Analysis of chloramphenicol in honey

Using the SCIEX Triple Quad 3500 system

Santosh Kapil Gorti[1], Chandrasekar M[1], Aman Sharma[1], Anoop Kumar[1], Manoj Pillai[1] and Jianru Stahl-Zheng[2]
[1]SCIEX, India; [2]SCIEX, Germany

Introduction

This technical note describes a sensitive LC-MS/MS method for analyzing chloramphenicol in honey using the SCIEX Triple Quad 3500 system. The method development was performed to meet the minimum required performance limit (MRPL) of 0.3 ng/mL as established by the EC Commission Decision 657/2002.[1]

Chloramphenicol has a wide spectrum of antimicrobial activities. It is effective against Gram-positive and Gram-negative cocci and bacilli (including anaerobes), rickettsia, mycoplasma and chlamydia. It is widely used as a human antibiotic and as a veterinary drug. Using the SCIEX Triple Quad 3500 system, multiple reaction monitoring (MRM) was used to quantify chloramphenicol in honey and the ion ratio was used to confirm its identification. The method was successfully applied to the analysis of store-bought honey samples (Figure 1).

The design of the Turbo V ion source and curtain gas interface used on the SCIEX Triple Quad 3500 system provides exceptional robustness and ruggedness. The advanced eQ electronics and the curved LINAC collision cell were designed for ultra-fast MRM detection and fast polarity switching for comprehensive multi-component analysis.

SCIEX Triple Quad 3500 system.

Key features of the SCIEX Triple Quad 3500 system for the analysis of chloramphenicol

- A sensitive, specific, rugged and reproducible LC-MS/MS method was developed for the analysis of chloramphenicol in honey using a simple extraction technique
- High accuracies (85 to 115%) and precision (%CV <5%) were achieved for matrix-matched calibration standards prepared in honey
- Mean recovery of 88% was achieved for chloramphenicol spiked at the MRPL concentration of 0.3 ng/mL in honey

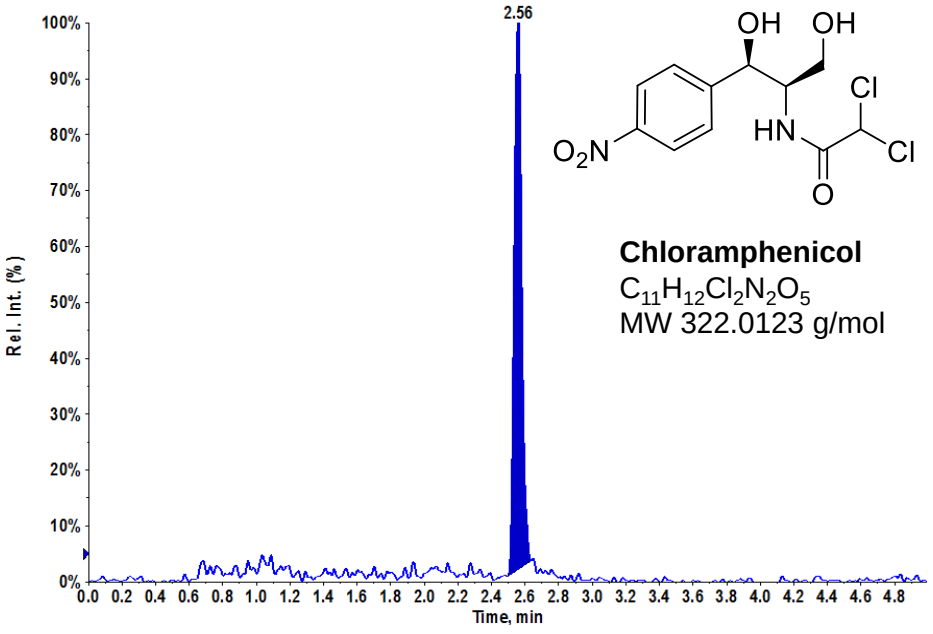


Figure 1. Representative extracted ion chromatogram (XIC) of chloramphenicol in a 0.1 ng/mL standard spiked in honey.

Experimental methods

Chemicals and samples: A neat standard of chloramphenicol (with chemical purity ≥99%) was purchased from Clearsynth. All other chemicals used were of LC-MS grade.

Honey samples were purchased from the local market in Delhi and Gurgaon, India and were kept at 2–8°C until analysis.

Sample preparation: A 1 g aliquot of honey was added to 5 mL of water, then vortexed for 2 minutes. Upon adding 10 mL of acetonitrile and 1 g of sodium chloride, the solution was mixed well and then centrifuged for 5 minutes at 4000 rpm. The acetonitrile layer was transferred to another tube and then evaporated to dryness under nitrogen. The residue was reconstituted with 1 mL of 90:10 (v/v), water/acetonitrile diluent for LC-MS/MS analysis.

