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Short communication

Improved sensitivity in detection of chlormequat by liquid chromatography–mass spectrometry

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Abstract

Two published separations, using electrospray mass spectrometry (ES-MS), exhibit significant differences in limits of detection (LODs) for chlormequat cation in pear. Separation on ODS1, confirmed to result from ion-exchange, gives shorter analysis times and calibration over a wider concentration range than on an SCX cation-exchange column. The superior LOD using ODS1 (0.04 ng ml^{-1} vs. 1.0 ng ml^{-1}) results mainly from better chromatographic peak shape. Separation on ODS1 combined with optimised ES-MS detection allows direct quantification of chlormequat on an ion trap instrument at levels lower than those required for residue analysis in foods and also in drinking water. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chlormequat, the 2-chloroethyltrimethylammonium ion (Fig. 1a, inset), is widely used as its chloride as a plant growth regulator [1], and its measurement is required to verify compliance with maximum residue limits (MRLs) in foods [2] and in drinking water supplies [3]. Because it lacks a UV chromophore, analytical methods often involve derivatisation. Recently, direct methods employing liquid chromatography–tandem mass spectrometry (LC–MS–MS) have been developed and applied to the analysis of pears [4–6] and cereals [6–8]. A strong cation-exchange (SCX) column has been used in the analysis of pear matrix samples [4,5], and a C_{18} column in the analysis of chlormequat in cereals [7]. Both methods employ electrospray (ES) which produces a simple mass spectrum that shows no fragment ions [4,5].

Recently, we demonstrated the benefit of optimisation of ion trap parameters for MS–MS detection of chlormequat using ion-exchange chromatography (IEC) on an SCX column [5]. Optimisation of MS conditions improved the overall sensitivity of the method to a level where the ion trap instrument performs comfortably within the working range required for measuring chlormequat in foods, and where its performance compares favourably with that of a triple quadrupole instrument. This report compares the chromatographic behaviour of chlormequat

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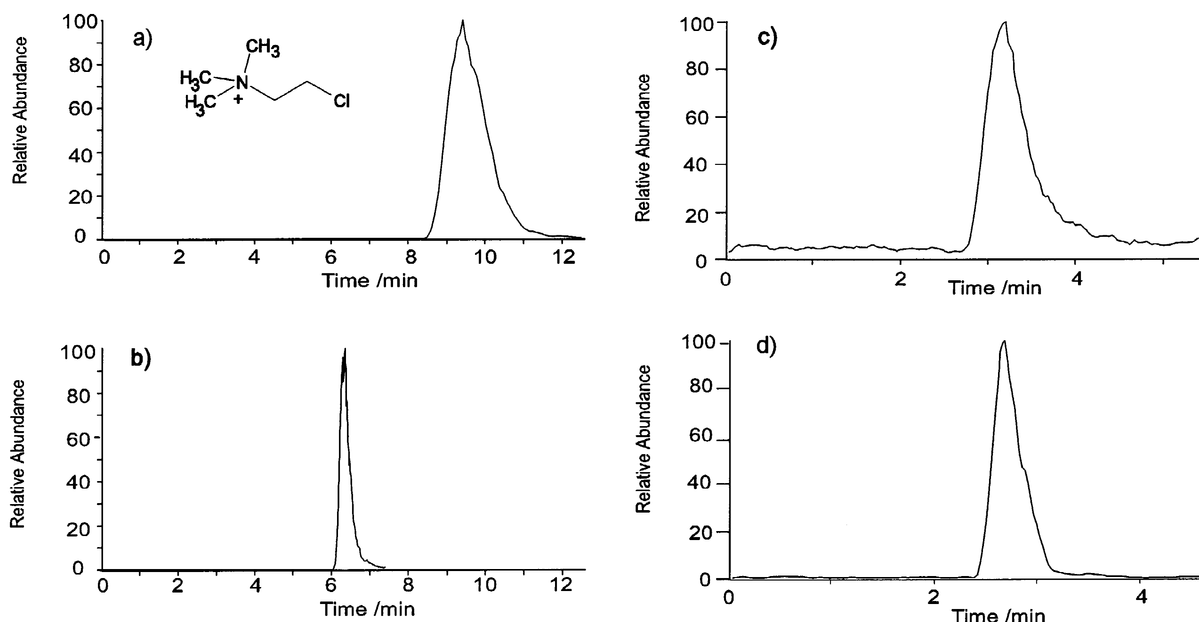

