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A Confirmatory LC-MS/MS Method for the Determination of Streptomycin in Honey

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Abstract

In this application note, a rapid and sensitive method is described for the determination and confirmation of streptomycin in honey using a Hydrophilic Interaction Chromatography (HILIC) column, weak cation exchange Solid Phase Extraction (SPE) cartridges and the Waters Micromass Quattro micro API Tandem Quadrupole Mass Spectrometer.

Benefits

The Waters Micromass Quattro micro API Tandem Quadrupole Mass Spectrometer provides sensitivity and selectivity, and allows confirmation in a single injection

Introduction

Honey, like other foods, is subject to strict quality control measures before it can be sold commercially. It is a natural product, produced solely by bees from flower and tree pollen. Human actions on agricultural land can

directly affect honey quality.

One method for controlling a highly contagious fruittree disease, "fire blight," is to spray infected trees with streptomycin, even though this action is banned in many countries. It can be difficult to check for illegal use since a heavy rain shower is sufficient to wash this antibiotic away. Additionally, use can be localized only to those trees in an orchard that are infected. A more efficient way to detect the use of streptomycin for disease control is the analytical monitoring of honey. Bees collect the pollen, including any tainted with streptomycin, and convert it to honey. Measurement of streptomycin in honey from suspected areas will confirm its illegal use, as well as ensuring that the honey is fit for human consumption.

Streptomycin itself does not pose any direct human health risk, but antibiotics should not be present in honey as they could impact the effectiveness of antibiotics used in human medicine. An adult would have to eat eight jars of honey every day for a potential health risk.[1] Currently, no European Union Maximum Residue Limit (MRL) is legislated, although the Foods Standards Agency in the UK has an action limit of 50 µg/kg, and Switzerland and Germany have imposed an MRL in honey of 20 µg/kg.

Streptomycin detection has previously been reported by both biological and chemical methods.[2] Many of the reported methods either suffer from interference, lack of confirmatory evidence, lengthy extraction procedures, or use ion-pairing reagents to retain the otherwise very polar streptomycin. In this application note, a rapid and sensitive method is described for the determination and confirmation of streptomycin in honey using a Hydrophilic Interaction Chromatography (HILIC) column, weak cation exchange Solid Phase Extraction (SPE) cartridges and the Waters Micromass Quattro micro API Tandem Quadrupole Mass Spectrometer.

Experimental

Extraction Procedure

The procedure was developed from the published method by Kaufmann *et al.*[2]

- 20 g of honey was dissolved in approximately 75 mL water
- The solution was made up to a total volume of 100 mL with water, before filtering through a fluted filter
- Streptomycin was eluted with 2 x 5 mL SPE elution 2 x 5 mL SPE conditioning solvent (2% acetic acid) was

passed through a Waters Sep-Pak Vac 6 cc Accell Plus CM cartridge (p/n: WAT054545 < https://www.waters.com/nextgen/us/en/shop/sample-preparation--filtration/wat054545-sep-pak-accell-plus-cm-6-cc-vac-cartridge-500-mg-sorbent-per-car.html>)

- The cartridge was rinsed with 2 x 5 mL water and not allowed to dry out
- 50 mL of honey solution was loaded at approximately 2 drops per second
- The cartridge was rinsed with 2 x 5 mL water
- Streptomycin was eluted with 2 x 5 mL SPE elution solvent (80:20, 2% acetic acid/acetonitrile) into a volumetric flask
- The final volume was adjusted to 10.0 mL with the addition of water to produce a matrix equivalent of 1 g/mL

LC Conditions

LC:	Alliance 2795 HPLC System
Mobile phase A:	200 mM HCO_2NH_4 + 100 mM HCO_2H
Mobile phase B:	Acetonitrile + 100 mM HCO_2H
Column:	Atlantis HILIC Silica, 2.1 x 50 mm, 3 µm at 30 °C (Part No. 186002011)
Guard column:	Atlantis HILIC Silica, 2.1 x 10 mm, 3 µm (Part No. 186002005)
Flow rate:	0.3 mL/min
Injection volume:	20 µL

