

# 109年度檢驗方法推廣訓練班

禽畜水產品中乃託文之檢驗方法開發

食品化學檢驗科 (動物用藥群組)

黃志能、晏家元、劉誌成、賴宥勳、陳珮瑄、曾衡宇

時間: 109年11月12日



衛生福利部  
食品藥物管理署  
Taiwan Food and Drug Administration

<http://www.fda.gov.tw/>

## 大綱

- 前言
- 檢驗方法評估過程
- 確效結果
- 建議方法與公告方法比較
- 補充



衛生福利部  
食品藥物管理署  
Taiwan Food and Drug Administration

<http://www.fda.gov.tw/>

# 前言

## 2019~2020年動物用藥研究主題

Year	Method Development
2019	禽畜水產品中動物用藥 Closantel 殘留之檢驗方法開發
	禽產品中動物用藥弗雷拉納殘留之檢驗方法開發
	鮑魚中mequindox及其代謝物之鑑別方法
2020	蛋類中Prednisolone之檢驗方法開發
	蛋類中Flavophospholipol之檢驗方法開發
	蛋類中Piperazine之檢驗方法開發
	禽畜水產品中乃託文之檢驗方法開發
	蛋類中左美素之檢驗方法開發
	Toltrazuril(妥適諸力)及其代謝物之檢驗方法開發
	Halquinol等4項羥基奎諾酮類藥物之同步分析方法
	Sulpyrin(斯路比林)等非固醇類抗發炎用藥之同步分析方法



## Paper review

1. Class
2. Enzyme Hydrolysis
3. Acid hydrolysis
4. Alkaline hydrolysis
5. Extraction solvent
6. Polarity

2  
Method for  
single or few  
compounds

1  
**Multi-  
residue  
method**

**The same class**



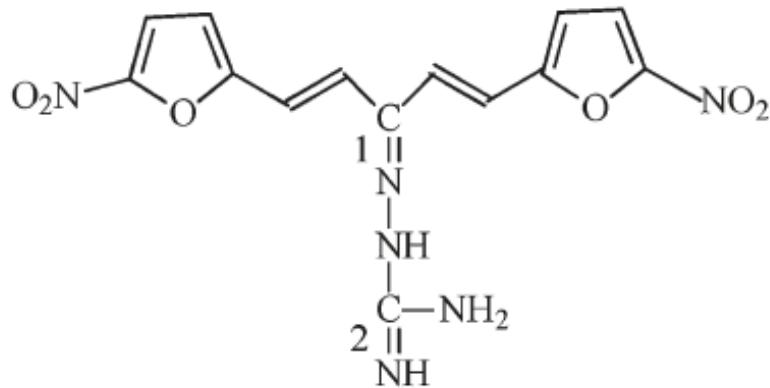
## Paper review

Multi-residue  
method

Multi-  
Screenin  
g  
method

# Introduction

## Nitrovin (乃託文)之結構、名稱、外觀、用途、分類



Nitrovin

- Nitrovin (NIT)
  - Synonyms
    - 1,5-bis(5-nitro-2-furanyl)-1,4-pentadien-3-one
    - Difurazone

- Molecular formula : C<sub>14</sub>H<sub>12</sub>N<sub>6</sub>O<sub>6</sub>
- Molecular weight : 360.28
- Appearance
  - A kind of orange yellow or red stable powder
- Application
  - An antibacterial growth promoter used in animal feeds
- Classification
  - Furans drug (呋喃類)
  - What comes to mind when you see “furans drug”?
    - Ans: Nitrofuran metabolites

# Introduction (cont.)

## Mechanism of Action

Type	Mechanism	Example
1	Inhibition of cell wall synthesis	$\beta$ -lactam, bacitracin, vancomycin
2	Damage to cell membrane function	Polymyxins
3	Inhibition of nucleic acid synthesis or function	nitroimidazoles, <b>nitrofurans</b> , quinolones, fluoro-quinolones
4	Inhibition of protein synthesis	aminoglycosides, phenicols, lincosamides, macrolides, streptogramins, pleuromutilins, tetracyclines
5	Inhibition of folic and folinic acid synthesis	Sulfonamides, trimethoprim

# Introduction (cont.)

## Nitrovin 與 Nitrofuran metabolites

- We are familiar with four nitrofuran metabolites as follows:

No.	Nitrofuran compound	Nitrofuran metabolites
1	Nitrofurantoin	AHD
2	Furazolidone	AOZ
3	Nitrofurazone	SEM
4	Furaltadone	AMOZ



- Now we have to learn more about two nitrofuran drugs

No.	Nitrofuran compound	Nitrofuran metabolite
5	Nifursol <sup>1</sup>	DNSAH
6	Nitrovin <sup>2</sup>	-

<sup>1</sup> Commission regulation(EU) 2019/1871

<sup>2</sup> 防檢局建請本署優先開發項目

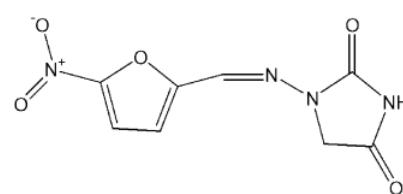
# Introduction (cont.)

## Nitrovin 與 Nitrofuran metabolites

- Parent drug or metabolites ?
  - The nitrofuran antibiotics are rapidly biochemically transformed in still toxic metabolites which have the property to be highly bound to proteins and thus stable for longer periods (several weeks or even months) in the food producing animals.
    - Nitrofurantoin, Furazolidone, Nitrofurazone, Furaltadone, Nifursol

