Application Note

Food Testing & Agriculture

Agilent

Trusted Answers

Detection, Confirmation, and Quantitation of Chloramphenicol in Honey

Using the Agilent 1260 Infinity II LC system coupled with the Agilent Ultivo LC/TQ

Author

Vikrant Goel
Agilent Technologies, Inc.

Abstract

This Application Note demonstrates the use of the Agilent 1260 Infinity II LC system coupled with the Agilent Ultivo LC/TQ to achieve very low picogram quantities of chloramphenicol (CAP) in honey samples. The method was developed on an Agilent Ultivo LC/TQ, which provides uncompromising results, despite the miniaturized form factor. This application is ideal for routine analysis in the food industry during the manufacturing, processing, and commercial testing of honey samples, or for academic purposes. Using simple liquid-liquid extraction (LLE)-based sample preparation, a limit of quantitation (LOQ) of 50 ppt can successfully be achieved in matrix.

Introduction

CAP is a broad-spectrum antibiotic that inhibits protein synthesis. Prolonged exposure causes a rare yet serious blood disorder (aplastic anemia) and damage to bone marrow. Since CAP has displayed significant toxicological effects on humans, its presence is banned from foods at levels higher than 0.3 ppb minimum required performance limit (MRPL).[1,2]

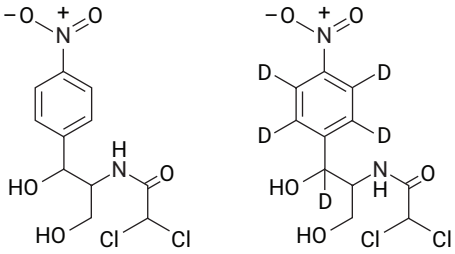

Figure 1. CAP and deuterated CAP.

The triple quadrupole LC/MS system is the gold standard as per US, EU, FSSAI, and other country guidelines for the confirmation of CAP in honey. An Ultivo LC/TQ, the ultimate evolution triple quadrupole LC/MS system, was used in this application. The sensitivity obtained on the Ultivo LC/TQ exceeded the MRPLs established by EU regulation for food.

This workflow used LLE only, while traditional methods use a combination of LLE and solid phase extraction (SPE). Removal of the SPE step provides a simple, cost-effective, and time-saving solution (Figure 2).[3,4,5]

Using CAP-d_5 as a structurally similar internal standard to reduce variations, the proposed solution using the Ultivo LC/TQ demonstrated specific, linear, robust, and reliable results.

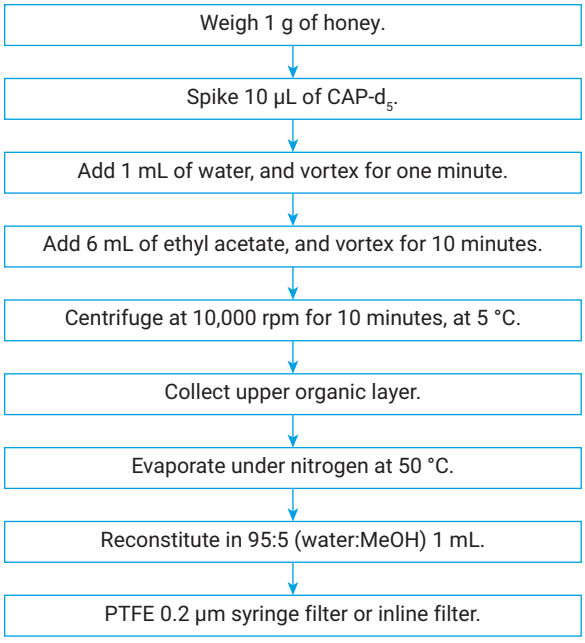

Figure 2. LLE-based sample preparation.

Experimental

The following solvents were used: acetonitrile (Honeywell, LC/MS, part number 34967); methanol (Honeywell, LC/MS, part number 34966); water (Millipore, Milli-Q); ethyl acetate (AR Grade, Rankem); and chloramphenicol (Agilent Technologies, part number 5091-0591). All working dilutions of CAP were prepared in 100% methanol.

