Analusis, **2000**, *28*, 155-158

Determination of chlormequat in pears by high-performance thin layer chromatography and high-performance liquid chromatography with conductimetric detection

J.-P. Lautié*, V. Stankovic and G. Sinoquet

Laboratoire Interrégional de la Direction Générale de la Concurrence et de la Répression des Fraudes, 25, avenue de la République, 91305 Massy, France

Abstract. The purpose of this paper is to present two simplified methods which involve inexpensive equipment and which can be applied by all analyst who have to determine chlormequat residues in pears. The two methods use the extraction with methanol and purification by formation of ion-pair with sodium tetraphenylborate. A first method is a high-performance thin layer chromatography (HPTLC), the second is high-performance liquid chromatography (HPLC) with conductimetric detector. The results obtained for the same samples by the two methods are quite comparable. These two methods are rapid and particularly the thin-layer chromatography.

Key words. Chlormequat detection – pesticide – plant growth regulator – pears – HPTLC – HPLC.

Introduction

The chlormequat (2-chloroethyl trimethylammonium) is a plant growth regulator, which acts mainly on the length of the internodes and leaf-stalks and on the leaves'chlorophyll content. It is used as a stabilizer of the length of the cereal stems as well as a flowering increase and organisation regulator of fruits and vegetables. In France the chlormequat is not authorized for the treatment of the pears, however a maximum residue limit of 3 ppm has been admitted (Arrêté du 5/8/92, *Journal Officiel de la République Francaise* and Directive 90-642 CEE). The origin of pears from various countries where this treatment is authorized and the possible confusion between national and foreign origin for the shares, have caused an emphatic request for the research of this product. Very quickly, it appeared that the methods described in the literature were not suitable to a systematic research of this pesticide. Many methods exist for the research of the quaternary amines, but a very few are workable with an ordinary material, good sensibility and on a fast procedure. For the most part the determination of this type of growth regulator needs to pass through several stages of purifications before any analysis is carried out. So G. Petrosini [1] uses cation exchange resin and alumina columns. The colorimetric methods like the one run by R.P. Mooney [2]. Using hexanitrodiphenylamine are not specific for the chlormequat determination and give positive reaction with the amines naturally present in plants.

Methods using thin-layer chromatography are generally easy to use, however the proposed purification before plate-deposit usually takes a long time and are costly with solvent. Stijve [3] purifies thus by 25 g of alumina and 190 ml of solvent. The gas chromatography through derivation with thiophenol [4] or pentafluorophenol [5] does not give satisfactory results. In fact Mortimer [6] criticises the method suggested by Allender [5] in underlining that the product obtained after derivation is not specific of chlormequat and shows that the parathion methyl gives the same product of derivation with pentafluorothiophenol.

The high resolution liquid chromatography often used for the herbicides like the paraquat and the diquat, could be used under certain restriction for the chlormequat research [7-8-9]. However, the detection by absorption within the UV is not usable for some compounds having a weak absorption level even at short wave lengths.

Liquid chromatography associated with mass spectrometry is no doubt the best adapted method for the research of this type of herbicide [10-11]. However, it is a heavy technique little adapted at present time for small size laboratories.

The present work describes the way for extraction and two methods for the research and the quantification of chlormequat in the pears. The residues are extracted from plant material with sodium tetraphenylborate solution. Sodium tetraborate is a general precipitation reagent for

*Correspondence and reprints.
Received November 19, 1999; revised February 29, 2000; accepted March 8, 2000.

compounds containing nitrogen. The salt is soluble in dichloromethane and it can be used for separating chlormequat from matrix. The first method for analysis is a high-performance thin layer chromatography technique.The second one uses high-performance liquid chromatography and a conductimetry detector. The thin layer method is fast and easy while liquid chromatography method requires an higher investment. We compared the results obtained by these two different quantification methods.

Material and methods

Sample preparations

Weigh 25 g of pear in a 250 ml flask, add 50 ml of methanol, homogenize and extract through filtration after a waiting of 3 minutes. Take back the residue and extract all over again using 20 ml of methanol. Filter through a filter paper and put together the filtrates in a round bottomed flask. Evaporate to the maximum with rotatory evaporator at 45 °C under vacuum and measure the volume (less than 10 ml). Adjust the volume with water to a determined amount for example 10 ml (test coutaining 2.5 g/ml), then purify by extraction with the help of sodium tetraphenylborate.

Extraction with 2% sodium sodium tetraphenylborate in water. Pour 1 ml of the last solution in a glass tube with screw cap (16 × 100 mm), add 0.1 ml of tetraphenylborate solution and 3 ml of dichloromethane and mix well on Vortex mixer for 1 min and leave to decant. The under organic phase was drawn off with Pasteur pipet or glass syringue (5 ml). Transfert to a second glass tube. Add to the aqueous phase again 3ml of dichloromethane, shake and remove the organic phase. Put together the organic phases in the second glass tube and evaporate them under nitrogen current at 45 °C. The evaporated tubes can be preserved for more than one week to -18 °C.

