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ORIGINAL ARTICLE

Comprehensive detection of 120 additives in food using nontargeted MS data acquisition

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Abstract

The compliance assessment on the labeling of food additives is a hard job, because there are nearly thousand legal food additives can be used in food, and countless illegal additives must also deal with. This study developed a non-targeted data acquisition screening method based on liquid chromatography high resolution mass spectrometry (HRMS) in which a precursor ion and two product ions of each analyte are able to be recorded. The high throughput screening method worked as foodomics that characterized and identified every food components as long as they were ionized in terms of theory. The data acquisition method called data independent acquisition (DIA) was achieved by a full scan form *m/z* 70−1050, and then followed wide window fragmentations of product ions recording. A full scan and the followed fragmentations generated 21 spectra in 2.6 s contributed about 6 data points for a typical 0.2−0.3 min width peak in HPLC. A detection database list of 120 additives included 79 colorants, 13 sweeteners, 12 preservatives and 7 antioxidants was established. Thirty-three commercial samples including beverages, candies, and sauces were surveyed for testing additives. Sweeteners (rebaudioside A) and flavoring agents (malic acid and fumaric acid) were found the most under declared additives. HPLC column often do not provide adequate retention for highly polar compounds such as organic acids (flavoring agents). In this study they were coeluted, but were able to be separated and determined by HRMS worked as the secondary separation tool. The surveillance results showed there is still room for food manufacturers to improve the connection between their product information and consumers.

Keywords: Additives, DIA, Fast screening, HRMS, Nontargeted

1. Introduction

Food additives are required to be labeled on the package of food. Most direct additives belong to parts of the ingredient labels of food products, and some trace amounts of indirect additives are not required to be labeled. Additives used in food commonly include colorants, sweeteners, preservatives, antioxidants, and flavoring agents. These natural and/or artificial additives need to be regulated and monitored before addition, treating, or processing. Food additives are evaluated by authorities such as European Food Safety Authority's (EFSA) or Food and Drug Administration (FDA) to be represented as legal additives, while others not listed in food additive status list are recognized as illegal. The regulations of nations applicable to food additives are vary that force exporters to adjust their product compositions to the importers' markets. Failure to comply may result in adulteration, misbranding, non-compliance and rejection [1]. Therefore, the monitoring and detection of additives in foods has to include both legal and illegal additives depend on their legal states. Besides, some additives such as Sudan dyes, dimethyl yellow and ethoxyquin are illegal or not permitted to use in food for human consumption,

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but may be found in contaminated foods [2–4]. It is necessary to develop a simultaneous detection method for broadly screening of additives in foods. However, additives with various functions to foods present differences in their physicochemical properties, these make the monitoring of a large group of additives difficult in every step for a method development such as sample preparation, separation and detection.

Various analytical methods reported the detections of a single class of additives. Sagdic [5] detected 18 phenolic compounds including antioxidants by high performance liquid chromatography (HPLC) in grape. Two natural antioxidants were monitored in enriched virgin olive oil trough capillary electrophoresis method [6]. Seven preservative were simultaneously determined in cosmetics by dispersive liquid–liquid microextraction coupled with capillary electrophoresis [7]. Multiclass determinations of additives in a single run were developed in recent year. Boyce [8] used micellar electrokinetic chromatography to separate and detect 6 antioxidants and 3 sweeteners in beverages. Ultra performance liquid chromatography (UPLC) coupled with electrospray ionization tandem mass spectrometry (ESI-MS/MS) was applied for rapid determination of 5 preservatives, 6 artificial sweeteners and 9 synthetic dyes in kimchi. The rapid separation was performed in 5.5 min and can be used for rapid quality control [9]. Methods focused on the sample pretreatment and cleanup were applied in accurate determination of preservatives and artificial sweeteners in juice by HPLC to remove matrix interference prior to chromatographic determination [10].

High resolution mass spectrometry (HRMS) such as orbitrap provided full-scan spectra with resolution 70,000 FWHM (full width at half maximum), which gave chance to look into the composition of a sample. The higher resolution made the assigned masses more accurate and background peaks better resolved. Studies had proved HRMS produced similar results in terms of reliability, specificity and sensitivity to the most wildly used low resolution MS/MS [11,12]. A validated approach analyzed 43 additives including sweeteners, flavor agents, antioxidants and preservatives in dairy product by quadrupole orbitrap mass spectrometry. Sample was prepared through quick, easy, cheap, effective, rugged and safe (QuEChERS) method prior to a 15-min fast analysis [13]. Another similar approach described the detection of 69 dyes in wines [14]. The results proved HRMS was a powerful method for screening analytes in foods. Additional capabilities of HRMS beyond the current MS/MS were by confirming the presence of an analyte not included in the target list. The measure of analytes without previous compound-specific tuning and selection enabled retrospective data analysis for a broad list of compounds, so called nontargeted data acquisition [15]. Compared to tandem mass only applicable to around 200 targets [16], nontargeted data acquisition carried out larger compound database, and widely used as metabolic fingerprinting and profiling approaches [17]. Wang [18] generated a non-target data acquisition for target analysis called nDATA workflow for screening of 845 pesticide residues in fruits and vegetables by UHPLC/Q-Orbitrap. The detection was based on the retention time, mass accuracy of a precursor and fragment ions which significantly expanding the number of pesticides currently being screened by traditional MS/MS approaches.

The aim of this study was designed to build a screening method for 120 additives by HRMS. The major areas of routine monitoring of food were pesticide residues, veterinary residues and natural toxins. Methods for these three classes of residues were well developed for detection of multiple residues in a single commodity, and be able to quantitate. However, the multi-class analytical method for additives such as dyes, sweeteners, preservatives, antioxidants and flavoring agents was few. A nontargeted method called variable data independent acquisition (vDIA) was developed for comprehensive sample detection of 120 additives in this study.

2. Methods

2.1. Chemicals

Methanol, acetonitrile, ethyl acetate, acetone, formic acid and ammonia acetate were purchased from Sigma–Aldrich (St. Louis, MO, US). Reference standards including (name and purity%) allura red AC (98%), alizarin (95%), alizarin green (95%), amaranth (95%), auramine O (95%), azorubine (95%), benzyl violet 4B (95%), brilliant blue FCF (95%), carminic acid (95%), chrysoidine G (95%), citrus Red 2 (95%), crocein orange G (95%), curcumin (95%), diethyl yellow (95%), erythrosine (95%), fast green FCF (95%), indigo carmine (95%), light green SF yellowish (95%), lissamine green B (95%), malachite green (95%), metanil yellow (95%), methyl yellow (95%), α-naphthol orange (95%), naphthol yellow S (95%), new coccin (95%), orange G (80%), orange II (95%), para red (95%), patent blue V (95%), phloxine (95%), ponceau SX (98%), quinoline yellow

