MEMS-enabled miniature triple quadrupole mass spectrometer

Steven Wright,^{*,(1)} Andrew Malcolm,⁽¹⁾ Christopher Wright,⁽¹⁾ Shane O'Prey,⁽¹⁾ Edward Crichton,⁽¹⁾ Neil Dash,⁽¹⁾ Richard W. Moseley,⁽¹⁾ Wojciech Zaczek,⁽¹⁾ Peter Edwards,⁽¹⁾ Richard J. Fussell,⁽²⁾ Richard R. A. Syms.⁽³⁾

⁽¹⁾ Microsaic Systems plc, Unit 2, GMS House, Boundary Rd, Woking, Surrey, GU21 5BX, UK.

⁽²⁾ Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK.

⁽³⁾ Department of Electrical and Electronic Engineering, Imperial College, Exhibition Rd, London, SW7 2AZ, UK.

*Corresponding author:

Tel +44 (0)1483 751 577 Email swright@microsaic.com

Abstract

Miniaturized mass spectrometers are becoming increasingly capable, enabling the development of many novel field and laboratory applications. However, to date, triple quadrupole tandem mass spectrometers, the workhorses of quantitative analysis, have not been significantly reduced in size. Here, the basis of a field-deployable triple quadrupole is described. The key development is a highly miniaturized ion optical assembly in which a train of six microengineered components are employed to generate ions at atmospheric pressure, provide a vacuum interface, effect ion guiding, and perform pre-filtering, fragmentation and mass analysis. Despite its small dimensions, the collision cell efficiently fragments precursor ions and yields product ion spectra that are very similar to those recorded using conventional instruments. The instrument has been used to detect Thiabendazole, a common pesticide, in apple pulp at a level of 10 ppb.

Introduction

Modern tandem mass spectrometers have femtogram detection limits [1-3], but are bulky, expensive, and power-hungry instruments that are often operated as central facilities. However, there are important applications that do not demand such high sensitivity and could potentially be better served by smaller, portable instruments with high selectivity. Well-known examples include point-of-care diagnostics, environmental monitoring, security, and drugs detection [4-9]. Another application is food safety, where there is a need for portable instrumentation capable of rapid, accurate, low-cost, detection of pesticides, veterinary drugs, and adulterants [10]. The increasing globalization of food supply, high profile food contamination cases, and regional variations in food safety standards have focussed attention on the need for testing at all points in the supply chain [11-13]. It is impractical for central facilities to analyze more than a small fraction of the foods reaching consumers. As the levels of contamination significant for human health are relatively high, more widespread testing could be provided by field-deployable, miniaturized tandem mass spectrometers.

Tandem mass spectrometry involves selection of a precursor ion, fragmentation of the precursor, and analysis of the fragment ions. Selectivity and sensitivity are enhanced, compared with a single-stage instrument, because the fragmentation pathways are characteristic of a particular molecule or class of molecule, and the initial precursor selection rejects other matrix components that would otherwise contribute to the chemical noise floor. Analysis may be performed using a tandem-in-space arrangement, such as a triple quadrupole, in which precursor selection, fragmentation, and mass analysis are each performed in separate sub-systems. Alternatively, the same processes can all be performed within an ion trap as a tandem-in-time sequence.

The origins of tandem mass spectrometry can be traced to studies of ion-molecule collisions in the 1960s and 1970s [14]. Early systems used magnetic sector analyzers [15], and collisions with a target gas occurred in drift sections or gas cells. Triple quadrupoles were initially developed to study the photofragmentation of selected precursors [16-17]. Apart from their reduced size, a key feature was the use of an intermediate rf-only quadrupole to guide product ions. Triple quadrupoles evolved into their modern form through the work of Yost and Enke in collaboration with Morrison and co-workers [18, 19], who introduced a collision gas into the rf-only quadrupole. They observed efficient fragmentation and rich, structurally diagnostic product ion spectra at low collision energies, leading to widespread use of the technique. Later, it was shown that fragmentation could also be performed in an ion trap, when Lourié et al. [20] demonstrated that energetic collisions between selected trapped ions and the buffer gas could be induced by applying an auxiliary ac waveform.

Previous attempts at miniaturizing tandem mass spectrometry have involved ion traps with simplified geometries [4, 21-23]. Although the miniaturization of quadrupole filters has been challenging [24-32], compact single quadrupole instruments have now been developed [33, 34], but so far there have been no attempts to extend the technology to a triple quadrupole. This is a potentially significant deficiency, as triple quadrupoles are preferred for some applications. The relative merits of triple quadrupoles and ion traps have been extensively discussed elsewhere [35, 36] and are briefly summarized below.

If a full product ion spectrum is required, ion traps offer better sensitivity than triple quadrupoles, whose sensitivity is compromised by the need to step through the signal at each m/z value in turn, while ions of all other m/z values are rejected. If, however, the analysis may be targeted, triple quadrupoles may be operated using the multiple reaction monitoring

(MRM) mode in which only the m/z values of specific product ions are monitored, leading to a duty cycle that may be better than that of an ion trap.

