



# **A New Multiresidue Method for the Analysis of Pesticides in Fruit and Vegetables Using LC-MS/MS Detection**

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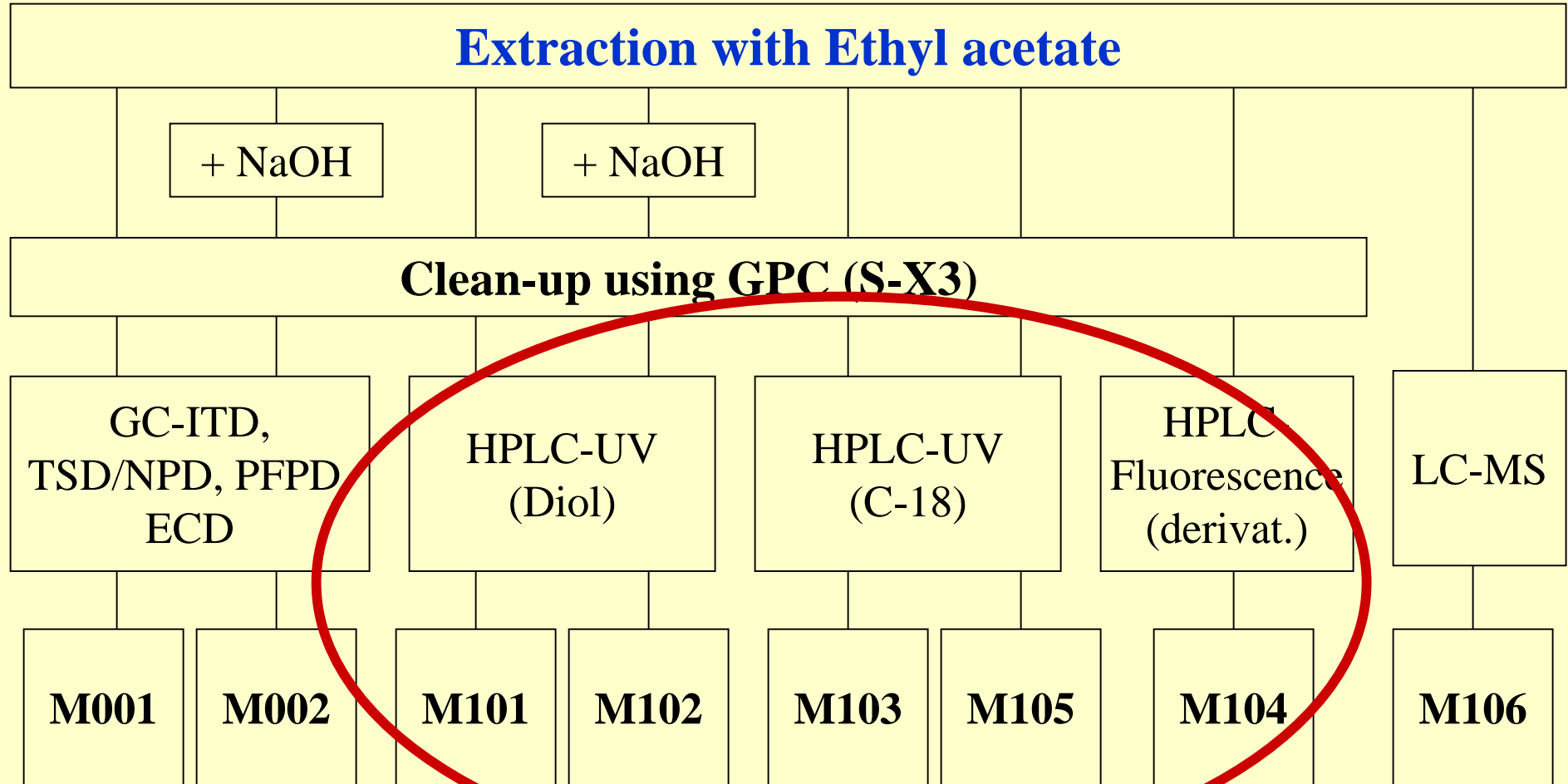
Livsmedelsverket

# Overview

## A new multiresidue method for pesticides using LC-MS/MS

- What does the analytical system look like today
- The new LC-MS/MS system
- Matrices
- Matrix effect
- Results
- Robustness

