农药残留检测技术
Pesticide Residue Test Technology

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农药残留限量标准
Pesticide Residue Limit Standards

- 中国食品中农药残留最大限量标准GB 2763-2014中，共涉及农药种类有387种，限量值有3650个。**Under the Pesticide Residue Limit Standard GB2763-2014 for Chinese food, there are 387 pesticides and 3650 limit values thereof are included.**
- 这个标准还在不断完善中，农药种类和限量值还在增加。**This Standard is still under improvement with more pesticide categories and their limit values being added.**

农药残留检测主要过程
Major Steps of Pesticide Residue Test

- 1、检测前工作准备；**Preparation Prior to Test;**
- 2、试样制备；**Make Samples;**
- 3、提取；**Extract;**
- 4、净化；**Purify;**
- 5、测定；**Test;**
- 6、检测过程的质量控制。**Quality Control over Test Progress.**

检测前工作准备
Preparation Prior to Test

- 环境的检查 **Environment Check**
  - 应对实验室、样品储藏室、前处理室和仪器室等环境进行控制，保证环境的温湿度符合检测的要求。**Environment of the lab, the sample storage, the preliminary processing room and the instrument room should be controlled to guarantee the temperature and humidity of the environment satisfying test requirements.**
  - 样品前处理室要进行控温，防止试剂的过度挥发，影响结果的准确性。湿度较大的地区，仪器室要除湿。**Temperature of the preliminary processing room should be controlled to prevent reagents from over evaporation which may impact the result accuracy. Instruct rooms in heavy humidity regions should be dehumanized.**

检测前工作准备
Preparation Prior to Test

- 试剂和药品的检查 **Reagent and Chemical Check**
  - 每进一批试剂和药品，在使用前按相应检验所要求的标准进行检测，对其进行杂质的检查，排除试剂对检测结果的干扰，如有必要应进行处理后再使用。**Reagents and chemicals under each order should be checked of it’s impurities as per relevant requirements before they are applied, to avoid any potential interference to the test result from such reagents. Treat the reagents and chemicals before use when necessary.**
  - 一般试剂浓缩**50~100**倍后测定，检查是否对被测组分有干扰。**Under normal circumstances, reagents are concentrated 50 to 100 times before applied to test, to determine if interference to the objective element happens.**

试样制备（样品取样部位）
Make Samples (Parts from Samples)

- 谷物 Grains
- 稻谷、小麦、大麦、玉米、高粱、薏仁、绿豆、豌豆等，取整粒。**Rice, wheat, barley, maize, sorghum, coix seed, green been, pea etc, take the whole item.**
- 油料 Oil
- 油菜籽、芝麻、棉籽、大豆、花生、葵花籽等，取整粒。 **Rapeseed, sesame, cottonseed, soybean, peanut, sunflower seed etc, take the whole item.**

试样制备（样品取样部位）
Make Samples (Parts from Samples)

- 芸薹薯类蔬菜 **Brassica Vegetables**
- 结球芸薹属：结球甘蓝、球茎甘蓝、抱子甘蓝等，取整棵。**Cabbage brassica: head cabbage, brassica oleracea, brussels sprout etc., take the whole item.**
- 头状花序芸薹属：花椰菜、青花菜，去除叶，取整棵。**Capitulum brassica: cauliflower, broccoli, remove the leaves then take the whole item.**
- 茎类芸薹属：芥蓝、菜薹、茎芥菜等，去除根，取整棵。**Root brassica: cabbage mustard, brassica campestris, stem mustard etc, remove the leaves then take the whole item.**
- 瓜类 **Melons**
- 代表作物：黄瓜、西葫芦、冬瓜、苦瓜、南瓜、丝瓜等。**Representatives: cucumber, summer squash, wax gourd, bitter gourd, pumpkin, sponge cucumber etc.**
- 去掉柄后的全瓜。**The whole item except pedicels.**

试样制备（样品取样部位）
Make Samples (Parts from Samples)

- 豆菜类蔬菜 **Bean Vegetables:**
  - 荚可食类：豇豆、菜豆、豌豆、四棱豆、刀豆等，取全荚。**Pod edible: cowpeas, beans, peas, asparagus peas, sword beans etc., take the whole item with pods.**
  - 荚不可食类：菜用大豆、蚕豆、豌豆等，去荚后的全豆。**Pod unedible: soybeans, horsebeans, peas etc., take the whole item without pods.**

  茄果类 **Solanaceous Fruits**

  代表种类有：茄子、西红柿、茄子、黄秋葵等 **Representatives: eggplant, tomato, eggplant, okra etc.**
  - 除去果柄后的整个果实。**The whole fruit without carpopodiums.**