Triple quadrupoles are also better suited to chromatography applications. Traps are susceptible to space-charge effects that manifest as resolution degradation and peak position errors at elevated ion densities. Consequently, to prevent over-filling as the ion flux changes during a chromatographic peak, ion traps must perform a short pre-scan before each sample to determine the flux and set the trapping period accordingly. The resulting irregular sampling leads to a high density of points at the peak but sparse sampling and poor definition of its leading and trailing edges. Triple quadrupoles are less susceptible to space charges, allowing a constant sampling rate and more accurate peak integration.

Triple quadrupoles are also able to perform precursor and neutral loss scans. These useful scan modes allow highly selective detection of similar compounds, and may for example be used to detect a series of drug metabolites in plasma [37]. Ion traps are unable to execute precursor and neutral loss scans, but achieve high selectivity by deconstructing molecules in a controlled sequence. As fragmentation is initiated by resonant excitation of specific precursor ions, the process is well controlled and may be applied to a selected fragment in successive generations of product ions (MS^n). In triple quadrupoles, however, fragmentation occurs through a collision cascade involving numerous uncontrolled pathways.

Ion traps are unable to retain product ions with m/z values below the molecular weight cut-off, which is typically one-third of the precursor ion m/z value. This complicates the analysis of some compounds, including tramadol, for example, which yields a diagnostic fragment ion at m/z 58 following selection of the $[M+H]^+$ precursor ion at m/z 264 [38]. There are no fundamental limits to the lowest m/z value that can be accessed by triple quadrupoles.

A miniaturized triple quadrupole would be particularly suited to applications requiring chromatographic separation and targeted quantitative analysis of known analytes. Here we demonstrate a linear triple quadrupole employing microengineered components to carry out all the key steps in tandem mass spectrometry, namely atmospheric pressure ionization, coupling into vacuum, ion guiding, collision-induced fragmentation, and mass analysis. A total of 6 microengineered devices are involved (not including pre- and post-filters), the highest number combined together in any instrument to date. In the following sections, the design, characteristics, and analytical performance of a highly miniaturized ion optical bench - the core of a future compact system - will be described.

Experimental Section

Vacuum system and ion optics bench

Fig. 1a is a schematic of the ion optics bench. The microspray ion source, vacuum interface, quadrupole ion guides (Q0 and Q2), and quadrupole ion filters (Q1 and Q3) are separate microengineered components. The pneumatically assisted source generates a plume of ions that impinges on the vacuum interface. The ion flux is drawn into the first vacuum stage through an entrance capillary, together with neutrals. The differentially pumped interface is a flow partitioning device, allowing a fraction of the gas and entrained ions to pass through an exit capillary. Ions transmitted to the second vacuum stage pass first through two electrostatic focussing elements, L0 and L1. The ion beam is then collisionally cooled and focussed in a 20 mm long quadrupole ion guide, Q0. While most of the ions are retained, neutrals are removed using the pump that evacuates the second stage. A narrow and thermalised ion beam passes through the inter-quad lens, L2, and into the first of the resolving quadrupoles, Q1. L2 also serves as a pressure bulkhead between the second and third vacuum stages. Precursor ions are selected by Q1 and then enter the collision cell

through an aperture, where they undergo energetic collisions with Ar atoms. A 20 mm long quadrupole ion guide, Q2, retains precursor and product ions as they transit the cell. The ions exit through a second aperture and are analysed by the second quadrupole, Q3. The ion detector consists of a conversion dynode and a conventional channeltron.

As the ions are thermalised during transit of the first ion guide, Q0, the collision energy is determined by the potential difference between Q0 and the collision cell. Hence, the collision energy is varied by applying a bias offset to Q0 and the vacuum interface optics while keeping the collision cell bias fixed.

A 5 L/min diaphragm pump evacuates the vacuum interface, and two turbomolecular pumps backed by a second 5 L/min diaphragm pump evacuate the second and third vacuum stages. The availability of compact, lightweight pumps has been key to the development of miniature mass spectrometers [22, 33, 34, 39]. However, since the sensitivity obtained using atmospheric pressure ionization is set by the amount of gas and entrained ions that can be drawn into vacuum, the limits of detection must reflect their small size. The most sensitive conventional tandem instruments can detect less than a femtogram, but only using bulky floor standing rotary pumps, typically with a speed of 500-1000 L/min and weighing over 40 kg.

Photographs of the ion source, vacuum interface, quadrupole mass filter, and quadrupole ion guide are shown in Fig. 1(b). These components were fabricated using micro-electro-mechanical systems (MEMS) technology; this generally involves a series of planar processing steps (often silicon-related) that selectively add or remove material. Complex structures can be built-up by etching both sides of the wafer, growing insulating oxide layers, selective metalization, and anodic bonding to glass wafers. As the design, fabrication, and performance characteristics of the MEMS components used here have been described in detail elsewhere [32, 33, 40, 41], only a brief overview is given.

Ions are generated by a low-voltage microspray ion source operating at a flow rate of 0.25 to 2 $\mu\text{L}/\text{min}$. In conventional systems, the vacuum interface serves as the counter electrode. The device used here incorporates etched silicon features to align a metal spray capillary relative to an integrated counter electrode. As the emitter tip is fixed at the optimum position, no further adjustment is necessary. For convenient handling, the microspray chip is mounted on a polymer cartridge. A threaded stainless steel fitting provides a fluidic coupling and a connection to the high voltage supply, while a stainless steel collar connects the counter electrode to ground. Nebulizing gas is supplied via a coaxial channel.