WS (95%), rhodamine B (95%), rose Bengal (95%), scarlet GN (95%), solvent green 3 (95%), Sudan black B (95%), Sudan I (95%), Sudan II (95%), Sudan III (95%), Sudan IV (95%), Sudan orange G (95%), Sudan red 7B (95%), Sudan red G (95%), Sudan red B (95%), sulforhodamine B (95%), sunsent yellow FCF (95%), tartrazine (95%), xylene fast yellow 2G (60%), pigment orange 5 (98%), disperse orange 37 (96%), oil orange SS (98%), red 2G (98%), fumaric acid (99%), succinic acid (99%), L-glutamic acid (99%), tartaric acid (99%), sodium lactate (99%), DL-malic acid (99%), caffeine (98%), L-theanine (98%), glucono-δ-lactone (98%), propyl gallate (98%), nordihydroguaiaretic acid (97%), 4-hexylresorcinol (98%), ethoxyquin (99%), methyl p-hydroxybenzoate (99%), ethyl p-hydroxybenzoate (99%), propyl p-hydroxybenzoate (99%), butyl p-hydroxybenzoate (99%), benzyl 4-hydroxybenzoate (99%), phenyl 4-hydroxybenzoate (98%), n-heptyl 4-hydroxybenzoate (99%), natamycin (99%), sorbic acid (99%), aspartame (98%), neohesperidin dihydrochalcone (98%), neotame (98%), rebaudioside A (96%), rebaudioside B (95%), sucralose (98%), L-cysteine hydrochloride (98%) and cyclamate (100%, SUPELCO) were from Sigma–Aldrich. Saccharin (99%) and acesulfame potassium (99%) were from Fluka (Charlotte, NC, US). Gentian violet (100%), stevioside (100%) and glycyrrhizic acid (100%) were purchased from USP (Rockville, MD, US). Dulcin (99%) and acid green 3 (unknown purity) were form Kanto-Kasel LTD. (Tokyo, Japan). Alitame (98%) was from Toronto Research Chemicals (North York, ON, Canada). Eosin Y (85%), fluorescein (95%), brilliant yellow (70%), disperse orange 3 (96%), basic fuchsin (unknown purity), astrazon orange G (unknown purity), 2-methoxy-4-nitroaniline (98%), alizarin yellow GG (50%), fast yellow AB (97%), disperse yellow 3 (96%), o-aminoazotoluene (99.3%), 1-(methylamino)-anthraquinone (98%), 4-aminoazobenzene (98.9%), lithol rubine BK (unknown purity) and new fuchsin (unknown purity) were from Uni-Onward Co. (Taipei, Taiwan). Astrazon orange R (unknown purity) was form TCI (Tokyo, Japan). Dyes with unknown purity of 4-amino carminic acid, solvent yellow 21, solvent orange 62, solvent red 8 and solvent yellow 16 were from BOC Sciences (Shirley, NY, US). Copper chlorophylls with unknown purity of Cu(II) chlorin e4 and Cu(II) chlorin e6 were from Frontier Scientific (Logan, UT, USA). Chemical each 100 mg was dissolved in appropriate solvent (methanol, ethanol, acetonitrile, ethyl acetate or acetone) in a 10-mL volumetric flask and diluted to volume as individual standard stock solution. Mixed solutions were prepared at 1, 5 or 10 μg/mL in 50% methanol solution to generate target list of additives.

2.2. Instrumentation for generating target list of additives

An UHPLC-ESI-Orbitrap MS system consisted of an UltiMate 3,000 pump, a Q Exactive mass spectrometer and a accucore aQ C18 column (2.1 × 150 mm, 2.6 μm particle size, Thermo Fisher Scientific, Rockford, IL, US) were utilized. The column was maintained at 35 °C and the flow rate was set at 0.5 mL/min. A gradient elution containing 0.1% formic acid with 20 mM ammonium acetate (A), and acetonitrile with 0.1% formic acid (B) was applied. The gradient was hold on 1% (B) for first 1 min and then increased from 1 to 99% (B) over the next 8 min. The eluent was remained for the next 7 min, and then mobile phase (B) was retained to 1% over the next 0.1 min, and this was followed by a 2.9 min re-equilibration period at 1% (B) prior to the next injection. The injection volume was 5 μL. The mass spectrometer was operated at ESI positive (3.5 kV) and negative (2.5 kV) mode. Resolution was set at 70,000 (defined at $m/z = 200$ and was set at full width at half maximum, FWHM). The precursor list was built by direct infusion mass spectrometry of each individual additive standard at concentration of 1 ppm.

2.3. Evaluation of limits of detection (LODs) by data independent acquisition (DIA)

Candy and carbonated sparkling water were used as blank sample for the evaluation of LODs for 79 colorants. Pork jerky and carbonated sparkling water were used for 13 sweeteners. Twelve preservatives were tested in pork jerky. Seven antioxidants and 9 flavoring agents were evaluated in carbonated sparkling water. Carbonated sparkling water was ultrasonicated for 15 min, and 10 g was transferred into a 100-mL volumetric flask. Additive standard stock solutions were spiked separately at the concentration 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 5 and 10 mg/kg, and made up to volume with 50% methanol solution (v/v). Solutions were filtered with membrane filters prior to analysis. Candy and pork jerky were ground separately and 10 g each was transferred into a 100-mL volumetric flask. Additive standard stock solutions were then spiked into sample for 30 min, and made up to volume with 50% methanol solution. The volumetric flask was untrasoncated for 15 min, and then centrifuged at 5,000×*g*. The filtrate was passed through a polyvinylidene fluoride (PVDF) membrane filter as sample solution.

Mass spectrometer worked in DIA mode and the parameters were as following. A full scan form *m*/*z* 70–1,050 was conducted, and then precursors in wide windows were selected to obtain 21 MS/MS spectra. Isolation window 27 amu was applied for *m*/*z* 100 to 500 in every 25 amu increment (eg. 100–125, 125–150, …, 475–500 amu). Isolation window 104 amu was utilized for *m*/*z* 500 to 1,000 in every 100 amu increment. The ions in every collection were then sent to high energy dissociation (HCD) cell for fragmentation, and the followed C-trap prior to orbitrap analysis. The cycle time for a full scan and 21 fragments spectra was about 2.6 s. The samples were required to run HPLC HRMS/MS twice, one for positive ESI and another for negative ESI due to the long cycle time of data acquisition.

2.4. Surveillance

Candies, beverages and soy sauces in total 33 samples were purchase from local market. Samples were separated into liquid and solid samples, and followed the same procedures described in sample preparation for LODs evaluation. The detection and data processing of non-target data acquisition for target analysis were performed using TraceFinder 3.3 software (Thermo Fisher Scitific). In a positive sample, the precursor ion of an analyte was extracted form full scan spectra, and the product ions were extracted form fragment spectra associated to the precursor segment.

3. Results and discussion

The precursor ion and two product ions along with retention time of each analyte were shown in Table 1. The ionization of general chemicals were either $[M+H]^+$ or $[M-H]^-$. However, for colorants, some were exceptional. $[M + H-Na]^-$, $[M-Na]^-$, $[M+H_2-Na]^+$, $[M+H_3-Na_2]^-$, $[M+H_3-Na]^-$, $[M-Cl]^+$ and $[M + H-Ca]^-$ might happen depended on the chemicals and the pH of sample solution and eluent. The target list was generated in parallel reaction monitoring (PRM) mode of mass spectrometry. A full scan at scan range *m*/*z* 70–1,050 was first conducted, and then the scan data was compared with the established precursor list. If there were a match, the mass spectrometer would isolate a target precursor ion (window was 4 amu), then fragmented the targeted precursor ion in the collision cell (30 eV and 60 eV stored in C-trap), and then sent to Orbitrap for detection. The resulting product ions were obtained as MS/MS spectrum, and all spectra of standard compounds were listed in the supplemental file (https://www.jfda-online.com/cgi/editor.cgi?article=3366&window=additional_files&context=journal). The dead time, no interaction between the sample and the column, was estimated to be 0.7 min [$(0.105\ cm)^2 \times \pi \times 15\ cm \times 70\%/0.5\ cm^3$ per min, 70% was an estimate of interstitial void space in porous silica particles]. Most additives obtained good retentions, except flavoring agents such as L-cysteine, fumaric acid, L-glutamic acid, tartaric acid, sodium lactate, DL-malic acid, glucono-δ-lactone, which obtained retention time between 0.6 and 0.8 min. These compounds co-eluted with other no retention food matrix. The mass spectrometer worked as the secondary separation tool. The low-resolution mass spectrometer (LRMS) basically separate two ions differing by one mass unit along the whole range scanned. The high-resolution mass spectrometer (HRMS), such as Orbitrap, measures ion oscillation frequencies and transfer into mass-to-charge ratio (*m*/*z*) with resolution 70,000 FWHM. The instrument provides accurate mass with detecting error below 5 ppm which enable the secondary separation of analytes in complex co-elute food matrix. Fig. 1b showed a chromatogram (total ion chromatogram, TIC) of a soy sauce (sample 32) at RT between 0 and 2.5 min. There was a big and broad peak indicated no/poor retention and co-elute in the first 2 min. The ESI negative spectrum of RT = 0.7 was showed in Fig. 1a that dressed there were a few compounds co-elute around the column dead time. These compounds were high polar chemicals performed no retention on a typical C18 column. However, lactate (Fig. 1c) and glutamic acid (Fig. 1d) were able to be separated from the co-elute matrix by selecting theoretical accurate masses of 89.0244 and 146.0459, respectively. HRMS was powerful and enable the secondary separation of analytes from very complex co-elute without interference. In Fig. 1a, obtained accurate mass of lactate *m*/*z* 89.02403 and glutamic acid *m*/*z* 146.04531 showed differences of −4.15 and −4.04 ppm, respectively, to their assigned value in Table 1. The advantage of HRMS enabled the detection and separation of analytes even in dead time range of chromatographic separation.