试样制备（样品取样部位）
Make Samples (Parts from Samples)

- 柑桔类水果 **Citrus Fruits**
  - 代表种类有：橙、柚、橘、柑、柠檬等。 **Representatives: orange, grapefruit, tangerine, mandarin, lemon etc.**
  - 取整个果实。**Take the whole fruit.**

- 核果类水果 **Drupe Fruits**
  - 桃、油桃、杏、枣、樱桃、李子。**Peach, nectarine, apricot, jujube, cherry, plum.**
  - 除去果柄及核的整个果实，但残留计算包括果核。**The whole item without capopodiums and drupes, but the residual calculation will have the drupes included.**

试样制备
Make Samples

- 将实验室样品混合后用四分法缩分，按以下方法预处理样品：**Mix all lab samples and split in quarters, preliminarily treat samples as per below:**
- ——对于个体小的物品（如苹果、坚果、虾等），去掉蒂、皮、核、头、尾、壳等，取出可食部分；**As for small size items (such as apple, nuts, prawn etc), remove the pedicle, skin, drupe, head, tail, shell etc and take only the edible part.**
- ——对于个体大的基本均匀物品（如西瓜、冬瓜等）可在对称轴或对称面上分割或切成小块；**As for large size items with even shapes (such as water melon, wax gourd etc), they can be cut into halves or chopped into blocks along the symmetry axis or symmetry face.**

试样制备
Make Samples

- ——对于不均匀的个体样（如鱼、菜等），可在不同部位切取小片或截成小段。**As for samples with uneven shapes (such as fish, vegetables etc), small sections can be taken from different parts, or the whole sample can be chopped into sections.**
- ——对于谷类和豆类等粒状、粉状或类似的样品，应使用圆锥四分法（堆成圆锥体—压成扁平形—划两条交叉直线分成四等分—取对角部分）进行缩分。**As for grain and beans in granular, powder or similar shapes, samples can be split in Cone Quartering (dump samples into a cone-press and flat-quarter the dump by two crossing lines-take the diagonal parts).**

农残检测方法
Pesticide Residue Test Method

目前蔬菜和水果农药残留检测方法用的最为广泛的是：**The current most popular methods adopted in testing pesticide residue from vegetables and fruits are:**

1、 **NY/T 761-2008，使用气相色谱和液相色谱，一 次能检测105种农药；NY/T 761-2008, using gas chromatography and liquid chromatography, which can have 105 pesticides inspected in one go;**

2、**GB/T 19648-2008，使用气相色谱串联质谱，一 次能检测500种农药；GB/T 19648-2008, using gas chromatography tandem mass spectrometry, which can have 500 pesticides inspected in one go;**

农残检测方法
Pesticide Residue Test Method

3、GB/T 20769-2006，使用液气相色谱三重四级串联质谱，一 次能检测450种农药；GB/T 20769-2006, using liquid-gas chromatography triple quadrupole tandem mass spectrometry, which can have 450 pesticides inspected in on go;

以上三种方法，在提取、浓缩步骤上基本一样，只是在净化步骤上有所不同，NY/T 761的方法测定有机磷时 All the three methods above share the same extraction and concentration steps, but have different filtration processes. When NY/T 761 is adopted in testing organic phosphorus,

4、目前国际上使用最多的QuEChERS 方法。The most worldwide popular method nowadays is QuEChERS.

称量
Weigh

- 称取25.0g试样于150mL烧杯中。**Weigh to take 25.0g sample and place into a 150ml beaker.**

- 样品称量时应充分搅匀，避免样品粘到容器壁上。**When weigh, sample should be stirred evenly to prevent it from sticking onto the container wall.**

提取
Extract

- 加入50mL乙腈，用高速组织匀浆机提取2min。**Add 50ml acetonitrile, and extract with a high speed tissue homogenate machine for 2 min.**

组织捣碎法：又称匀浆提取法，优点是简便、快速、效果好。一般操作是样本加提取剂后高速捣碎，使溶剂与微细试样反复紧密接触、萃取，从而提取出检测成分的方法。

Tissue Stirring also know as Homogenizing takes advantages of convenience, quick progressing and excellent efficiency. Normally it vigorously smashes the sample with extractant added, to let the solvent and fine samples frequently contacted and leached, then extract elements for test.