Gradient

Time(min)	%A
0	10
6	60
10	60
10.1	10
16	10

MS Conditions

MS:	Waters Micromass Quattro micro API Electrospray mode with positive polarity
Capillary voltage:	1.0 kV
Extractor:	4 V
RF lens:	0 V
Source temp.:	120 °C
Desolvation temp.:	500 °C
Cone gas flow:	60 L/hr
Desolvation gas flow:	1200 L/hr
Collision gas pressure:	Argon at $2.5e^{-3}$ mBar

Multiplier:	650 V

The quadrupole resolution was tuned so that the precursor and product ions were resolved with a half height peak width of <0.7 Da. The Multiple Reaction Monitoring (MRM) transitions, along with the collision energies and dwell times for the method are listed in Table 1. Four MRM transitions were monitored, a quantification and a confirmation transition for both streptomycin and the internal standard, dihydrostreptomycin.

	MRM Transition (eV)	Dwell Time (s)	Cone Voltage (V)	Collision Energy
Streptomycin	582 → 263	0.3	45	35
	582 → 176	0.3	45	38
Internal Std	584 → 263	0.1	40	30
	584 → 246	0.1	40	38

Table 1. MRM Method Parameters.

A series of matrix-matched calibration standards, matrix blanks and recovery samples were analyzed in order to determine method accuracy, linearity, precision, repeatability and recovery. The Limit of Determination (LOD) was also estimated from the lowest concentration matrix-matched standard. The internal standard, dihydrostreptomycin was spiked at 50 µg/kg in all samples. Matrix-matched calibration standards were made up at 1, 2, 5, 10, 20, 50, and 100 µg/kg. Recovery samples were spiked at 20 µg/kg prior to extraction.

Results and Discussion

LC-MS and LC-MS/MS methods for this determination have previously revolved around the use of ion-pairing

reagents to retain the very polar streptomycin.[2] In this instance, streptomycin was retained using a HILIC column.

Hydrophilic Interaction Chromatography (HILIC) is a variation of normal phase chromatography where the organic portion of the mobile phase is the weak solvent and the aqueous portion is the strong solvent. Elution is in the order of increasing hydrophilicity and the technique can be used for polar compounds un-retained by conventional reverse-phase HPLC. HILIC columns provide a practical alternative to ion-pairing reagents, using electrospray-friendly solvents that can enhance electrospray sensitivity due to the highly organic mobile phases that promote better desolvation. There is the possibility of decreasing the sample preparation time, as the evaporation and reconstitution steps can be eliminated because the organic fraction can be injected directly onto the HILIC column. Figure 1 illustrates the retention of streptomycin on a HILIC column compared to that on a reverse-phase column with 100% water.

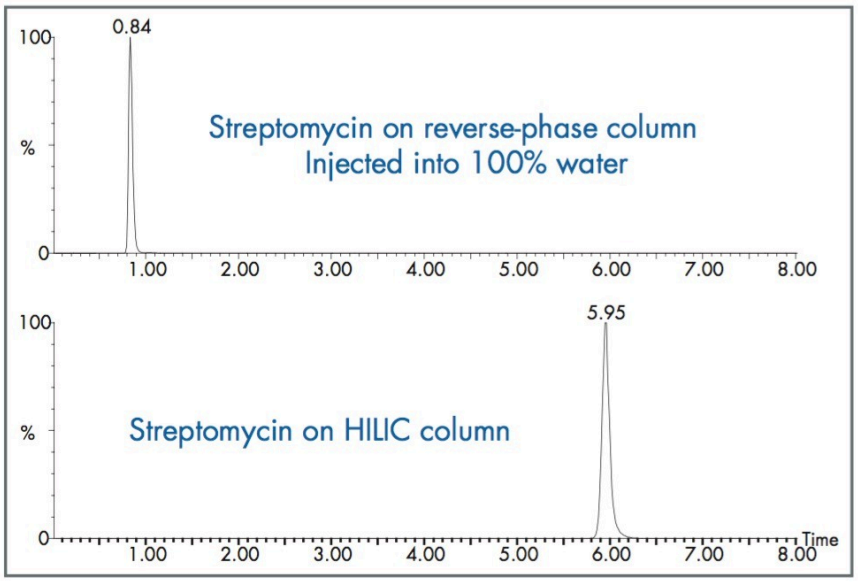

Figure 1. Comparison of retention for streptomycin.