3.1. DIA for precursor selection and product ions detection

DIA required no selection of a special precursor in a narrow range of typically 1 amu (conventional MRM), instead a wide window such as 27 or 104 amu in this study was selected. Various wide windows were selected to cover all potential analytes in the range between *m*/*z* 100 to *m*/*z* 1,000, and the followed fragmentation generated product ions for

Table 1. The ionization mode, detection ions, retention time and LODs of target compounds were determined.

	Compound	Elemental composition	Ionization mode	Precursor Ion (theoretical)	Delta mass[a]	Product ions	Retention time	LODs (ppm)	
								Candy	Beverage
1	Tartrazine	$C_{16}H_9N_4Na_3O_9S_2$	[M+2H−3Na]-	466.9973	2.7	197.9872 172.0068	3.02	0.5	0.5
2	Amaranth	$C_{20}H_{11}N_2Na_3O_{10}S_3$	[M+2H−3Na]-	536.9738	2.4	316.9669 237.0101	3.17	0.5	0.5
3	Indigo carmine	$C_{16}H_8N_2Na_2O_8S_2$	[M+H−2Na]2-	209.9867	6.7	79.9559 154.9584	3.38	5	5
4	Cochineal red A, Ponceau 4R	$C_{20}H_{11}N_2Na_3O_{10}S_3$	[M+2H−3Na]2-	267.9833	3.2	145.0280 208.9909	3.48	5	5
5	Sunset yellow	$C_{16}H_{10}N_2Na_2O_7S_2$	[M+H−2Na]-	407.0013	1.4	205.9917 327.0445	3.68	0.5	0.5
6	Naphthol yellow S	$C_{10}H_4N_2Na_2O_8S$	[M+H−2Na]-	312.9772	1.3	233.0203 295.9744	3.68	0.5	0.5
7	Orange G	$C_{16}H_{10}N_2Na_2O_7S_2$	[M+H−2Na]-	407.0013	1.7	301.9560 158.0373	3.74	0.1	0.1
8	Allura red AC	$C_{18}H_{14}N_2Na_2O_8S_2$	[M+H−2Na]-	451.0275	1.6	205.9917 79.9559	3.95	0.5	0.5
9	Carminic Acid	$C_{22}H_{20}O_{13}$	[M−H] -	491.0831	1.2	357.0610 327.0510	4.06	0.5	0.5
10	Xylene fast yellow 2G	$C_{16}H_{10}Cl_2N_4Na_2O_7S_2$	[M+H−2Na]2-	251.9689	1.7	170.9996 107.0377	4.1	5	5
11	Scarlet GN	$C_{18}H_{14}N_2Na_2O_7S_2$	[M+H−2Na]+	437.0472	2.3	201.0454 118.0651	4.23	0.1	0.1
12	Azorubine	$C_{20}H_{12}N_2Na_2O_7S_2$	[M+H−2Na]-	457.0170	3.9	377.0601 221.0151	4.41	0.5	0.5
13	Ponceau SX	$C_{18}H_{14}N_2Na_2O_7S_2$	[M+H−2Na]$^-$	435.0326	2.8	355.0748 199.0308	4.46	0.1	0.1
14	Light green SF	$C_{37}H_{34}N_2Na_2O_9S_3$	[M+H−2Na]$^-$	747.1510	1.1	683.1891 170.0043	4.49	0.5	0.5
15	G3 (Fast green FCF	$C_{37}H_{34}N_2Na_2O_{10}S_3$	[M+H−2Na]$^-$	763.1459	1.4	497.1547 577.1115	4.53	0.5	0.5
16	Lissamine green B	$C_{27}H_{25}N_2NaO_7S_2$	[M+2H−Na] $^+$	555.1254	1.5	392.1883 377.1648	4.56	0.5	0.5
17	Brilliant blue FCF	$C_{37}H_{34}N_2Na_2O_9S_3$	[M+2H−2Na]$^-$	747.1510	2.6	561.1166 260.0512	4.6	0.5	0.5
18	α-Naphthol orange	$C_{16}H_{11}N_2NaO_4S$	[M−Na]$^-$	327.0445	2.4	170.9995 247.0876	4.76	0.1	0.1
19	Quinoline yellow S	$C_{18}H_{13}NaNO_5S$	[M—3H−Na]$^-$	352.0285	2.7	288.0666 272.0717	4.77	0.1	0.1
20	Sulforhodamine B	$C_{27}H_{30}N_2O_7S_2$	[M+H] $^+$	559.1567	2.5	515.0941 501.0910	5.21	0.05	0.05
21	Crocein orange G	$C_{16}H_{11}N_2NaO_4S$	[M−Na]$^-$	327.0445	2.6	206.9995 142.0298	5.26	0.1	0.1
22	Orange II (Acid orange 7)	$C_{16}H_{11}N_2NaSO_4$	[M−Na]$^-$	327.0445	1.5	170.9995 155.9874	5.26	0.1	0.1
23	Patent blue V	$C_{27}H_{31}N_2NaO_7S_2$	[M+2H−Na] $^+$	561.1724	2.8	479.1999 435.1346	5.28	0.5	0.5
24	Alizarin	$C_{14}H_8O_4$	[M−H] $^-$	239.0350	3.8	211.0400 195.0451	5.42	0.5	0.5
25	Alizarin green (Patent green)	$C_{37}H_{34}ClN_2NaO_6S_2$	[M+2H−Na] $^+$	703.1698	2.5	517.1342 533.1655	5.48	0.5	0.5
26	Chrysoidine G	$C_{12}H_{12}N_4 \cdot HCl$	[M−Cl] $^+$	213.1135	4.2	121.0634 94.0525	5.49	0.1	0.1
27	Curcumin	$C_{21}H_{20}O_6$	[M−H] $^-$	367.1187	6.7	134.0368 173.0608	5.8	5	5
28	Benzyl violet 4B	$C_{39}H_{40}N_3NaO_6S_2$	[M−Na]$^-$	710.2364	2.7	630.2810 540.2310	5.8	0.1	0.1
29	Auramine O	$C_{17}H_{21}N_3$	[M+H] $^+$	268.1808	1.2	147.0916 131.0603	5.99	0.1	0.1
30	R7 (Erythrosine)	$C_{20}H_6I_4Na_2O_5$	[M+3H−2Na]$^-$	836.6623	3.1	582.8521 329.0435	6.4	0.5	0.5

(continued on next page)

Table 1. (continued)

	Compound	Elemental composition	Ionization mode	Precursor Ion (theoretical)	Delta mass[a]	Product ions	Retention time	LODs (ppm)	
								Candy	Beverage
31	Sudan orange G	$C_{12}H_{10}N_2O_2$	$[M+H]^+$	215.0815	2.5	93.0573 95.0127	7.09	0.1	0.1
32	Malachite green	$C_{23}H_{25}ClN_2$	$[M-Cl]^+$	329.2012	4.9	313.1699 208.1120	7.52	0.5	0.5
33	Rhodamine B	$C_{28}H_{31}ClN_2O_3$	$[M-Cl]^+$	443.2329	1.4	399.1703 355.1077	7.58	0.05	0.05
34	Phloxine	$C_{20}H_2Br_4Cl_4Na_2O_5$	$[M-Na]^-$	784.5406	4.4	658.6242 704.5731	7.72	5	5
35	Rose bengal	$C_{20}H_2Cl_4I_4Na_2O_5$	$[M-Na]^-$	970.4919	2.9	672.6936 890.5253	7.75	5	5
36	Para red	$C_{16}H_{11}N_3O_3$	$[M+H]^+$	294.0873	1.4	277.0843 128.0494	8.53	0.5	-[b]
37	Methyl yellow	$C_{14}H_{15}N_3$	$[M+H]^+$	226.1339	2.7	95.0494 105.0447	8.54	0.1	–
38	Sudan red G	$C_{17}H_{14}N_2O_2$	$[M+H]^+$	279.1128	4.0	123.0678 108.0443	8.88	0.1	0.1
39	Citrus Red 2	$C_{18}H_{16}N_2O_3$	$[M+H]^+$	309.1234	2.6	138.0549 153.0784	8.9	0.5	0.5
40	Sudan I	$C_{16}H_{12}N_2O$	$[M+H]^+$	249.1022	1.2	128.0494 232.0992	8.94	0.5	–
41	Dimethyl yellow	$C_{16}H_{19}N_3$	$[M+H]^+$	254.1652	2.7	95.0494 134.0943	9.05	0.1	–
42	Sudan II	$C_{18}H_{16}N_2O$	$[M+H]^+$	277.1335	3.2	121.0886 106.0651	9.95	5	5
43	Sudan black B	$C_{29}H_{24}N_6$	$[M+H]^+$	457.2135	4.5	193.0760 211.1104	10.28	5	5
44	Sudan III	$C_{22}H_{16}N_4O$	$[M+H]^+$	353.1397	2.9	196.0869 128.0494	10.69	0.5	–
45	Solvent green 3	$C_{28}H_{22}N_2O_2$	$[M+H]^+$	419.1754	2.3	327.1128 401.1648	10.79	0.5	–
46	Sudan red 7B	$C_{24}H_{21}N_5$	$[M+H]^+$	380.1870	2.7	169.0760 183.0916	10.95	5	5
47	Sudan IV	$C_{24}H_{20}N_4O$	$[M+H]^+$	381.1710	3.8	224.1175 276.1121	11.46	0.5	–
48	Metanil yellow	$C_{18}H_{14}N_3NaO_3S$	$[M-Na]^-$	352.0761	0.9	171.9817 155.9881	5.77	0.1	0.1
49	Eosin Y	$C_{20}H_8Br_4Na_2O_5$	$[M+H-2Na]^-$	646.6992	0.6	522.7816 442.8743	6.26	10	10
50	Fluorescein	$C_{20}H_{12}O_5$	$[M-H]^-$	331.0612	0.9	286.0635 243.0815	5.8	0.2	0.2
51	Brilliant yellow	$C_{26}H_{18}N_4Na_2O_8S_2$	$[M+H-2Na]^-$	579.065	0.4	499.1082 369.9836	4.59	10	10
52	Disperse orange 3	$C_{12}H_{10}N_4O_2$	$[M-H]^-$	241.0731	1.5	122.0248 211.0751	7.22	0.5	0.5
53	Gentian violet (Crystal violet)	$C_{25}H_{30}N_3Cl$	$[M-Cl]^+$	372.2434	1.1	356.2121 340.1808	7.89	0.1	0.1
54	Basic fuchsin	$C_{20}H_{20}ClN_3$	$[M-Cl]^+$	302.1652	1.0	209.1072 195.0917	5.38	0.1	0.2
55	Astrazon orange G	$C_{22}H_{23}ClN_2$	$[M-Cl]^+$	315.1856	1.6	300.1617 285.1386	6.31	0.1	0.1
56	Astrazon orange R	$C_{28}H_{27}ClN_2$	$[M-Cl]^+$	391.2169	1.9	376.1934 361.1699	7.53	0.1	0.1
57	Basic violet 2	$C_{22}H_{24}N_3Cl$	$[M-Cl]^+$	330.1965	1.8	300.1495 223.123	5.93	0.1	0.1
58	2-Methoxy-4-nitroaniline (Fast red B Base)	$C_7H_8N_2O_3$	$[M+H]^+$	169.0608	1.2	152.058 72.0444	5.26	0.2	0.2
59	Alizarin yellow GG	$C_{13}H_8N_3NaO_5$	$[M-Na]^-$	286.0469	0.9	242.0571 156.0581	6.23	0.1	0.1
60	Fast yellow AB	$C_{12}H_{11}N_3O_6S_2$	$[M-H]^-$	356.0016	0.6	276.0448 248.0387	3.1	10	10

(continued on next page)

Table 1. (continued)