Fig. 1. LC–MS total ion chromatogram obtained monitoring SIM *m/z* 122 during the analysis of chlormequat on (a) the Partisil 10 SCX column at 600 ng ml^{-1}, inset shows the structure of chlormequat; (b) the PhaseSep S5 ODS1 column at 600 ng ml^{-1}; (c) the Hypersil 5 μm ODS column and (d) the Hypersil BDS C_{18} column.

on SCX IEC and C_{18} stationary phases and examines their suitability for the quantification of chlormequat by LC–ES-MS.

2. Experimental

2.1. Chemicals and preparation of standard solutions

A standard solution of 0.01 mg ml^{-1} chlormequat chloride (Qmx, Halstead, UK) was prepared by diluting a stock solution of concentration 1 mg ml^{-1} in water–methanol (50:50, v/v) (HPLC-grade; Fisons, Loughborough, UK). Standards for calibration and determination of limits of detection (LODs) were prepared by dilution of the standard solution in extracts of pear matrix and covered the range 0.0016 to 1.62 μg ml^{-1} of chlormequat cation (to convert μg ml^{-1} to the usual units for reporting pesticide residues, mg kg^{-1}, multiply by a factor of 5). For determination of LOD and calibration plots in water, the standard solution was diluted in either tap water or deionised water (FiStreem RO60 reverse osmosis; Fisons). The extraction procedure has been described previously [4].

2.2. LC–MS analysis

The LC–MS system comprised a Thermo Separations liquid chromatograph (P4000) and an autosampler (AS3000) coupled via an electrospray source to a Finnigan LCQ mass spectrometer operated in the positive ion mode using Finnigan Navigator software version 1.2. Finnigan developmental software was used to access the advanced controls on the ion trap (Finnigan, Hemel Hempstead, UK).

Selected ion monitoring (SIM) of both *m/z* 122 and *m/z* 124 was performed during the same analysis. MS–MS analyses were performed during a separate acquisition, as MS–MS experiments required the LC and autosampler trigger signals to be disconnected from the LCQ [5]. Optimised MS ion trap parameters were as follows: isolation window 1.5 *m/z*, activation amplitude (AA) 0.96 V, activation Q (AQ) 0.354 and activation time (AT) 35 ms [5].

2.3. Ion-exchange chromatography

The mobile phase comprised 50 m*M* ammonium ethanoate (analytical grade; Fisons) in methanol–water (50:50, v/v) (HPLC grade, Fisons). A Partisil 10 SCX column, 150 mm×2.1 mm (Capital HPLC, Broxburn, UK) was used at ambient temperature at a flow-rate of 0.2 ml min^{-1}. A 20-μl injection loop was fitted and overfilled five times. The column, which had been used almost exclusively for the analysis of chlormequat, was equilibrated with fresh mobile phase for an hour prior to LC experiments and the sample syringe was flushed with solvent (1 ml) before each injection to prevent carry-over. The column dead volume was measured using β-carotene as unretained species. MS conditions were: sheath and auxiliary gas flows of 55 and 30 arbitrary units, respectively, capillary temperature 200°C, capillary voltage 46 V, spray voltage 4.0 kV. SIM and MS–MS experiments used the divert valve for the first 7.5 min to avoid caramel formation due to the build up of sugars in the heated capillary.

