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Simple ion chromatographic method for the determination of chlormequat residues in pears

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Abstract

Current methods for quantitative determination of chlormequat residues in food crops are characterized by rather low recoveries and the need for derivatization (in case of gas chromatography, GC), or by high capital investment (in case of liquid chromatography–mass spectrometry, LC–MS). We propose a cation-exchange chromatography method for the analysis of chlormequat in pears. The method is based on extraction of the target compound with 40 m*M* HCl, followed by centrifugation and filtration. The filtrate is directly injected into an ion chromatograph equipped with a commercially available cation-exchange column and a suppressed conductivity detection system. While the limit of detection (LOD) (0.5 mg/kg) may not be small enough to allow dietary analysis, the method meets all validation requirements and is an alternative for the existing GC and LC–MS methods in quality control.

Keywords: Food analysis; Fruits; Chlormequat; Pesticides; Quaternary ammonium compounds

1. Introduction

(2-Chloroethyl)trimethylammonium chloride or chlormequat is a plant growth regulator or dwarfing agent used in several food crops, especially cereal grains, grapes and pears. Acute oral LD_{50} for rats is 883 mg/kg [1]. As the effect of this substance on human health is not sufficiently documented [2], maximal residue levels (MRLs) have been established. MRLs in Belgium are 2–5 mg/kg in cereal grains, 1 mg/kg in grapes and 3 mg/kg in pears [3]. Different analytical methods for chlormequat residues in crops have been reported. Pasarela and Orloski described the spectrophotometric measurement of a yellow dipicrylamine–chlormequat complex [4]. Gas chromatographic (GC) analysis methods are based on derivatization with benzenethiolate [5] or pentafluorothiophenolate [6], or on conversion to acetylene by heating with alkali [7]. As GC is not very suitable for analysis of quaternary ammonium compounds, liquid chromatographic (LC) methods

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have been proposed. Recently, a method consisting of a single clean-up using a solid-phase extraction (SPE) C_{18} cartridge, chromatographic separation by a standard C_{18} high-performance liquid chromatography (HPLC) column and quantification by coupling of the LC to a mass spectrometer was proposed for the analysis of chlormequat in grain [8]. However, the high capital investment results in a high cost per analysis, which is not favorable for routine quality control.

Since ion chromatography (IC) was introduced in 1975, great progress has been made in sensitivity as well as in detection selectivity [9]. The suppression systems now can very well differentiate between the conductometric signals of analyte and eluent. A laborious analytical method for determination of the quaternary ammonium compound mepiquat chloride in animal and plant matrices was published in 1991 [10]. Here we propose a new IC method for chlormequat residue analysis. Our method requires minimal sample preparation, and provides a well resolved analyte peak without any interferences. The method is suitable for routine quality control.

2. Experimental

2.1. Ion chromatography instrumentation

Analyses were performed with a Dionex DX120 ion chromatograph equipped with a high-pressure pump, an ED40 conductometric detector and a Rheodyne injection valve (25-μl sample loop). For separation, an IonPac CG12A guard column (50×4 mm) was coupled to an IonPac CS12A analytical column (250×4 mm). For suppression, a Dionex CSRS ultra self-regenerating suppressor was employed. Peaknet 5.0 software was used for system control and data acquisition. All IC related equipment was supplied by Dionex (Mechelen, Belgium).

2.2. Chemicals

All chemicals were supplied by Sigma–Aldrich (Bornem, Belgium), and were of analytical-reagent grade unless specified otherwise. HCl was used to prepare the extraction solution. H_2SO_4, HCl and acetonitrile (analytical-reagent grade for HPLC) were used to prepare the eluents. Tetrabutylammonium hydroxide was used as chemical regenerant. Chlormequat was used to prepare standard solutions. The eluents were prepared with ultrapure water obtained by Milli-Q-plus filtration (Millipore); all other solutions were made with deionized water.