	Compound	Elemental composition	Ionization mode	Precursor Ion (theoretical)	Delta mass[a]	Product ions	Retention time	LODs (ppm)	
								Candy	Beverage
61	Disperse yellow 3	$C_{15}H_{15}N_3O_2$	$[M-H]^-$	268.1092	0.7	134.0611 92.0506	7.31	0.1	0.1
62	o-Aminoazotoluene	$C_{14}H_{15}N_3$	$[M+H]^+$	226.1339	1.5	121.076 91.0542	7.74	0.1	0.1
63	Pigment orange 5	$C_{16}H_{10}N_4O_5$	$[M-H]^-$	337.0578	1.2	125.0118 167.0098	8.11	0.5	0.5
64	1-(Methylamino)-anthraquinone	$C_{15}H_{11}NO_2$	$[M+H]^+$	238.0863	0.6	223.0628 165.0699	7.8	0.2	0.5
65	4-aminoazobenzene	$C_{12}H_{11}N_3$	$[M+H]^+$	198.1026	0.3	95.0478 105.0447	6.9	0.1	0.1
66	Lithol rubine BK	$C_{18}H_{12}CaN_2O_6S$	$[M+H-Ca]^-$	385.05	0.8	187.0401 143.0502	5.55	10	10
67	Sudan red B	$C_{24}H_{20}N_4O$	$[M+H]^+$	381.171	1.7	224.1196 106.0651	11.29	1	1
68	Solvent yellow 21	$C_{34}H_{25}CrN_8O_6$	$[M-H]^-$	692.123	1.3	475.0616 648.1332	6.92	1	1
69	Solvent orange 62	$C_{32}H_{23}CrN_{10}O_8$	$[M-H]^-$	726.1033	1.5	447.0146 540.0361	7.96	1	1
70	Solvent red 8	$C_{32}H_{26}CrN_{10}O_8$	$[M-H]^-$	729.1268	2.2	246.9691 447.0151	7.84	1	1
71	Solvent yellow 16	$C_{16}H_{14}N_4O$	$[M-H]^-$	277.1095	1.1	117.0458 172.0642	8.58	1	1
72	Fast brown RR	$C_{16}H_{14}N_4$	$[M+H]^+$	263.1291	0.7	143.0730 246.1025	7.22	0.02	0.02
73	Oil orange SS	$C_{17}H_{14}N_2O$	$[M+H]^+$	263.1179	0.4	246.1152 107.0729	9.3	0.02	0.02
74	Red 2G (Azophloxine)	$C_{18}H_{13}N_3Na_2O_8S_2$	$[M+H-2Na]^-$	464.0228	1.1	358.9775 263.9972	4.06	2	2
75	Cu(II) Chlorin e4	$C_{33}H_{32}CuN_4Na_2O_4$	$[M+H-2Na]^-$	612.1803	2.3	481.1459 553.1670	9.19	10	20
76	Cu(II) Chlorin e6	$C_{34}H_{31}CuN_4Na_3O_6$	$[M+2H-2Na]^-$	656.1702	3.1	522.1850 507.1615	10.39	10	20
77	4-amino carminic acid	$C_{22}H_{21}NO_{12}$	$[M-H]^-$	490.0991	3.7	356.0776 326.0670	4.12	0.5	0.5
78	Disperse orange 37	$C_{17}H_{15}Cl_2N_5O_2$	$[M+H]^+$	392.0676	4.3	351.0410 165.0784	8.2	0.1	0.1
79	Guinea green B	$C_{37}H_{35}N_2NaO_6S_2$	$[M-Na]^-$	667.1942	4.5	497.1893 587.2363	5.49	10	10
80	Saccharin	$C_7H_5NO_3S$	$[M-H]^-$	181.9917	4.2	105.9598 92.9186	2.83	0.02	0.02
81	Cyclamate	$C_6H_{13}NO_3S$	$[M-H]^-$	178.0543	4.1	79.9568 94.9955	3.02	0.1	0.1
82	Aspartame	$C_{14}H_{18}N_2O_5$	$[M+H]^+$	295.1288	2.3	120.0813 103.0547	4.03	0.1	0.1
83	Stevioside	$C_{38}H_{60}O_{18}$	$[M-H]^-$	803.3706	1.6	641.3231 317.2116	5.58	0.1	0.1
84	Acesulfame potassium	$C_4H_5NO_4S$	$[M-H]^-$	161.9866	5.2	82.0292 77.9649	1.36	0.02	0.02
85	Dulcin	$C_9H_{12}N_2O_2$	$[M+H]^+$	181.0971	4.3	108.0449 65.0391	4.5	0.02	0.02
86	Neohesperidin dihydrochalcone	$C_{28}H_{36}O_{15}$	$[M+H]^+$	613.2127	0.9	137.0602 179.0708	4.89	1	1
87	Glycyrrhizin	$C_{42}H_{61}O_{16}$	$[M-H]^-$	821.3959	0.3	351.0563 113.0239	5.62	0.1	0.1
88	Neotame	$C_{20}H_{30}N_2O_5$	$[M+H]^+$	379.2227	2.9	172.1337 259.181	5.52	0.1	0.1
89	Alitame	$C_{14}H_{25}N_3O_4S$	$[M+H]^+$	332.1638	3.3	129.0738 159.0769	4.3	0.1	0.1
90	Rebaudioside A	$C_{44}H_{70}O_{23}$	$[M-H]^-$	965.4235	2.1	803.3755 641.3168	5.13	10	10

(continued on next page)

Table 1. (continued)

	Compound	Elemental composition	Ionization mode	Precursor Ion (theoretical)	Delta mass[a]	Product ions	Retention time	LODs (ppm)	
								Candy	Beverage
91	Rebaudioside B	$C_{38}H_{60}O_{18}$	$[M-H]^-$	803.3707	0.7	641.3237 317.2122	5.55	10	10
92	Sucralose	$C_{12}H_{19}Cl_3O_8$	$[M+NH_4]^+$	414.0484	1.4	198.9920 216.0629	3.88	2	1
93	Propyl gallate	$C_{10}H_{12}O_5$	$[M-H]^-$	211.0612	3.3	125.0233 169.0131	4.95		0.1
94	2,4,5-Trihydroxybutyrophenone	$C_{10}H_{14}O_2$	$[M-H]^-$	195.0662	3.1	95.0128 125.0233	5.43		0.1
95	Nordihydroguaiaretic acid	$C_{18}H_{22}O_4$	$[M-H]^-$	301.1445	0.7	122.0362 109.0284	6.29		0.5
96	4-Hexyl resorcinol	$C_{12}H_{18}O_2$	$[M-H]^-$	193.1234	1.7	125.0225 151.0025	7.03		10
97	4-Hydroxymethyl-2,6-di-tert-butylphenol (HMBP)	$C_{15}H_{24}O_2$	$[M-H]^-$	235.1703	2.1	160.0883 217.1587	7.26		10
98	Ethoxyquin	$C_{14}H_{19}NO$	$[M+H]^+$	218.1539	0.5	174.0913 160.0757	7.41		0.2
99	L-Cysteine hydrochloride	$C_3H_7NO_2S{\cdot}HCl$	$[M-Cl]^+$	122.0270	0.4	76.0215 86.9899	0.61		10
100	Fumaric acid	$C_4H_4O_4$	$[M-H]^-$	115.0037	0.0	71.0133 68.9977	0.73	10	
101	Succinic acid	$C_4H_6O_4$	$[M-H]^-$	117.0193	0.0	73.0290 99.0082	1.08	10	
102	L-Glutamic acid	$C_5H_9NO_4$	$[M-H]^-$	146.0459	4.0	128.0348 102.0555	0.68	1	
103	Tartaric acid	$C_4H_6O_6$	$[M-H]^-$	149.0092	4.0	87.0082 72.9926	0.68	10	
104	Sodium lactate	$C_3H_5NaO_3$	$[M-H]^-$	89.0244	4.2	59.0133 71.0133	0.79	10	
105	DL-Malic acid (Hydroxysuccinic acid)	$C_4H_6O_5$	$[M-H]^-$	133.0142	3.0	115.0031 71.0133	0.72	0.5	
106	Caffeine	$C_8H_{10}N_4O_2$	$[M+H]^+$	195.0877	2.2	107.0497 59.0497	3.93	0.1	
107	L-Theanine	$C_7H_{14}N_2O_3$	$[M-H]^-$	173.0932	4.0	155.0821 84.0449	0.90	10	
108	Glucono-δ-lactone	$C_6H_{12}O_7$	$[M+H]^+$	195.051	2.6	129.0188 75.0082	0.68	10	

	Compound	Elemental composition	Ionization mode	Precursor Ion (theoretical)	Delta mass	Product ions	Retention time	LODs (ppm) Pork jerky
109	Methyl p-hydroxybenzoate	$C_8H_8O_3$	$[M+H]^+$	153.0546	3.2	110.0965 110.0965	4.90	0.1
110	Ethyl p-hydroxybenzoate	$C_9H_{10}O_3$	$[M+H]^+$	167.0702	3.5	95.0496 121.0289	5.51	0.1
111	Propyl p-hydroxybenzoate	$C_{10}H_{12}O_3$	$[M-H]^-$	179.0713	4.1	136.0160 93.0340	6.08	0.1
112	Iso-propyl p-hydroxybenzoate	$C_{10}H_{12}O_3$	$[M-H]^-$	179.0713	3.9	137.0238 93.0340	6.00	0.1
113	Butyl p-hydroxybenzoate	$C_{11}H_{14}O_3$	$[M-H]^-$	193.087	3.6	93.0340 136.016	6.60	0.1
114	Iso-butyl p-hydroxybenzoate	$C_{11}H_{14}O_3$	$[M-H]^-$	193.087	3.6	136.016 92.0262	6.55	0.1
115	Sec-butyl p-hydroxybenzoate	$C_{11}H_{14}O_3$	$[M-H]^-$	193.087	3.8	93.0340 137.0238	6.46	0.1
116	Natamycin	$C_{33}H_{47}NO_{13}$	$[M-H]^-$	664.2974	2.9	137.0238 111.0446	5.36	1
117	Sorbic acid	$C_6H_8O_2$	$[M+H]^+$	113.0597	2.1	58.0658 55.0549	6.5	0.1
118	benzyl 4-hydroxybenzoate	$C_{14}H_{12}O_3$	$[M-H]^-$	227.0714	1.8	136.0155 108.0206	6.49	0.1
119	Phenyl 4-hydroxybenzoate	$C_{13}H_{10}O_3$	$[M-H]^-$	213.0557	2.3	93.0346 62.0009	6.32	0.1
120	n-heptyl 4-hydroxybenzoate	$C_{14}H_{20}O_3$	$[M-H]^-$	235.1340	2.3	108.0217 136.0166	7.99	0.1