# Multiresidue methods of pesticides in fruit and vegetables used in Swedish monitoring today



## Pesticides included in the multiresidue methods today, covered by the new LC-MS/MS method

### M101/102

Carbendazim  
Imazalil  
Linuron  
Thiabendazole  
Thiophanate-methyl

### M103

Clofentezine  
Fenoxycarb  
Hexythiasox

### M105

Dinocap

### M104

#### N-methyl carbamates

Aldicarb**	Isoprocarb
Bendiocarb	Methiocarb **
Butocarboxim*	Methomyl
Butoxycarboxim	Oxamyl
Carbaryl	Oxamyl-oxim
Carbofuran	Promecarb
Carbofuran-3-OH	Propoxur
Ethiofencarb**	Thiodicarb
	Trimethacarb

#### **Together with its:**

\* -sulphoxide

\*\* -sulphoxide and - sulphone

### New pesticides

Acetamiprid  
Carbosulfan  
Demeton  
Demeton-S-methyl\*\*  
Disulfoton\*\*  
Furathiocarb  
Imidacloprid  
Phorate\*\*  
Phorate-O-analogue  
Terbufos  
Terbufos-O-sulphone  
Thiometon\*\*  
Vamidotion\*\*

# LC-MS/MS: Sample preparation

- Extraction with ethyl acetate (+ NaOH for basic compounds)
- Concentration and filtration
- Evaporation of an aliquot to dryness
- Dissolve in methanol and filtrate
- No additional clean-up
- Inject on LC-MS/MS (2.5 g/ml)
- Quantify with matrix matched standards, or by standard addition (co-chromatography)

# LC-MS/MS: Analysis

## HPLC:

- **Column:** Genesis C18, 100 x 3 mm, 4 $\mu$ m
- **Mobile phase:** Gradient MeOH / 10 mM ammonium-formate  
pH 4.1

## LC-MS/MS:

- **System:** Micromass QuattroLC
- **Interface:** Electrospray in pos/neg mode
- One fragment ion for each pesticide
- All 57 pesticides are analysed in one single injection (both pos/neg)
- 30 minutes / injection

# Method validation

- Recoveries from spiked matrix
- 0.01 mg/kg and 0.05 mg/kg
- 4 recoveries at each level from 11 commodity groups
- Totally 44 recoveries / each pesticide / level
- Test of matrix effect in each run

# Matrices from commodity groups according to EU commission

EU no	Commodity	Species			
1.1	Citrus fruits	Grapefruits	Lemons	Mandarins	Oranges
1.3	Pome fruits	Apples	Pears		
1.4	Stone fruits	Apricots	Nectarines	Peaches	Plums
1.5	Berries and small fruits	Grapes	Strawberries		
1.6	Various fruits	Bananas	Kiwi fruits	Mangoes	Pineapples
2.1	Root/tuber vegetables	Carrots	Sweet potatoes		
2.2	Bulb vegetables	Onions			
2.3	Fruiting vegetables	Cucumbers	Peppers	Melons	Tomatoes
2.4	Brassica vegetables	Cabbages	Cauliflower	Brussels sprouts	
2.5	Leafy vegetables	Lettuce	Spinach		
2.7	Stalk/stem vegetables	Celery	Leeks		



