Occurrence of Perchlorate in Foodstuffs:

Analytical Methodology Development and the Chinese Dietary Exposure

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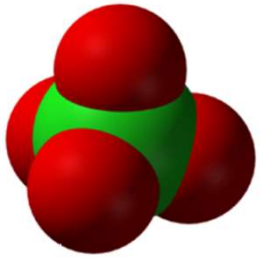
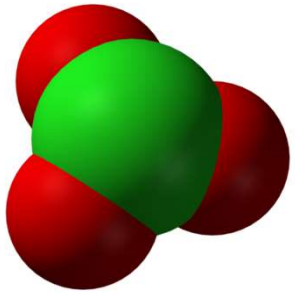
Background



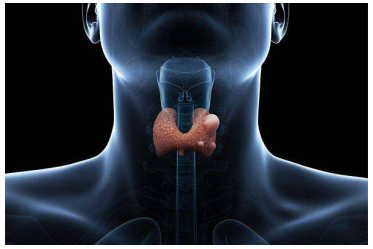
ClO_4^-

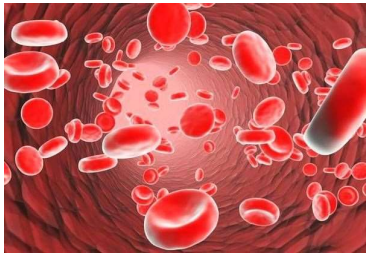
Perchlorate & Chlorate

ClO_3^-



- Concern because ClOx are competitive inhibitors of iodine uptake in the thyroid.
- Can cause damage to red blood cells.
- Its presence in food a potential health concern for vulnerable groups, particularly infants, pregnant women and people with thyroid dysfunction.





Background

June 29, 2020

Mandatory maximum residue levels for chlorate and perchlorate

On 04.06.2020, the European Commission published Regulation (EU) 2020/749 amending Annex III of Regulation (EC) No. 396/2005. According to this regulation, new maximum residue levels for chlorate in food will apply starting from 28.06.2020 without transitional regulation.

Chlorate is the salt of chloric acid. It is released as a by-product when disinfectants are used and when drinking water is treated, causing residues of chlorate to appear in food. Repeated ingestion of chlorate can lead to inhibited iodine absorption in humans, which can have health effects, especially for risk groups such as thyroid patients, newborns and children.

Residues of chlorate are frequently found in frozen vegetables, fruit juices and salads. It occurs as a result of glazing frozen vegetables, diluting concentrates for juice, and

Regulation (EU) 2020/685 ClO_4^- 0.01-0.75 mg/kg

	Foodstuffs (1)	Maximum level (mg/kg)
9.	**Perchlorate**	
9.1.	Fruits and vegetables with the exception of:	0,05
	— *Cucurbitaceae* and kale	0,10
	— leaf vegetables and herbs	0,50
9.2	Tea (*Camellia sinensis*), dried Herbal and fruit infusions, dried	0,75
9.3	Infant formula, follow-on formula, foods for special medical purposes intended for infants and young children and young child formula (3)(4)(*)	0,01
	Babyfood (3)(4)	0,02
	Processed cereal based food (3)(29)	0,01

(*) young child formula are milk based drinks and similar protein based products intended for young children. These products are outside the scope of Regulation (EU) No 609/2013 (Report from the Commission to the European Parliament and the Council on young child formulae (COM/2016/0169 final) (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52016DC0169&qid=1559628885154&from=EN).'

Regulation (EU) 2020/749 ClO_3^- 0.05-0.7 mg/kg

COMMISSION REGULATION (EU) 2020/749

of 4 June 2020

amending Annex III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for chlorate in or on certain products

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC (1), and in particular Article 14(1)(a) and Article 16(1)(a) thereof,

Whereas:

(1) According to Commission Decision 2008/865/EC (2) all authorisations for plant protection products containing chlorate have been revoked following the non-inclusion of chlorate in Annex I to Council Directive 91/414/EEC (3).

Background

- Determination of ClOx in food is a challenge due to the trace level and their high polar property.

- Current methodology：

  Ion chromatography (IC),

  IC coupled with tandem mass spectrometry (IC-MS/MS)

  HPLC-MS/MS （ion-exchanging based column were commonly used）

low-sensitivity, high-cost, short column life and relatively complicated operation

- We establish an accurate analytical method for the determination of chlorate and perchlorate in drinking and foodstuffs using ^{18}O stable isotope labelled internal standards calibration.

Analytical Method Development

Waters UPLC-Xevo TQ-XS MS/MS

Ionization mode: ESI(-)

Capillary: 1.00 KV

Source Temp. : 150ºC

Desolvation Temp.: 450ºC

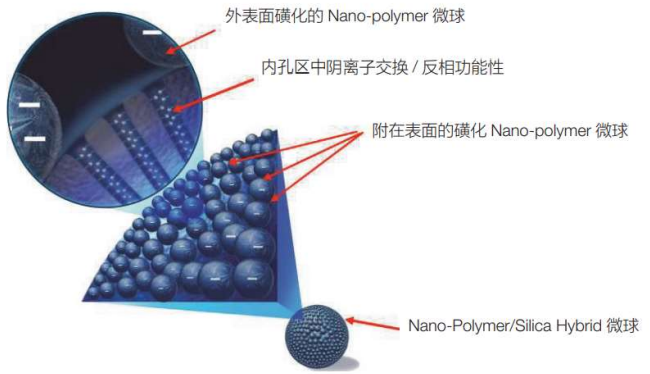
Gas flow: 1000 L/h;

Target	Parent ion （m/Z）	Product ion （m/Z）	Cone （V）	CE （eV）
Chlorate	83.0	67.0*	25	18
Perchlorate	85.0	69.0	25	17
	99.0	83.0*	18	18
	101.0	85.0	18	18
Chlorate-IS	89.0	71.0*	25	18
	91.0	73.0	25	17
Perchlorate-IS	107.0	89.0*	18	18
	109.0	91.0	18	18

Analytical Method Development

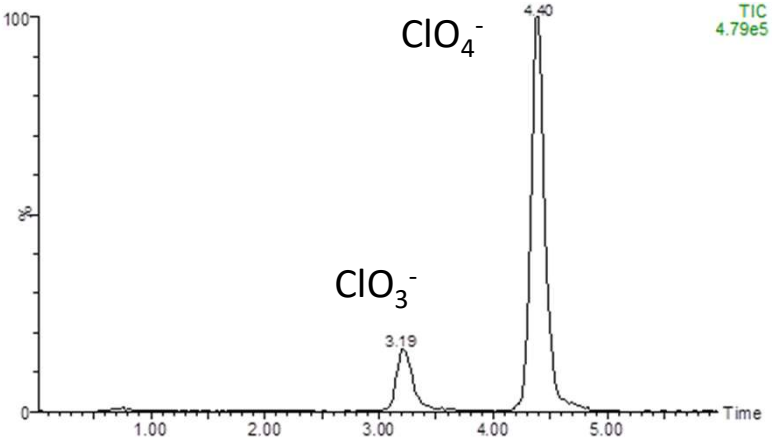
➢ Selection of LC Cloumn

Cloumn	:	Thermo Trinity P1 (2.1*100mm, 3 μm)
Mobile	:	A-20 mM NH_4FA, B-acetonitrile （ACN）
Flow	:	0.5 mL/min
Temp	:	40 ℃
Injection Vol	:	3 μL