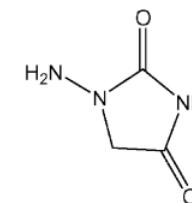
Nitrofuran Compound

Nitrofurantoin

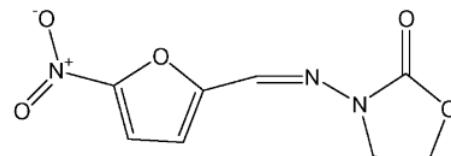


Nitrofuran Metabolite

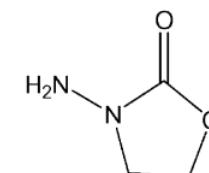
AHD



Furazolidone



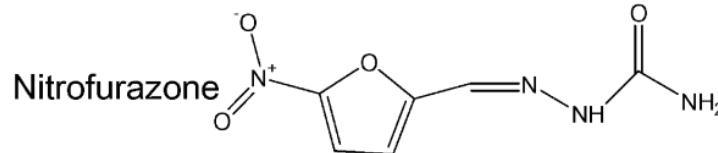
AOZ



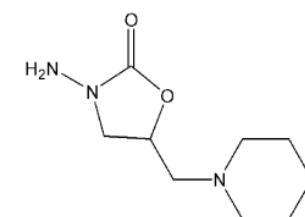
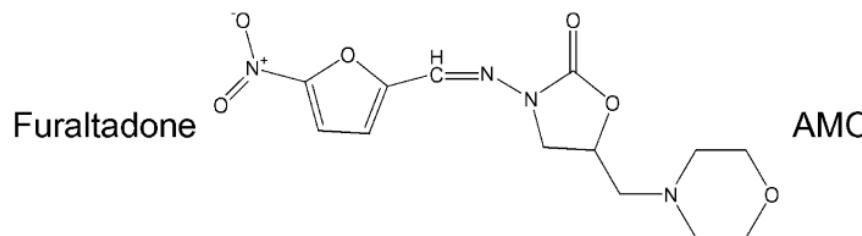
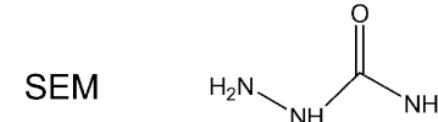
# Introduction (cont.)

## Nitrovin 與 Nitrofuran metabolites

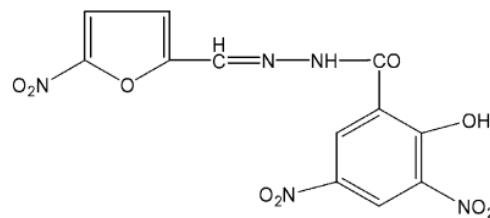
**Nitrofuran Compound**



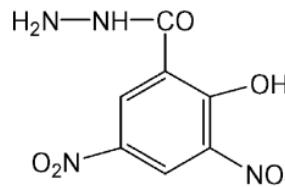
**Nitrofuran Metabolite**



Nifursol



DNSAH



# Introduction (cont.)

## *Parent drug or Metabolites ?*

*Food Additives and Contaminants*, May 2005; 22(5): 406–414



### **Depletion of four nitrofuran antibiotics and their tissue-bound metabolites in porcine tissues and determination using LC-MS/MS and HPLC-UV**

K. M. COOPER<sup>1</sup>, P. P. J. MULDER<sup>2</sup>, J. A. VAN RHIJN<sup>2</sup>, L. KOVACSICS<sup>3</sup>, R. J. MCCRACKEN<sup>4</sup>, P. B. YOUNG<sup>4</sup>, & D. G. KENNEDY<sup>4</sup>

<sup>1</sup>Queen's University Belfast, Northern Ireland, <sup>2</sup>RIKILT-Institute of Food Safety, Wageningen, The Netherlands,

<sup>3</sup>National Food Investigation Institute, Budapest, Hungary, and <sup>4</sup>Chemical Surveillance Department, Department of Agriculture and Rural Development, Stoney Road, Stormont, Belfast BT4 3SD, Northern Ireland

實驗中將豬隻分群(停藥期及無停藥期)，餵食含furazolidone, furaltadone, nitrofurantoin, nitrofurazone等硝基呋喃藥物之飼料10天，屠宰後分析藥物原體及代謝物含量，結果顯示主要以代謝物為主，代謝物半衰期介於5~15天左右。

# Metabolism and depletion of nifursol in broilers

T. Zuidema<sup>a,\*</sup>, P.P.J. Mulder<sup>a</sup>, J.A. van Rhijn<sup>a</sup>, N.G.M. Keestra<sup>a</sup>,  
L.A.P. Hoogenboom<sup>a</sup>, B. Schat<sup>a</sup>, D.G. Kennedy<sup>b</sup>

<sup>a</sup> RIKILT – Institute of Food Safety, Bornsesteeg 45, P.O. Box 230, 6700 AE Wageningen, The Netherlands

<sup>b</sup> Chemical Surveillance Department, Veterinary Sciences Division, Department of Agriculture and Rural Development, Stoney Road, Stormont, Belfast BT4 3SD, Northern Ireland, UK

Received 14 June 2004; received in revised form 17 August 2004; accepted 17 August 2004

Available online 18 December 2004

Table 2

Average residue of nifursol in µg/kg in liver, kidney, muscle, bile and plasma of broilers treated with feed containing 50 mg/kg nifursol during a period of 7 consecutive days

	Days after cessation of medication				
	0	3	7	14	21
Liver	<2	<2	<2	<2	<2
Kidney	<1	<1	<1	<1	<1
Muscle	<2	<2	<2	<2	<2
Bile	16285	20	<2	<2	<2
Plasma	281	0.3	<0.25	<0.25	<0.25

