Briefing Session on Analysis of Sweeteners in Food

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110 Years 百 煉精粹 足信賴 of Professional Services

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Background : What is Sweetener?

According to Cap 132U Sweeteners in Food Regulations

《食物內甜味劑規例》

- ***sweetener*** means any chemical compound which is sweet to the taste, but does not include any sugars or other carbohydrates or polyhydric alcohols.
- **甜味劑** 指任何帶甜味的化合物，但不包括糖或其他碳水化合物或多羥醇.

According to Cap 132W Food and Drugs (Composition and Labelling) Regulations《食物及藥物(成分組合及標籤)規例》

Schedule 3

2. List of ingredients

(1) Prepackaged food shall be legibly marked or labelled with a list of ingredients, headed or preceded by an appropriate heading consisting of or including any of the words “ingredients”, “composition”, “contents” or words of similar meaning.

2. 配料表

(1) 預先包裝食物須加上可閱的標記或標籤，用以表列食物的配料，該表並須冠以適當標題，而標題中須載有或包括任何“配料”、“成分組合”、“內含物質”的字樣或具類似意思的文字。

According to Cap 132W Food and Drugs (Composition and Labelling) Regulations《食物及藥物(成分組合及標籤)規例》

- "ingredient" means any substance, including any additive and any constituent of a compound ingredient, which is used in the manufacture or preparation of a food and which is still present in the finished product, even if in altered form

  “配料”指用於製造或配製食物並繼續存在於製成品中的任何物質(即使形態已有所更改)，包括任何添加劑或合成配料的任何成分

- (6) The functional classes of additive for the purpose of sub-paragraph (5) are - Sweetener

  (6) 就第(5)節而言，添加劑的作用類別為 – 甜味劑

Background : Testing Scope

English name	Chinese name	INS No.	Relative Sweetness
Acesulfame potassium	醋磺內酯鉀	950	200
Alitame	縮二氨酸基酰胺	956	2000
Aspartame	天冬酰胺	951	180
Cyclamic acid (and Sodium, Potassium, Calcium Salts)	環己基氨基磺酸 (和鈉、鉀、鈣鹽)	952	30-50
Saccharin (and Sodium, Potassium, Calcium Salts)	糖精 (和鈉、鉀、鈣鹽)	954	300
Sucralose	三氯半乳蔗糖	955	600
Neotame	紐甜	961	7,000 – 13,000
Steviol Glycosides*	甜菊醇糖苷	960	200-300

* Steviol Glycosides contains high percentage of Stevioside and Rebaudioside A.

Ref: https://www.cfs.gov.hk/english/multimedia/multimedia_pub/multimedia_pub_fsf_46_02.html

Background : Sample Types

Wide range of food samples:

- Milk, cheese
- Drink
- Meat, preserved meat products
- Fish, seafood
- Ice-cream, yogurt
- Fruit
- Seeds, nuts, rice
- Biscuit, cake
- Sugar confectionery
- Etc…

According to 2023 Policy Address - Policy Measures (2023年施政報告 -政策措施)

