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Applikationsbericht

Application of ACQUITY TQD for the Analysis of Chloramphenicol Residues in Honey Extracts

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Abstract

This application note will evaluate the suitability of the Waters ACQUITY TQD for tandem quadrupole based analysis of chloramphenicol with its deuterated internal standard using Multiple Reaction Monitoring (MRM) experiments. Honey extracts will be used for this analysis.

Introduction

Chloramphenicol is used by beekeepers to control American Foulbrood Disease within hives which is believed to be caused by Paenibaccilus larvae. See Figure 1. This is used even though there are no approved antibiotic treatments for American Foulbrood.

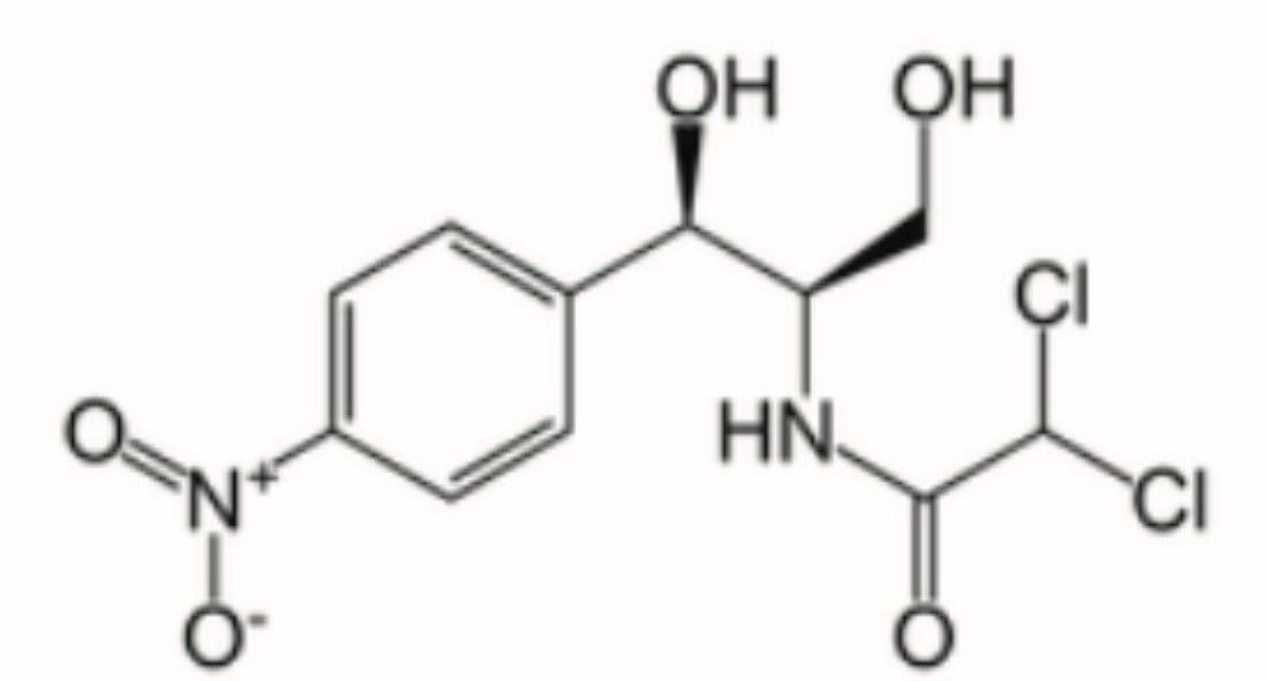


Figure 1. Chloramphenicol molecule.

Concern with antibiotic therapies is that they may remove the symptoms but a low level of the bacteria may persist, and can then be spread to otherwise healthy hives. Chloramphenicol is a potent antibiotic that may

eliminate the disease in hives. It had been in worldwide use before being widely withdrawn due to restrictions upon its entry into food products1. Residues of chloramphenicol can be found in honey if bees are treated during the harvesting season.[2]

The current EU legislation that governs chloramphenicol is EU decision 2003/181/EC.[3] A Minimum Required Performance Limit (MRPL) has been set at 0.3 µg/kg for honey as it falls into the category of animal derived food. The Japanese Positive List[4] and FDA[5] both state that chloramphenicol should not be present in honey and it is banned from food in both countries.

