1.0 INTRODUCTION

1.1 Scope and chemical structures

Analytical method GRM035.03A is suitable for the determination of triasulfuron and its metabolite CGA150829 (Figures 1-2) in water. The limit of quantification (LOQ) of the method has been established at 0.01 µg/L.

This method complies with OECD guidance document ENV/JM/MONO(2007)17, US EPA guidelines EPA OPPTS 860.1340 and OPPTS 850.7100 and EU guidelines SANCO/3029/99 rev. 4, SANCO/825/00 rev. 7.

1.2 Method summary

Water samples are acidified then taken through a solid-phase extraction (SPE) procedure using Oasis ^{TM} MCX cartridges. The SPE cartridges are washed with orthophosphoric acid 1% and the compounds are eluted with methanol/35% ammonia solution (95/5, v/v). The column eluates are evaporated to remove the solvents and then diluted with ultra pure water.

Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 0.01 µg/L for triasulfuron and CGA150829.

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted.

2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted provided that acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix 2.

2.3 Preparation of analytical standard solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

2.3.1 Stock solutions

Weigh out accurately, using a five-figure balance, sufficient triasulfuron and CGA150829 analytical standards and carefully transfer into separates "Class A" volumetric flasks. Dilute to the mark with acetonitrile (for triasulfuron) or methanol (for CGA150829) to give 200 µg/mL stock solutions.

Alternatively, the appropriate volume of solvent (acetonitrile for triasulfuron and methanol for CGA150829) to add to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity.

$$V = \frac{W \times P}{C} \times 1000$$

P	=	Standard purity in decimal form (P(%)/100)
V	=	Volume of acetonitrile required
W	=	Weight, in mg, of the solid analytical standard
C	=	Desired concentration of the final solution, (µg/mL)
1000	=	Unit conversion factor

In this case, the standard material is weighed directly into a volumetric flask.

2.3.2 Fortification solutions

It is recommended that, as a minimum, 10 µg/L and 1 µg/L solutions are prepared by serial dilution in ultra pure water/acetonitrile (80/20,v/v) for triasulfuron and CGA150829. The preparation of LC-MS/MS calibration standards is discussed in Section 3.4.

2.3.3 Standard solution storage and expiration

The stock solutions must be stored in a refrigerator or in a freezer when not in use to prevent decomposition and/or concentration of the standard and should be prepared each 4 months for triasulfuron and 6 months for CGA150829. All solutions used for this study were stored between 0 and 9°C.

2.4 Safety precautions and hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S G Luxon, The Chemical Society, London (Reference 4).

Solvent and Reagent hazards

	35% Ammonia solution	Acetonitrile	Orthophosphoric acid	Methanol
Harmful Vapour	✓	✓	✓	✓
Highly Flammable	✗	✓	✗	✓
Harmful by Skin Absorption	✓	✓	✓	✓
Causes burns	✓	✗	✓	✗
Irritant to eyes and respiratory tract	✓	✓	✓	✓
Syngenta Divisional Toxicity Class	SHC-C, S	SHC-C, S	SHC-C, S	SHC-C, S
OES Short Term (mg/m^3)	24	105	*	310
OES Long Term (mg/m^3)	17	70	*	260

* No data available

In all cases avoid breathing vapour. Avoid contact with eyes and skin.

Triasulfuron has been designated Syngenta Hazard Category SHC-A. There are currently insufficient data to assign a Syngenta Hazard Category (SHC) to CGA150829. CGA150829 is therefore assumed to be SHC-D until further information becomes available. Suitable precautions must be taken when handling the solid compound and solutions. The toxicity classification scale rates highly toxic chemicals as SHC-E and non-toxic chemicals as SHC-A. An additional hazard category of S indicates the compound is a severe skin and eye irritant.

3.0 ANALYTICAL PROCEDURE

The method is summarized in flow chart form in Appendix 5.