Chromatography: LC separation was performed on a Shimadzu system using a Phenomenex Synergi Fusion RP C18 column (50 x 2.6 mm, 2.5 µm). A flow rate of 0.4 mL/min and an injection volume of 20 µL were used. The 5-minute gradient used is presented in Table 1.

Table 1. Chromatographic gradient.

Time (min)	%A	%B
0.01	*85*	*15*
0.30	*85*	*15*
0.50	*75*	*25*
1.00	*70*	*30*
1.50	*15*	*85*
3.00	*15*	*85*
4.00	*85*	*15*
5.00	*Controller*	*Stop*

Mobile phase A: *Water*
Mobile phase B: *Acetonitrile*

Mass spectrometry: Analysis was performed using the SCIEX Triple Quad 3500 system with a Turbo V ion source in negative electrospray ionization mode at an ion spray voltage of 2800 V. Data were acquired in MRM mode. Table 2 shows the 2 MRM transitions used to monitor chloramphenicol and their corresponding compound-dependent parameters.

Table 2. MRM transitions and compound-dependent parameters for chloramphenicol.

Analyte	Q1 > Q3	DP (V)	CE (V)
Chloramphenicol 1	*320.8 > 151.8*	*-85*	*-15*
Chloramphenicol 2	*320.8 > 256.8*	*-85*	*-23*

Chloramphenicol 1 was used as the quantifier transition and chloramphenicol 2 used as the qualifier transition.

Data acquisition and processing: Analyst software, version 1.6.2 was used for data acquisition. All data were processed using MultiQuant software, version 3.0.1. The peak areas from the quantifier and qualifier transitions were used to calculate the ion ratio for the identification of chloramphenicol (Figure 2).

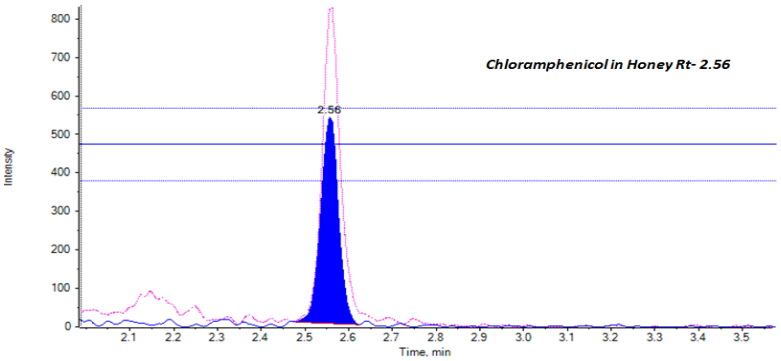


Figure 2. XIC of chloramphenicol in honey. Chloramphenicol eluted at a retention time of 2.56 min. The MRM ratio was <1.0. The quantifier ion is shown in blue and the qualifier ion is shown in pink.

Calibration performance

A calibration curve was prepared with levels ranging from 0.1 ng/mL to 5.0 ng/mL spiked concentration, achieving a regression coefficient (*r*) of 0.99 using a weighting factor of $1/x^2$ (Figures 3 and 4).

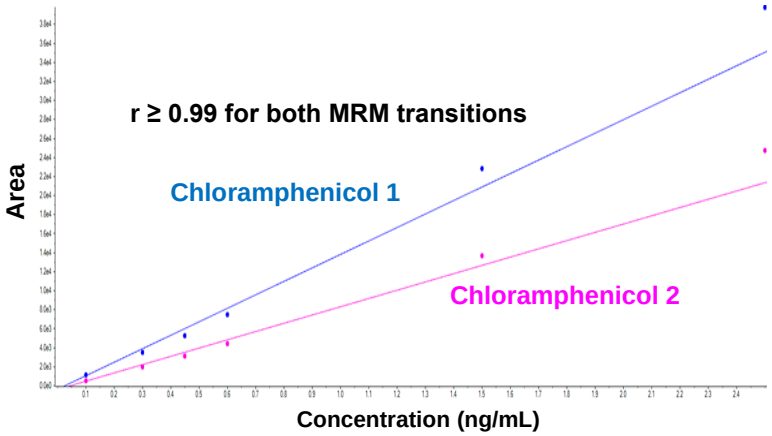
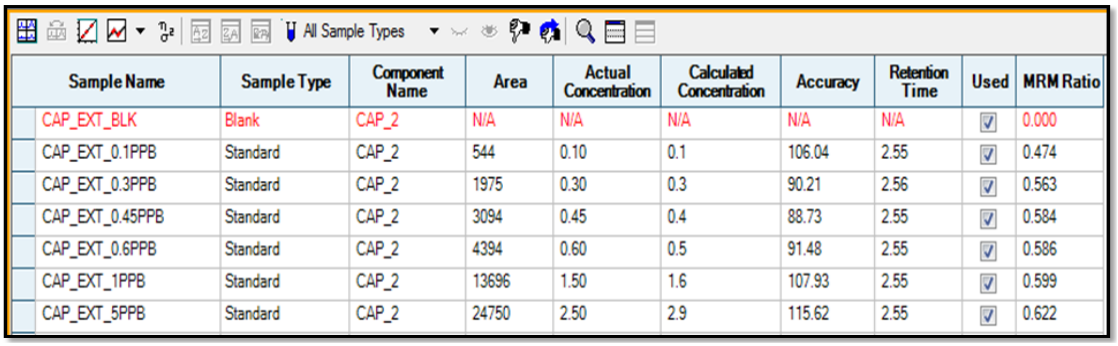


