EUCHINASAFE
中欧食品安全

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EU-China-Safe aims at reducing food fraud and improving food safety through focusing on improving food legislation, food inspection and increasing access to information across Europe and China. State-of-the-art technologies including a virtual laboratory will create a unique space to share and demonstrate best practice. The use of innovative technologies will result in improved detection of adulteration of food products as well as increased traceability and transparency of global supply chains.

The project runs from September 2017 to August 2021. It involves 33 partners and is coordinated by QUB (The Queen’s University of Belfast, UK).

More information on the project can be found at www.euchinasafe.eu (website in construction)

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Nature of the deliverable

ORDP	Open Research Data Pilot	
R	Document, report (excluding the periodic and final reports)	x
DEM	Demonstrator, pilot, prototype, plan designs	
DEC	Websites, patents filing, press & media actions, videos, etc.	
E	Ethics	
OTHER	Software, technical diagram, etc.	

Dissemination Level

PU	Public, fully open, e.g. web	
CO	Confidential, restricted under conditions set out in Model Grant Agreement	x
CI	Classified, information as referred to in Commission Decision 2001/844/EC	

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1. SUMMARY

Deliverable D4.1 was to deliver the report on LC-MS/MS methods for ClOx and NOx residues in diary powders established

2. INTRODUCTION

ClOx (chlorate and perchlorate) and NOx (nitrate and nitrite) in food are of concern due to their adverse effects on human and animal health. ClOx is important inorganic contaminants in milk and infant formula, while NOx (Nitrates and nitrites) residues can occur in milk due to cleaning processes on-farm and at processing plants. However，the methodology available in the literature for the analysis of these ionic targets is much limited. Hence, we stipulated relevant content in Horizon 2020 EU-China Safe Project. According to the Grant Agreement, Teagasc will transfer their existing non-published methods for ClOx analysis to CFSA. In parallel, Chinese methods for NOx analysis will be transferred from CFSA to Teagasc.

3. CHLORATE AND PERCHLORATE

As a foundation of this study, CFSA has organised the members of China national food safety risk monitoring alliance, BJCDC, established a comprehensive analytical method using ion chromatography-tandem mass spectrometry (IC-MS/MS) with stability isotope dilution technology for the simultaneous determination of perchlorate and bromate in a variety of foodstuffs, including vegetable, animal producing foods, cereals and dietary samples. The food samples surrogated with 18O marked internal standard were extracted by water and acetonitrile. The extracts were then purified by an Envi™ Carb SPE cartridge. With the hydrophilic Ion Pac AS19 column and linear gradient potassium hydroxide used as eluent, perchlorate was analyzed by a triple quadrupole mass spectrometer. Good linearities were achieved for bromate and perchlorate over the range of 0.1-50 µg/L and 0.05-50 µg/L, respectively, with correlation coefficients R2>0.998. The quantification limits of the method were 0.5 µg/kg-1.0 µg/kg for perchlorate, Mean recoveries ranged from 80.3% to 123.8%, with precisions no more than 20% which complied with the regulations for determination of trace contaminants residues in food matrix. The 144 mixture dietary samples collected from 12 provinces in Chinese Total Diet Study in 2007 were analyzed and perchlorate was detected 91.7 per cent of dietary samples, with a concentration range of 0.14 µg/L-70.91 µg/kg. Relatively high level of perchlorate occurred in vegetables, fruits and grains, corresponding to the concentration range of 3.11-46.44 µg/kg, 4.03-12.28 µg/kg and 3.14-70.91 µg/kg, respectively.

Then, CFSA and BJCDC developed a method for determination of chlorate and perchlorate in tea by UPLC-MS/MS based on isotopic internal standard method. The ClOx in tea was extracted with 0.2% acetic acid and then centrifuged. The supernatant was cleaned-up with Carb SPE. The separation of perchlorate was carried out on a Thermo Acclaim TRINITY P1 column, Calibration curve was linear in the concentration range of 0.25~50.0 µg/L (r=0.9991). The average recoveries at the spiked levels of 0.05~0.80 mg/kg were 94.6%~110.2% and the relative standard deviations (RSDs) were 1.8%~9.6%, respectively. Using this method, perchlorate was detected at 0.012~0.664 mg/kg in 40 tea samples collected from the market in Beijing.