Enhancing food safety

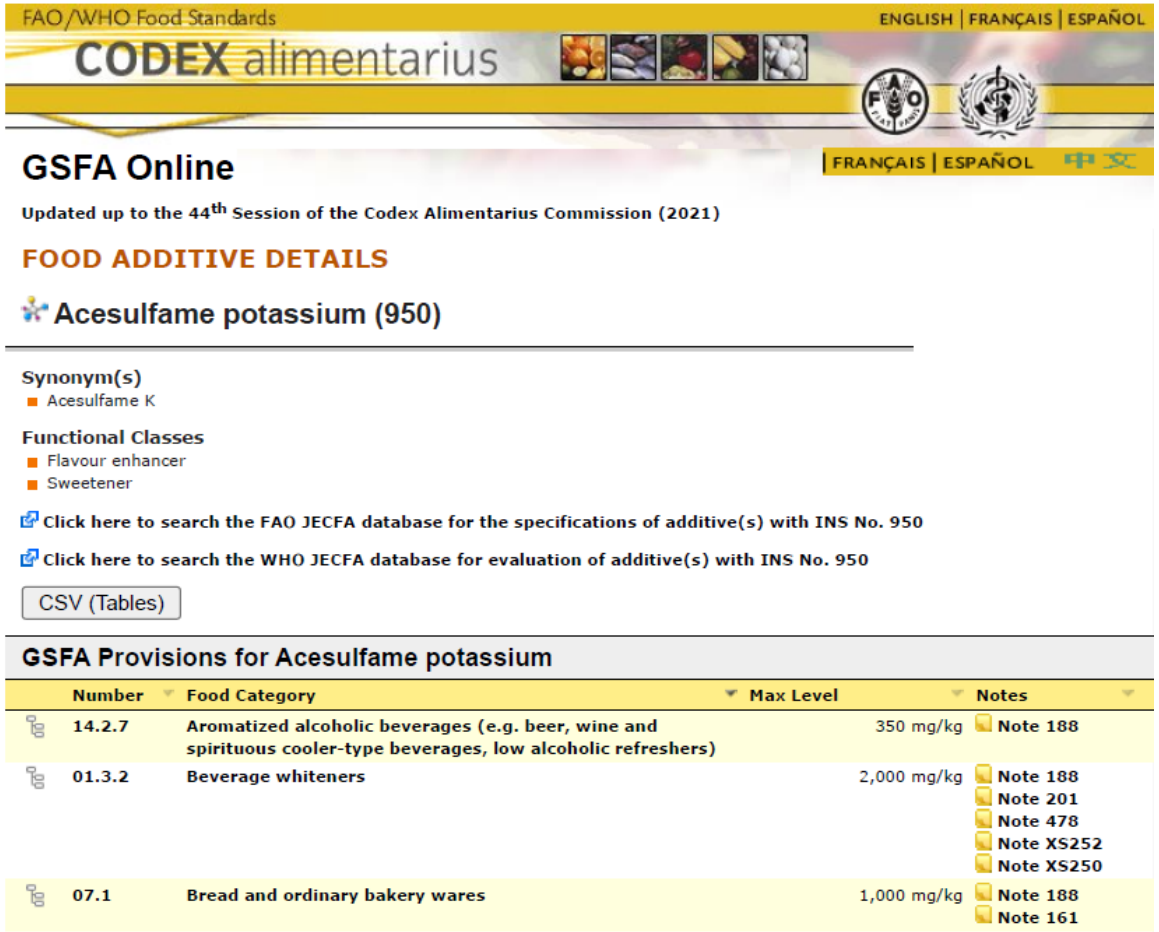
- Review the food safety standards for sweeteners in food in 2024 and conduct public consultation in 2024-25. (EEB)

提升食物安全

- 在2024 年內檢視食物中甜味劑的食物安全標準，並在2024 –25 年進行公眾諮詢。

（環境及生態局）

Regulatory Control [CODEX] (1)



FAO/WHO Food Standards

ENGLISH | FRANÇAIS | ESPAÑOL

CODEX alimentarius

FRANÇAIS | ESPAÑOL 中文

GSFA Online

Updated up to the 44th Session of the Codex Alimentarius Commission (2021)

FOOD ADDITIVE DETAILS

Acesulfame potassium (950)

Synonym(s)
- Acesulfame K

Functional Classes
- Flavour enhancer
- Sweetener

Click here to search the FAO JECFA database for the specifications of additive(s) with INS No. 950

Click here to search the WHO JECFA database for evaluation of additive(s) with INS No. 950

CSV (Tables)

GSFA Provisions for Acesulfame potassium

Number	Food Category	Max Level	Notes
14.2.7	Aromatized alcoholic beverages (e.g. beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)	350 mg/kg	Note 188
01.3.2	Beverage whiteners	2,000 mg/kg	Note 188 Note 201 Note 478 Note XS252 Note XS250
07.1	Bread and ordinary bakery wares	1,000 mg/kg	Note 188 Note 161

https://www.fao.org/gsfaonline/additives/details.html?id=104

Regulatory Control [CODEX] (2)