提取过程中注意事项
Notes When Extract

- 样本在提取过程中，应保证样品提取时间、提取过程与质控样品尽量相同，使质控更清楚的反应前处理操作的准确性。
- **During the extraction process, the extract time, extract process and quality control of each sample should be maintained as similar as possible, to guarantee quality control clearly reflects the accuracy of the preliminary treatment operation.**

过滤
Filter

- 将过滤纸连续两次对折，叠成90°圆心角形状；把叠好的滤纸按一侧三层，另一侧一层打开，成漏斗状；把漏斗状滤纸装入漏斗内，滤纸边要低于漏斗边，加少许水润湿，沿玻璃棒向漏斗口内倒入均质好的试样。**Fold the filter paper in half twice consecutively into 90° radius angle shape. Open the folded paper into a funnel with three layers at one side and one layer at the other. Place this filter paper into the funnel. Top of the paper should be lower then edge of the funnel. Moist the paper with a little water then dump the stirred samples into the funnel guided by a glass rod.**
- 过滤到最后，用玻璃棒将滤纸四边挑起折叠，用塞子压一下，将滤液收集到装有5 克～7 克氯化钠的100 毫升具塞量筒中。**To the end of the filtering, peel up all sides of the filter paper then fold them up, followed with pressing with a plug. Collect the filtered solution into a 100ml cylinder with stopper that contains 5ml to 7ml sodium chloride.**