Nifrusol主要以代謝物DNSAH存在



WORLD HEALTH ORGANIZATION

## INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC, LYON, FRANCE

July 1983

### *Absorption, distribution, excretion and metabolism*

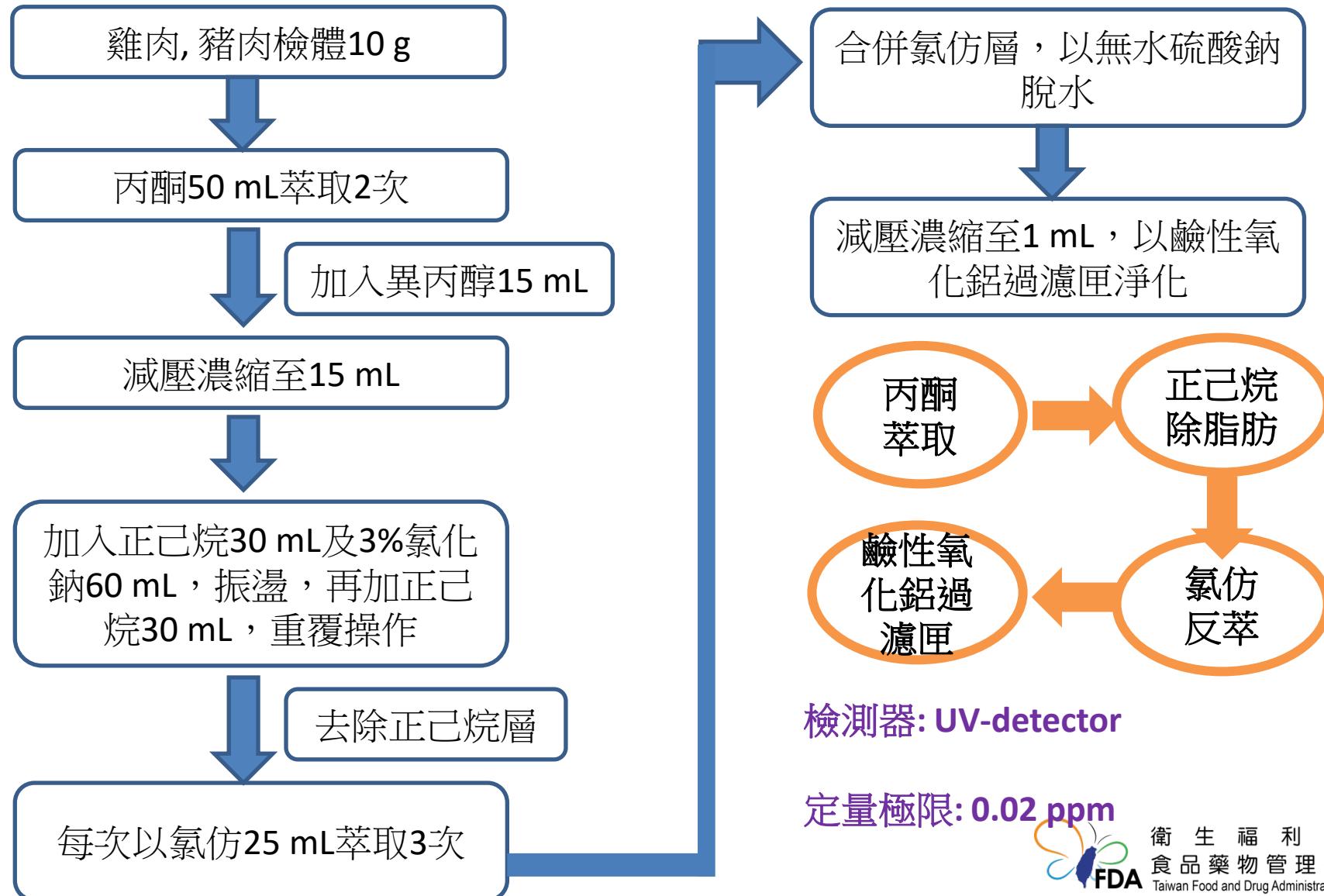
Following oral administration of  $^{14}\text{C}$ -nitrovin to rats, 0.6% of the radioactivity was absorbed through the intestine, the highest amount being found in the liver and kidneys. Activity was excreted rapidly in the faeces, with approximately 90% recovery within 48 hours. Only 1% of the radioactivity was found in the urine, and traces of radioactivity were detected in exhaled carbon dioxide. A small amount of radioactivity was still detected in animal bodies 12 days after its administration (Struck *et al.*, 1980a).