Sweetener	Number	Food Category	Max Level
Alitame (956)	14.1.4	Water-based flavoured drinks, including “sport,” “energy,” or “electrolyte” drinks and particulated drinks	40 mg/kg
Aspartame (951)	4.1.2.3	Fruit in vinegar, oil, or brine	300 mg/kg
Acesulfame potassium (950)	12.5	Soups and broths	110 mg/kg
CYCLAMATES (952) 952(i), 952(ii) and 952(iv)	14.2.7	Aromatized alcoholic beverages (e.g. beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)	250 mg/kg
SACCHARINS 954(i), 954(ii), 954(iii) and 954(iv)	14.2.7	Aromatized alcoholic beverages (e.g. beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)	80 mg/kg
Sucralose (Trichlorogalactosucrose) (955)	9.4	Fully preserved, including canned or fermented fish and fish products, including mollusks, crustaceans, and echinoderms	120 mg/kg
Neotame (961)	9.3	Semi-preserved fish and fish products, including mollusks, crustaceans, and echinoderms	10 mg/kg
STEVIOL GLYCOSIDES (960) 960a, 960b, 960c and 960d	12.2.2	Seasonings and condiments	30 mg/kg

Accreditation Criteria (1)

HOKLAS Supplementary Criteria No.37
'Food' Test Category – Chemical Testing

Calibration curve

- At least 3 standards (excluding blank) should be used to establish a linear calibration graph.
- The standards used shall bracket the entire range of concentration of test samples.
- Criteria for the correlation coefficient of linear calibration graph should be set and implemented.
- Should be checked at suitable intervals using calibration standard (frequency of around 5%).
- Acceptance criteria shall be established and shall commensurate with the measurement uncertainty.

QC requirement

- Blank, duplicate, spike, laboratory control sample, control charts, etc.

Accreditation Criteria (2)

Validation of methods (Non-standard methods)

- Laboratories shall verify their competence to perform the test by the use of certified reference materials and participation of proficiency testing programmes.

Important method performance characteristics:

- Limit of detection
- Limit of quantitation
- Precision
- Bias (assess using CRMs)
- Applicable concentration ranges
- Sample matrices

  If a method is to be accredited under ‘general foodstuffs’, satisfactory validation data shall be obtained for at least 5 different common food matrices (protein, carbohydrate, oil, dietary fibre and water), and at least three food types representative of each food matrix.

Testing Methods – International Standard Methods (1)

Standards	Testing Methods	Techniques
AOAC 969.27	Non-nutritive Sweeteners in Nonalcoholic Beverages Qualitative Thin-layer Chromatographic Method	TLC
AOAC 957.09	Cyclohexylsulfamate (Cyclamate) Salts in Nonalcoholic Beverages	$BaSO_4$ precipitation test (Qualitative)
AOAC 969.28	Sodium Cyclamate and Calcium Cyclamate in Canned Fruit Colourimetric Method	Acid hydrolysis, colorimetric method
AOAC 973.29	Saccharin in Food (Gravimetric Method)	$BaSO_4$ precipitation
AOAC 980.18	Saccharin in Food Differential Pulse Polarographic Method	Differential Pulse Polarographic Method
AOAC 947.10	Saccharin in Food Sublimation Method	Sublimation
AOAC 934.04	Saccharin in Nonalcoholic Beverages	Colormetric Test with NH_3-free H_2O and Nessler reagent

Testing Methods – International Standard Methods (2)

Standards	Methods	Techniques
NMKL 122 (1997) 2nd Ed.	Saccharin. Liquid chromatographic determination in beverages and sweets .	HPLC
NMKL 123 (1998) 2nd Ed.	Cyclamate. Spectrophotometric determination in foods.	Spectrometry
GB 5009.28-2016	食品中苯甲酸、山梨酸和糖精鈉的測定	HPLC-UV
GB 5009.247-2016	食品中紐甜的測定	HPLC-UV
GB 5009.97-2023	食品中環己基氨基磺酸鈉的測定	GC-FID, HPLC-UV, LC-MSMS
EN 1379 : 1997	Foodstuffs - Determination of cyclamate and saccharin in liquid table top sweetener preparations. Method by high performance liquid chromatography	HPLC-UV
EN 12856:1999-04	Foodstuffs- Determination of acesulfame-K, aspartame and saccharin – High performance liquid chromatographic method	HPLC-UV/PDA
EN 12857:1999-04	Foodstuffs - Determination of cyclamate – High performance liquid chromatographic method	Derivatization, HPLC-UV