On April 16th 2018, Teagasc transferred their non-published methods for ClOx analysis to BJCDC and CFSA. Briefly, the analytes are extracted by a solution of water/acidified acetonitrile, centrifuged and concentrated under nitrogen. The extracts are filtered and transferred to an autosampler vial. Chlorates residues are determined on a PFP column using UPLC-MS/MS. In following one month, CFSA and BJCDC confirmed and evaluated the method with respect to its linearity, the recovery as well as the precision (represented to the RSDs). The results are shown in the Appendix I. In summary, good linearity was achieved in the concentration range of 0.10~10.0 μg/L (r=0.9997). The average recoveries at two spiked levels in milk and formula were 85.6%~123.7% and the RSDs (n=6) were less than 12%. The method was simple, rapid and highly sensitive, and suitable for the determination of ClOx in dairy samples.

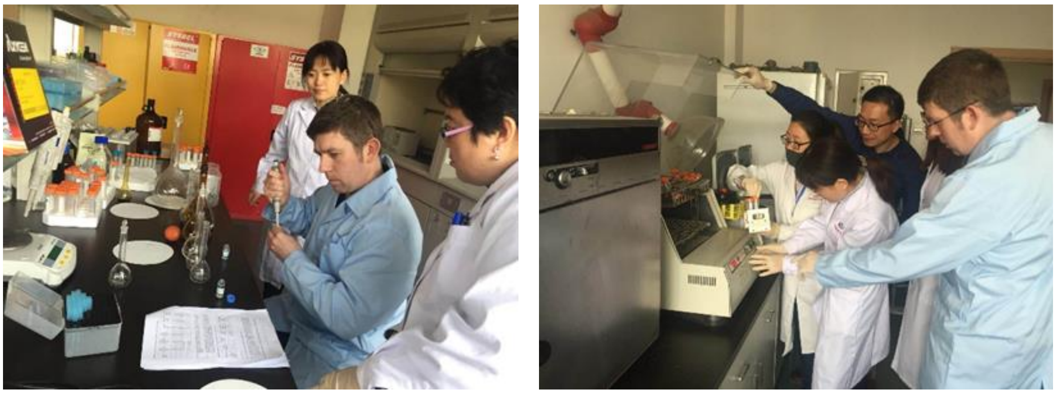

Fig 1. Dr. Martin Danaher from Teagasc transferred the ClOx method to BJCDC and CFSA

In May 2018, BJCDC submitted a proposal to set the national standard method for ClOx analysis in food based on the UPLC-MS/MS technique. This proposal was approved by the Expert Committee on National Standards for Food Safety just one week ago (Sep 11th, 2018). That means BJCDC will improve the ClOx method and draft the national standard method in following 18 months. It will also be a meaningful achievement of our project.

4. NITRATE AND NITRITE

As for the NOx, currently, the National Standard method used for the analysis of both nitrite and nitrate in food involved two parts: one is the spectrophotometric method based on the Griess reaction, while the other is ion chromatography/conductivity detection (IC/CD) technique. There are many enterprise and/or academies used other methods for NOx analysis, e.g. amperometry, capillary electrophoresis, spectrophotometry with or without flow injection analysis, as well as the optical sensor technology.

In the past few months, CFSA tried to develop an IC-MS/MS method for simultaneous determination of nitrite and nitrate in dairy samples, particularly the formula sample. A Dionex Ionpac AS 19 column was used for the target ions separation and linear gradient potassium hydroxide used as eluent. nitrite and nitrate were analyzed by a triple quadrupole mass spectrometer (Agilent 6495) under negative electrospray ionization mode. Good linearities were achieved for over the range of 10-500 μg/L for the neat standard solution. However, when a GCB cartridge was used for the purification of acetonitrile-based formula extracts, severe signal depression was found, usually as higher as 80%+. So, the method development is not available yet. We will continue to optimize the whole method.

5. CONCLUDING REMARKS

BJCDC and CFSA validated the LC-MS/MS method for ClOx detection transferred from Teagasc. The results showed that the method was simple, rapid and highly sensitive, and suitable for the determination of ClOx in dairy samples. BJCDC will draft the national standard method for ClOx detection in following 18 months. NOx analytical method based on LC-MS/MS is not available yet, which need further optimization.

6. ACKNOWLEDGEMENT

We wish to acknowledge the contribution of all project partners who contributed to the completion of this deliverable.