The residue was dissolved in 0.3 ml of methanol and submitted to the determination by the two following methods:

– by high-performance thin layer chromatography (HPTLC);

– by high-performance liquid chromatography and conductimetric detector (HPLC).

Chlormequat determination

Chlormequat determination by high-performance thin layer chromatography

– Plate of HPTLC cellulose (Merck 5787).

– Chamber of CAMAG development to double tank with lid in stainless steel.

– Linomat IV (CAMAG TLC applicator) for the quantitative application of the sample on plate.

– Scan for reading plates TLC Scanner II (CAMAG).

– Development solution: 7 ml of dichloromethane, 4 ml of methanol and 4 ml of hydrochloric acid (1N).

– Introduce this mixture in the development chamber 30 minutes before putting a plate.

– Detection reagent: reagent of Munier and Machebœuf [12]:

• Solution A: dissolve 1.7 g nitrate of basic bismuth in 100 ml hydrochloric acid 1/2.

• Solution B: dissolve 40 g potassium iodide in 100 ml water.

Prepare the final spraying solution immediately before use. Take a graduated cylinder of 1 liter. Fill it to half with water and add to it 40 ml of A solution, 40 ml of B solution and 150 ml of acetic acid. Adjust to 1 liter with water.

Deposit on the plates

Working standard and sample solution were applied to the HPTLC plates. Standard solution of chlormequat: 5, 10, 20, 40 microlitres of working stock solution at 50 mg/l in methanol were spotted on the plates. Sample solutions are dissolved in 0.3 ml of methanol and these solutions (20 or 40 microlitres) were spotted too.

Plate development

Carefully place the plates in the development chamber. Allow the solvent to move up to a height of 8 cm. Take the plates and let them dry for about 30 minutes under a hood at room temperature.

Quantitation

The plates are visualized by spraying the Munier and Machebœuf solution and allow 15 minutes at room temperature before evaluating (the plates can be also immersed 2 seconds in a revelation tank containing 1 liter of reagent and then are dried for 1 hour at room temperature). The colored spots were stable several hours.

The chlormequat is revealed as a red spot which is examined to a wave length of 510 nm with Linomat apparatus.

Chlormequat determination by high performance liquid chromatography

Column ZORBAX SCX granulometry 5 µm, length 25 cm × 4.6 mm

Mobile phase: 90 % (oxalic acid 0.22 g and ethylenediamine 0.075 g in 100 ml of water), 10 % of acetone.

Room temperature.

Injection volume: 50 microlitres.

Pump Merck L6200, flow rate: 1 ml/minute.

Detector conductimetric Waters 431 Range 500 microS sensitivity 0.005.

Standard solution: 10 mg/litre in methanol.

Results and discussion

Chlormequat determination by high performance thin-layer chromatography

The routine limit of quantification (LOQ) is 0.5 mg/kg for pear. This limit was calculated from the peak areas by use the equation:

$$LOQ = 10 \times N/B$$

where N, is the standard deviation of the peak areas (6 spotting) and B is the slope of corresponding calibration curve.

Figure 1 shows two chromatograms obtained through this method.

The obtained chromatograms show a very separated chlormequat peak from other peaks of the samples.

This method used for the preparation of pear samples by the tetraphenylborate gives correct purified extracts for this type of analysis.

Chlormequat determination by high performance liquid chromatography

The routine limit of of quantification (LOQ) calculated in the same way as in method "a" is 0.1 mg/kg. The obtained chromatograms show a very separed chlormequat peak from other peaks of the samples (Fig. 2).

The method used for the preparation of pear samples by the sodium tetraphenylborate gives enough purified extract for this type of analysis. It appears like a good alternative method for purifying extracts containing chlormequat. The simplicity and the speed allow to proceed to an important number of determinations in a short time.

Recovery studies

Samples were fortified with working standard solution. Average recoveries of triplicate analysis are presented in table I.

Table I. Recoveries of chlormequat.

Sample	*spike (ppm)*	*HPTLC meth.a*	*HPLC meth.b*
a	0.5	78 (13)	86 (8)
b	1.5	87 (11)	92 (15)
c	3.0	85 (12)	91 (7)
d	6.0	86 (12)	90 (10)

Values in parentheses are coefficients of variation (%).

At present, there is no normalized method for the research of chlormequat and we have compared the two quantification methods we used.

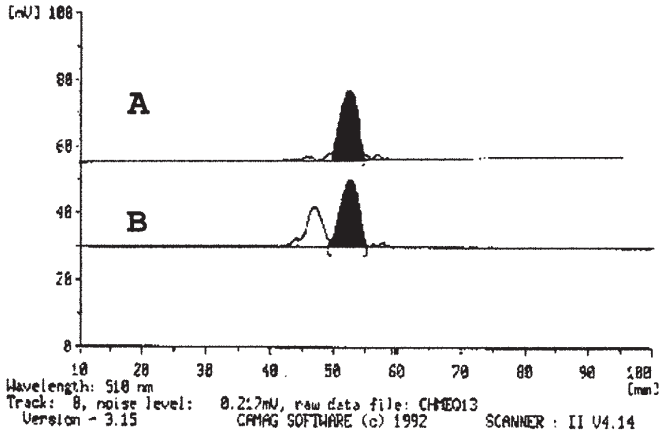

Figure 1. HPTLC of standard of 5.5 mg/kg Chlormequat (a) ; pear sample incurred with 3 mg/kg (b).

