



Agence fédérale
pour la Sécurité
de la Chaîne alimentaire



ISP-WIV

NRL - Workshop Tranquilizers 21st June 2007

Scientific Institute of Public Health - Food Section

Jànosi Amaya



Programme



ISP-WIV

- 9h00 – 9h30
 - Welcome and introduction to the workshop
- 9h30 – 10h30
 - Overview of analytical methods (litt. + lab)
- 10h30 – 10h45
 - Coffee break
- 10h45 – 12h00
 - Proficiency test on tranquilizers and b-blockers in pig kidney
- 12h00 – 12h30
 - Workshop conclusion





ISP-WIV

Welcome to the NRL workshop

Analysis of tranquilizers and beta-blockers in pig kidney



Why this workshop? – NRL tasks



ISP-WIV

- Development and validation of analytical methods
- *Organisation of workshops*
- Organisation of ringtests
- Delivery of standards or reference material and address of suppliers
- Help, support and guide for other labs in Belgium
- Representation as expert in the EC



Objectives



ISP-WIV

To get the opportunity to discuss with other scientists about :

- Extraction and detection methods used
- Determine and solve problems encountered during the analysis of tranquilizers and beta-blockers
- The proficiency test results (April 2006)
- Find an harmonisation of analytical methods and performance limits between laboratories



Overview



ISP-WIV

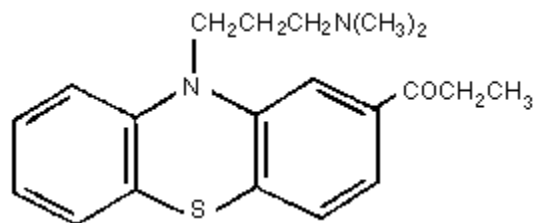
- tranquilizers :neuroleptics
 - agent acting on central nervous system (sedatives)
 - » butyrophenone : azaperone, azaperol
 - » phenothiazines : promazine
 - » thioxanthenes: chlorprotixène
- beta-blockers : anti-adrenergics
 - agent acting on autonomic nervous system (cardio-vascular)
 - » carazolol



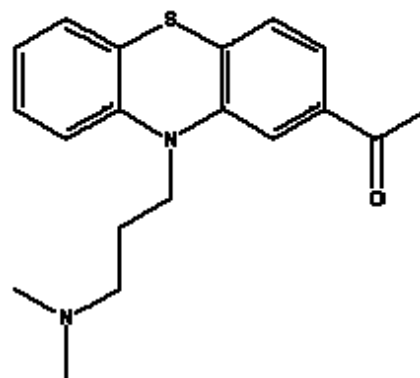


ISP-WIV

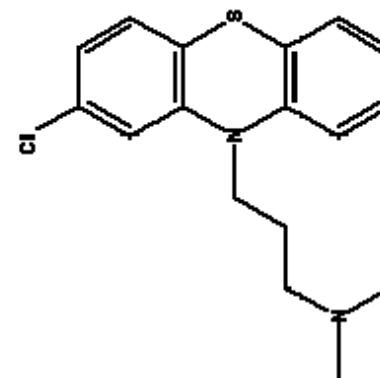
Structure of tranquilizers (1)



Propionylpromazine
Sigma
(C₂₀H₂₄N₂S)



Acepromazine
CRL- RIVM
(C₁₉H₂₂N₂O₂S)



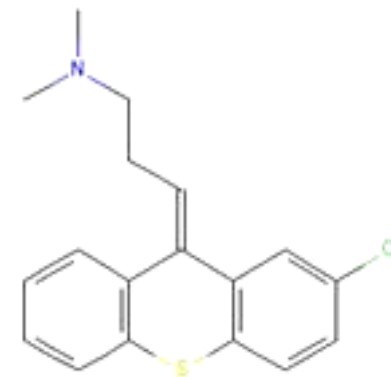
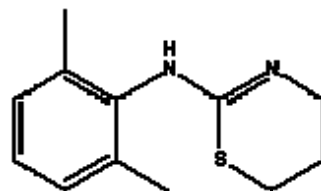
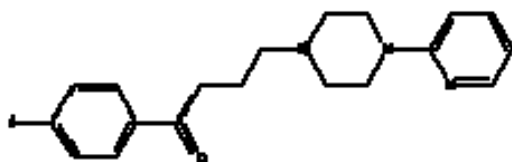
Chlorpromazine
Sigma
(C₁₇H₁₉ClN₂S)



Structure of tranquilizers (2)



ISP-WIV



Azaperone

CRL-RIVM

(C₁₉H₂₂FN₃O)

Xylazine

Sigma

(C₁₂H₁₆N₂S)

Chlorprothixene

Sigma

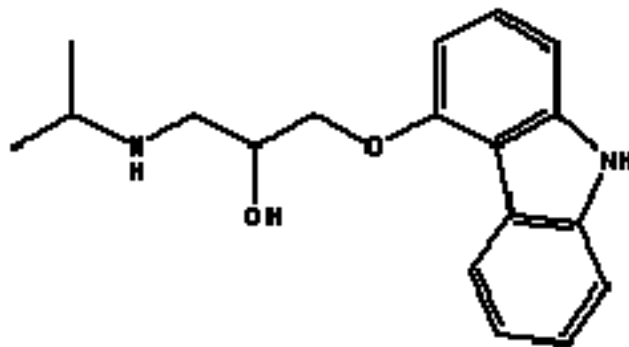
(C₁₈H₁₈ClNS)





ISP-WIV

Structure of beta-blocker (3)



Carazolol
CRL-BVL
(C₁₈H₂₂N₂O₂)



Biotransformation



ISP-WIV

- Hydroxylation and reduction of the ketone function (acepromazine, azaperone, propionylpromazine) → major metabolic pathway in female pigs
 - azaperone → azaperol

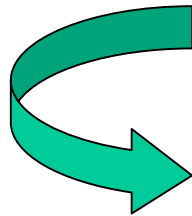


Use



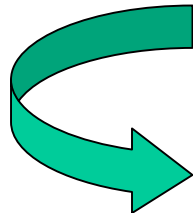
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- To prevent stress in animals (pigs) during transport from the farm to the slaughterhouse



loss of meat quality

- Tranquilizers and beta-blockers frequently used in veterinary medicine.
- Administration before slaughter



residues in edible tissues





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Health's consumer

→ Potential risk !!!

Regulation and surveillance are required !!!

→ Legislation:

→ Council Directive 96/23/EC

→ Monitoring residues



Legislation for pig kidney



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- MRL - EC Regulation 2377/90 : Annex I

Carazolol	25 ppb
Azaperone	$\Sigma = 100$ ppb
Azaperol	

- MRPLs proposed by CRL Bilthoven : Banned substances

Xylazine	50 ppb
Chlorprothixene	50 ppb
Acepromazine	50 ppb
Propionylpromazine	50 ppb
Chlorpromazine	10 ppb

Annex IV





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ISP-WIV

Overview

Analytical Methods

Tranquilizers and beta-blockers

Scientific Institute of Public Health - Food Section

Jànosi Amaya



Plan



ISP-WIV

- **Overview literature**
- ISP/WIV
- FLVVG
- VAHDB
- CER





ISP-WIV

Literature : Screening methods

Mainly Carazolol (other tranquilizers)

Blood, Urine, Serum, muscle, kidney, liver

- RIA
- ELISA
- TLC
- HPLC-UV
- HPLC-Fluo : + derivatisation





ISP-WIV

Reference : screening methods

- E. Rattenberger, P. Matzke, J. Neudegger, Arch. Lebensmittelhyg. 36 (1985) 85.
- N. Haagsma, E.R. Bathelt, J.W. Engelsma, J. Chromatogr. 436 (1988) 73.
- L.A. Van Ginkel, P.L.W.J. Schwillens, M. Olling, Anal. Chim. Acta 225 (1989) 137.
- H.J. Keukens, M.M.L. Aerts, J. Chromatogr. 464 (1989) 149.
- J. Hadrlich, S. Rieger, Dtsch. Lebensm.-Rundsch., 90 (1994) 273.
- W. Arneth, Z. Lebensm.-Unters. Forsch., 201 (1995) 261.
- Cerkvenik-Flajs, V. Anal.Chim.Acta, 586 (2007) 374



Literature Confirmatory methods



ISP-WIV

- MS most efficient technique, multi residues
Tranquilizers + beta-blockers
- Pig kidney, muscle
- lower concentrations (around MR(P)L)
 - GC/MS
 - LC/MS/MS



Reference : confirmatory method



ISP-WIV

- H. Hoogland, W.M.J. Beek, H.J. Keukens, M.M.L. Aerts, Arch. Lebensmittelhyg. 42 (1991) 77.
- Y. Govaert, P. Batjoens, K. Tsilikas, J.M. Degroot, S. Srebrnik, Anal. Chim. Acta 123 (1998) 2507-2512.
- P. Delahaut, P.Y. Brasseur, M. Dubois, J. Chromatogr. A. 1054 (2004) 373.
- D. Van Doorn, P.R. Kootstra, Sterk S.S., poster communication, 5th int. Symp. on Hormone and Veterinary Drugs, Antwerp, Belgium (2006)



Plan



ISP-WIV

- In general (litterature)
- **ISP/WIV**
- FLVVG
- VAHDB
- CER



ISP/WIV : Sample treatment



ISP-WIV

2.5 gr minced sample + IS
chlorpromazine-d3 + haloperidol

extraction with 10 ml of acetonitrile by shaking and centrifugation

defatting the supernatant with hexane

evaporate the extract to dryness under vacuum

dissolution in 10 ml of acetonitrile/water (50/50; v/v)

SPE on OASIS MCX
(Waters)

evaporation until dryness
dissolution in 500 μ l of
ACN/ ammonium acetate buffer

LC-MS/MS





ISP-WIV

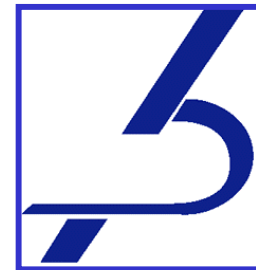
Experimental conditions : HPLC

System: Waters 2695
Alliance

- Column: Symmetry C_{18}
150x2.1 mm, 5 μm
(Waters)
- Autosampler T° : room T°
- Column T° : 30 $^\circ\text{C}$
- Gradient elution:
 - mobile phase A : ACN
 - mobile phase B
:AcONH₄ 0.01 M
 - flow rate of 0,4ml/min