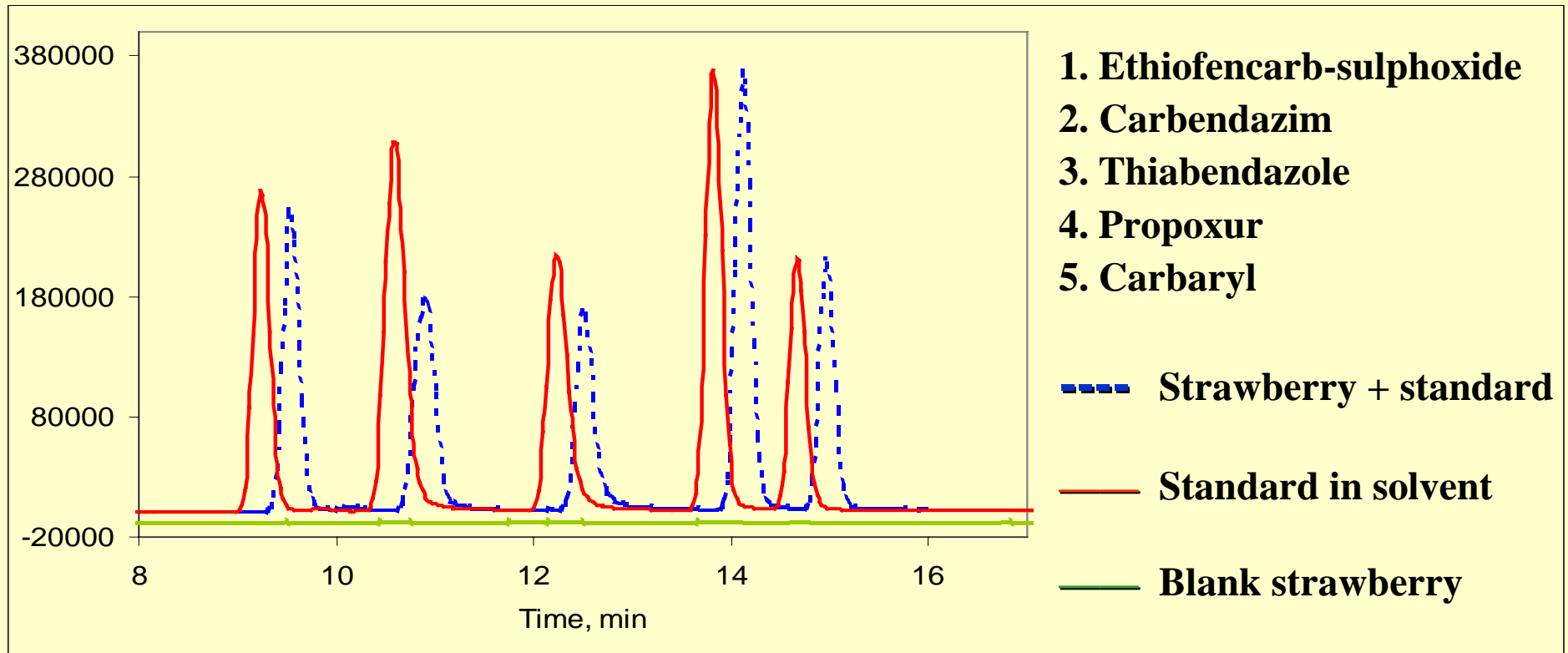
# Matrix effect

= **Signal from the pesticide in matrix compared to signal in solvent**

- Can be a problem in LC-MS
- Often due to competition between matrix component and analyte for charge
- Differs from matrix to matrix and from pesticide to pesticide
- Differs from time to time
- Can be corrected for by matrix matched standards or by addition of isotopically labelled internal standards
- Isotopically labelled standards are unpractical for multiresidue methods and/or are not available

# Matrix effect: **Suppression** ES+/MSMS

## Strawberries



- Standard addition 0.05 mg standard / kg strawberries
- Suppression for carbendazim and thiabendazole

# Matrix effect: **How to correct?**

## **External matrix matched standards:**

- Standards in the same matrix as the sample
  - Can be difficult when analysing many different matrices

## **Standard addition (co-chromatography):**

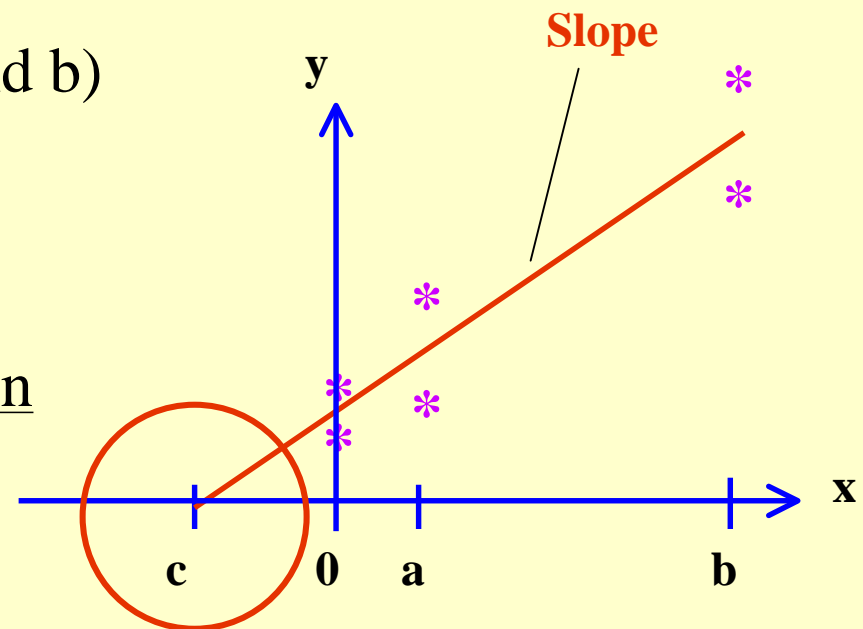
- Addition to the same extract
- Probably the best correction
- Possible to use when analysing many different matrices
- Single-level or multi-level addition