The MRPL is the target for laboratories with the least sensitive analytical techniques. Achieving and going below this level will lead to an increase in food safety as contaminated batches which do not meet the relevant regulations can be destroyed. Chloramphenicol toxicity in humans has a wide range of effects. Bone marrow depression is common but the effects can be reversed if levels in the body are lowered or eliminated. Aplastic anemia gives a more serious reaction which is not considered to be dose related. It has a frequency of one incidence in every 24,000–40,000 courses of treatment, with a mortality rate greater than 50% of cases.[6]

The introduction of the ACQUITY TQD detector allows scientists to perform chloramphenicol analysis while harnessing all the benefits that this instrument brings to the laboratory. See Figure 2. The IntelliStart technology in this instrument is designed to reduce the burden of complicated operation, time-intensive troubleshooting, and upkeep. Its small footprint will give any laboratory an advantage as this powerful tool removes the need for larger instrumentation.

Figure 2. ACQUITY TQD featuring the TQ Detector.

This note describes chloramphenicol analysis, using a solution which is able to exceed the requirements of worldwide regulation, the lowest level being the current EU legislation.

Experimental

Blank honey extracts and solvent solutions of chloramphenicol and deuterated chloramphenicol (internal standard) were kindly provided by the Central Science Laboratory, (CSL) York, UK. This CSL procedure involves an overall 2x dilution during the extraction from honey to in-vial sample. Samples for analysis were prepared by spiking the blank extract at varying concentrations to achieve a matrix matched calibration curve.

UPLC Conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18} Column 2.1 x 50 mm, 1.7 µm
Column temp:	30 °C
Flow rate:	450 µL/min.
Mobile phase A:	98:2 (v/v) water:methanol
Mobile phase B:	Methanol
Total run time :	3.5 min
Injection volume :	20 µL, Full loop injection

Gradient:

Time 0.00 min	95% A
Time 0.50 min	95% A

Time 1.60 min	10% A
Time 1.90 min	10% A
Time 2.05 min	95% A

MS Conditions

MS system:	Waters ACQUITY TQ Detector
Ionization mode:	ESI negative polarity
Capillary voltage:	3 kV
Cone voltage:	30 V
Desolvation gas:	Nitrogen, 800 L/Hr, 450 °C
Cone gas:	Nitrogen, 20 L/Hr
Source temp:	120 °C
Acquisition:	Multiple Reaction Monitoring (MRM)
Collision gas:	Argon at 3.5 x 10^{-3} mBar

Acquisition and Processing Methods

The data were acquired using Waters MassLynx Software v. 4.1. Incorporated into MassLynx is the IntelliStart technology which was designed specifically for the SQ and TQ detectors. IntelliStart automates optimization of MS parameters for your sample and also monitors the health of the MS system, reducing the time for operator-intensive troubleshooting and upkeep. The data was processed using TargetLynx Application Manager. This quantification package is capable of automating quality control checks such as calculating ion ratios, flagging analytical results above/below thresholds set by the user, plus other features.

Results and Discussion

Chloramphenicol was chromatographed successfully. Figure 3 shows chloramphenicol and its internal standard at a concentration equivalent to ten times below the MRPL – 0.03 µg/kg in matrix. This gives a signal to noise figure of 55:1, peak to peak. The ACQUITY UPLC method described gives a retention time of 1.66 minutes for chloramphenicol.

