Determination of Free and Bound Maleic Hydrazide Residues in Tobacco by High Performance Liquid Chromatography

by:

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SUMMARY

A new method is described for the qualitative and quantitative determination of both free and bound maleic hydrazide residues in tobacco leaves and cigarette filler by high performance liquid chromatography. Analyses were carried out by hydrolyzing samples of ground tobacco with 4 N hydrochloric acid for 40 minutes under reflux followed by sample chromatography, running isocratic elutions with a dilute solution of phosphoric acid. The quantitative determination of maleic hydrazide was performed by light absorption at 320 nm, by the calibration curve method. Recoveries of maleic hydrazide added to tobacco samples were greater than 90%. The detection limit of the method, determined on ground tobacco leaves, was at least 5 ppm. The results obtained by this procedure and by the ISO standard method no. 4876 are in good accordance.

ZUSAMMENFASSUNG

Cette étude décrit une nouvelle méthode d'analyse qualitative et quantitative des résidus d'hydrazide maléique libres et liés dans les feuilles de tabac et les charges de cigarettes et ce, au moyen de la chromatographie haute performance en phase liquide. Les analyses sont réalisées en hydrolysant des échantillons de tabac râpé avec de l'acide chlorhydrique 4 N pendant 40 minutes à reflux. Cette opération est suivie d'une chromatographie des échantillons et d'élutions isocratiques avec une solution diluée d'acide phosphorique. Le dosage de l'hydrazide maléique a été effectué par absorption de la lumière à 320 nm en faisant appel à la méthode de la courbe d'étalonnage. Les taux de récupération de l'hydrazide ajouté aux échantillons de tabac ont été supérieurs à 90%. La limite de détection, déterminée sur les feuilles de tabac râpé, s'est avérée de 5 ppm au moins. Les résultats obtenus selon cette procédure présentent une bonne concordance avec ceux que donne la méthode ISO standard N° 4876.

INTRODUCTION
Maleic hydrazide (3, 6-dioxo-1, 2-dihydropyridazine) is a systemic plant growth-regulator widely used in agriculture as a sprout inhibitor for stored products as well as for tobacco sucker control. Although the maleic hydrazide is usually applied only to the top of the tobacco plant, it diffuses and is transferred throughout the plant, where it can be found as free or bound compound.

Results in the literature show that high residues level are present in tobacco leaves as well as in cigarettes. Due to the fact that some authors (1-2) report different effects and levels of toxicity for the maleic hydrazide and that the German Government has stated a tolerance level of 80 ppm in cigarette tobacco, a fast, easy and safe analytical procedure is required for the determination of this substance.

Many papers have been published concerning the analysis of maleic hydrazide in tobacco leaf and in cigarette filler.

The standard method ISO 4876 and the official method of the Association of the Official Analytical Chemists for the determination of total maleic hydrazide, free and bound, are based on an acidic digestion followed by a hydrolytic reduction of the free compound to hydrazine by zinc in an aqueous sodium hydroxide solution. The hydrazine is then steam distilled into an acidic p-dimethylaminobenzaldehyde solution and the resulting yellow compound has an absorption maximum at 355 nm (3-5).

Some authors determine the maleic hydrazide in tobacco and in tobacco smoke by gas chromatographic procedures, using methyl or trimethylsilyl or alkyl carbonate derivatives (6-13). Others describe HPLC methods involving purifications by passages through several different resins (14-17).

The purpose of this work is to find the best conditions for detecting and determining in a quick and easy way the total maleic hydrazide in tobacco leaf and in cigarette filler by the aid of HPLC.

EXPERIMENTAL
Reagents
- Maleic hydrazide
- Hydrochloric acid
- Phosphoric acid

Apparatus
- Perkin Elmer series 4 liquid chromatograph equipped with:
  - Pumping system Series 4
  - 1.4 µl microcell
  - LC autocontrol system series 4
  - Spectrophotometric detector mod. LC85B
  - Diode array detector mod. 235C
  - Sample injection valve with a 6 µl sample loop
  - 15 cm (5 µp particle size) reverse phase Perkin Elmer C8 column
  - Hewlett-Packard 3396A integrator
  - Varian 1200 recorder

Operating conditions
- Temperature: room temperature in the range 18 - 23 °C
- Meter scale: 10 mV
- Chart speed: 1.0 cm/min
- Isocratic elution
- Eluent: H₃PO₄ 0.3% w/v
- Flow rate: 0.5 ml/min

Analyses of tobacco
5 g of cigarette filler or tobacco leaves is ground and introduced into a 250 ml flask, 100 ml of 4N HCl is added and the mixture is boiled under reflux for 40 minutes. After cooling, the extract is filtered through a folded filter. A few ml of the solution are passed through a 3 ml SPE disposable column packed with C-bonded silica gel by Supelco. The extract is then injected directly into the chromato-
RESULTS AND DISCUSSION

The maleic hydrazide at the pH of the eluent (about 2.0) has an ultraviolet absorption maximum at 302 nm. The values of the optical density are read at 313 nm and sometimes at 319 nm, where the absorbance is 8-10% lower, because in the chromatograms of some tobacco samples there is a peak not perfectly separated from the maleic hydrazide one (Figure 1) and which has practically no absorbance at 319 nm. In Figure 2 the graph shows the variation of the signal intensities of maleic hydrazide and the interfering compound vs wavelength. This kind of graph, obtained measuring the height of both the peaks of the same tobacco sample at different wavelengths, is an example of the method which can be used for any kind of tobacco to choose the most suitable wavelength. Also the absorbance due to the background disappears at wavelengths higher than 310-315 nm. Figure 3 shows the absorption spectra taken, by subtracting the background using a diode array detector, at the upslope, apex and downslope of the chromatographic peak, as well as the overlay spectra. In particular from the overlay and apex spectra it is possible to see that the background absorbance is practically negligible at wavelengths higher than 315 nm and therefore the maleic hydrazide peak can be perfectly detected and measured.

