Analysis hazards in food : from one-by-one determination to class-by-class screening and finally to chemometrics-based discrimination

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Chinese Academy of Inspection and Quarantine(CAIQ)

Content

1. Background

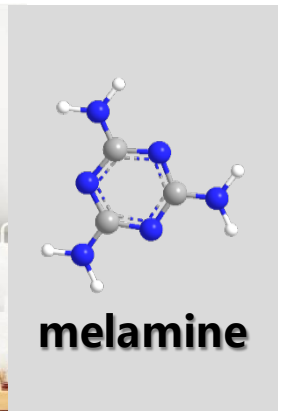
Food safety incidents occur frequently

Plasticizer everywhere

Melamine in milk powder

Recycled oil incident

……



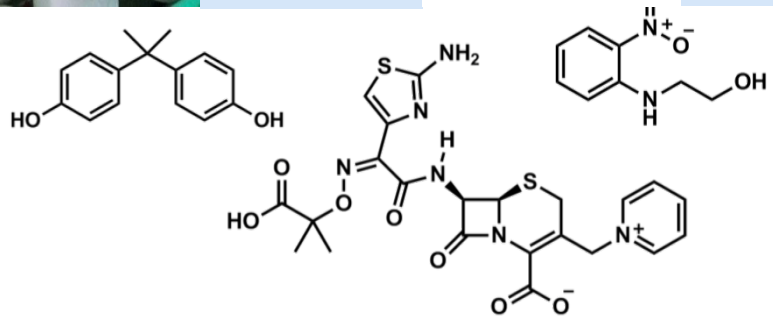
clenbuterol

cyclamate

Sudan Red

malachite green

More and more novel chemical hazardous substances are emerging



1. Background

hazardous substance

- **Known :** pesticide, veterinary drug, illegal substance ;
- **Novel :** new structural analogues to known hazardous substances with similar toxicity ;
- **Unknown :** unknown substance produced during food processing or food storage.

The challenge to detect

- **Known:** how to increase the throughput of the method ?
- **Novel:** how to the explore the new structural analogues?
- **Unknown:** how to explore it and determine the food’ s safety or not ?

The solution

- **Known:** One-by-One Determination/screening Method ;
- **Novel:** Class-by-Class Screening Method ;
- **Unknown:** Discrimination method based on chemometrics .

Content

2. The One-by-One Determination Method Based on Database Searching

Classic multi-residues determination method:

400~500 compounds are determinated using MS in MRM mode

Disadvantages:

Time-cousuming

Unstable for standard solvent

Wasteful for the organic reagent

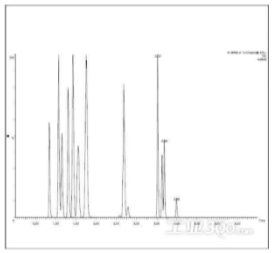
2. The One-by-One Determination Method Based on Database Searching

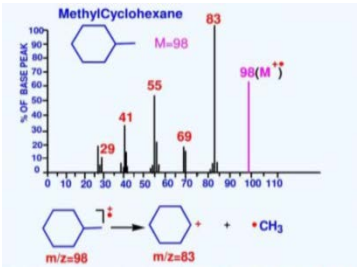
The workflow of One-by-One Determination Method Based on Database Searching

Pesticides 1000

Veterinary drug 500

Illegal substances 200





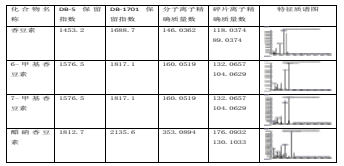
The Targets

Standards Preparation

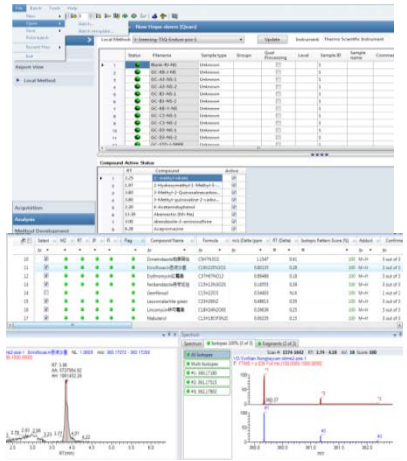
Data Acquisition

The LC-HRMS information

*about 2000



MS Database

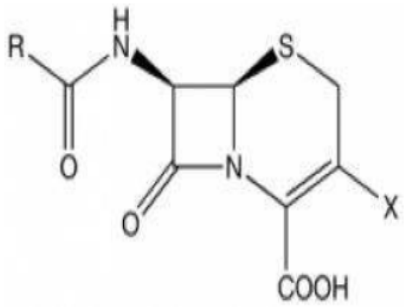


High Throughput Screening Method

Screening Result

2. The One-by-One Determination Method Based on Database Searching

- ✓ **Advantage : high throughput**
- ✓ **Disadvantage : hazards which are not in the database can not be detected, even the novel structural analogues of known hazardous substances**



For example : cephalosporin

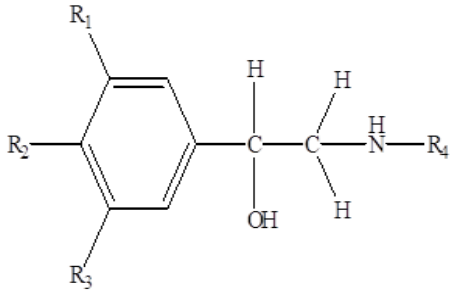
More and more novel derivatives have appeared

How to explore the structural analogues ?