[a] Difference between the measured mass/charge (m/z) of procurer and the exact m/z of that ion. Expressed as δ ppm.
[b] Compound is not soluble in water.

the mixed precursors in every wide window. Theoretically, DIA was able to record all chemicals with the required information of precursors and product ions in a sample as long as they were able to be ionized by ESI. The identification of analytes was performed according to SANTA/11813/2017 guidelines [19]. Mass accuracy less than 5 ppm was applied for 2 fragments, and less than 10 ppm was for a precursor. Matched retention time was limited in 0.2 min. Signal was required to have S/N ratio larger than 3 or at least 5 subsequent scans presented (in some case S/N ratio can not be calculated due to S/N ratio = infinity). A total ion chromatogram of sample 22 as an example was shown in Fig. 2a, and the presence of allura red AC was shown in Fig. 2b which data was processed by selecting a product ion *m*/*z* 205.9917 from a group of precursors in the range of *m*/*z* 449–476 (accurate mass of allura red AC was 451.0275). The scan spectrum of RT = 4.05 (from full scan chromatogram, Fig. 2a) was showed in Fig. 2c. There were various compounds co-elute and each one can be a precursor. In DIA, isolation window 27 amu was applied for *m*/*z* 100 to 500 in every 25 amu increment. Therefore, the precursor ion of allura red AC was selected along with other ions presented in the range of *m*/*z* 449 to 476 (Fig. 2d). Multiple precursors were fragmented together, and the mixed product ions were shown in Fig. 2e. Two characteristic ions 79.95695 and 205.99075 indicated the positive identification of allura red AC.

3.2. LODs for the screening method

Colorants were tested in candy and carbonated sparkling water for LODs evaluation. The obtained LODs ranged between 0.02 and 20 part per million (ppm), and mostly 0.5 ppm in Table 1. Antioxidants and flavoring agents observed LODs at 1–10 ppm range. Preservatives were tested in pork jerky

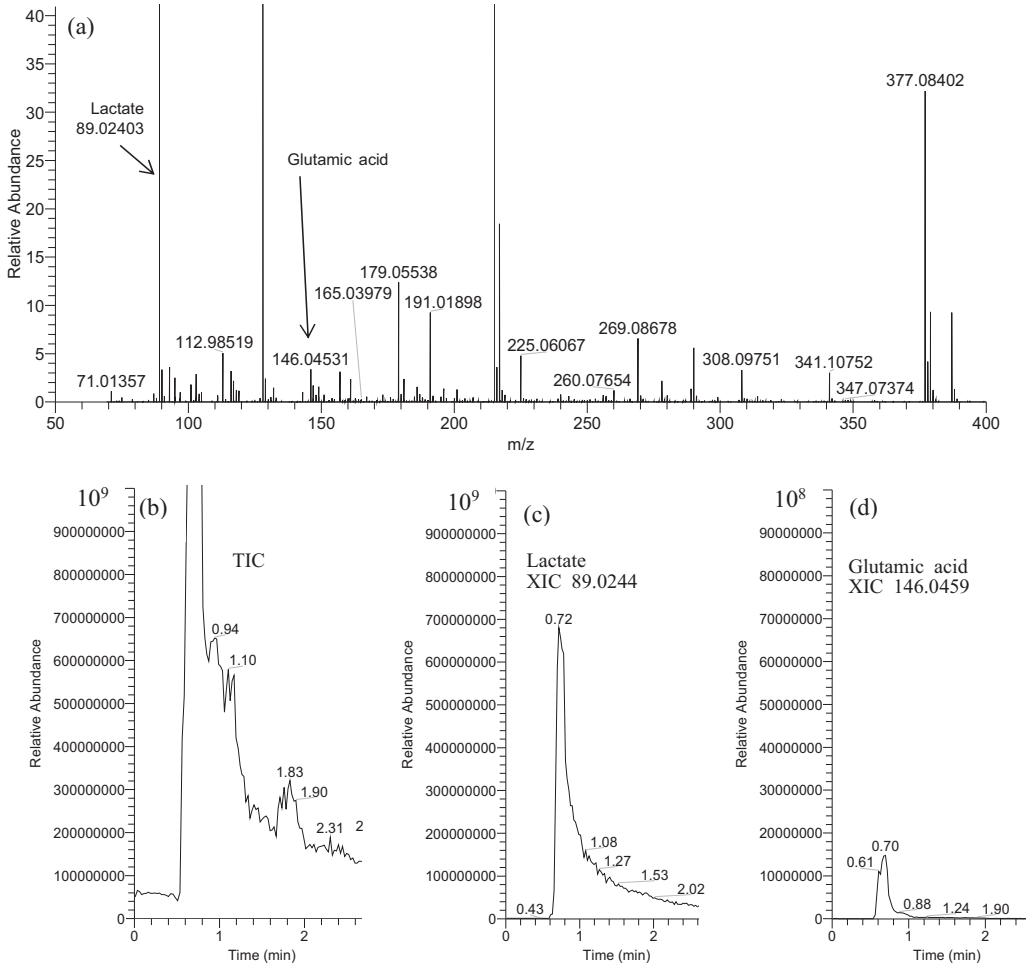

Fig. 1. Chromatograms and MS spectrum of a soy sauce sample. a) ESI negative spectrum of RT = 0.7 min; b) chromatogram of RT around dead time (0.7 min); c) chromatogram of lactate utilizing XIC 89.0244; d) chromatogram of glutamic acid utilizing XIC 146.0459.