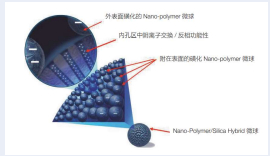
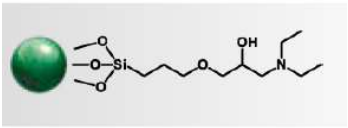
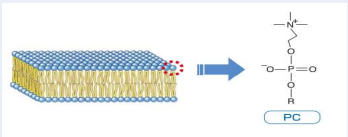
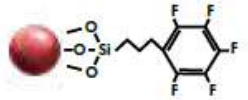
梯度洗脱程序

Time (min)	Module	Command	Value
0.50	Pumps	Pump B Conc.	35
4.00	Pumps	Pump B Conc.	65
5.00	Pumps	Pump B Conc.	90
7.00	Pumps	Pump B Conc.	90
7.10	Pumps	Pump B Conc.	35
10.00	Controller	Stop	

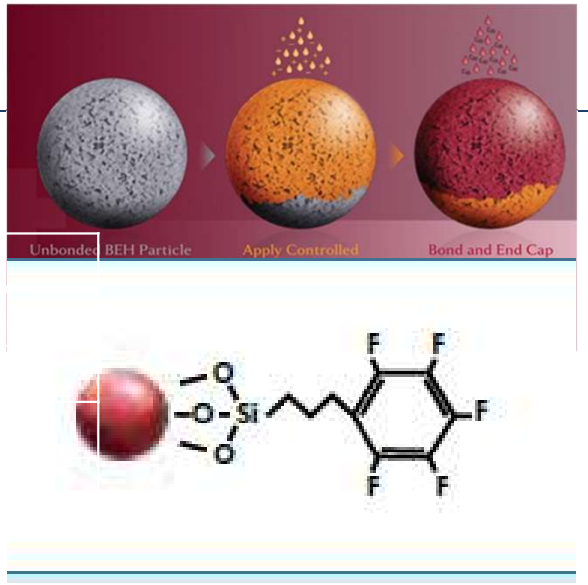


Analytical Method Development

➢ Selection of LC Cloumn

Cloumn	Stationary phase	Moblie phase	Peak width/min	Concern
Thermo Trinity P1		ACN-20 mM NH4FA	~0.3	RT shifting, peak tailing
Waters Torus DEA		ACN-20 mM NH4FA	~0.15/0.3	Poor results of chlorate
Shiseido HILIC PC		ACN-20 mM NH4AC	~0.25	Poor retention of perchlorate
Waters CSH PFP		ACN-1% FA	~0.20	/

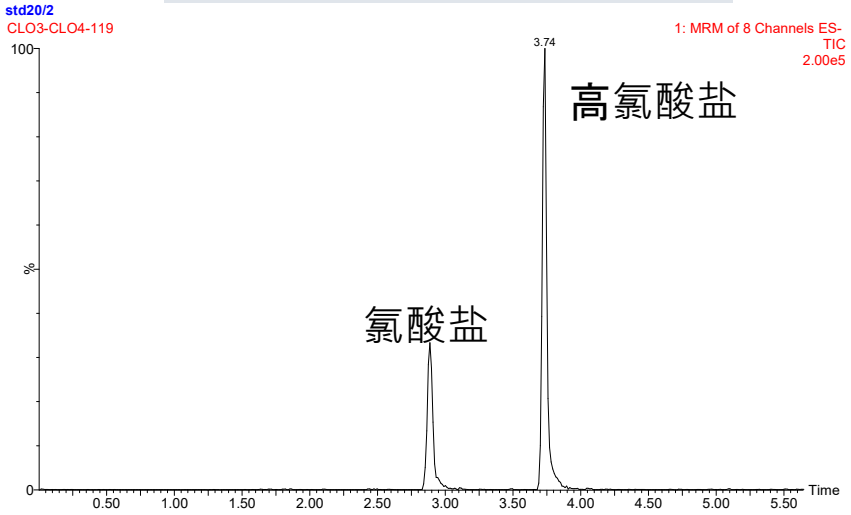
Analytical Method Development



➢ Selection of LC Cloumn

Cloumn : CSH PFP column(2.1*100mm, 1.7 μm)
Mobile : A-acetonitrile （ACN）， B-0.1% FA
Flow : 0.4 mL/min
Temp : 40 ℃
Inj Vol : 5 μL

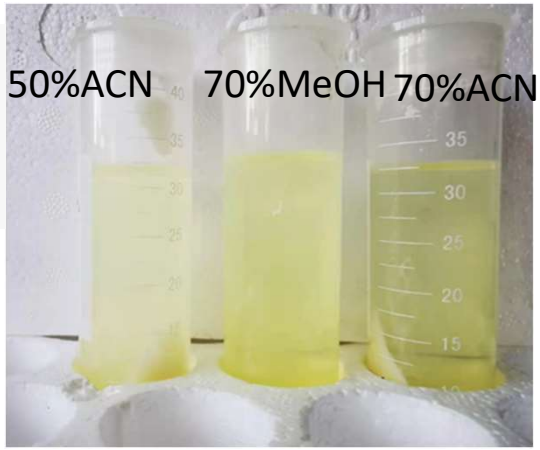
时间（min）	流速（mL/min）	%A（ACN)	%B（0.1%FA）
0.0	0.4	25	75
0.5	0.4	25	75
4.0	0.4	65	35
5.0	0.4	95	5
7.0	0.4	95	5
7.5	0.4	25	75
10	0.4	25	75

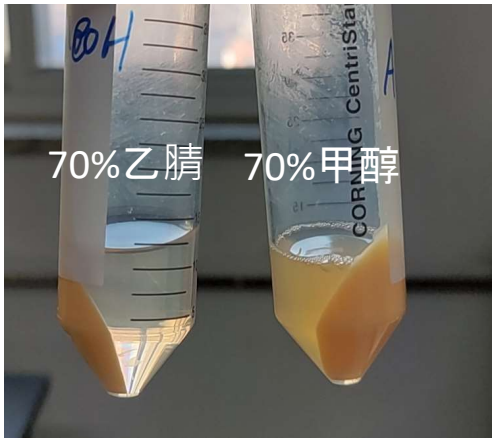


Analytical Method Development

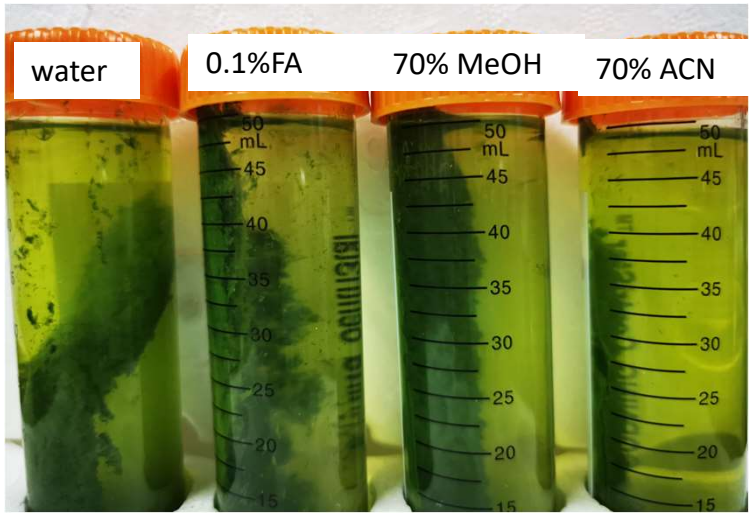
- Selection of extraction solution
  - water
  - Water-Methanol (MeOH)
  - Water-ACN
  - 0.1% formic acid -ACN