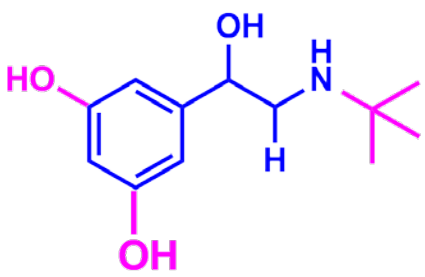
Content

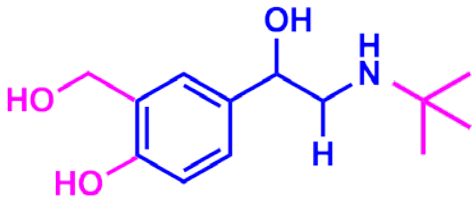
3.The Class-by-Class Screening Method Based on Fragmentation Markers

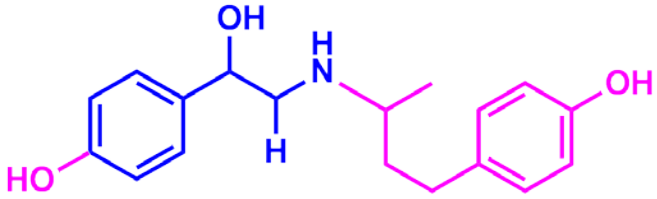
Phenylethanolamines

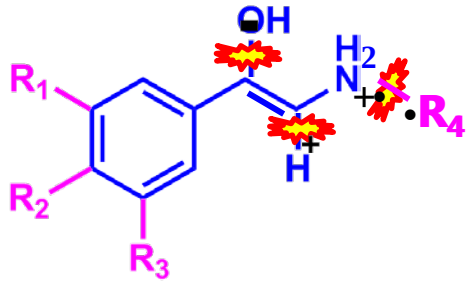


ß-receptor agonists have a phenylethanolamine structure, which benzene ring has an alkaline ß-hydroxyl side chains, so that they are easy to dehydration.



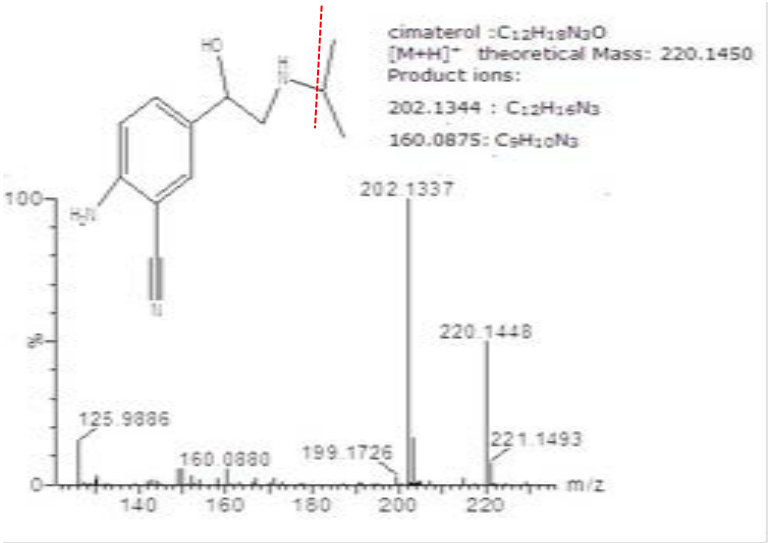




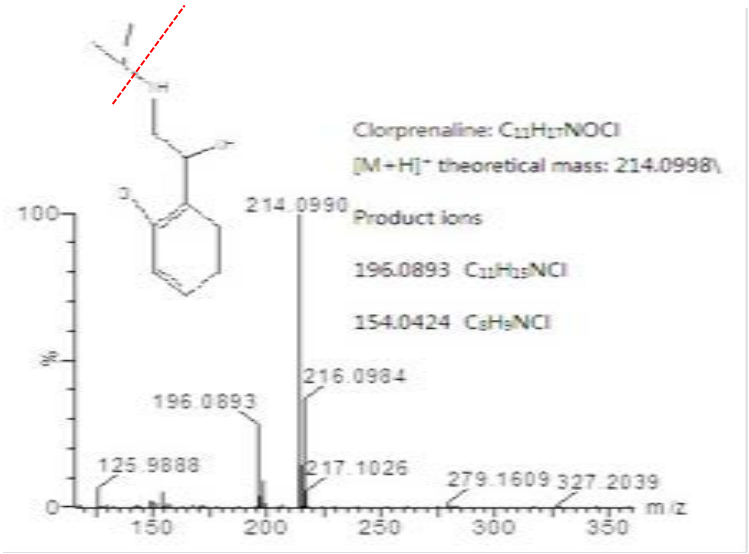


H_2O

fragmentation mechanism of ß-receptor agonists



Cimaterol(西马特罗)