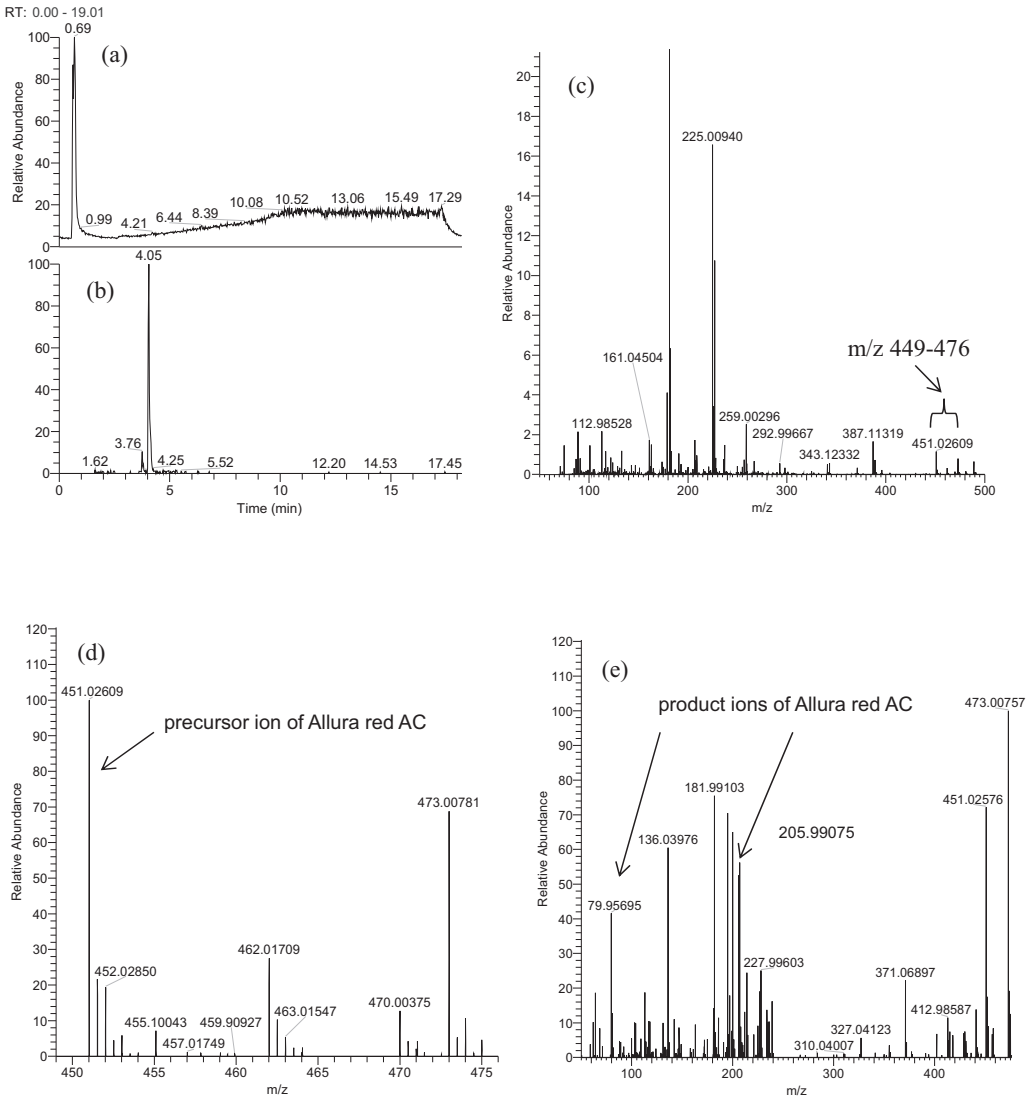

Fig. 2. Chromatograms and MS spectra of a mints candy sample. a) total ion chromatogram; b) chromatogram of product ion m/z 205.99075 from precursors in the range of m/z 449−476; c) ESI full scan MS spectrum of RT = 4.05; d) multiple precursors in a wide window selected in the range of m/z 449−476; e) spectrum of mixed product ions for multiple precursors.

matrix and obtained LODs between 0.1 and 1 ppm. In the comparison to other multi-residue studies for additives by targeted acquisition method [9], this nontargeted data acquisition provided 10 to 100-fold higher LODs. Conventional targeted method optimized ionization and fragmentation parameters for each analyte such as cone voltage for precursors and collision energy for product ions. However, nontargeted method was not able to optimize MS parameter for "non target", instead a basic or typical MS parameter was applied for a broad compound screening or called high throughput screening. Colorants such as rhodamine B observed similar LOD to a targeted MRM method. Cu(II) Chlorin e4 and Cu(II) Chlorin e6 observed LODs as 20 ppm in this study, but a targeted MRM method reported 20 ppb as the LODs and 50 ppb as the LOQs [20]. Therefore, nontargeted method utilizing DIA is able to screen a broad list of analyte, and also suitable for food additive screening in which the detection level is around ppm level. However, for trace level analysis such as pesticide or veterinary drug residues, DIA screening may not applicable in such low detection level (10−100 ppb), and traditional optimized targeted MRM method with sample preparation for limited analytes is still suggested.

3.3. Surveillance results of samples form commercial market

Thirty three foods were tested utilizing the DIA method. The detected additives and labeled

Table 2. Surveillance results of food sample form local market.

No.	Product	Product form	Labeled additives	Detected additives
1	Sparkling water (lemon)	Taiwan	Citric acid, Flavoring (Gum arabic, Sucrose acetoisobutyrate, Medium-chain triglyceride), Sodium citrate, Vitamin C, Carithamine, Vitamin B6	Rebaudioside A, DL-Malic acid, Fumaric acid, Glucono-δ-Lacton, Sodium Lactate
2	Orange soda	Taiwan	Citric acid, Gum arabic, Flavoring, Glycerol, Vitamin C, Vitamin E, Stevia, Medium-chain triglyceride, Glycerol ester of wood rosin, Carotene, sodium carbonate	Rebaudioside A, DL-Malic acid, Fumaric acid, Glucono-δ-Lactone, L-Glutamic acid, Sodium Lactate, Tartaric acid
3	Sparkling water (grape)	Taiwan	Flavoring, Citric acid, Vitamin C, Tartaric acid, Sodium citrate, Allura red AC, Brilliant blue FCF	Tartaric acid, Glucono-δ-Lactone, Sodium Lactate, DL-Malic acid, Fumaric acid, Succinic acid, Allura red AC, Brilliant blue FCF
4	Apple soda	Taiwan	Flavoring, DL-Malic acid, Caramel colors, Stevia	DL-Malic acid, Fumaric acid, Glucono-δ-Lactone, L-Glutamic acid, Sodium Lactate, Tartaric acid, Succinic acid
5	Berry vinegar drink	Taiwan	Citric acid, flavoring, Sodium erythorbate, Sodium metaphosphate, Sodium polyphosphate, Anthocyanin, Sodium pyrophoaphate	Sodium Lactate, DL-Malic acid, Fumaric acid, Tartaric acid
6	Apple soda	Taiwan	Apple flavoring, Caramel colors, Citric acid, Natural apple flavoring	Rebaudioside A, DL-Malic acid, Fumaric acid, Glucono-δ-Lactone, Sodium Lactate
7	Apple vinegar drink	Taiwan	Citric acid, flavoring, Anthocyanin, Sodium erythorbate, Sodium citrate, Sodium metaphosphate, Sodium polyphosphate, Sodium pyrophoaphate	DL-Malic acid, Fumaric acid, Glucono-δ-Lactone, Sodium Lactate, Succinic acid
8	Soft drink	Taiwan	Citric acid, Sodium citrate, Vitamin C, Flavoring, Acesulfame potassium, Aspartame, Beta-carotene	Aspartame, Acesulfame potassium, Fumaric acid, Sodium Lactate
9	Sparkling grape drink	Taiwan	Grape falvoring, Citric acid, Grape skin pigment	DL-Malic acid, Fumaric acid, Glucono-δ-Lactone, L-Glutamic acid, Sodium Lactate, Tartaric acid, Succinic acid
10	Zero soda	Taiwan	Flavoring, Citric acid, Erythritol, Vitamin C, DL-Malic acid, Monosodium L-aspartate, Sucralose, Acesulfame potassium, Vitamin B1, Vitamin B2, Vitamin B6	DL-Malic acid, Acesulfame potassium, Sucralose
11	Mints (grape)	Taiwan	Sorbitol, Aspartame, Acesulfame potassium, Malic acid, Grape flavoring, Magnesium stearate, Silicon dioxide, Erythrosine, Brilliant blue	Tartaric acid, DL-Malic acid, Sodium Lactate, Aspartame, Acesulfame potassium, Erythrosine, Brilliant blue FCF
12	Mints (soda)	Taiwan	Sorbitol, Aspartame, Acesulfame potassium, Malic acid, Soda flavoring, Magnesium stearate, Silicon dioxide, Brilliant blue	DL-Malic acid, Fumaric acid, Sodium Lactate, L-Glutamic acid, Aspartame, Acesulfame potassium, Brilliant blue FCF
13	Mints (peach)	Taiwan	Sorbitol, Aspartame, Acesulfame potassium, Peach flavoring, Malic acid, Magnesium stearate, Silicon dioxide, Erythrosine	DL-Malic acid, Sodium Lactate, Succinic acid, Aspartame, Acesulfame potassium, Erythrosine
14	Mints (peach, no sugar)	Taiwan	Sorbitol, Acesulfame potassium, Sucralose, Magnesium stearate, Flavoring, DL-Malic acid, Citric acid, Allura red AC	DL-Malic acid, Acesulfame potassium, Sucralose, Allura red AC
15	Mints	Taiwan	Sorbitol, Acesulfame potassium, Sucralose, Magnesium stearate, Flavoring, Lactic acid, Calcium lactate, Indigo carmine	Sodium Lactate, Acesulfame potassium、Sucralose, Indigo carmine
16	Mints	Taiwan	Sorbitol, Acesulfame potassium, Sucralose, Flavoring, Magnesium stearate, Brilliant blue FCF	Aspartame, Acesulfame potassium, Sucralose, Brilliant blue FCF