Infant formula





Eggs

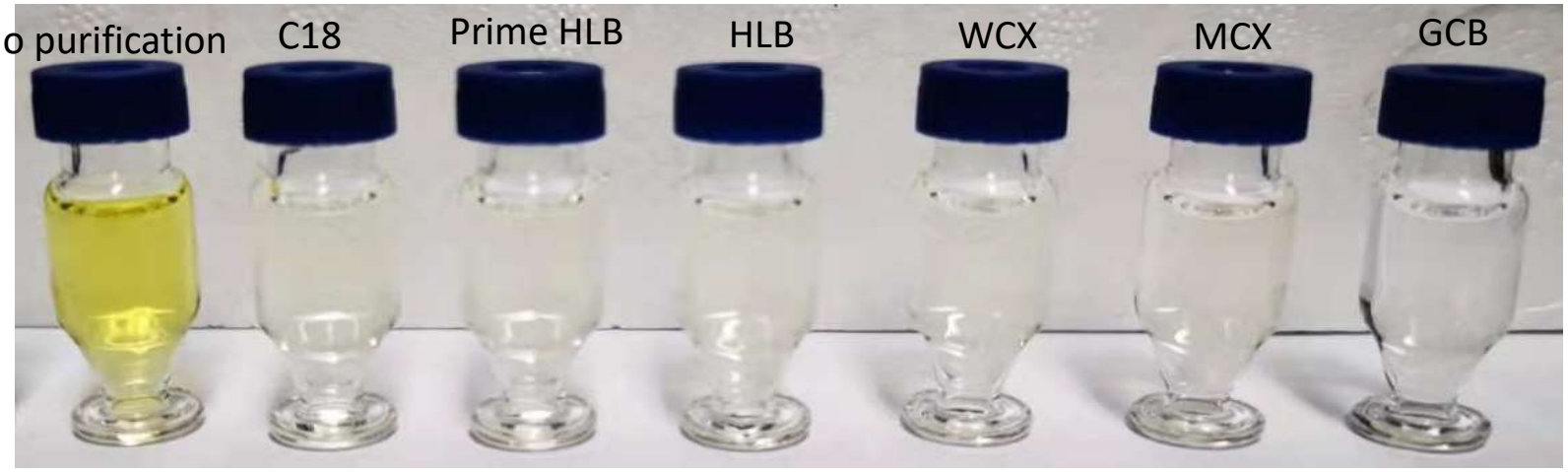


Spinach

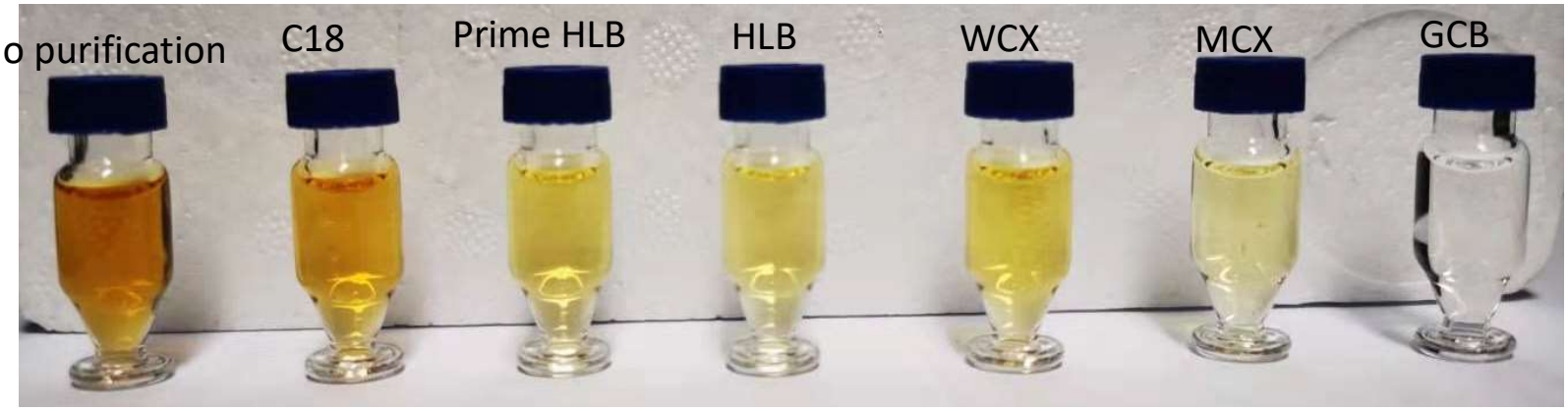
Analytical Method Development

- SPE purification

Spinach

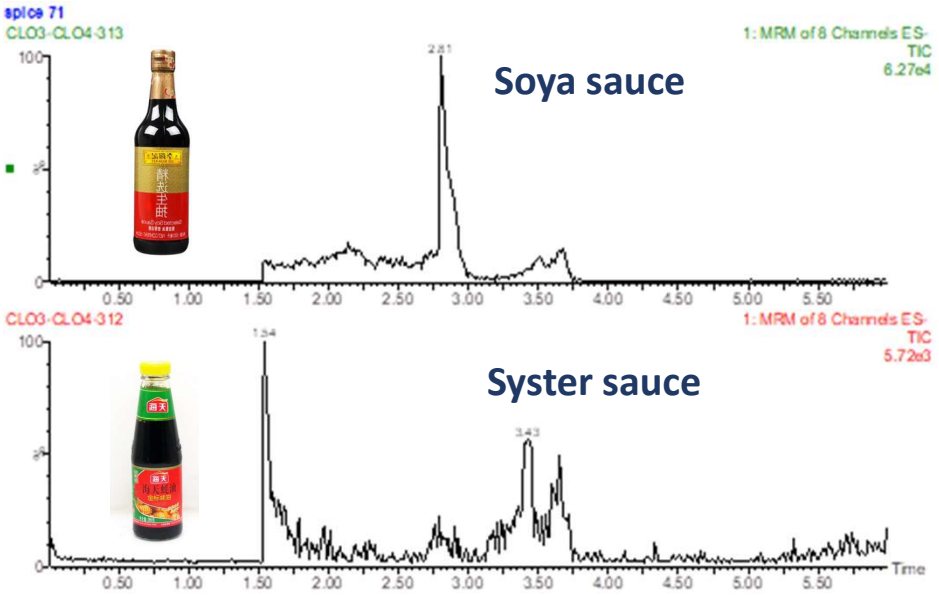


Composite spice

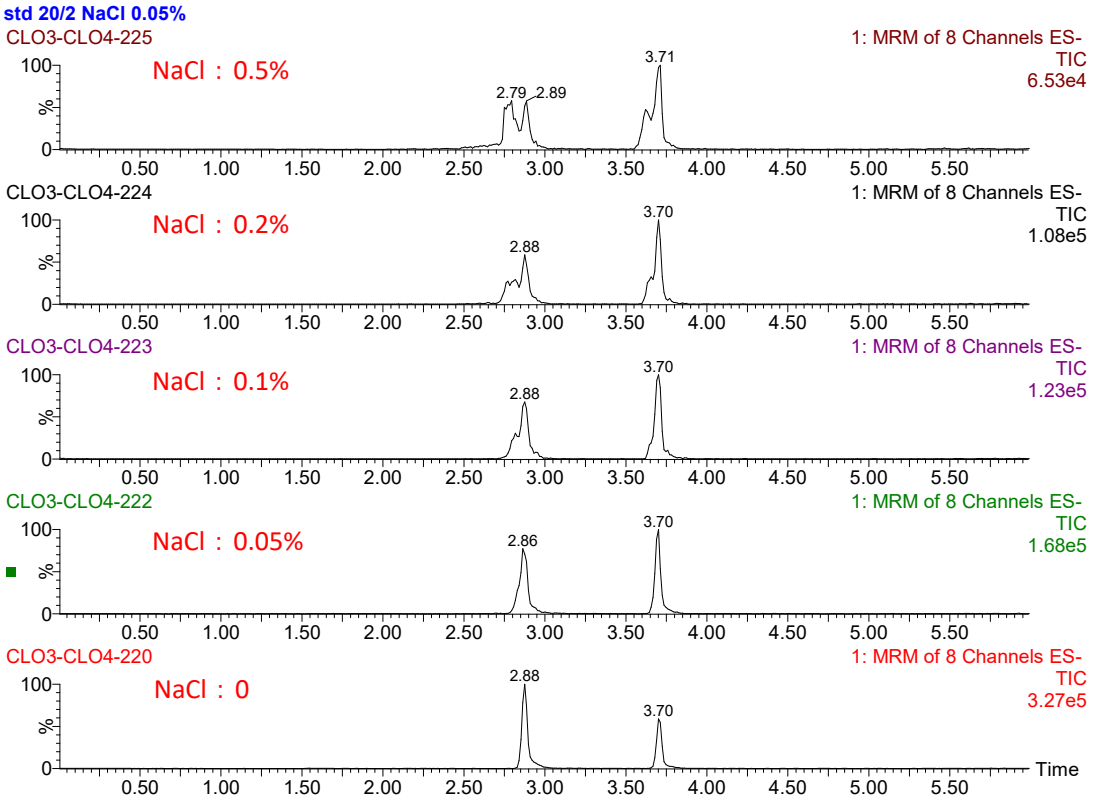


Analytical Method Development

➢ High-Salt Sample



The effect of NaCl ？

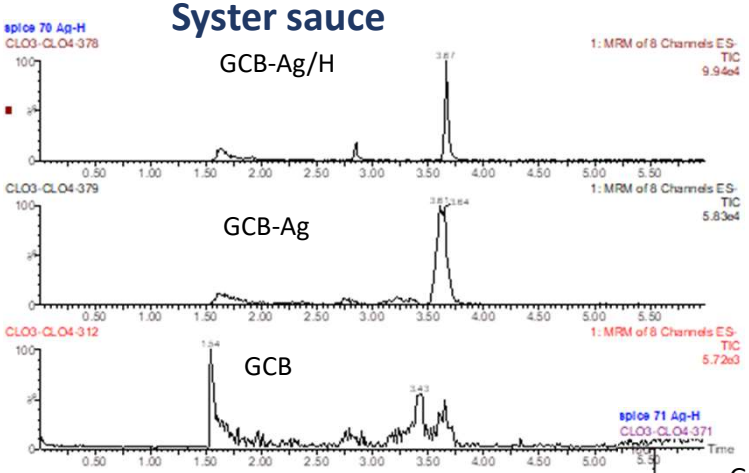


➢ **A further purification step is necessary for the high salt samples**

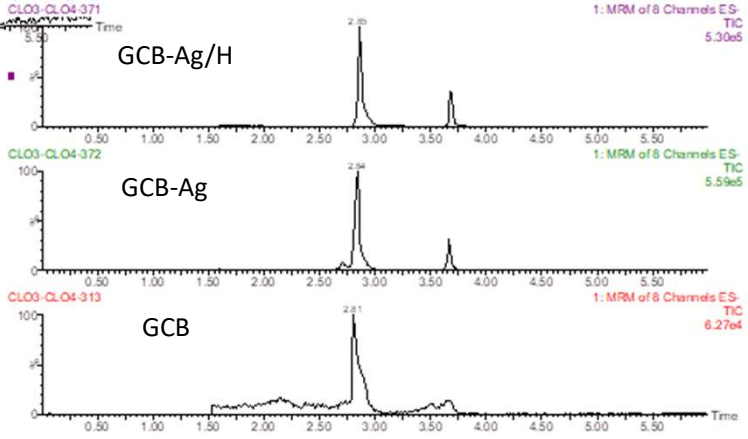
Analytical Method Development

