1. INTRODUCTION

1.1 Summary

This SOP describes a method for the screening (ref. 1) of samples of urine, bile, meat and kidney of bovine origin for the presence of zearalenone, zearalanone and a quantitative screening method (ref. 1) for ? -zearalanol (zeranol), ? -zearalanol (taleranol), ? -zearalenol and ß-zearalenol in samples of urine, bile, meat, liver and kidney of bovine origin.
A combination of solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS) is used.
The method comprises the following steps: Hydrolysis of the urine, bile, liver and kidney samples using Suc Helix Pomatia, or in case of meat, enzymatic digestion with protease; Liquid-liquid extraction (LLE) with tert-Butyl methyl Ether (TBME); purification using SPE on reversed-phase C_{18} cartridges and straight-phase amino (NH_2) cartridges. Detection is performed by GC-MS in the negative chemical ionisation mode (NCI).

1.2 Field of application.

The method is used to perform qualitative screening analyses for the determination of zearalenone, zearalanone and quantitative screening analyses for ? -zearalanol, ? -zearalanol, ? -zearalenol, ß-zearalenol, in samples of urine, bile, meat, liver and kidney of bovine origin. The limit of detection is 0.5 ng/ml-g for each analyte, based on the detection of the most abundant ion, with a response at the correct retention time exceeding the signal to noise ratio of 3. Additional confirmation analyses will in all positive cases be necessary in order to derive a final conclusion with respect to the presence or absence of the analytes.

1.3 References.

1.3.1 A.A.M. Stolker, Validation of analytical methods for the identification and quantification of organic resides and contaminants, Draft 991103, November 1999, RIVM.

1.3.2. H.A.Herbold, S.S. Sterk, R.W. Stephany, L.A. van Ginkel. Multi residue method using coupled-column HPLC and GC-MS for the determination of anabolic agents in samples of urine. RIVM report n.389002 - 035, September 1997.

1.3.3. H.J. van Rossum et al, Multi residue analysis anabolic agents, SOP ARO/113, revision 4, 21 januari 1997, RIVM.

1.3.4. Draft Commission Decision laying down performance criteria for the analytical methods to be used for detecting certain substances and residues thereof in live animal and animal and animal products according to Council Directive 96/23/EC repealing Commission Decision 90/515/EC, 93/256/EC and 93/257/EC, SANCO/1805/2000.

1.3.5. M.H.Blokland, S.S.Sterk, L.A. van Ginkel, Report: 24 months Progress Report on project FAIR-CT97-3443, Period 1999.06.04-2000.05.31.

1.3.6. J.C.Miller and J.N.Miller, statistics for analytical chemistry (second edition), ISBN 0-13-845439-6.

2 MATERIALS[1]

The used water is Milli-Q water.

2.1 Chemicals.

2.1.1 SPE extraction column: 6 ml disposable C_{18} cartridge (Alltech, 2005430).

2.1.2 SPE extraction column: 6 ml disposable amino (NH_2) (Alltech, 211153).

2.1.3 Methanol (Baker, 8045).

2.1.4 Ethanol (Baker, 8006).

2.1.5 Acetone (Baker, 8001).

2.1.6 Iso-octane (Baker, 8715).

2.1.7 Hexane (Merck, 4367)

2.1.8 Derivatisation reagent: Heptafluorobutyric Acid Anhydride (HFAA) (Pierce, 63164).

2.1.9 Acetic acid (Merck, 63).

2.1.10 Sodium acetate (Merck, 6268).

2.1.11 Acetate buffer, 2 mol/l, pH 5.2. Dissolve 25.2 g of acetic acid and 129.5 g of sodium acetate in 800 ml water. Adjust the pH with a pH-meter to 5.2±0.1; add water to a final volume of 1000 ml.

2.1.12 Beta-glucuronidase/sulfatase (suc d'Helix Pomatia containing 100.000 units ß-glucuronidase and 100.000 units sulfatase per ml, France, code IBR 213473).

2.1.13 tert-Butylmethylether (TBME) (Merck, 1845).