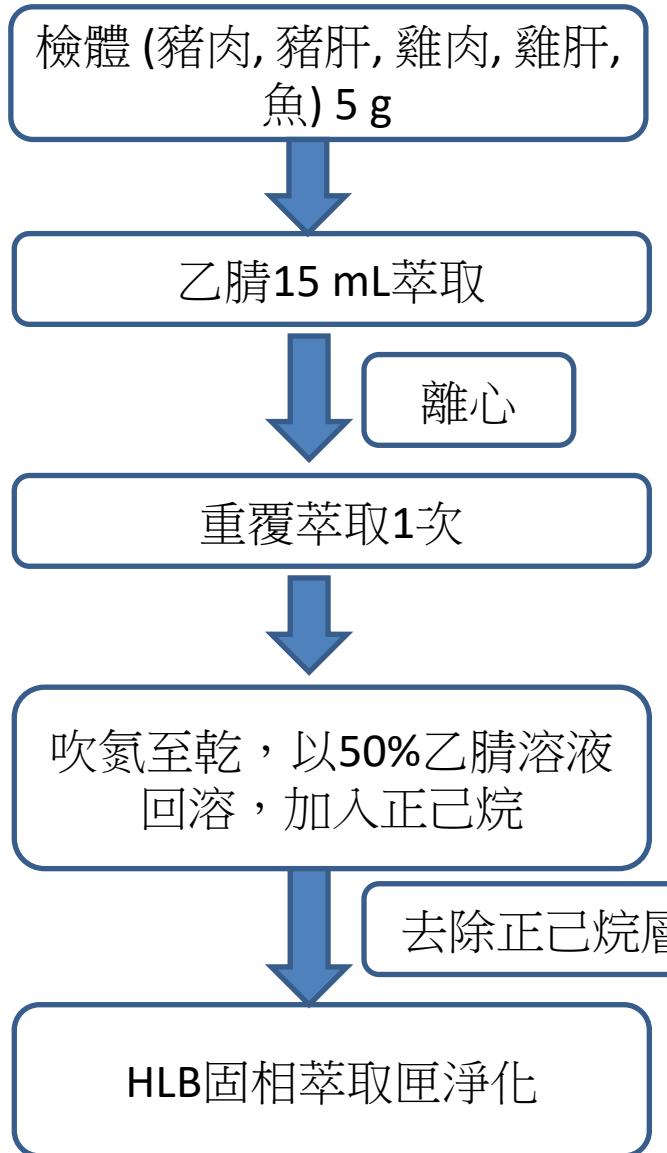
Nitovin主要以藥物原體之形式存在



衛生福利部  
食品藥物管理署  
Taiwan Food and Drug Administration

# 食品中動物用藥殘留量檢驗方法 - 乃託文之檢驗

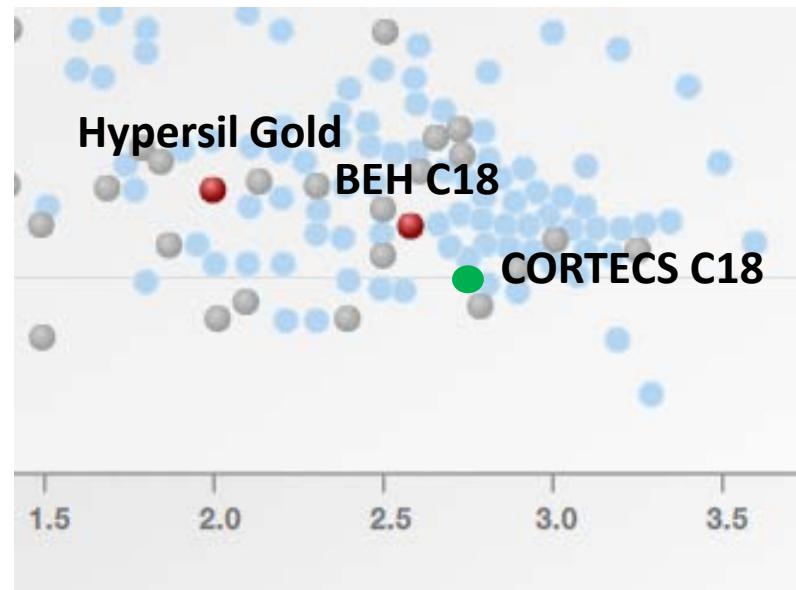




- Detector: LC-MS/MS
- Column: Thermo Hypersil Gold C18
  - Fully porous
  - Reverse phase column
- Mobile phase: ddH<sub>2</sub>O, Acetonitrile
- LOQ: 0.0005 ppm

## Method development

- Detector: LC-MS/MS
- Column: Thermo Hypersil Gold C18
  - Fully porous
  - Reverse phase column
- Mobile phase: ddH<sub>2</sub>O, Acetonitrile
- LOQ: 0.0005 ppm



- Column: CORTECS C18
  - Solid Core
  - Reverse phase
  - Be used for analyzing Nitrofurans metabolites in TFDA's method
- Mobile phase: ddH<sub>2</sub>O, MeOH ; containing 0.1%FA

## □ LC parameters

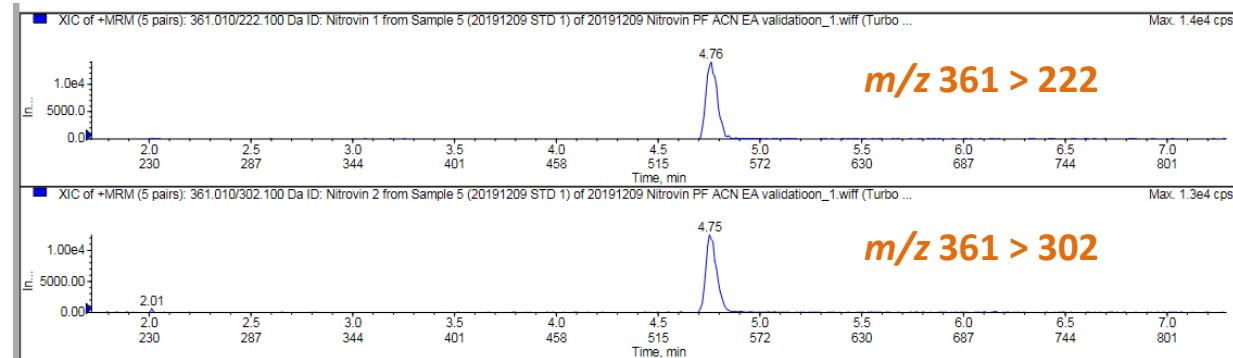
- Mobile phase :
  - A: 0.1% FA in ddH<sub>2</sub>O
  - B: 0.1% FA in MeOH
- Injection volume : 10 μL
- Flow rate: 0.3 mL/min



時間 (min)	A (%)	B (%)
0.0 → 1.0	80 → 80	20 → 20
1.0 → 5.0	80 → 0	20 → 100
5.0 → 10.0	0 → 0	100 → 100
10.0 → 10.5	0 → 80	100 → 20
10.5 → 13.5	80 → 80	20 → 20

## □ MS parameters

- ESI<sup>+</sup>
- Ion spray voltage : 5.5 kV
- Ion source temperature : 100 °C
- Turbo heater temperature : 500 °C
- Nebulizer gas, GS1 : 50 psi
- Heated gas, GS2 : 50 psi



定量離子對

定性離子對

分析物	定量離子對			定性離子對		
	前驅離子(m/z)>	去集簇電壓(V)	碰撞能量(eV)	前驅離子(m/z)>	去集簇電壓(V)	碰撞能量(eV)
乃託文	361 > 222	200	27	361 > 302	200	29



