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Determination of the plant growth regulator chlormequat in food by liquid chromatography–electrospray ionisation tandem mass spectrometry

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Abstract

A confirmatory method for the determination of trace levels of chlormequat in a variety of different food matrices was developed. It entails a single clean-up step over a solid-phase cation exchange resin and subsequent liquid chromatography–electrospray ionisation tandem mass spectrometry using a stable isotopically labelled internal standard. Mass spectral acquisition was done in selected reaction monitoring mode, selecting the transitions from both the ^{35}Cl and the ^{37}Cl isotope of chlormequat. Recoveries after extraction and clean-up, determined with radio-labelled chlormequat and averaged over the spiking range (16–65 $\mu g\,kg^{-1}$) in four different commodities, were within 88–96%, with a coefficient of variation better than 8%. The method can be applied to pears, pear juice concentrates, fruit purées, and cereal products, with typical limits of detection for chlormequat estimated at 2–5 $\mu g\,kg^{-1}$. A survey of different food commodities revealed that chlormequat was detectable — albeit at very low levels — in many of the food samples analysed, with the highest concentration recorded in pears purchased in Switzerland and of South African origin (5.5 $mg\,kg^{-1}$). Measurements were also conducted on two LC–MS instruments and demonstrate the versatility and robustness of the method and its applicability to instruments of different ion source design. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Food analysis; Chlormequat; Pesticides

1. Introduction

Chlormequat chloride [(2-chloroethyl) trimethylammonium chloride, also known as chlorocholine chloride, Cycocel, and often abbreviated as CCC] is a plant growth regulator and is used to reduce the risk of lodging and increase yields of wheat, rye, oats and barley. It also promotes flower formation, improves fruit setting in pears, almonds, vines, olives and tomatoes, and prevents premature fruit drop of pears, apricots and plums [1]. Chlormequat is employed extensively in agriculture, primarily in food grains and on pears. Data published by national pesticide surveys in Europe clearly reflect the intense usage of this compound, particularly in wheat and rye [2]. In the case of pears, residues may be found at significant levels even if treatment is carried out under Good Agricultural Practice. Supervised trials with pears in The Netherlands led to residue levels ranging from 3.5 to 8 $mg\,kg^{-1}$ [3], whereas the

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present *Codex Alimentarius* Maximum Residue Limit (MRL) for pears is set at 3 mg kg^{-1} [4].

Chlormequat is a strongly polar, cationic molecule and lacks any chromophore, making its detection by conventional analytical techniques rather cumbersome. Earlier methods for the determination of chlormequat include thin-layer chromatography [5], headspace gas chromatography [6], gas chromatography after derivatisation with sodium benzenethiolate [7] and colorimetry [8]. The disadvantages of these methods have been addressed in recent publications [9,10], the major criticisms being the lack of specificity and the presence of other quaternary ammonium compounds that may interfere in the analysis and thus lead to false-positive results. Furthermore, in the case of gas chromatographic methods, the efficacy of the derivatisation step in a complex food matrix is difficult to assess.

Due to the polar and non-volatile nature of the molecule, separation by liquid chromatography (LC) or capillary electrophoresis and detection by mass spectrometry (MS) are the most promising strategies for confirmatory analysis and quantitation at low $\mu g\ kg^{-1}$ levels. The use of capillary electrophoresis coupled to mass spectrometry for the separation and detection of quaternary ammonium herbicides has been described, but analyses were performed either at high concentrations in formulation products [11] or only in water [12].

So far, two LC–MS methods using electrospray ionisation (ESI) have been published to determine chlormequat residues at trace levels in grain [9] and pears [10]. The latter method employs concertedly selected ion monitoring (SIM) and selected reaction monitoring (SRM) techniques for quantification, but without the use of a stable isotopically labelled internal standard. A ^{13}C-enriched internal standard was introduced in the former method but suffered from the apparent interference of matrix components and an impure internal standard.

The aim of the present study was to develop a confirmatory, quantitative method for the analysis of chlormequat that can achieve low $\mu g\ kg^{-1}$ detection levels in a variety of food commodities (pears, pear purée, pear juice concentrate, and cereals). Radiolabelled chlormequat chloride was used to optimise the clean-up step, and an isotope labelled internal standard was used for quantitative determination. Furthermore, identical samples were analysed on two different LC–MS instruments allowing us to assess the robustness and versatility of the method and to compare the performance of two different LC–MS interface designs.

2. Experimental

2.1. Materials and reagents

All solvents were of analytical grade and were purchased from Merck (Dietikon, Switzerland). Water was either purified in-house using a Millipore Milli-Q water purification system (Millipore, Volketswil, Switzerland) or was HPLC-grade (Merck). Ammonium acetate was from Merck; the scintillation fluid was Ultima Gold from Packard Bioscience (Groningen, The Netherlands).