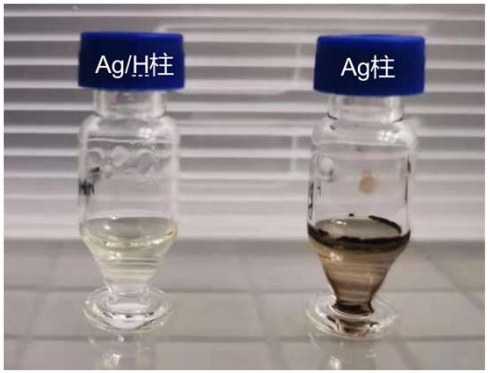
- Further purification

Syster sauce



Soya sauce

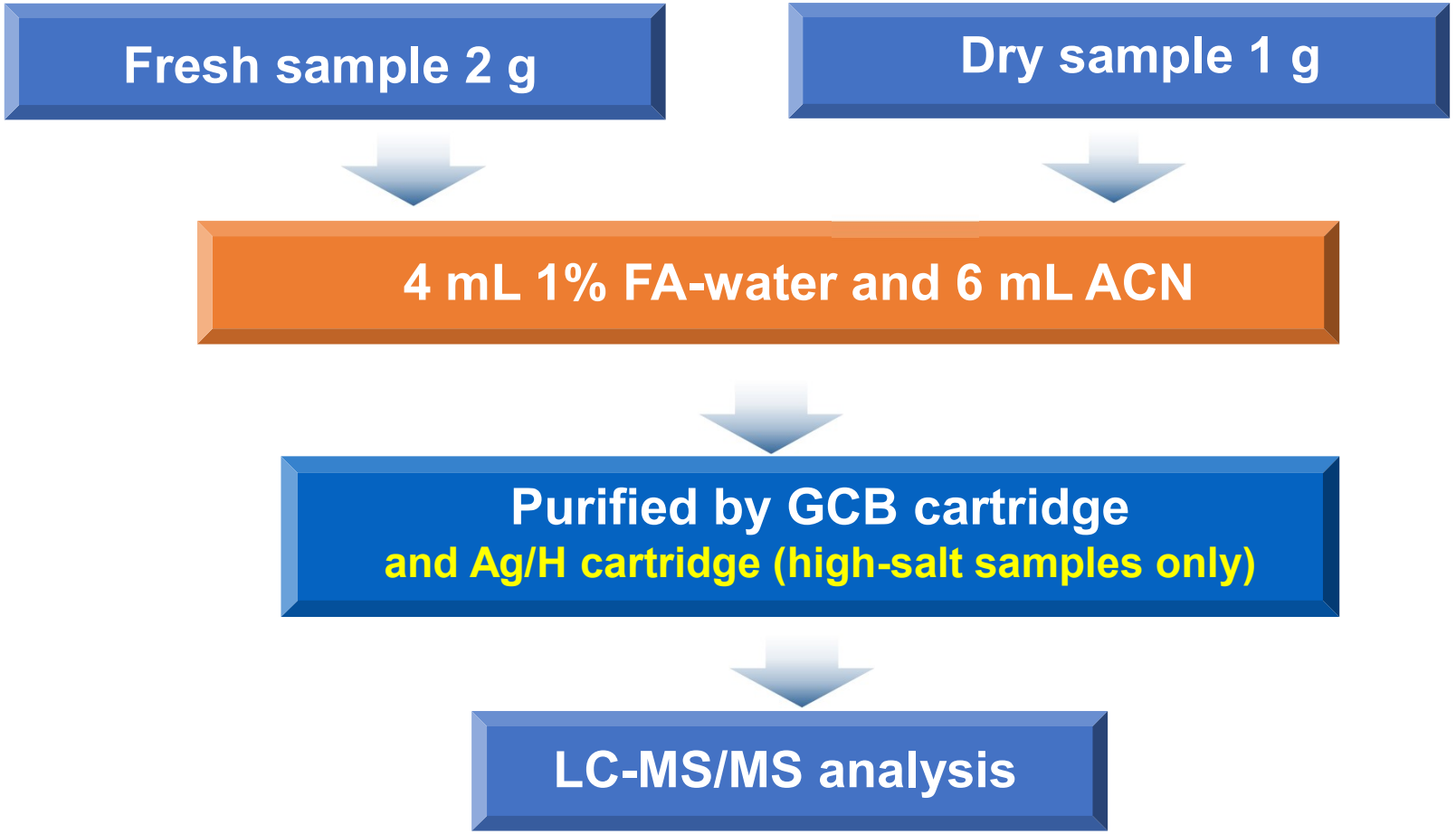




- In general, Ag/H cartridge presents better results than the Ag cartridge

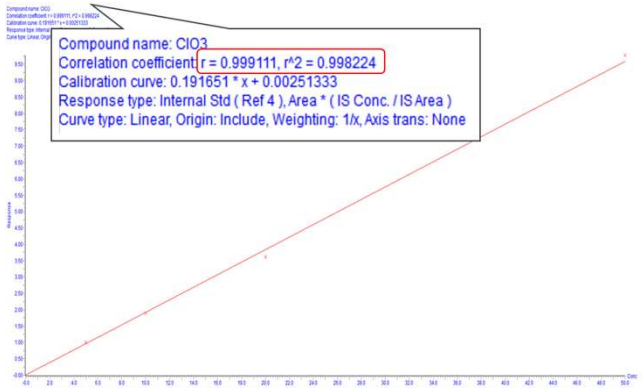
Analytical Method Development

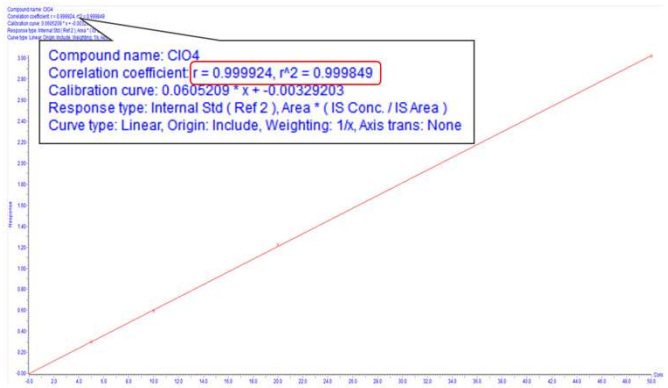
- Sample pretreatment procedure



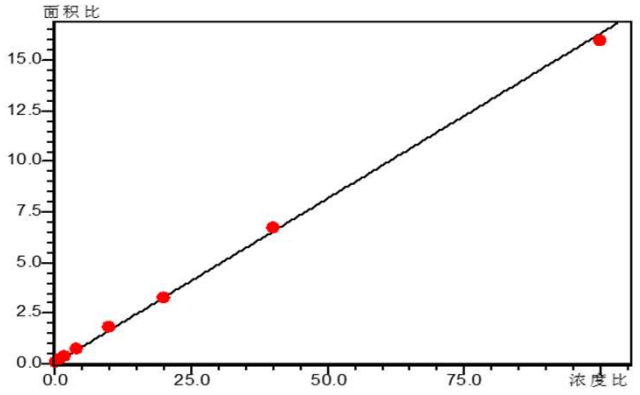
Analytical Method Development

- Linearity: isotope labelled internal standards calibration

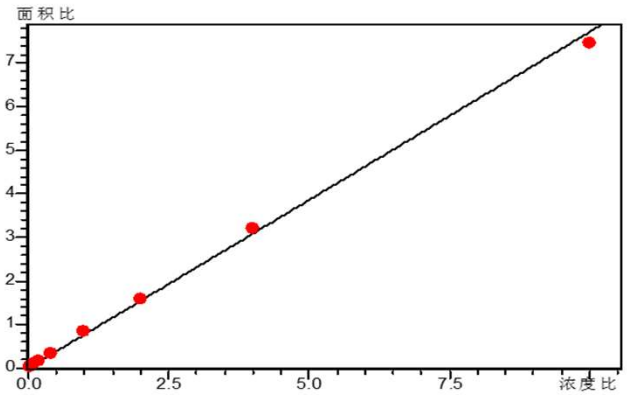