3.1 Solid Phase Extraction Procedure

a) If water samples are received deep frozen they should be allowed to defrost completely at room temperature. Defrosted samples should be shaken thoroughly to ensure sample homogeneity prior to analysis.

b) Transfer 25 mL of the water sample to be analysed into a polypropylene centrifuge tube (50 mL size). Sample fortification, if required, should be carried out at this point. At least one untreated control and two control samples fortified with a known amount of triasulfuron and CGA150829 should be analysed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired.

c) Add concentrated orthophosphoric acid (50 µL) to each sample. Cap the centrifuge tubes securely and shake gently to mix.

d) Take one Waters Oasis MCX cartridge (60 mg/3 mL size) for each sample to be analysed and place on a suitable vacuum manifold. Add methanol (2 mL) and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at a rate of approximately 1 mL/min, discarding the column eluates. Do not allow the cartridges to become dry. Add ultra pure water (2 mL) to the top of each cartridge and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluates. Do not allow the cartridges to become dry.

e) Load water samples from Section 3.1 (b) onto the SPE cartridges via a suitable column reservoir and allow to percolate through under gravity or under low vacuum, at a rate of approximately 1-2 mL/min, to the level of the top frit. Do not allow cartridges to become dry.

Note: It is recommended that water with visible particulate matter is filtered through a polypropylene frit placed in the column reservoir before loading on to the SPE cartridge, to prevent blockage of the SPE frit.

f) On completion of loading, remove the column reservoir and connector. Add 1% orthophosphoric acid solution (2 mL) to centrifuge tubes and apply the rinsing solution to the top of the SPE cartridge and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluates. Remove the excess acid under vacuum by application of high vacuum for a few seconds.

g) Place suitable disposable, plastic graduated centrifuge tubes (15 mL size) under each port, as required, in the manifold rack. Add 95/5 v/v methanol/35% ammonia solution (3 mL) to the top of each cartridge and allow to percolate through under gravity. Collect the column eluate containing triasulfuron and CGA150829. Remove the excess solvent from the cartridges by application of positive pressure or vacuum, collecting the column eluate.

h) Evaporate the collected eluates to 0.5 mL under a stream of nitrogen in a sample concentrator with the heating block set at 40 °C to evaporate solvents.

i) Adjust the final volume to 2 mL with ultra pure water and sonicate the extracts.

j) Transfer the samples into suitable autosampler vials ready for final determination by LC-MS/MS, using the chromatographic conditions described in Section 4.0.

3.2 Time required for analysis

The methodology is normally performed with a batch of 15 or more samples. One person can complete the analysis of 15 samples in 1 day (8 hour working period).

3.3 Method stopping points

It is recommended that the analytical procedure be completed in one day. Acceptable method recoveries will validate any work flow interruptions. Samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

3.4 Preparation of calibration standards for LC-MS/MS

Enhancement (quantification chromatographic conditions) and suppression (confirmatory chromatographic conditions) of the instrument response for CGA150829 were observed in the ground water tested and matrix-matched standards were used for calibration and quantification for this matrix. For triasulfuron, only the confirmatory transition exhibited an enhancement of the instrument response in the ground water tested and matrix-matched standards were used for both transitions for this matrix.

No significant matrix effects were observed for surface water and drinking water and non-matrix matched standards were used for calibration and quantification for both matrices.

Matrix and non-matrix matched calibration standards should be prepared as described below.

LC-MS/MS calibration standards for triasulfuron and CGA150829 should be prepared in water or, where appropriate, in matrix extract within each analysis batch. For example, to prepare a 0.5 µg/L calibration standard, transfer 500 µL of a 100 µg/L standard in acetonitrile/ ultra pure water (20/80, v/v) in a volumetric flask (10 mL). Add acetonitrile/ ultra pure water (20/80, v/v) to the 10 mL mark. Stopper the flask securely and shake to mix thoroughly, then dilute 10-fold the solution further with ultra pure water or matrix.

Transfer aliquots into a suitable autosampler vials for analysis by LC-MS/MS.

