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Screening and Low-Level Quantitation of Chloramphenicol in Commercial Honey Samples Using Miniaturized LC/MS System

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Introduction

Abstract:

This poster demonstrates the usage of Agilent 1260 Infinity II LC system coupled with Ultivo LC/TQ Mass Spectrometry system to achieve sensitivity in very low picogram quantity of Chloramphenicol in various honey samples.

The method developed on miniaturized Ultivo LC/TQ provides highest confidence in results for routine analysis for food industry whether involved in manufacturing or processing or commercial testing of honey samples or for academic purpose.

In this methodology, by using a simple Liquid-Liquid Extraction (LLE) based sample preparation, LOQ of 50ppt (pg/ml) has been demonstrated in honey.

Introduction:

Chloramphenicol (CAP) inhibits protein synthesis in bacteria and is a broad-spectrum antibiotic. Its prolonged exposure causes rare yet serious blood disorder - aplastic anemia, a damage of bone marrow. Since CAP has displayed significant toxicological effects on humans, its presence is banned from foods at levels higher than 0.3 ppb (ng/ml) (MRPL)[1].

Quadrupole LC/MS system are the gold standards as per US, EU, FSSAI and other country guidelines for confirmation of CAP in Honey[2].

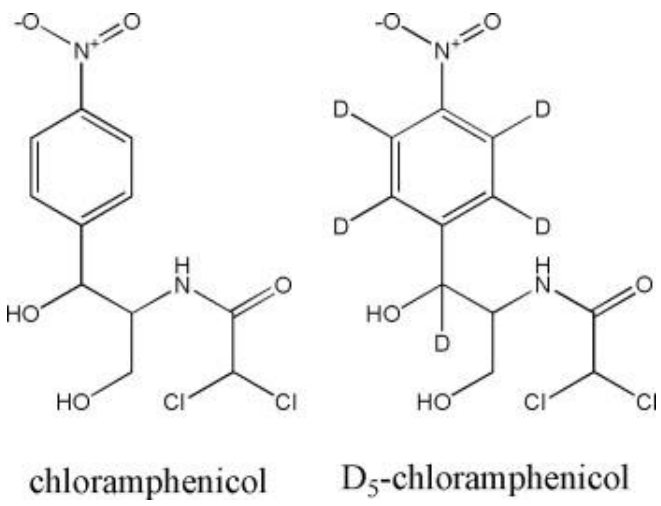

Figure 1 : Chloramphenicol and Deuterated Chloramphenicol using Ultivo LC/TQ

Experimental

Sample Preparation:

The workflow makes usage of only LLE instead of LLE as well as SPE (Solid Phase Extraction)[4,5,6]. Therefore providing a cost-effective and low time consuming solution (fig 2).

The proposed solution using Agilent LC/MS has demonstrated specific, linear, robust results and uses CAP-D5 as structurally similar Internal Standard to nullify variations.

Figure 2 : Workflow for Sample Preparation

Reagents and Instruments:

Acetonitrile (Honeywell, LC/MS, 34967), Methanol (Honeywell, LC/MS, 34966), Water (Millipore, milliQ), Ethyl Acetate (AR Grade, Rankem), Chloramphenicol (Agilent Technologies, P No 5091-0591). All working dilutions of CAP were prepared in 100% Methanol.

Agilent 1260 Infinity II Quaternary Pump (G7104C); Agilent 1260 Infinity II Vialsampler (G7129C); Agilent 1260 Infinity II MCT (G7116A); Ultivo LC/TQ.