Figure 3. Calibration curves for chloramphenicol. The linear dynamic range spanned 0.1 to 5.0 ng/mL with an r value >0.99.



Sample Name	Sample Type	Component Name	Area	Actual Concentration	Calculated Concentration	Accuracy	Retention Time	Used	MRM Ratio
CAP_EXT_BLK	Blank	CAP_2	N/A	N/A	N/A	N/A	N/A	☑	0.000
CAP_EXT_0.1PPB	Standard	CAP_2	544	0.10	0.1	106.04	2.55	☑	0.474
CAP_EXT_0.3PPB	Standard	CAP_2	1975	0.30	0.3	90.21	2.56	☑	0.563
CAP_EXT_0.45PPB	Standard	CAP_2	3094	0.45	0.4	88.73	2.55	☑	0.584
CAP_EXT_0.6PPB	Standard	CAP_2	4394	0.60	0.5	91.48	2.55	☑	0.586
CAP_EXT_1PPB	Standard	CAP_2	13696	1.50	1.6	107.93	2.55	☑	0.599
CAP_EXT_5PPB	Standard	CAP_2	24750	2.50	2.9	115.62	2.55	☑	0.622

Figure 4. Accuracy and ion ratio data for matrix-matched calibration standards of chloramphenicol spiked in honey.

Method performance in honey spikes

Recovery was assessed in replicates (n=6) of honey samples spiked at the MRPL concentration of 0.3 ng/mL. The mean recovery of chloramphenicol was 88% (Table 3).

Table 3. Recovery of chloramphenicol spiked in honey matrix at the MRPL concentration level.

Replicates (*n* = 6)	Concentration (ng/mL)
1	*0.300*
2	*0.250*
3	*0.250*
4	*0.250*
5	*0.270*
6	*0.260*
Average concentration (ng/mL)	*0.263*
Original concentration (ng/mL)	*0.300*
Recovery (%)	*87.78*

Conclusions

The method and data presented here showcase a fast and accurate solution that meets regulatory requirements for the quantitation and identification of chloramphenicol in honey samples by LC-MS/MS. The SCIEX Triple Quad 3500 system provided excellent sensitivity and selectivity and minimal sample preparation was performed to maximize throughput. Automatic MRM ratio calculation in MultiQuant software can be used for confirmation in compound identification.

References

1. European Commision. (2003) 2003/181/EC: Commission Decision of 13 March 2003 amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin (Text with EEA relevance). *Official Journal of the European Union.*
2. ECFA, Who Food Additives Series, 53, 1–40, (2014)
3. FSSAI manual of methods of analysis of food. http://fssai.gov.in/Portals/0/Pdf/15Manuals/ANTIBIOTICS%20AND%20RESIDUES.pdf
4. A. Schreiber: 'MultiQuant™ Software Version 3.0 - Improving Data Quality and Processing Throughput with Better Peak Integration, Quantitative and Qualitative Compound Review for the Analysis of Food, Drinking Water, and Environmental Samples' Application Note AB SCIEX (2013) #8160213-01
5. Letícia R. Guidi, Luiza H. M. Silva, Christian Fernandes, Nicki J. Engeseth and Maria Beatriz A. Gloria; LC-MS/MS determination of chloramphenicol in food of animal origin in Brazil. Scientia Chromatographica 2015; 7(4): 287-295
6. EC European Commission, Commission Decision 2002/657/EC of 12 August 2002. Off. J Eur. Communities, L221, 8–36 (2002)

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices