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Determination of Acrylamide Monomer in Hydroponically Grown Tomato Fruits by Capillary Gas Chromatography–Mass Spectrometry

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ABSTRACT

Tomato (Lycopersicon esculentum *L*) *fruits from plants grown hydroponically on polyacrylamide gel were obtained in order to assess any possible uptake of acrylamide monomer from the nutrient solution to the fruit during cultivation. Analysis of acrylamide in the gel itself involved aqueous extraction, bromination and then capillary GC determination with nitrogen-specific detection. By standard addition a level of* $0{\cdot}18 \pm 0{\cdot}01$ *g kg*$^{-1}$ *residual monomer was found to be present in a sample of gel used by an experimental horticultural station. Tomato fruits were analysed by extraction of the aqueous phase, bromination, silica-gel cartridge clean-up and capillary GC–MS determination by selected ion monitoring. The recovery of the method was 26–62% but losses throughout were compensated for by use of 2,3-dibromo-2-dimethylpropionamide internal standard. No acrylamide monomer could be detected in tomato fruits from plants grown hydroponically on polyacrylamide gel at a limit of detection of* 1×10^{-6} *g kg*$^{-1}$*, demonstrating that the monomer is not transferred from the growing medium into tomato fruits.*

Key words: Acrylamide, polyacrylamide gel, tomatoes, monomer, hydroponic, capillary GC–MS.

INTRODUCTION

Hydroponics is an established horticultural technique offering the advantage of higher crop yields than conventional methods, and can be employed in desert,

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rocky and stony areas provided a water supply is available. Crops can be grown more quickly and are more consistent in quality than conventionally grown produce. An inert matrix is used in hydroponics to support the root system of the plant, and traditionally materials such as sand, vermiculite or treated peat mixed with small pebbles are utilised. This medium should be such that it does not suffocate the plants by preventing aeration of the roots and should be capable of holding moisture. Polyacrylamide gel potentially has a number of advantages as such a medium for use in hydroponic cultivation. It is essentially inert, has a very large capacity to absorb water, and is a medium which does not require sterilisation and should not therefore be a source of plant disease. However, gels contain residual acrylamide monomer which is readily extracted into water. In view of the known toxicity of acrylamide (Le Quesne 1980) it is important to establish whether there is any uptake of this monomer by plants and ultimate accumulation into fruit and vegetables destined for human consumption.

A number of papers have reported methods for the determination of acrylamide monomer in polyacrylamide and copolymers (Frind and Hensel 1984; Garofalo *et al* 1988), in water samples treated with polyacrylamide coagulant aid (Croll and Simpkins 1972; Hashimoto 1976), in biological samples (Poole *et al* 1981), and in sugar where polyacrylamide anti-incrustation agents are employed (Tenkel *et al* 1989). Methods both for analysis of the gel and for monitoring contamination of other materials at trace levels rely upon ionic bromination of acrylamide, then extraction of the derivative into ethyl acetate, prior to GC or HPLC determination. The methods appear to work well, although difficulties have been reported with poor and irreproducible recoveries due to the instability of the brominated derivative, but these can be overcome with adequate precautions. By capillary GC using an alkali flame ionisation detector the analysis of acrylamide in sugar was restricted to a limit of detection of 1×10^{-5} g kg^{-1} due to interferences present at around 4×10^{-6} g kg^{-1} (Tenkel *et al* 1989). Using multidimensional reversed phase HPLC with thermospray LC–MS employing selected ion monitoring detection, a limit of detection of 2×10^{-7} g kg^{-1} was achieved for sugar analysis, although recoveries were low and somewhat variable (Cutie and Kallos 1986).

In this paper we have adopted the general analytical approach used elsewhere (for example Tenkel *et al* 1989), taking note of reported problems of light and heat instability of the acrylamide derivative. We have also employed internal standards of close chemical similarity to acrylamide to compensate for recovery losses and therefore improve the analytical precision. Although analysis of residual acrylamide monomer in gel material can be readily carried out using GC with a nitrogen-specific detector, for the monitoring of fruits such as tomato at the 10^{-6} g kg^{-1} limit of detection GC–MS was chosen as the approach offering greater specificity and sensitivity.

EXPERIMENTAL

Materials

Tomato plants (*Lycopersicon esculentum* L) were grown on polyacrylamide 'jelly bags' in Stockbridge House Experimental Horticultural Station, Selby, North

Yorkshire and ripe fruit were stored at −18°C until analysed. Control samples of tomatoes grown on rockwool, perlite and soil were also provided together with unused polyacrylamide growing bags.