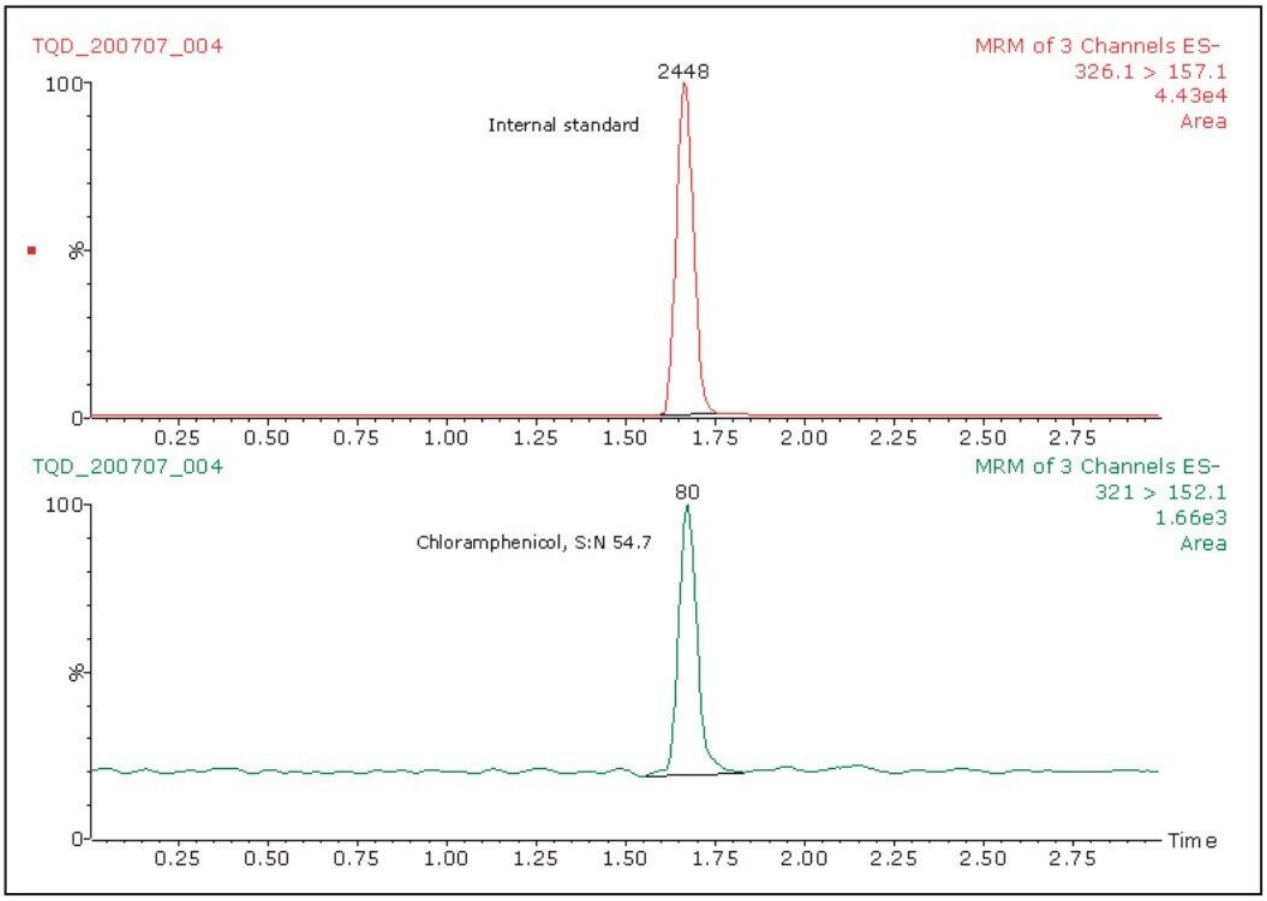
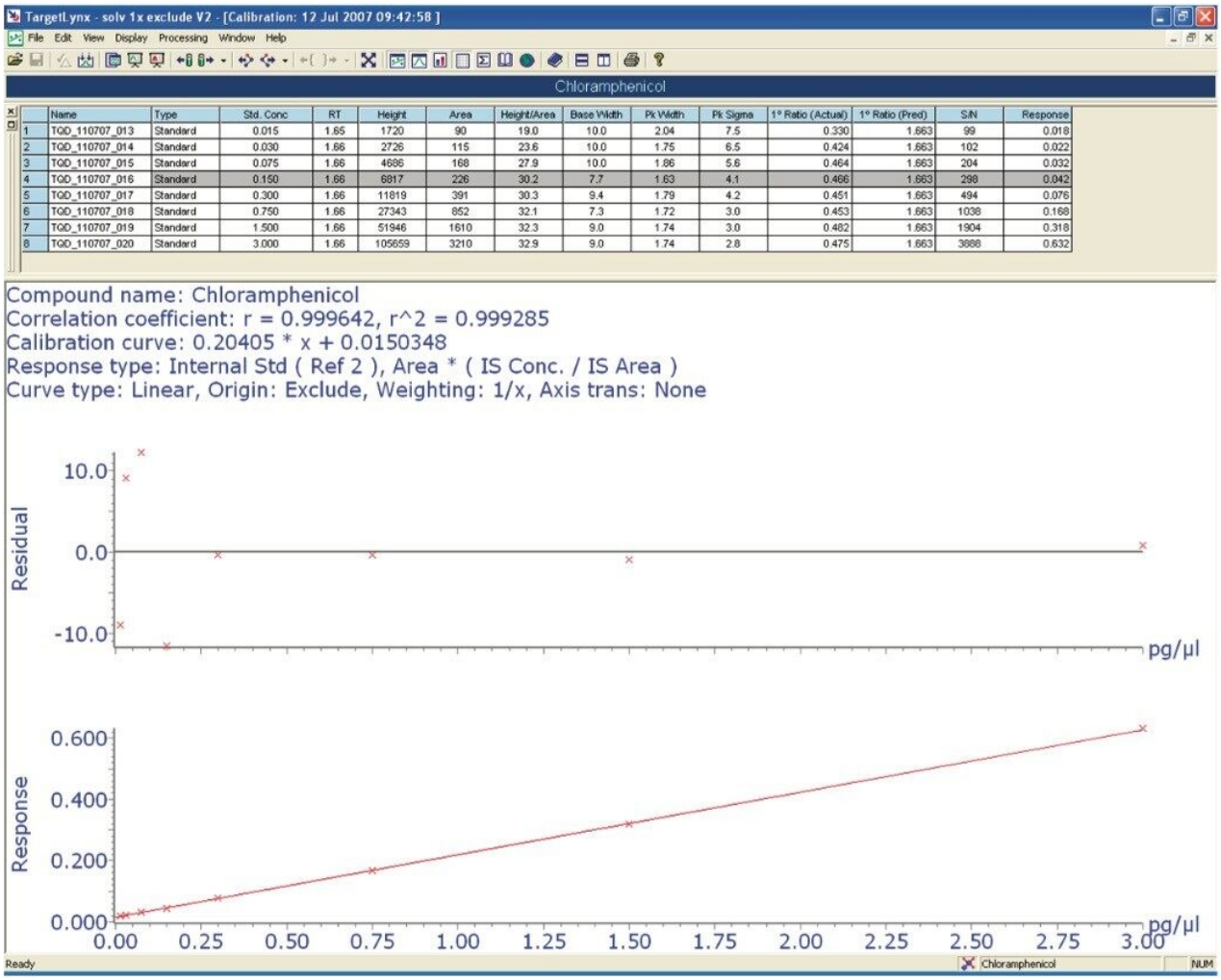