Testing Methods – Other Literature Methods

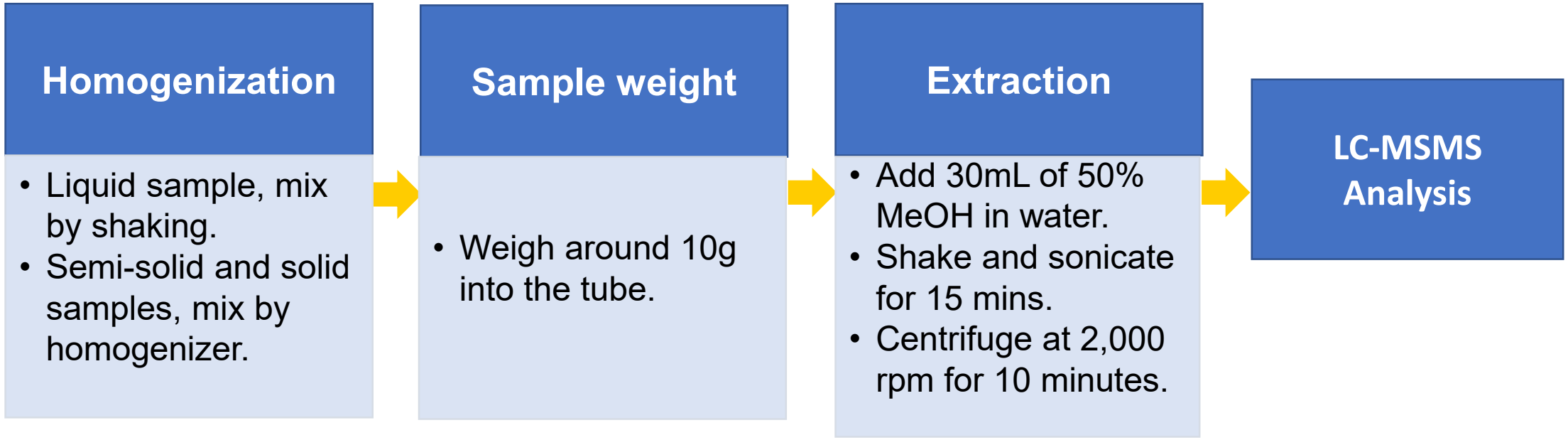
Methods	Techniques
Determination of Sucralose in Foods *LGC Sucralose SOP*	HPLC-RI
Simultaneous Determination of Nine intense Sweeteners in foodstuffs by High Performance Liquid Chromatography and Evaporative Light Scattering Detection – Development and Single-laboratory Validation *Andrzek Wasik, Josephine McCourt, Manuela Buchgraber, Journal of Chromatography A 2007, 1157, 187-196. (IRMM Validated Method)*	HPLC-ELSD
Simultaneous Determination of Twelve Sweeteners and Nine Preservatives in Food by Solid-Phase Extraction and LC-MS/MS *Sayuri Tsuruda, Tomonori Sakamoto, Kouichi Akaki, Journal of the Food Hygienic Society of Japan 2013, 54, 204-212.*	LC-MS/MS
Detection of 10 Sweeteners in Various Foods by Liquid Chromatography/Tandem Mass Spectrometry *Chui-Shiang Chang, Tai Sheng Yeh, Journal of Food and Drug Analysis 2014, 22, 318-328.*	LC-MS/MS
Simultaneous determination of sweeteners in beverages by LC-MS/MS *Hiroaki Sakai, Azusa Yamashita, Masayoshi Tamura, Atsuo Uyama &Naoki Mochizuki, Food Additives &Contaminants: Part A 2015, 32(6), 808-816.*	LC-MS/MS

Testing Methods: GL Methods

Sweetener	Analysis Technique	Reference method
Acesulfame potassium, Aspartame, Alitame, Saccharin	HPLC (Quantification)	EN 12856:1999
Sucralose	HPLC (Quantification)	uses RI as detector
Cyclamate	HPLC (Quantification)	EN12857:1999
Acesulfame potassium, Saccharin, Cyclamate, Aspartame, Sucralose, Alitame, Neotame and Steviol Glycosides	LC-MS/MS (Alternative Confirmation)	-

Alternative Confirmation Technique: LC-MS/MS (1)

Sample Preparation



Ref: Sayuri Tsuruda, Tomonori Sakamoto, Kouichi Akaki, Simultaneous Determination of Twelve Sweeteners and Nine Preservatives in Food by Solid-Phase Extraction and LC-MS/MS, *Journal of the Food Hygienic Society of Japan* 2013, 54, 204-212.