Acrylamide monomer (99% pure), methacrylamide (98% pure) and *N*,*N*-dimethyl acrylamide (99% pure) were from Aldrich Chemical Company Ltd, Gillingham. The solvents methanol (HPLC grade), ethyl acetate (HPLC grade redistilled) and hexane (glass distilled grade) were from Rathburn Chemicals, Walkerburn, and the derivatising reagents potassium bromide and hydrobromic acid (48%) were from Aldrich Chemical Company Ltd, Gillingham; saturated bromine water was prepared by stirring distilled water with bromine (99%) for 1 h at 5°C, from which the aqueous phase was used.

Precautions

Samples and standards were prepared avoiding contact with plastic materials at all times. The centrifuge tubes were capped with aluminium foil, and glass wool was used to filter the tomato extracts to avoid possible contamination from filter paper. The brominated acrylamide derivative is both heat and light sensitive and thus the brominating flasks were wrapped in foil. Derivatised samples were kept in amber vials and stored in the freezer prior to analysis.

Analysis of acrylamide in polyacrylamide

Polyacrylamide (0·1 g) was weighed into a conical flask and an internal standard solution (1 g litre^{-1} of each of methacrylamide and *N*,*N*-dimethylacrylamide in water) was added by syringe (100 μl) to the gel followed by 25 ml of distilled water. The gel was allowed to stand for 5 h to absorb all the water, then methanol (250 ml) was added and allowed to stand overnight. This procedure drew the water from the polyacrylamide gel along with the contaminating monomer and the internal standards, and left the gel virtually desiccated in 90% methanol solution. In this way the free monomer was released from the polymer. An aliquot (20 ml) of the resulting solution was transferred to a 20-ml vial and was evaporated to 1 ml under a stream of nitrogen at 50°C. The vial was wrapped with aluminium foil, and potassium bromide (3·75 g), hydrogen bromide (0·2 ml), bromine water (1·4 ml) and distilled water (14 ml) were added. The vial was capped with PTFE-faced septa and allowed to stand overnight at 0°C. When the reaction was completed, the excess bromine was decomposed by adding sodium thiosulphate solution (1 M) dropwise until the yellow colour disappeared. The resulting solution was transferred to a 100-ml separating funnel, the reaction vial was washed twice with 2 ml portions of distilled water and the rinsings were transferred into the separating funnel. The aqueous solution was extracted with two 10-ml portions of redistilled ethyl acetate. The organic phase was dried with anhydrous sodium sulphate, evaporated to 0·5 ml under nitrogen at 50°C, and stored in an amber vial prior to GC analysis.

Gas chromatography

GC analysis was carried out using a Carlo Erba 4160 gas chromatograph (Fisons Instruments, Crawley) equipped with an NPD 40 nitrogen-specific alkali flame

ionisation detector and a split/splitless injector. Data were processed using a Spectra-Physics 4290 integrator. The GC column was a fused silica 17 m × 0·25 mm ID coated with a bonded-phase methyl silicone CP SIL 19CB, 0·20-μm film thickness (Chrompack, London) operated at 1·5 ml min^{-1} hydrogen carrier gas flow rate under the following conditions: Temperature programme from 80°C (1 min) at 15°C min^{-1} to 200°C, injector temperature (180°C). Injection (1 μl) were in the splitless mode. Quantification was by standard addition, spiking the gel with acrylamide monomer over the range 0–1 g kg^{-1}.

Analysis of acrylamide in tomatoes

Tomato fruits (600 g fresh wt) were homogenised in a stainless steel Waring blender, and subsamples (50 g) were transferred to 250-ml centrifuge tubes. A solution of internal standard (0·103 g litre^{-1} aqueous methacrylamide) was added by syringe (5 μl) and mixed with an Ultra Turrax blender. The tubes were capped with aluminium foil and the samples were centrifuged for 15 min at 2000 × *g*. The supernatant liquid was decanted (through a funnel with glass wool) into a 250-ml conical flask. The solids pellet was resuspended in water (25 ml) and recentrifuged. The combined supernates contained in a flask wrapped in aluminium foil were treated with 82 ml of brominating solution (152 g KBr, 8 ml HBr, 50 ml bromine water and 600 ml water). The flasks were allowed to stand overnight at 0°C. Excess bromine was decomposed by adding thiosulphate solution (1 M) dropwise until the yellow colour disappeared. The resulting solution was transferred to a 250-ml separating funnel with rinsing and extracted with ethyl acetate (1 × 20 ml and 1 × 10 ml). The organic phase was centrifuged for 5 min at 1500 × *g*, water was removed by Pasteur pipette and the organic phase was dried over sodium sulphate. The extract was evaporated to about 100 μl under nitrogen at 50°C.