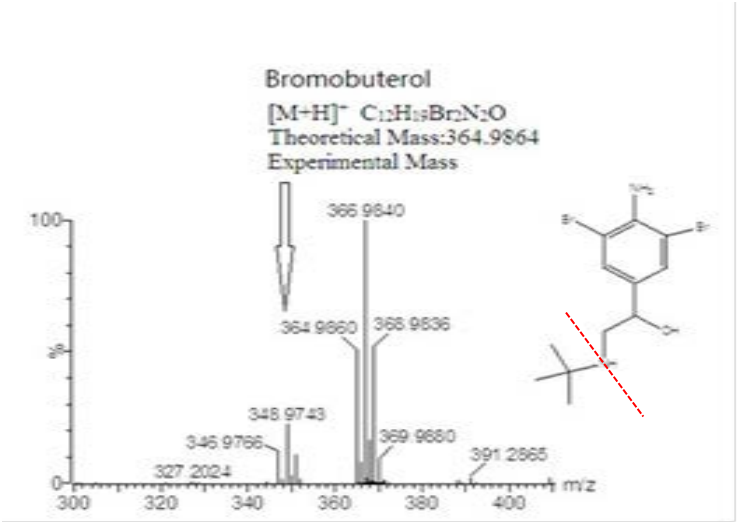


Clorprenaline(氯丙那林)

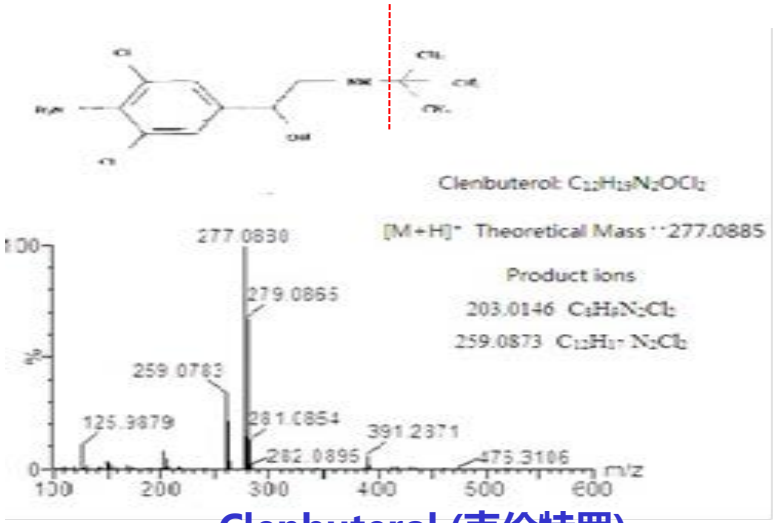
Dehydration - propyl group loss

3.The Class-by-Class Screening Method Based on Fragmentation Markers

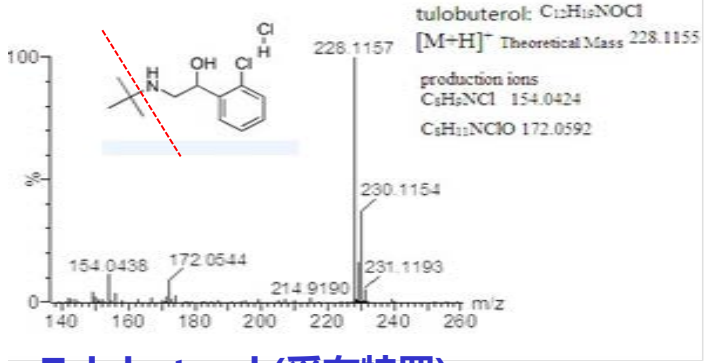
fragmentation mechanism of ß-receptor agonists



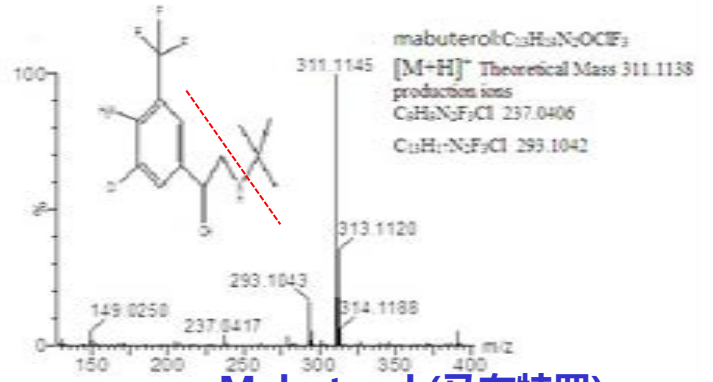
Bromobuterol (溴布特罗)