Figure 3. Chloramphenicol in honey matrix ten times below the MRPL at 0.03 µg/kg.

Figure 4 shows the linearity of the ACQUITY TQD. Here the quantifiable range is from 0.015 pg/µL to 3 pg/µL equivalent to 0.03 µg/kg to 6 µg/kg in pre extracted honey. This calibration curve shows good linearity with a correlation coefficient of r^2 >0.999. The variance between the calibration points in the line is within acceptable values as all points lie within 15% of their nominal value. The linear range achieved with this assay is equivalent to 0.03 µg/kg to 6 µg/kg in honey.



	Name	Type	Std. Conc	RT	Height	Area	Height/Area	Base Width	Pk Width	Pk Sigma	1° Ratio (Actual)	1° Ratio (Pred)	S/N	Response
1	TQD_110707_013	Standard	0.015	1.65	1720	90	19.0	10.0	2.04	7.5	0.330	1.663	99	0.018
2	TQD_110707_014	Standard	0.030	1.66	2726	115	23.6	10.0	1.75	6.5	0.424	1.663	102	0.022
3	TQD_110707_015	Standard	0.075	1.66	4686	168	27.9	10.0	1.86	5.6	0.464	1.663	204	0.032
4	TQD_110707_016	Standard	0.150	1.66	6817	226	30.2	7.7	1.63	4.1	0.466	1.663	298	0.042
5	TQD_110707_017	Standard	0.300	1.66	11819	391	30.3	9.4	1.79	4.2	0.451	1.663	494	0.076
6	TQD_110707_018	Standard	0.750	1.66	27343	852	32.1	7.3	1.72	3.0	0.453	1.663	1038	0.168
7	TQD_110707_019	Standard	1.500	1.66	51946	1610	32.3	9.0	1.74	3.0	0.482	1.663	1904	0.318
8	TQD_110707_020	Standard	3.000	1.66	105659	3210	32.9	9.0	1.74	2.8	0.475	1.663	3886	0.632

Figure 4. TargetLynx screenshot showing linear range of chloramphenicol in honey matrix (0.015 pg/µL to 3 pg/µL equivalent to 0.03 µg/kg to 6 µg/kg in pre-extracted honey).