Experimental

Ultivo LC/TQ Conditions

Ionization Mode = ESI-AJS (-ve)

Nebulizer Gas = 35psi

Drying Gas = 10L/min at 350Deg C

Sheath Gas = 12L/min at 400Deg C

Capillary Voltage = 2000 V

Nozzle Voltage = 1500 V

Fragmentor = 90 V

Analyte	Transition	CE (V)	CAV (V)	Dwell Time
CAP	321/151.9	9	9	50 ms
CAP	321/257.1	2	9	50 ms
CAP	321/194	3	9	50 ms
CAP-D5	326/157	9	9	50 ms

Table 1: MRM Parameters

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

Parameter	Value
Column	Poroshell EC C18, 2.1 x 100mm x 2.7µm (P/N 685775-902)
Flow Rate	500 µl/min
Injection Vol	25 µL
Column Temp.	50^0 C

Table 2: HPLC Gradient and Method

Results and Discussion

Considering 300ppt as the desired MRPL, most of analytical laboratories keep 100ppt as routine LOQ. The suggested method has LOD of 25ppt, however looking at diverse nature of honey resources the LOQ of 50ppt is being recommended. (seen in fig 3).

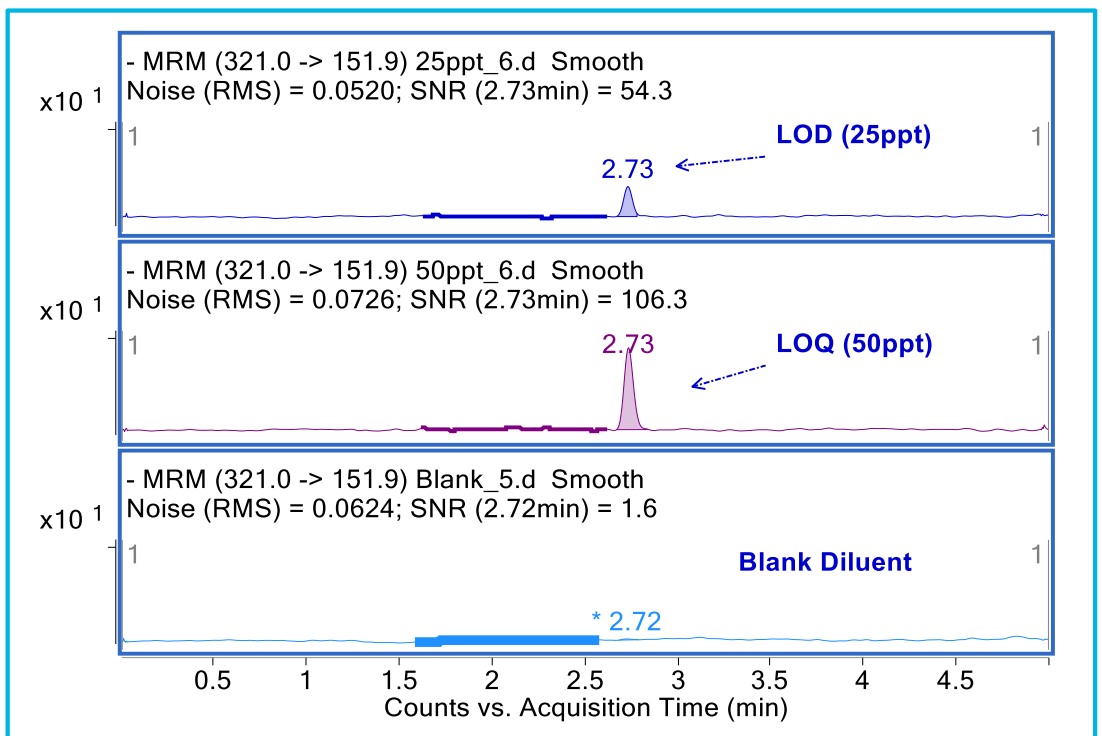

Figure 3. Sensitivity of Chloramphenicol on Ultivo LC\TQ

Calibration and Linearity

A linearity plot was generated for Relative Response (area ratio of CAP vs CAP-D5) across concentration levels from 50ppt to 600ppt (fig 4). For a rugged data, tri-plicate were obtained at each concentration level and at LOQ level, 6 replicates were submitted. The calibration table with 1 Quantifier, 2 Qualifiers, MRM Ratio is shown in table 3, in accordance to regulations.