2.4. High-performance liquid chromatography on C_{18} phases

A Spherisorb S5 ODS1, 250×4.6 mm column (Phase Separations, Deeside, UK) was used at ambient temperature with the mobile phase reported previously [7], comprising methyl cyanide–methanol–water–ethanoic acid (53:21:25:1) containing a final concentration of 50 m*M* ammonium ethanoate, flowing at 1 ml min^{-1}. A 20-μl injection loop was fitted and overfilled five times. Except for the manufacturer's tests, the column had been used solely for the analysis of chlormequat. It was equilibrated with fresh mobile phase for an hour prior to LC experiments and the sample syringe flushed with solvent (1 ml) before each injection to prevent carry-over.

Further experiments to examine the retention mechanism of chlormequat on C_{18} phases were carried out using either a Hypersil ODS 5 μm fully endcapped column, 250×4.6 mm (HPLC Technology, Macclesfield, UK) or a Hypersil BDS C_{18} 5 μm column, 250×4.6 mm (Shandon, available from Alltech, Carnforth, UK) with the same eluent composition as for ODS1. Column dead volumes were measured using sodium nitrite [9] (analytical grade; Fisons) as unretained species.

The MS conditions were: sheath and auxiliary gas flows 65 and 20 arbitrary units, respectively, capillary temperature 230°C, capillary voltage 18 V, spray voltage 3.5 kV. SIM and MS–MS experiments used the divert valve for the first 4.5 min.

3. Results and discussion

3.1. Retention mechanism

SIM analysis (m/z 122; M^+) [5] of chlormequat (ca. 600 ng ml^{-1} in pear matrix; equivalent to the UK MRL for pears [2] of 3 mg kg^{-1}) on SCX and C_{18} ODS1 columns show chlormequat eluting at 9.4 and 6.4 min, respectively (Fig. 1a and b). Retention of chlormequat on C_{18} ODS1 has been suggested to result from interaction of the cation with residual silanol groups in the stationary phase [7]. To examine this, chlormequat was analysed using base-deactivated silica (BDS) C_{18} and fully end-capped Hypersil ODS columns (Fig. 1c and d). All three stationary phases have similar hydrophobicities but exhibit markedly differing degrees of silanophilic character [10,11], Hypersil BDS being the lowest and ODS1 the greatest [12]. Retention factors, k, calculated from the retention times and measured dead times of all three columns, show chlormequat to be retained on ODS1, only slightly retained on Hypersil ODS and unretained on Hypersil BDS (Table 1), corresponding to the decrease in the silanophilic character of the three phases. Thus, the high silanol content of ODS1 and limited shielding of silanols at the silica surface is responsible for retention of chlormequat on this stationary phase.

To ascertain if, as suggested by Vahl et al., the silanol sites on the ODS1 act via cation exchange [7], the ionic strength and pH of the mobile phase was varied. Reduction in ionic strength, resulting from a tenfold decrease in the salt concentration at a constant buffer ratio $[CH_3COO^-]/[CH_3COOH]=0.29$, led to a threefold increase in k ($k=4.9$) from that obtained using the original conditions ($k=1.6$; Table 1). At an ammonium ethanoate concentration of 5 m*M* a decrease in the buffer ratio to 0.029,

Table 1
Column properties and characteristics for the columns used in the RP-HPLC and IEC methods

Column	Dead time (t_M)	Retention time (t_R)	Retention factor (k)	LOD (ng ml^{-1})	
				SIM m/z 122	MS–MS m/z 122 to 58
Hypersil BDS C_{18}	2.62	2.63	0.0		
Hypersil ODS	2.58	2.84	0.1		
PhaseSep ODS1	2.47	6.52	1.6	0.04	0.06[a]
Partisil SCX	2.67	9.42	2.5	1.0	1.0[b]

[a] For MS–MS calibration [intercept (c)=$1.5\pm4.6\cdot10^5$; slope (m, ml μg^{-1})=$1.72\pm0.05\cdot10^8$; R^2=0.958].
[b] See Ref. [5].