Clenbuterol (克伦特罗)



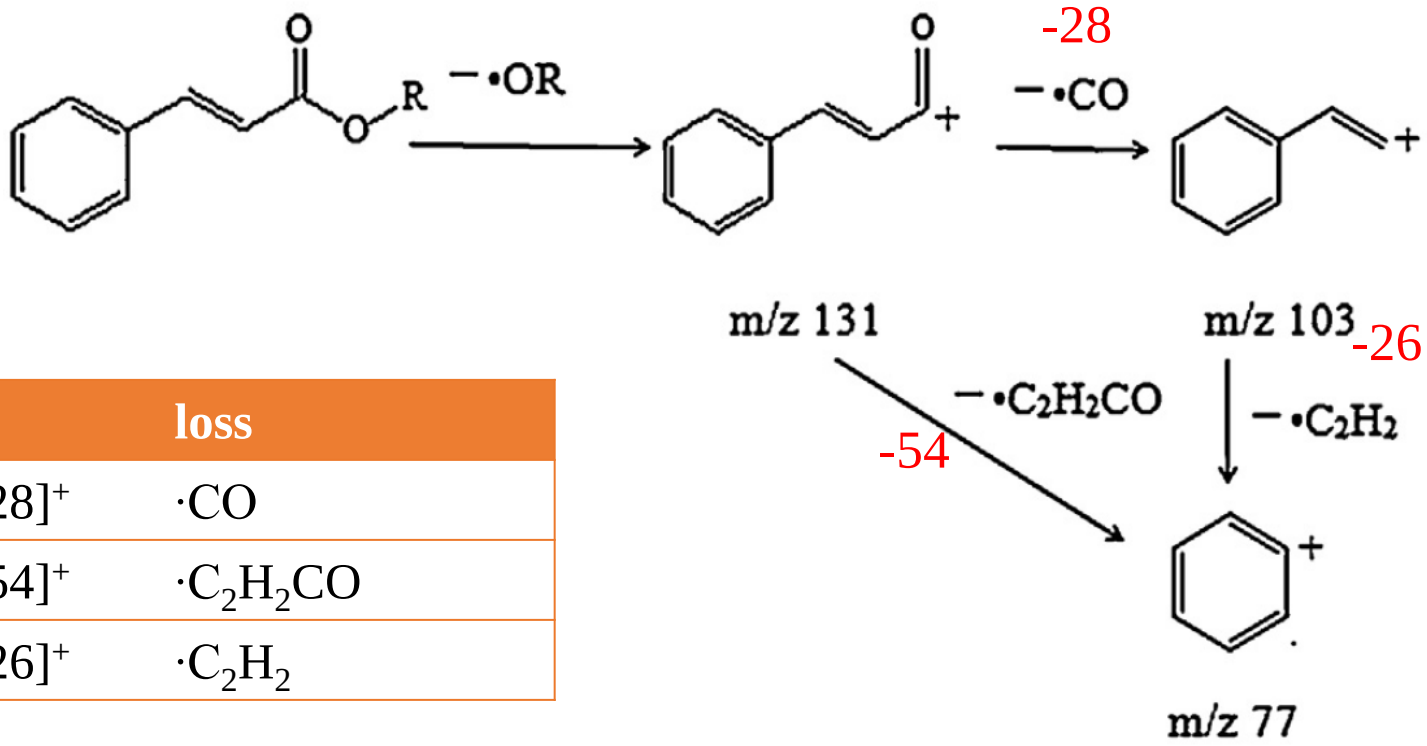
Tulobuterol (妥布特罗)



Mabuterol (马布特罗)