All these graphs confirm that the values of the optical density could be read at wavelength in the range 310-320 nm.

Table 1.
Recoveries of maleic hydrazide spiked into tobacco samples.

<table>
<thead>
<tr>
<th>Amount added (µg/g)</th>
<th>Amount found (µg/g)</th>
<th>Recoveries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>20.2</td>
<td>101.0</td>
</tr>
<tr>
<td>30</td>
<td>28.6</td>
<td>95.3</td>
</tr>
<tr>
<td>50</td>
<td>48.9</td>
<td>97.8</td>
</tr>
<tr>
<td>100</td>
<td>101.0</td>
<td>101.0</td>
</tr>
<tr>
<td>150</td>
<td>144.1</td>
<td>96.6</td>
</tr>
<tr>
<td>200</td>
<td>193.5</td>
<td>98.7</td>
</tr>
</tbody>
</table>

Average recovery: 98%
R.S.D. (%): 2.6

However, to avoid any possible interference due to coeluting components and/or to the background of tobacco samples, the 320 nm wavelength is recommended. In this case the little sensitivity variation can always be compensated for by a higher injection volume.

Quantitative determinations were carried out by the calibration curve method, which is a straight line within the range 0 to 200 ppm. The correlation coefficient is 0.999994. The detection limit, corresponding to a signal to noise ratio better than 10, is at least 5 ppm (Figure 4) and the recoveries of maleic hydrazide added to the ground tobacco samples at level of 10-100 ppm were greater than 90% (Figure 5, Table 1). The values of the relative standard deviation obtained from different tobacco samples, each tested five times or more, were in the range 6-8% (Table 2).

Figure 6 shows the correlation diagram between the results obtained by the ISO standard method and the present one; the results are in good accordance. The correlation coefficient of this diagram is 0.977 (Table 3).

CONCLUSIONS

Due to its general insolubility in most organic solvents, maleic hydrazide cannot be extracted from aqueous solutions and due to its very high melting point cannot be easily analyzed by gas chromatography as free compound. It must be derivatized. Other analysts used the HPLC or ion chromatography. However, these methods involve different procedures of purifications and/or separation by column chromatography. Some of these methods are difficult to handle...
Figure 1.
Chromatogram of a tobacco sample hydrolyzed with 4 N HCl for 40 minutes.

Figure 2.
Variation of the signal intensities of maleic hydrazide residues and the interfering compound versus wavelength.

--- M.H.; --- Interfering Compound.
Figure 3.
Absorption spectra of maleic hydrazide residues, taken by using the diode array detector.

Figure 4.
Quantitative determinations of maleic hydrazide; detection limit: 5 ppm.
Figure 5.
Untreated tobacco sample ——
The same tobacco sample after addition of 10 ppm of maleic hydrazide ———
Table 3.
Comparison with ISO method 4876.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MH residues (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPLC</td>
</tr>
<tr>
<td>Cut tobacco</td>
<td>36.1</td>
</tr>
<tr>
<td>Burley no. 1</td>
<td>49.9</td>
</tr>
<tr>
<td>Burley no. 2</td>
<td>51.8</td>
</tr>
<tr>
<td>Burley no. 3</td>
<td>81.3</td>
</tr>
<tr>
<td>Burley no. 4</td>
<td>33.1</td>
</tr>
<tr>
<td>Flue cured no. 1</td>
<td>37.0</td>
</tr>
<tr>
<td>Flue cured no. 2</td>
<td>62.2</td>
</tr>
<tr>
<td>Flue cured no. 3</td>
<td>78.6</td>
</tr>
<tr>
<td>Flue cured no. 4</td>
<td>88.4</td>
</tr>
<tr>
<td>Flue cured no. 5</td>
<td>131.6</td>
</tr>
<tr>
<td>Flue cured no. 6</td>
<td>116.7</td>
</tr>
<tr>
<td>Flue cured no. 7</td>
<td>135.2</td>
</tr>
<tr>
<td>Flue cured no. 8</td>
<td>82.5</td>
</tr>
<tr>
<td>Flue cured no. 9</td>
<td>37.7</td>
</tr>
</tbody>
</table>

Correlation line: $y = 0.959 \times + 4.273$
Correlation coefficient = 0.977

The present method is completely safe, specific, easy, quick to be performed and shows good sensitivity. Furthermore the reduction of the hydrolysis time to only 40 minutes allows analysis of at least thirty samples per day.

REFERENCES

2. Epstein, S.S., and N. Mantel: Hepatocarcinogenicity of the herbicide maleic hydrazide following parenteral administration to infant Swiss mice; J. Cancer 3 (1968) 325.


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