To test the extraction method described, four recovery experiments were performed for streptomycin in honey spiked at 20 µg/kg, the MRL in Switzerland and Germany. Each sample was analyzed in duplicate and compared to a calibration curve of matrix-matched standards. The mean recovery was determined to be 84.5% with a relative standard deviation of 4.1%.

As this method is defined as a confirmatory method, two MRM transitions per compound were

monitored. Chromatograms corresponding to the four transitions are illustrated in Figure 2. The ion ratio between the quantification transition (28049) and the confirmation transition (17121) for streptomycin is 1.64. The presence of streptomycin is considered confirmed if the observed ion ratio from any extract does not deviate by more than 20% from this expected value.[3]

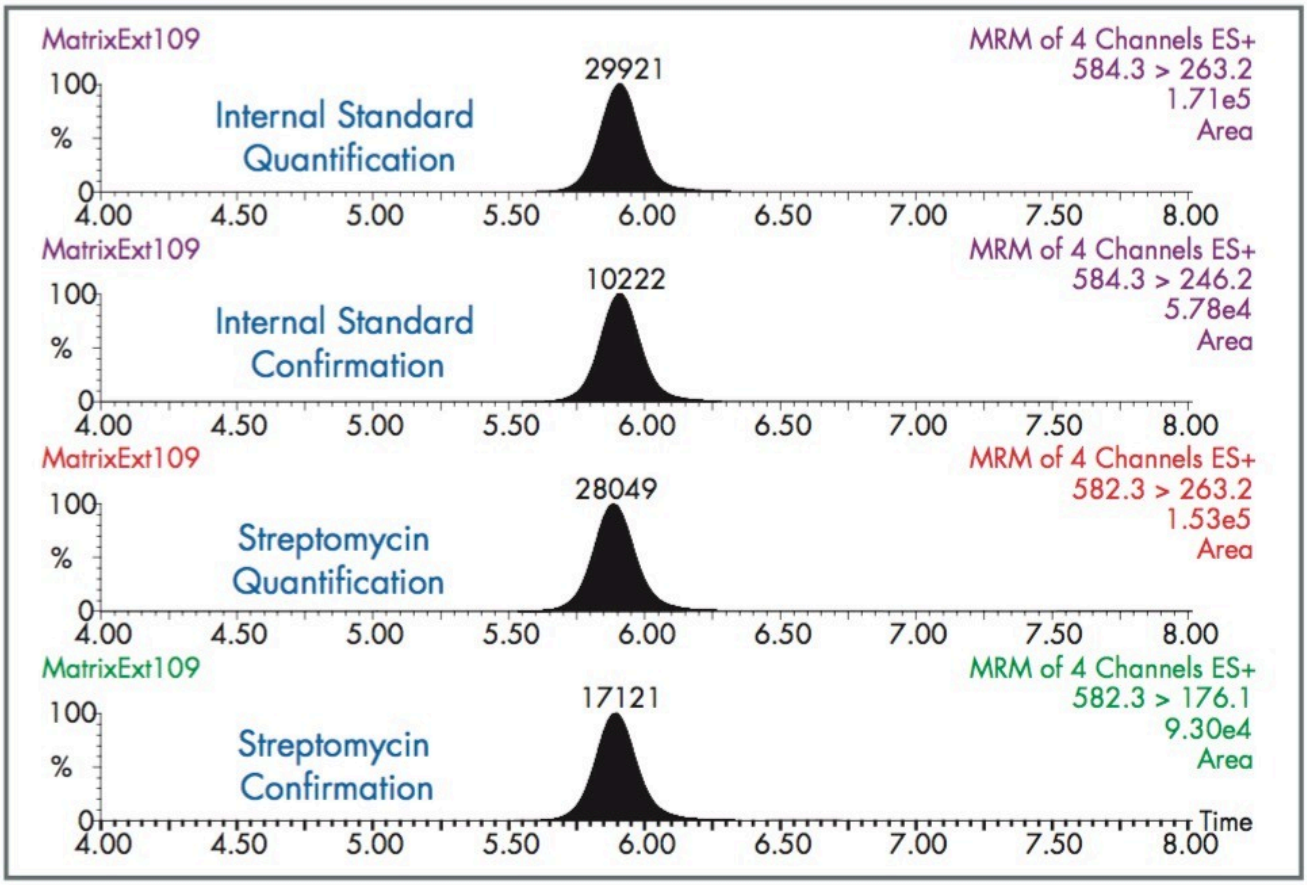

Figure 2. MRM chromatograms for streptomycin and the internal standard.

The quantification (461) and confirmation (280) chromatograms for a honey extract spiked with 1 µg/kg streptomycin are illustrated in Figure 3. Even at this low concentration, 20 times lower than the MRL, the confirmation ion ratio of 1.65 is easily within the confirmation criteria of 1.64 ± 20%. By extrapolation, the confirmation LOD, where both MRM transitions have a signal-to-noise (S/N) ratio of > 3:1, can be estimated as <0.1 µg/kg. This compares favorably with previously reported methods using ion-pairing reagents.[2]