Temps (min)	A %	B %
0	0	100
3	0	100
5	30	70
6	45	55
7	60	40
9	70	30
10	100	0
12	100	0
17	0	100
20	0	100





ISP-WIV

Experimental conditions : MS

System: triple quadrupole Quattro *Micro* (Waters)

- Ionisation: ES with + ion mode
- Capillary: 3.8 kV
- Source T°: 120°C
- Desolvation T°: 400°C
- Cone and collision : see molecules
- Monitoring: MRM mode



Ionic transitions (1)



ISP-WIV

Analytes	Precursor Ion (MH ⁺) (m/z)	Daughter Ions (m/z)	Cone (V)	Collision Energy (eV)
carazolol	299.20	115.7	15	20
		222.1	15	20
azaperol	330.30	120.6	30	23
		312.3	30	15
azaperone	328.30	165	25	20
		120.7	25	21
xylazine	221.15	89.4	20	22
		164	20	24
Haloperidol (SI)	376.20	165.00	40	25



Ionic transitions (2)



ISP-WIV

Analytes	Precursor Ion (MH ⁺) (m/z)	Daughter Ions (m/z)	Cone (V)	Collision Energy (eV)
chlorprothixène	316.10	271.1	25	21
		231	25	33
acepromazine	327.20	85.5	35	20
		57	35	30
propionylpromazine	341.20	85.5	32	22
		57	32	25
chlorpromazine	319.20	85.5	35	18
		57	35	40
Chlorpromazine-d3 (SI)	322.20	88.32	32	19



Chromatogram of a sample

blank pig kidney spiked at

- the MRL level for carazolol, azaperone and azaperol and at

- the proposed MRPL level for xylazine, chlorprothixene, chlorpromazine, acepromazine and propionylpromazine

spike MRL/MRPL (2)21-Feb-200621:41:12

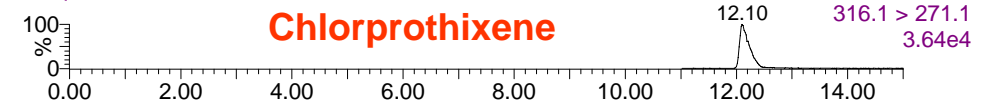
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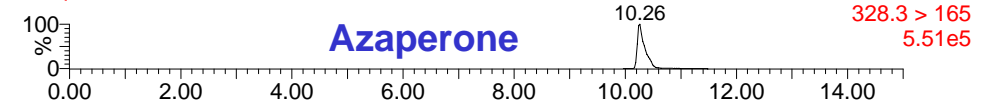
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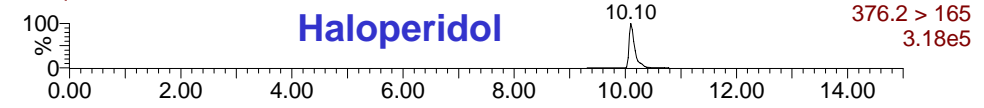
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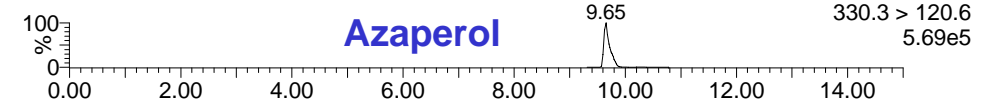
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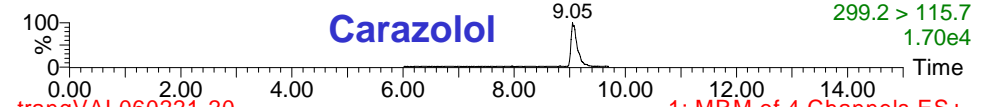
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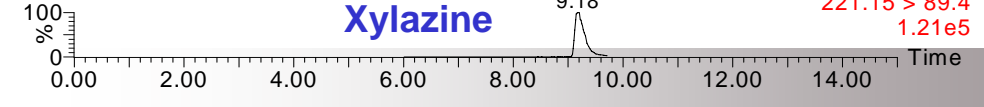
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tranqVAL060221-20



tranqVAL060221-20



Quantitation

- Matrix effect in pig kidney : except for propionylpromazine
- Matrix-matched std calibration curve (5 points)
- Lineaire : except for azaperone
- Recovery correction with a spiked sample except for chlorpromazine (chlorpromazine-d3)
- Recovery range between 80 and 106% at MR(P)L level



Validation parameters



ISP-WIV

Compound	MRL ($\mu\text{g kg}^{-1}$)	CC_{α} ($\mu\text{g kg}^{-1}$)	CC_{β} ($\mu\text{g kg}^{-1}$)
Carazolol	25	30.39	35.76
Azaperone	$\Sigma = 100$	122.81	145.61
Azaperol		115.14	130.24
Proposed	MRPL ($\mu\text{g kg}^{-1}$)		
Xylazine	50	14.78	26.21
Chlorprothixene	50	28.01	49.68
Acepromazine	50	10.98	< 25
Propionylpromazine	50	7.25	< 25
Chlorpromazine	25	5.08	< 12.5





ISP-WIV

ISP/WIV method : conclusion

- multiresidues method for 6 tranquilizers and 1 beta-blocker in pig kidney
- qualitative method:
 - chlorprotixene → screening
- quantitative method:
 - carazolol, azaperol, azaperone (MRL)
 - xylazine
 - acepromazine, propionylpromazine, chlorpromazine



Plan



ISP-WIV

- In general (litterature)
- ISP/WIV
- **FLVVG**
- VAHDB
- CER





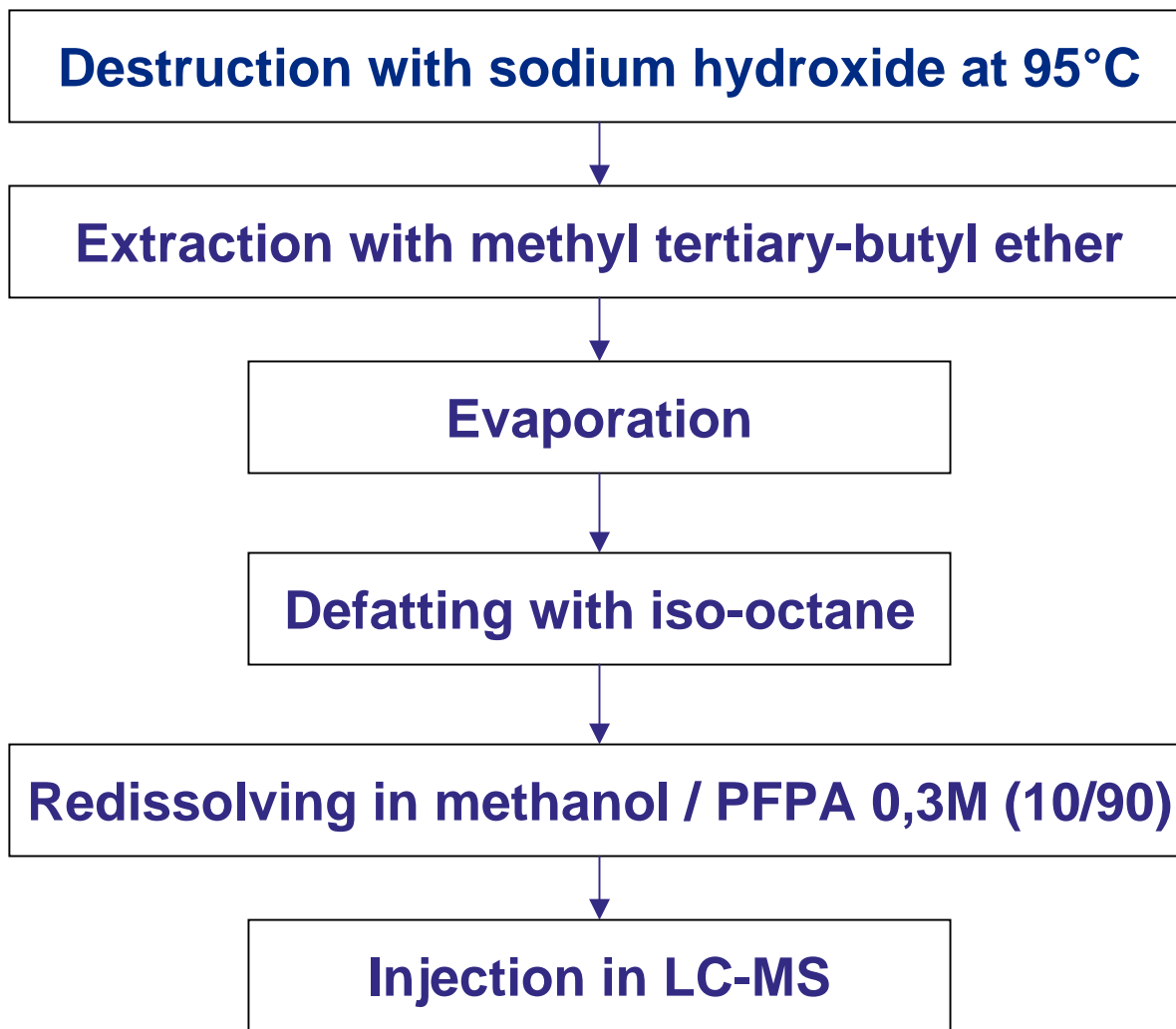
Federaal Agentschap
voor de veiligheid
van de Voedselketen