## Paper review

2

Method for  
single or few  
compounds

1

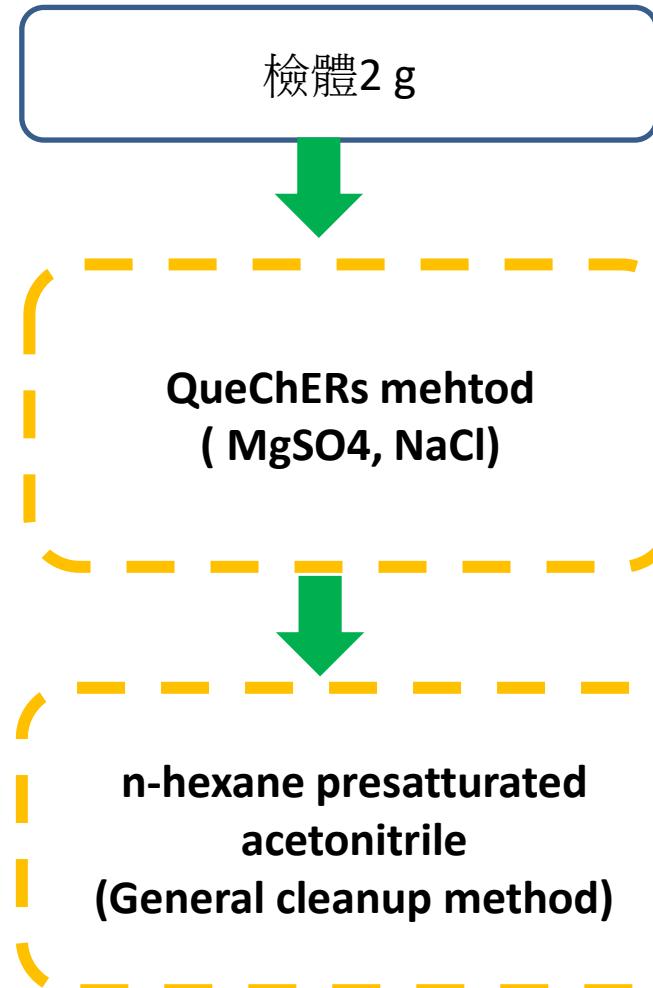
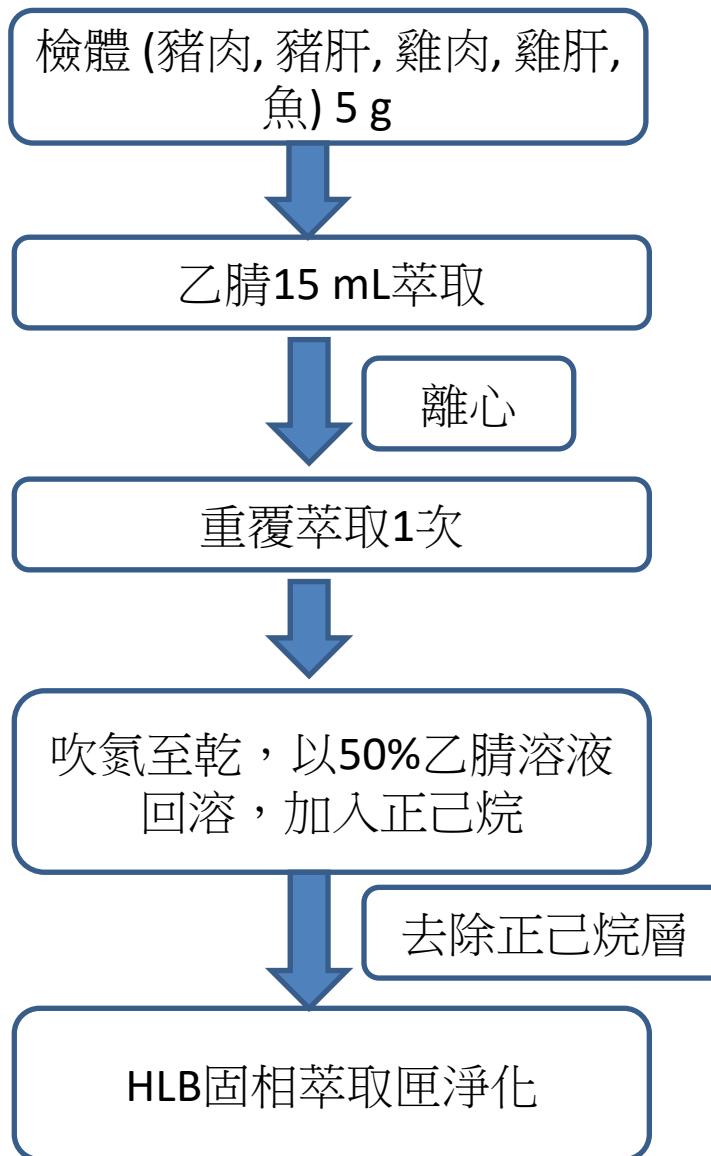
Multi-  
residue  
method