4.0 FINAL DETERMINATION

The following instrumentation and conditions have been found to be suitable for this analysis. Other instrumentation can also be used, though optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use. The method has been developed for use on the Applied Biosystems API 4000 LC-MS/MS.

4.1 Instrument Description

HPLC system	:	Shimadzu LC20AD
Detector	:	Applied Biosystems API 4000 triple quadrupole mass spectrometer with Analyst™ software version 1.4.2
Gas Supply	:	Local nitrogen supply (from liquid nitrogen)

4.2 Chromatography Conditions for triasulfuron and CGA150829 (Quantification Method)

Column	:	Zorbax SB-C8, 3.5 µm, 150 × 3 mm
Column Oven Temperature	:	40 ± 5°C
Injection volume	:	100 µL
Stop Time	:	6 minutes
Injection protocol	:	Analyse calibration standard after 4 sample injections
Mobile phase	:	Solvent 1 = Methanol/Acetonitrile (50/50, v/v) Solvent 2 = ultra pure water + 0.2% acetic acid

Mobile Phase Gradient

Time (min)	% Solvent 1	% Solvent 2	Flow (mL/min)
0.0	20	80	0.75
2.0	50	50	0.75
4.0	50	50	0.75
4.1	20	80	0.75
6.0	20	80	0.75

Under these conditions the retention times of triasulfuron and CGA150829 are approximately 4.85 minutes and 1.9 minutes respectively.

4.3 Chromatography Conditions for CGA150829 (Confirmatory Method)

Column	:	Zorbax SB-CN, 5 µm, 150 × 4.6 mm
Column Oven Temperature	:	40 ± 5°C
Injection volume	:	100 µL
Stop Time	:	6 minutes
Injection protocol	:	Analyse calibration standard after 4 sample injections
Mobile phase	:	Solvent 1 = Methanol/Acetonitrile (50/50, v/v) Solvent 2 = ultra pure water + 0.2% acetic acid

Mobile Phase Gradient

Time (min)	% Solvent 1	% Solvent 2	Flow (mL/min)
0.0	20	80	0.75
2.0	50	50	0.75
4.0	50	50	0.75
4.1	20	80	0.75
6.0	20	80	0.75

Under these conditions the retention time of CGA150829 is approximately 3.45 minutes.

4.4 Mass Spectrometer Conditions for Triasulfuron and CGA150829

Interface	:	TurboIonSpray
Polarity	:	Positive
Curtain gas (CUR)	:	25
Temperature (TEM)	:	500°C
Ionspray voltage	:	5500V
Collision gas setting (CAD)	:	4
Gas 1 (GS1)	:	45
Gas 2 (GS2)	:	55
Interface heater (ihe)	:	On
Scan type	:	Multiple reaction monitoring (MRM)

MRM Conditions		Triasulfuron primary transition	Triasulfuron confirmatory transition
Q1 *m/z*	:	402.1	402.1
Q3 *m/z*	:	167.3	141.2
Dwell time	:	200 ms	200 ms
Resolution Q1	:	Unit	Unit
Resolution Q3	:	Unit	Unit
Declustering potential (DP)	:	55 V	55 V
Entrance potential (EP)	:	10 V	10 V
Collision energy (CE)	:	25 V	26 V
Collision cell exit potential (CXP)	:	10 V	8 V

MRM Conditions		CGA150829 primary condition	CGA150829 confirmatory condition
Q1 *m/z*	:	141.1	141.1
Q3 *m/z*	:	58.1	58.1
Dwell time	:	200 ms	1000 ms
Resolution Q1	:	Unit	Unit
Resolution Q3	:	Unit	Unit
Declustering potential (DP)	:	60 V	60 V
Entrance potential (EP)	:	10 V	10 V
Collision energy (CE)	:	35 V	35 V
Collision cell exit potential (CXP)	:	10 V	10 V

Typical chromatograms are presented in Figures Section.

