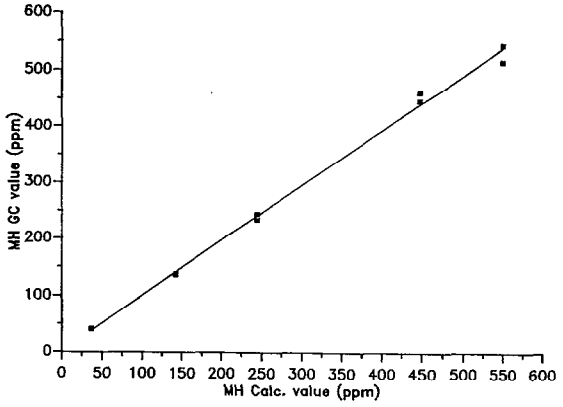

Fig. 3. Linearity of MH detection in fortified tobacco extracts.

The method was further validated by comparing the amounts of MH detected by the GC procedure with the amounts detected by the ISO reference method [9]. Thirty tobaccos with MH contents ranging from 0 to 300 ppm were analysed and, as shown in Fig. 4, an excellent correlation was obtained (r^2 = 0.9775).

DISCUSSION

The very low solubility of MH in non-polar organic solvents and the necessity to hydrolyse the

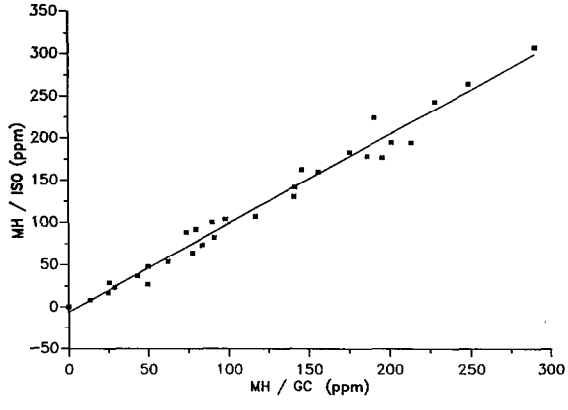

Fig. 4. Correlation between MH residues determined by GC and by spectrophotometry by the ISO 4876 method [9].

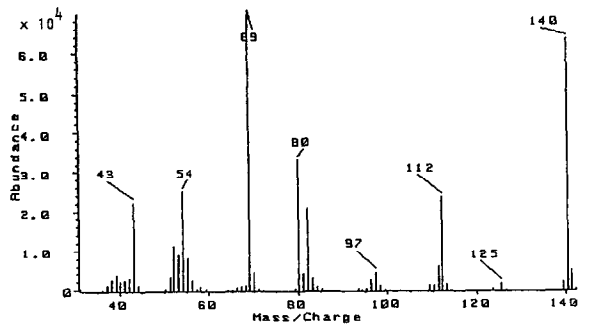

Fig. 5. Electron impact mass spectrum of the methylated derivative of MH.

MH glycoside require and aqueous medium for the initial step. Under such conditions, the tobacco material gives a very complex mixture that is not compatible with any direct chromatographic analysis. Therefore, the "*in situ*" methylation with dimethyl sulphate presented several advantages: free MH is converted into its dimethyl derivative, which can be quantitatively extracted from the aqueous phase with chloroform; the partitioning into the organic phase, associated with specific GC detection, was sufficient to ensure an interference-free analysis. On decreasing its polarity by methylation, MH was also made more suitable for GC analysis.

Although MH can exist in three tautomeric forms (**I–III**) [1], methylation with dimethyl sulphate produces only the derivative of one isomer (**II**, 6-methoxy-2-methylpyridazin-3-one). Positive proof of the identity of the derivative was obtained by GC–MS and ^{1}H NMR.

In the ^{1}H NMR spectrum, the two signals (singlets) at 3.6 and 3.8 ppm correspond to methyl groups in different environments, *i.e.*, CH_3–N (3.6 ppm) and CH_3–O (3.8 ppm). In addition, the measured chemical shifts are in accordance with values reported by Katritzky and Waring [1], *i.e.*, 3.53 and 3.78 ppm for CH_3–N and CH_3–O, respectively, in 6-methoxy-2-methylpyridazin-3-one.

The mass spectral data (Fig. 5) are in agreement with the fragmentation of pyridazine reported by Porter [12]: The molecular ion at *m/z* 140 loses a molecule of CO, producing the signal at *m/z* 112, and this ion loses CH_3N_2, resulting in the base peak at *m/z* 69 and the signal at *m/z* 43. Fragments at *m/z* 80 and 82 orginate from the ion at *m/z* 112 by loss of methanol and formaldehyde, respectively.

CONCLUSIONS

A GC method for the routine determination of MH residues in tobacco has been presented and validated. The method is specific, rapid and requires little manual labour. Including the hydrolysis step, which is the most time-consuming step in the method, 20 samples can be processed daily. The use of an internal standard added to the hydrolysis solution at the beginning of the procedure guarantees a high precision, as it allows corrections for occasional losses during sample preparation, variations in injection volume and changes in detector sensitivity. However, owing to the efficiency of the extraction procedure (>95% recovery) and the stability of the NP detector, these corrections were minor.

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REFERENCES

1 A. R. Katritzky and A. J. Waring, *J. Chem. Soc.*, (1964) 1523.
2 G. U. N. Towers, A. Hutchinson and W. A. Andreae, *Nature (London)*, 181 (1958) 1535–1536.
3 A. F. Haeberer and O. T. Chortyk, *J. Assoc. Off. Anal. Chem.*, 62 (1979) 171–175.
4 P. R. Wood, *Anal. Chem.* 25 (1957) 1879–1883.
5 C. Anglin and J. H. Mahon, *J. Assoc. Off. Agric. Chem.*, 41 (1958) 177–182.
6 J. R. Lane, D. K. Gullstrom and J. E. Newell, *J. Agric. Food Chem.*, 6 (1958) 671–674.
7 I. Hofmann, *J. Assoc. Off. Agric. Chem.*, 44 (1961) 723–725.
8 M. Ihnat, J. Robert and I. Hoffman, *J. Assoc. Off. Anal. Chem.*, 56 (1973) 1164–1172.
9 *International Standard, ISO 4876*, International Standards Organization, Geneva, 1980.
10 A. F. Haeberer, W. S. Schlotzhauer and O. T. Chortyk, *J. Agric. Food Chem.*, 22 (1974) 328–330.
11 Y. Y. Liu and D. Hofman, *Anal. Chem.*, 45 (1973) 2270–2273.
12 A. N. Porter, *Mass Spectrometry of Heterocyclic Compounds*, Wiley, New York, 1985, p. 715.