TRANQUILIZERS AND β -BLOCKERS IN MEAT

FLVVG



Extraction and clean-up

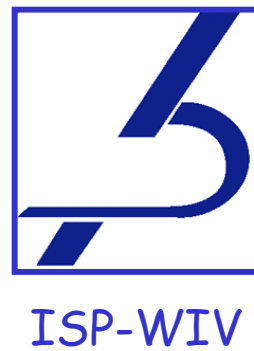


Analysis: LC-MSⁿ -method

- **Column:**
Varian Pursuit Diphenyl 3 μ 150 mm x 2.0 mm
- **Mobile phase:**
PFPA 10 mM / ACN (60/40) - 0,25 ml/min
- **Ionisation method:**
ESI
- **Recorded ions:**
Full scan MS² of the protonated molecular ion



Plan



- In general (litterature)
- ISP/WIV
- FLVVG
- **VAHDB**
- CER



LIMITS



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azaperone	+ azaperol	Porc	100 µg/kg*	Meat
			100 µg/kg*	Kidney
carazolol		Bovine	5 µg/kg*	Meat
			15 µg/kg*	Kidney
		Porc	5 µg/kg*	Meat
			25 µg/kg*	Kidney

*<http://pharmacos.eudra.org> 12 october 2005

- **haloperidol, propionylpromazine, xylazine, acepromazine, chlorprothixene 10 µg/kg**
- **chlorpromazine MRPL 25 µg/kg**



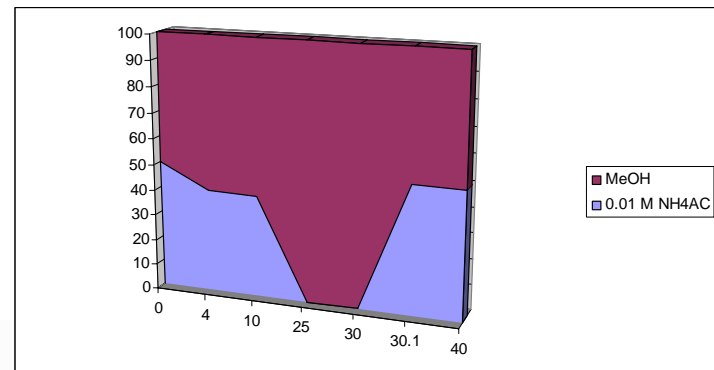
METHOD



FACULTY OF VETERINARY MEDICINE
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- Extraction with AcN
- Clean-up: Oasis HLB
- LC-MS-MS
- Nucleodur C18 Gravity, 5 μm (Macherey-Nagel)
- 0.01 M Ammoniumacetate and MeOH

Delahaut et al., 2003 & 2004



MS-PARAMETERS



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		Parent ion	Product ion
azaperol	azl	330	149, 192, 312
carazolol	car	299	116, 196, 222,
Azaperone	aza	328	121, 147, 165
Propionylpromazine	prp	341	240, 268, 296
Xylazine	xyl	221	147, 164
Acepromazine	acp	327	240, 254, 282
Chloorpromazine	chlprom	319	274, 246, 239,
Chloorprothixene	chlp	316	231, 271, 273
<i>Isobutcar 61 (=IS)</i>		313	130, 196, 222
<i>Haloperidol (=IS)</i>	<i>Hpd</i>	376	123, 165, 358

LC-MS²: STANDARD

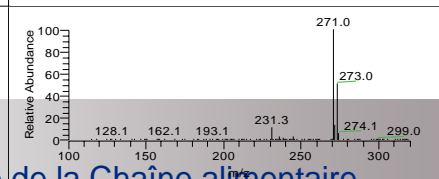
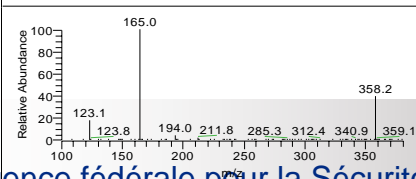
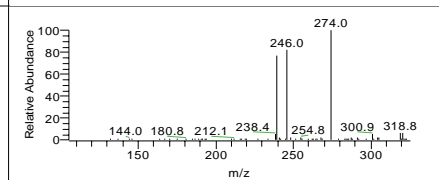
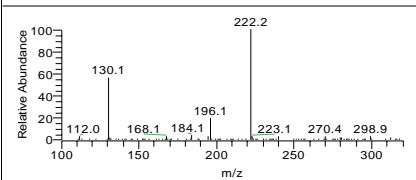
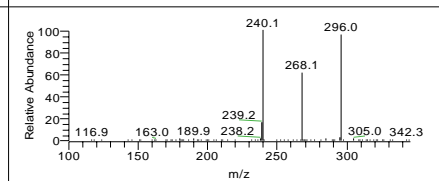
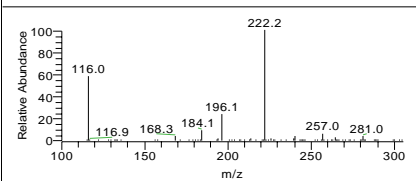
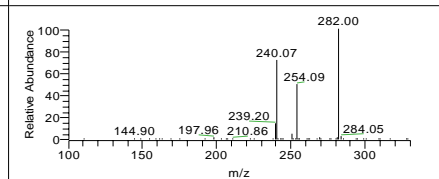
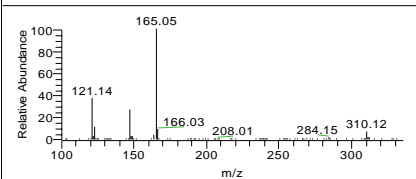
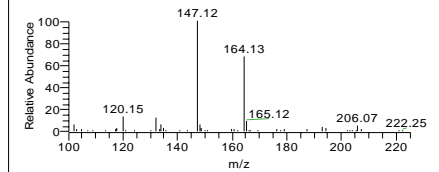
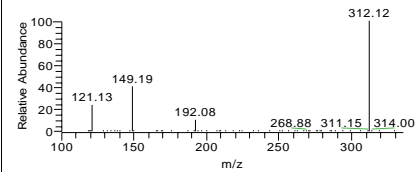
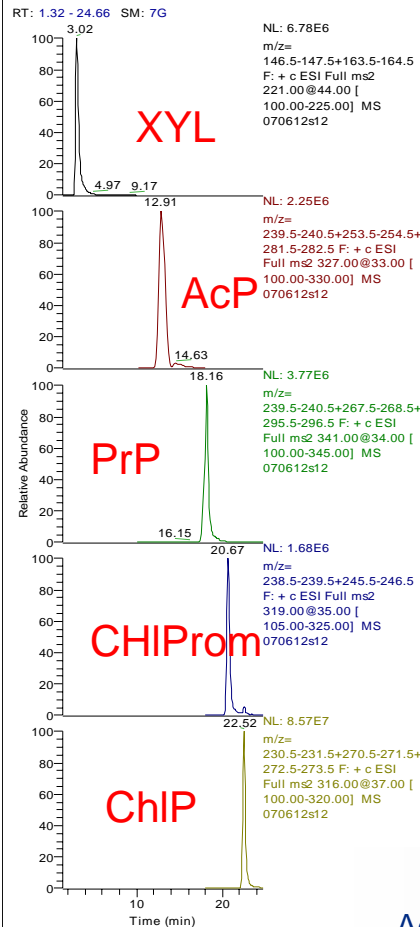
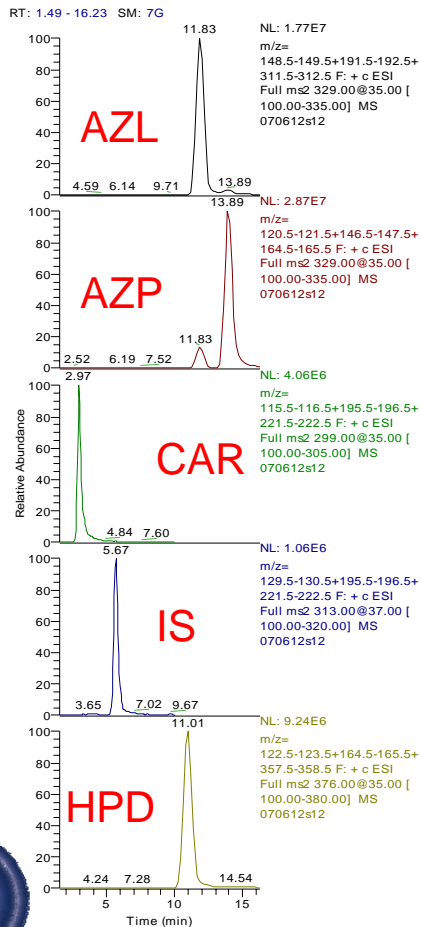


