Gas chromatography–high-resolution time-of-flight mass spectrometry in pesticide residue analysis: advantages and limitations

Tomáš Čajka, Jana Hajšlová*

Department of Food Chemistry and Analysis, Institute of Chemical Technology, Technická 3, 16628 Prague 6, Czech Republic

Available online 11 September 2004

Abstract

In this study, 20 modern pesticides representing various chemical classes with a broad range of physico-chemical properties were selected for demonstration of applicability of exact mass orthogonal acceleration time-of-flight (TOF) mass spectrometer GCT (Micromass, UK) in GC analysis of their residues in purified peach extracts. The influence of experimental chromatographic conditions as well as various detector settings and data processing strategies on the performance characteristics of analytical procedure achieved during optimisation process were critically discussed. As documented in this study unbiased identification and reliable quantification of target analytes is possible due to: (i) application of narrow mass window (0.02–0.05 Da) for extracting analytical ions; (ii) availability of full spectral information even at very low levels of target analytes. With only a few exceptions, the limits of quantification for most of the pesticides involved in this study were fair below 0.01 mg/kg level, which represents the maximum EU residue limit set recently for pesticide residues in cereal-based foods and baby foods for infants and young children.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Pesticide residues; Food analysis; Performance characteristics

1. Introduction

Until recently gas chromatography coupled to mass spectrometry (GC–MS) has been used by many analytical laboratories for confirmation of analytes tentatively identified by element/group selective detectors such as ECD, NPD and/or FPD. Nowadays, this powerful technique has become a routine analytical tool for the determination of pesticide residues in food and many other matrices [1]. Quadrupole is probably the most popular mass analyser; however, to obtain sufficiently low detection limits required for regulation purpose the selected ion monitoring (SIM) mode has to be employed. Unfortunately, using this sensitive detection set-up a part of spectral information is lost. Similar drawback is encountered when ion trap analyser is operated in MS/MS mode to obtain high detection selectivity enabling improved signal-to-noise ratio. Under these circumstances, the use of MS analysers

based on time-of-flight technology provides an innovating approach to overcoming the above drawbacks limiting full exploitation of mass spectrometry potential to determine analytes at trace levels.

Contrary to scanning instruments equipped by quadrupole, ion trap and/or magnetic sector analysers that employ for separation of ions of diverse m/z values changing setting of electrical or magnetic field, ions originated from analyte in TOF MS source are in the first phase accelerated to get constant kinetic energy and then ejected into a mass analyser using pulsed electric-field gradient oriented orthogonally to the ion beam (orthogonal acceleration TOF). The flight times of ions separated in a field free region are proportional to the square root of respective m/z value [2].

The most challenging features of time-of-flight MS technique coupled to gas chromatography, can be summarised as follows [2–10]:

(i) *Acquisition speed*. Time needed to obtain one mass spectrum is in the range of tens microseconds. 5000–40 000

primary spectra that are hereby obtained in 1 s are summed and, as the final result, 1–500 spectra/s are then stored in computer depending on the type of TOF MS instrument. Such a high acquisition rate of "fast" TOF MS instruments predetermines their use as detectors coupled to very fast and ultra fast GC separation.

(ii) *Mass resolution*. Good resolution is achieved by orthogonal sampling of generated ions, which is important for their spatial focusing (ions are ejected to a mass analyser at practically same instant). Further improvement of mass resolution is obtained using reflectron for energy focusing. This "ion mirror" consists of a series of ring electrodes with linearly increasing voltage creating retarding fields. After reaching the reflectron area, ions with higher energy penetrate more deeply inside, what extends the time until they are reflected. As a consequence of this phenomenon, the ions of the same m/z value with different initial energies reach detector at almost the same time. In addition, the mass resolution is substantially improved by making the ions to pass twice along a TOF flight tube before reaching the detector.

While the "fast" TOF MS instruments provide only a unit mass resolution (typically about 1400 FWHM) its value for those systems employing high-resolution TOF analysers may be as high as 7000 FWHM and even more. It should be noted that in the latter case the spectral acquisition rate is typically limited by approx. 10 spectra/s.

(iii) *Mass accuracy*. In the case of high-resolution TOF MS system, mass accuracy 5–10 ppm is attainable by using a lock mass approach, i.e. an exact mass of selected ion of reference compound, which is continuously supplied into the ion source during analyses. On the basis of previously performed mass calibration over a given mass range and defined value (ion) of a lock mass, the software automatically corrects the values of all masses in the acquired spectra. Measurement of an accurate mass of a particular ion enables both calculation of its elemental composition, i.e. makes possible identification of unknown compound in chromatogram, and confirmation respective target analyte identity.

(iv) *Acquisition of complete spectra*. Contrary to scanning instruments that provide enhanced selectivity and sensitivity only when operated in a selected ion monitoring mode (quadrupole) or when a measurement of daughter fragmentation ions in MS/MS mode is employed (ion trap), TOF MS instruments allow acquisition of full mass spectra even at these very low concentration levels thanks to higher mass-analyser efficiency. This efficiency is for a quadrupole mass analyser scanning over a 500 amu mass range only about 0.1% while 25% efficiency is obtained for oa-TOF instrument. This enables to use full capabilities of library reference spectra search for identification/confirmation of trace analytes identity.

(v) *Absence of spectral skew*. Since discrete packages of ions are sampled and analysed in the flight tube with a very fast repetition, no changes in the ratios of analyte ions across the peak occur during the acquisition of the mass spectrum and, consequently, no spectral skew, which is commonly observed by scanning instruments, is encountered. Utilization of a special computer software (deconvolution function) allows resolution of partially overlapped peaks on the basis of increasing/decreasing intensity of ions in collected spectra hence identification/confirmation of compounds using library search is possible.

(vi) *Extended mass range*. The upper mass limit of common mass analysers on the market ranges between m/z 600–1050, nevertheless, it might be fairly higher since only microseconds in case of TOF MS are required to get an extended mass range. Actually, the most of TOF analysers coupled to GC operate up to m/z 1000–1500.

The aim of presented study was to demonstrate the application potential of GC–TOF MS technique in analysis of pesticide residues at levels 10 μg/kg and lower. The control of these concentrations is required by the EU legislations specifying maximum residue limit for cereal-based foods and baby foods for infants and young children [11]. Pesticides representing various chemical classes, hence, possessing a wide range of physico-chemical properties as volatility, polarity, etc. were involved in our study.