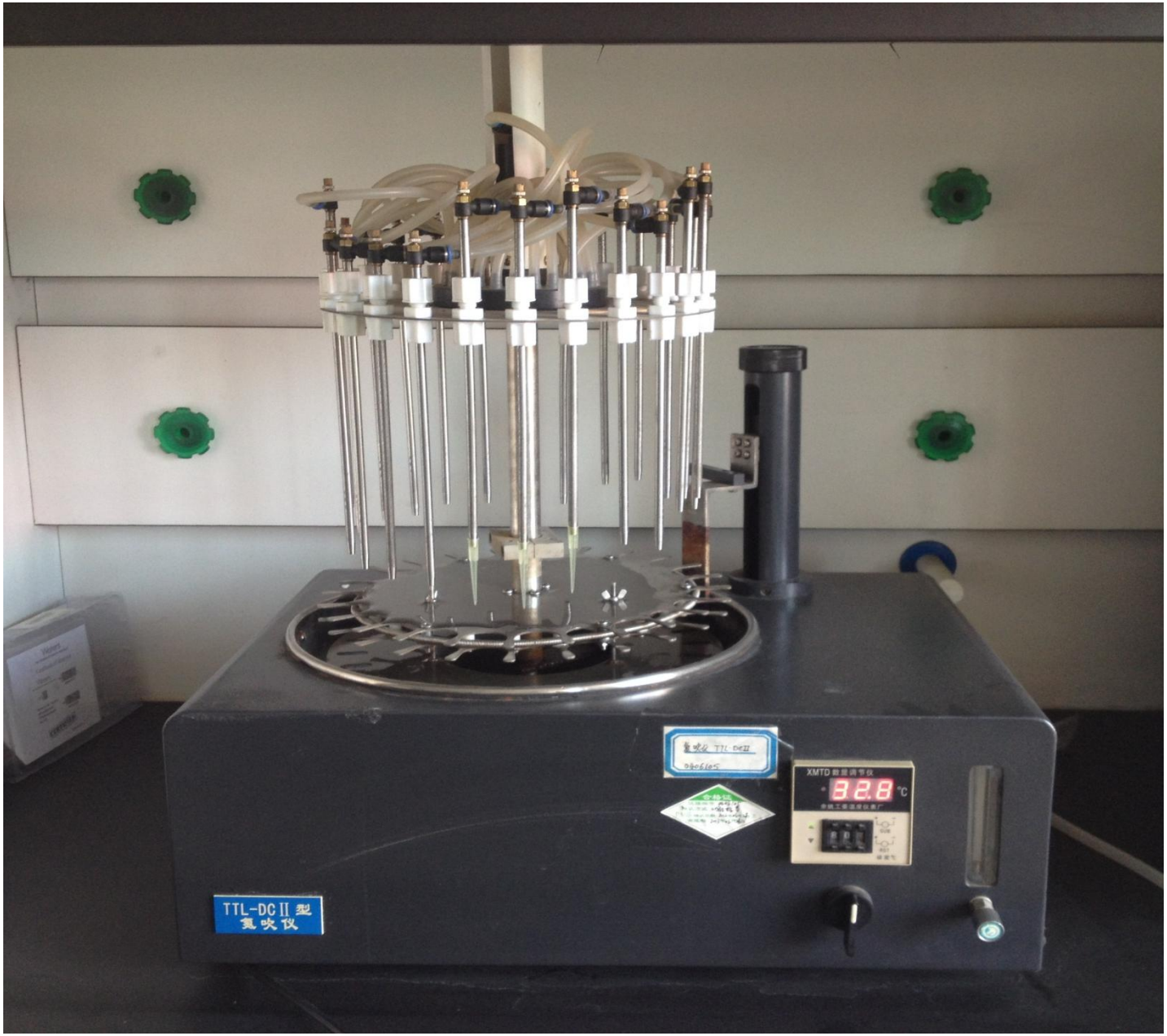
盐析
Salt Out

- 盐析过程中振荡频率和振幅应一致，保证样品的平行性。**Shaking frequencies and swings during the salt out need to be in consistency to guarantee parallelism of the samples.**
- 盐析过程中，要充分摇匀后再静置。如果摇匀过程中发生乳化现象，则长时间静置或者加少量试验用水，直到分层清晰。**During the salt out, completely shake the sample until even before let it sit. Should emulsification happens when shaking, sample needs to sit longer or extra little water is added until clear layers appear.**
- 静止时间应足够，一般不应小于**0.5**小时。在静止过程中，应不时轻摇容器，使器壁上的水珠下落。**Sitting time should be sufficient, normally it’s longer than 0.5 hour. Shake the container from time to time during the sitting and let any water drops from the container wall fall.**

浓缩
Concentrate

- 旋转蒸发器 Rotatory Evaporator
  - 其特点是可以边减压边旋转，故温度变化不大时，热量传递较快，使蒸馏能快而平稳地进行，而不发生暴沸；在使用中还可根据浓缩液体积，更换各种容量（10毫升～l升）的烧瓶。旋转蒸发器的特点是浓缩速度快，且回收率高。**Its characteristics include hardly temperature change out of simultaneous decompression when rotating, quick heat transfer promoting fast and even distillation without boiling. Depending on volume of the concentration, flasks in different volumes (10ml-1ltr) are changeable during the process. Rotatory evaporators also have fast concentration progress nad high recovery rate facts.**
- 气流吹蒸浓缩装置 Air Flow and Steam Concentration Device
  - 利用空气或氮气流吹带出溶剂的浓缩方法，适用于体积小、易挥发的提取液，但对于蒸汽压较高的农药就比较容易损失。**This device is used for concentration by blowing the air or nitrogen out from the solution, suitable for extract solution in small volume but high evaporation. However, pesticides with high steam pressure are easily lost with it.**