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standaard PT



LC-MS²: PL-SPIKE

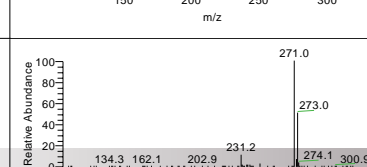
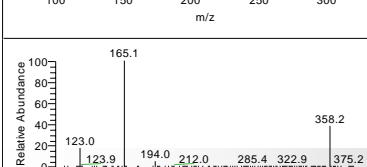
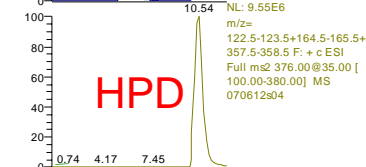
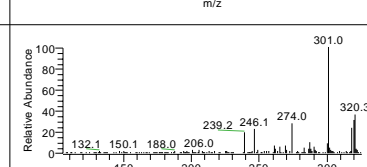
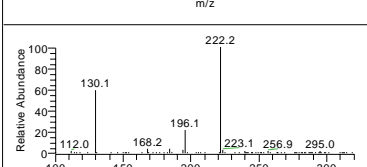
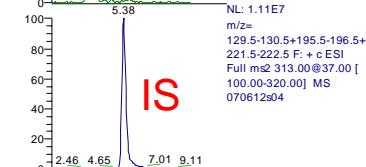
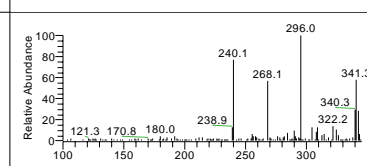
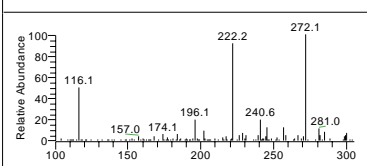
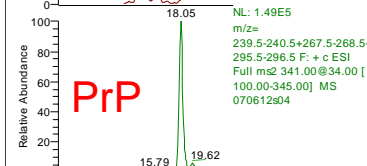
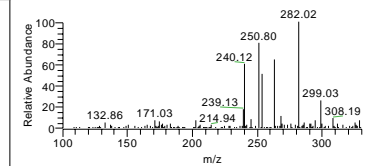
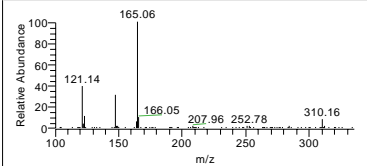
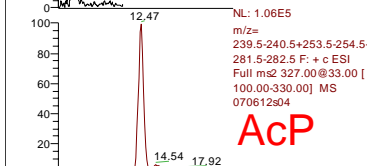
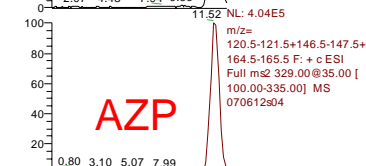
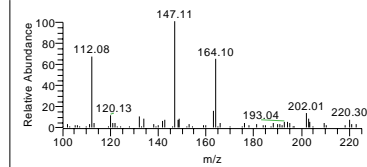
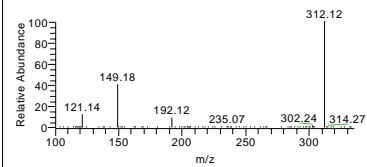
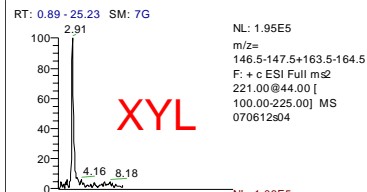
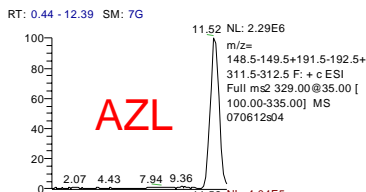


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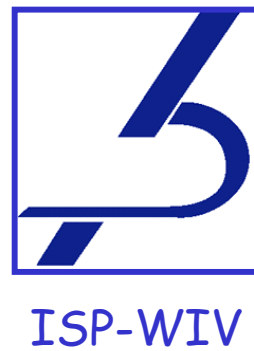
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nucleodur+pre-mei 07

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Q195B



Plan



- In general (litterature)
- ISP/WIV
- FLVVG
- VAHDB
- **CER**





ISP-WIV

Extraction methods: comparison

Sample amount	2.5g	5g	2g	
Extraction solvent	ACN	ACN	NaOH 95°C MTBE	ACN
Defatting	Hexane	/	Isooctane	
SPE	Oasis MCX	/	/	Oasis HLB
dissolution	ACN/AcO NH ₄ 0,01M	ACN/H ₂ O	MeOH/PFP A 0,3M	MeOH/AcO NH ₄ 0,01M



Analytical methods: comparison



ISP-WIV

Inj. vol	10 µl	100 µl	100 µl	
HPLC column	Waters Symmetry C18 (5µm)	Biomatrix + Lichrochart Purospher RP18	Varian Pursuit Diphenyl (3µm)	Nucleodur C18 Gravity (5µm)
Mobile phase	AcONH4 → ACN gradient	AcONH4 → ACN gradient	PFPA 10mM / ACN isocratic	AcONH4 0,01M / MeOH
Run time	15 min	12 min		25 min
MS mode	ESI + MRM	ESI + MRM	ESI + Full scan MS/MS	ESI + MRM



Détection des tranquillisants, de la xylazine et des beta bloquants dans le rein, le muscle par LC-MS/MS

P.-Y. BRASSEUR



1. Description

- Molécules recherchées :
 - ❑ promazines (propionylpromazine, acépromazine et chlorpromazine)
 - ❑ dérivés de la butyrophénone (azapérol et azapérone)
 - ❑ carazolol (beta bloquants)
 - ❑ chlorprothixène
 - ❑ xylazine
- promazines et chlorprothixène : interdits (MRPL)
- carazolol et dérivés de la butyrophénone (LMR)

1. Description

- promazines et chlorprothixène : interdits (MRPL)
- carazolol et dérivés de la butyrophénone (LMR)

TABLEAU DES LMR			
<i>MATRICE</i>	<i>AZAPERONE AZAPEROL ($\mu\text{g}/\text{kg}$) porc et bovin</i>	<i>CARAZOLOL ($\mu\text{g}/\text{kg}$) porc</i>	<i>CARAZOLOL ($\mu\text{g}/\text{kg}$) bovin</i>
Muscle	Somme = 100	5	5
Rein	Somme = 100	25	15

2. Matériel

- Réactifs
 - ❑ Acétonitrile HPLC (Acros Organics)
 - ❑ H₂O HPLC (Acros Organics)
 - ❑ Acétate ammonique P.A. (NH₄Ac) (VWR)
 - ❑ Ethanol P.A. (VWR)

2. Matériel

- Standards

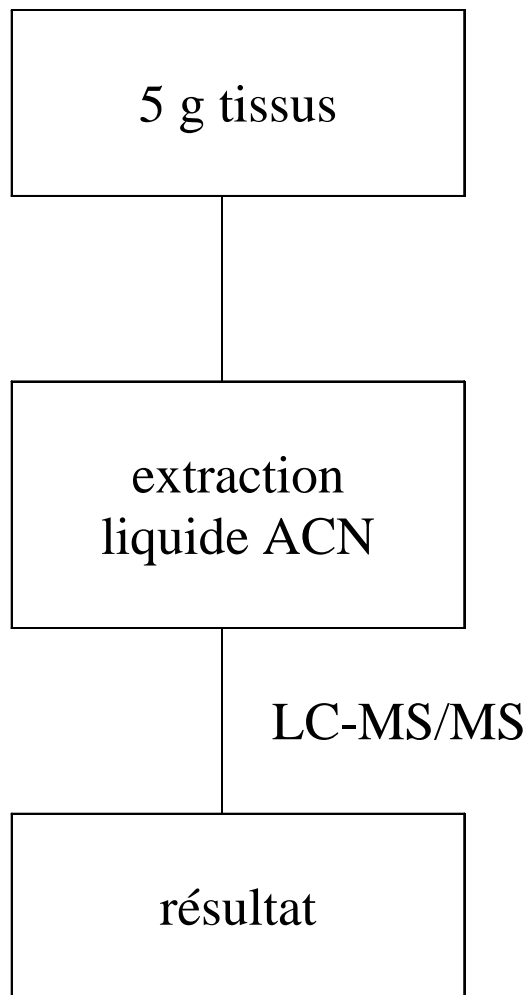
-azapérol	Janssen, lot V897-24
-azapérone	Janssen, lot V880268
-carazolol	Boehringer Mann.lot 438393000
-chlorpromazine	Sigma, lot 48H1403
-acépromazine	Sigma, lot 47F0403
-propionylpromazine	Sigma, lot 34H0942
-chlorpromazine-d ₃ (SI)	Radian, lot MMP-24543-45D
-halopéridol (SI)	Sigma, lot 18H0408
-isobutcar 61* (SI)	molécule synthétisée chez H. De Brabander
-chlorprothixène	Sigma, lot 40H0346
-xylazine	Bayer R-159-1

- Echantillons biologiques
muscles, reins provenant d'animaux non traités

2. Matériel

- Matériel pour la détection
 - ❑ Chaîne HPLC Hewlett Packard série 1100
 - Colonne Biomatrix (50 x 4,6 mm) (Chrompack 29230)
+ précolonne Biomatrix (Chrompack 28168)
 - Colonne Merck Lichrochart Purospher RP-18 (Merck 1-5079980)
125 X 3 mm + précolonne Merck Purospher RP-18
(Merck 1-50167)
 - ❑ Spectromètre de masse - Quattro Ultima Platinum
(triple quadruple) (Waters)

3. Principe général



4. Description de la méthode

4.1. Extraction

- ❑ 5 g de tissu + IS
- ❑ ajout de 20 ml ACN (agitation 15')
- ❑ centrifugation (10' à 4000 RPM)
- ❑ prélèvement d'1 ml de surnageant
- ❑ évaporation au bain-marie à 60 °C sous flux d'azote
- ❑ dissolution de l'extrait dans 0,8 ml de mélange ACN/H₂O (20/80)

4. Description de la méthode

4.2. Analyse de l'extrait par LC-MS/MS

□ Conditions HPLC

- débit d'élution : 1 ml/min
- volume d'injection de l'extrait purifié : 100 µl
- température du four : 50 ± 2 °C
- gradient appliqué

Temps (min.)	ACN (%)	Acétate d'Ammonium 0,1M
0,0	0	100
3,0	0	100
5,0	30	70
6,0	45	55
7,0	60	40
9,0	70	30
10,0	100	0
11,0	0	100
12,0	0	100

4. Description de la méthode

4.2. Analyse de l'extrait par LC-MS/MS

□ Conditions MS/MS

- source ESI en mode +, MRM, t° de désolvation de ± 300 °C
- t° de la source : ± 120 °C
- gaz de cône : 150 l/h
- gaz de désolvation : 750 l/h
- split sur le flux HPLC : 1/3

4. Description de la méthode

4.2. Analyse de l'extrait par LC-MS/MS

MRMs utilisés

Molécules	Ion parent (m/z)	Ion fille (m/z)	Voltage de cône (V)	Energie de collision (eV)
Azapérol	330,0	148,9	50	20
		120,9	50	20
Azapérone	328,0	164,9	50	18
		120,9	50	18
Carazolol	299,0	116,0	50	18
		222,0	50	18
Chlorpromazine	319,0	86,1	50	18
		58,2	50	19
Acépromazine	327,0	86,1	50	18
		58,2	50	19
Propionylpromazine	341,0	86,1	50	18
		58,2	50	21
Chlorprothixène	316,0	270,8	50	17
		230,8	50	21
Chlorpromazine-d ₃	322,0	89,1	50	17
Halopéridol	376,0	164,9	50	20
Isobutcar 61 *	313,0	130,0	50	18
Xylazine	221	90/147	50	18/18