The vacuum interface measures 1 cm in diameter and is constructed from two circular silicon dies that are etched so that an internal cavity is formed when they are bonded together back-to-back. Radial channels leading to the perimeter allow differential pumping of the cavity. Ions generated by the microspray are drawn in through a central capillary together with nebulizing gas. Much of the gas and entrained ions is pumped away from the cavity while the remainder passes through an exit capillary into the second vacuum stage.

The 53 mm long quadrupole mass filter consists of conventionally manufactured cylindrical rods held in a precision mount fabricated using MEMS techniques. The four main rods are mounted in pairs on two glass plates that are accurately positioned using six ball-and-socket mounts. Etched silicon features bonded to the glass plates support the rods and engage with the spacer balls. The radius of the kissing circle touching the four rods is 1 mm. The glass plates additionally support sets of short pre-filter and post-filter rods, which prevent defocusing of the ion trajectories by fringing fields. The 20 mm long ion guides are of a similar construction, but do not incorporate pre- and post-filters.

A photograph of the ion optics bench is shown in Fig. 1(c). This piston-like structure is accommodated in the circular bore of the vacuum manifold, and may be entirely removed

as all electrical connections are made at the rear with pin and socket connectors. Flat rubber gaskets and O-rings separate the different pumping stages when the piston is inserted. The central O-ring provides support only and is not a pressure bulkhead.

The microspray cartridge screws into an elbow receiver with mating electrical, fluidic and gas connectors. This assembly engages with the vacuum interface flange and is held in place by a collar nut (far left of Fig. 1(c)). A static fluidic split is also provided to allow coupling to liquid chromatography systems operating at conventional flow rates. The microengineered vacuum interface is concealed within the stainless steel interface flange, but may be accessed without removing the piston assembly.

Ceramic printed circuit boards (PCBs) support the quadrupoles and ion detector. Each PCB carries printed metal tracks on top and bottom surfaces that provide electrical connections, laid out to minimize electrical interference and breakdown. The use of PCBs to support mass spectrometry components has proven to be a convenient means of providing electrical connections and secure, accurate mountings [42]. Standard push-fit electrical connectors allow modular construction and easy replacement of individual components.

Q2 is located in the stainless steel collision cell between Q1 and Q3. A fluorinated ethylene propylene (FEP) tube supplies Ar gas from a supply line routed through the piston frame. Ions enter and exit the cell through 0.7 mm diameter apertures. Diaphragm lenses are provided immediately before and after the cell to focus the ion beam. For the m/z 195 fragment of reserpine, transmission through Q2 is optimized when the rf amplitude is set at 100 V (0-pk), which corresponds to q (the Mathieu stability parameter) equal to ≈ 0.25 . There is no provision for an axial electric field in Q2, which is often used to sweep ions out of the collision cell. Several methods can be used to generate a suitable field, including segmented,

tilted, or conical rods [43-46]. A segmented-rod quadrupole of a similar size to Q2 has previously been used to accelerate and fragment ions in a time-of-flight instrument [47].

Test system

Testing was undertaken using a breadboard development platform. A commercial single quadrupole (4000 MiD, Microsaic Systems plc, Woking, UK) was used as a basis, providing a built-in computer, high voltage supplies for the dynode, channeltron detector, and microspray, a nebulizing gas flow controller, turbomolecular pump controllers, an rf supply for Q3, and dc supplies for the vacuum interface. The vacuum chamber, turbomolecular pumps, and rf amplifiers for Q1 and Q2 were suspended on a frame above the donor chassis.

Further dc power supplies and an auxiliary computer control additional functions. A rack of dc power supplies (MST 36-5M, Kepco, Flushing, NY) controlled through a GPIB interface (PCI-GPIB, National Instruments Corporation, Austin, TX) hosted by the auxiliary PC provided biases to the prefilters, postfilters and collision cell. Additional voltages from a digital-to-analogue converter (PCIM-DDA06/16, Measurement Computing Corporation, Norton, MA) control the rf amplitudes and main rod biases for Q1 and Q2. The built-in computer retained control of Q3 scanning and data acquisition. All three rf amplifiers were driven at the same frequency using a signal generator. The frequency was tuned to the resonance frequency of Q3 in combination with its rf amplifier whereas the Q1 and Q2 amplifiers were brought into resonance by high voltage trim capacitors.

Preparation of apple extracts

Samples were prepared using the QuEChERS (Quick-Easy-Cheap-Rugged-Safe) technique [48]. Whole fruit was diced, frozen and homogenized with a food blender. A 10 g sample was mixed with 10 mL of acetonitrile and shaken vigorously for 1 minute. Solid

reagents (4 g magnesium sulphate, 1 g sodium chloride, 0.5 g sodium citrate dibasic sesquihydrate, 1 g sodium citrate tribasic dehydrate) was added to adjust the pH and induce phase separation of the organic and aqueous components. After shaking for 1 minute, the mixture was centrifuged. The supernatant organic layer was mixed with magnesium sulphate (0.9 g) and a dispersive solid phase extraction agent (0.15 g PSA) to remove unwanted co-extractives. After shaking for 30 seconds, the mixture was centrifuged again. A sample of the clear acetonitrile solution was then taken for analysis.