A Bond-Elut silica gel column was preconditioned with 20% v/v ethyl acetate/hexane. The derivatised sample (75 μl) was added and the column was washed with 20% ethyl acetate/hexane (2·5 ml) and 35% ethyl acetate (5 ml) from which the first 2 ml was discarded and the next 2 ml fraction was collected. The chromatographic internal standard solution (7·2 × 10^{-2} g litre^{-1} of 2,3-dibromo,*N*,*N*-dimethylpropionamide in ethyl acetate) was added (0·25 ml) and the resulting solution was evaporated to 50 μl and analysed by GC–MS.

Capillary gas chromatograhy–mass spectrometry

Combined GC–MS was carried out using a Carlo Erba 4160 GC coupled directly to a VG 12000 quadrupole mass spectrometer. Tomato extracts were analysed using a J & W 30 m × 0·25 mm DB 17 fused silica capillary column (0·2 μm film thickness) programmed from 65 to 250°C at 15°C min^{-1}. Scanned spectra were obtained over the range 400–40 Da with a 1-s scan. For selected ion monitoring, eight ions were monitored: four from the 2,3-dibromopropionamide (m/z 106, 108, 150, 152) and two each from the internal standards 2,3-dibromo 2-methylpropionamide (m/z 120 and 122) and 2,3-dibromo N,N-dimethyl-propionamide (m/z 178 and 180). Quantification was by standard addition on the basis of peak areas.

RESULTS AND DISCUSSION

The analysis of the polyacrylamide used for the hydroponic cultivation of tomatoes was carried out by the method of standard additions, making five additions to the gel to cover the range 0·2 to 1 g kg^{-1}. Calibration lines were drawn based on each of the two different internal standards, and these showed a common intercept which corresponded to a level of $0{\cdot}18 \pm 0{\cdot}01$ g kg^{-1} of residual acrylamide monomer in the polyacrylamide. Replicate analyses of the polymer without additions ($n=4$) showed the relative standard deviation of the method to be 5–6%. Polyacrylamide sold for domestic horticultural use was also analysed and found to contain 0·48 g kg^{-1} residual monomer. Figure 1 shows a typical GC chromatogram for the analysis of polyacrylamide using nitrogen specific detection.

For the analysis of tomatoes in order to achieve 10^{-6} g kg^{-1} sensitivity and adequate specificity, a silica gel clean-up stage was introduced and the determination was by selected ion monitoring capillary GC–MS. The specificity of the procedure and absence of interferences at the 1×10^{-6} g kg^{-1} level was demonstrated by the analysis of control tomatoes grown hydroponically on rockwool, perlite and conventionally on soil. Spiking these tomatoes at 1×10^{-6} g kg^{-1} showed the method to have a recovery of 26–62% ($n=4$). These recovery figures relate to the combined efficiencies of the entire method (extraction, bromination, re-extraction and clean-up) and are calculated using the 2,3-dibromo-N,N-dimethylpropionamide added as a chromatographic standard. The use of methylacrylamide as an internal standard, added at the outset, compensated for the rather variable recoveries. The origin of this variability was