5.0 CALCULATION OF RESULTS

5.1 Single point calibration procedure

Residues may be calculated in µg/L for each sample using a mean standard response from each of the injections bracketing the sample as follows.

a) Make repeated injections of each standard containing triasulfuron and CGA150829 as required at an appropriate concentration into the LC-MS/MS operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for the analytes.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to the analytes.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

d) Calculate the residues in the sample, expressed as µg/L, using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue (µg/L)} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}}$$

PK area (SA) = Peak response for sample
PK area (STD) = Average peak response for bracketing standards
Standard Conc. = Concentration of standard (µg/mL)
Sample Conc. = Sample concentration (L/mL)

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \text{ (µg/L)}$$

Although single point calibration may be used to quantify residues it is recommended that a calibration curve is generated with each analytical run to demonstrate the linearity of instrument response (Reference 5).

5.2 Multi point calibration procedure

Residues may be calculated in µg/L for each sample as follows.

a) Make repeated injections of a standard containing triasulfuron and CGA150829 as required over a concentration range appropriate to the expected residues in the samples (for example, 50% LOQ to 20 x LOQ). An appropriate number of different concentrations within this range should be prepared (at least four).

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to each analyte. Calibration standard solutions should be interspersed throughout the analysis, after a maximum of four injections of sample solutions.

c) Generate calibration curve parameters using an appropriate regression package.

d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard concentration, m is the gradient of the line of best fit ("X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the "R-Squared" value for the regression.

Re-arrangement for x gives

$$x = \frac{y - c}{m}$$

e) Alternatively (depending on the regression analysis software available) a quadratic equation may be used to fit the data. In this case the following general equation should be re-arranged and used to calculate residues:

$$y = a + bx + cx^2$$

Where y is the instrument response value, x is the standard concentration and a, b, c are constants.

f) Calculate the residues of each compound in the sample, expressed as µg/L, as follows

$$\text{Residue (µg/L)} = \frac{\text{Analyte found (µg/mL)}}{\text{Sample conc. (L/mL)}}$$

Where analyte found (µg/L) is calculated from the standard calibration curve and sample conc. is the final sample concentration in L/mL.

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \ (\mu g/L)$$

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be completed as detailed in Section 3.0 for each set of samples analysed to verify that the water is free from contamination. A minimum of one control should be analysed with each batch of samples.

At least two recovery samples (control samples accurately fortified with a known amount of triasulfuron and CGA150829) should also be analysed alongside each set of samples Provided the recovery values are acceptable they may be used to correct any residues found. The fortification levels should be appropriate to the residue levels expected.

Recovery efficiency is generally considered acceptable when the mean values are between 70% and 110% and with a relative standard deviation of $\leq$20%.

7.0 SPECIFICITY

It is recommended that reagent blank samples be included in a sample set if contamination is suspected.

7.1 Matrix Interference

LC-MS/MS is a highly specific detection technique. Interference arising from the ground water samples tested has been observed for triasulfuron and CGA150829. Interference arising from the drinking and surface water samples tested has not been observed for triasulfuron and CGA150829.

7.2 Reagent and solvent interference

Using high purity solvents and reagents no interference has been found.

7.3 Labware interference

This method uses disposable labware. All reusable glassware should be detergent washed and then rinsed with HPLC-grade methanol, acetone or acetonitrile prior to use.

8.2 Matrix Effects

Enhancement (quantification chromatographic conditions) and suppression (confirmatory chromatographic conditions) of the instrument response for CGA150829 were observed in the ground water tested and matrix-matched standards were used for calibration and quantification for this matrix. For triasulfuron, only the confirmatory transition exhibited an enhancement of the instrument response in the ground water tested and matrix-matched standards were used for both transitions for this matrix.

No significant matrix effects were observed for surface water and drinking water and non-matrix matched standards were used for calibration and quantification for both matrices.

8.6 Final Extract stability

Residues of triasulfuron and CGA150829 in final extracts have been shown to be stable when stored refrigerated between 0 and 9°C for up to 9 days when reanalysed against freshly prepared calibration standards.