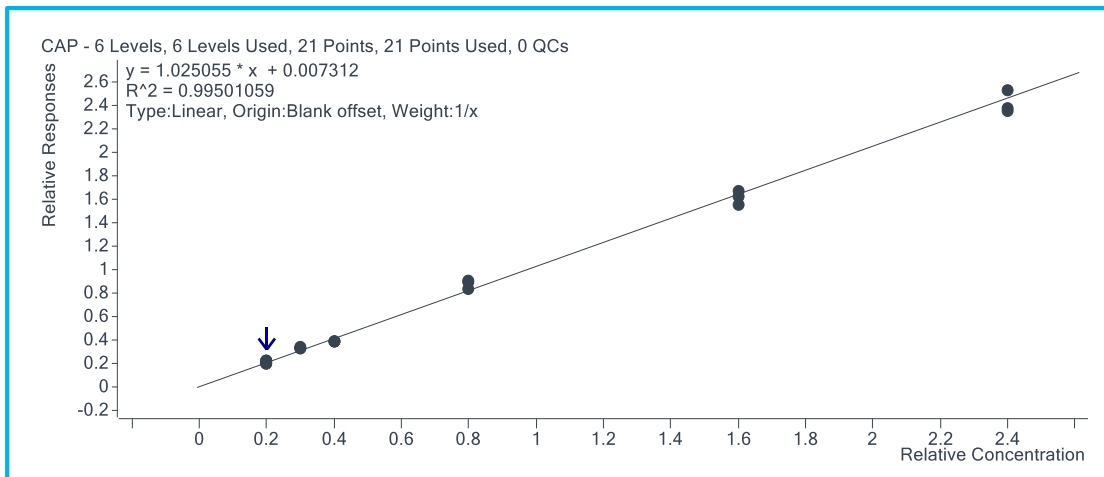

Figure 4: Linearity Plot from 50ppt to 600ppt (R^2 = 0.995)