Waters UPLC-Xevo TQ-XS MS



Y=0.163191X+0.0184304
R=0.9994



Y=0.770577X+0.00968475
R=0.9991

Shimadzu 30A 8060 LCMS

Analytical Method Development

➢ Method Validation

MLOQ:

ClO_4：0.5 μg/kg； ClO_3：5 μg/kg

Linearity：

ClO_4：0.1~100 μg/kg； ClO_3：1~1000 μg/kg

Recovery：

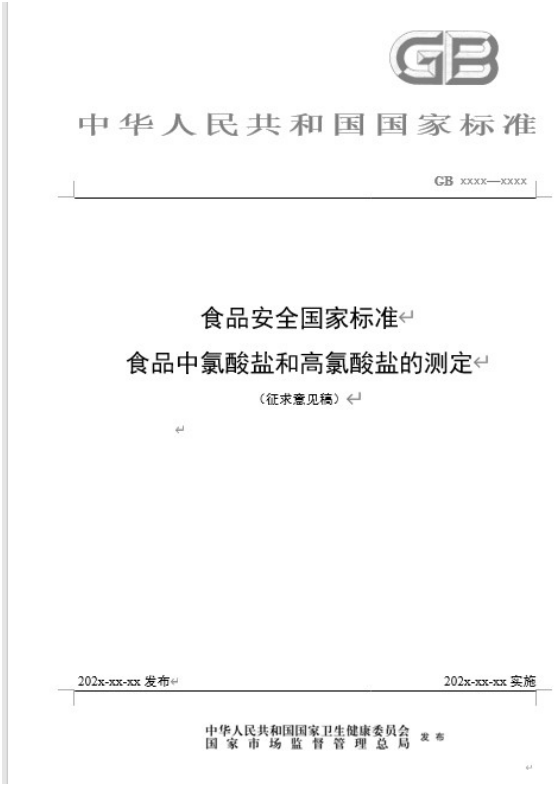
82.3-109.6%

Accuracy：

RSD<15%

➢ **Suitable for chlorate and perchlorate determination in a variety of food samples, e.g. animal producing foods, cereals, vegetable, fruits, drinking, dairy products, and seasoning samples……**