2. Experimental

2.1. Materials

Pesticide standards (acephate, captan, carbaryl, chlorpyrifos, deltamethrin, dichlorvos, dimethoate, endosulfan I, endosulfan II, endosulfan sulfate, heptachlor, lindane, methamidophos, methiocarb, permethrins, phosalone, pirimiphosmethyl, procymidone, propargite, and thiabendazole) were supplied by Dr. Ehrenstorfer GmbH (Germany). The purity was not less than 95%. Stock as well as set of working pesticide solutions in concentration range 0.5–500 ng/ml were prepared in ethyl acetate. All solvents used in experiments (ethyl acetate, cyclohexane) were of analytical grade (Scharlau, Spain; Merck, Germany). Residue-free peaches were obtained from common market.

2.2. Methods

2.2.1. Matrix-matched standards

Twenty-five grams of homogenised peach sample were mixed with 100 ml of ethyl acetate. After addition 75 g of anhydrous sodium sulphate the sample was homogenised using a Turrax macerator at 10 000 rpm for 2 min. The crude extract was filtred through a layer of anhydrous sodium sulphate and the filter cake was rinsed three times with 25 ml of ethyl acetate. The combined filtrates were evaporated to a volume of ca. 25 ml and after a transfer into a volumetric flask made up to 50 ml with cyclohexane.

Two millilitres of this crude extract were purified by high performance gel permeation chromatography (HPGPC) using a PL gel column (600 mm × 7.5 mm, 50 Å). Cyclohexane/ethyl acetate (1:1, v/v) mixture was used as a mobile phase at a flow rate 1 ml/min. Collected "pesticide" fraction (eluted in the range 14.5–31.0 ml) was evaporated, the remaining solvent was removed by a gentle stream of nitrogen and the residue was re-dissolved in 1 ml pesticide standard solution. Concentrations of each of 20 pesticides in standards in peach extract were as follows: 0.5, 1, 2.5, 5, 10, 25, 50, 100, 250 and 500 μg/kg of matrix (in analysed extracts these values correspond to ng/ml). Blank sample was prepared in the same way by dissolving the residue left after evaporation of HPGPC fraction in 1 ml of ethyl acetate.

2.2.2. Instrumentation

Analyses were performed by gas chromatograph GC System 6890 Series (Agilent Technologies, Palo Alto, CA, USA) coupled to a GCT high-resolution time-of-flight mass spectrometer (Micromass, Manchester, UK). The GC system was equipped with an electronic pressure control (EPC), a split/splitless injector and a PAL Combi autosampler (CTC Analytics, Zwingen, Switzerland).

2.2.2.1. Gas chromatography. A DB-5 MS (20 m × 0.18 mm × 0.18 μm) capillary column used for separation was operated under following conditions: oven temperature program: 70 °C for 1.0 min, 25 °C/min to 200 °C, 10 °C/min to 280 °C (9.8 min); helium flow rate: 1.0 ml/min; injection mode: splitless 1.0 min; injection temperature: 250 °C; injection volume: 1 μl.

2.2.2.2. Mass spectrometric detection. Acquisition rate: 1 Hz; pusher interval: 33 μs (30 303 raw spectra/s); inhibit push value: 14; time-to-digital converter: 3.6 GHz;

mass range: m/z 45–500; ion source temperature: 220 °C; transfer line temperature: 280 °C; detector voltage: 2650 V.

The instrument was manually tuned using 2,4,6-tris-fluoromethyl-[1,3,5]-triazine. The mass resolution was calculated from continuum data using the highest mass from reference compound (m/z 285) and the full width at half maximum of this peak. Generally, the mass resolution was more than 7000 FWHM in all experiments. For exact mass calibration nine fragments (obtained in an electron ionisation mode) of this reference compound in centroid mode were used. Once this calibration was made the m/z 284.9949 was used as an internal reference mass (lock mass). The exact mass calibration was considered successful with maximum differences between measured and theoretical masses of 1.0 mDa. In both cases, 30 final spectra (i.e. 30 points) were used for calculation of mass resolution and for exact mass calibration during the tuning procedure. The reference compound was continuously introduced into the ion source also during the real analysis. MassLynx 3.5, MassLynx 4.0 and ChromaLynx were employed for data processing.

3. Results and discussion

While performance characteristics of GC–MS methods attainable by common quadrupole and/or ion trap analysers routinely used in trace analysis have been thoroughly characterised in many studies [12–17] only very limited information is available on the quality of data generated by high-resolution TOF MS [18]. In the following paragraphs results obtained by high-resolution TOF MS instrument are described in details. Both advantages and limitations of this novel technology only recently introduced into pesticide residue analysis are discussed.

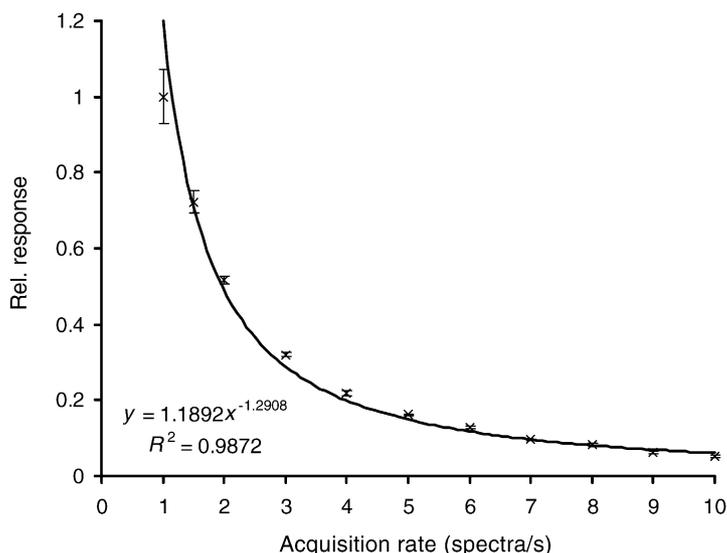$$y = 1.1892x^{-1.2908}$$
$$R^2 = 0.9872$$

Fig. 1. Relationship between average ($n = 3$) relative response (area) and spectral acquisition rate. Phosalone in matrix-matched standard as an example, 50 pg of analyte injected.
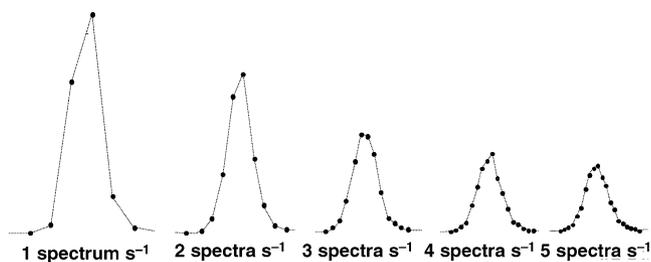
Fig. 2. The influence of acquisition rate on peak shapes. Phosalone in matrix-matched standard as an example, 50 pg of analyte injected.