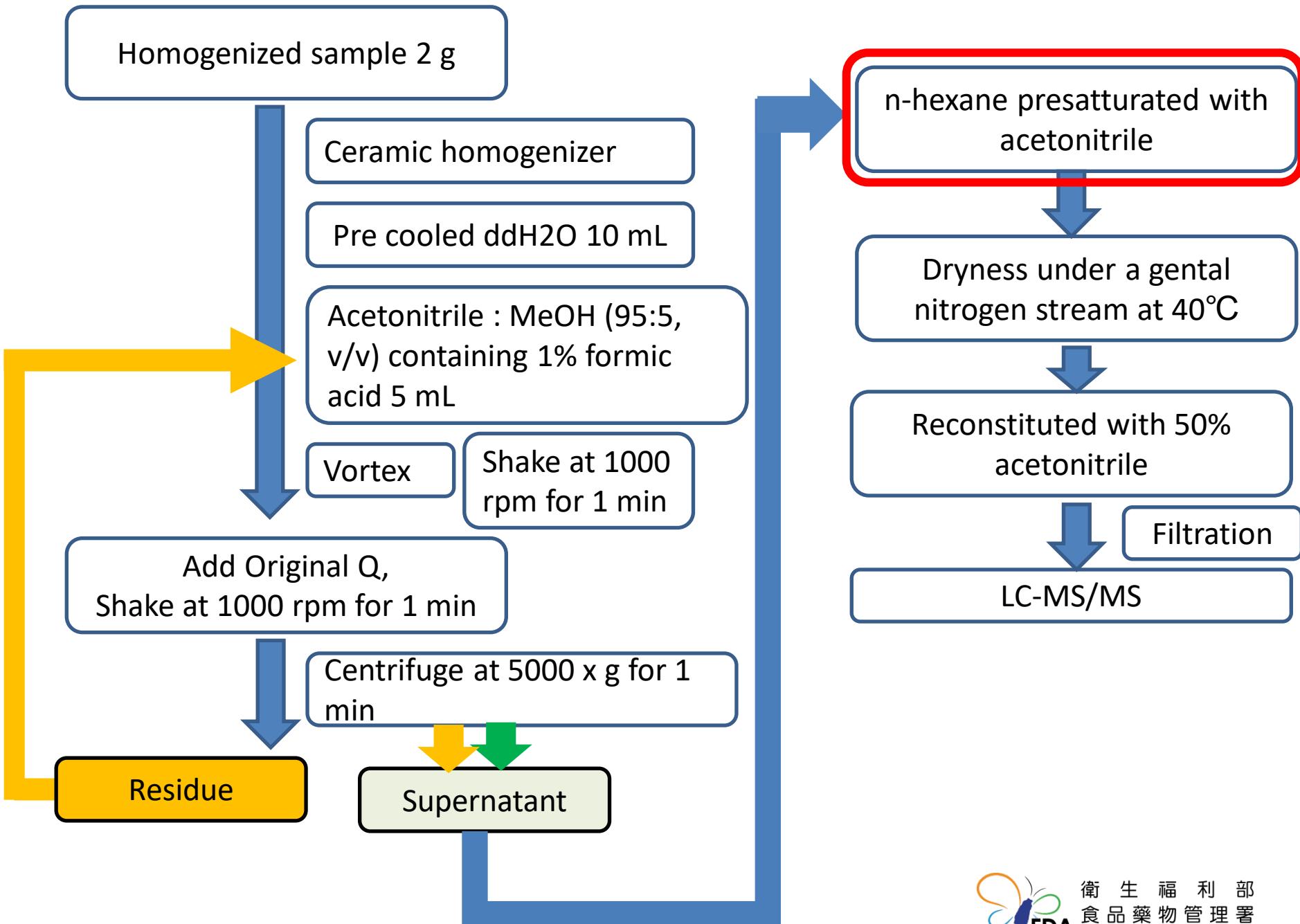
The same class

### 針對Nitrovin之分析

Item	Check
Class	Nitrofurans
Enzyme hydrolysis	No
Acid hydrolysis	No
Alkaline hydrolysis	No
Extraction solvent	Acetonitrile (no special reagent)
Polarity	Reverse column can achieve good separation

## Method development





Homogenized sample 2 g

Ceramic homogenizer

Pre cooled ddH<sub>2</sub>O 10 mL

Acetonitrile : MeOH (95:5,  
v/v) containing 1% formic  
acid 5 mL

Vortex

Shake at 1000  
rpm for 1 min

Add Original Q,  
Shake at 1000 rpm for 1 min

Centrifuge at 5000 x g for 1  
min

Residue

Supernatant

Dryness under a gental  
nitrogen stream at 40°C

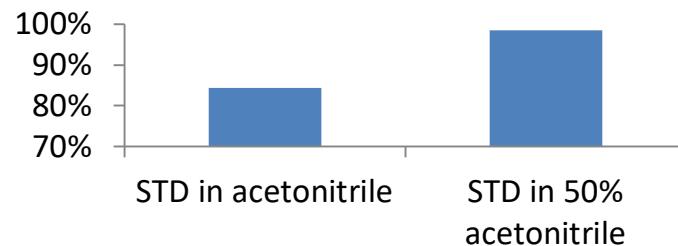
Reconstituted with 50%  
acetonitrile

n-hexane presaturated with  
acetonitrile

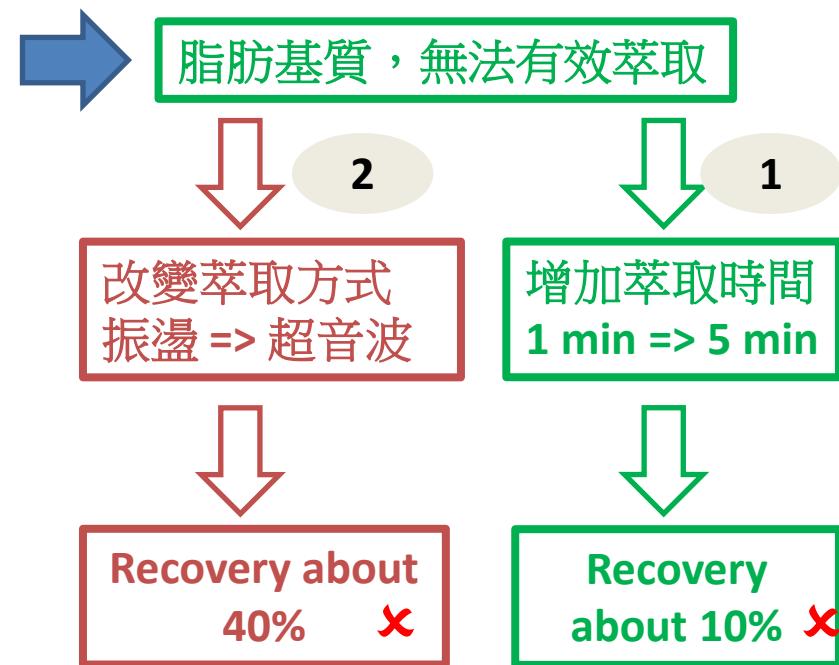
Filtration

LC-MS/MS

Recovery for cleanup by n-  
hexane presaturated with  
acetonitrile



Intra-Lab	Chicken muscle	Pig Liver
	0.01 µg/g	
QC-1	99.8	94.5
QC-2	98.8	99.6
QC-3	96.9	97.2
QC-4	98.5	93.3
QC-5	95.1	91.7



超音波萃取

3 延長萃取時間 ✕

更改萃取溶劑

4

ACN : MeOH (95:5, v/v)+1%FA

40%

✕

ACN : MeOH (80:20, v/v)+1%FA

29~35%

✕

ACN : Acetone (80:20, v/v)+1%FA

9~23%

✕

ACN+1%FA

30%

✕

ACN

60%

✕

ACN : EA (8:2, v/v)