# Matrix effect: **Standard addition**

## Calculation:

- Determine the slope of the curve from the standard additions (a and b)
- True conc. = **-c**

$$c = \frac{\text{signal in sample without addition}}{\text{slope}}$$



## Matrix effect: **Results**

Matrix effect has been measured at 0.05 mg/kg together with every recovery test

<b>Mean value:</b>	<b>104%</b>
<b>RSD, %:</b>	<b>23%</b>
<b>n:</b>	<b>2193</b>

- All recovery values have been corrected for matrix effect
- For more information, see **Poster 95 and 96**

## Recovery: Results

Recoveries from 57 pesticides in 31 different commodities

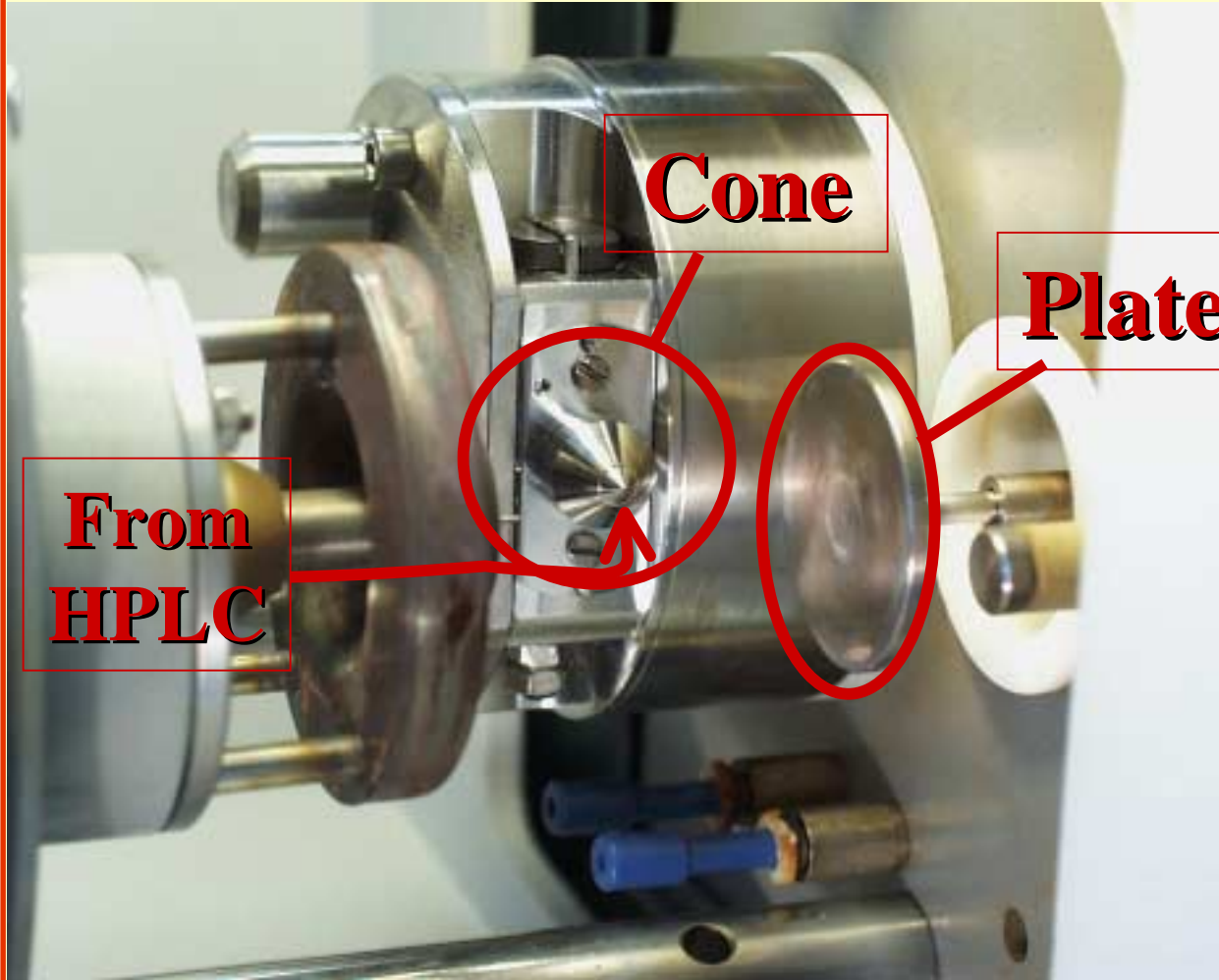
	<u>0.01 mg/kg</u>	<u>0.05 mg/kg</u>
<b>Mean recovery:</b>	<b>87%</b>	<b>87%</b>
<b>RSD, %:</b>	<b>22%</b>	<b>19%</b>
<b>n:</b>	<b>2383</b>	<b>2202</b>