Analytical Method Development



中华人民共和国国家标准

GB xxxx—xxxx

食品安全国家标准

食品中氯酸盐和高氯酸盐的测定

（征求意见稿）

202x-xx-xx 发布　　202x-xx-xx 实施

中华人民共和国国家卫生健康委员会
国家市场监督管理总局 发布

食品安全国家标准公开征求意见

目前正在公开征求意见的标准列表

请选择您要发表意见的标准：　标准名称：　类别：请选择　搜索

序号	标准名称	标准类别	征集截止时间	操作
1	食品安全国家标准 食品中三氯蔗糖(蔗糖素)的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
2	食品安全国家标准 食品中果糖、葡萄糖、蔗糖、麦芽糖、乳糖的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
3	食品安全国家标准 食品中乳铁蛋白的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
4	食品安全国家标准 食品中膳食纤维的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
5	食品安全国家标准 食品中维生素A和E的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
6	食品安全国家标准 食品中维生素D的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
7	食品安全国家标准 化学分析方法验证通则	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
8	食品安全国家标准 调理肉制品生产卫生规范	生产经营规范	2021-10-22-2021-11-20	发表意见
9	食品安全国家标准 食品微生物学检验培养基和试剂的质量要求	微生物检验方法与规程	2021-10-22-2021-11-20	发表意见
10	食品安全国家标准 微生物学检验方法验证通则	微生物检验方法与规程	2021-10-22-2021-11-20	发表意见
11	食品安全国家标准 食品中生物素的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
12	食品安全国家标准 食品中二噁英及其类似物毒性当量的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
13	食品安全国家标准 食品中氯酸盐和高氯酸盐的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
14	食品安全国家标准 食品中N-亚硝胺类化合物的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
15	食品安全国家标准 食品中钼的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
16	食品安全国家标准 食品中铬的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
17	食品安全国家标准 食品中镉的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
18	食品安全国家标准 食品接触材料及制品 邻苯二甲酸酯的测定和迁移量的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
19	食品安全国家标准 食品中过氧化值的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
20	食品安全国家标准 酒和食用酒精中乙醇浓度的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
21	食品安全国家标准 食品接触材料及制品 多元素的测定和多元素迁移量的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
22	食品安全国家标准 食品接触材料及制品 9种抗氧化剂迁移量的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
23	食品安全国家标准 食品中淀粉的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
24	食品安全国家标准 食品接触材料及制品 纸、纸板及纸制品中荧光性物质的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见
25	食品安全国家标准 食品接触材料及制品 异噻唑啉酮类化合物迁移量的测定	理化检验方法与规程	2021-10-22-2021-11-20	发表意见

Method Application

- Occurrence of chlorate and perchlorate in seasoning samples

Classification	Parameters	Chlorate	Perchlorate
Sauce （n=13）	Detectable	8	13
	Occ.（%）	61.5	100
	Max（mg/kg）	17.13	0.114
	Min（mg/kg）	<LOD	0.018
	Average（mg/kg）	1.943	0.035
	Medium（mg/kg）	0.067	0.025
Vinegar （n=13）	Detectable	12	13
	Occ.（%）	92.3	100
	Max（mg/kg）	2.335	4.087
	Min（mg/kg）	<LOD	0.014
	Average（mg/kg）	0.204	0.365
	Medium（mg/kg）	0.022	0.063
Spice （n=36）	Detectable	23	32
	Occ.（%）	63.9	88.9
	Max（mg/kg）	2.164	10.84
	Min（mg/kg）	<LOD	<LOQ
	Average（mg/kg）	0.150	1.075
	Medium（mg/kg）	0.052	0.185
Others （n=22）	Detectable	14	18
	Occ.（%）	63.6	81.8
	Max（mg/kg）	0.327	0.680
	Min（mg/kg）	<LOD	<LOQ
	Average（mg/kg）	0.123	0.245
	Medium（mg/kg）	0.090	0.042

Dietary Exposure Analysis

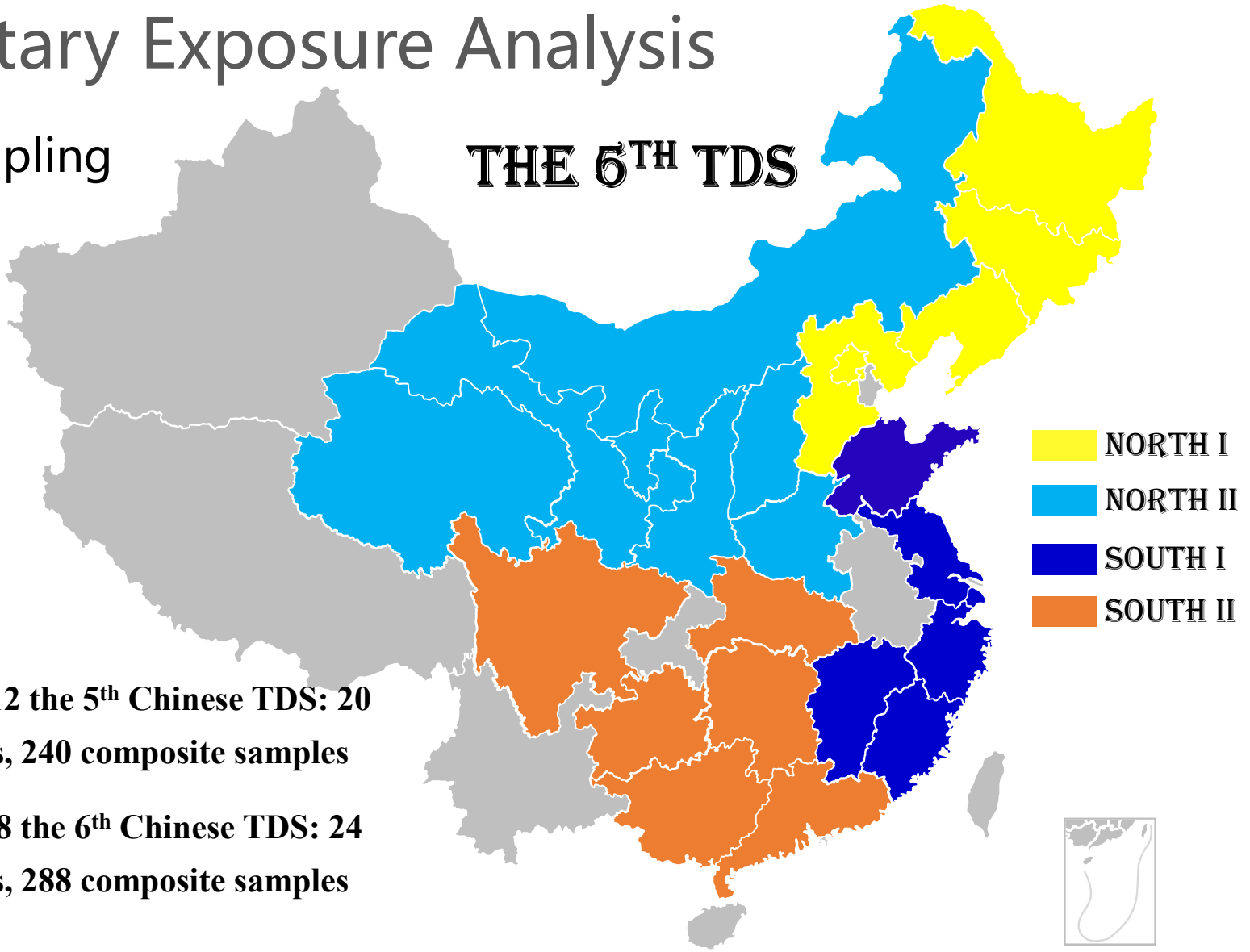
- Total dietary study (TDS)

膳食是人体暴露化学污染物的主要途径
Dietary intake: the major source of contaminants exposure for humans