80%

✕

EA

50%

✕



Homogenized sample 2 g

Fat

Ceramic homogenizer

Acetonitrile : Ethyl acetate (4:1, v/v) 10 mL

Vortex

Ultrasonic shake at 50°C for 15 min

Centrifuge at 5000 x g for 1 min

Supernatant

Dryness under a gental nitrogen stream at 40°C

Reconstituted with 50% acetonitrile

n-hexane presaturated with acetonitrile

Filtration

LC-MS/MS

面對脂肪基質，  
採用超音波萃取  
並降低萃取溶劑  
極性



衛生福利部  
食品藥物管理署  
Taiwan Food and Drug Administration

# Method development for nitrovin

## Validation data

Matrix	Intra-day precision <sup>a</sup>						Inter-day precision <sup>b</sup>	LOQ (ppm)	r			
	0.001 µg/g		0.002 µg/g		0.005 µg/g							
	Recovery (%)	CV (%)	Recovery (%)	CV (%)	Recovery (%)	CV (%)						
Pork	87.6	5.1	99	2.1	93.8	3	8.4	0.001	0.9996			
Fish muscle	72	4.8	82.1	7.1	90.3	2.4	12.8	0.001	0.9998			
Pig liver	98.9	11.9	92	5.3	93.2	2.9	9.4	0.001	0.9993			
Chicken egg	95	2.2	88.9	4.1	89.2	2.9	4.8	0.001	0.9986			
Milk	94.2	5	83.9	10.7	91	4	5.6	0.001	0.9995			
Honey	110.1	5.9	108.3	5.3	101.5	4.4	11.7	0.001	0.9993			
Pig Fat	91	5.4	87.5	3.7	90.3	5.5	8.6	0.001	0.9994			