Full details are presented in Tables 12 and 13.

9.0 LIMITATIONS

The method has been tested on representative water types. It can reasonably be assumed that the method can be applied to other water types not tested in this study, provided successful recovery tests at the relevant levels validate the suitability of the method.

10.0 CONCLUSIONS

This procedure has been demonstrated to be a reliable and accurate procedure for the determination of triasulfuron and CGA150829 residues in water. Only commercially available laboratory equipment and reagents are required. The analysis of a batch of 15 samples can be completed by one person in 1 day (8 working hour period). Untreated and fortified samples should be analysed with each set of samples to demonstrate absence of any interference and adequate recovery, if possible. The limit of quantification of the method is 0.01µg/L.

This method complies with OECD guidance document ENV/JM/MONO(2007)17, US EPA guidelines EPA OPPTS 860.1340 and OPPTS 850.7100 and EU guidelines SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 7.

11.0 REFERENCES

1. S. Oppillliart (2010): Triasulfuron -Validation of an Analytical Method for the Determination of Residues of Triasulfuron and its Metabolite CGA150829 in Water. Eurofins|ADME Bioanalyses Report S09-03244.
2. Watson S. A., Residue Analytical Method for the Determination of Residues of Triasulfuron in Laboratory Tank Water. Syngenta Method RAM 469/01
3. S. L. Hargreaves (2006). Residue Analytical Method for the Determination of Residues of Prosulfuron (CGA152005) Metabolites CGA150829, CGA159902, CGA325025, CGA349707 and SYN542604 in Soil. Syngenta Method RAM 490/01.
4. Luxon S G (1992): Hazards in the Chemical Laboratory 5th Edition. The Royal Society of Chemistry. Thomas Graham House, The Science Park, Cambridge CB4 4WF, UK. ISBN 0-85186-229-2.
5. Cardone M J, Palermo P J and Sybrand L B (1980): Potential error in single point ratio calculations based on linear calibration curves with a significant intercept. Anal Chem., 52 pp 1187-1191

Figure 1: Structure of Triasulfuron

Compound Code Number	:	Triasulfuron
CAS Number	:	82097-50-5
IUPAC Name	:	1-[2-(2-chloroethoxy)phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea
Molecular Formula	:	$C_{14}H_{16}ClN_5O_5S$
Molecular Weight	:	401.8 g/mol

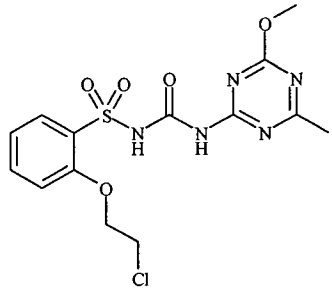
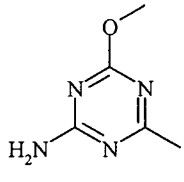


Figure 2: Structure of CGA150829

Compound Code Number	:	CGA150829
CAS Number	:	1668-54-8
IUPAC Name	:	4-methoxy-6-methyl-[1,3,5]triazine-2-ylamine
Molecular Formula	:	$C_5H_8N_4O$
Molecular weight	:	140.1 g/mol



APPENDIX 1 APPARATUS

UK Suppliers

General glassware, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough Leicestershire LE11 5RG.

Polypropylene centrifuge tubes, 50 mL and 15 mL capacity, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG.

Column connectors, available from Argonaut Technologies, Tir-y-Berth Industrial Estate, New Road, Hengoed, Mid Glamorgan, CF8 8AU.

Column reservoirs, 70 mL size, available from Agilent Technologies UK Limited Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire SK8 3GR.

Polyethylene 70 µm frits for 70 mL reservoir, available from Agilent Technologies UK Limited Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire SK8 3GR.

Oasis MCX solid phase extraction cartridges 60 mg, 3 mL size, available from available from Waters Ltd, 730-740 Centennial Court, Elstree, Hertfordshire WD6 3SZ.