To mimic contaminated fruit samples, organically produced, pesticide free apples (*malus pumila*) were spiked with known amounts of thiabendazole at the start of the extraction process, *i.e.* when the homogenized sample is first agitated with acetonitrile. For example, a sample spiked at 100 ppb is prepared by adding 1 µg of thiabendazole to the initial 10 g sample of homogenized apple pulp.

Chemicals

Reserpine ($\geq 98\%$), thiabendazole (99.8%), verapamil hydrochloride ($\geq 99\%$), formic acid (98%), methanol (HPLC grade), acetonitrile (HPLC grade), and water (HPLC grade) were all purchased from Sigma-Aldrich, Poole, UK. The QuEChERS extraction reagents were also obtained from Sigma-Aldrich as pre-mixed formulations (Supel QuE citrate (EN) and PSA (EN) tubes). Argon (99.998%) was supplied by Air Liquide UK Ltd, Birmingham, UK.

Results and Discussion

Precursor selection

To achieve high selectivity, it is essential that the desired mono-isotopic precursor ion is transmitted by Q1 while other components are rejected. In Fig. 2, the left-hand spectrum corresponds to mass analysis of the reserpine $[M+H]^+$ ion with the instrument configured as a single quadrupole. The Ar supply to the collision cell was turned off and Q1 was operated in rf-only (all-pass) mode while Q3 was scanned. The mono-isotopic ion appears at m/z 609, while the ^{13}C isotope peaks are observed at m/z 610 and m/z 611 with the expected intensities. The right-hand spectrum was obtained with the instrument configured as a tandem mass spectrometer. Q1 was operated in mass resolving mode and set to transmit m/z 609 with unit resolution (FWHM ~ 0.7 m/z units) while Q3 was scanned, also with unit resolution. Ar was introduced into the collision cell, but the collision energy was kept low to limit fragmentation. No evidence of m/z 610 or m/z 611 can be seen in the spectrum, indicating that the desired precursor had been correctly isolated by Q1.

Collision cell

The key parameters governing fragmentation and product ion yields are the collision energy and the target gas pressure. It is demonstrated below that the miniaturized collision cell yields rich product ion spectra at conventional collision energies and can transmit ions with high efficiency when the gas pressure is optimized.

A series of product ion spectra from the fragmentation of reserpine at collision energies from 20 to 35 eV are shown in Fig. 3. Both Q1 and Q3 were operated at unit resolution. At 20 eV collision energy, there is little evidence of fragmentation and the spectrum is dominated by the precursor ion at m/z 609. However, at 30 and 35 eV,

fragmentation is extensive and prominent product ions are observed at m/z 174, 195, 236 and 397. Essentially identical spectra have been recorded using conventional triple quadrupoles at similar collision energies [49, 50]. It should be noted that the low-mass product ions appear below one third of the precursor ion m/z value and would therefore not be seen with an ion trap in MS/MS mode.

Fig. 4 shows how precursor and product ion intensities depend on the collision cell gas pressure. To simplify the analysis, thiabendazole, which yields only two major product ions, was used. The collision energy was set at 24 eV, as this results in an approximately uniform distribution of precursor and product ions at the optimum pressure. No attempt was made to measure the collision cell pressure, as the additional fittings and electrical connections would add considerable complexity. Instead, the pressure was calculated using the relationship $\Delta P = Q/C$, where ΔP is the pressure difference between the collision cell and the surrounding vacuum, C is the combined conductance of the entrance and exit apertures, and Q is the Ar gas flow. Assuming molecular flow (valid for cell pressures less than ≈ 0.1 Torr), the conductance may be calculated using $C=0.25vA$, where A is the area of the apertures, and v is the average velocity of argon. Q may be determined from the pressure increase in the third vacuum stage and the pumping speed of the turbomolecular pump. **What about v ?**

Also shown in Fig. 4 is the signal recorded with Q3 operated in rf-only mode. With the dc components of the drive waveform removed, all ions with q values less than 0.908 are simultaneously transmitted and the signal can be taken as a measure of the total ion flux leaving the collision cell. An rf amplitude (0-pk) of just 75 V was used for these measurements, and consequently, only ions with $m/z < 40$ were excluded.

When the Ar pressure is increased, the total ion and precursor ion intensities initially decrease sharply, following the Beer - Lambert law $I = I_0 \exp(-\sigma nL)$, where σ is the collision cross-section, n is the number density, and L is the path length. In this low pressure regime, ions are scattered and lost from Q2. However, as the pressure is increased further, the intensities of all the peaks increase and pass through a maximum at approximately 0.05 Torr. This overall increase in transmitted ion flux is characteristic of collisional focusing [50-52], a process by which ions lose kinetic energy through collisions with the target gas. Under some circumstances, focusing may be interpreted as a viscous drag rather than a series of discrete collisions [53]. As the ions lose energy, their harmonic oscillations within the pseudopotential well generated by the ion guide become damped, causing accumulation of ions close to the axis. The resulting narrow beam is well suited for efficient transmission through the small aperture of the collision cell and subsequent analysis by Q3.