2.1.14 Tris(hydroxymethyl)-amino-methane (Merck, 8382).

2.1.15 Tris buffer, 0.1 mol/l, pH 9.5. Dissolve 12.1 g of Tris(hydroxymethyl)-amino-methane in 800 ml of water. Adjust the pH to 9.5 ? 0.1 and add water to a volume of 1000 ml.

2.1.16 Subtilisin (Protease), (Sigma, P-5380).

2.2 Standards

2.2.1 Internal Standards.

Relevant internal standards are ? -zearalanol-d4, ? -zearalanol-d4, ? -zearalenol-d4, ? -zearalenol-d4 (source RIVM) and zearalenone-d6 (source Veterinary Science Division). Stock solutions containing 1 mg/ml are prepared by dissolving the appropriate amount of the analyte in ethanol. Quality control includes the registration of a mass spectrum (identity). These solutions are stored in the dark at -20°C for a maximum period of 5 years. Working solutions are prepared by 10-fold dilutions of the stock solutions with methanol. These solutions are stored in the dark at 4°C (range 1-10°C) for a maximum period of 6 months.

2.2.2 Standards.

Relevant standards are ? -zearalanol, ? -zearalanol, ? -zearalenol, ? -zearalenol, zearalenone and zearalanone. Relevant data of the analytes are listed in Table 1. Stock solutions containing 1 mg/ml are prepared by dissolving the appropriate amount of the analyte in ethanol. Quality control includes the registration of a mass spectrum (identity). These solutions are stored in the dark at -20°C for a maximum period of 5 years. Working solutions are prepared by 10-fold dilutions of the stock solutions with methanol. These solutions are stored in the dark at 4°C (range 1-10°C) for a maximum period of 6 months.

[1] Reference to a product or company is for purposes of identification and information only and does not imply any recommendation by the CRL or RIVM of the company.

Table 1. Information about analytes.

Analytes	CAS #	Formula	Mol. Weight
? -zearalanol	26538-44-3	$C_{18}H_{26}O_5$	322.4
? -zearalanol	42422-68-4	$C_{18}H_{26}O_5$	322.4
? -zearalenol	36455-72-8	$C_{18}H_{24}O_5$	320.4
ß-zearalenol	71030-11-0	$C_{18}H_{24}O_5$	320.4
Zearalanone	17924-92	$C_{18}H_{24}O_5$	320.4
Zearalenone	597578-0	$C_{18}H_{22}O_5$	318.4

2.3 Equipment

2.3.1 Glass derivatisation vials, (Hewlett Packard , 5182-0553).

2.3.2 Automatic pipettes (Gilson P20, P100, P200, P1000 and P5000).

2.3.3 Injection vials, Wide Mouth Crimp (Alltech, 98213), with micro inserts (200 µl) (Alltech EK-1022.395) and aluminium caps, (Chrompack, 10210).

2.3.4 GC-MS equipment. Hewlett Packard (HP) 5973 mass-spectrometer equipped with a HP 7673 automatic sampler and a HP computer with HPChem data acquisition software.

2.3.5 Fused silica capillary column ZB-1, length 30 m, i.d. 0.25 mm, 0.25 ? film thickness, (Phenomenex, 7HG-G001-11).

2.3.6 Vortex, (Vortex-genie).

2.3.7 pH-meter, (Applikon).

2.3.8 Electric waterbath with thermostat adjustable ± 5°C with nitrogen facility, (TurboVap, Zymark).

2.3.9 Centrifuge Varifuge 3.0R, (Heraeus).

2.3.10 Plastic centrifuge tubes 50 ml, (Omnilabo).

2.3.11 Incubator oven TH15, (Edmund Bühler).

2.3.12 Rotator REAX-2, (Heidolph).

3. GC-MS CONDITIONS

The GC-MS conditions in table 2 can be used as a starting point for this analysis.