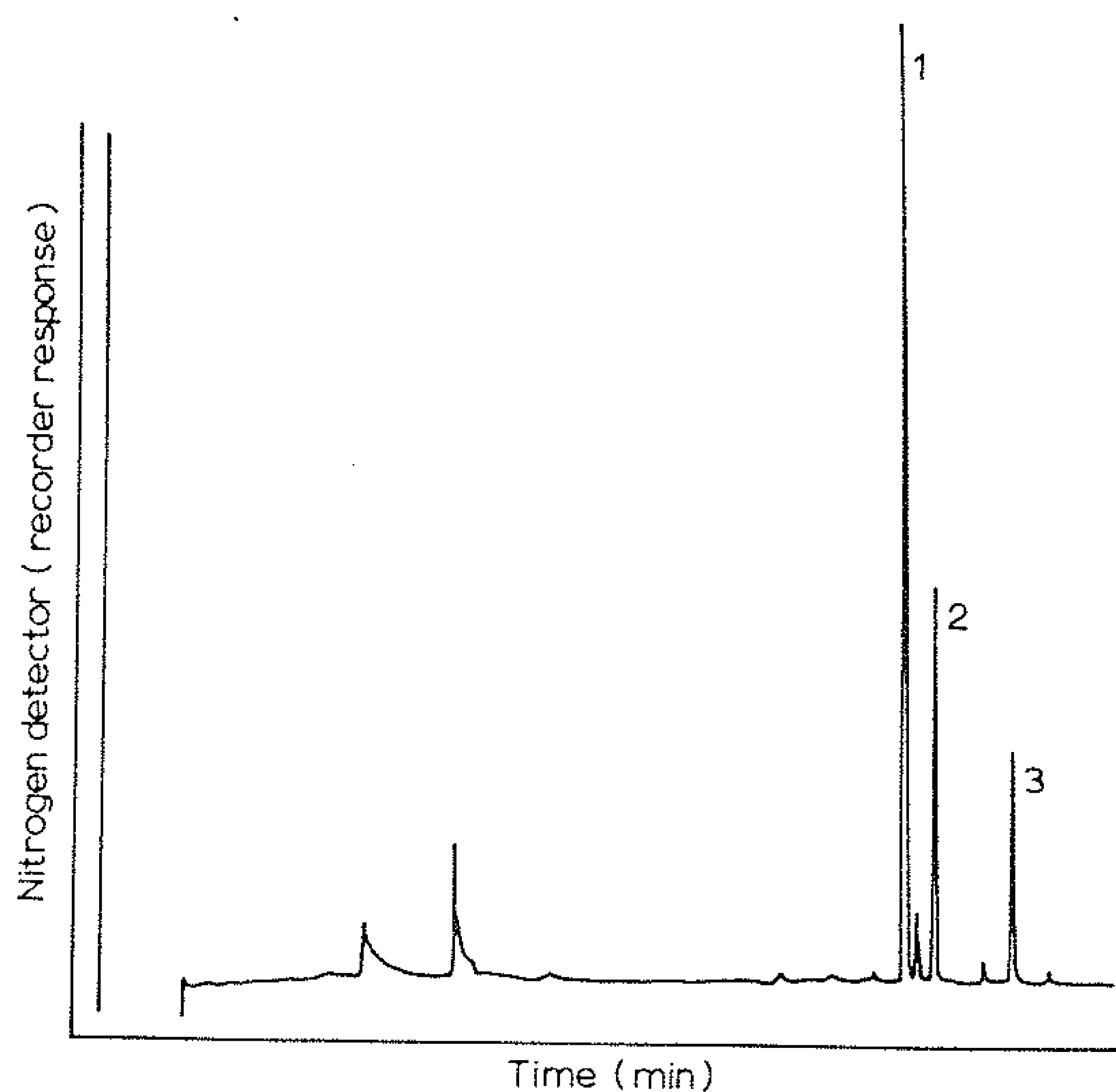

Fig 1. Chromatogram illustrating the analysis of acrylamide in polyacrylamide gel. Capillary GC (CP SIL 19CB) temperature programmed from 80°C (1 min) at 15°C min^{-1} to 200°C. Peaks: (1) 2,3-dibromo-*N*,*N*-dimethylpropionamide; (2) 2,3-dibromo 2-methylpropionamide; (3) 2,3-dibromo-propionamide.