[Methyl-^{14}C]-chlormequat chloride was custom synthesised by Moravek Biochemicals (Brea, CA, USA) with a radiochemical purity >99.9% and a specific activity of 30 mCi $mmol^{-1}$. Non-labelled chlormequat chloride was purchased from Dr. Ehrensdorfer (Augsburg, Germany). d_9-Chlormequat chloride, isotopic purity >98%, was custom synthesized by Deutero (Kastellaun, Germany). Stock solutions of chlormequat were prepared at 0.1 mg ml^{-1} in water and stored at +4°C if not used; they were found to be stable over a period of at least 3 months. Working standards were prepared fresh with every batch of analyses by adequate dilution of the stock solution in water. Pears were purchased in various groceries in Switzerland and in France. Pear and apple–pear purées were of different brands and, according to the declaration on the labels, contained 92–95% fruit. Ready-to-eat cereals and dried fruit of various brands were purchased off-the-shelf. Samples of pear juice concentrates with incurred amounts of chlormequat were obtained from Nestlé Germany.

2.2. Sample extraction and clean-up

2.2.1. Cereals, pear juice concentrates, and fruit purées

If required, cereals were ground in a laboratory mill (Model 3303, Perten Instr., Hamburg, Germany) to achieve a particle size of 0.5–1 mm. A test portion

(10 g) of the sample material was weighed in a 150-ml screw-capped bottle and a fixed amount of d_9-chlormequat chloride in water was added to achieve a final concentration of the cation of 31 μg kg^{-1}. The sample material was suspended in approximately 75 ml of a methanol–water mix (1:1, v/v) and stirred with a magnetic stirrer for 15 min at ambient temperature.

2.2.2. Pears

Unpeeled pears (three to six pears, suspected homogeneous lot, approx. 500 g) were cut into slices and homogenised for ~10 s at room temperature in a Buechi 440 laboratory mixer (Buechi, Flawil, Switzerland). An aliquot of the sample (10 g) was taken and prepared as described above.

The suspensions were made up to 100 ml with methanol–water (1:1, v/v). An aliquot of 10 ml, equivalent to 1 g of sample, was applied to solid-phase extraction (SPE) cartridges (LiChrolut SCX, 500 mg, Merck) that were positioned on a Supelco Visiprep vacuum manifold (Supelco, Buchs, Switzerland) and preconditioned with consecutively two bed volumes each of methanol, water, and 10 mmol l^{-1} hydrochloric acid. If necessary, particularly with solid food samples, the 10 ml aliquot was transferred to conical polypropylene tubes (15 ml) and centrifuged at 3600 *g* for 5 min in a benchtop centrifuge (MSE Scientific Instruments, Leicestershire, UK). The clear supernatant was charged onto the column as described above. After penetration of the extract (flow-rate ~0.5 ml min^{-1}), the column was rinsed with 4 ml of each methanol and acetonitrile. The analyte was then eluted with 5 ml of a 1:1 mixture (v/v) of methanol and 0.25 mol l^{-1} aqueous ammonium acetate (pH 6.8), prepared fresh before use. Care was taken that the columns did not run dry during the clean-up procedure. Methanol was removed in vacuo at 35°C, and the samples were lyophilised overnight. The residues were redissolved in methanol (0.5 ml) and stored at −20°C until analysed by LC–MS–MS as described below.

2.3. Determination of extraction recovery using radio-labelled standard

A solution of ^{14}C-chlormequat chloride in ethanol (51 μCi ml^{-1}) was diluted 1:100 in water, and samples were fortified with the radio-labelled analyte (40 μl, 5.27 ng μl^{-1}) to achieve a final spiking level of 16.3 μg chlormequat cation per kg. Extraction was then performed as described above. Typically, the volume of each column effluent was determined and an aliquot (500 μl) analysed in a 10-ml liquid scintillation cocktail to determine recovery. Radioactivity was measured with a 1219 Rackbeta liquid scintillation counter (LKB-Wallac, Turku, Finland).

2.4. Liquid chromatography and mass spectrometry

Two different triple quadrupole mass spectrometers were used in this study, a Finnigan TSQ 700 (Finnigan, San Jose, CA, USA), and a Micromass Quattro-LC (Micromass, Manchester, UK). On both instruments, LC separations were performed under almost identical conditions by ion exchange chromatography on a Spherisorb SCX column (150×2 mm with guard column 30×2 mm; Bischoff, Leonberg, Germany), injecting 10 μl of sample. All runs were performed under isocratic conditions at a flow-rate of 0.3 ml min^{-1} and 0.25 ml min^{-1} for the TSQ 700 and Quattro-LC, respectively, using methanol–water 1:1 (v/v) containing a final concentration of 50 mmol l^{-1} ammonium acetate (pH 6.8, not adjusted). The column temperature was set to 35°C, and the LC flow was introduced into the ion source of the MS without a split.