Alternative Confirmation Technique: LC-MS/MS (2)

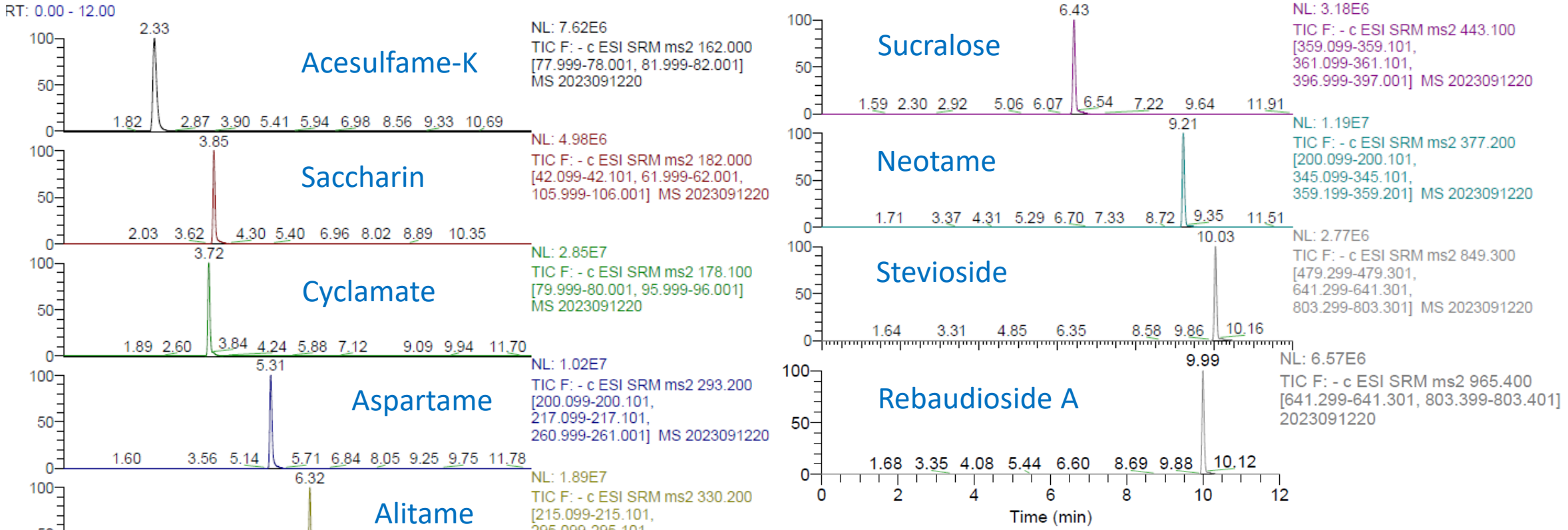
LC setting	
LC column	C18 column, 3.5μm, 2.1 x 100mm
Flow rate	0.3 mL/min
Solvent	A: 0.1% formic acid in water
	B: 0.1% formic acid in acetone

MS Setting	
Ionization mode	Negative ion mode
Spray voltage	4.5kV
Capillary temperature	350 ℃

Solvent programme

Time (min)	A (%)	B (%)
0	98	2
0.5	98	2
12	30	70
13	30	70
14	98	2
25	98	2

Alternative Confirmation Technique: LC-MS/MS (3)



Certified Reference Materials

CRM are available from the following reference material producers:

LGC
Supelco
National Institute of Metrology, China

Proficiency Testing Programmes

Proficiency Test	Start Date	Matrix	Analytes
FAPAS 03164	2021	Cola drink	-Acesulfame-K -Saccharin -Benzoic Acid -Caffeine
FAPAS 03165	2021	Soft drink	-Acesulfame-K -Aspartame -Cyclamate -Saccharin
FAPAS 03172	2022	Soft drink	-Acesulfame-K -Aspartame -Cyclamate -Saccharin
FAPAS 03179	2023	Soft drink	-Acesulfame-K -Aspartame -Cyclamate -Saccharin -Sucralose
FAPAS 03180	2023	Cola drink	-Acesulfame-K -Saccharin -Benzoic Acid -Caffeine

Thank you!