Dehydration – butyl group loss

Cinnamic esters

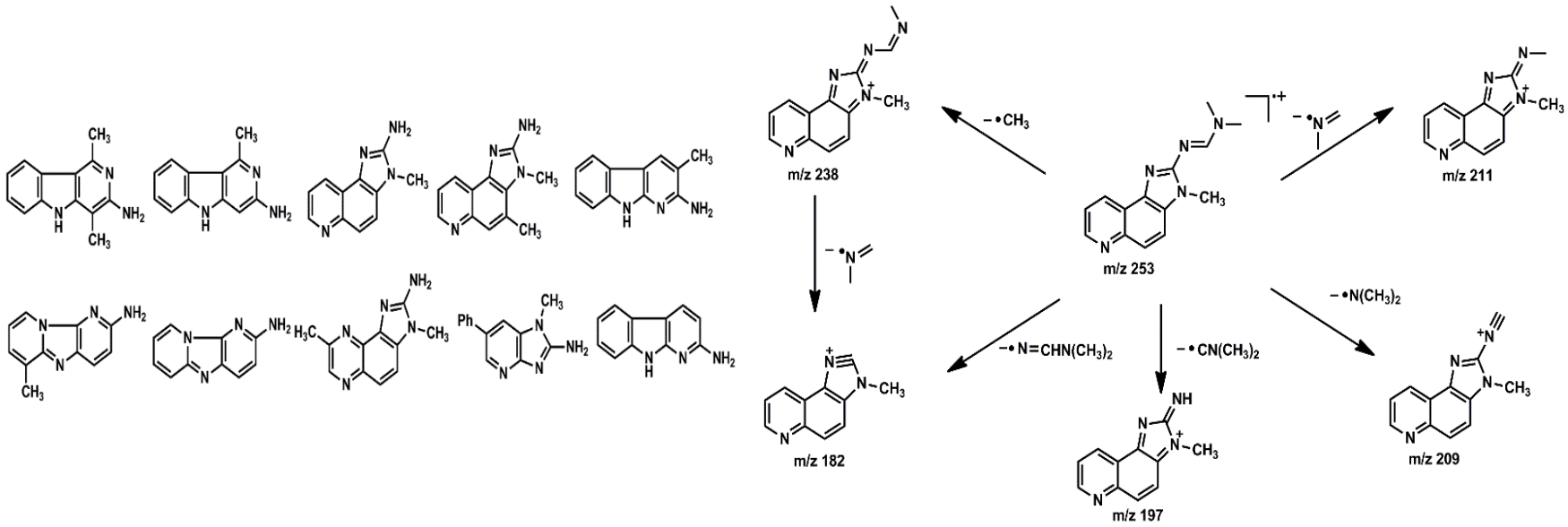


m/z	loss
$[M-28]^+$	·CO
$[M-54]^+$	$\cdot C_2H_2CO$
$[M-26]^+$	$\cdot C_2H_2$

If the MSMS produces stable peak m/z131, m/z103, m/z77, we can infer that it may be cinnamate esters

3.The Class-by-Class Screening Method Based on Fragmentation Markers

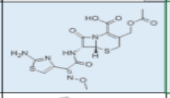
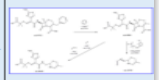
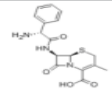
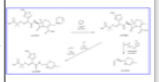
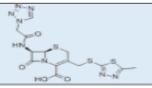
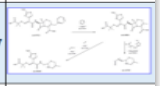
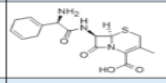
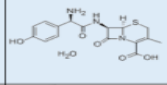
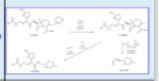
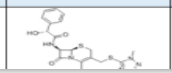
heterocyclic amines



Compounds with the same skeleton have the similar fragmentation pathway. It could be used for the exploration of novel derivative in food.

3.The Class-by-Class Screening Method Based on Fragmentation Markers

MS fragmentation pathway database was constructed.

NO.	Compound Name	Formula	Structure	Ionization mode	Mass [M/Z]	MS^2			Fragmentation pathway
列1	列2	列3	列4	列5	列6	列7	列8	列9	列10
111	Cefotaxime	C16H17N5O7S2		[M+H]+	456.06422	167.02698	324.05771	396.04221	
112	Cephalexin	C16H17N3O4S		[M+H]+	348.10125	158.02681	174.05466	106.06541	
113	Cefazolin	C14H14N8O4S3		[M+H]+	455.03729	156.01086	323.05505	295.06027	
114	Cefradine	C16H19N3O4S		[M+H]+	350.11690	176.07030	158.02684	108.08105	
115	Cefadroxil	C16H17N3O5S		[M+H]+	364.09617	192.06534	174.05476	347.06909	
116	Cefamandole	C18H18N6O5S2		[M+H]+	463.08529	158.02701	347.06937	185.03789	

- capacity: 1092 compounds
- includes: fragment ions, neutral loss, fragmentation pathway
- accuracy of mass number: 0.0001Da

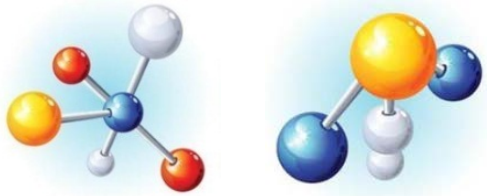
3.The Class-by-Class Screening Method Based on Fragmentation Markers

Similar structural substances have the same MS fragmentation mechanism, MS fragmentation markers of a class compounds can be used to establish a "**class-by-class** " screening method.

data base

NO.	Compound Name	Formula	Structure	Ionization mode	Mass (M/Z)	MS^2			Fragmentation pathway	Neutral Loss
111	Cefotaxime	C16H17N5O7S2		[M+H]+	456.06422	167.02698	324.05771	396.04221		
112	Cephalexin	C16H17N3O4S		[M+H]+	348.10125	158.02681	174.05466	106.06541		
113	Cefazolin	C14H14N8O4S3		[M+H]+	455.03729	156.01086	323.05505	295.06027		
114	Cefradine	C16H19N3O4S		[M+H]+	350.11690	176.07030	158.02694	108.08105		
115	Cefadroxil	C16H17N3O5S		[M+H]+	364.09617	192.06554	174.05476	347.06909		
116	Cefamandole	C18H18N6O5S2		[M+H]+	463.08529	158.02701	347.06937	185.03789		
117	Cefaclor	C15H14ClN3O4S		[M+H]+	368.04683	114.01112	332.06915	191.08112		
118	Cephalonium	C20H18N4O5S2		[M+H]+	459.07914	152.01617	158.02675	123.05527		
119	Ceftazidime	C22H22N6O7S2		[M+H]+	547.10641	167.02708	396.07910	468.06351		

Determination principle of markers



Marker1 Marker2

- All the compounds in the same class have the marker fragmentation ions
- Stable abundance >50%

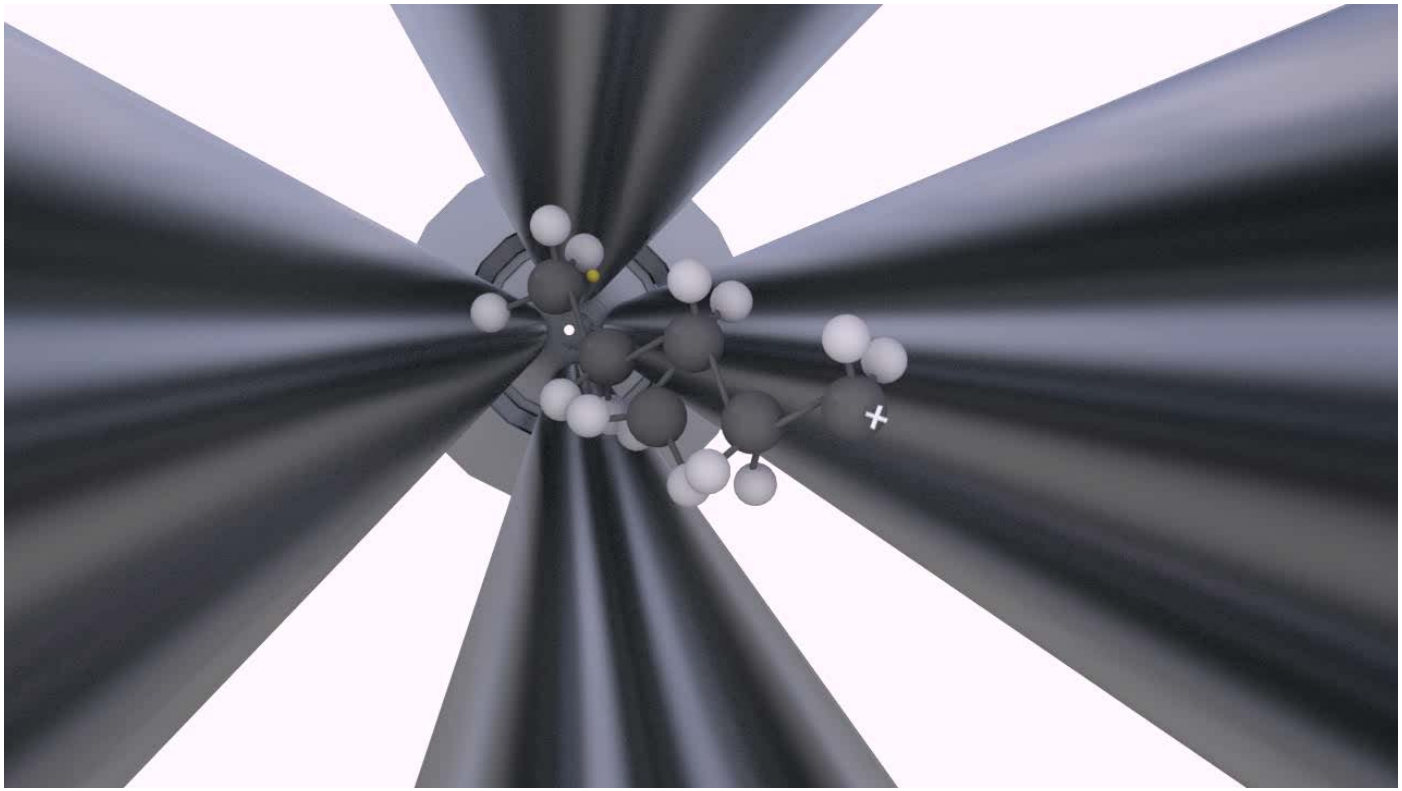
fragmentation markers

m/z	标志中性丢失（碎片）	
$[M-15]^+$	$\cdot CH_3$	β-lactams
$[M-42]^+$	$\cdot N(CH_2)_2$	
$[M-44]^+$	$\cdot N(CH_3)_2$	
$[M-56]^+$	$\cdot CN(CH_3)_2$	HCAs
$[M-71]^+$	$\cdot N{=}CHN(CH_3)_2$	
m/z 28	CO	phenyleth-anolamines
m/z 44	CO2	
m/z79	C5H5N+	
m/z 42	CH2CH2CH2	bisphenols
m/z 94	C6H6O	
m/z 54	C2H2CO	
m/z 26	•C2H2	sulfon-amides
m/z 228	C8H10N3O3S	

52 MS fragmentation markers were determied.

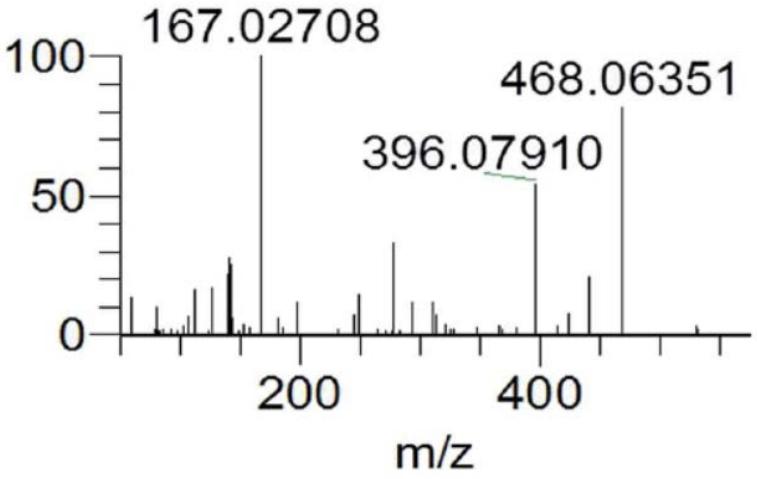
3.The Class-by-Class Screening Method Based on Fragmentation Markers

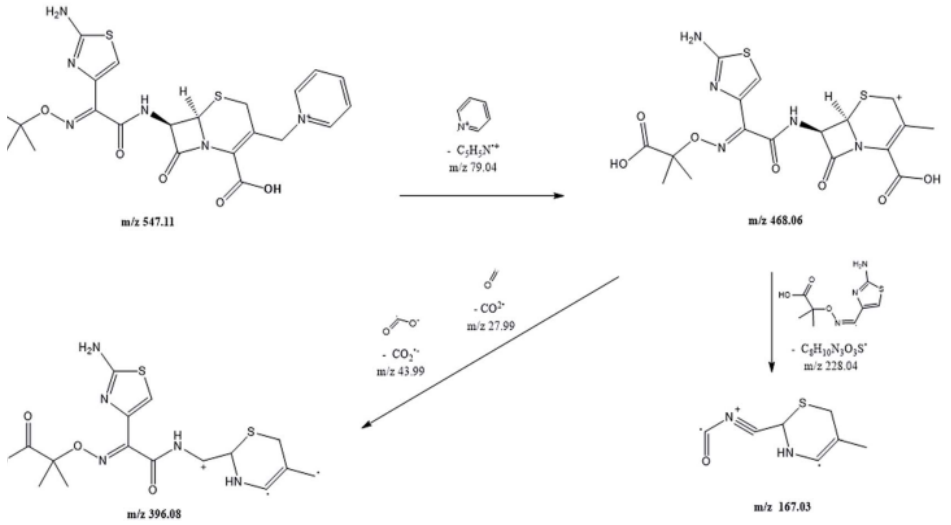
A class of compounds can be found by scanning fragmentation marker



3.The Class-by-Class Screening Method Based on Fragmentation Markers

Example: the substance in pork was screened class by class Based on fragmentation marker





new banned β-lactam antibiotics in pork: ceftazidime

Analytical Methods, 2017, 9(2): 6534-6548

3.The Class-by-Class Screening Method Based on Fragmentation Markers

- ✓ **Advantage : structural analogues can be screened class by class, which supply the solution to the abuse of novel structural analogues with similar toxicity to know hazardous substances.**

- ✓ **Disadvantage : the unknown substances produced during food process or storage can not be explored by this method.**

The abuse of novel structural analogues

The risk during food process and storage

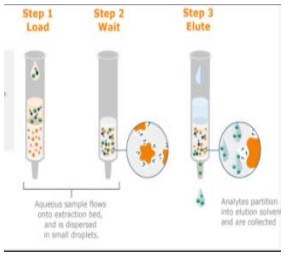
Content

4. The Discrimination Method Based on Chemometrics

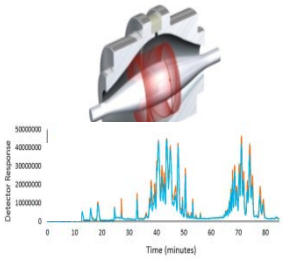
The workflow of discrimination method in milk overheating