At 0.05 Torr, the collision gas thickness (the product of gas density and the cell length) is $3.2 \times 10^{15} \text{ cm}^{-2}$, close to the value of $4 \times 10^{15} \text{ cm}^{-2}$ established as optimum for a cell with a 15 cm long conventional ion guide [50]. This result suggests that the optimum collision gas thickness may be universal, even when the cell length and pressure are changed by an order of magnitude. The same optimum has also been established for a quadrupole used to guide ions introduced into vacuum [51], but other investigations have yielded discordant results.

For example, the optimum collision gas thickness for a 40 cm long quadrupole beam cooler in a nuclear physics experiment [54] was $6.9 \times 10^{16} \text{ cm}^{-2}$, but this high value may be attributed to the use of He rather than Ar as the collision target. More intriguing is an investigation of the characteristics of a 7 cm long quadrupole ion guide [54], which showed that the optimum pressure for transmission of angiotensin III and its fragments is in excess of

0.3 Torr, equivalent to a collision gas thickness of $6.8 \times 10^{16} \text{ cm}^{-2}$. Although the use of dry air as the buffer accounts for some loss of efficiency, this value still seems anomalous.

It can be concluded from the rf-only signal that at 0.05 Torr the total ion flux recovers to 75% of the value measured with no Ar supply. At 0.05 Torr, the ion flux contains fragment and surviving precursor ions whereas only precursor ions are present at base pressure. Although the transmission should then be high, it is unlikely to be 100%, as some of precursor ions are lost through scattering with the residual gas. A previous study [50] has reported efficiencies of 30-50% for a conventional collision cell. However, the calculation was based on resolved peak heights rather than total ion measurements, which convolutes the transmission and filtering characteristics of Q3 with the ability of the collision cell to transmit ions.

Sensitivity and dynamic range

Flow injection analysis (FIA) was used to assess sensitivity and dynamic range. A loop injection valve was used to inject 2 μL aliquots of stock reserpine solutions into a solvent stream flowing at 200 $\mu\text{L}/\text{min}$. A 70:30:0.1 mixture of acetonitrile, water, and formic acid was used as the carrier solvent and the diluent for stock solutions. The fluidic split was set for a ratio of 300:1. Seven injections were performed for each stock solution, including a blank solution with no reserpine. The intensity of the m/z 609 $\rightarrow$ 195 transition was monitored with a dwell time of 200 ms and the collision energy of 30 eV. Each signal transient was 3-4 s wide. Fig. 5 shows a plot of signal transient area against concentration. The detection limit is 5 ng/mL ($S/N=3$, peak-to-peak definition) and the response is linear over four orders of magnitude (5 ng/mL to 30 $\mu\text{g}/\text{mL}$). At the detection limit, 10 pg of reserpine was injected, but only 30 fg was passed to the mass spectrometer.

Coupling to liquid chromatography

There has been considerable progress in ambient ionization techniques and direct analysis protocols that allow mass analysis without chromatographic separation. This is particularly important for field applications, since the overall system size is significantly reduced and sample preparation becomes less onerous. However, at present, the vast majority of methods, including those relating to food safety, dictate the use of separation prior to mass spectrometry. For field use, a miniaturized triple quadrupole should ideally be coupled to a miniaturized separator. However, for initial evaluation, a conventional high performance liquid chromatography (HPLC) system (1100 Series, Agilent, Santa Clara, CA, USA) with an Ascentis Express C18 LC column (10 cm x 0.46 cm, Sigma-Aldrich) was used.

Fig. 6 shows a chromatogram obtained following injection of 1 ng of verapamil. The signal corresponds to the intensity of the m/z 455 $\rightarrow$ 165 transition (34 eV collision energy), which was monitored with a dwell time of 200 ms. The solvent gradient of acetonitrile and water, both with 0.1% formic acid, was set so that the organic component was 10% at 0 mins, 10% at 2 mins, 100% at 9.5 mins, 100% at 11.5 mins, 10% at 13.5 mins, and 10% at 16.5 mins. Five consecutive injections yielded peak areas with a relative standard deviation (RSD) of 5.1%. The signal-to-noise ratio is 100:1 using the peak-to-peak definition, and 540:1 using the rms definition. Of the 1 ng injected, only 1 pg was passed to the mass spectrometer, as the static split was reduced to 1000:1 to accommodate a column flow rate of 0.8 mL/min. From the area of the peak in Fig. 6, which gives the total number of ion counts resulting from 1 pg of verapamil in solution, the probability of detection is 2×10^{-5} at m/z 165, within the range of values reported for conventional instruments [50, 56].

Detection of pesticide in apple pulp

A potential application of a field-deployable triple quadrupole is the monitoring of pesticide residues. To demonstrate this application, the instrument was used to detect and quantify thiabendazole (a common pesticide used to control fungal disease) in apples. Acetonitrile extracts were prepared from whole apple pulp using the earlier QuEChERS technique.