Instrumentation

- Agilent 1260 Infinity II flexible pump (G7104C)
- Agilent 1260 Infinity II vialsampler (G7129C)
- Agilent 1260 Infinity II multicolumn thermostat (G7116A)
- Agilent Ultivo LC/TQ with AJS ion source (G6465A)

Table 1. HPLC gradient method.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 685775-922)
Mobile Phase	A) Water B) Methanol; 500 µL/min
Injection Volume	25 µL
Column Temperature	50 °C
Gradient	Time (Min) / Water (100%) / Methanol (100%) 0.0 / 95 / 5 2.5 / 2 / 98 3.0 / 2 / 98 3.5 / 95 / 5 5.0 / 95 / 5

Results and discussion

Considering that 300 ppt is defined as the desired MRPL, a generalized level of 100 ppt is set as the routine LOQ in most analytical laboratories. The suggested method has a limit of detection (LOD) of 25 ppt. However, looking at the diverse nature of honey resources, an LOQ of 50 ppt is recommended. The MRM chromatogram shown in Figure 3 demonstrates blank, LOD, and LOQ in diluent. Additionally, a reproducible elution profile was obtained by injecting various concentrations of CAP in diluent, as shown in Figure 4. Table 2 shows the coefficient of variation (%CV) data of CAP for a calculated concentration of its six replicates.

Table 2. Agilent Ultivo LC/TQ conditions.

Parameter	Setting	
Ionization Mode	AJS (negative)	
Nebulizer Gas	35 psi	
Drying Gas	10 L/min at 350 °C	
Sheath Gas	12 L/min at 400 °C	
Capillary Voltage	2,000 V	
Nozzle Voltage	1,500 V	
Fragmentor Voltage	90 V	
CAV	9 V	
Dwell Time	50 msec	
Resolution	Unit/Unit	
Analyte	**MRM Transition**	**CE (V)**
CAP	321/151.9	9
CAP	321/257.1	2
CAP	321/194.0	3
CAP-d_5	326/157.0	9

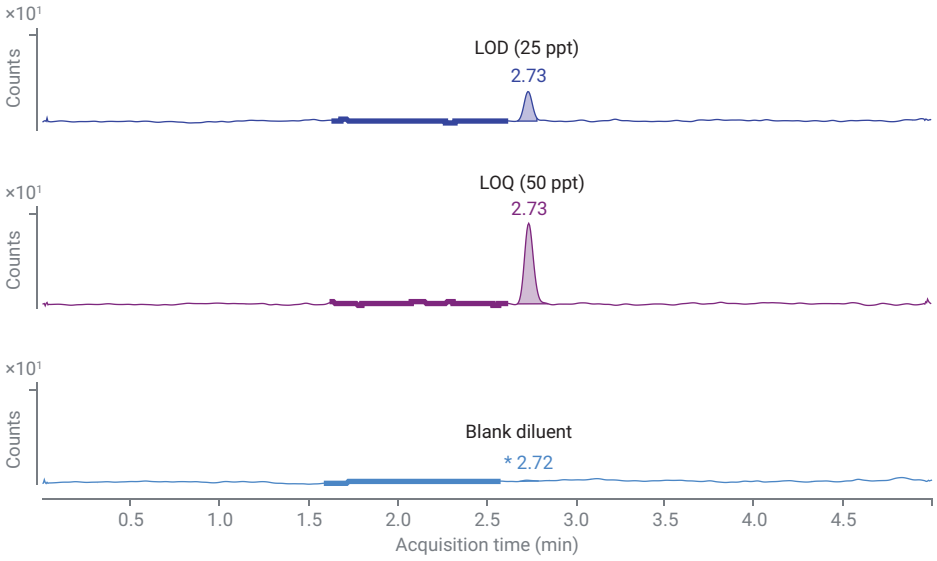

Figure 3. Sensitivity of CAP on the Agilent Ultivo LC/TQ.