- Results from both basic and neutral extraction
- All recovery values have been corrected for matrix effect
- For more information, see **Poster 95 and 96**

# Robustness

- The same HPLC-column has been used for about 4000 injections
- The pre column can be used for about 1000 injections before replacement
- No loss in performance after 48 hours
- Very little deposit in the interface

## Robustness: LC-MS interface



Deposit on the interface may alter the signal

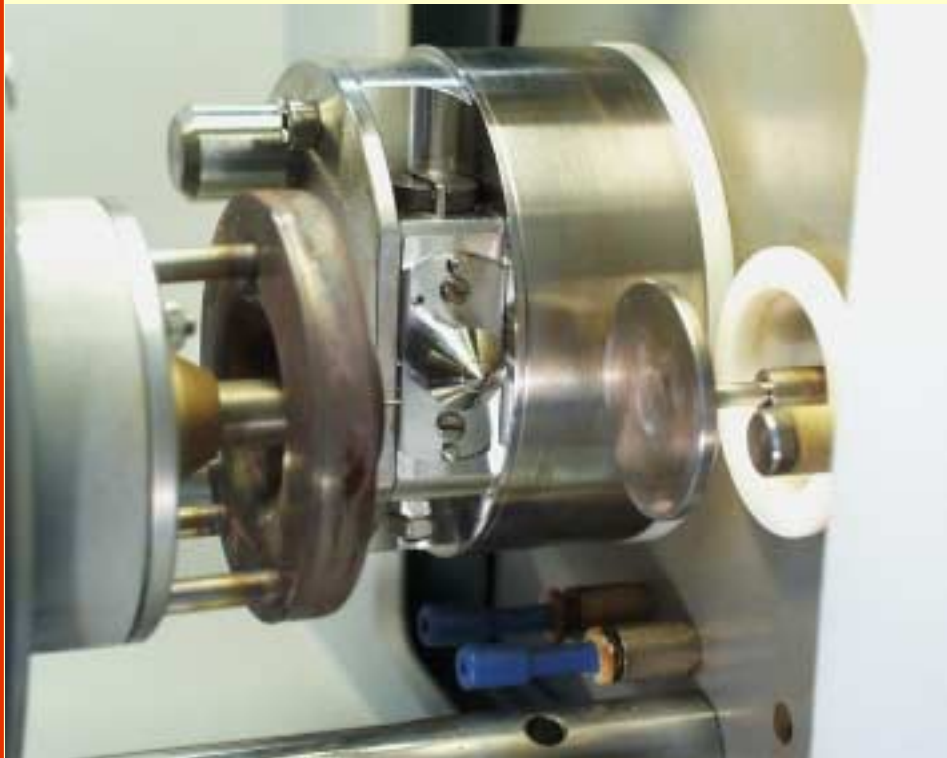
More or less deposit depending on system (extraction and clean-up)