5. Résultats de validation

- viande

ECHANTILLONS (matrices)	SUBSTANCES RECHERCHEES	CC α (ppb)	CC β (ppb)
MUSCLE (prise d'essai : 5 g)	azapérol	56	64,0
	azapérone	56	64,2
	somme azaperone et azaperol	111	121
	carazolol	5,6	6,1
	chlorpromazine	1,75	2,00
	chlorprothixène	1,77	2,00
	xylazine	1,38	2,00
	acépromazine	1,72	2,00
	propionylpromazine	1,81	2,00

5. Résultats de validation

- **rein**

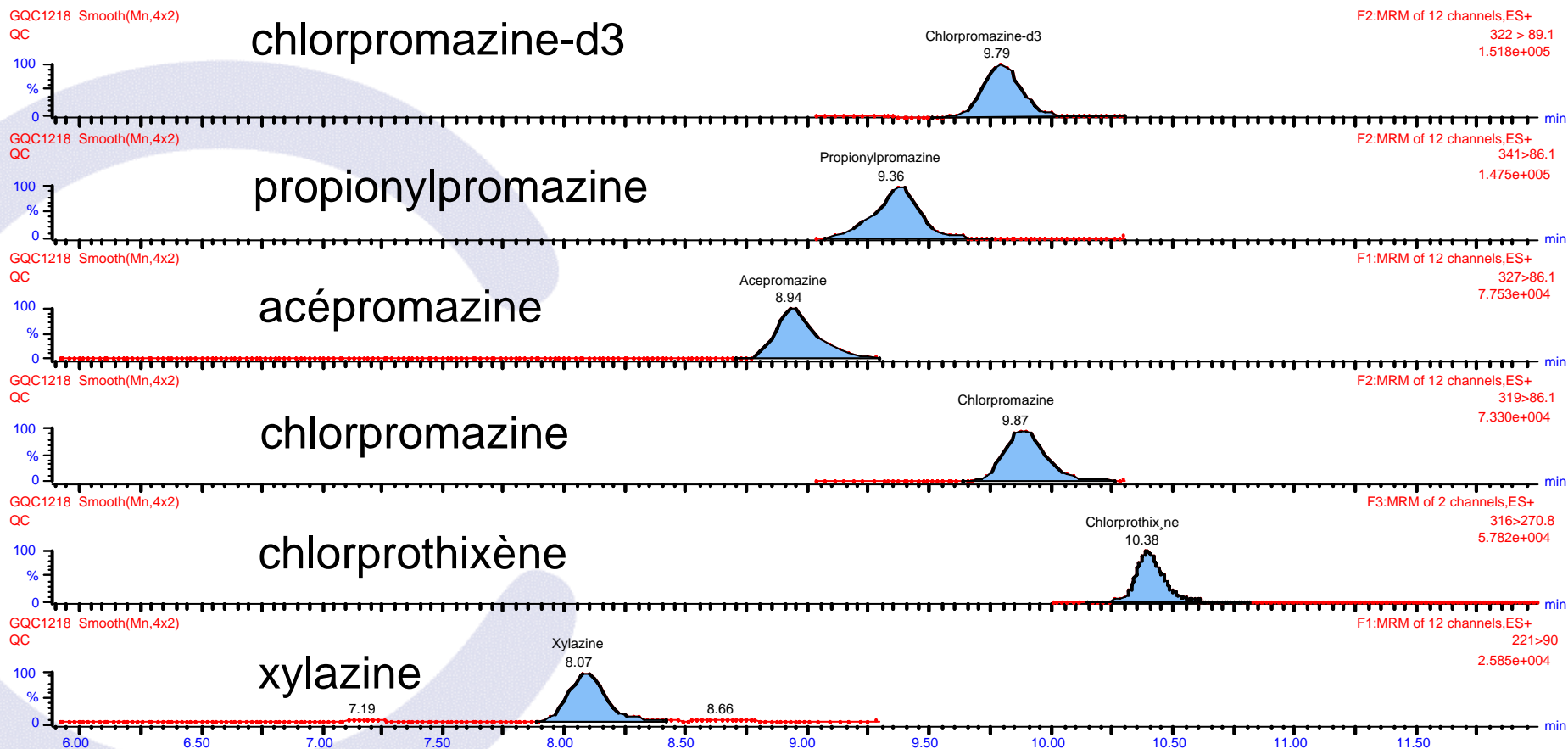
ECHANTILLONS (matrices)	SUBSTANCES RECHERCHEES	CC α (ppb)	CC β (ppb)
REIN (prise d'essai : 5 g)	azapérol	54	59,0
	azapérone	56	62,0
	somme azaperone et azaperol	109	118
	carazolol	26	28,0
	chlorpromazine	1,80	2,00
	chlorprothixène	1,65	2,00
	xylazine	1,48	2,00
	acépromazine	1,76	2,00
	propionylpromazine	1,83	2,00

6. Quantification

- Courbe standard en 6 points extraite dans la matrice
- Composés et standard interne correspondant

Composé	SI correspondant	MRL/MRPL
azapérol	halopéridol	MRL
azapérone		MRL
carazolol	isobutcar 61*	MRL
chlorpromazine	chlorpromazine-D ₃	MRPL
acépromazine		
propionylpromazine		
xylazine		
chlorprothixène		

Chromatogrammes des promazines



Chromatogrammes des tranquillisants et beta bloquants

GQC1218 Smooth(Mn,2x2)
QC

halopéridol



F1:MRM of 12 channels,ES+
376>164.9
2.838e+006

GQC1218 Smooth(Mn,2x2)
QC

azapérol



F1:MRM of 12 channels,ES+
330>120.9
5.314e+005

GQC1218 Smooth(Mn,2x2)
QC

azapérone



F2:MRM of 12 channels,ES+
328>164.9
1.751e+005

GQC1218 Smooth(Mn,2x2)
QC

isobutcar 61*



F1:MRM of 12 channels,ES+
313>130
7.357e+005

GQC1218 Smooth(Mn,2x2)
QC

carazolol



F1:MRM of 12 channels,ES+
299>116
5.387e+004

Kits ELISA disponibles en notre laboratoire pour la détection des tranquillisants

- Generic promazine (réf. E.D.1)
- Azaperone-azaperol 2 hours (réf. E.D.2)
- Carazolol 2 hours (réf. E.D.3)

CER Groupe

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Publications

- Determination of a method for detecting and quantifying azaperone, azaperol and carazolol in pig tissues by liquid chromatography-tandem mass spectrometry.
 FLUCHARD D., KIEBOOMS S., DUBOIS M., DELAHAUT P.
 Journal of Chromatography, 2000, 744, 139-147.
- Validation of a method for detecting and quantifying tranquillisers and a beta-blocker in pig tissues by liquid chromatography-tandem mass spectrometry.
 DELAHAUT P., LEVAUX C., ELOY P., DUBOIS M.
 Analytica Chimica Acta, 2003, 483, 335-340.
- Multiresidue method for the detection of tranquillisers, xylazine, and a β -blocker in animal production by liquid chromatography-tandem mass spectrometry.
 DELAHAUT P., BRASSEUR P.-Y., DUBOIS M.
 Journal of Chromatography A, 2004, 1054, 373-378.

Merci de votre bonne attention!



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ISP-WIV

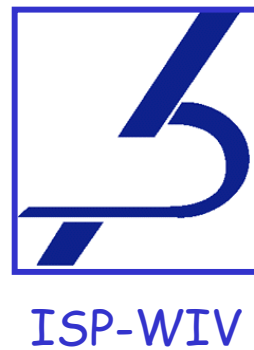
NRL - Interlaboratory study Tranquilizers and beta-blockers April 2006

Scientific Institute of Public Health - Food Section

Jànosi Amaya



Inter-laboratory study



- ILS has been organized in April – June 2006
- Identification of tranquilizers and beta-blockers residues in pig kidney
- invitations (11 labs) – participation (4 labs)
- screening and/or confirmatory method
- materials:
 - 2 treated animals
 - + 4 spiked tissues



Planning of the study (2006)



ISP-WIV

- February : 11 invitations
- April : samples dispatched to 4 labs
- Analyses done within 3 weeks (stability)
- June : reception of results + report



Materials : pig kidney



ISP-WIV

	analytes	Concentration	MRL / MRPL
SAMPLE 1 / 5	/	/	
SAMPLE 2	chlorpromazine	26 ppb	10 ppb
<u>SAMPLE 3</u>	carazolol	13 ppb	25 ppb
<u>SAMPLE 4</u>	Acepromazine Azaperol Azaperone	65 ppb 122 ppb 44 ppb	50 ppb $\Sigma = 100$ ppb
SAMPLE 6	Xylazine Azaperol Azaperone	60 ppb 40 ppb 40 ppb	50 ppb $\Sigma = 100$ ppb