Fig. 7(a) shows MRM chromatograms for the m/z 202 $\rightarrow$ 175 and m/z 202 $\rightarrow$ 131 transitions. The intensities were monitored cyclically using a dwell time of 200 ms and a collision energy of 24 eV. The mass spectrometer was coupled to an Agilent 1100 HPLC as described above, but methanol was used in place of acetonitrile as the solvent since thiabendazole elutes very early with acetonitrile. The gradient was programmed so that the organic component was 10% at 0 mins, 10% at 2 mins, 100% at 9.5 mins, 100% at 11.5 mins, 10% at 13.5 mins, and 10% at 16.5 mins. The samples were diluted with water (1:1) to improve the peak shapes and better match the sample solvent to the initial gradient composition. Five injections were performed for each sample.

Distinct peaks are observed for both transitions at a retention time of 6.2 minutes, even for the 10 ppb sample. Despite the complex nature of the biological matrix, the noise floor is very low (less than 20 counts per minute for the m/z 202 $\rightarrow$ 175 transition). The peak area repeatability for five consecutive injections, expressed as the relative standard deviation (RSD), is 5.3 % for the 100 ppb sample and 17.0 % for the 10 ppb sample. Retention times are stable to within ± 2 seconds.

To check the recovery efficiency of the QuEChERS extraction, matrix-matched calibration samples were prepared by adding known amounts of thiabendazole to unspiked apple extract. A calibration graph covering the range of interest is shown in Fig. 7(b). As an internal standard was not used, absolute peak areas rather than peak area ratios are plotted

against concentration. Using this calibration, the concentration of thiabendazole obtained from apple pulp spiked at 100 ppb was 117 ng/mL. Given that the volume of acetonitrile used was 10 mL, it follows that a total of 1170 ng was present in the final extract. Hence, the calculated recovery is 117%, since 1000 ng was originally added to 10 g of apple pulp. This is within the range (60-140%) specified in the EU guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed [57].

Biological matrices are known to cause contamination of vacuum interfaces, resulting in a loss of sensitivity over time. During the testing described above, fifty-four injections of apple extract, each of 5 μ L, were performed over a period of two weeks. A regular check revealed that the sensitivity decreased by 25% during this time. On removal of the vacuum interface component, some light fogging of the originally pristine surface could be seen.

Conclusions

A miniaturised triple quadrupole has been demonstrated for the first time, using MEMS components. The instrument has all the characteristics of conventional triple quadrupoles, and allows precursor ions to be selected with unit resolution and fragmented with high efficiency, yielding the expected product ion spectra. The key new feature – a MEMS collision cell – has been investigated and it has been shown that short length can be compensated using high gas pressure to achieve the required collision gas thickness.

One consequence of miniaturization is reduced sensitivity, because small pumps limit the flow of gas and entrained ions that may be drawn into vacuum. Despite this, it has been demonstrated that a pesticide can be detected at a level of 10 ppb in a biological matrix. Because most maximum residue limits are higher, this result implies the instrument is potentially suitable for food safety testing. In any case, the applications that could be

addressed by a portable triple quadrupole should be viewed from the perspective of the complete analytical process, including sample preparation. In many cases, significant analyte enrichment and stripping of matrix components can be achieved through the use of solid phase extraction media, which are often available in formats suitable for field use.

Acknowledgements

This work was funded by the European Commission as part of the FP7 Programme (ARROWS project). Helpful discussions with Charles Smith, Consultant, are gratefully acknowledged.

References

1. Thomson, B. *Genet. Eng. Biotechnol. N.* **2012**, 32, 17.
2. Bayen, S.; Yi, X.; Segovia, E.; Zhou, Z.; Kelly, B. C. *J. Chromatogr. A*, **2014**, 1338, 38-43.
3. Sun, L.; Li, H.; Willson, K.; Breidinger, S.; Rizk, M. L.; Wenning, L.; Wolf, E. J. *Anal. Chem.* **2012**, 84, 8614-8621.
4. Li, L.; Chen, T.-C.; Ren, Y.; Hendricks, P. I.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* **2014**, 86, 2909-2916.
5. Chen, T.-C.; Ouyang, Z. *Anal. Chem.* **2013**, 85, 1767-1772.
6. Kumano, S.; Sugiyama, M.; Yamada, M.; Nishimura, K.; Hasegawa, H.; Morokuma, H.; Inoue, H.; Hashimoto, Y. *Anal. Chem.* **2013**, 85, 5033-5039.
7. Smith, J. N.; Noll, R. J.; Cooks, R. G. *Rapid Commun. Mass Spectrom.* **2011**, 25, 1437-1444.