2.4.1. TSQ 700

The TSQ 700 was equipped with a Finnigan ''ESI II'' electrospray ion source. The MS was coupled to a Waters (Rupperswil, Switzerland) LC system, consisting of a type 757 autosampler, a 600-MS pump with system controller and column oven and a type 486-MS UV detector. A column-switching valve (Valco ECM5625, from a Finnigan TSP-II Thermospray interface) was used after the LC column and the flow directed to waste during the initial phase of the run prior to elution of the analyte. MS conditions were as follows: electrospray voltage, 4.2 kV; transfer capillary temperature, 200°C; sheath gas (nitrogen), 0.52 MPa; auxiliary gas, 20 ''units''. The capillary and lens voltages were set to 3.9 and 41.4 V for chlormequat and to 21.0 V and 58.9 V for the isotope-labelled standard, respectively. MS–MS data were acquired at a collision energy of −35 eV in the

laboratory framework using argon at a pressure of 4 hPa (3.0 mTorr) as the collision gas. Quantitation data were acquired in neutral loss mode using a cycle time of 0.50 s and a tolerance of ±0.5 mass units. For non-labelled chlormequat, the transitions *m/z* 122→58 and *m/z* 124→58 were observed; for the deuterated analogue, the transitions *m/z* 131→66 and *m/z* 133→66 were observed. Data acquisition was performed on a DECstation 5100 running under Ultrix 4.4 (Digital Equipment, Maynard, MA, USA) using the Finnigan software package ICIS2, Ver. 8.3.0 SP1.

2.4.2. Quattro-LC

The Quattro-LC mass spectrometer, equipped with a ''Z-Spray'' electrospray ion source, was coupled to a Waters 2690 ''Alliance'' separation module. The chromatographic conditions were described above. Instrument control and data processing were performed using MassLynx NT software, Ver. 3.3 (Micromass). The needle voltage was typically set to +2.77 kV, the cone voltage to 39 V, and the RF lens to 0.12 V. Source block and desolvation temperatures were set at 150°C and 400°C; nebuliser and desolvation gas flows to 97 and 620 l N_2 h^{-1}, respectively. The ion energy of the first and second quadrupole was 0.6 V and 1.0 V. Chlormequat was detected using the same SRM channels as described above, except that a third transition using *m/z* 122→63 was observed, thus giving added confidence to analyte identification. All data were acquired at a collision energy of −30 eV except for the transition *m/z* 122→63 whose optimum was −20 eV. Argon was used as collision gas at a pressure of 2.5 hPa (1.9 mTorr). The dwell time was set to 1 s.

2.5. Quantitation

Calibration curves were established using spiked matrix standards. Concentrations between 3.9 and 780 μg kg^{-1} of non-labelled chlormequat were used together with a fixed amount of d_9-chlormequat as internal standard (31 μg kg^{-1}). Each sample was prepared in duplicate, and each injected at least three times in an arbitrary order during a series of analyses, which included samples, standards and blanks. Quantitation data were obtained using the transition *m/z* 122→58 for chlormequat and *m/z* 131→66 for the internal standard. All quantitative calculations were done using robust statistics [13].

3. Results and discussion

3.1. Sample extraction and clean-up

A major challenge in this study was to develop a common clean-up method for the food commodities of interest (pears, pear juice concentrates, pear purées, and cereals). Thus, radio-isotope dilution, which allows determination of the absolute recovery of the ^{14}C-labelled tracer, was employed to screen a number of disposable SPE cartridges. Surprisingly, the only resin that performed consistently well with all food matrices was LiChrolut SCX. Elution of the analyte was found best with a final concentration of 0.125 mol l^{-1} ammonium acetate in the solvent mixture.

Food samples without detectable levels of chlormequat, as confirmed by techniques described in [14], were chosen to determine recovery. However, in the case of pear juice concentrates, no chlormequat-''free'' samples were available, so recovery experiments were done on the sample with the lowest chlormequat levels (~0.1 mg kg^{-1}). As illustrated in Table 1, recoveries of chlormequat in all four food commodities and at three spiking levels were above 80%, with a coefficient of variation (C.V.) better than 10%. When averaged over the whole spiking range for each food matrix, recoveries are within the range 88–96%, and the C.V. is better than 8%. These results demonstrate that this SPE

Table 1
Recoveries±C.V. (%) of ^{14}C-chlormequat in various foods after sample extraction and solid-phase clean-up on LiChrolut SCX[a]

Food commodity	Spiking level of chlormequat (μg kg^{-1})		
	16.3	*32.6*	*65.2*
Pears	83±5.3	98±9.2	94±7.6
Pear juice concentrate[b]	92±7.9	88±5.5	84±5.0
Pear purée	95±9.6	91±4.5	103±3.5
Cereals	94±8.0	94±7.6	83±5.3

[a] Entries represent an average of three extractions, performed on different days and each with two independent determinations ($N=6$).

[b] Contains incurred residues (122 μg kg^{-1}).

procedure enables efficient extraction of chlormequat at the $\mu g\,kg^{-1}$ level in a number of different food matrices.