The low point of the calibration curve allows quantitation to ten times below the MRPL. A further increase in sensitivity can be achieved using ACQUITY TQD by injecting more sample onto the column or modifying the sample extraction method. The extraction used includes an overall dilution of 2 resulting in a matrix equivalent of 0.5 g/mL. A sample concentration step would give lower levels of quantitation with this instrument.

Instrument ruggedness was tested using multiple injections of matrix-matched standard at the MRPL level. The amount of matrix injected onto column was 700 mg when seventy injections were made in succession.

The plot of peak area against injection number shows that there is no drop off in response when considering the analyte peak area only. See Figure 5. The percent relative standard deviation (%RSD) is shown in the

header of the graph and is 5.1% over the 70 injection run.

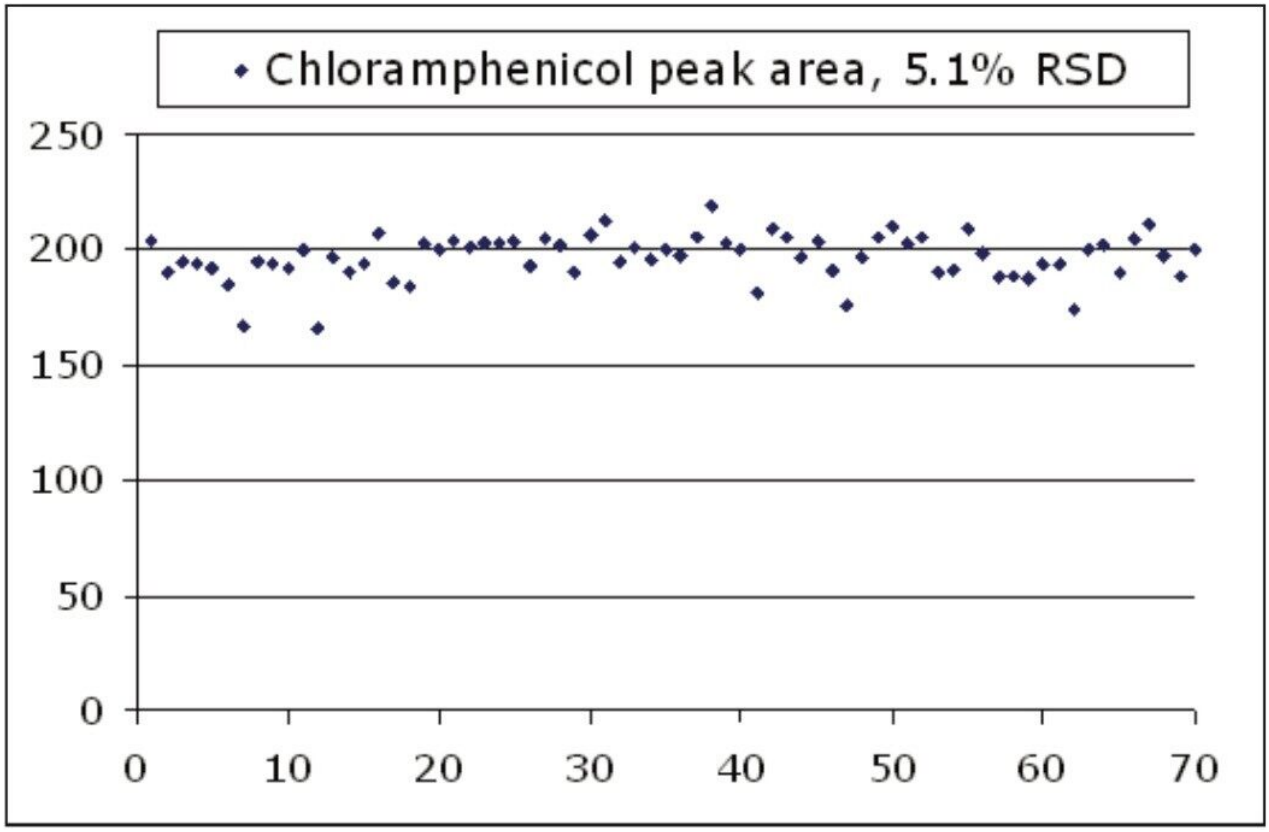


Figure 5. Plot showing changes in chloramphenicol peak area over a 70 injection run. The amount of compound injected onto the column from the sample extract is equivalent to the MRPL level of 0.3 µg/kg in honey.