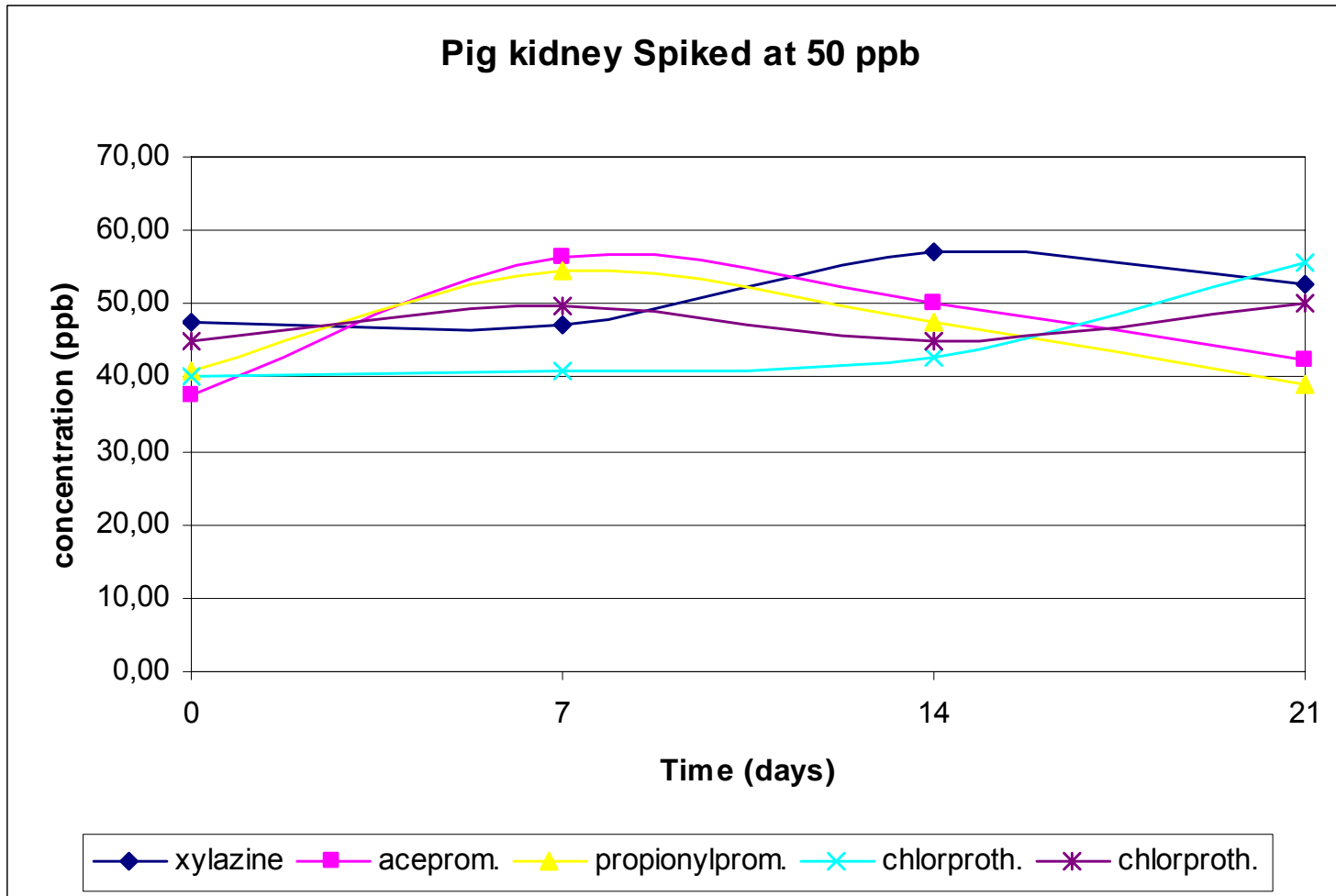


Stability Study		Xylazine	carazolol	aceprom.	azaperol	azaperone	propionylpro.	chlorproth.	chlorproth.	chlorpro.
		MRL / MRPL	50µg/kg	25µg/kg	50µg/kg	Σ = 100µg/kg		50µg/kg	50µg/kg	50µg/kg
IS		halop.	halop.	chlor-d3	halop.	halop.	chlor-d3	halop.	chlor-d3	chlor-d3
Day 0	1	49,33	27,65	40,01	93,04	104,79	37,11	58,69	38,3	24,4
	2	43,07	21,25	37,71	93,13	92,88	45,24	30,94	74,19	23,39
	3	50,6	30,67	35,51	104,25	111,46	40,73	30,31	21,9	22,91
	mean	<u>47,67</u>	<u>26,52</u>	<u>37,74</u>	<u>96,81</u>	<u>103,04</u>	<u>41,03</u>	<u>39,98</u>	<u>44,80</u>	<u>23,57</u>
	std dev	4,03	4,81	2,25	6,45	9,41	4,07	16,21	26,74	0,76
	CV (%)	8,5	18,1	6,0	6,7	9,1	9,9	40,5	59,7	3,2
D0 +7days	1	47,77	20,84	53,61	79,73	91,2	51,8	45,51	47,65	22,27
	2	44,63	21,35	55,66	83,34	81,42	53,2	34,5	43,7	18,61
	3	48,55	22,31	59,9	83,4	90,79	58,64	42,38	57,84	23,28
	mean	<u>46,98</u>	<u>21,50</u>	<u>56,39</u>	<u>82,16</u>	<u>87,80</u>	<u>54,55</u>	<u>40,80</u>	<u>49,73</u>	<u>21,39</u>
	std dev	2,08	0,75	3,21	2,10	5,53	3,61	5,67	7,30	2,46
	CV (%)	4,42	3,47	5,69	2,56	6,30	6,62	13,91	14,67	11,49
D0 +14days	1	59,23	25,64	44,98	106,19	104,75	43,78	43,52	38,2	21,91
	2	54,97	25,16	54,04	94,46	81,95	48,19	39,68	47,32	22,82
	3	57,38	25,83	51,61	106,45	106,97	50,41	45,09	49,53	23,08
	mean	<u>57,19</u>	<u>25,54</u>	<u>50,21</u>	<u>102,37</u>	<u>97,89</u>	<u>47,46</u>	<u>42,76</u>	<u>45,02</u>	<u>22,60</u>
	std dev	2,14	0,35	4,69	6,85	13,85	3,37	2,78	6,01	0,61
	CV (%)	3,73	1,35	9,34	6,69	14,15	7,11	6,51	13,34	2,72
D0 +21days	1	56,63	23,18	32,93	89,02	95,32	30,96	65,62	49,09	24,77
	2	51,53	20,74	36,99	81,59	85,43	35,55	54,76	49,72	24,59
	3	49,39	21,07	57,12	84,58	85,87	51,03	46,82	51,28	24,38
	mean	<u>52,52</u>	<u>21,66</u>	<u>42,35</u>	<u>85,06</u>	<u>88,87</u>	<u>39,18</u>	<u>55,73</u>	<u>50,03</u>	<u>24,58</u>
	std dev	3,72	1,32	12,95	3,74	5,59	10,52	9,44	1,13	0,20
	CV (%)	7,08	6,11	30,59	4,39	6,29	26,84	16,93	2,25	0,79

Stability study (1)



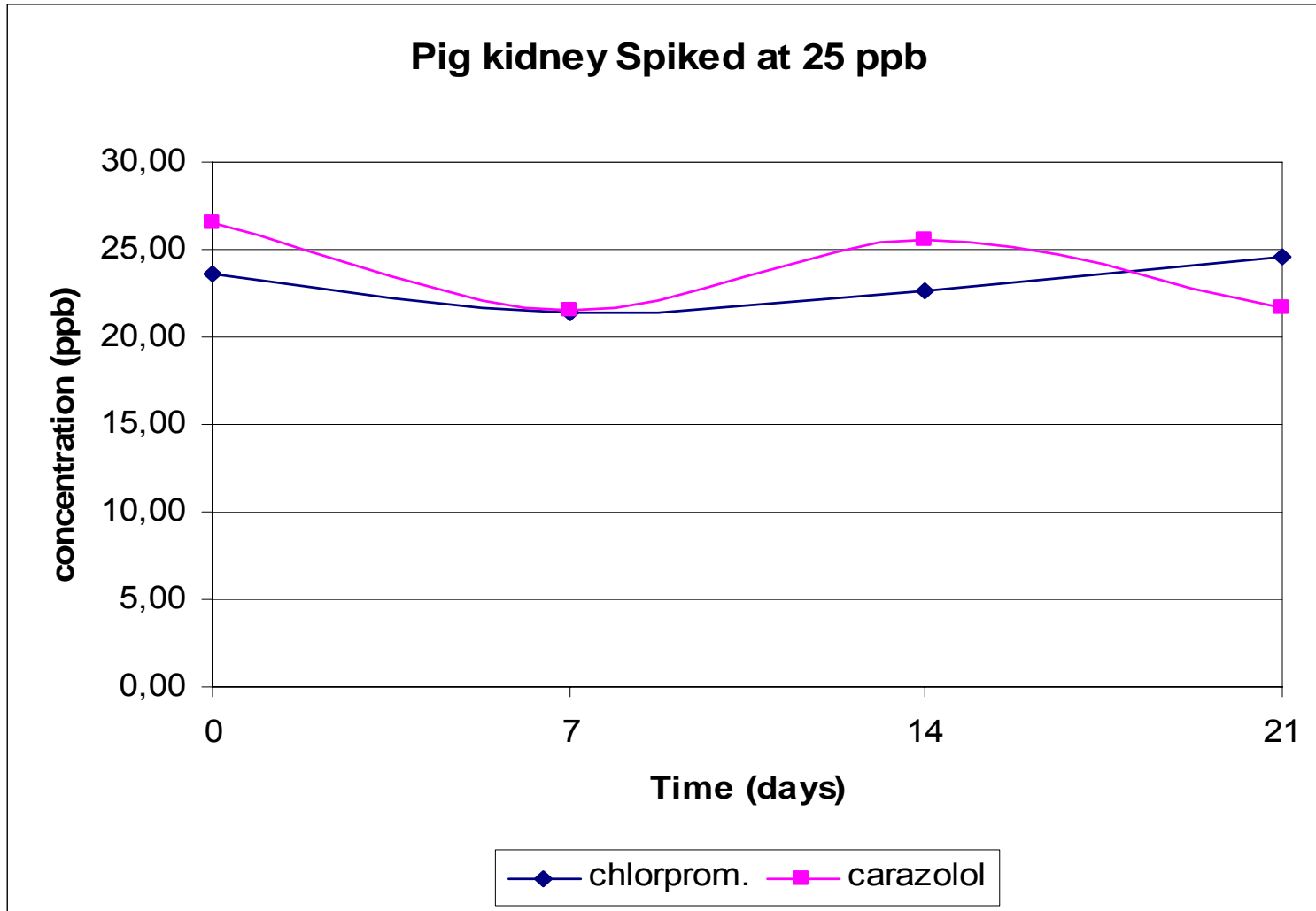
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Stability study (2)