achieved by increasing ethanoic acid to 1%, caused a decrease in k by 40% (k=2.3). Conversely, increasing the buffer ratio to 2.9 caused an increase in k by 40%. An increase in pH will favour dissociation of the silanol groups, thereby increasing the practical specific capacity [11]. Thus, the increases in retention of chlormequat on increasing pH and on decreasing ionic strength are consistent with ODS1 exhibiting ion-exchange capacity [9,13]. Although ODS1 is not designed as a stationary phase for IEC the results presented show that the free silanol sites, which may represent up to 6.5 μmol m^{-2} [11,12,14], cause it to be an effective cation-exchange medium. Notably, under the conditions published for separation of chlormequat on the SCX and ODS1 phases, the ODS1 phase exhibits a similar practical specific capacity to that of SCX and gave reproducible retention times over ca. 300 analyses.

The ODS1 and SCX columns also exhibit significant differences in the peak profiles for chlormequat, peak width at half height (W_h)=0.3 for ODS1 and 1.2 min for SCX, and both methods give asymmetric tailing peaks at all concentrations. Peak asymmetry [9] (A_s) at 10% peak height=2.14 on SCX and 1.83 on ODS1. The lower A_s value and narrower peak width on ODS1 reveals better chromatography than on SCX. A concentrated solution of the polycyclic aromatic hydrocarbon pyrene (100 μg ml^{-1}) gave a more symmetrical peak on the ODS1 column (A_s= 1.36) but with the same peak width as chlormequat. Thus, better peak symmetry results from the hydrophobic interactions than from the silanol interactions and homogeneity in the particle size distribution of the ODS1 stationary phase is good. Accordingly, the higher A_s value for chlormequat indicates inhomogeneity within the cation-exchange sites and may reflect the presence of different types of silanol site: both very acidic and less acidic (cf. Refs. [11,12,15]). The SCX stationary phase comprises irregular porous silica with sulfonate functionalities chemically bonded as strong cation-exchange sites. Overall, the difference in A_s values for chlormequat on ODS1 and SCX suggest greater inhomogeneity within cation-exchange sites in the latter.

The acidity of silanol groups depends on their local environment within the silica matrix [11,12,15]. Metal ions within the silica matrix may cause neighbouring silanol groups to be more acidic due to electron withdrawal [12]. The ODS1 stationary phase contains a high sodium content (1500 ppm) [14] and so is likely to contain some highly acidic silanol sites, though these are estimated to represent less than 1% [15]. Both types of silanol site will contribute to the retention of chlormequat on ODS1, and it appears likely that peak tailing is due mainly to the interaction of chlormequat with the more acidic silanol sites. The chlormequat peak obtained using Hypersil ODS, which has fewer residual silanol groups than ODS1 due to end-capping, also exhibited tailing (Fig. 1c). Thus, the peak tailing indicates some silanol activity, which is most likely caused by very acidic silanol groups. By contrast, chlormequat did not exhibit tailing on Hypersil BDS (Fig. 1d). There was, however, a slight degree of peak broadening compared with the unretained species, indicating partial activity. The smaller peak width on ODS1 compared with SCX probably results mainly from a combination of the smaller size and more spherical nature of the stationary phase particles. In both cases the columns were eluted at, or close to, their maximum flow-rates (and optimal ES-MS conditions were used for the respective flow-rates). Given the narrower peak width and lower asymmetry on ODS1, detection of lower concen-

trations of chlormequat should be possible due to the concentration dependence of the response in ES-MS [16].

3.2. Analytical performance

LODs (three times the S/N), for both methods, measured using serial dilution in pear matrix from the calibration standard of lowest concentration (0.0016 μg ml^{-1}), were found to be ca. 20-times lower for ODS1 than for SCX (Table 1). This improvement is attributed mainly to the better peak shape, i.e., the narrower peak width on ODS1, and consequent increase in concentration at peak centre than on SCX. The results compare favourably with the LOD (0.5 μg kg^{-1}=0.1 ng ml^{-1}) obtained independently on an SCX column following sample pre-treatment by solid-phase extraction [6]. The LOD on ODS1 is an order of magnitude better than that obtained previously using the same separation and a triple quadrupole instrument.