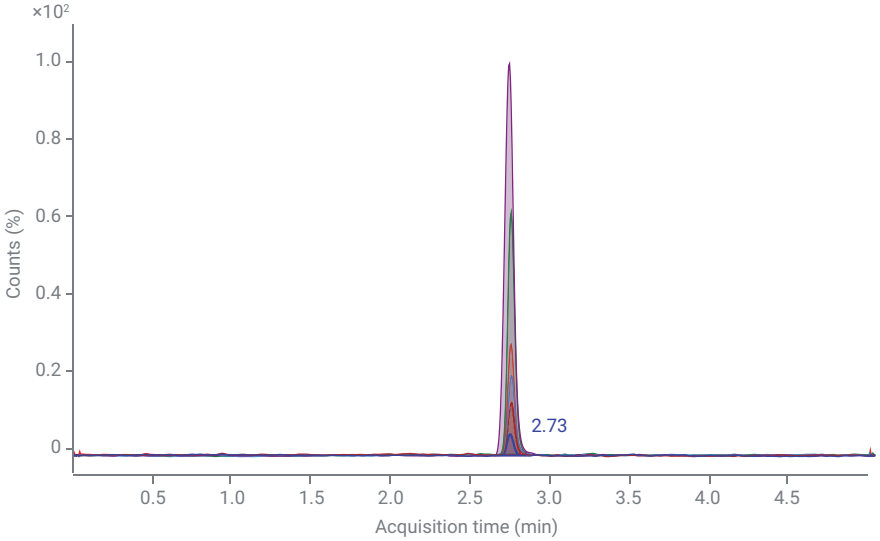

Figure 4. Overlay of various concentrations of CAP.

Calibration and linearity

A calibration linearity plot was generated for relative response (area ratio of CAP versus CAP-d_5) across concentration levels from 50 to 600 ppt (Figure 5). For rugged data, three replicates were obtained at each concentration level, and at the LOQ level, six replicates were submitted. A screenshot of the calibration table with one quantifier, two qualifiers, and the MRM ratio is shown in Figure 6, in accordance with regulations.

Quantitation in honey samples

The suggested method was extended to commercial honey samples. Honey was purchased from local shops (brand 1, brand 2, and brand G) and road-side vendors (local and local 2) of Delhi, India. All samples were submitted in triplicate. Figure 7 shows that the results reported CAP to be at a level lower than the EU-MRPL level of 300 ppt.

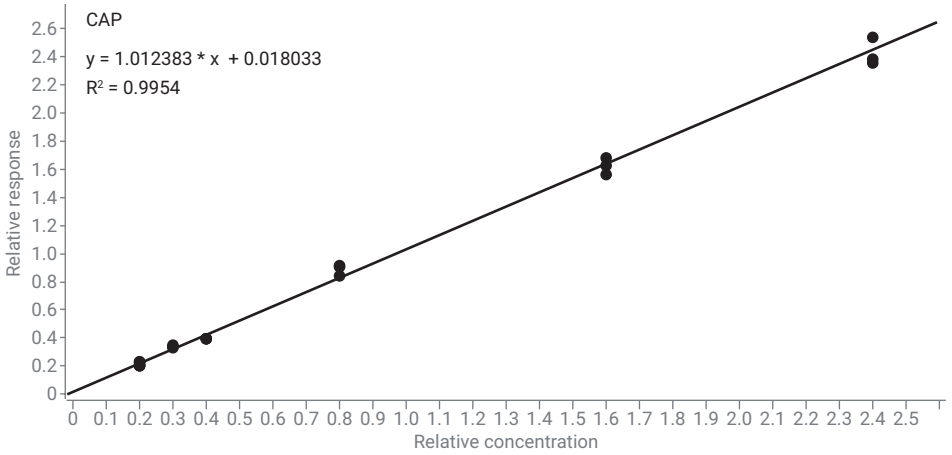

Figure 5. Linearity plot from 50 to 600 ppt (R^2 = 0.9953).