Solid phase extraction vacuum manifold, available from Varian Limited, 6 Mead Road, Oxford Industrial Park, Yarnton, Oxford OX5 1QU, UK.

Plastic disposable pipettes, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG.

Ultrasonic bath e.g. Ultrawave U300/D, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG.

Techne Dri-block 3D heating block, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG

Crimp cap autosampler vials and caps, available from Agilent Technologies UK Limited Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire. SK8 3GR.

API 4000 LC-MS/MS system equipped with a TurboIonSpray source, available from Applied Biosystems, 120 Birchwood Boulevard, Warrington, Cheshire WA3 7PB.

Shimadzu LC20AD HPLC system equipped with autosampler, quaternary pump, vacuum degasser and column compartment with column switching valve, available from Shimadzu UK Limited, Mill Court, Featherstone Road, Wolverton Mill South, Milton Keynes MK12 5RD.

HPLC column, Zorbax SB-C8, 3.5µm, 150x3 mm, available from Agilent Technologies UK Limited Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire SK8 3GR.

HPLC column, Zorbax SB-CN, 5µm, 150x4.6 mm, available from Agilent Technologies UK Limited Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire SK8 3GR.

Peak Scientific NM20ZA gas station, available from Peak Scientific Instruments Ltd., Fountain Crescent, Inchinnan Business Park, Inchinnan, Renfrew PA9 4RE.

US Suppliers

General glassware, available from Fisher Scientific Fisher Scientific, Liberty Lane, Hampton NH 03842.

Polypropylene centrifuge tubes, 50 mL and 15 mL capacity available from Fisher Scientific, Liberty Lane, Hampton NH 03842.

Oasis™ MCX solid phase extraction columns, 3 mL 60 mg size, available from Waters Corporation, 34 Maple Street, Milford, Massachusetts, 01757-3696.

Solid phase extraction vacuum manifold, available fromVarian Inc. 24021 Frampton Avenue, Harbor City, CA 90710, USA.

Column connectors, available from Argonaut Technologies, Order Processing, 1101 Chess Drive, Foster City, CA 94404.

Column reservoirs, 70 mL size, available from available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304.

Polyethylene 70 µm frits for 30 mL reservoir, available Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304.

Plastic disposable pipettes, available from Fisher Scientific, Liberty Lane, Hampton NH 03842.

Ultrasonic bath e.g. Ultrawave U300/D, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842.

Techne Dri-Block heating block, available from Techne Incorporated, 3700 Brunswick Pike, Princeton, New Jersey 08540-6192.

Crimp cap auto sampler vials and caps, available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304.

API 4000 LC-MS/MS system equipped with a TurboIonSpray source, available from Applied Biosystems, 850 Lincoln Center, Foster City, CA 94404-1128.

Shimadzu LC20AD HPLC system equipped with autosampler, quaternary pump, vacuum degasser and column compartment with column switching valve, available from Shimadzu Scientific Instruments, 7102 Riverwood Drive, Columbia, MD 21046, U.S.A.

HPLC column, Zorbax SB-C8, 3.5µm, 150x3 mm, available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304.

HPLC column, Zorbax SB-CN, 5µm, 150x4.6 mm, available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304.

Peak Scientific NM20ZA gas station, available from Peak Scientific Instruments, 1300 West Belmont Ave., Chicago IL 60657.

APPENDIX 2 REAGENTS

UK Suppliers

Solvents: Ultra pure water (HPLC grade), methanol and acetonitrile available from Rathburn Chemicals Ltd., Walkerburn, Scotland EH43 6AU

Analytical grade ammonia solution 35%, concentrated orthophosphoric acid and acetic acid available from Sigma-Aldrich, The Old Brickyard, New Road, Gillingham, Dorset SP8 4XT or www.sigmaaldrich.com

Triasulfuron and CGA150829 analytical standards available from Syngenta, GLP Testing Facility, Syngenta,CH-4333 Munchweilen, Switzerland.