(continued on next page)

Table 2. (continued)

No.	Product	Product form	Labeled additives	Detected additives
17	Mints (lemon)	Thailand	Isomalt, Citric acid, Lactic acid, Flavoring, Sucralose, Tartrazine, Brilliant blue FCF	Sodium Lactate, Sucralose, Allura red AC, Tartrazine, Brilliant blue FCF
18	Mints (berries)	Thailand	Isomalt, Flavoring, Citric acid, DL-Malic acid, Sucralose, Acefulfame potassium, Allura red AC, Tartrazine	DL-Malic acid, Sucralose, Acesulfame potassium, Allura red AC
19	Mints (honey)	Thailand	Isomalt, Citric acid, Flavoring, Sucralose, Tartrazine	Sucralose, Tartrazine
20	Mints (honey and lemon)	India	Isomalt, Aspartame, Acefulfame potassium, Flavoring, Citric acid, Beta-carotene	Aspartame, Acesulfame potassium
21	Mints	India	Isomalt, Aspartame, Acefulfame potassium, Flavoring, Curcumin, Brilliant blue FCF	Aspartame, Acesulfame potassium, Curcumin, Brilliant blue FCF
22	Mints (fruits)	Australia	DL-Malic acid, Starch acetate, Flavoring, Sodium citrate, Xanthan gum, Titanium dioxide, Starch sodium succinate, Palm wax, Sunset yellow FCF, Allura red AC, Brilliant blue FCF	DL-Malic acid, Fumaric acid, Tartrazine, Sunset yellow FCF, Allura red AC, Brilliant blue FCF
23	Lollipop (apple)	Vietnam	Lactic acid, DL-Malic acid, Citric acid, Flavoring, Curcumin	Sodium Lactate, DL-Malic acid, Curcumin
24	Mints (strawberry)	Vietnam	Lactic acid, DL-Malic acid, Citric acid, Flavoring, Betarubin	Sodium Lactate, DL-Malic acid
25	Chewing gum (mint)	Taiwan	Sorbitol, Maltitol, D-Xylitol, D-Mannitol, Aspartame, Acesulfame potassium, Sucralose, Glycerol, Flavoring, Sodium carboxymethyl cellulose, Palm wax, Sodium alginate, BHT, Brilliant blue FCF,	Aspartame, Acesulfame potassium, Sucralose, Brilliant blue FCF
26	Grape C	Taiwan	Grape powder (oxidized starch, citric acid, flavoring, grape skin pigment), Vitamin C, Hydropropyl methylcellulose, Citric acid, Cellulose, Grape flavoring, Magnesium stearate, Ferric pyrophosphate, Folic acid, Vitamin B12 (sodium citrate, citric acid, vitamin B12)	Tartaric acid, L-Glutamic acid, Sodium Lactate, DL-Malic acid
27	Mints	Australia	Starch acetate, Flavoring, Xanthan gum, Palm way, Starch sodium succinate, Titanium dioxide, Brilliant blue FCF	Fumaric acid, Succinic Acid
28	Mints	Taiwan	Sorbitol, Maltitol, Maltitol syrup, D-Mannitol, Aspartame, Acesulfame potassium, Flavoring, Sodium carboxymethyl cellulose, Palm wax, Sodium alginate, BHT, BHA, Brilliant blue FCF,	Aspartame, Acesulfame potassium, Brilliant blue FCF
29	Soy sauce	Taiwan	Glycyrrhizin	Tartaric Acid, Glucono-δ-Lactone, DL-Malic Acid, Fumaric Acid, L-Glutamic Acid, Sodium Lactate, Succinic Acid, Glycyrrhizic acid
30	Soy sauce	Taiwan	Acetylated distarch adipate, Corn syrup, Glycin, Sodium succinate	L-Glutamic Acid, Fumaric Acid, DL-Malic Acid, Sodium Lactate, Succinic Acid
31	Soy sauce	Taiwan	Maltitol syrup	L-Glutamic Acid, Fumaric Acid, DL-Malic Acid, Sodium Lactate, Succinic Acid
32	Soy sauce	Taiwan	None	Glucono-δ-Lactone, L-Glutamic Acid, Sodium Lactate, DL-Malic Acid, Succinic Acid
33	Soft dring	Taiwan	Flavoring, Citric acid, Vitamin C, DL-Malic acid, L-Aspartic acid, Vitamin B2, Vitamin B6, Vitamin B1	DL-Malic acid

additives were listed in Table 2. Sample 1, 6 were sparkling water and soda found under declared rebaudioside A as sweetener, which might come from the flavoring agent in their ingredients. For other drinks and candies, fumaric acid, glucono-δ-lacton, sodium lactate, DL-malic acid, L-glutamic acid, tartaric acid and succinic acid were the most often flavorings under declared, which might come from natural extracts, flavoring agent mixture or artificial additives in their ingredients. Some colorants such as tartrazine (in sample 18, red candy in appearance) and Brilliant blue FCF (in sample 27, white mints in appearance) were labeled in product package, but not detected. Artificial sweeteners such acefulfame potassium, sucralose and aspartame which belong to regular sweetener testing list were all clear labeled in the package and detected. This surveillance results showed that all tested samples were all complied with regulation, but there is still room for manufacture to improve clearly declaration of product information to consumer.

4. Conclusions

This study established a nontargeted DIA analytical method and a detection database list of 120 food additives for rapid screening of food products. The database included 79 colorants, 13 sweeteners, 12 preservatives and 7 antioxidants, enclosed the chromatographic retention time, accurate mass of molecular ion, accurate mass of two product ions, LODs in matrix (candy, beverage or jerky). HRMS enabled secondary separation. Some high polar flavoring agent, such as lactate, L-theanine and glutamic acid were able to be separated and determined by HRMS in coelutant. However, due to the normal sample preparation and the neglect of matrix effects, DIA offered higher LODs for most compounds compared to traditional targeted MRM method. Mass spectrometer worked in full scan and wide window fragmentations that recorded all components in theoretical enabled retrospective analysis for unknowns and increasable target lists. A survey of commercial products of beverages, candies and sauces showed this method can efficiently screen food for illegal additives or false labeling. The results revealed sweeteners and flavoring agents were the most under declared additives. For example, no-calorie sweetener rebaudioside A, about 150–400 times sweeter than sugar, was found in some soda. The surveillance results showed the current labeling is good, but still needed for improvement. The detection database will continually be expand to increase the detection range of food additives for this rapid screening method.

Conflict of interest

There is no potential conflict of interest to declare.

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