	Sample			CAP Met...	CAP Results				Qualifier...	Qualifier...	CAP-IS (ISTD) R...
	Type	Level	Acq. Date-Time (Inst.)	Exp. Conc.	RT	Calc. Conc.	Accuracy	ISTD Resp. Ratio	Ratio	Ratio	RT
	Blank		8/24/2018 4:18 AM		2.59	0.00		0.0061		208.3	2.72
	Cal	1	8/24/2018 1:54 AM	50.00	2.73	43.47	86.9	0.2061	87.3	47.7	2.73
	Cal	1	8/24/2018 2:06 AM	50.00	2.73	42.73	85.5	0.2031	110.6	44.4	2.73
	Cal	1	8/24/2018 2:12 AM	50.00	2.73	48.06	96.1	0.2244	92.0	38.5	2.73
	Cal	1	8/24/2018 2:18 AM	50.00	2.73	48.88	97.8	0.2277	84.6	40.7	2.72
	Cal	1	8/24/2018 2:24 AM	50.00	2.73	49.16	98.3	0.2288	104.3	47.7	2.73
	Cal	1	8/24/2018 2:30 AM	50.00	2.73	42.17	84.3	0.2008	80.7	39.1	2.73
	Cal	2	8/24/2018 2:42 AM	75.00	2.73	86.21	114.9	0.3768	88.0	41.5	2.72
	Cal	2	8/24/2018 2:48 AM	75.00	2.73	82.51	110.0	0.3621	105.0	43.3	2.73
	Cal	2	8/24/2018 2:54 AM	75.00	2.73	81.06	108.1	0.3563	87.4	47.4	2.72
	Cal	3	8/24/2018 3:06 AM	100.00	2.73	89.71	89.7	0.3908	96.7	48.9	2.72
	Cal	3	8/24/2018 3:12 AM	100.00	2.73	89.93	89.9	0.3917	100.9	39.7	2.72
	Cal	3	8/24/2018 3:18 AM	100.00	2.73	91.81	91.8	0.3992	90.0	38.6	2.72
	Cal	4	8/24/2018 3:24 AM	200.00	2.73	219.27	109.6	0.9086	103.6	47.5	2.72
	Cal	4	8/24/2018 3:36 AM	200.00	2.73	217.76	108.9	0.9025	104.5	46.3	2.72
	Cal	4	8/24/2018 3:42 AM	200.00	2.73	205.06	102.5	0.8518	109.8	46.2	2.72
	Cal	5	8/24/2018 3:48 AM	400.00	2.72	399.33	99.8	1.6281	90.5	43.9	2.71
	Cal	5	8/24/2018 3:54 AM	400.00	2.72	380.87	95.2	1.5543	95.3	46.6	2.71
	Cal	5	8/24/2018 4:00 AM	400.00	2.71	412.19	103.0	1.6795	94.3	43.7	2.71
	Cal	6	8/24/2018 11:48 AM	600.00	2.73	583.31	97.2	2.3633	94.6	42.2	2.72
	Cal	6	8/24/2018 11:54 AM	600.00	2.72	584.87	97.5	2.3696	98.3	43.3	2.71
	Cal	6	8/24/2018 12:00 PM	600.00	2.71	626.66	104.4	2.5366	95.1	41.4	2.71

Figure 6. Calibration table for CAP from 50 to 600 ppt.

Recovery in honey samples

A sample without a chromatographic peak RT of 2.73 ±0.1 minutes and an ion ratio beyond EU guidelines are considered negative samples.[6] Sample brand-G had CAP levels above the LOD value, and sample local-2 had CAP levels above LOQ value. In addition, a spike experiment was performed by adding 50 ppt CAP to honey samples, shown in Figure 7. In the spike study shown in Table 3, good recovery (80 to 120%) was reported in all five samples, confirming that the suggested method is suitable for routine CAP analysis in honey.

Conclusion

The LOQ is 1/6 times the EU-MRPL. The LC method offers UHPLC separation at low pressure using an Agilent InfinityLab Poroshell 120, 2.7 µm column. The LLE-based sample preparation method uses easy and less time-consuming steps. True honey samples were successfully analyzed for CAP, in accordance with EU norms.