3.1. Signal intensity vs. acquisition rate

As a typical example, the decrease of phosalone signal occurring with increasing of a spectral acquisition rate is shown in Fig. 1. One hertz, the lowest possible setting of this value, obviously provided the highest analyte signal (i.e. the lowest LOD values). It should be noticed that the intensity of detector response is not the only parameter considered when the optimal acquisition rate setting is selected. In a real life situation, the high signal-to-noise (S/N) ratio is an essential parameter for obtaining low detection limits. The decrease of S/N ratio was also achieved with increased acquisition rate, however, in the case of using narrow mass window setting the measurement of noise (N value) become rather difficult, since its value was poorly reproducible contrary to analyte signal (S value).

As shown in Fig. 2, the use of a low spectral acquisition rate unavoidably resulted in rather poor peak shapes that obviously do not represent classic Gaussian curve. In our study, the width at the baseline of pesticide peaks ranged (depending on their position in chromatogram and concentration of analyte) between 3 and 8 s, hence, relatively few data points per peak were collected for some compounds. Regardless

that fact, reliable quantification of most peaks was still possible (for details see Section 3.6). These results are in line with practical experiments described by Baumann et al. [19], who showed that in spite of 7–8 points per peak required for obtaining the 99.99% peak recovery, having available only 3–4 points resulted only in a small degradation (1.4%) of peak recovery, i.e. the uncertainty of peak area measurement is almost negligible. Similar results were reported also by Amirav, who found 2–3 points per peak still acceptable for accurate measurement [20]. Additionally, the Guidelines for Residues Monitoring in the EU [21] require the minimum three data points per peak. Although this criterium was not met in our study at too low concentrations (only two points per peak were often achieved), the measurement as discussed above was still possible.

3.2. Mass resolution and mass accuracy

The mass resolution (R) of particular measurement is described by equation [2]:

$$R = \frac{m}{\Delta m}, \tag{1}$$

where m is a measured mass and Δm is a full width at half maximum (FWHM) of respective peak. Supposing mass resolution 7000 FWHM declared for GCT instrument and choosing ion m/z 120.000 as an example, its calculated full width at half maximum is 0.0171. In theory, this means that resolving of two coeluting peaks is only possible when the difference between their centres is approx. $2 \times 0.0171 \approx 0.034$. In other words, ions m/z 120.000 and 120.034 could be still resolved. Considering this in a context of mass accuracy attainable by instrument used in this study, i.e. supposing target accuracy 0.002 Da for a single peak, one should be aware that separation of two coeluting masses 120.000 and
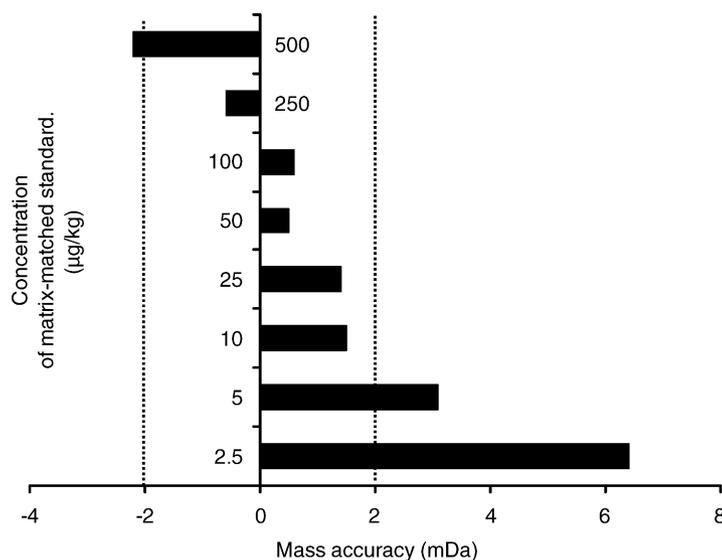

Fig. 3. Mass accuracy of fragmentation ion of dimethoate (theoretical mass 124.9826 Da) in dependence on concentration of analyte in matrix-matched standards.

120.002 is practically not possible, although for a single peak the precision to which its centre can be determined is as low as parts per million (ppm).

In the next step, the feasibility of achieving the above mentioned mass accuracy 0.002 Da under experimental conditions was tested. Worth to notice that according to the GCT manufacture's specification [5], mass accuracy 0.001 Da for masses below 200 Da should be attainable. However, using 30 spectra (30 points) for exact mass calibration and considering peak under GC condition used in this study (2–7 points per peak), i.e. 2–7 spectra for determination of the mass accuracy of particular analyte, we decided to use 0.002 Da as the upper limit for reporting the (exact) ion mass value.