7. APPENDICES

Appendix I: Results of Teagasc method for ClOx detection Operated by CFSA and BJCDC

SOP: provided by Teagasc

Operator: Congrong Fang (CFSA), Jing Zhang (BJCDC)

Instrument: Agilent 1290 UHPLC coupled with 6495 TQ-MS

Column: Infinity poroshell 120 PFP（1.9 μm, 2.1 mm×50 mm）Part: 699675-408; Batch:17331

The results are as follows:

Table 1 Calibration curves of chlorate and perchlorate

	Target ion	Range (μg/L)	Calibration	R	LOQ（μg/kg）
1	ClO_3^-	0.1~10	Y = 1.231137X-0.005162	0.9998	0.1
2	ClO_4^-	0.1~10	Y = 5.282368X-0.019012	0.9997	0.2

Table 2 concentrations of chlorate and perchlorate in 2 dairy product samples (*n*=6)

Sample	Target ion	Concentration（μg/kg）	Mean μg/kg	RSD (%)

		1	2	3	4	5	6		
Sample 1 Liquid milk (SanYuan)	ClO_3^-	3.30	3.41	3.39	3.32	3.09	3.30	3.30	3.47
	ClO_4^-	10.92	10.77	10.62	10.61	10.71	10.94	10.76	1.33
Sample 2 Formula (Heinz)	ClO_3^-	309.55	295.46	305.13	304.94	307.78	312.66	305.92	1.92
	ClO_4^-	14.11	14.22	15.09	14.65	14.46	14.44	14.49	2.41

Table 3 Recovery and RSD at 2 spiked levels

Sample	**Target ion**	**Spiked (µg/kg)**	**Recovery %**						**Mean (%)**	**RSD (%)**
			1	**2**	**3**	**4**	**5**	**6**		
Sample 1 Liquid milk	ClO_3^-	10	98.0	102.5	100.7	105.8	97.1	100.8	100.8	3.1
		100	101.8	105.5	108.3	103.2	105.8	104.6	104.9	2.2
	ClO_3^-	10	106.8	100.8	104.2	100.4	100.9	103.0	102.7	2.4
		100	107.0	103.9	106.7	104.7	106.0	103.5	105.3	1.4
Sample 2 Formula	ClO_3^-	15	85.6	103.1	104.7	123.7	108.5	108.2	105.6	11.6
		500	106.7	93.8	107.1	101.1	103.1	104.6	102.7	4.8
	ClO_3^-	15	97.6	97.0	100.0	98.2	99.6	98.2	98.4	1.2
		500	103.5	98.0	102.5	106.3	104.9	102.2	102.9	2.8