Table 2, Parameters GC-MS

Parameters	
Carrier gas is helium	1 ml/min (EPC system for constant flow)
Column	Fused silica ZB-1, 30 m, 0.25 mm i.d., 0.25 ? film
Detector temperature	250°C MS-source, 120°C quadrupole.
Oven temperature	80 °C for 1 min, increased at 25°C/min. to 300°C and held at this temp. for 4 min.
Ionisation	NCI (methane), SIM mode
Injection volume	1 ?l
Mass monitored (m/z)	Zearalanol-? /ß 713 and for the internal st. 717 Zearalenol-? /ß 711 and for the internal st. 715 Zearalenone 513 and for the internal st. 519 Zearalanone 515
Tune	Manual tune optimised for the ions monitored

4 ANALYTICAL PROCEDURES

4.1 Spiking procedure

A portion of 5 ml of urine or bile or 5 gram of homogenised liver, kidney or meat is transferred to a 50 ml plastic tube. The samples are spiked with a mixture of internal standards (? /ß-zearalanol-d4, ? /ß-zearalenol-d4 and zearalenone-d6) at the level of 10 ng (corresponding to 2 ng/ml-g). Also control samples are prepared. These control samples consist of blank material and spiked material at 1 ng/ml-g, see table 3.

Table 3, Pipetting scheme for the control and the samples

Identity (ID).	Standards (2.2.2)	Internal standard (2.2.1)
Blank control sample	0 µl	100 µl of 0.1 ng/µl
Control sample spiked on 1 ng/ml-g	50 µl of 0.1 ng/µl	100 µl of 0.1 ng/µl
Sample	0 µl	100 µl of 0.1 ng/µl

4.2 Hydrolysis of urine, bile, kidney and liver

The pH of the samples of urine and bile is adjusted to 5.2 by adding 2 ml of 2 mol/l acetate buffer. For liver and/or kidney 5 ml of 2 mol/l acetate buffer pH 5.2 are added. Subsequently, 40 µl of Suc Helix Pomatia is added. The mixture is vortexed for 30 sec. and the pH is checked (when necessary the pH is adjusted to 5.2 with acetic acid). The mixture is hydrolysed during 2 hours at 37°C in an oven under constant shaking (300 motions/min).

4.3 Enzymatic digestion of meat

To a test portion of 5 g meat, 5 ml of Tris buffer are added. To this mixture 5±1 mg of protease is added. Subsequently the mixture is incubated during 2 hours at 50°C in an oven under constant shaking (300 motions/min).

4.4 Liquid/liquid extraction.

To the mixture of 4.2/4.3 10 ml of TBME is added. After mixing head over head for 10 min., the mixture is centrifuged for 3 min. at 2700 g. The organic layer is collected in a glass tube and evaporated at 50°C under a gentle stream of nitrogen. The dry residue is dissolved in 5 ml of 50/50 v/v-% methanol/water. This mixture is washed with 1 ml of hexane by thorough mixing during 30 sec. The mixture is centrifuged for 3 min. at 2700 g. The hexane layer is separated from the methanol/water fraction and discarded. The washing procedure is repeated once. After the washing procedure 2 ml of water is added. The methanol/water layer in the tube is further processed over a C_{18} disposable column (SPE).

4.5 Solid Phase Extraction

4.5.1 SPE C_{18}

The C_{18} column (6 ml) is preconditioned with 5 ml of methanol and 5 ml of Milli-Q water. The sample is passed through the column. The C_{18} column is washed with 5 ml of 40/60 v/v-% methanol/water. The analytes are eluted with 5 ml of 80/20 v/v-% methanol/water. The eluate is collected and evaporated at 50°C under a gently stream of nitrogen until dryness and re-dissolved in 5 ml of 80/20 v/v-% acetone/methanol.

4.5.2 SPE NH_2

The NH_2-column is preconditioned with 5 ml 80/20 v/v-% acetone/methanol after which the extract is passed through the amino column. The eluate is collected and evaporated at 50°C under a gently stream of nitrogen until dryness. The residue is dissolved in 0.5 ml of ethanol.

4.6 Detection.

A calibration curve is prepared by pipetting the volumes given in table 4 into derivatisation vials.

Table 4, Preparation of calibration curve.