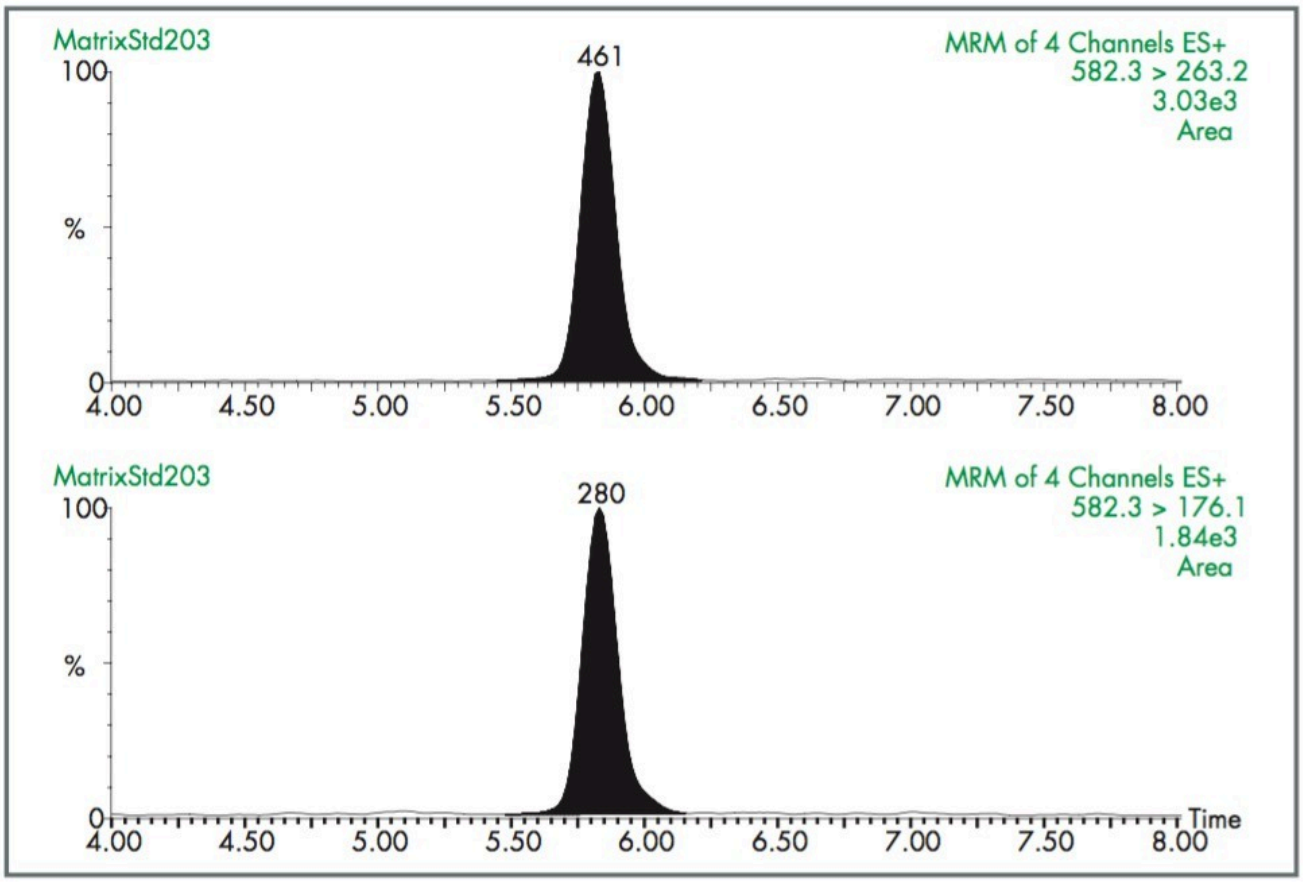

Figure 3. Streptomycin confirmed at 1 µg/kg.

Matrix-matched standards were generated at the 1, 2, 5, 10, 20, 50, and 100 µg/kg levels in honey. These standards were each injected five times in a typical batch analysis. The data was then processed using Waters TargetLynx Application Manager. A representative calibration curve with a correlation coefficient of $r^2 = 0.9998$ is illustrated in Figure 4.