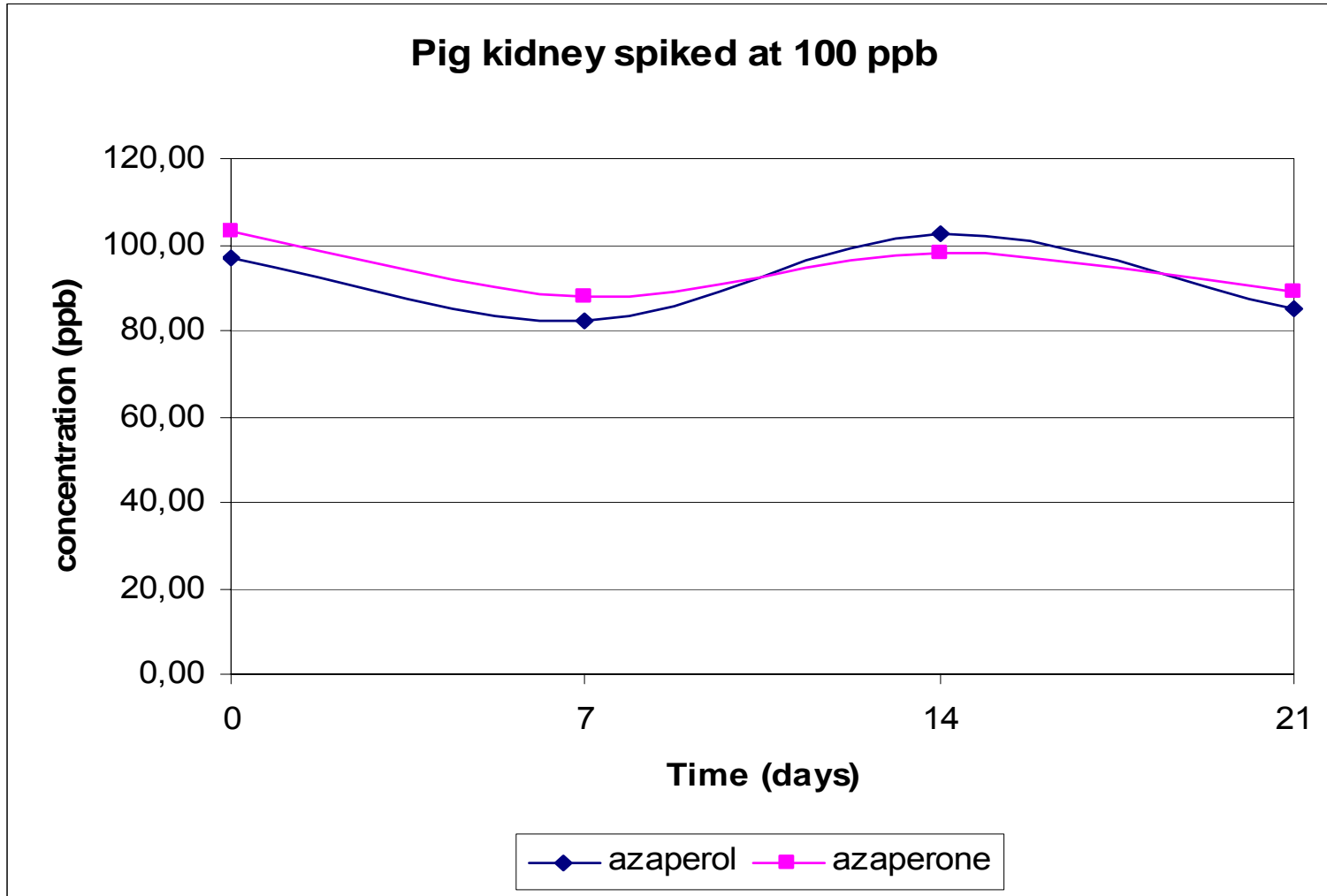
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Stability study (3)



ISP-WIV





ISP-WIV

Homogeneity : treated pig kidney

SAMPLE 3	MRL = 25ppb	SAMPLE 4	MRPL = 50ppb	MRL Σ = 100 μ g/kg (MRL)		MRL = 25ppb
	Carazolol μ g/kg		Acepromazine μ g/kg	Azaperol μ g/kg	Azaperone μ g/kg	Carazolol μ g/kg
Day 1	12,14	Day 1	62,30	131,36	47,36	0,53
Day 1	12,68	Day 1	67,44	131,81	48,81	0,71
Day 1	11,88	Day 1	60,96	118,69	42,90	0,44
Day 1+10	13,03	Day 1+10	66,03	112,75	41,65	0,96
Day 1+10	12,74	Day 1+10	(101,51)	120,15	40,77	1,05
Mean (n=5)	12,49		64,18 (71.65)	122,95	44,30	0,74
Std dev	0,47		3,05	8,36	3,58	0,26
CV (%)	3,76		4,76 (23.59)	6,80	8,07	35,82



Analytical method



ISP-WIV

Code labo	IS used	IS for which molecule	Calibration curve	# point curve	LCMS/MS	Correction for recovery	screening
A	haloperidol		matrice	6	yes	by IS	no
B	Isobutcar 61 Haloperidol Chlorpromazine d3	carazolol azaperone – ol promazines	matrice	6	yes	by IS	yes
C	haloperidol		matrice	9	yes	by spikes	no
D	Haloperidol Chlorpromazine d3	xylazine carazolol azaperone – ol Promazine chlorprotixene	matrice	6	yes	by spikes By IS	no



Results



ISP-WIV

- Banned / not authorized substances:
 - quantitative evaluation ($<$ or $>$ MRPL suggested)
- MRL substances:
 - quantification ($<$ or $>$ MRL) carazolol and azaperol + azaperone
- 4 labs \rightarrow no statistics !



Qualitative : Identification

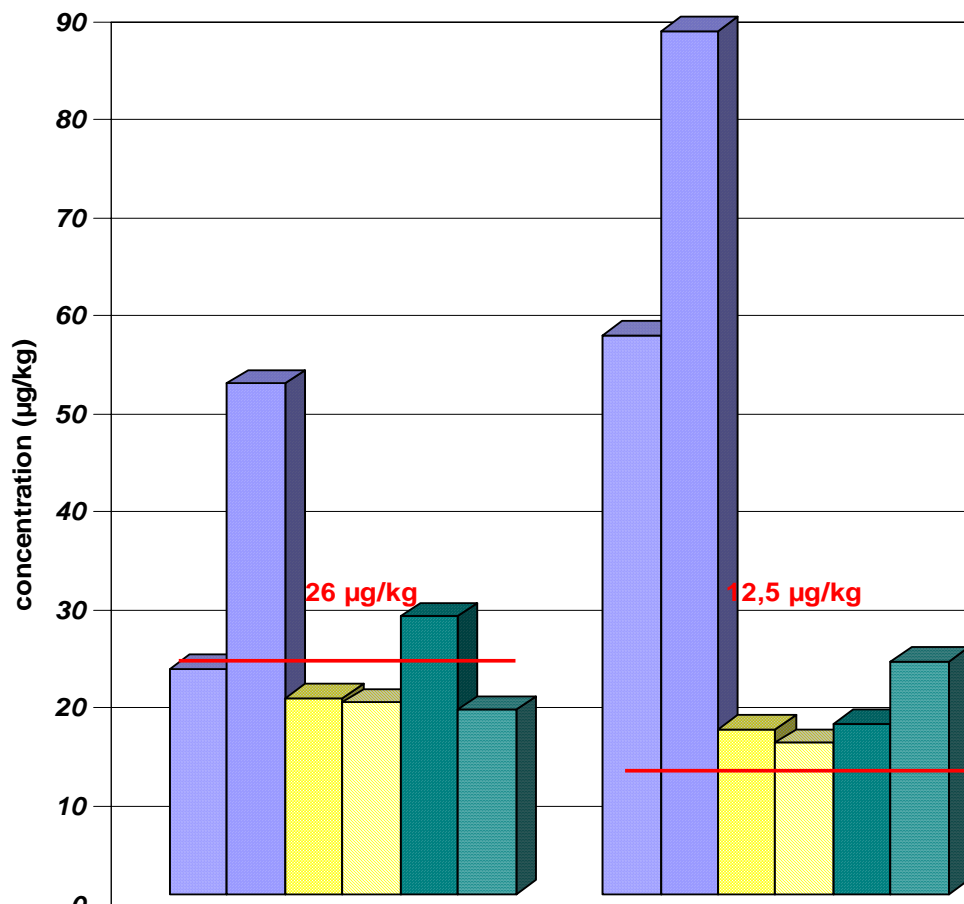


ISP-WIV

Labo	A LCMS/MS	B LCMS/MS	B ELISA	C LCMS/MS	D LCMS/MS	Assigned result
SAMPLE 1	/ C	/ C	/ C	/	/ C	/ C
SAMPLE 2	Chlorpromazine NC	Chlorpromazine NC	Promazine group > 1ppb	Chlorproma zine	Chlorproma zine NC	Chlorproma zine NC
SAMPLE 3	Carazolol NC	Carazolol C	Carazolol > 12.5ppb	Carazolol	Carazolol C	Carazolol C
SAMPLE 4	acepromazine azaperol Azaperone NC	acepromazine azaperol azaperone Carazolol NC	Promazine group > 1ppb Aza- ol+one >50ppb	acepromazine azaperol Azaperone	acepromazine azaperol azaperone Carazolol NC	acepromazine azaperol azaperone Carazolol NC
SAMPLE 5	/ C	/ C	/ C	/	/ C	/ C
SAMPLE 6	xylazine azaperol Azaperone NC	xylazine azaperol Azaperone NC	Aza- ol+one >50ppb	xylazine azaperol Azaperone	xylazine azaperol Azaperone NC	xylazine azaperol Azaperone NC

Quantitative (1)