raw material

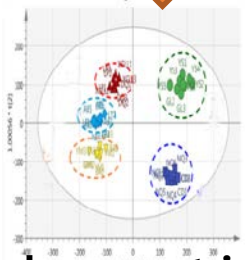
Heating in different conditions



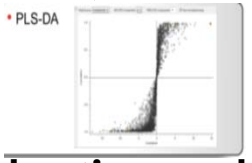
sample pretreatment



data acquisition

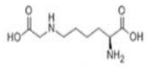


chemometric analysis



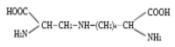
overheating markers determination

A



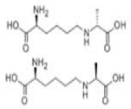
CML

B

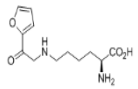


LAL

C

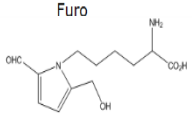


D



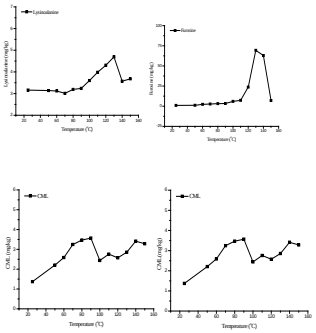
Furo

E



Pyrra

structural analysis



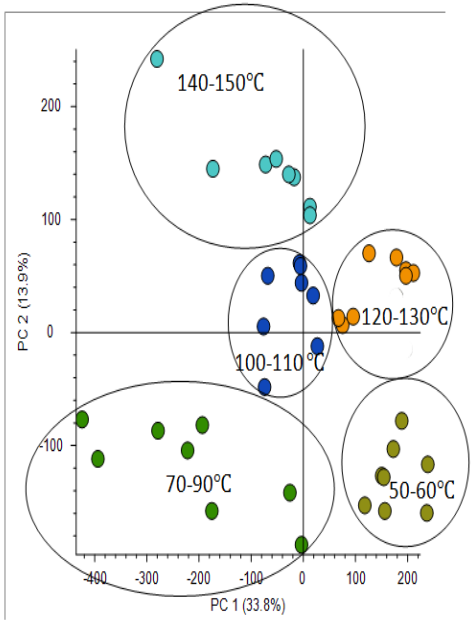
validation the markers

4. The Discrimination Method Based on Chemometrics

Determination of overheating markers

The groups of sample

Group	Heat Temp.	Number
1	50°C	20
2	60°C	20
3	70°C	20
4	80°C	20
5	90°C	20
6	100°C	20
7	110°C	20
8	120°C	20
9	130°C	20
10	140°C	20
11	150°C	20

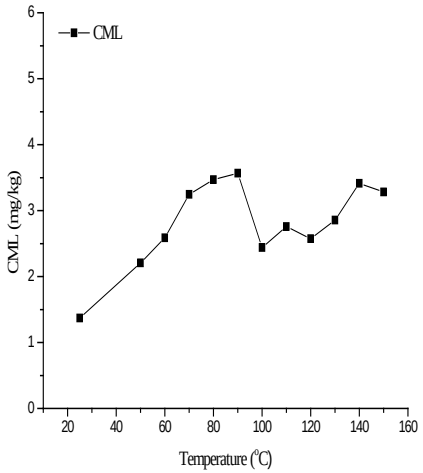


Five markers were determined

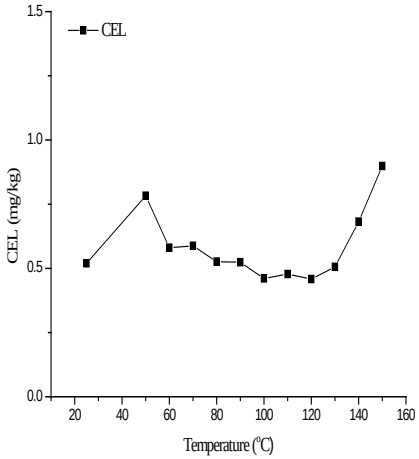
No	name	Molecular Formula	Molecular Weight
1	Lysinoalanine	$C_9H_{19}O_4N_3$	233
2	Carboxymethyl-L-lysine	$C_8H_{16}O_4N_2$	204
3	Carboxyethyl-L-lysine	$C_9H_{18}O_4N_2$	218
4	Furosine	$C_{12}H_{18}O_4N_2$	254
5	Pyrraline	$C_{12}H_{18}O_4N_2$	254

4. The Discrimination Method Based on Chemometrics

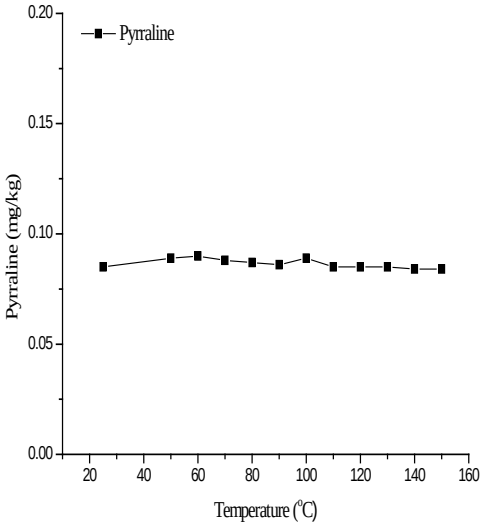
The correlationship between these markers amount with temperature



Carboxymethyl-L-lysine



Carboxyethyl-L-lysine



pyrraline

Content

5. Summary

Summary

- For **the known compounds listed in the standard or regulations**, a one-by-one determination method based on database searching was developed.
- For novel **structural analogues similar to known compounds**, a class-by-class screening method based on fragmentation markers was developed.
- For **unknown compounds procued during food process or storage**, a discrimination method based on chemometrics was developed.

Acknowledgements

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