<sup>a</sup>n=5 ; <sup>b</sup>n=10

上述七種基質

本署食品化學檢驗方法確效規範

回收率介於72.0 ~ 110.1%

濃度範圍0.001 ~ 0.01, 回收率60 ~ 125%

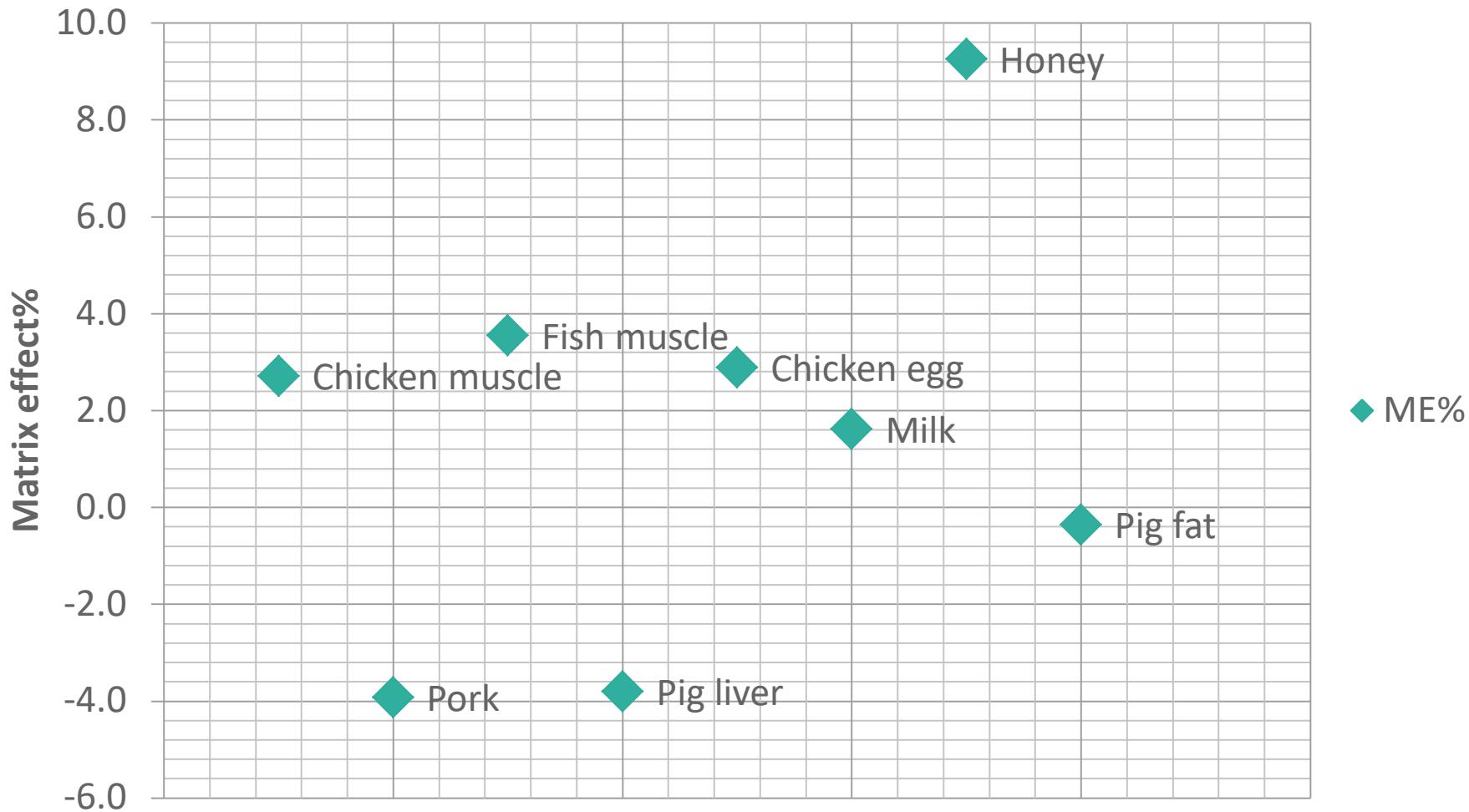
重複性 2.1 ~ 11.9%

濃度範圍 0.001 ~ 0.01, 重複性 30%, 中間精密度 32%

中間精密度 4.8 ~ 12.8%

# Method development for nitrovin

Matrix effect scatter plot



# 目前乃託文建議方法與公告方法之比較

方法	食品中動物用藥殘留量檢驗方法 —乃託文之檢驗 (MOHWV0021.01)	食品中動物用藥殘留量檢驗方法—乃託文 之檢驗(二)
基質	雞肉、豬肉	肌肉、內臟、蛋、蜂蜜、乳汁、脂肪
萃取	丙酮萃取 ↓ 正己烷除脂 ↓ 氯仿萃取	肌肉、內臟、蛋、蜂蜜、乳汁 QuEChERs方法萃取(含1%甲酸之乙腈:甲醇(95:5,v/v)及無水硫酸鎂、氯化鈉粉劑)  脂肪 溶劑萃取(乙腈:乙酸乙酯(8:2, v/v)+超音波振盪
淨化	鹼性氧化鋁過濾匣	乙腈飽和正己烷
稀釋倍數	5 g / 1 mL	2 g / 20 mL
檢測器	紫外光檢出器	質譜
定量極限	0.02 ppm	0.001 ppm



109年8月11日預  
告廢止



109年8月11日預  
公告



## Paper review

2

Method for  
single or few  
compounds

1

Multi-

The same ch...

## 針對Nitrovin之分析

Item	Check
Class	Nitrofurans
Enzyme hydrolysis	No
Acid hydrolysis	No
Alkaline hydrolysis	No
Extraction solvent	Acetonitrile (no special reagent)
Polarity	Reverse column can achieve good separation

乃託文未併入其它方法原因:

1. 優先開發項目，原考慮併入硝基呋喃代謝物方法。但性質不太一樣。
2. 無乃託文殘留標準，此呋喃類藥物國際方法定量極限較低
3. 先從舊方法優化改善，並保留後續方法整併彈性

想法:

感度沒有到很差，以中高階質譜儀來看，執行建議方法定量極限至少可達0.001 ppm。未來會評估併入多重方法可行性。

# 補充說明

Commission regulation (EU) 2019/1871

動物用藥多重方法整併進度

## COMMISSION REGULATION (EU) 2019/1871

of 7 November 2019

on reference points for action for non-allowed pharmacologically active substances present in food of animal origin and repealing Decision 2005/34/EC

(Text with EEA relevance)

ANNEX

Reference points for action (RPA)



行動參考  
指標值



最低檢測能力要求  
(MRPL)

Substance	RPA ( $\mu\text{g}/\text{kg}$ )	Other provisions
Chloramphenicol	0,15	
Malachite green	0,5	0,5 $\mu\text{g}/\text{kg}$ for the sum of malachite green and leucomalachite green
Nitrofurans and their metabolites	0,5 <sup>(1)</sup>	0,5 $\mu\text{g}/\text{kg}$ for each of the metabolites of furazolidone (AOZ or 3-amino-2-oxazolidinone), furaltadone (AMOZ or 3-amino-5-methylmorpholino-2-oxazolidinone), nitrofurantoin (AHD or 1-aminohydantoin), nitrofurazone (SEM or semicarbazide) and nifursol (DNSH or 3,5-dinitrosalicylic acid hydrazide)

<sup>(1)</sup> Due to the natural occurrence of SEM in crayfish at levels above the RPA, only levels of AOZ, AMOZ, AHD and DNSH above the RPA are a clear indication of the illegal use of nitrofurans and their metabolites. The RPA of 0,5  $\mu\text{g}/\text{kg}$  for SEM in crayfish shall only be applied, when the illegal use of nitrofurazone on crayfish has been established.

# 氯黴素、硝基呋喃及孔雀綠檢驗方法日後方向

目前方法	檢驗項目	檢驗基質	定量極限 ( $\mu\text{g}/\text{kg}$ )	MRPL ( $\mu\text{g}/\text{kg}$ )	RPA ( $\mu\text{g}/\text{kg}$ )
				Commission decision 2002/657/EC Annex II	Commission regulation(EU) 2019/1871
氯黴素類抗生素之檢驗 (MOHWV0043.00)	Chloramphenicol	肌肉、蛋類、內臟、乳汁及蜂蜜	0.3	0.3	0.15
硝基呋喃代謝物之檢驗 (MOHWV0040.06)	AMOZ	肌肉、內臟、乳汁及蜂蜜	1	1	0.5 <sup>1</sup>
	AOZ				
	AH				
	SC				
孔雀綠及其代謝物之檢驗 (MOHWV0003.01)	Malachite green	水產品	0.5	sum of malachite green and leucomalachite green	
	Leucomalachite green			2	0.5

<sup>1</sup> Commission regulation(EU) 2019/1871所列硝基呋喃代謝物，包含AMOZ, AOZ, AH, SH及nifursol共五項。

# 動物用藥方法開發策略與標的

## ✓ 動物用藥

→ 針對動物用藥方法進行整併，預計擴增檢驗品項至100項左右，涵蓋基質範圍為禽畜水產品之肌肉、內臟、乳汁、蛋類、蜂蜜及脂肪。本方法暫定為「食品中動物用藥殘留量檢驗方法 - 多重殘留分析(三)」。

目前測試整併包括：5篇公告方法(多重殘留分析(二)、賽滅淨之檢驗、氟苯並嘧啶氨基甲酸之檢驗、Benzimidazole 類多重殘留分析、Triclabendazole及其代謝物之檢驗)及2篇建議方法(抗原蟲劑多重殘留分析(二)、弗雷拉納之檢驗)及部分優先開發項目\*。

\*防檢局&食品組請辦優先開發項目

防：Arprinocid, Bephenium Hydroxynaphthoate, Coumaphos, Nitrovin.

食：Toltrazuril, Coumaphos, Gamithromycin, Tildipirosin



衛生福利部  
食品藥物管理署  
Taiwan Food and Drug Administration

# Thanks for your attention

- 問題與討論



衛生福利部  
食品藥物管理署  
Taiwan Food and Drug Administration

<http://www.fda.gov.tw/>