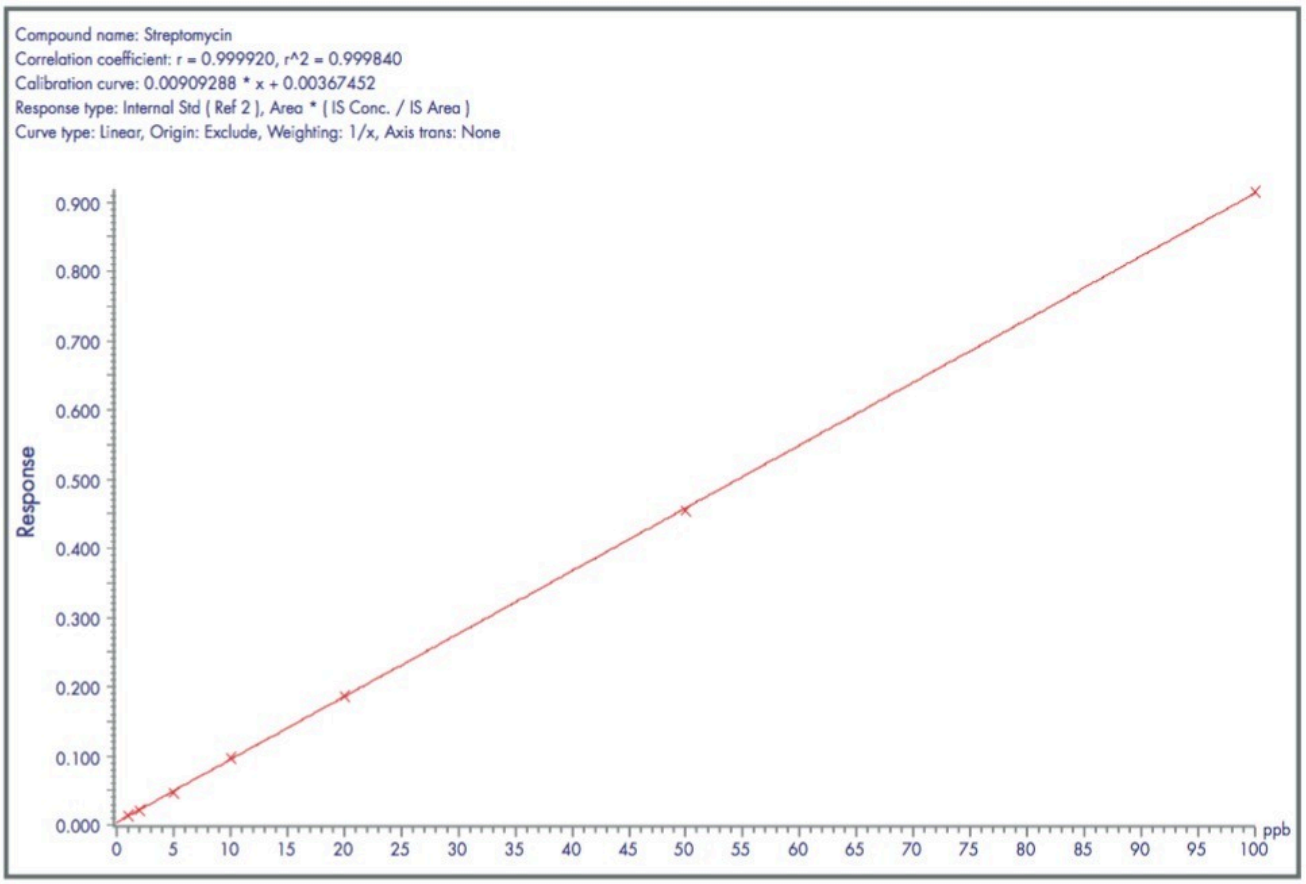

Figure 4. Calibration curve for streptomycin in honey, 1–100 µg/kg.

The method accuracy and precision are listed in Table 2. Five injections were performed on day one and two injections on days two and three. These matrix spikes formed part of three batch analyses totalling 115 matrix injections. Good instrumental accuracy (mean) and precision (%RSD) were obtained at ten times less than the MRL, and five times greater than the MRL.

Spiked Concentration µg/kg	Mean	Std. Dev.	% RSD
2.0	1.99	0.09	4.5
20.0	20.17	0.76	3.7
100.0	100.91	3.75	3.7

Table 2. Method accuracy and precision over three days.

The method repeatability for three batches of matrix-matched calibration standards is illustrated in Figure 5. This graph shows response factor (peak area/concentration) against injection number. Twenty five injections were performed on day one and ten injections on days two and three. No instrument maintenance was performed between each day other than flushing the column with acetonitrile and water without buffer. The graph indicates the repeatability of the method with a good relative standard deviation of 4.6% across all forty five injections. The response factor will remain constant if the response is linear and the source robustness is good.