Sample 2 and 3

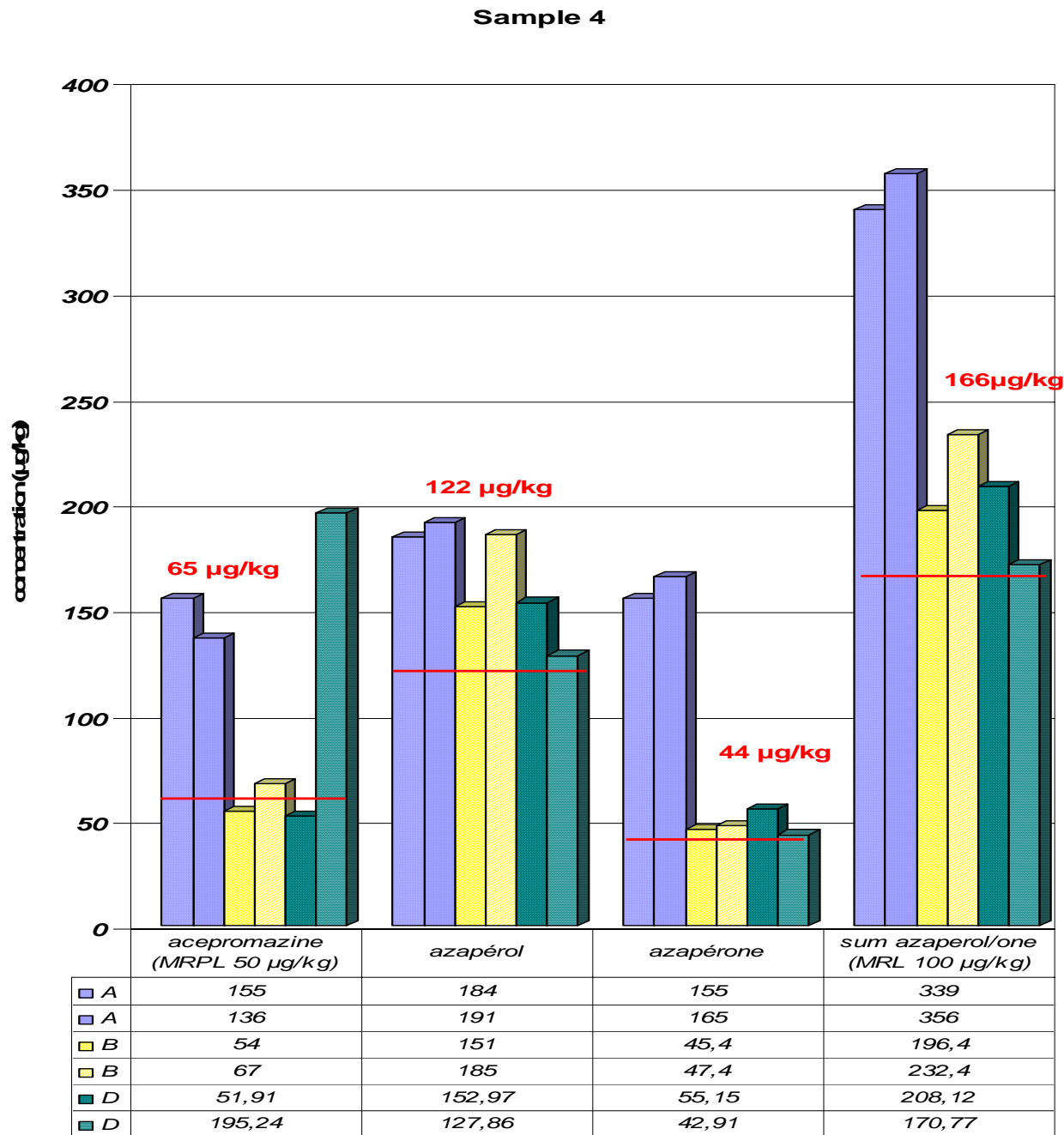


	chlorpromazine (MRPL 25 µg/kg)	carazolol (MRL 25 µg/kg)
■ A	23	57
■ A	52	88
■ B	20	16,8
■ B	19,5	15,4
■ D	28,28	17,32
■ D	18,78	23,71

lab code / molecules



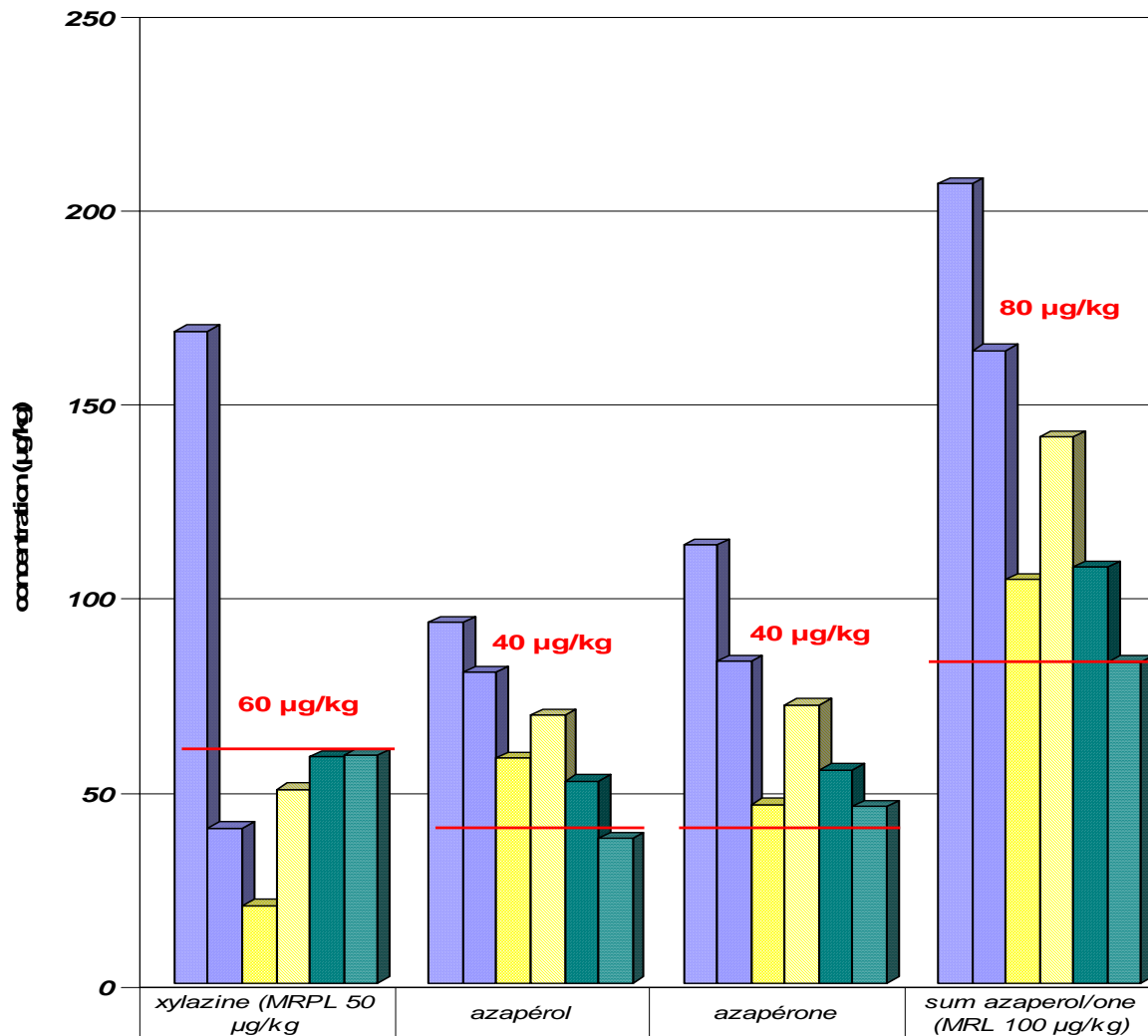
Quantitative (2)



lab code / molecules

Quantitative (3)

Sample 6



	<i>xylazine (MRPL 50 µg/kg)</i>	<i>azapérol</i>	<i>azapérone</i>	<i>sum azaperol/one (MRL 100 µg/kg)</i>
■ A	168	93	113	206
■ A	40	80	83	163
■ B	20	58	46	104
■ B	50	69	71,7	140,7
■ D	58,35	52,15	54,91	107,06
■ D	58,73	37,28	45,48	82,76

lab code / molecules



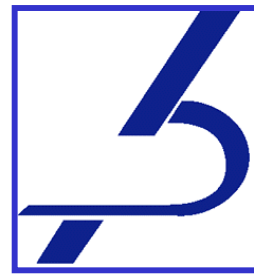
ISP-WIV

Interlaboratory study: Conclusion

- 6 samples : 2 incurred + 2 blancs + 2 spiked
- sample 2: chlorpromazine >oldMRPL(25ppb) >> newMRPL (10ppb)
- sample 6: xylazine > MRPL; azaperone + azaperol < MRL
- treated animals:
 - sample 3: carazolol 1/2MRPL
 - sample 4: acepromazine >MRPL
 - azaperol + azaperone > MRL
- 4 labs
- 6 molecules to identify
- results: identification : C or NC
quantification : OK except for lab C (technical problems)



ILS: Conclusion (2)



ISP-WIV

- Qualitative:
 - No false negative
 - One false positive (A3)
- Quantitative:
 - very few results → no z-score
 - over estimation of concentrations by lab A
 - carazolol < MRL → ok except lab A
 - Over-estimation for azaperol + azaperone → sum > MRL → false!
- Acepromazine not distributed homogeneously (?) in treated animals
→ strange results in the homogeneization study and analysis of the samples
- Banned substances → well detected





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ISP-WIV

Conclusions of the workshop

Scientific Institute of Public Health - Food Section



Conclusions



ISP-WIV





ISP-WIV

Closing remarks

- Thank you for your attention !
- Thanks
 - for participating
 - FAVV/AFSCA for financial support
 - H.Noppe, I.Heymans, PY Brasseur and A.Hauters
- Important
 - Please sign the attendance list
 - Please fill in the questionnaire