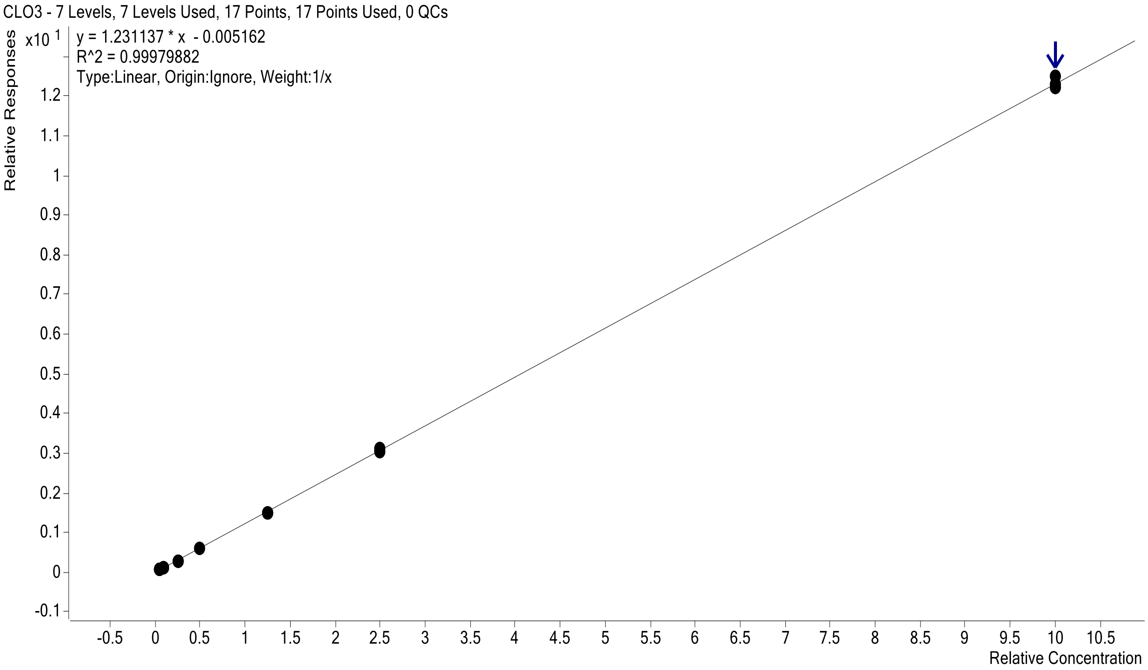

Fig A1. Calibration curve of ClO_3^-

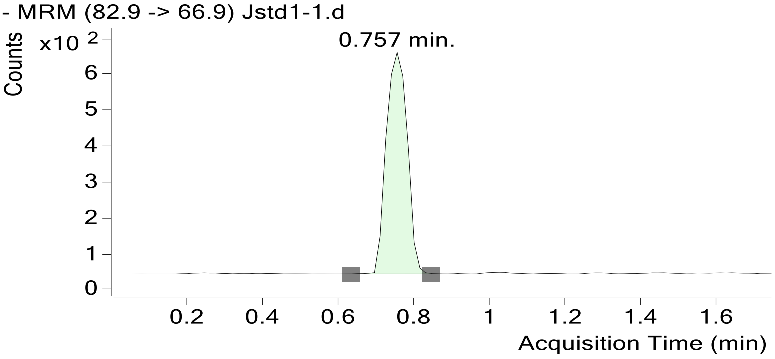

Fig A2.LC-MS/MS chromatograms of chlorate（1 μg/L）

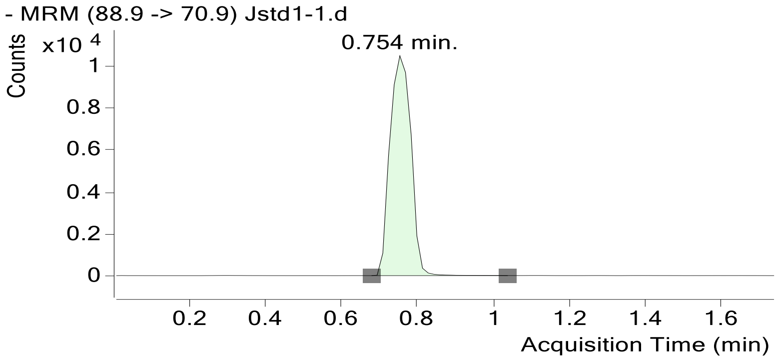

Fig A3. LC-MS/MS chromatograms of $^{18}O_3$-chlorate （1 μg/L）

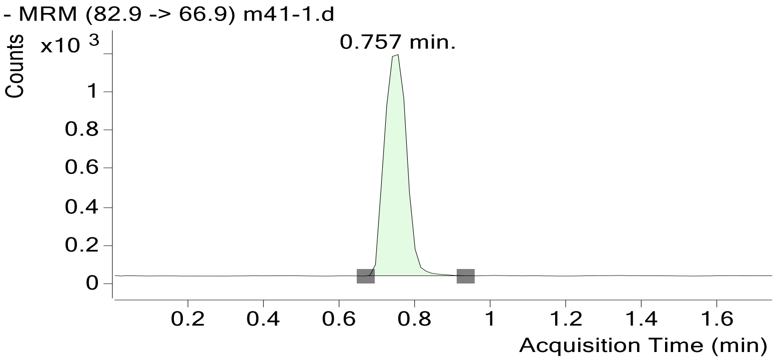

Fig A4. LC-MS/MS chromatograms of chlorate in milk