3.2. Mass spectrometry

3.2.1. Electrospray ionisation (ESI) and MS–MS of chlormequat

Under ESI conditions, chlormequat shows an abundant M^+ ion without fragmentation (Fig. 1). As tetraalkyl ammonium compounds are already charged in solution, they are easily amenable to ionisation directly from the liquid phase. The daughter ion spectrum obtained upon dissociation of the M^+ ion of chlormequat is shown in Fig. 2, both for the labelled and non-labelled compound. As reported previously [10,12], the main fragmentation of chlormequat in positive ESIMS is the loss of the chloroethyl moiety, leading to a product ion with m/z 58 (Fig. 3). With respect to the two most abundant chlorine isotopes, this corresponds to a loss of 64 and 66 amu, respectively [Fig. 2(a) and (b)]. Analogously, the fragmentation of d_9-chlormequat leads to a major fragment at m/z 66 by loss of 65 and 67 amu, respectively [Fig. 2(c) and (d)]. An interesting aspect is that the ratio of the ions with m/z 58 and 59 is inverted compared to m/z 66/68. This effect depends on the collision energy; however, we did not perform a detailed study of this phenomenon.

For quantitative work, all four transitions from both isotope peaks of the M^+ ion were recorded in the SRM mode. The area ratio of the ^{35}Cl and ^{37}Cl isotope peaks, which should be approximately 3:1, was additionally used to verify the presence of the analyte.

3.3. Selected reaction monitoring (SRM) vs. single ion monitoring (SIM)

Fig. 4 shows a comparison of chromatograms recorded on the TSQ 700 obtained using SRM and SIM on the same sample (pear purée). Although the absolute signal intensity in the SRM run is lower, the signal-to-noise (S/N) ratio of the ^{35}Cl trace was found to be at least three times higher than using the SIM mode. The S/N ratio of the ^{35}Cl to the ^{37}Cl peaks follows this trend closely; we observed, for example, $S/N=18$ for the transition m/z 122→58, $S/N=7$ for m/z 124→58, and $S/N=6$ and $S/N=1$ for SIM of m/z 122 and m/z 124, respectively.

3.4. Separation by LC

Under the conditions given here, chlormequat elutes at approximately 11 min. The overall LC run time was set at 15 min, so that 96 injections could be made per day. Retention times were very stable and varied less than 0.1 min within one batch of solvent, although a distinct dependency on solvent composition (ammonium acetate concentration) was noted. The chromatograms are free from interference (Fig. 4, left; Fig. 5). The use of acetonitrile instead of methanol did not improve separation or peak shape.

The importance of sample preparation is apparent when data from the present study are compared to those reported by Startin et al. [10]. The authors

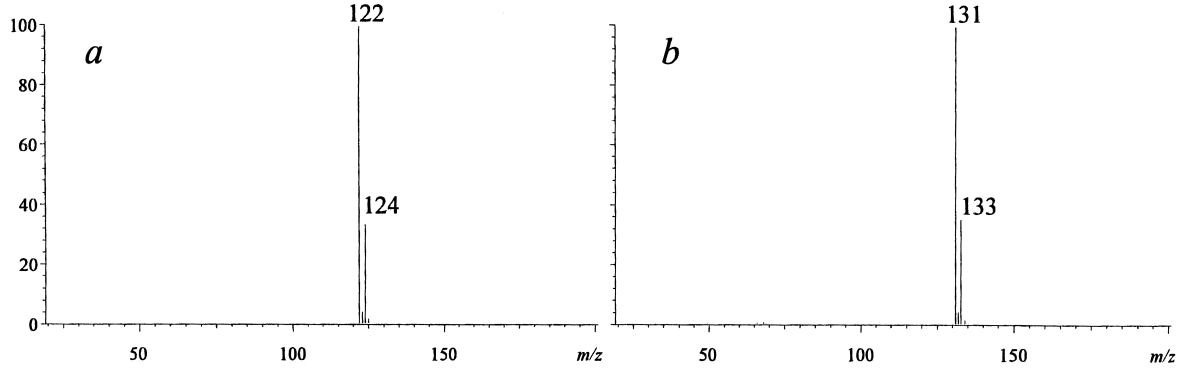

Fig. 1. ESIMS of non-labelled chlormequat (a) and its d_9-cognate (b).