8. Hendricks, P. I.; Dalglish, J. K.; Shelley, J. T.; Kirleis, M. A.; McNicholas, M. T.; Chen, T. C.; Chen, C.-H.; Duncan, J. S.; Boudreau, F.; Noll, R. J.; Denton, J. P.; Roach, T. A.; Ouyang, Z.; Cooks, R. G. *Anal. Chem.* **2014**, *86*, 2900-2908.
9. Wells, J. M.; Roth, M. J.; Keil, A. D.; Grossenbacher, J. W.; Justes, D. R.; Patterson, G. E.; Barket Jr, D. J. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1419-1424.
10. Krska, R.; Suman, M.; Janssen, H.-G.; Pico, Y.; Nielen, M. *The Analytical Scientist*, Issue 10, **2013**, 34-39.
11. Ensuring Safe Foods and Medical Products Through Stronger Regulatory Systems Abroad, Eds. Riviere, J. E.; G. J. Buckley, G. J., National Academies Press, Washington DC, **2012**.
12. Xin, H.; Stone, R. *Science*, **2008**, *322*, 1310-1311.
13. Commission decision of 20 June 2003 on emergency measures regarding hot chilli and hot chilli products (2003/460/EC), *Official Journal of the European Union*, L154/114.
14. Shukla, A. K.; Futrell, J. H. *J. Mass Spectrom.* **2000**, *35*, 1069-1090.
15. Futrell, J. H.; Miller, C. D. *Rev. Sci. Instrum.* **1966**, *37*, 1521.
16. McGilvery, D. C.; Morrison, J. D. *J. Chem. Phys.* **1977**, *67*, 368-369.
17. Vestal, M. L.; Futrell, J. H. *Chem. Phys. Lett.* **1974**, *28*, 559-561.
18. Yost, R. A.; Enke, C. G. *J. Am. Chem. Soc.* **1978**, *100*, 2274-2275.
19. Yost, R. A.; Enke, C. G.; McGilvery, D. C.; Smith, D.; Morrison, J. D. *Int. J. Mass Spectrom. Ion Phys.* **1979**, *30*, 127-136.
20. Louris, J. N.; Cooks, R. G.; Syka, J. E. P.; Kelley, P. E.; Stafford, G. C.; Todd, J. F. J. *Anal. Chem.*, **1987**, *59*, 1677-1685.
21. Laughlin, B.C.; Mulligan, C.C.; Cooks, R.G. *Anal. Chem.* **2005**, *77*, 2928-2939.
22. Gao, L.; Song, Q.; Patterson, G.E.; Cooks, R.G.; Ouyang, Z. *Anal. Chem.* **2006**, *78*, 5994-6002.

23. Jiang, D.; Jiang, G.-Y.; Li, X.-X.; Xu, F.-X.; Wang, L.; Ding, L.; Ding, C.-F. *Anal. Chem.* **2013**, *85*, 6041-6046.
24. Gear, M.; Syms, R. R. A.; Wright, S.; Holmes, A. S. *J. Microelectromech. Syst.* **2005**, *14*, 1156-1166.
25. Orient, O.J.; Chutjian, A.; Garkanian, V. *Rev. Sci. Instrum.* **1997**, *68*, 1393-1397.
26. Holkeboer, D.H.; Karandy, T.L.; Currier, F.C.; Frees, L.C.; Ellefson, R.E. *J. Vac. Sci. Technol. A* **1998**, *16*, 1157-1162.
27. Ferran R. J.; Boumsellek S. *J. Vac. Sci. Technol. A* **1996**, *14*, 1258-1265.
28. Cheung, K.; Vélasquez-García, L. F.; Akinwande, A. I. *J. Microelectromech. Syst.* **2010**, *19*, 469-483.
29. Taylor, S.; Tindall, R. F.; Syms, R. R. A. *J. Vac. Sci. Technol. B* **2001**, *19*, 557-562.
30. Vélasquez-García, L. F.; Cheung, K.; Akinwande, A. I. *J. Microelectromech. Syst.* **2008**, *17*, 1430-1438.
31. Wright, S.; Syms, R. R. A.; O' Prey, S.; Hong, G.; Holmes, A. S. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 146-156.
32. Wright, S.; O'Prey, S.; Syms, R. R. A.; Hong, G.; Holmes, A. S.; *J. Microelectromech. Syst.* **2010**, *19*, 325-337.
33. Malcolm, A.; Wright, S.; Syms, R. R. A.; Moseley, R. W.; O' Prey, S.; Dash, N.; Pegus, A.; Crichton, E.; Hong, G.; Holmes, A. S.; Finlay, A.; Edwards, P.; Hamilton, S. E.; Welch, C. J. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 3281-3288.
34. Malcolm, A.; Wright, S.; Syms, R. R. A.; Dash, N.; Schwab, M.-A.; Finlay, A. *Anal. Chem.* **2010**, *82*, 1751-1758.
35. Soler, C.; Mañes, J.; Picó, Y. *J. Chromatography A*, **2005**, *1067*, 115-125.