2.3. Analytical methods

2.3.1. Sample preparation and spiking

Pear samples (Conférence, Beurré Hardy) were randomly collected at auctioneers and retail trade. Pear samples free of chlormequat were obtained from private growers. Pears without stem and crown were mixed in a Moulinette blender (Moulinex). Samples (12.5 g) were extracted with 15 ml 40 m*M* HCl in 50-ml centrifuge tubes (Falcon) for 1 min using an Ultra-Turrax at maximum speed (Janke and Kunkel). The homogenized samples were centrifuged at 12 520 *g* (Jouan-BR4I) for 5 min. The supernatant was decanted and vacuum filtered (Ederol, quality 12). The residue was washed with 15 ml 40 m*M* HCl and homogenized in a Vortex (Scientific Industries). Following centrifugation, the supernatant was again removed by vacuum filtration. The total volume of the combined filtrates was adjusted to 50 ml with 40 m*M* HCl. Spiked samples were used in recovery experiments. Known amounts of chlormequat equivalent to 2.0, 4.0 and 8.0 mg/kg (i.e., 65, 130 and 260% of the tolerance level) were added to pear samples free of chlormequat in six replications. These samples then were subjected to the same extraction and separation processes.

Contaminated samples at approximately 65, 110 and 175% of the tolerance level were analyzed in sixfold to determine the precision. The different levels of contamination were obtained by mixing mash of contaminated pears with that of non-contaminated pears.

2.3.2. Ion chromatography

All samples were passed through 0.22-μm Acrodisc LCPVDF filters (Gelman) before injection. The injection volume was 25 μl. The mobile phase consisted of 20 m*M* H_2SO_4 in 4.0% acetonitrile. The flow-rate was 1.0 ml/min and total analysis time was 12 min. 100 m*M* tetrabutylammonium hydroxide

was used as regenerant. Quantification was done by area measurement (Peaknet 5.0).

2.3.3. Calibration curve

Calibration curves were generated by plotting peak areas against the concentration of the standards injected. Six chlormequat standards between 0.2 and 2.0 mg/l (equivalent to 0.8 and 8.0 mg/kg, respectively, for the pear sample) in 40 m*M* HCl were prepared in triplicate with a 3-month time interval.

2.3.4. Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were defined as the amount, expressed in ppm, equivalent to three and 10 times, respectively, the background noise contributed by the matrix blank [11]. The values were obtained by four repeated analyses.

2.3.5. Mass spectrometry

Mass spectra were recorded with a Finnigan LCQ-Deca with direct infusion of the samples into the electronspray ionization probehead. Instrument parameters were automatically tuned to maximize the intensity of the m/z 122.1 ion in the positive ion mode.

3. Results and discussion

Different eluents were tested to obtain well-resolved chlormequat signals in a pear matrix. With 40 m*M* HCl, chlormequat eluted after 5.0 min. Decreasing the HCl concentration to 30 m*M* HCl resulted in an increase of the retention time with 1.0 min, and in the appearance of a shoulder on the peak, which indicates that the separation was not complete. 20 m*M* H_2SO_4 provided complete separation with elution of chlormequat at 8.0 min. Decreasing the H_2SO_4 concentration to 15 m*M* resulted in an increase of the retention time with 1.5 min. No additional peaks or shoulders appear in the chromatogram close to the chlormequat peak. Chromatograms of blank pear matrix (Conférence), pear samples spiked with 3.0 mg/kg chlormequat and contaminated with 7.0 mg/kg are shown in Fig. 1. Similar chromatograms were obtained for Beurré Hardy. Some minor peaks appear in the chromatograms before and after the chlormequat peak, even in samples free of pesticides, but these peaks do not disturb the chlormequat analysis. Choline chloride, which is a chemically closely related compound, elutes already after 5.0 min when analyzed under the same experimental conditions and does not interfere with the analysis of chlormequat.