Results and Discussion

Sample				CAP M...	CAP Results					Quali ...	Quali ...	CAP-IS...
Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	MI	Calc. Conc.	Accuracy	RR	Ratio	Ratio	RT
Blank_2.d	Blank		8/24/2018 12:30 AM		2.9		0.2		0.008	592.1	64.0	2.7
50ppt_5.d	Cal	1	8/24/2018 2:00 AM	50.0	2.7		48.5	97.1	0.206	116.5	44.1	2.7
50ppt_6.d	Cal	1	8/24/2018 2:06 AM	50.0	2.7		48.1	96.2	0.205	95.5	44.4	2.7
50ppt_7.d	Cal	1	8/24/2018 2:12 AM	50.0	2.7		53.0	106.0	0.225	92.0	38.5	2.7
50ppt_8.d	Cal	1	8/24/2018 2:18 AM	50.0	2.7		53.7	107.5	0.228	84.6	40.7	2.7
50ppt_9.d	Cal	1	8/24/2018 2:24 AM	50.0	2.7		54.2	108.4	0.230	84.9	47.7	2.7
50ppt_10.d	Cal	1	8/24/2018 2:30 AM	50.0	2.7		47.1	94.3	0.201	80.7	39.1	2.7
75ppt_2.d	Cal	2	8/24/2018 2:42 AM	75.0	2.7		84.0	112.0	0.352	88.0	41.5	2.7
75ppt_3.d	Cal	2	8/24/2018 2:48 AM	75.0	2.7		81.0	108.0	0.339	97.2	43.3	2.7
75ppt_4.d	Cal	2	8/24/2018 2:54 AM	75.0	2.7		80.1	106.8	0.336	87.4	47.4	2.7
100ppt_2.d	Cal	3	8/24/2018 3:06 AM	100.0	2.7		94.2	94.2	0.394	96.7	48.9	2.7
100ppt_3.d	Cal	3	8/24/2018 3:12 AM	100.0	2.7		93.7	93.7	0.391	100.9	39.7	2.7
100ppt_4.d	Cal	3	8/24/2018 3:18 AM	100.0	2.7		95.3	95.3	0.398	90.0	38.6	2.7
200ppt_1.d	Cal	4	8/24/2018 3:24 AM	200.0	2.7		220.2	110.1	0.910	103.6	47.5	2.7
200ppt_3.d	Cal	4	8/24/2018 3:36 AM	200.0	2.7		218.7	109.3	0.904	104.5	46.3	2.7
200ppt_4.d	Cal	4	8/24/2018 3:42 AM	200.0	2.7		203.4	101.7	0.841	104.4	46.2	2.7
400ppt_1.d	Cal	5	8/24/2018 3:48 AM	400.0	2.7		395.0	98.8	1.627	90.5	43.9	2.7
400ppt_2.d	Cal	5	8/24/2018 3:54 AM	400.0	2.7		378.2	94.5	1.558	95.3	46.6	2.7
400ppt_3.d	Cal	5	8/24/2018 4:00 AM	400.0	2.7		407.9	102.0	1.680	94.3	43.7	2.7
600ppt_1.d	Cal	6	8/24/2018 11:48 AM	600.0	2.7		578.6	96.4	2.380	94.6	42.2	2.7
600ppt_2.d	Cal	6	8/24/2018 11:54 AM	600.0	2.7		572.6	95.4	2.355	98.3	43.3	2.7
600ppt_3.d	Cal	6	8/24/2018 12:00 PM	600.0	2.7		617.6	102.9	2.539	95.1	41.4	2.7
Blank_3.d	Blank		8/24/2018 12:06 PM		2.6		0.0		0.006		208.3	2.7
Brand1_1.d	Sample		8/24/2018 12:12 PM		3.0		9.2		0.045			2.7
Brand1_Spike...	Sample		8/24/2018 12:30 PM		2.7		43.0		0.184	101.6	47.1	2.7
Brand2_1.d	Sample		8/24/2018 12:48 PM		3.0		93.3		0.390	15.6	3.3	2.7
Brand2_Spike...	Sample		8/24/2018 1:06 PM		2.7		59.8		0.253	108.4	37.1	2.7
BrandG_1.d	Sample		8/24/2018 1:24 PM		2.7		30.6		0.133	106.7	47.1	2.7
BrandG_Spike...	Sample		8/24/2018 1:42 PM		2.7		77.8		0.326	90.6	49.7	2.7
Local_1.d	Sample		8/24/2018 2:00 PM		2.7		3.2		0.021	318.5		2.7
Local_Spike_...	Sample		8/24/2018 2:18 PM		2.7		52.7		0.223	77.2	39.2	2.7
Local2_1.d	Sample		8/24/2018 2:36 PM		2.7		130.7		0.543	95.7	40.3	2.7
Local2_Spike...	Sample		8/24/2018 2:54 PM		2.7		187.0		0.774	100.3	44.1	2.7

Table 3: Calibration Table for CAP with MRM Ratios and Quantitation of commercial samples

Quantitation in honey samples and Recovery at LOQ

Honey was purchased from local shops (Brand 1, Brand 2 and Brand 3) and also from local vendors (Local 1 and Local 2) of Delhi, India. All the samples were submitted in triplicates. All sample reported CAP lower than MRPL, as seen in table 3.

Sample	Pre-spike conc. (a in ppt)	Post-spike conc. (b in ppt)	% Recovery = 100(b-a)/50
Brand 1	ND	43.0	86 %
Brand 2	ND	59.8	119.6 %
Brand G	30.6	77.8	94.4%
Local 1	ND	52.7	105.4 %
Local 2	130.7	187.0	112.6 %

Table 4: Recovery calculation (un-spike vs LOQ spike)

Samples not having Chromatographic RT of 2.73 ± 0.1 min and Ion Ratio beyond SANTE guidelines are considered as negative samples [3]. Further, a spike experiment was performed by adding 50ppt CAP in honey samples. Average recovery values were within 80-120% as seen in table 4.

Conclusions

- LOQ is 1/6 times of EU-MRPL.
- The LC method offers UHPLC separation at low pressure by using Poroshell 2.7um column.
- The LLE based sample preparation method uses easy and less time consuming steps with 81-101% recovery.
- Commercial Honey samples are analyzed for CAP, in accordance to EU-norms.

References

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