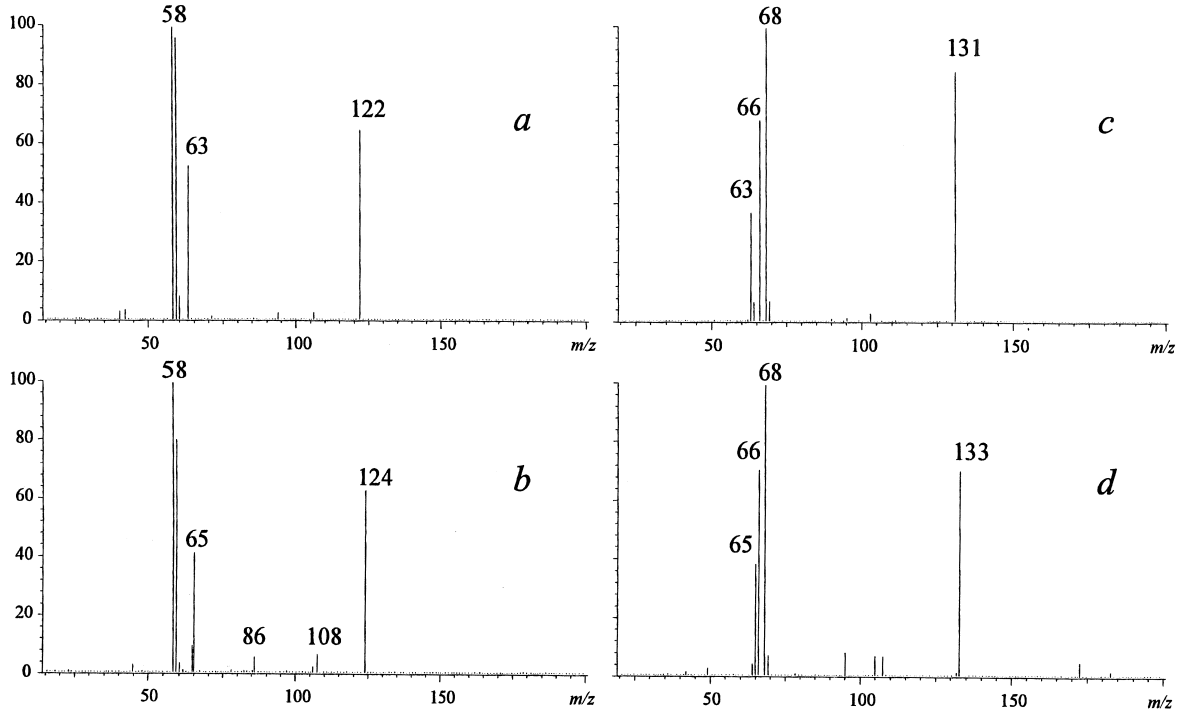

Fig. 2. ESIMS–MS daughter ion spectra of chlormequat, showing (a) the ^{35}Cl isotope peak and (b) the ^{37}Cl isotope peak of unlabelled chlormequat; (c) the ^{35}Cl isotope and (d) the ^{37}Cl isotope peak of d_9-chlormequat.

chose direct analysis of a methanol–water pear extract without a prior clean-up step, with the result that the chromatographic behaviour degraded proportionally to the increasing number of samples injected onto the column.

In contrast to this, using the conditions described herein, column performance did not deteriorate even after more than 1200 injections. We observed, however, that a fair amount of solid matter precipitated in the ion source region of both instruments, blocking the sampling orifices. A first approach to avoid blockage on the TSQ 700 was to use a home-made ''orthogonal flow'' device at the heated capillary, which consisted essentially of a small metal bar of 1-mm width that was fixed ca. 1 mm in front of the sampling orifice and blocked the line-of-sight pene-

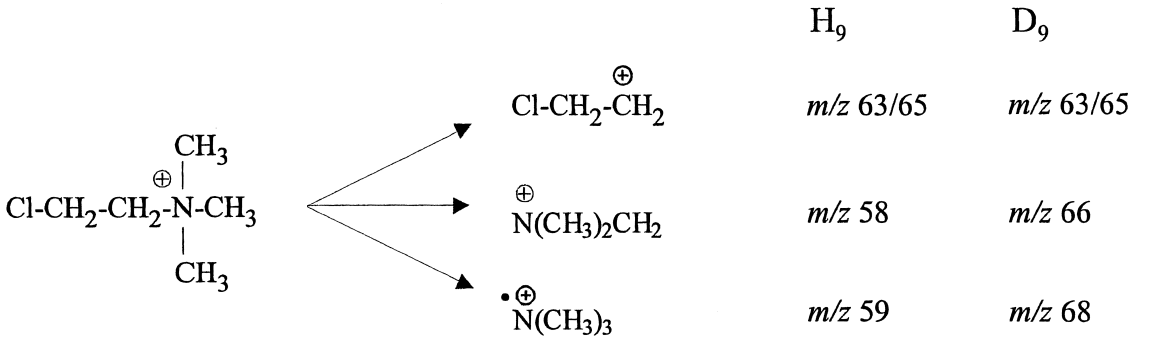

Fig. 3. Fragmentation of chlormequat (see also Fig. 2). Given are the m/z of the fragments for the non-labelled (left column) and the d_9-compound (right column).