IWAKI
キムワイプ


TTL-DCⅡ型
氮吹仪
XMTD数显调节仪
℃

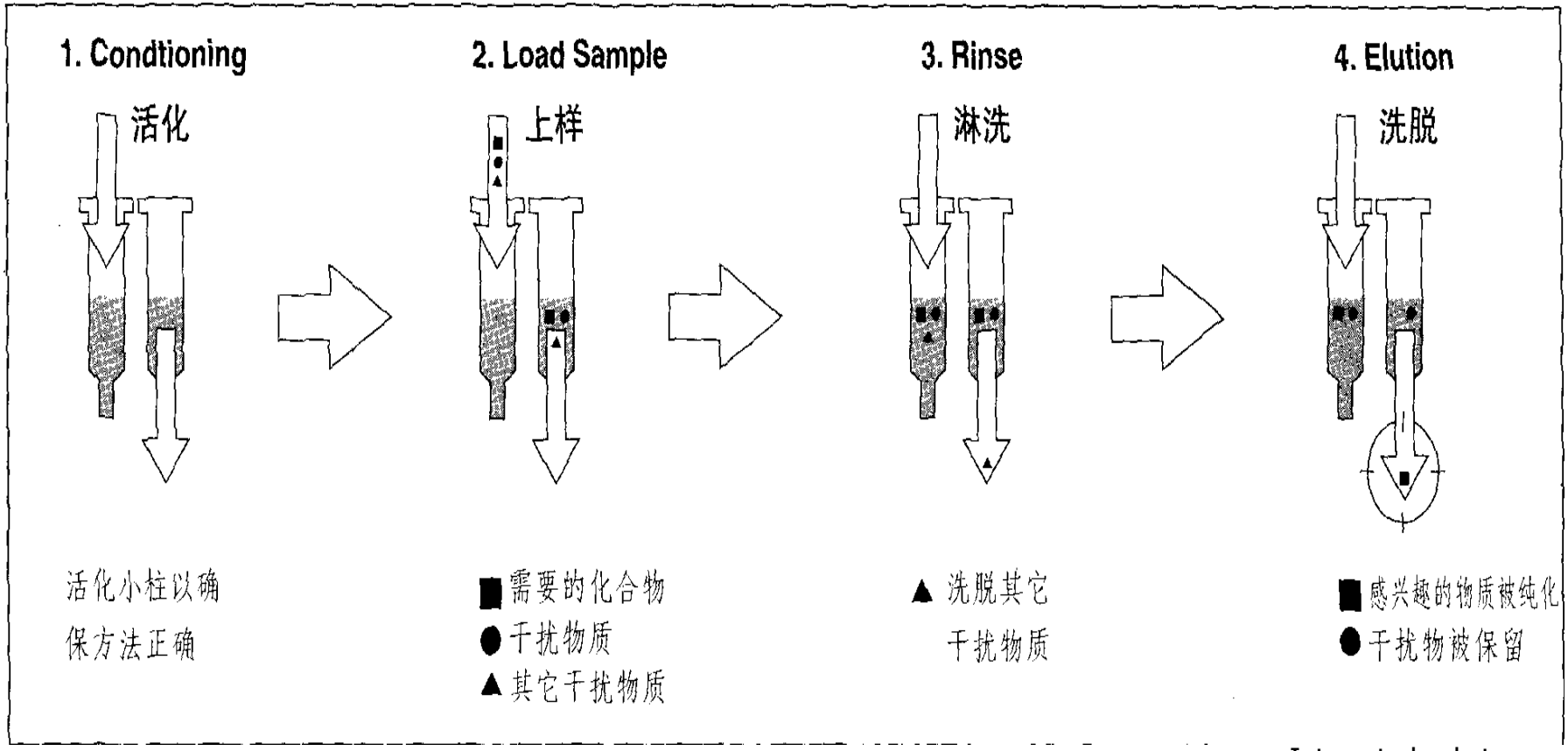
浓缩注意事项
Notes When Concentrate

- 使用氮吹浓缩时，加热温度不宜过高，一般控制在**80 ℃**以下。氮气流量不能过大，不能将样品吹干，在近干状态下取离水浴锅，自然晾干，若一定要干涸时，则操作必须细心，可以用橡皮球慢慢吹入干燥空气。**Heating temperature should be moderate and normally lower than 80 ℃ when concentrate by nitrogen blowing. Overflow of nitrogen may dry the sample up, which is prohibited. Remove the nearly dry sample away from the water bath and dry it up naturally. When compulsory drying is required, blow dry air into the container with rubber balls carefully.**
- 若易氧化的样品，还必须使用氮气，否则会导致某些农药回收率偏低。**Easy oxidation samples need nitrogen applied to avoid low pesticide recovery.**
- 氮吹时，氮吹管不能离液面太近，防止污染。**To prevent pollution, don’t keep the nitrogen blowing tube too close to the liquid surface.**

净化
Purify

- 使用有机溶剂提取样本中的农药时，样本中的油脂、蜡质、蛋白质、叶绿素及其他色素、胺类、酚类、有机酸类、糖类等会同农药一起被提取出来，会严重干扰残留量的测定。故必须将农药与上述杂质进行分离，然后才能对痕量农药进行分析测定。这一操作步骤就是所谓净化。
- **When pesticides are extracted from organic solutions, the grease, wax, protein, chlorophyll and other pigments, amines, phenols, organic acids, sugars may also be extracted and its will significantly impact the residual test, therefore, before the pesticide is trace analysed any abovementioned impurities must be removed. This process is known as Purify.**

固相萃取过程示意图
Solid Phase Leaching Flow Chart



Condition the column to guarantee the right method

Chemical Compound Required
Interference Substances
Other Interference Substances

Elute other interference substance

Interested substances are purified
Interference substances are kept

15g样品加15mL乙腈（含1%乙酸），6g无水硫酸镁和1.5g 醋酸钠后剧烈振摇1min。3450r/m离心1min，取1mL上层液加入50mg PSA ,50mg C_{18}和150mg无水硫酸镁混合20s，3450r/m离心1min。取上层液两份。一份稀释后进LC-MS/MS，另一份加保护剂后进GC/MS。

Add 15ml acetonitrile (contains 1% acetic acid), 6g magnesium sulphate and 1.5g natrium aceticum into 15g sample, shake vigorously for 1 min. Centrifugation at 3450r/m for 1 min, take 1ml liquid from the upper layer then add 50mg PSA, 50mg C_{18} and 150mg anhydrous magnesium sulphate to blend for 20s, Centrifugation at 3450r/m for 1 min. Take two sets of liquid from the tope layer, with one set diluted into LC-MS/MS, and the other charged into GC/MS with protection agent added.