The response ratio was also measured over the run (analyte peak area/internal standard peak area). Figure 6 shows a similar pattern to Figure 5 with regard to outliers and overall %RSD. This shows that this assay can be run either with or without internal standard but will give better results when it is included.

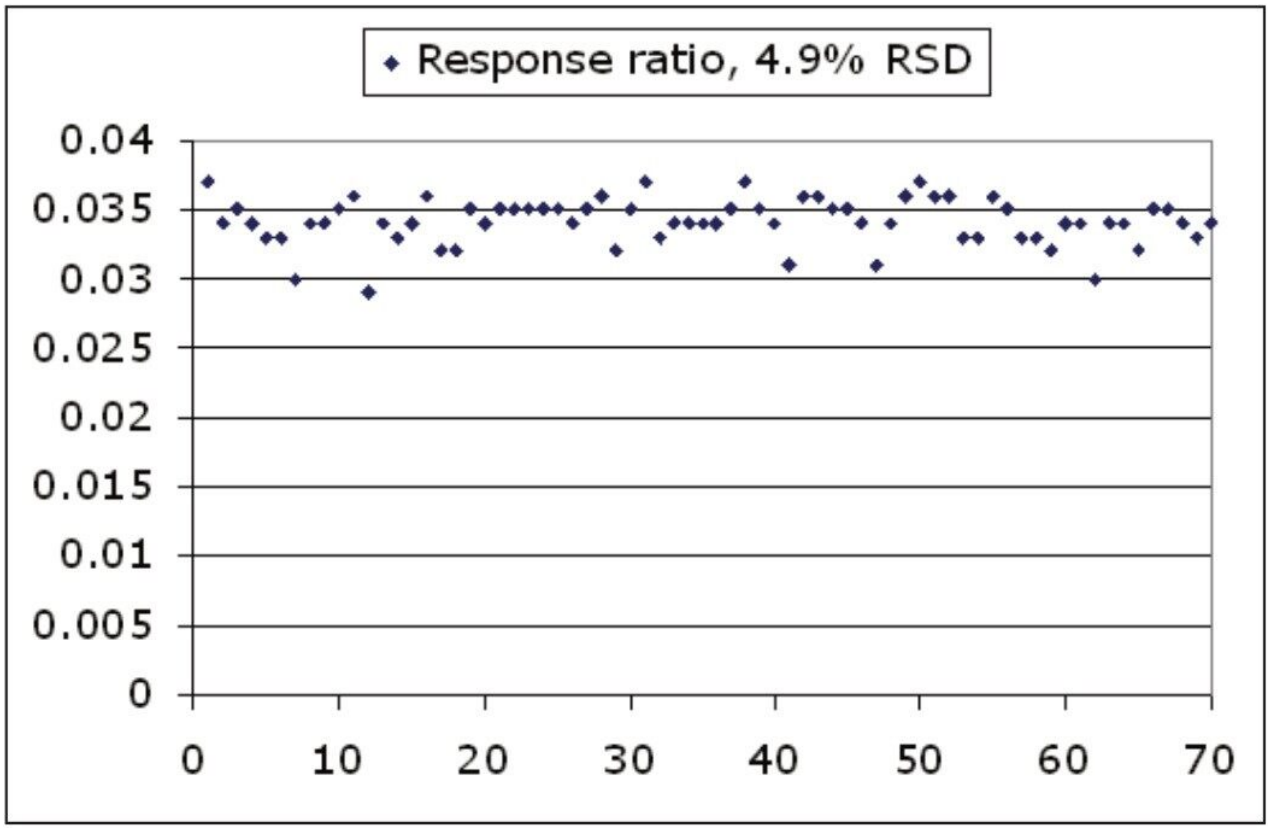


Figure 6. Plot showing changes in chloramphenicol response ratio over a 70 injection run. The amount of compound injected onto the column from the sample extract is equivalent to the MRPL level of 0.3 µg/kg in honey.