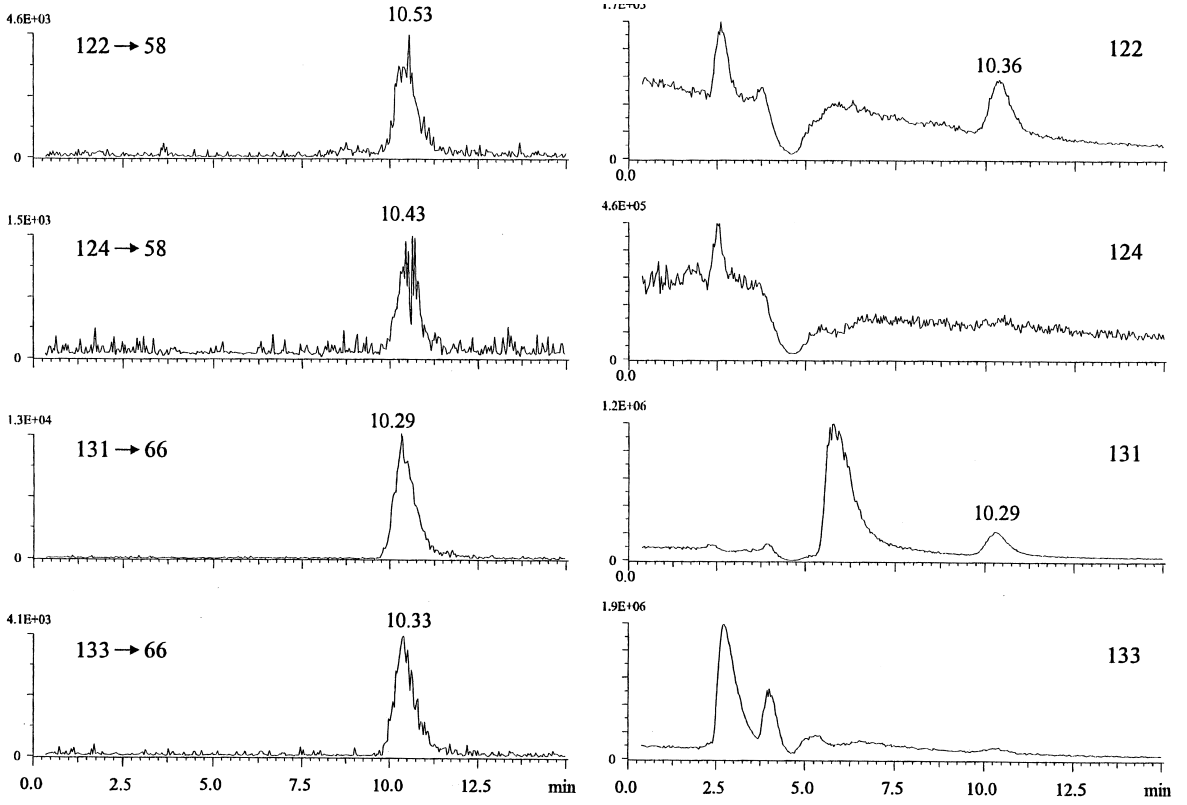

Fig. 4. LC–ESIMS–MS selected reaction monitoring chromatogram traces (left) and selected ion monitoring traces (right) of a pear purée sample spiked with 7.8 μg kg^{-1} chlormequat. Data were recorded on the TSQ 700 and are shown without smoothing.

tration of particles into the sampling system. Although this device prolonged the uptime of the instrument, the loss of sensitivity was too important for this study. Therefore, a column switching valve was used which was controlled by the mass spectrometer, so that the LC flow was directed to waste during the initial phase of a run and switched to the MS before the expected elution of the analyte peak. The device proved to operate reliably and allowed continuous trouble-free operation overnight and over weekends. Furthermore, the use of an LC column with 2-mm I.D. was fully compatible with the high matrix loads experienced in this study.

3.5. Calibration and method performance characteristics

All calibration curves were prepared in the same, or at least a very similar, matrix as the actual samples to compensate for matrix influences [15,16]. Linear calibration curves were obtained from the limit of detection (LOD) to 780 μg kg^{-1}, in all cases with coefficients of determination $r^2 > 0.999$. Calibrations were found to have slopes between 0.6 and 1.2, depending on the food matrix (data not shown). The goodness of fit (accuracy) of the measured values with the theoretical data was verified by plotting the measured deuterium enrichment against the calculated values as described in [17], achieving correlation coefficients of 0.998 or better, and slopes in the range 0.93–1.04. This is close to the theoretical value of 1.00 and indicates good agreement between measured and calculated values.