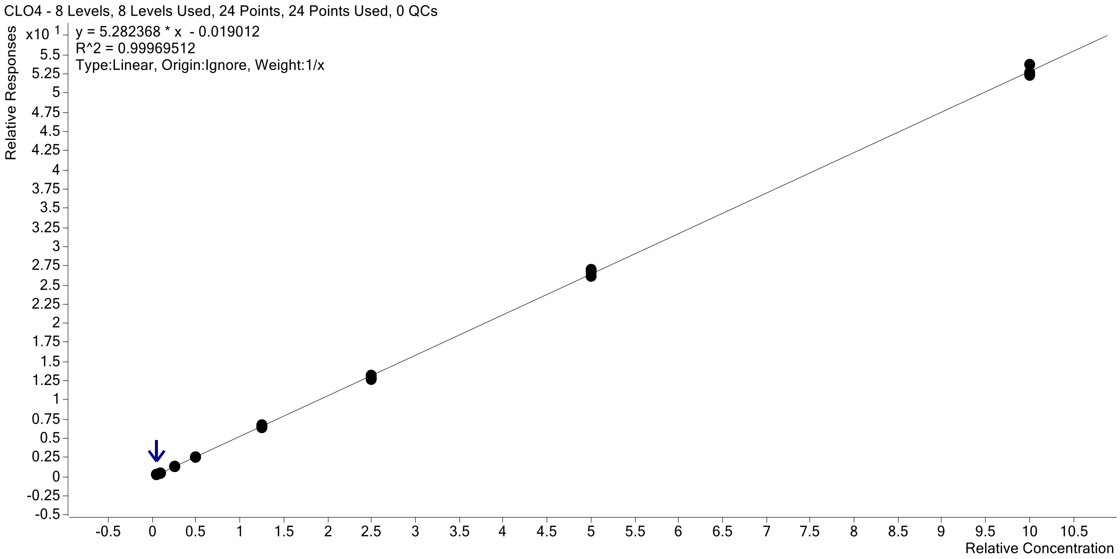

Fig A5. Calibration curve of ClO_4^-

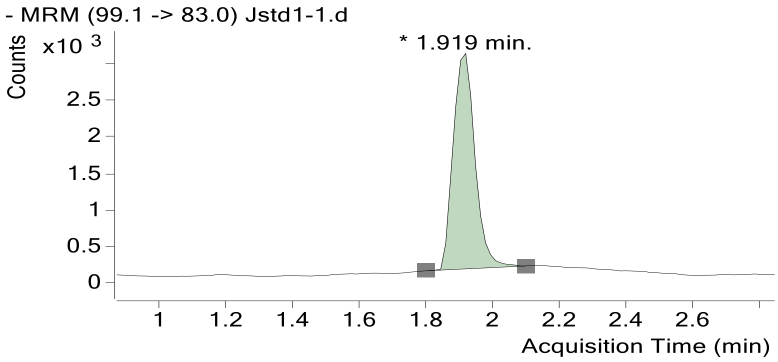

Fig A6.LC-MS/MS chromatograms of perchlorate（1 μg/L）

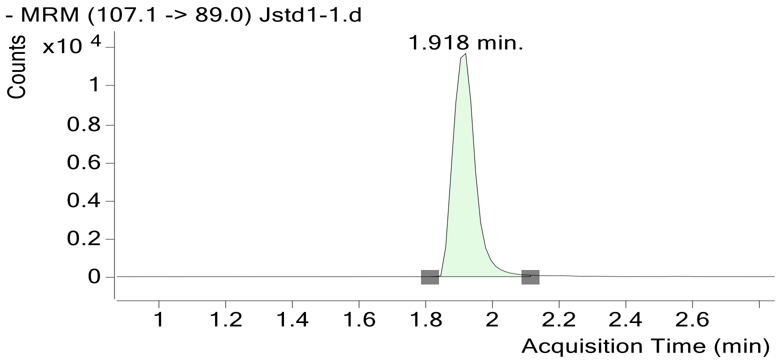

Fig A7. LC-MS/MS chromatograms of $^{18}O_4$-perchlorate（1 μg/L）

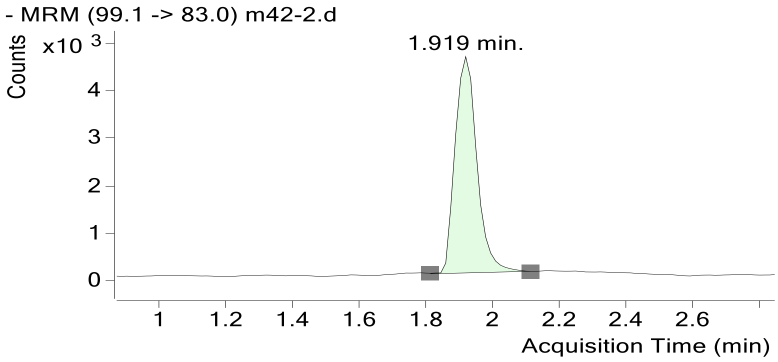

Fig A8. LC-MS/MS chromatograms of perchlorate in formula sample