36. Ramanathan, R.; Çömezoğlu, S. N.; Humphreys, W. G. *Using Mass Spectrometry For Drug Metabolism Studies*, Chpt. 5, Ed.: Korfmacher, W. A., 2nd ed., CRC Press, Taylor and Francis Group, 2010.
37. Clarke, N. J.; Rindgen, D.; Korfmacher, W. A.; Cox, K. A. *Anal. Chem.* **2001**, *73*, 430A-439A.
38. Hakala, K. S.; Kostianen, R.; Ketola, R. A. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2081-2090.
39. Contreras, J. A.; Murray, J. A.; Tolley, S. E.; Oliphant, J. L.; Tolley, H. D.; Lammert, S. A.; Lee, E. D.; Later, D. W.; Lee, M. L. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1425-1434.
40. Wright, S.; Syms, R. R. A.; Moseley, R.; Hong, G.; O'Prey, S.; Boxford, W. E.; Dash, N.; Edwards, P. J. *Microelectromech. Syst.* **2010**, *19*, 1430-1443.
41. Syms, R. R. A.; Zou, H.; Bardwell, M.; Schwab, M.-A. *J. Micromech. Microeng.* **2007**, *17*, 1567-1574.
42. Borman, S.; *Chem. Eng. News*, **2006**, *84*, 45-51.
43. Javahery, G.; Thomson, B. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 697-702.
44. Mansoori, B. A.; Dyer, E. W.; Lock, C. M.; Bateman, K.; Boyd, R. K.; Thomson, B. A. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 775-788.
45. Lock, C. M.; Dyer, E. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 432-448.
46. Loboda, A.; Krutchinsky, A.; Loboda, O.; McNabb, J.; Spicer, V.; Ens, W.; Standing, K. *Eur. J. Mass Spectrom.* **2000**, *6*, 531-536.
47. Dodonov, A.; Kozlovsky, V.; Loboda, A.; Raznikov, V.; Sulimenkov, I.; Tolmachev, A.; Kraft, A.; Wollnik, H. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1649-1656.
48. Anastassiades, M.; Lehotay, S. J.; Štajnbaher, D.; Schenck, F. J. *J. AOAC Int.* **2003**, *86*, 412-431.

49. Hopley, C.; Bristow, T.; Lubben, A.; Simpson, A.; Bull, E.; Klagkou, K.; Herniman, J.; Langley, J. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 1779-1786.
50. Thomson, B. A.; Douglas, D. J.; Corr, J. J.; Hager, J. W.; Joliffe, C. L. *Anal. Chem.* **1995**, *67*, 1696-1704.
51. Douglas, D. J.; French, J. B. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 398-408.
52. Morris, M.; Thibault, P.; Boyd, R. K. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 1042-1063.
53. Lock, C. M.; Dyer, E. W. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 422-431.
54. Nieminen, A.; Huikari, J.; Jokinen, A.; Äystö, J.; Campbell, P.; Cochrane, E. C. A. *Nucl. Instr. Meth. Phys. A* **2001**, *469*, 244-253.
55. Hang, W.; Lewis, C.; Majidi, V. *Analyst*, **2003**, *128*, 273-280.
56. Page, J. S.; Kelly, R. T.; Tang, K.; Smith, R. D. *J. Am. Soc. Mass Spectrom.* **2007**, *18*, 1582-1590.
57. Document No. SANCO/12571/2013, European Commission, Health and Consumer Protection Directorate-General, 19 November 2013.

Figure Captions

Fig.1. (a) Schematic of the miniaturized ion optics bench (not to scale). Q0 and Q1 are quadrupole ion guides, Q1 and Q3 are quadrupole mass filters, and L0 – L4 are electrostatic elements. D is a multiplying detector. (b) Microengineered microspray, vacuum interface, quadrupole mass filter, and quadrupole ion guide. (c) Complete ion optics bench.

Fig. 2. Demonstration of precursor ion selection. The reserpine spectrum on the left-hand side was obtained with the instrument configured as a single quadrupole (Q1 in rf-only mode). The spectrum on the right-hand side was obtained with the instrument configured as a tandem mass spectrometer (Q1 set to pass m/z 609 with unit resolution).

Fig. 3. Product ion spectra resulting from the fragmentation of reserpine at collision energies from 20-35 eV. Product ions are observed at m/z 174, 195, 236 and 397 when the collision energy (CE) exceeds 25 eV. The spectra have not been centroided or otherwise processed.

Fig. 4. Dependence of thiabendazole precursor and product ion intensities on the Ar collision cell pressure. The $[M+H]^+$ ion appears at m/z 202 and major product ions at m/z 131 and 175. Also shown is the signal recorded with Q3 in rf-only mode, which corresponds to the total ion count. Collisional cooling and fragmentation are optimal at 0.05 Torr.

Fig. 5. Evaluation of sensitivity and dynamic range by flow injection analysis of reserpine stock solutions. The limit of detection is 5 ng/mL (10 pg injected) and the response is approximately linear over four orders of magnitude. Weighted ($1/\sigma^2$) linear regression yields $y = 1.005 \times 10^{10} x - 0.652$ (—) and $r^2 = 0.990$

Fig. 6. Chromatogram corresponding to 1 ng of verapamil on-column. The intensity of the m/z 455 $\rightarrow$ 165 transition was monitored at a collision energy of 34 eV. The data have not been smoothed or otherwise processed.

Fig. 7. (a) MRM chromatograms corresponding to thiabendazole concentrations of 10 and 100 ppb in apple pulp. The m/z 202 $\rightarrow$ 175 transition is used for quantitation whereas the m/z 202 $\rightarrow$ 131 transition is used as the qualifier. The data have been smoothed using a five point moving average, and the m/z 202 $\rightarrow$ 175 chromatograms have been offset to aid presentation. (b) Calibration graph obtained by adding known amounts of thiabendazole to apple extract. Weighted ($1/\sigma^2$) linear regression yields $y = 10.682 x + 33.87$ (—) and $r^2 = 0.998$.