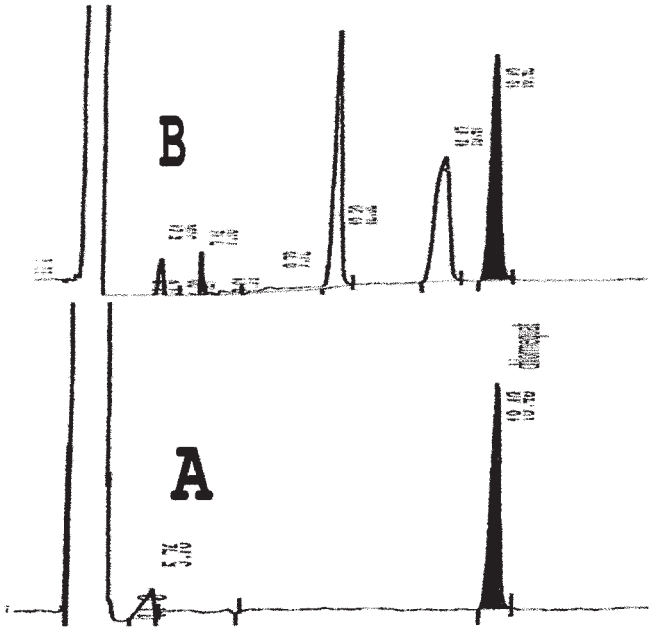

Figure 2. HPLC of standard of 5.5 mg/kg Chlormequat (a); pear sample incurred with 3 mg/kg (b).

We have compared the two methods of analysis by the matched pair Student test [13]. The data are obtained on 30 samples of naturally contaminated pears, measured by the HPTLC method and the HPLC method. The data are considered as paired data. The comparison of the two analytical methods used for the determination of chlormequat is made by the test of equality of the means proposed by P. Dagnelie [13]. We have calculated the observed "T".

$$T(obs) = \frac{|\bar{d}|}{\sqrt{\frac{\Sigma di^2 - 1/n\left(\Sigma di\right)^2}{n\left(n-1\right)}}}$$

d_i = difference between two pairs measurements for the same observation *i*.

For 30 pairs of analysis the observed T is 0.802. For a risk of 5 %, the Student distribution table gives a value of T29,0.975 = 2.045. T(obs) is inferior to 2.045. So, the difference of the means is not significant.Therefore it is certain that the two methods give identical values with a risk of 5 %.

Conclusion

Search for pesticides and particularly of those containing a polar group in their formula like quarternary amine is generally difficult. The main reasons are the low quantity of residues present in more or less complex matrix. However, in the case of chlormequat, no literature method recorded gave us any full satisfaction. On general way the indispensable purifications after extraction induce a loss of time and cause losses of product which give back their hazardous evaluation.

The HPLC method which deals with a higher sensibility does not bring a meaningful improvement. It appears that the results of chlormequat analysis through these two methods are quite comparable. So the extraction method proposed here, as well as the quantification by HPTLC, seem to be a very interesting solution: speed of analysis with an appropriate sensibility and a less important invesment**.** Therefore this procedure may be useful for monitoring residual Chlormequat in pears.

References

1. Petrosini, G.; Busilli, M.; Tafuri, F. *Analyst* **1969**, *94*, 674-677.
2. Mooney, R.P.; Pasarela, N.R. *J. Agric. Food. Chem.* **1970**, *15*, 869-871.
3. Stijve, T. *Deutsche Lebensmittel Rundschau* **1980**, *76*, 234-237.
4. Tafuri, F.; Businelli, M.; Giusquiani, P.L. *Analyst* **1970**, *95*, 675-679.
5. Allender, W.J. *Pest. Sci.* **1992**, *35*, 265-269.
6. Mortimer, R.D.; Weber, D.F. *Pestic. Sci.* **1994**, *40*, 31-38.
7. Assoc. Off. Anal. Chem. **1990**, 992-17, 15e edition.
8. Worobey, B.L. *Pest. Sci.* **1987**, *18*, 245-257.
9. Carneiro, M.C.; Puignou, L.; Galceran, M.T. *J. of Chromatogr. A* **1994**, *669*, 217-224.
10. Juhler, R.K.; Vahl, M. *J. Assoc. Off. Anal. Chem.* **1999**, *82*, 331-336.
11. Castro, R.; Moyano, E.; Galceran, M.T. *J. Chromatogr. A* **1999**, *830*, 145-154.
12. Munier, R.; Machebœuf, M. *Bull. Soc. Chim. Biol.* **1951**, *33*, 846- 849.
13. Dagnelie, P. *Théorie et méthodes statistiques;* Presse agronomique de Gembloux, 1975.