在加入乙腈萃取溶剂后加入**NaCl**和**MgSO4,**此添加顺序防止过度放热，保证热不稳定农药的稳定性。**During leaching, add NaCl and MgSO4 following adding acetonitrile. This adding sequence is to prevent over heat releasing, to guarantee stability of unstable pesticides.**

提取步骤加入无水硫酸镁及氯化钠帮助农药向乙腈层分配，提高回收率！
During extract process, add of anhydrous magnesium sulphate and sodium chloride is to assist distributing the pesticides into the acetonitrile layer, to improve recovery rate!

多数蔬菜和水果: **PSA+MgSO4**，去除极性有机酸, 一些糖和脂肪类；**Most vegetables and fruits: PSA+MgSO4, remove polarity organic acids, some sugars and fats;**

蔬菜和水果**>1% Fat, PSA+C_{18}+$MgSO_4$**, 去除极性有机酸，糖类，脂肪和一些类固醇；**Vegetable and fruits > 1% Fat, PSA+C_{18}+$MgSO_4$, remove polarity organic acids, sugars, fats and some steroids.**

带色素的蔬菜和水果：**PSA+GCB+$MgSO_4$**, 去除极性有机酸，糖类，脂肪+类胡萝卜素和叶绿素，不能用于平面结构农药；**Vegetables and fruits with pigments: PSA+GCB+$MgSO_4$, remove polarity organic acids, sugars, fats + carotenoid and chlorophyl, not suitable for parallel structured pesticides;**

共面农药，可以在萃取溶剂中加入一定量甲苯。**As for coplanarity pesticides, certain amount of methylbenzene can be added into the leaching solution.**

标准溶液的配制
Formulate Standard Reagent

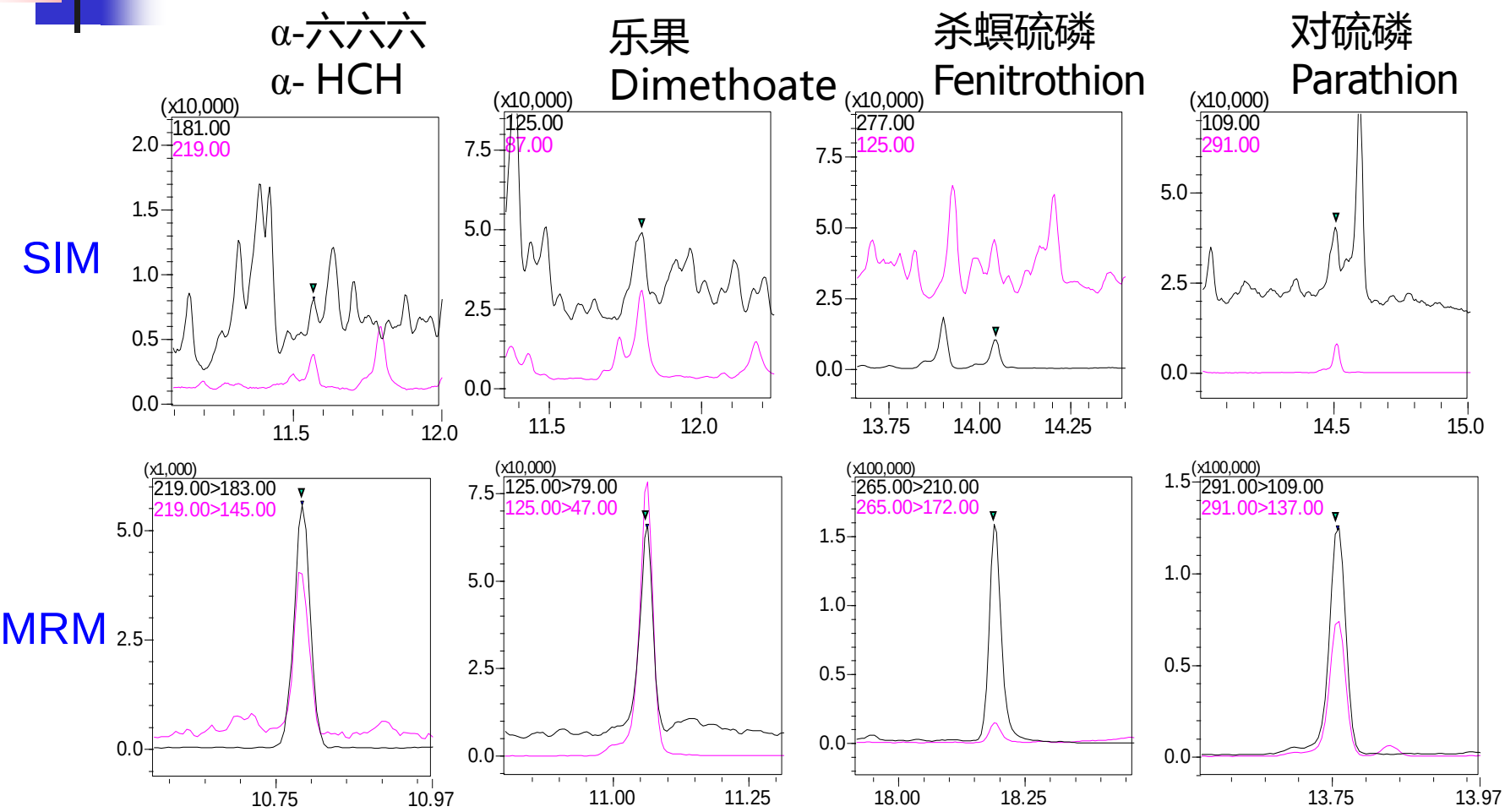
- 由于不同样品存在一定的基质效应，因此用试剂配制标准溶液测定样品往往会造成检测结果偏高或偏低的现象。**Due to the fact that certain substrate effect happens to different samples, standard solution formulated from reagents may cause higher or lower test result when testing samples.**
- 用气相色谱仪和气相色谱-串联质谱测定时，一般产生基质增强效益，用液相色谱-串联质谱测试时，一般产生基质抑制效应。**Substrate enhancement effect occurs when test with gas chromatography and gas chromatography tandem mass spectrometry, while substrate restrain effect occurs when test with liquid chromatography tandem mass spectrometry.**
- 采用样品空白提取液配制标准溶液，可以有效弥补基质效应带来的定量偏差。**Standard solution from blank sample extraction liquid could effectively offset tolerance caused by substrate effect.**