The variabilities within and between series were calculated from sets of results of triplicate (Quattro-LC) or five to eight replicate (TSQ 700) analyses of a sample with incurred residues (203 μg kg^{-1}), and are depicted in Table 2. The limit of detection

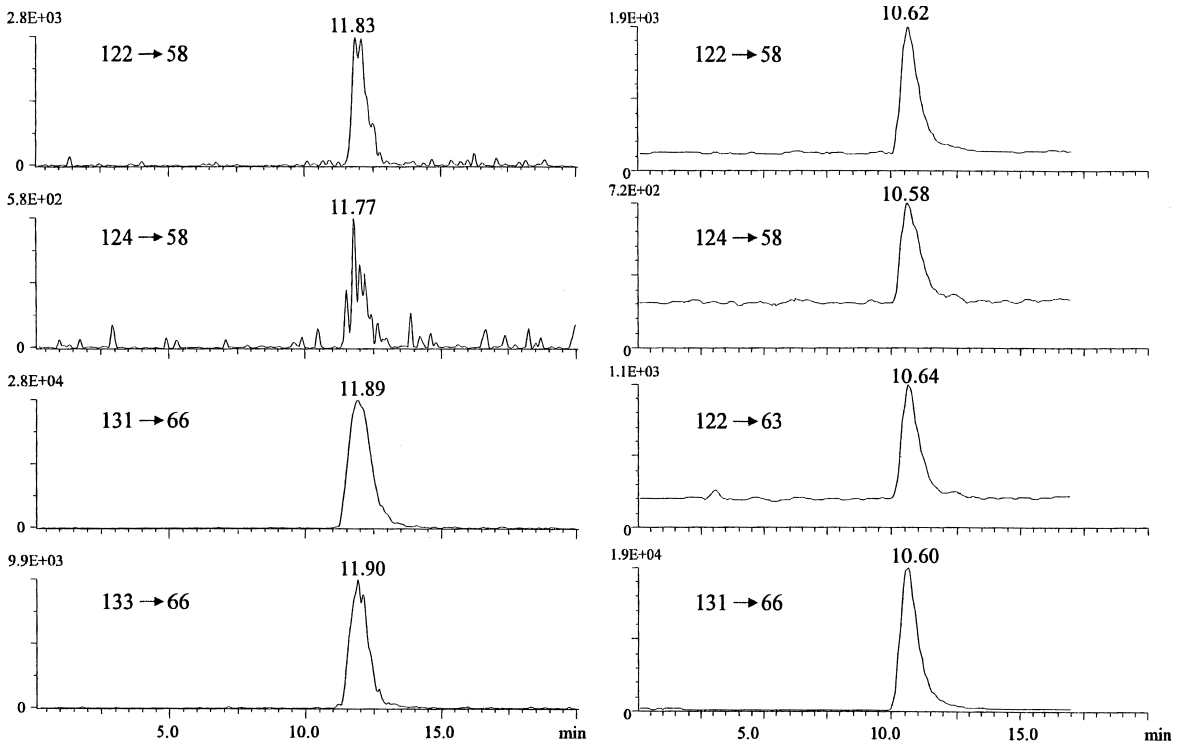

Fig. 5. LC–ESIMS–MS chromatograms of a naturally incurred cereal sample. Data were acquired using the TSQ 700 (left traces) and the Quattro-LC (right traces) and are shown with a five-point smooth. The amounts calculated were 3.6 μg kg^{-1} and 4.3 μg kg^{-1}, respectively.

(LOD) was estimated to be 2 and 5 μg kg^{-1} for the Quattro-LC and TSQ 700, respectively, using quantitation on both the ^{35}Cl and ^{37}Cl peaks. This calculation is based on a signal-to-noise ratio of 3:1 and is valid for the principal food commodities that were analysed (Table 2). If only the ^{35}Cl transition is considered for positive identification, then an LOD below 1 μg kg^{-1} is achieved for the Quattro-LC. It is evident that the Quattro-LC is about three-fold more ''sensitive'' than the TSQ 700 and provides better intra- and inter-assay precision. This difference is not surprising as the TSQ 700 is a relatively old instrument that was originally developed for GC–MS–MS applications and later retrofitted to LC–MS operation, while the Quattro-LC is a recent design, developed specifically for LC–MS–MS work. In spite of the instrumental differences, the performance of both instruments is clearly within the same order of magnitude and is sufficient for the detection of chlormequat at low μg kg^{-1} levels in all food commodities investigated here.

Table 2
Key analytical parameters for the present method, given with the transition m/z 122→m/z 58

Parameter	Instrument	
	TSQ 700	Quattro-LC
LOD (μg kg^{-1})	1.5	0.5
LOQ (μg kg^{-1})	4.0	1.5
Within series C.V.[a]	6.8%	1.9%
Between series C.V.[b]	10.6%	2.1%

[a] df=8.
[b] df=4.