As shown in Fig. 3, using fragmentation ion of dimethoate as an example, mass accuracy better than 0.002 Da was achieved within the concentration range 10–250 μg/kg. While at lower concentrations too weak analytical signal did not allow exact mass measurement, saturation of a time-to-digital converter (TDC) was the cause of the failure to get
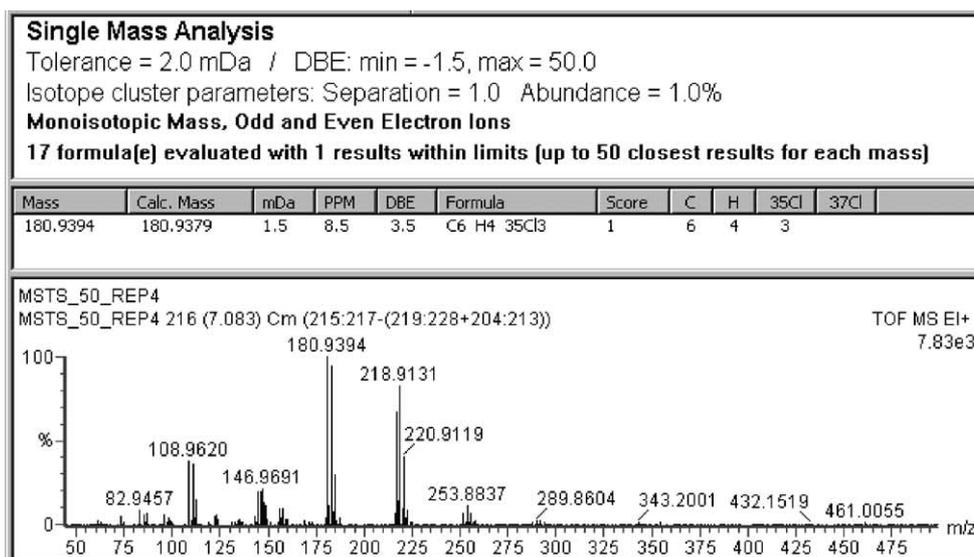
(A)
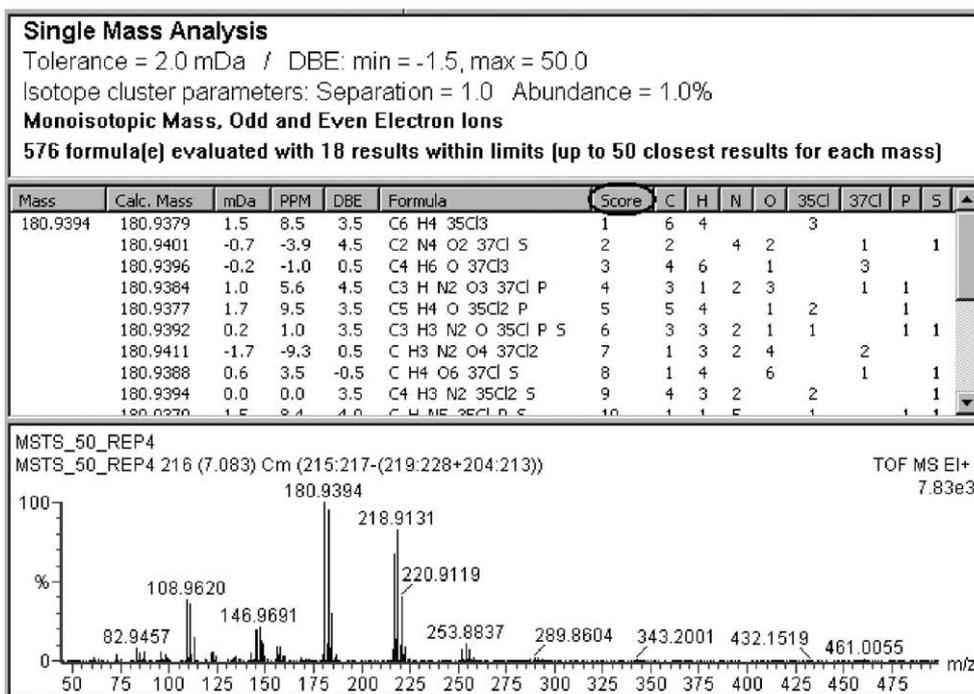
(B)

Fig. 4. Proposals for the elemental composition of fragmentation ion of lindane in peach extract at 50 μg/kg (theoretical mass 180.9379 Da). Selected entries for calculation: (A) carbon, hydrogen and chlorine; (B) carbon, hydrogen, chlorine, nitrogen, oxygen, phosphorus and sulphur.

satisfactory mass accuracy for this analyte at concentration above 250 μg/kg.

In addition to the possibility to confirm the target analyte identity, exact mass setting may serve for the calculation of elemental composition of "unknowns". It has to be emphasised that selection of the appropriate number of various elements potentially presented in the particular ion together with estimation of maximum/minimum number of each of these elements as well as choosing an acceptable mass tolerance (maximum difference between measured and theoretical masses) were the factors predetermining the success of a search assisted by MassLynx software. Worth to notice that for "unknowns" the estimation of elemental composition (especially that of fragmentation ions) represents a rather demanding task: good knowledge of fragmentation mechanism is required. However, in practice a mass measurement within 2 mDa gives a relatively short list of elemental compositions hence discrimination among the listed items is not so complicated. Unambiguous identification of unresolved isomers on the basis of separated mass spectra remains of course the problem.

An illustration of the result obtained by entering carbon, hydrogen and chlorine into dialogue window as elements presumed to be presented in the selected ion is shown in Fig. 4A. In particular case, lindane was unambiguously identified as the only conceivable compound. The difference between experimental and theoretical mass of fragmentation ion was as

low as 1.5 mDa. However, when more elements such as nitrogen, sulphur, phosphorus and oxygen were added on the list, the number of possible element compositions increased (see Fig. 4B). As an additional criterium, the MassLynx 4.0 version allots also the "score" value related to the fit of the reconstructed isotopic spectrum for each hit, to the original spectrum. Using this algorithm the highest priority (score 1) was given to lindane in spite of a worse agreement between the theoretical and measured mass as compared to other proposed combinations.

3.3. Selectivity of detection

Occurrence of interfering ions with masses close to those of target analyte is one of the main factors that limits achieving the low detection limits at any GC method employing mass spectrometric detector. This chemical noise originating from various sources (e.g. matrix coextracts, contamination from an ion source, column bleed, etc.) has an adverse effect on the S/N ratio. Reduced width of the mass window generally results in a significant elimination of background interferents of any origin and, consequently, leads to improving LODs of analytes. Fig. 5 illustrates the differences in performance of detection of low levels of chlorpyrifos (10 μg/kg) in matrix extract as obtained under high and low-resolution mode. While by using 1 Da mass window, S/N ratio was only 4:1, setting the mass window as narrow as 0.02 Da led to
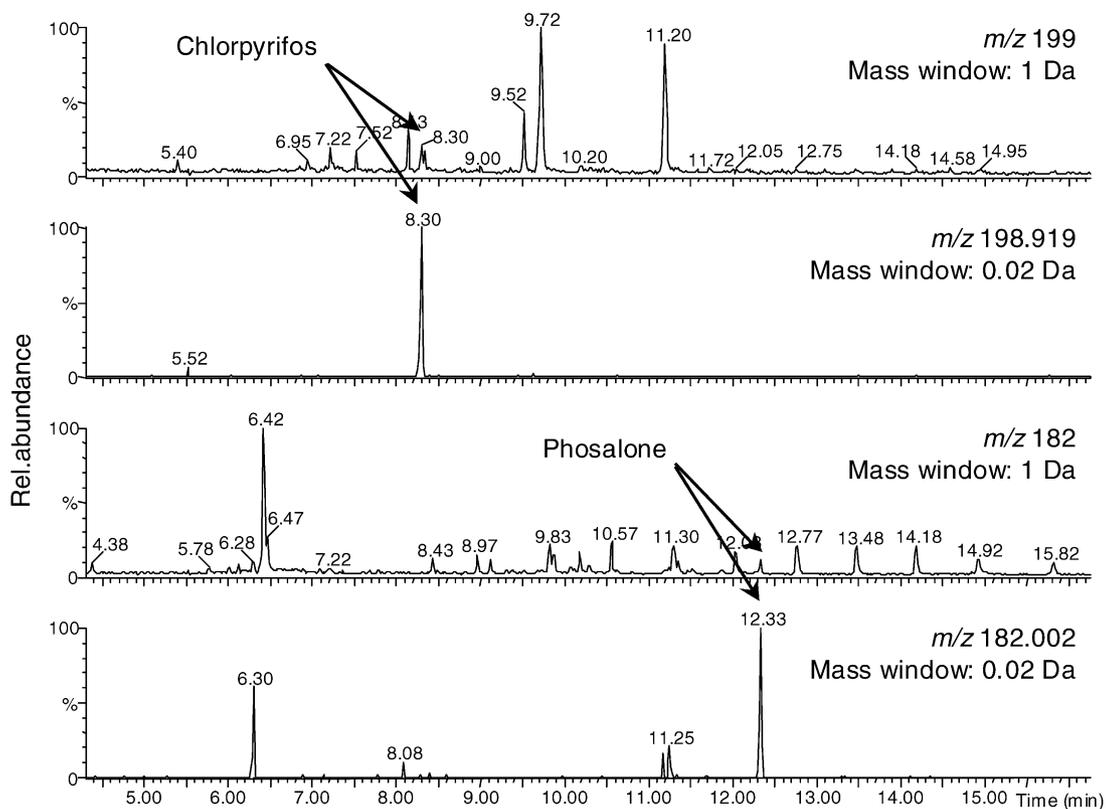

Fig. 5. Selectivity of target analytes detection in relation to the mass window setting (chlorpyrifos and phosalone at concentration 10 μg/kg).
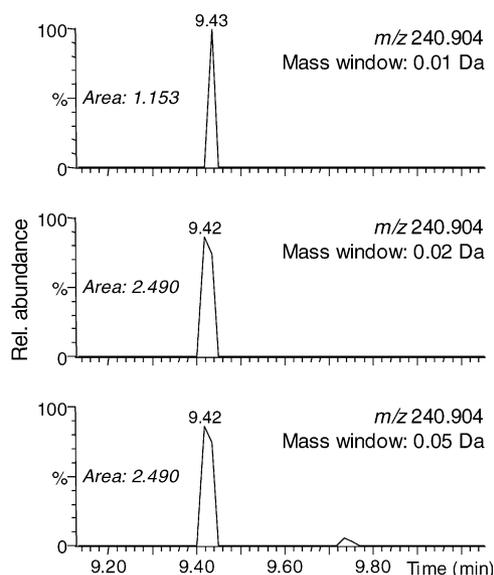
Fig. 6. The influence of width of mass window for detection of endosulfan I at concentration 5 μg/kg.

a remarkably higher value: 128:1. Due to a very low incidence of noise signals, their acquisition for S/N calculation required more than 1 min. Fig. 5 also shows an improvement of phosalone detectability. Exact mass setting enabled ten times higher S/N ratio (from 8:1 to 84:1), nevertheless, the selectivity of ion 182.002 was rather lower since more signals at different retention time appeared in chromatogram in comparison with chlorpyrifos at exact mass 198.919.

Worth to notice that setting of the mass window narrower than 0.02 Da may result, especially at low concentrations of target analytes, in an underestimation of area of representative peak (Fig. 6) because of lower mass accuracy at low ion intensity. In other words, the use of wider mass window setting (0.05–0.02 Da) is more suitable for extraction of particular ions for quantification purpose.

3.4. Limits of quantification/limits of detection

The lowest calibration levels of target analytes in peach extract varied between 0.5 and 25 μg/kg (see Table 1) and were considered as the limits of quantification (LOQs). Not only the technical potential of TOF MS detector but also other factors such as chromatographic behaviour of particular pesticide and its EI fragmentation pattern play an important role in this context.

Relatively poor LOQ obtained for instance for acephate (25 μg/kg) was obviously due to the lack of sufficiently specific ions in its mass spectrum (only low m/z are yielded by EI fragmentation) and also because of distinctly tailing peak of this (rather polar) analyte.

As regards dichlorvos, a coelution of matrix component obscured its base ion (m/z 109). For that reason the identification/quantification had to be performed using other, less intensive ion(s), which, however, did not result in increased LOQ of this analyte.

On the other hand, very good detectability of pesticide residues was achieved in the case of the latest eluting pesticide deltamethrine, which commonly represents troublesome

Table 1
Performance characteristics of optimised GC procedure employing GCT TOF MS for quantification of 20 modern pesticides in peach (ions used for quantification purposes in bold)

	Pesticide	m/z[a]			Working range (μg/kg)[b]	Correlation coefficient (R^2)	LOQ (μg/kg)	R.S.D. of areas (%)[c]
1	Methamidophos	94.007	95.014	**141.005**	2.5–250	0.996	2.5	2.7
2	Dichlorvos	**184.978**	186.976	219.948	1–100	0.999	1	4.3
3	Acephate	94.007	**136.018**	183.014	25–250	1.000	25	4.7
4	Dimethoate	87.015	93.011	**124.984**	2.5–250	0.995	2.5	7.7
5	Lindane	**180.940**	182.937	218.914	0.5–250	0.999	0.5	5.6
6	Carbaryl	115.055	116.062	**144.059**	2.5–100	0.998	2.5	5.6
7	Heptachlor	**271.811**	273.809	336.848	0.5–250	0.998	0.5	6.8
8	Pirimiphos-methyl	276.058	**290.072**	305.096	1–100	0.999	1	6.3
9	Methiocarb	153.038	**168.062**	225.085	2.5–100	0.995	2.5	6.0
10	Chlorpyrifos	**198.919**	257.894	313.956	0.5–250	0.999	0.5	4.6
11	Captan	**79.052**	149.043	263.956	10–250	0.997	10	4.7
12	Thiabendazole	174.026	**201.037**	202.048	10–250	1.000	10	7.5
13	Procymidone	96.055	**283.008**	284.013	2.5–250	1.000	2.5	7.0
14	Endosulfan I	194.948	**240.904**	338.873	2.5–250	1.000	2.5	5.8
15	Endosulfan II	194.948	**240.904**	338.873	2.5–250	0.991	2.5	7.5
16	Endosulfan sulfate	**271.812**	273.809	386.838	1–500	0.999	1	7.3
17	Propargite	135.081	**173.098**	350.153	10–500	0.999	10	4.2
18	Phosalone	**182.002**	184.000	366.984	0.5–250	0.999	0.5	7.0
19	Permethrin I	163.008	**183.083**	184.087	5–500	0.999	5	7.9
19	Permethrin II	163.008	**183.083**	184.087	2.5–250	0.999	2.5	8.1
20	Deltamethrin	181.067	**252.907**	254.906	2.5–500	1.000	2.5	7.5

[a] Ions for exact mass setting presented as mean value of six repetitive analyses of pesticide standards in pure solvent (ethyl acetate) at concentration 100 ng/ml.

[b] From four to nine data points in calibration curve.

[c] Repeatability of peak areas calculated from six repetitive analyses of spiked peach extract at 50 μg/kg.

compound (too high LOD) when determined by conventional GC–MS systems. Improved LOQ obtained in our study was not only the result of excellent detection performance of TOF MS detector (chemical noise was efficiently eliminated by exact mass setting of quantification ion) but also due to faster chromatographic separation that provided narrower, hence, higher peaks (the risk of exceeding linear range has to be considered for high concentration of analyte, see Section 3.5 (iii)).

Worth to notice that estimating the limits of detection (LODs) by extrapolation based on results obtained for the lowest measured concentration and pre-defined target S/N ratio was practically impossible. This fact can be illustrated for instance on base peak of endosulfan I (m/z 240.904): an S/N ratio 18:1 obtained for concentration 2.5 μg/kg provided when extrapolated to S/N = 3:1 estimated LOD 0.4 μg/kg. However, no perspicuous peak of this pesticide could be detected when matrix-matched standards corresponding to concentration levels 1 or 0.5 μg/kg were analysed. The reason for this phenomenon might be a use of narrow mass window for detection of target analytes. Under this condition, radical elimination of chemical noise occurred resulting in an enhancement of signal-to-noise parameter (compared to unit mass resolution instruments).

3.5. Working range

The linearity of GCT instrument was tested in the concentration range of analytes 0.5–500 μg/kg, which corresponds to conceivable contamination levels of real life samples. For most of our target analytes, the working range was relatively narrow, about two orders of magnitude (see Table 1). In addition to this limitation, the range of linear relationship changed during a longer time period because of a limited lifetime of MCP (which is, unfortunately, general drawback at any TOF MS instrument employing MCP for detection of ions). Therefore, its voltage should be regularly checked (preferably always during a tuning procedure), and increased if required to ensure that the majority of ion events is counted (at least 85% of all the single ion events). As shown in Fig. 7, while non-linear calibration plot encountered at low MCP voltage setting, the increase of this value resulted in linear concentration-signal relationship over the whole measured range. One should keep in mind that the correct setting of MCP voltage is important not only for unbiased calibration but has also effect on an isotope ratio of ions and exact mass measurement as well as resolution.

Using GCT TOF MS system for the measurement of real life samples practical limitation caused by saturation of time-to-digital converter (TDC) should be always considered. This unit measures ion arrival times, which are converted directly into nominal m/z values. After registration of any such event, the TDC requires fixed dead time (4–5 ns) to recover before it can register next event. With increasing concentration of
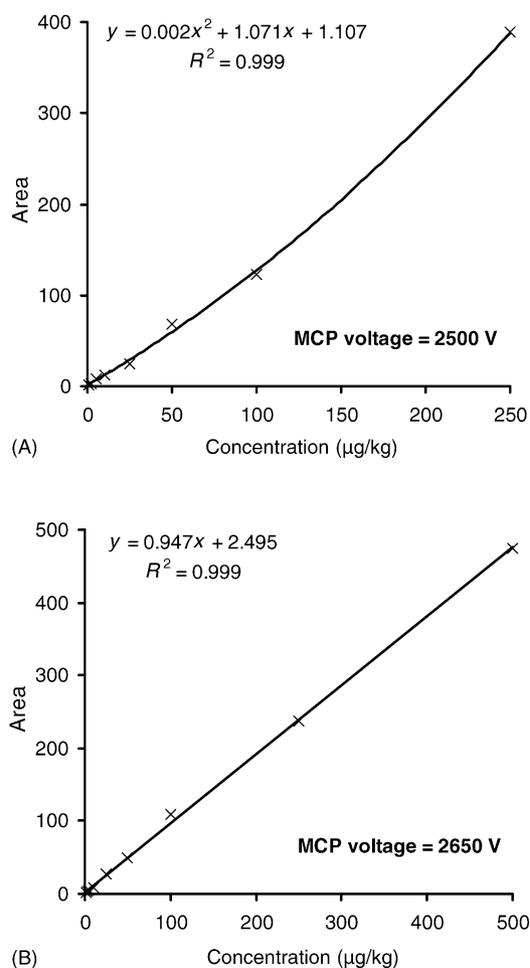

Fig. 7. Calibration curves of endosulfan sulfate at different MCP voltage setting. (A) The low MCP gain resulted in a nonlinear curve (best-fit by second-order polynom). (B) Increasing of MCP voltage provided linearity range 1–500 μg/kg.

analyte, it is more likely that some ions are not registered because of their arriving just within the dead time or simultaneously with the first recorded ion. Using dead time correction, this process shifts the centroid mass of a particular peak to higher mass by an amount resulting from its intensity and the instrument resolution. However, this correction process fails to work at too high concentration. On such conditions accurate quantification is impossible because of non-linear relationship between the intensity of analyte signal and its concentration.