总膳食研究（TDS）**是反映生活在一个国家或地区人群通**过食品实际摄入化学物量的最准确的方法
Total dietary study (TDS) is one of the most effective methods to assess the potential health risk to the general population posed by food contamination

Dietary Exposure Analysis

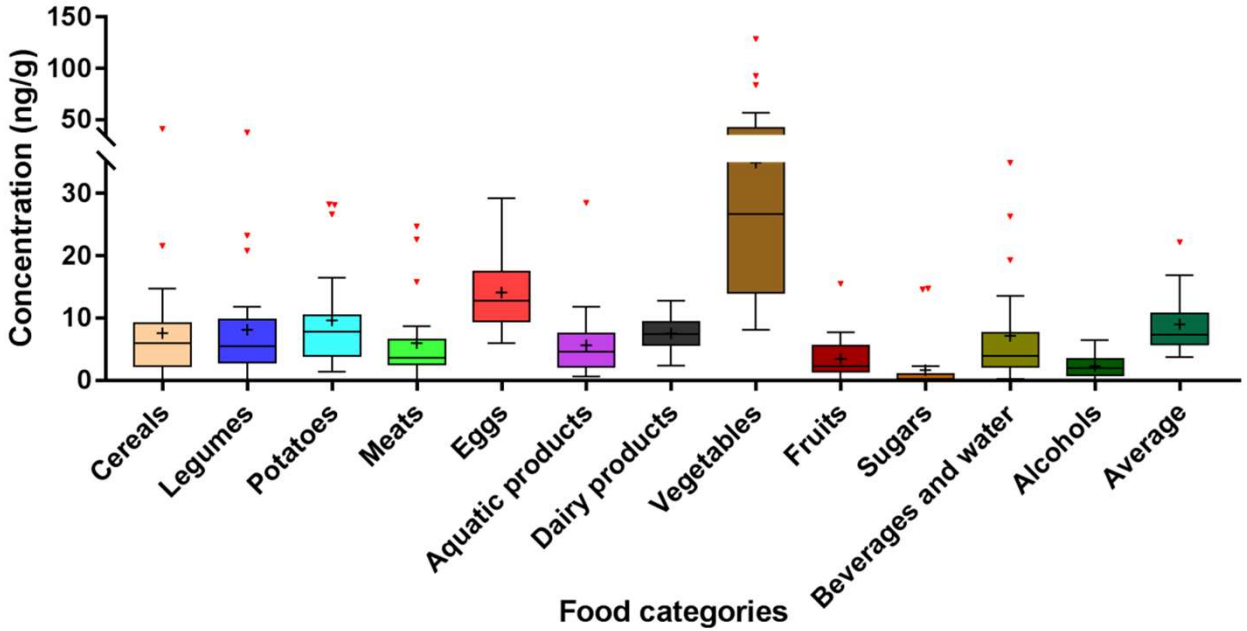
- Sampling



- **2010-2012 the 5th Chinese TDS: 20 provinces, 240 composite samples**
- **2016-2018 the 6th Chinese TDS: 24 provinces, 288 composite samples**

Dietary Exposure Analysis

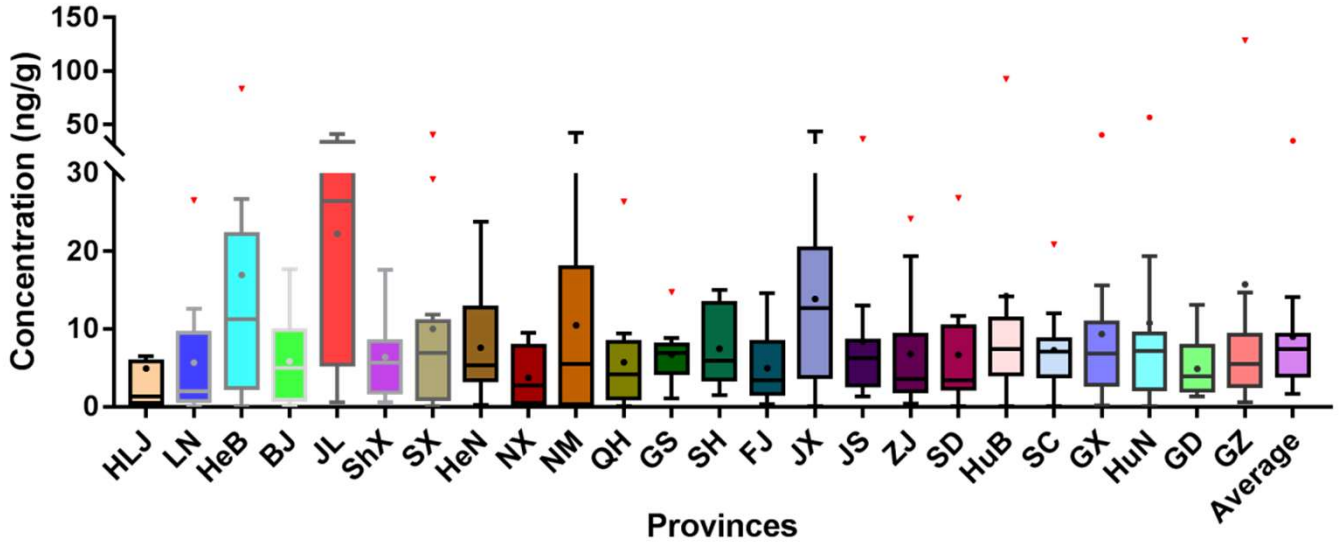
- Occurrence of perchlorate in the 6th TDS samples



- There are 273 out of total 288 samples detected containing perchlorate, with a concentration range of 0.50-129 μg/kg, among which the highest level occurred in the vegetables sample from GZ province

Dietary Exposure Analysis

- Occurrence of perchlorate in the 6th TDS samples



- Samples from Jilin province presented the maximum perchlorate level 0.58～41.19 μg/kg （average 22.20 μg/kg），followed that of Hebei and Jiangxi provices.