US Suppliers

Solvents: Analytical grade acetonitrile and methanol available from B & J Brand Solvents, from Scientific Products Division of Baxter Healthcare Corporation, USA.

Ultra pure HPLC grade water from e.g. Fluka via Sigma-Aldrich www.sigmaaldrich.com

Analytical grade ammonia solution 35%, concentrated orthophosphoric acid and acetic acid available from www.sigmaaldrich.com

Triasulfuron and CGA150829 analytical standards, available from Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419-8300.

Preparation of Reagents

1. 95:5 v/v methanol: 35% ammonia solution
   Mix 950 mL of methanol and 50 mL of ammonia solution 35% in a 1 L volumetric flask. Stopper flask securely and mix thoroughly by shaking.

2. 20:80 v/v acetonitrile:water.
   Mix 800 mL of ultra pure water and 200 mL of acetonitrile in a 1 L volumetric flask. Stopper flask securely and mix thoroughly by shaking.

3. 1% v/v orthophosphoric acid in ultra pure water.
   Add concentrated orthophosphoric acid (1 mL) to 100 mL ultra pure water in a 100 mL volumetric flask. Stopper flask securely and mix thoroughly by shaking.

4. 50:50 v/v methanol/acetonitrile
Mix 500 mL of methanol and 500 mL of acetonitrile in a 1 L volumetric flask. Stopper flask securely and mix thoroughly by shaking.

5. 0.2% v/v acetic acid in ultra pure water
Add concentrated acetic acid (2 mL) to 1 L ultra pure water in a 1 L volumetric flask. Stopper flask securely and mix thoroughly by shaking.

APPENDIX 3 API4000 MS/MS TUNING PROCEDURE

Calibration of Instrument

The instrument must be mass-calibrated on a regular basis using polypropylene glycol (PPG) solutions according to the manufacturer's instructions. Calibrate both mass-resolving quadrupoles (Q1 and Q3).

Tuning instrument for triasulfuron and CGA150829 in Positive Ionisation Mode

Infuse a standard solution triasulfuron and CGA150829 (0.1 to 1.0 µg/mL in mobile phase, see section 4.2 and 4.3) directly into the mass spectrometer interface at a rate at of about 10 µL/min. Roughly adjust interface parameters (sprayer position, spray, heater/auxiliary gas flows, as well as voltages of spray, orifice, and focusing ring) for a sufficiently high parent ion signal at under positive ionisation conditions.

Using the Analyst 1.4.2 software quantitative optimisation routine, tune the instrument for
triasulfuron and CGA150829 ensuring that the correct ions are selected. Alternatively, the instrument ion optics and collision energy may be tuned manually for triasulfuron and CGA150829 to ensure maximum sensitivity.

Analyte	Parent ion	Daughter ion Primary transition	Daughter ion Confirmatory transition
Triasulfuron	*m/z* 402.1	*m/z* 167.3	*m/z* 141.2
CGA150829	*m/z* 141.1	*m/z* 58.1	-

Note: If problems are encountered in tuning the instrument for these ions, the ions should be entered in the method as detailed above and tuning performed manually.

Finally, connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injections of triasulfuron and CGA150829 standards in mobile phase and at the flow rate to be used. Tune the interface parameters (sprayer position, spray and heater gas flows, spray, orifice, and focusing ring voltages) and the collision gas flow for maximum sensitivity.

In positive ionisation mode, cations of triasulfuron and CGA150829 generated in the ion source are selected and subjected to further fragmentation by collisional activation. The most sensitive daughter ions are then selected and used for quantitative analysis.

APPENDIX 5 METHOD FLOWCHART

Measure water sample (25 mL) into centrifuge tube and acidify with conc. orthophosphoric acid

↓

Oasis MCX procedure

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Elute with 3 mL of 95/5 v/v methanol/35% ammonia solution

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Evaporate to 0.5 mL and redissolve in UPW. Analyse by LC-MS/MS using appropriate HPLC column for triasulfuron and CGA150829