If deposit - it will be on the cone and the plate

**Clean interface**



## Robustness: LC-MS interface



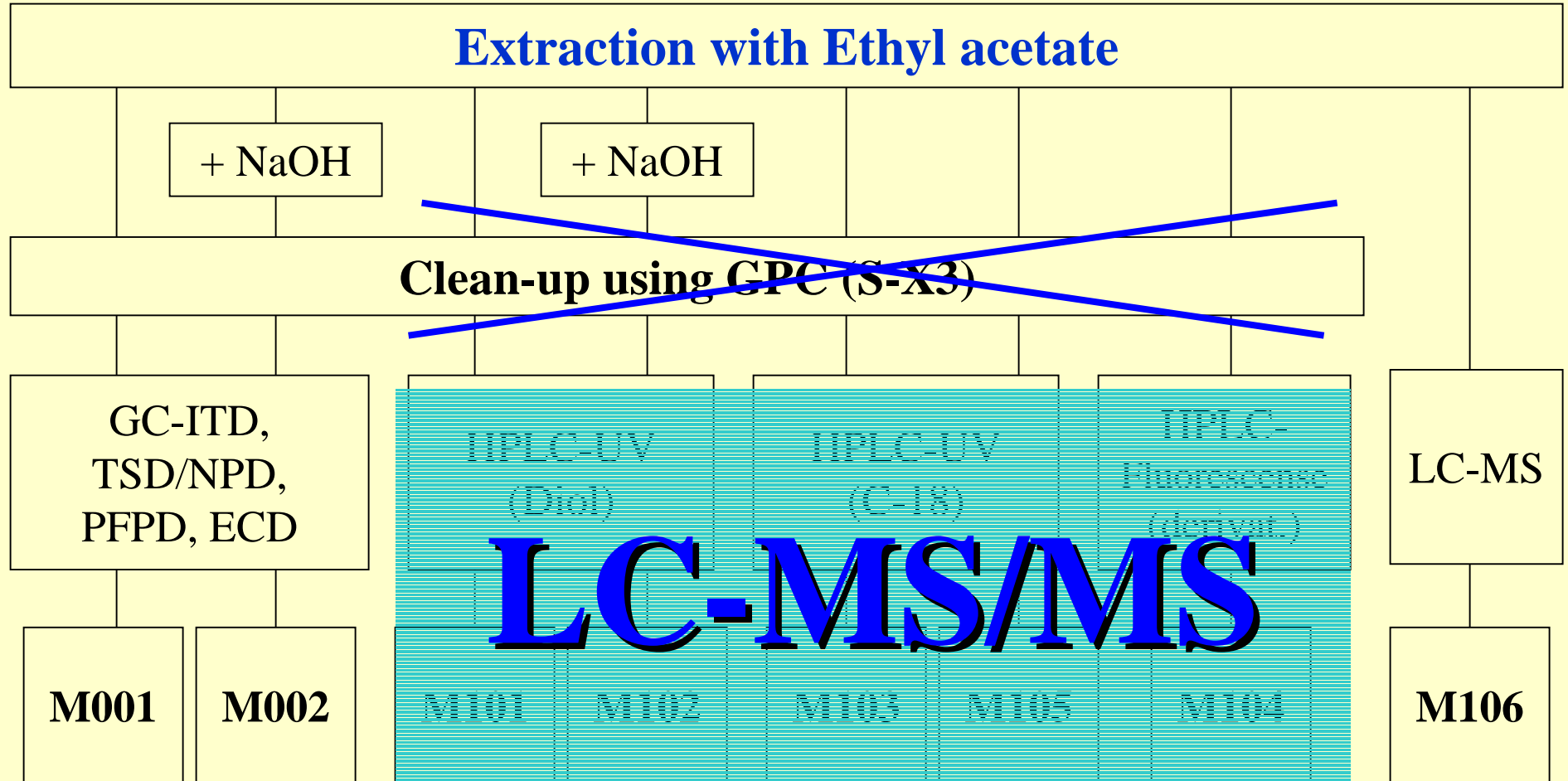
**Clean interface**



**... and after 45 hours / 85 injections**

**Only very little deposit!**

# Multiresidue methods of pesticides in fruit and vegetables used in Swedish monitoring ~~today~~ tomorrow





# Conclusions

- Use of the established ethyl acetate extraction makes it efficient and applicable for monitoring purposes
- It is possible to quantify and detect 57 different pesticides in one single run
- Confirmation of identity simultaneously
- Detection limit is  $< 0.01$  mg/kg ( $0.025$   $\mu$ g/ml) for all compounds studied
- A very good complement to GC-determination
- No sample clean-up necessary => High through-put

# The working team



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