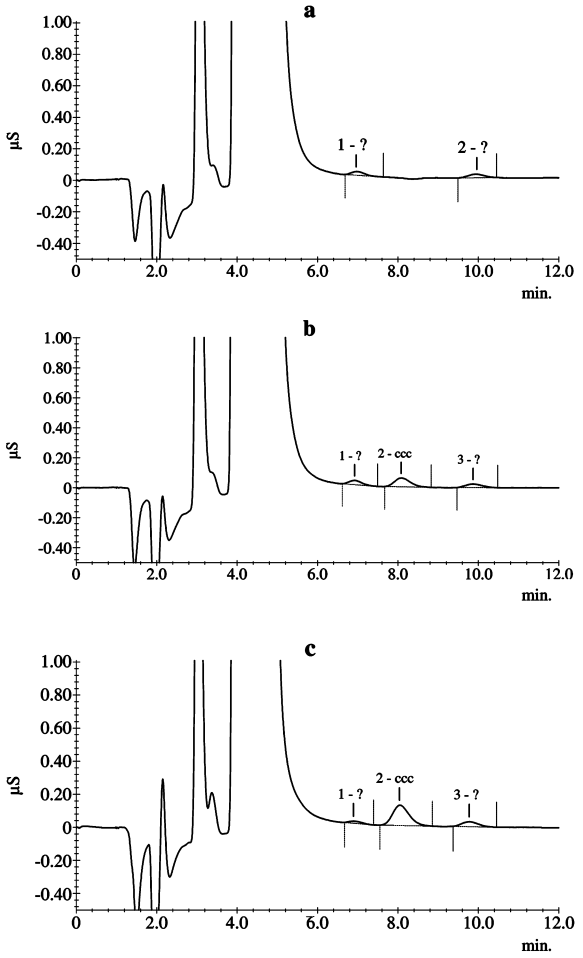

Fig. 1. IC determination of chlormequat (ccc) in blank pear matrix (a), pear sample fortified with chlormequat (3 mg/kg) (b) and pear sample contaminated with chlormequat (7 mg/kg) (c). Eluent: 20 m*M* H_2SO_4 in 4% acetonitrile, 1.0 ml/min; detection, conductivity (µS); columns, IonPac CG12A and CS12A.

The alkali and alkaline earth metal ions present in pears are not removed by the extraction. But these cations elute much earlier than chlormequat and therefore purification of the extract before injection, as is the case for other methods determining chlor-

mequat residues [4–8,10] is unnecessary. To maintain low background conductivity and constant sensitivity, the flow-rate of the chemical regenerant must be at least four times the eluent flow-rate; regular column and suppressor clean-up as described by the manufacturer are recommended.

A six-point chlormequat calibration curve in the range corresponding to 0–2 mg/l was used to quantify the correlation coefficient (R^2) of the linear regression. Values of R^2 were 0.9986, 0.9988 and 0.9946 at monthly time intervals.

To determine the recovery of chlormequat, samples fortified at three levels were analyzed in six replicates. An average recovery of 94% was obtained (Table 1). By GC analysis (after pyrolysis to acetylene) and colorimetric measurement of chlormequat dipicrylamine, recovery in pears was 79% and 87%, respectively [4,7]. With LC–MS–MS, chlormequat recovery in grain was 91% [8] and with GC–MS analysis (after formation of a pentafluorothiophenyl derivate), recovery in cotton seeds was 81% [6]. Most previous studies have routinely used methanol for extraction of chlormequat in plant samples [4–8,10] although chlormequat is characterized by a water solubility of 100% at 20°C [1]. The present excellent recovery results demonstrate that 40 m*M* HCl is indeed a good solvent for chlormequat extraction in pears.

Pear samples with 65, 110 and 175% of the tolerated chlormequat concentration (i.e., 2.0, 3.2 and 5.3 mg/kg) were analyzed in order to determine the precision of this method. The repeatability of the analysis expressed as the relative standard deviation was in the range of 6 to 11% (Table 2). With LC–MS–MS analysis, relative standard deviation in grain samples fortified with chlormequat was 11% [8]; with GC–MS analysis of derivated samples, standard deviation for 81% recovery in cotton seeds was 5.4 [6].

Table 1
Recovery of chlormequat in fortified pear samples

Fortification level (mg/kg)	Number of samples	Recovery (%)	Standard deviation
2	6	92.30	10.24
4	6	93.84	12.72
8	6	95.92	4.66

Table 2
Precision of chlormequat analysis in pears

Concentration (mg/kg)	Number of samples	Standard deviation
1.98	6	0.12
3.23	6	0.36
5.26	6	0.35

The instrumental detection limit for chlormequat was 0.125 mg/l. LOD and LOQ for the chlormequat analysis in pears were 0.50 mg/kg and 1.7 mg/kg, respectively. Better sensitivity for chlormequat was obtained by GC, colorimetric and LC–MS–MS methods [5–8,10]. The LOD for mepiquat chloride in plant matrices by ion chromatography with conductivity detection was 0.05 mg/kg, but this method requires extensive sample preparation and concentration [10]. Considering the present tolerance level, the here obtained sensitivity is sufficient for quality control.

The chromatograms of reagent blanks and sample matrix blanks of Conférence and Beurré Hardy pears were free of interfering peaks at the retention time of chlormequat suggesting the specificity of the method. The specificity was further confirmed by mass spectrometry (Fig. 2). Injection of a pear sample into an electron spray mass spectrometer in the positive mode allowed to directly observe the 122.1 and 124.1 m/z peaks of the chlormequat cation, in the characteristic Cl isotope ratio of 3 to 1. This supplementary analysis irrefutably proves the presence of chlormequat in the analyzed sample, and additionally validates the ion chromatographic analysis method.