Ion ratio stability was also assessed. The average difference is 7%. Figure 7 shows this plot with the mean value highlighted as a red line. All injections lie within a 20% tolerance limit as specified in EU legislation 2002/657/EC.[7]

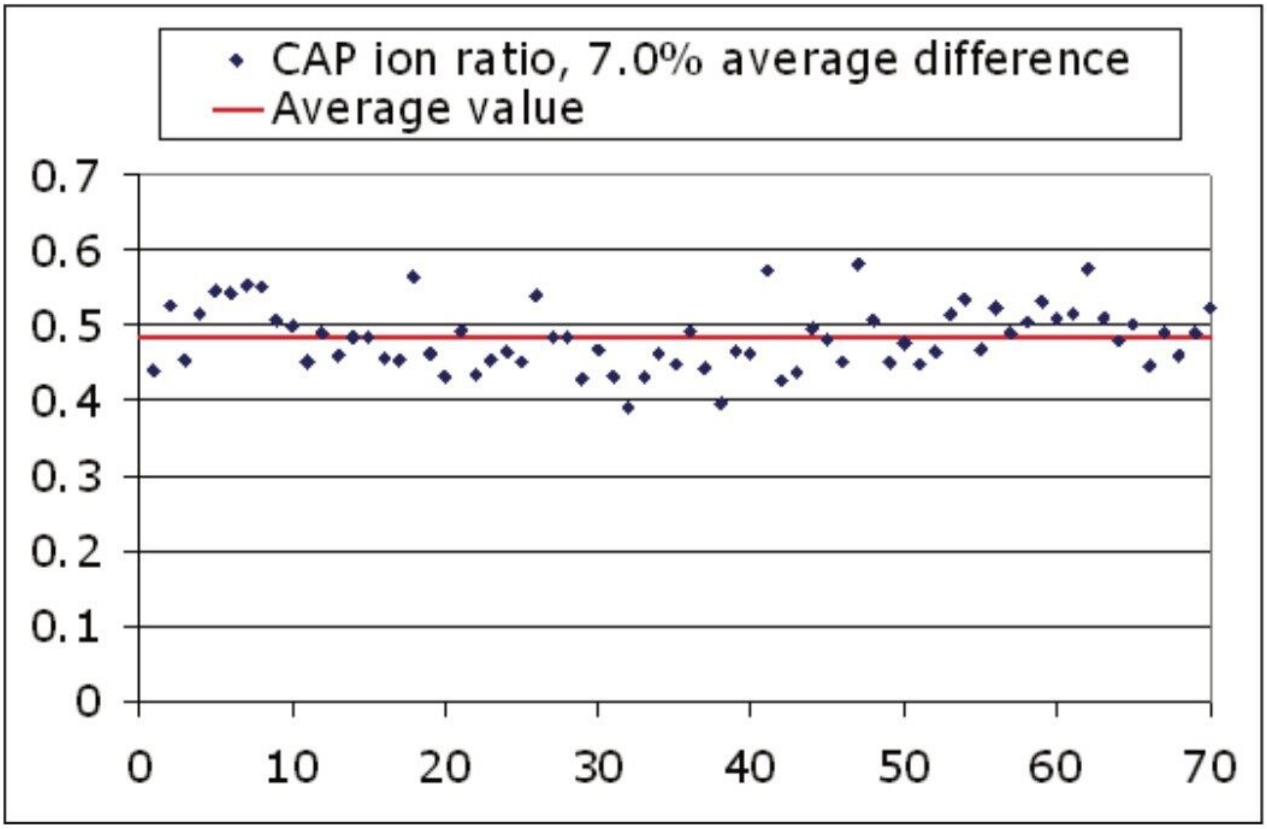


Figure 7. Plot showing changes in chloramphenicol ion ratio over a 70 injection run. The amount of compound injected onto the column from the sample extract is equivalent to the MRPL level of 0.3µg/kg in honey.

Conclusion

A method based on ACQUITY TQD was developed for the analysis of chloramphenicol in honey extract.

Chloramphenicol, which causes serious harm to human health, can be quantified in honey matrix using the method described at levels below the EU legislation limits with ACQUITY TQD.

ACQUITY TQD response remained constant throughout a run of 70 samples with a tight RSD of 5.1%.

Ion ratios for confirmation using two MRM transitions were shown to be stable which is important for quantification and confirmation.

The benefits of UPLC for a revenue conscious laboratory are shown with increased speed, along with reduced solvent usage and the associated costs of solvents and solvent disposal.

References

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ACQUITY UPLC System <https://www.waters.com/514207>

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