Sample			CAP Results			Qualifi...	Qualifi...	CAP-IS (...
Name	Type	Acq. Date-Time	RT	MI	Final Conc.	Ratio	Ratio	RT
Brand1	Sample	8/24/2018 12:12 P...	2.973		4.55			2.721
Brand1	Sample	8/24/2018 12:18 P...	2.978		15.36	102.6		2.721
Brand1	Sample	8/24/2018 12:24 P...	2.431		23.49	63.9		2.726
Brand1_Spike	Sample	8/24/2018 12:30 P...	2.732		40.89	101.6	47.1	2.726
Brand1_Spike	Sample	8/24/2018 12:36 P...	2.727		41.76	95.4	54.6	2.726
Brand1_Spike	Sample	8/24/2018 12:42 P...	2.732		41.39	77.4	51.9	2.726
Brand2	Sample	8/24/2018 12:48 P...	2.978		89.47	15.6	3.3	2.726
Brand2	Sample	8/24/2018 12:54 P...	2.431		67.75	55.8	146.5	2.726
Brand2	Sample	8/24/2018 1:00 PM	2.983		128.01	15.8	9.6	2.726
Brand2_Spike	Sample	8/24/2018 1:06 PM	2.732		44.81	93.7	36.4	2.721
Brand2_Spike	Sample	8/24/2018 1:12 PM	2.732		42.73	87.3	57.8	2.726
Brand2_Spike	Sample	8/24/2018 1:18 PM	2.727		38.41	93.1	49.7	2.726
BrandG	Sample	8/24/2018 1:24 PM	2.727		25.90	100.0	47.1	2.726
BrandG	Sample	8/24/2018 1:30 PM	2.732		30.59	96.2	49.4	2.726
BrandG	Sample	8/24/2018 1:36 PM	2.732		28.61	112.0	57.6	2.726
BrandG_Spi...	Sample	8/24/2018 1:42 PM	2.732		74.66	90.6	49.7	2.720
BrandG_Spi...	Sample	8/24/2018 1:48 PM	2.727		77.49	103.1	51.1	2.721
BrandG_Spi...	Sample	8/24/2018 1:54 PM	2.732		83.27	107.3	42.1	2.726
Local	Sample	8/24/2018 2:00 PM	2.743		0.00	318.5		2.726
Local	Sample	8/24/2018 2:06 PM	2.732		2.10		156.5	2.726
Local	Sample	8/24/2018 2:12 PM	2.620		0.00	390.9	72.5	2.726
Local_Spike	Sample	8/24/2018 2:18 PM	2.732		48.68	77.2	39.2	2.726
Local_Spike	Sample	8/24/2018 2:24 PM	2.732		41.78	113.2	53.8	2.726
Local_Spike	Sample	8/24/2018 2:30 PM	2.732		51.07	75.9	55.6	2.726
Local2	Sample	8/24/2018 2:36 PM	2.732		130.33	95.7	40.3	2.726
Local2	Sample	8/24/2018 2:42 PM	2.732		142.36	99.0	41.4	2.726
Local2	Sample	8/24/2018 2:48 PM	2.727		164.55	88.2	47.4	2.721
Local2_Spike	Sample	8/24/2018 2:54 PM	2.732		193.57	100.3	44.1	2.726
Local2_Spike	Sample	8/24/2018 3:00 PM	2.732		207.12	83.0	45.5	2.726
Local2_Spike	Sample	8/24/2018 3:06 PM	2.732		197.05	96.4	44.8	2.726

Figure 7. Market samples and market samples spiked at the LOQ level.

Table 3. Chloramphenicol in various samples of honey.

Market Sample	Prespike Concentration (a)	Postspike Concentration (b)	% Recovery = 100(b – a)/50
Brand 1	ND	41.35 ppt	82.69%
Brand 2	ND	41.98 ppt	83.96%
Brand G	28.37 ppt	78.47 ppt	100.20%
Local	ND	47.18 ppt	94.35%
Local 2	145.75 ppt	199.25 ppt	107.0%

References

1. Scientific Opinion on Chloramphenicol in Food and Feed. *EFSA Journal* **2014**, *12(11)*, 3907.
2. Commission Decision 2003/181/EC.
3. Fang, Y. *et al.* Detection, Confirmation, and Quantification of Chloramphenicol in Honey and Shrimp at Regulatory Levels Using Quadrupole and Ion Trap LC/MS Application. *Agilent Technologies Application Note*, publication number 5988-9920EN, **2007**.
4. Zhao, L. *et al.* Determination of Chloramphenicol, Florfenicol, and Thiamphenicol in Honey Using Agilent SampliQ OPT Solid-Phase Extraction Cartridges and Liquid Chromatography-Tandem Mass Spectrometry. *Agilent Technologies Application Note*, publication number 5990-3615EN, **2009**.
5. Jin-Lan, S. *et al.* Screening 36 Veterinary Drugs in Animal Origin Food by LC-MS/MS Combined with Modified QuEChERS Method. *Agilent Technologies Application Note*, publication note 5991-0013EN, **2012**.
6. SANTE/11813/2017.

www.agilent.com/chem

Printed in the USA, September 6, 2019
5994-1317EN

Agilent
Trusted Answers