During the evaluation of GCT performance three other factors were identified having influence on linear range of analytes:

(i) Number of primary (raw) spectra (*pusher interval*) per stored spectrum. Decrease of number of these transient spectra summed for each final mass spectrum (1 per second in this case) resulted in drift of linear range to higher concentration range; however, decrease of sensitivity occurred.
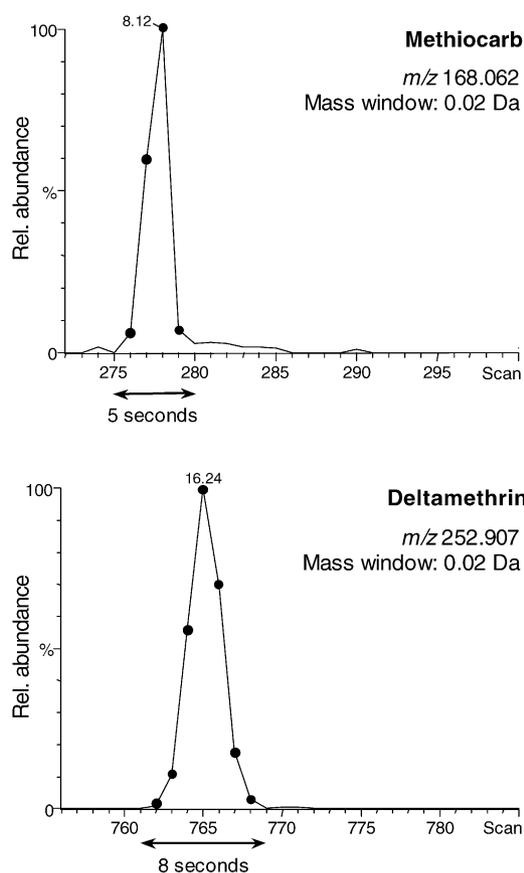
Fig. 8. Comparison of peak widths of methiocarb and deltamethrin, both analytes at concentration 100 μg/kg in peach extract.

(ii) Number of pushes multiplier (N_p *value*). This value (a sensitivity factor) is applied for correction of an inappropriate dead time correction algorithm of time-to-digital converter. Since this failure is systematic, it can be corrected supposing the used resolution of instrument is known and the number of pushes (calculated from the pusher rate and acquisition length) is modified. This can be done by experimentation (iteration) with the N_p value. Decrease of this value led in our experiments to reduction of the linear range.

(iii) Width of a chromatographic peak. The linear range is rather wider for analytes with wider width of peak. This fact is illustrated in Fig. 8 where methiocarb and deltamethrin are presented as examples. The narrower chromatographic peaks in the case of methiocarb resulted in a very narrow linear range. On the contrary, in the case of deltamethrin the wider chromatographic peaks led to increase of linear range (see Table 1).

Finally, it must be also noticed that linear range of calibration curve obtained for a single mass (quantification ion) was at least two orders of magnitude for most of analysed compounds. However, at the same time one must accept that for regulation purpose the use of only one identification ion of target analyte does not comply to quality requirements approved by the EU, which require the minimum two ions of $m/z > 200$, or three ions of $m/z > 100$ for confirmation purposes [20]. Adhering to these rules, the concentration working range available for confirmation purpose is rather limited and strongly depends on fragmentation pattern of particular compound. As far as ions with comparable intensity to that of quantification ion are lacking in mass spectrum (i.e. due to low intensities cannot be detected) then reliable confirmation can be achieved only at higher concentration of particular analyte. However, one should be aware of increase of risk of exceeding linear range for the most intensive ion at high concentrations.

3.6. Repeatability of responses

Repeatability of responses was determined as a relative standard deviation (R.S.D., %) from six repetitive analyses of peach matrix-matched standard (50 μg/kg) using 1 Hz, i.e. the lowest spectral acquisition rate. R.S.D.s of peak areas of target analytes were in range 2.7–8.1% (see Table 1). As discussed earlier, the slower is the acquisition rate the lower LOD can be attained. This study clearly demonstrates the possibility to employ 1 Hz acquisition rate for reliable measurements of pesticide residues.

3.7. Repeatability of retention times

Repeatability of retention times was determined within the same batch as a repeatability of responses. Except of methamidophos with R.S.D. 0.25%, the R.S.D.s of other analytes were below 0.04%.

3.8. Exact mass deconvolution

The ChromaLynx deconvolution software offers a unique opportunity for identification and semi-quantification of analytes in complex mixtures. Thanks to the absence of spectral skew (see Section 1) it enables a location, peak detection and generation of "clean" mass spectra of co-eluting peaks, therefore, their chromatographic resolution is not a limiting factor supposing the retention time and shape of overlapping peak is not absolutely identical. Library search against commercially available libraries is possible even for overlapping peaks. In addition, thanks to the exact mass measurement, calculation of elemental composition from deconvoluted spectra is possible (see Section 1).