测定
Test

- 农药残留检测常用仪器：**Common instrument used in pesticide residual test:**
- 气相色谱仪、液相色谱仪、气相色谱-串联质谱仪和液相色谱-串联质谱仪。**Gas chromatograph, liquid chromatograph, gas chromatography tandem mass spectrometry and liquid chromatography tandem mass spectrometry.**
- 由于蔬菜基质比较复杂，干扰多，液相色谱仪的灵敏度较低，气相色谱仪和单级气相色谱-串联质谱仪抗干扰能力差，因此目前使用最多的是气相和液相色谱三重四级串联质谱仪。**Due to complicated substrates from vegetables, abundant interference, low sensibility of liquid chromatograph, limited anti-interference function of gas chromatograph and single phase gas chromatography tandem mass spectrometry, the most instrument applied currently is gas and liquid chromatography triple quadrupole tandem mass spectrometry.**

分析结果(1)：油麦菜样品加标(5 μg/kg)
Test Results (1) Target (5 μg/kg) added into lettuce samples

GC-MS(SIM)与GC-MS/MS(MRM)测定对比
Test and compare GC-MS(SIM) and GC-MS/MS (MRM)

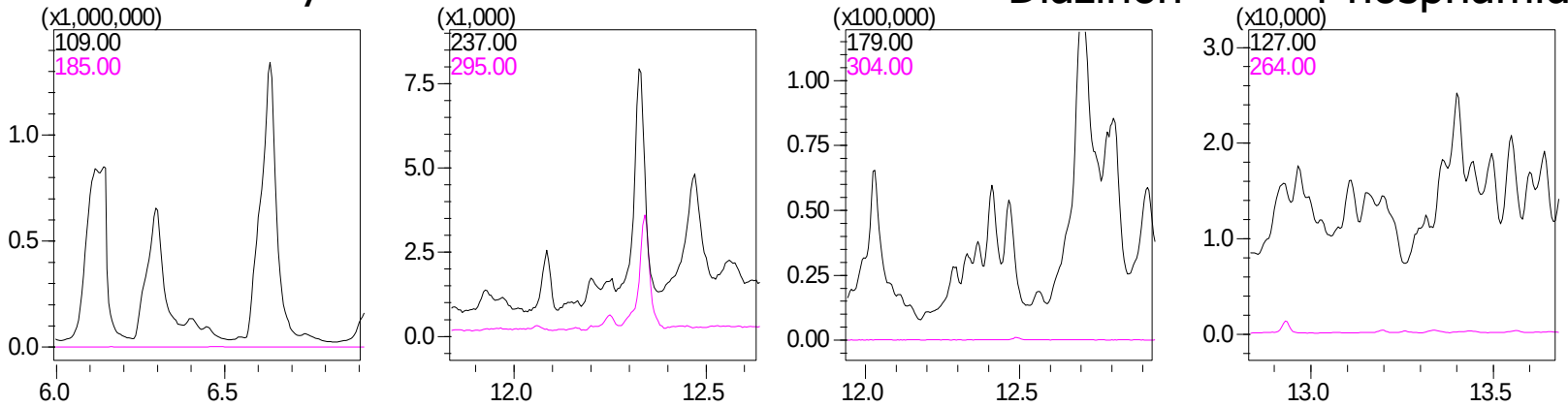


分析结果(1)：生姜的样品加标(5 μg/kg)
Test Results (1) Target (5 μg/kg) added into ginger samples