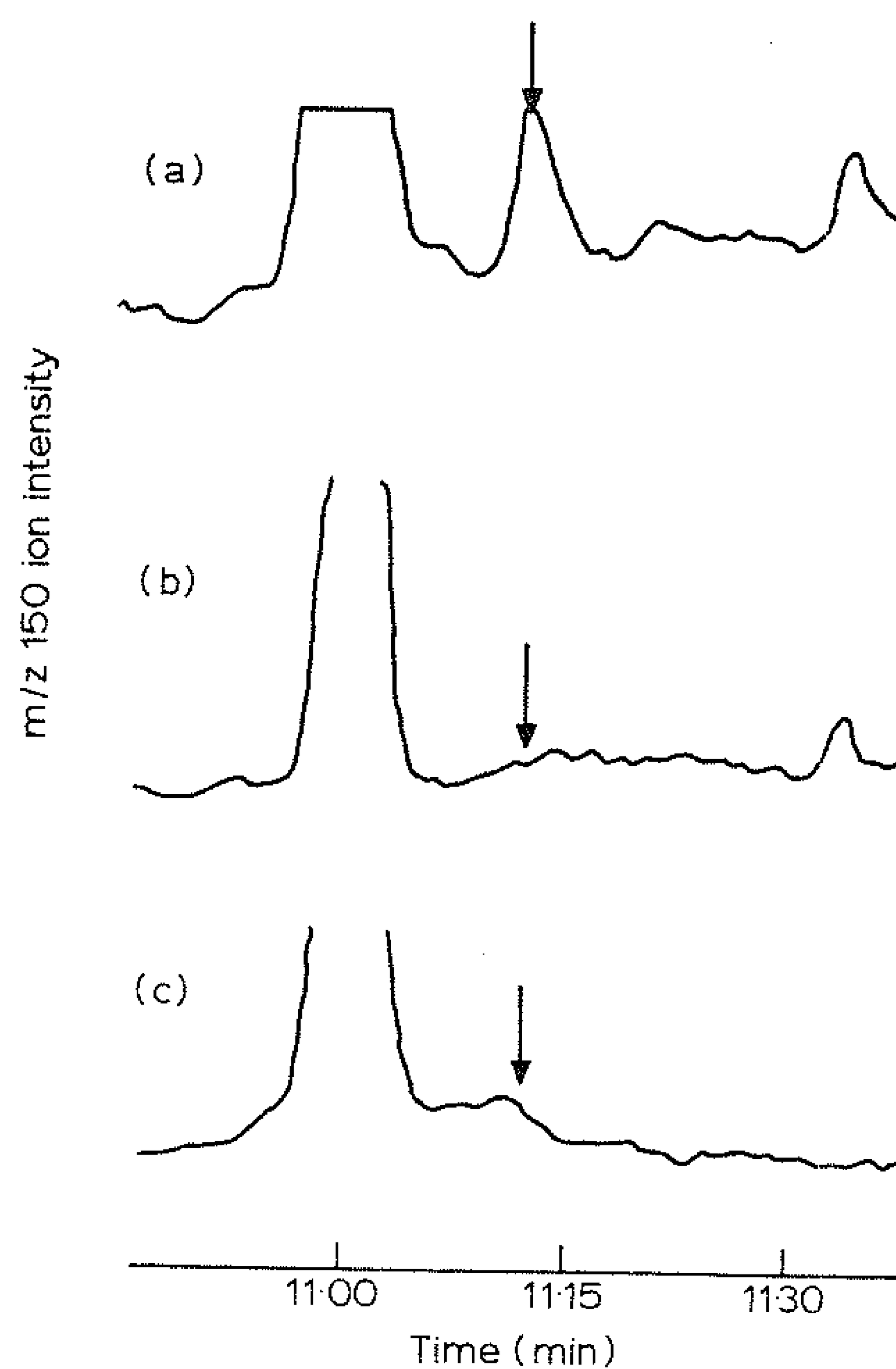

Fig 2. Chromatogram illustrating the selected ion monitoring of acrylamide in tomatoes. Capillary GC (DB 17) temperature programmed from 65°C to 250°C at 15°C min^{-1}. Monitoring m/z 150. (a) Acrylamide spike at 1×10^{-6} g kg^{-1} into control tomatoes; (b) tomatoes grown in polyacrylamide gel bags; (c) tomatoes grown conventionally on soil.

the need to take a narrow cut from the silica clean-up cartridge in order to avoid an interfering substance. When the analysis was carried out of tomatoes grown hydroponically on polyacrylamide gel, these were indistinguishable from the controls with acylamide being present below the limit of detection of 1×10^{-6} g kg^{-1}. Typical selected ion chromatograms for the analysis of tomatoes are shown in Fig 2. Ions of m/z 150, 152 (acrylamide derivative) and m/z 120, 122 (methacrylamide internal standard) were found to be the most reliable ions for quantification purposes. The expected 1:1 ratio of each of these two bromine containing fragment pairs was a useful check against interference in any of the four selected ion channels. Similarly, ions 178 and 180 were used to monitor the derivatised *N*,*N*-dimethylacrylamide and the expected 1:1 ratio of this pair used to ensure against interferences. This work has therefore demonstrated that, despite the relatively high levels of residual acrylamide monomer in polyacrylamide gels, the monomer is not transferred to tomato fruits when the gels are used as a hydroponic growing medium.

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