The LODs for chlormequat in pear matrix on ODS1 for SIM and MS–MS are lower than the limit (0.1 ng ml^{-1}) for individual pesticides in drinking water set by a recent European Directive [3]. Although a method has achieved the required LOD it involved a preconcentration step [17]. Due to the poor LOD achievable using existing methods, the analytical method for chlormequat is among those recommended for review by the UK Drinking Water Inspectorate [18]. Our results suggested that the ion trap LC–MS method operated under optimised SIM or MS–MS conditions [5] and using the ion-exchange capacity of Spherisorb ODS1 would be suitable for direct determination of chlormequat in water. Measurements of LODs in tap water (1.0 pg ml^{-1}) and deionised water (0.1 pg ml^{-1}) confirm this to be the case.

The precision of the two LC methods was examined using solutions (methanol–water, 50:50) containing 20 ng ml^{-1} of chlormequat. Better precision, reflected in lower relative standard deviations (RSDs), was obtained for SIM analysis on ODS1 (RSD=5.2%; n=20) than on SCX (RSD=10%; n=20). However, precision under MS–MS conditions was worse on ODS1 (RSD=17%; n=20) than on SCX (RSD=11%; n=20), probably as a result of fewer scans being obtained across the chromatographic peak.

Calibration standards comprising chlormequat in pear matrix were analysed in random order using both separation methods. The calibration plot obtained using ODS1 [intercept (c)=2.1±0.7·10^5; slope (m, ml μg^{-1})=1.42±0.01·10^7] reveals a greater linear dynamic range than with the SCX column [c=2.3±0.8·10^6; m, ml μg^{-1}=3.79±0.09·10^7]. This is due both to the lower limit of quantification and the improved linearity at high concentrations on ODS1. The coefficient of determination (R^2) for the SIM linear regression calibration plot on SCX over the range 0.008 to 1.04 μg ml^{-1} was found to be greater (R^2=0.995) [5] than over the range 0.008 to 1.62 μg ml^{-1} (R^2=0.987), indicating curvature at higher chlormequat concentrations. By contrast, the corresponding values obtained on ODS1 remained constant over the two ranges 0.008 to 1.04 μg ml^{-1} and 0.008 to 1.62 μg ml^{-1} (R^2=0.997 and 0.998, respectively). Calibration for selected reaction monitoring (m/z 122 to 58) also showed good linear dynamic range, though with greater scatter at higher concentrations. The calibration plots for chlormequat in water both revealed significantly greater slopes than the corresponding plot from pear matrix, suggesting an influence from the matrix.

For routine monitoring, it is advantageous for calibration plots to be linear over a wide range and to extend to high concentrations. For example, quantification of chlormequat in foodstuffs may require accurate measurements over a wide dynamic range. Calibration that is linear over a wide range and extends to high concentrations removes the need for dilution of such samples and so eliminates one source of uncertainty in the measurement.

4. Conclusions

The work presented shows, quantitatively, the influence of chromatographic conditions on the overall sensitivity of an LC–ES-MS method. The retention of chlormequat on ODS1 has been shown to result from ion-exchange behaviour involving the residual acidic silanol groups in the stationary phase, and the analysis of chlormequat highlights the effects

of improved peak shape on the LOD for ES-MS detection. In the analysis of chlormequat the ODS1 column offers lower detection limits and shorter analysis times than a dedicated IEC column, and the calibration exhibits a wider linear dynamic range. The method employed has been shown to be suitable for the analysis of chlormequat in foods and also to surpass the stringent detection limits required for the direct analysis of chlormequat in drinking water.

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