4. Conclusion

A simple, fast and economical method for chlormequat residue analysis in pears has been developed. It involves extraction with 40 m*M* HCl, filtration and ion chromatographic analysis without any further clean-up.

The validation data demonstrate that this method

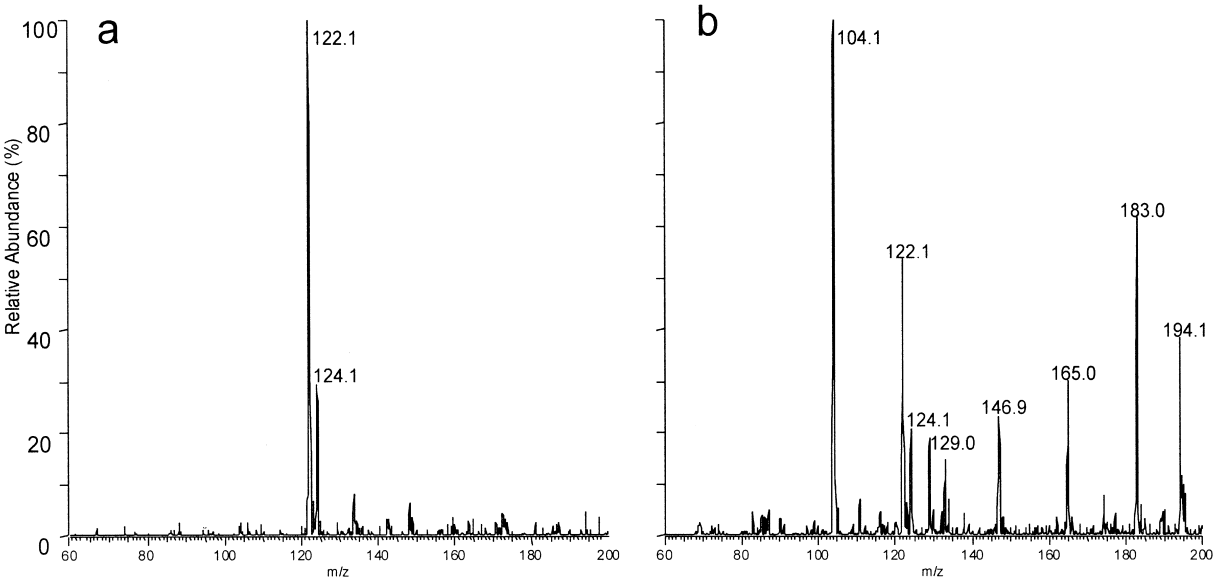

Fig. 2. ESI mass spectra of (a) 7 mg/l chlormequat in deionized water, (b) chlormequat in pear matrix. *y*-Units are arbitrary.

is convenient for routine analysis of chlormequat residues in pears.

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References

[1] C.R. Worthing, The Pesticide Manual, A World Compendium, British Crop Protection Council, 1979.
[2] International Chemical Safety Cards: 0781. International Programme on Chemical Safety, Commission of the European Community, 1993.
[3] Koninklijk Besluit tot vaststelling van de maximumgehalten aan residuen van bestrijdingsmiddelen toegelaten in en op voedingsmiddelen. Belgisch Staatsblad, 170(92), 2000.
[4] N.R. Pasarella, E.J. Orloski, in: G. Zweig (Ed.), Pesticides and Plant Growth Regulators, Academic Press, New York, 1974, p. 523, Chapter 31.
[5] F. Tafuri, M. Businelli, P.L. Giusquiani, Analyst 95 (1970) 675.
[6] W.J. Allender, Pestic. Sci. 35 (1992) 265.
[7] P.A. Greve, E.A. Hogendoorn, Med. Fac. Landbouw. Rijksuniv. Gent 52 (2b) (1987) 695.
[8] R.K. Juhler, M. Vahl, J. AOAC Int. 82 (2) (1999) 331.
[9] W.W. Buchberger, P.R. Haddad, J. Chromatogr. A 789 (1997) 67.
[10] A. Fegert, U. Schepers, B. Schwarz, Fresenius J. Anal. Chem. 339 (1991) 441.
[11] W.G. Fong, H.A. Moye, J.N. Seiber, J.P. Toth, Pesticides Residues in Foods, Chemical Analysis, Vol. 151, Wiley, 1999.