3.6. Analysis of various food samples for chlormequat

A number of food commodities containing pears, and some cereals and cereal flours (wheat, rye, rice), as well as cereal pre-mixes containing >50% wheat were analysed on both LC–MS instruments within the framework of a limited survey, the results of

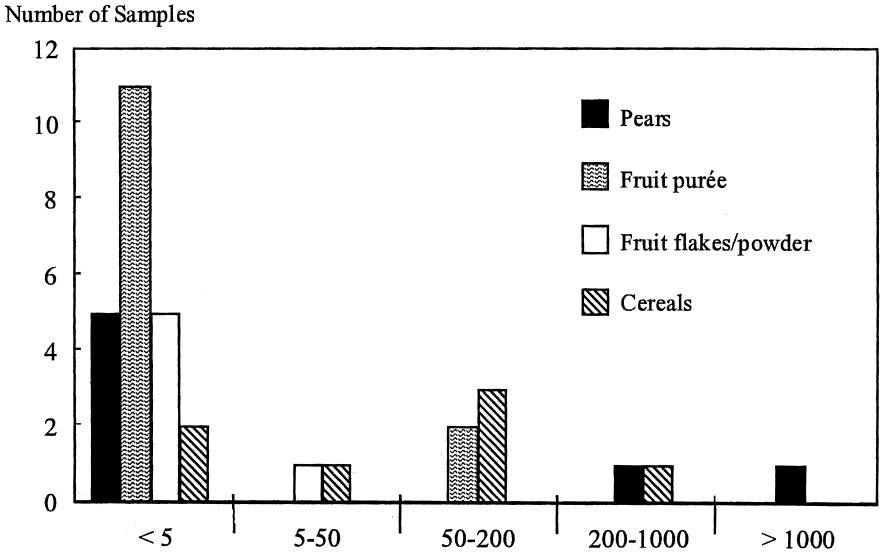

Fig. 6. Results of a limited survey expressed as concentration ranges of chlormequat residues (μg kg^{-1}) found in various food commodities. Each individual sample was determined in duplicate, with at least triplicate injections on both mass spectrometers.

which are summarised in Fig. 6. It can be seen that most of the samples investigated — particularly cereals — contain detectable levels of the residue. Of the food samples analysed, the highest level of chlormequat (5.5 mg kg^{-1}) was found in pears of South African origin, above the Codex MRL of 3 mg kg^{-1} [4]; it was particularly striking that the levels of chlormequat detected in pear were either very low (<5 μg kg^{-1}) or very high. This extensive usage and presence of chlormequat is well supported and further illustrated by data published in national pesticide surveys [2,18].

4. Conclusion

We have developed and validated a sensitive, quantitative analytical method for the detection and monitoring of chlormequat at low μg kg^{-1} levels. In contrast to existing methods, it is applicable to a wide variety of different food commodities and incorporates a single SPE clean-up step followed by isotope dilution LC–ESIMS–MS to quantify chlormequat. This method is suitable as a routine enforcement method with detection limits well below 10 μg kg^{-1} in a wide range of complex food matrices. The ruggedness of the method is demonstrated on two LC–MS instruments with different ion source design, both achieving comparable quality parameters.

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References

[1] C.D.S. Tomlin (Ed.), The Pesticide Manual, 11th ed., British Crop Protection Council, UK, 1997, p. 220.
[2] R.K. Julhler, M. Vahl, J. AOAC Int. 82 (1999) 331.
[3] Joint Meeting of the FAO Panel of Experts, Pesticide Residues in Food, Report on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues, FAO Plant Production and Protection Paper No. 127, FAO, Rome, 1994, p. 50.
[4] Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Report of the 31st Session of the

Codex Committee on Pesticide Residues, The Hague, 12–17 April 1999, ALINORM 99/24A.
[5] T. Stijve, Dt. Lebensm.-Rundsch. 76 (1980) 234.
[6] Ministry of Welfare, Health and Cultural Affairs, in: Analytical Methods for Residues of Pesticides, 5th ed., Rijswijk, The Netherlands, 1988, p. 43.
[7] F. Tafuri, M. Businelli, P.L. Giusquani, Analyst 95 (1970) 675.
[8] J. Sachse, Z. Lebensm. Unters. Forsch. 163 (1977) 274.
[9] M. Vahl, A. Graven, R.K. Juhler, Fresenius J. Anal. Chem. 361 (1998) 817.
[10] J.R. Startin, S.J. Hird, M.D. Sykes, J.C. Taylor, A.R.C. Hill, Analyst 124 (1999) 1011.
[11] D. Wycherly, M.E. Rose, K. Giles, T.M. Hutton, D.A. Rimmer, J. Chromatogr. A 734 (1996) 339.
[12] E. Moyano, D.E. Games, M.T. Galceran, Rapid Commun. Mass Spectrom. 10 (1996) 1379.
[13] K. Danzer, Fresenius Z. Anal. Chem. 335 (1989) 869.
[14] F. Tafuri, M. Businelli, P.L. Giusquani, Analyst 95 (1970) 675.
[15] P. Haefelfinger, J. Chromatogr. 218 (1981) 73.
[16] A.R.C. Hill, Quality Control Procedures for Pesticide Residue Analysis — Guidelines for Residues Monitoring in the European Union, European Commission, Brussels, Document 7826/VI/97, 1997.
[17] A.A. Stämpfli, I. Blank, R. Fumeaux, L.B. Fay, Biol. Mass Spectrom. 23 (1994) 642.
[18] J. Brueggemann, H.-D. Ocker, Chem. Mikrobiol. Technol. Lebensm. 10 (1986) 113.