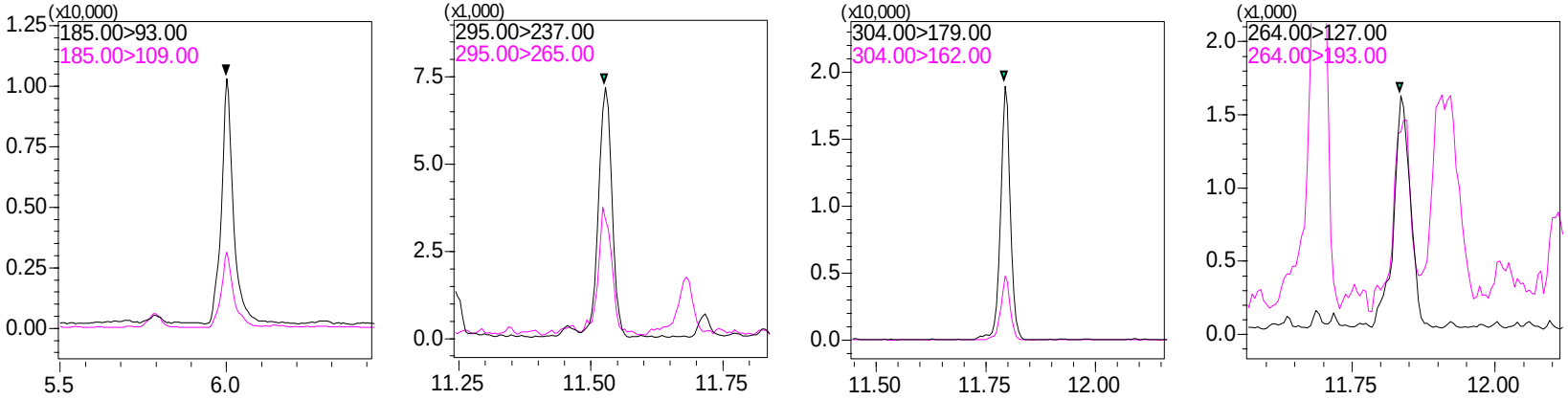
GC-MS(SIM)与GC-MS/MS(MRM)测定对比
Test and compare GC-MS(SIM) and GC-MS/MS (MRM)

敌敌畏 O-dimethyl-O-2 | 五氯硝基苯 Pentachloronitrobenzene | 二嗪农 Diazinon | 磷胺 Phosphamidon

SIM



MRM



检测过程的质量控制
Quality Control over Test Progress

- 首先做试剂空白，空白值过高应检查试剂是否有问题或器皿被污染。**Make reagent blanks at start. Check to see if the reagent is in question or the container is contaminated when the blank values are too high.**
- 每批次检测时应通过加标回收率或测定标准物质进行质量控制。农药残留检测每测定**10**个样品进一个标准溶液，每测定**24**个样品做一个添加回收率。**Quality control during each batch of test is achieved through standard addition recovery rate or testing the standard substance. As for pesticide residual test, each standard solution should be added every 10 sample tests, and each standard addition recovery rate should be made every 24 sample tests.**
- 如发现回收率超过**70 %~120 %**的范围时，该批次样品要重做。**Retest any batch of samples when the recovery rates is beyond range of 70 %~120 %.**

检测过程的质量控制
Quality Control over Test Progress

- 农药残留检测对超标或接近限量值的样品，应重新称样进行测定，并使用极性不同的柱子或不同检测器进行确认。经复测后仍超标的样品，用气质或液质联用仪进行定性检测。**During the pesticide residual test, any sample of which the content exceeded the standard or the value is close to the limit, that sample needs to be reprepared, retested and confirmed by using another column with different pole or different instrument. Samples of which the content still exceed the standard after retest need to be confined by a gas or Liquid chromatography-mass spectrometry.**
- 标准溶液应现用现配，放置一段时间后浓度有所改变，每次再用时应与以前的图谱进行比较，检查峰面积是否有较大的变化。**The standard solution should be freshly prepared for use. The concentration may change once being placed for a while, thus it’ s required to compare the diagram with previous ones to determine if significant changes happened to the peak area.**

谢谢聆听！
Thanks for your time!

这些菜放心食用吧！

Enjoy these tested vegetables!