The capability of this software to identify pesticides contained in matrix-matched standard at concentration 50 μg/kg was tested employing various acquisition rates (1, 2, 3, 4 and 5 spectra/s). Almost 50% of present pesticides were identified when the highest tested acquisition rate (5 spectra/s) was employed; as an example Fig. 9 shows identification of heptachlor. The decrease of spectral acquisition rates led to a reduced number of identified analytes. Considering all the
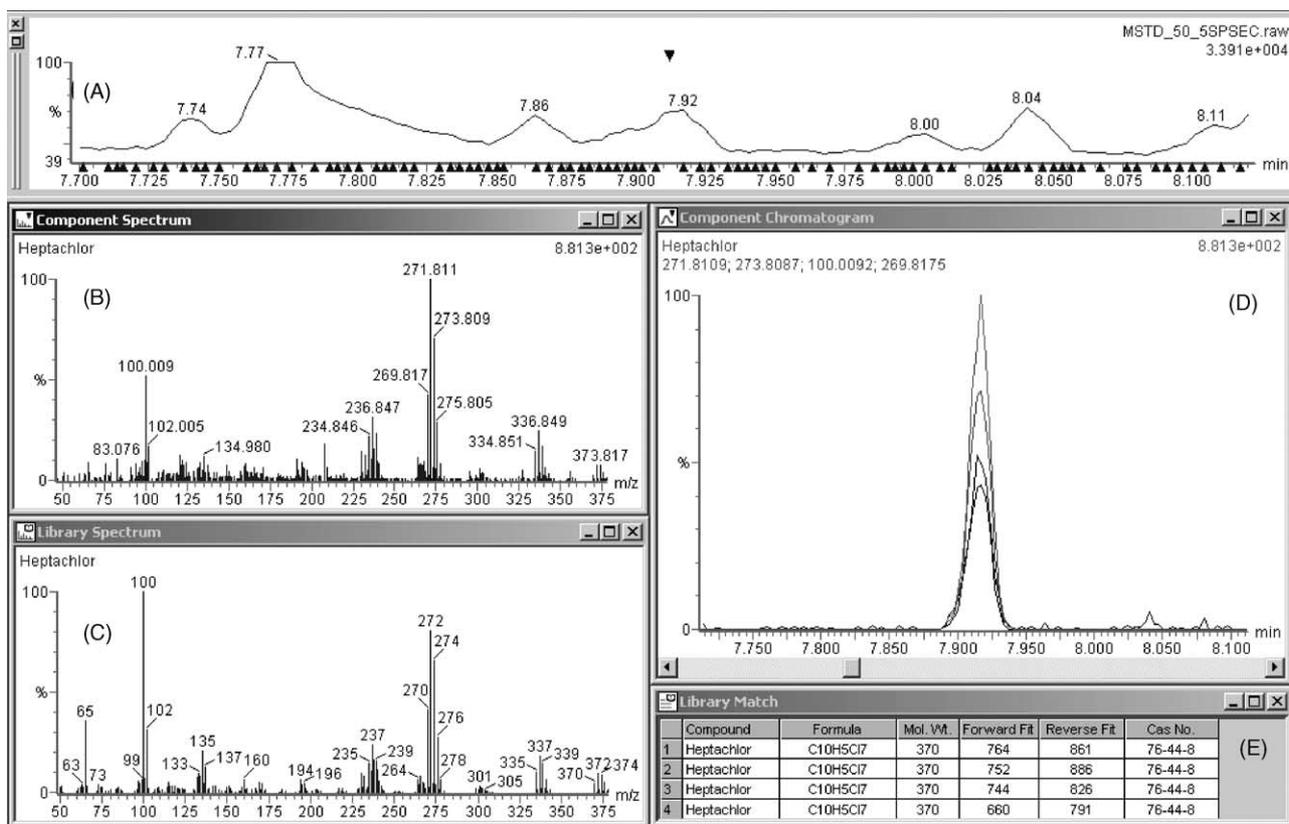
Fig. 9. Heptachlor in GPC purified peach extract spiked at 50 µg/kg. The data were acquired at 5 spectra/s. (A) Total ion chromatogram with identified compounds (marked as bottom triangles) and with selected heptachlor (marked as a top triangle); (B) deconvoluted mass spectrum of heptachlor from peach extract; (C) library mass spectrum of heptachlor; (D) overlap of four main ions of heptachlor used for deconvolution; (E) library match with reverse factor more than 800; (F) non-deconvoluted mass spectrum of heptachlor from peach extract.

relevant facts, it is obvious that the conditions required for reliable identification and settings required for highly sensitive detection of analytes at trace level are rather contradictory in this respect.

The unique features of employed software are seen mainly in non-target screening of components occurring in complex mixtures at sufficiently high levels that allow faster acquisition rates (5–10 spectra/s). Although the most of pesticide residues at concentration level 50 µg/kg were not automatically identified by the deconvolution software, the identity of many abundant matrix components not completely removed from crude peach extract by purification procedure was reported.

4. Conclusions

In this study, high-resolution time-of-flight mass spectrometer GCT has been demonstrated as a powerful tool for reliable detection and accurate quantification of pesticide residues even at very low concentration levels. The performance characteristics obtained by GC–TOF MS in many respects exceed those achievable by conventional MS analysers. Among many conceivable uses not only safe control of common multiresidue limits (MRLs) but also residue limits as low as 0.01 mg/kg required by the EU legislations for cereal-based foods and baby foods for infants and young children is possible.

Acknowledgements

References

[1] J. Hajšlová, J. Zrostlíková, J. Chromatogr. A 1000 (2003) 181.
[2] C. Herbert, R. Johnstone, Mass Spectrometry Basics, CRC Press, Boca Raton, FL, 2002.
[3] M. Powell, in: A.J. Handley, E.R. Adlard (Eds.), Gas Chromatographic Techniques and Applications, CRC Press, Boca Raton, FL, 2001, p. 164.
[4] LECO Pegasus GC–TOF MS, specification note, LECO Corporation, USA, 2001.
[5] Micromass GCT, Specification note, Waters, UK, 1999.
[6] Tempus TOF MS, Specification note, ThermoFinnigen, UK, 2002.
[7] M. Guilhaus, J. Mass Spectrom. 30 (1995) 1519.
[8] M. Guilhaus, Spectrochim. Acta B 55 (2000) 1511.
[9] K. Maštovská, S.J. Lehotay, J. Chromatogr. A 1000 (2003) 153.
[10] J. Hopkins, Anal. Chem. News Features (1999) 445A.
[11] Commission Directive 2003/13/EC of 10 February 2003 amending Directive 96/5/EC on processed cereal-based foods and baby foods for infants and young children, Off. J. Eur. Commun. L41 (2003) 33.
[12] P. Sandra, B. Tienpontand, F. David, J. Chromatogr. A 1000 (2003) 299.
[13] J.L. Martínez Vidal, F.J. Arrebola, M. Mateu-Sánchez, J. Chromatogr. A 959 (2002) 203.
[14] J. Quintana, I. Martí, F. Ventura, J. Chromatogr. A 938 (2001) 3.
[15] M. Krahmer, K. Fox, A. Fox, Int. J. Mass Spectrom. 190–191 (1999) 321.
[16] R.J.C.A. Steen, I.L. Freriks, W.P. Cofino, U.A.Th. Brinkman, Anal. Chim. Acta 353 (1997) 153.
[17] G.F. Bauerle Jr., K.L. Ray, J.S. Brodbelt, Anal. Chim. Acta 317 (1995) 137.
[18] J. Dallüge, P. Roose, U.A.Th. Brinkman, J. Chromatogr. A 970 (2002) 213.
[19] F. Baumann, E. Herlicska, A.C. Brown, J. Blesch, J. Chromatogr. Sci. 7 (1969) 680.
[20] A. Amirav, H. Jing, Anal. Chem. 67 (1995) 3305.
[21] European Commission, Quality control procedures for pesticide residues analysis — guidelines for residues monitoring in the European Union (prepared by A.R.C. Hill), second ed., European Commission document SANCO/3103/2000, Brussels, 2000.