Dietary Exposure Analysis

- Calculation of Dietary Intakes

$$D = \sum \frac{C \times F}{f \times W}$$

D：Daily intakes of perchlorate（μg/kg bw/day）

C：Concentration in one kind of food（μg/kg wet weight）

F：Consumption of one kind of food（g/day）

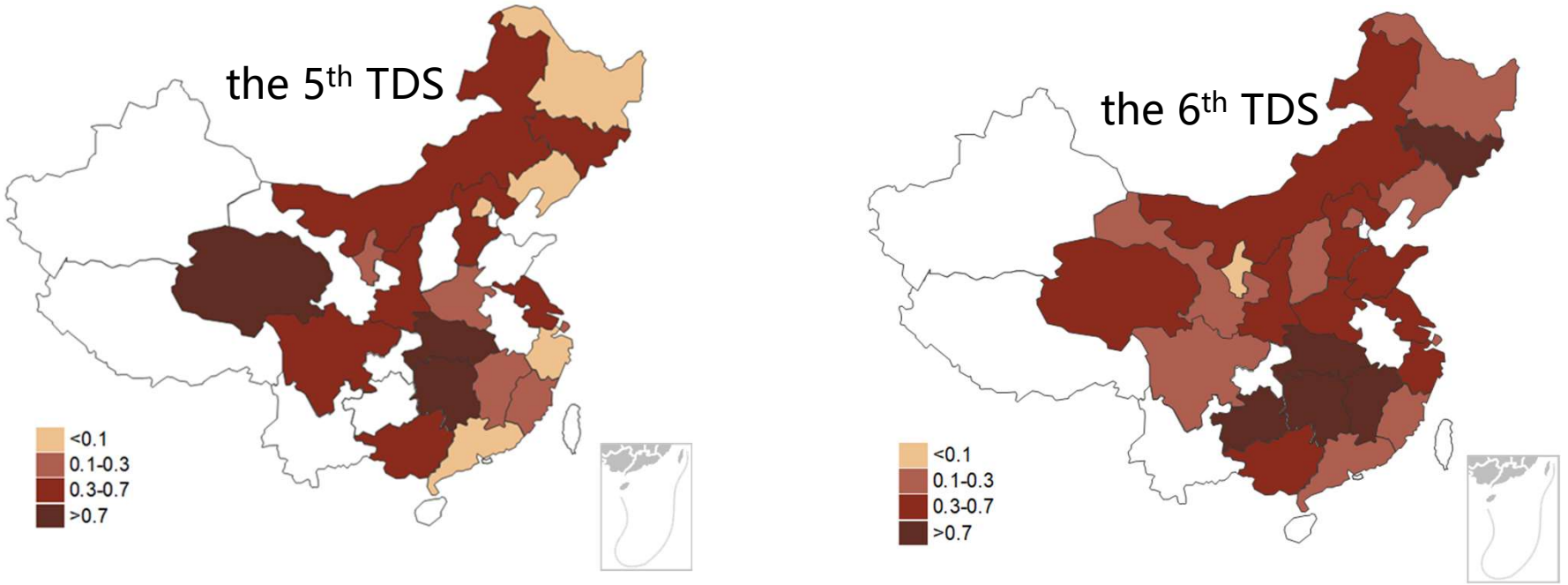
f： Ratio of raw and cooked of one kind of food

W：Body weight（kg）

For any residue below the MLOD, ½ MLOD were used

Dietary Exposure Analysis

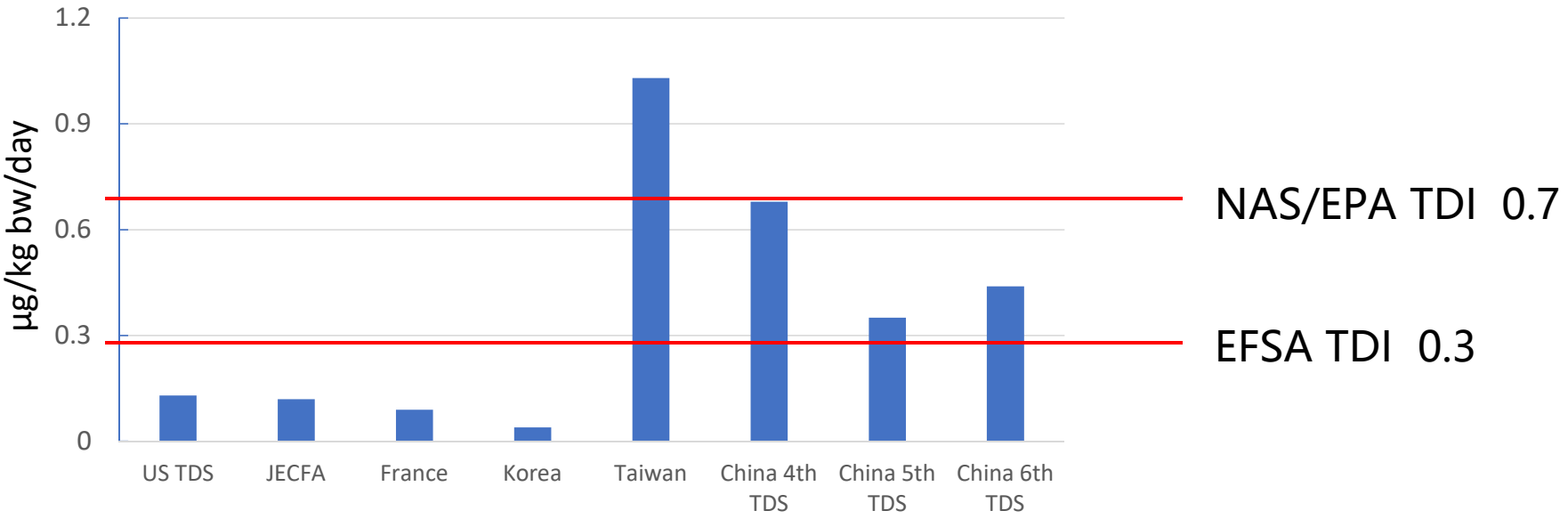
- The dietary exposures to perchlorate in the 5th and 6th TDS



Estimated mean perchlorate intakes from a few provinces are greater than the reference dose (0.70 μg/kg bw/day) recommended by National Academy of Science of US.

Dietary Exposure Analysis

- The dietary exposures to perchlorate: China VS the other country or regions

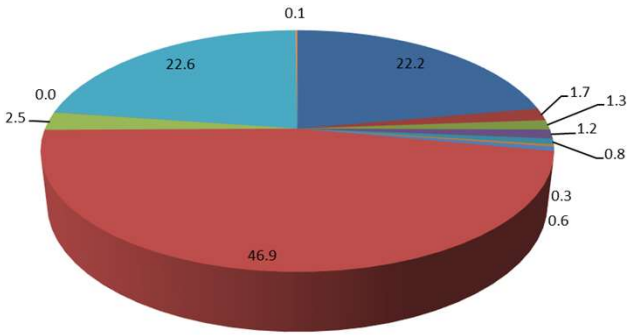


- Estimated mean perchlorate intake in this study were 0.351 µg/kg bw/day and 0.449 µg/kg bw/day, respectively, exceeding the EFSA's tolerance daily intake (0.30 µg/kg bw/day)

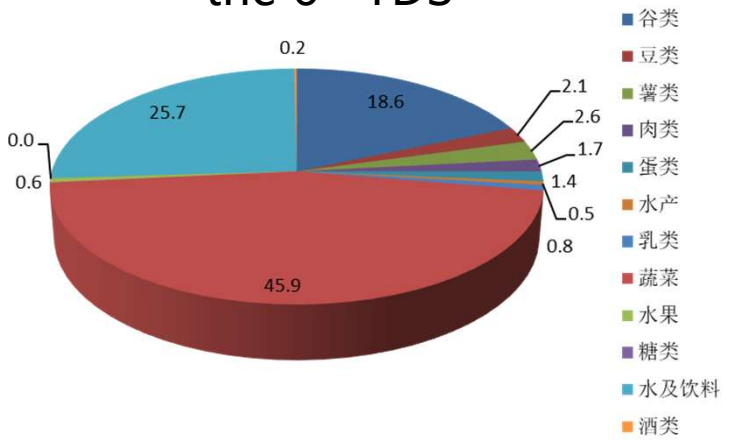
Dietary Exposure Analysis

- The distribution of daily intakes of perchlorate and iodine among food categories

the 5th TDS



the 6th TDS



Vegetables> Drinking > Cereals

Summary

- Analytical methods for simultaneous determination of chlorate and perchlorate in a variety of dietary samples were established based on SPE purification and LC-MS/MS analysis.
- The established method was used for the detection of real samples. Seasoning products presented a notable contamination of ClOx.
- The average intake to perchlorate of Chinese adults were 0.35 and 0.44 μg/kg bw/day for the 5^{th} and 6^{th} TDS, respectively, exceeding the EFSA's tolerance daily intake (0.30 μg/kg bw/day).

Thank you for your attention!