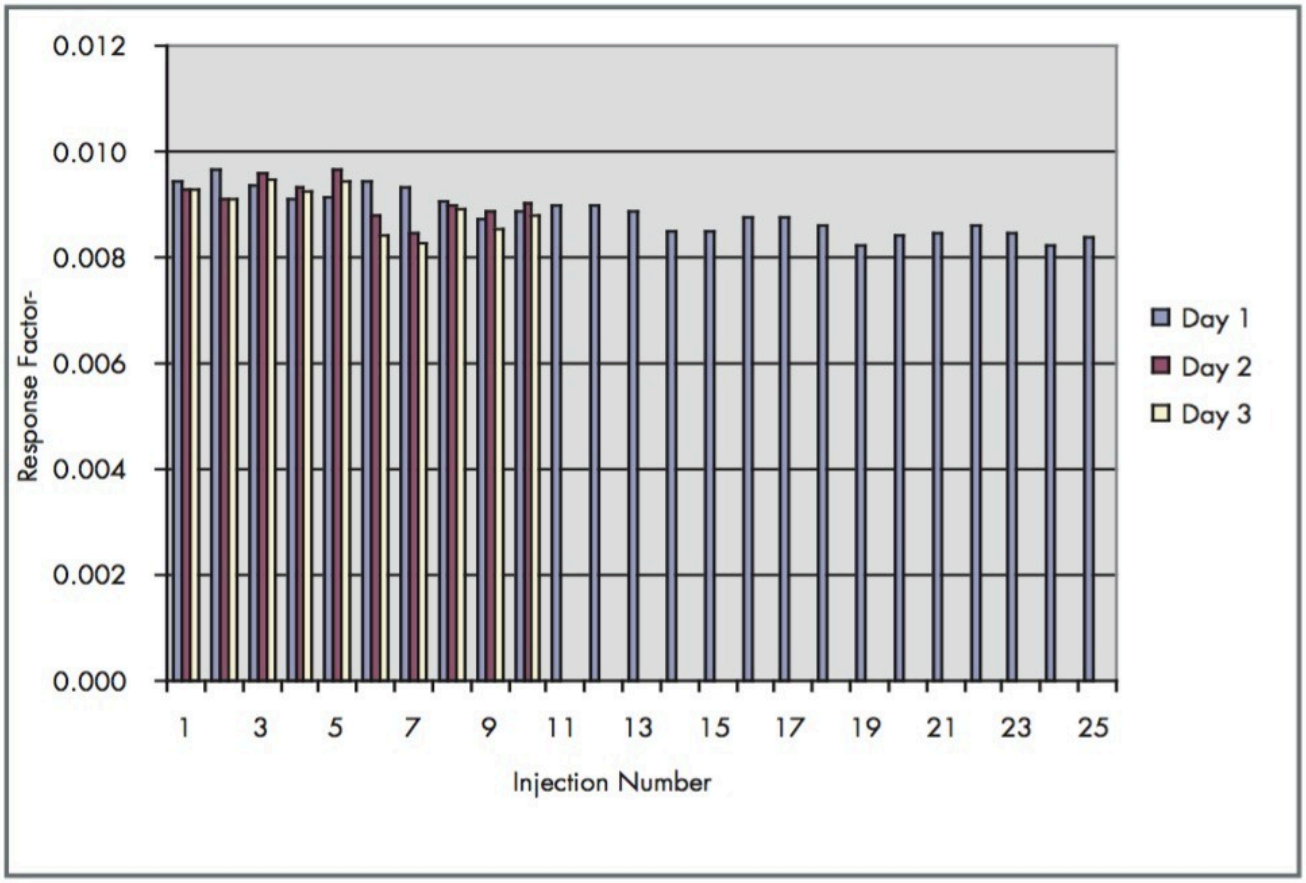

Figure 5. Response factor versus injection number over three days.

Ion ratios between the quantification transition and the confirmation transition are important as they provide the basis of confirmation. The ion ratio statistics are listed in Table 3 for sixty three matrix injections of streptomycin over a three-day period, in three separate batch analyzes. The relative standard deviation indicates good repeatability of confirmation ion ratios with a number significantly less than the EU regulation[3] for MRM transitions with an ion ratio of 1.63 (61%).

Mean	1.63
Std. Dev.	0.03
% RSD	1.9
EU Regulation 2002/657/EC	± 20 %

Table 3. Confirmation ion ratio repeatability over three days.

In this method, 10 g honey is dissolved in 50 mL water and extracted with a 500 mg Sep-Pak Cartridge, and eluted with 10 mL solvent. One possible method improvement could be to reduce the volumes and weights proportionally to decrease the extraction time, the solvent usage and cartridge size. Additionally, the method is based around the Sep-Pak technology that does not allow drying between steps. Another possible method enhancement could be to change to the Waters Oasis WCX (Weak Cation Exchange) cartridge to upgrade to the very latest, innovative SPE chemistry.

Conclusion

A rapid and sensitive method has been described for the determination and confirmation of streptomycin in honey. The Atlantis HILIC Column and electrosprayfriendly solvents provide good retention, peak shape and sensitivity. A simple extraction method using Sep-Pak weak cation exchange SPE cartridges provides good recovery. The Waters Micromass Quattro micro API Tandem Quadrupole Mass Spectrometer provides sensitivity and selectivity, and allows confirmation in a single injection. The limits of determination achieved are well below that required by legislation for any country in the European Union.

References

1. http://www.foodstandards.gov.uk/multimedia/faq/honeyfaq/
2. Kaufmann, A., Butcher, P. and Kolbener, P., Rapid Commun. *Mass Spectrom*, 17 (2003) 2575–2577.

3. Commission Decision 2002/657/EC, *Official Journal of the European Communities*, No. L221 (2002) 8–36.

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