ID.	Standards	Internal standard
Blank	0 µl	100 µl of 0.1 ng/µl
St 1.0 ng	100 µl of 0.01 ng/µl	100 µl of 0.1 ng/µl
St 2.0 ng	20 µl of 0.1 ng/µl	100 µl of 0.1 ng/µl
St 5.0 ng	50 µl of 0.1 ng/µl	100 µl of 0.1 ng/µl
St 10.0 ng	100 µl of 0.1 ng/µl	100 µl of 0.1 ng/µl
St 20.0 ng	200 µl of 0.1 ng/µl	100 µl of 0.1 ng/µl

The extract and standards are transferred into derivatisation vials and evaporated at 50°C under a gentle stream of nitrogen until dryness. The dry residue is derivatised instantly with 10 ?l of HFAA and 20 µl of iso-octane. The mixture is transferred into an injection-vial with micro insert. The vials are placed in the automatic sampler and 1 ?l is injected into the GC-MS, data acquisition is performed as described in table 2.

5 INTERPRETATION AND CALCULATION

5.1 Check

The first step is a performance check of the GC-MS system after tuning. This is done by injecting a 1 ng standard (table 4) and determination the S/N ratio for a-zearalanol. The S/N-ratio should be greater then 100.

5.2 Calculation

The area of the selected ion of the standard and internal standard are calculated and the ratio is the response variable. A calibration curve is constructed by linear curve fitting using least squares linear regression calculation. Unknown concentrations are calculated by interpolation.
Quantification is only valid if:

- The maximum of the signal originating from the analyte has a S/N ratio > 3.
- In the blank control samples all the internal standards should be visible. In the spiked control samples should also all the compounds be visible (S/N ratio > 10 for internal standards, > 6 for the non-deuterated compounds).
- The coefficient of correlation of the constructed calibration curve is better than 0.99.
- The numerical value of the intercept may not deviate more than ± 3 SD from zero.
- Calibration curves are calculated using calibration software.

6. VALIDATION OF METHOD

The method described in this SOP was validated according to our validation program (ref. 1). From this validation program it is concluded that this method is qualitative for zearalenone, zearalanone and quantitative for ? -zearalanol, ? -zearalanol, ? -zearalenol, ß-zearalenol in bovine urine, bile, meat, liver and kidney.

6.1 Decision limit (CC?) and Detection Capability (CCß)

A standard curve, of five points around 0.5 ng/ml, was prepared in blank urine and analysed. The CC? and CCß were determined from these curves.

Table 5, Determination (n=1) of the CCß and CC? (ng/ml)

Analytes	**CCa**	**CCß**
? -zearalenol	0.06	0.11
ß-zearalenol	0.07	0.12
? -zearalanol	0.07	0.11
ß-zearalanol	0.21	0.36
Zearalenon	0.35	0.60
Zearalanon	0.19	0.33

From the results for CCß and CC? it was concluded that analytes at 0.5 ng/ml can be reliably detected. To confirm this, analytes were spiked to a representative blank urine sample (n=9) at 0.5, 1.0 and 2.0 ng/ml and the accuracy was determined. No significant differences were observed between the different levels.

Table 6, Check of the analyses at 0.5, 1.0 and 2.0 ng/ml, in triplicate for each level (n=9)

ID	? -zearalanol	ß-zearalanol	? -zearalenol	ß-zearalenol	Zearalenone	Zearalanone
Average (%)	103.1	104.7	133.2	120.8	99.9	110.1
CV (%)	2.7	4.7	5.5	3.2	22.1	6.0

6.2 Trueness

There was no certified reference material available; therefor the trueness could not be determined. For an estimate of trueness, however, the recovery can be used.

6.3 Recovery

The recovery was determined at 1 ng/ml by measuring the absolute recovery. To determine the absolute recovery a blank sample of urine was spiked with a mixture of compounds at the level of 1 ng/ml (n=3) and the internal standard was added after clean up.

Table 7, Recovery (%) (n=3)

ID	? -zearalanol	ß-zearalanol	? -zearalenol	ß-zearalenol	Zearalenone	Zearalanone
Average	71.7	67.8	65.5	71.5	37.5	86.9
CV (%)	1.8	3.3	0.4	3.6	5.5	11.9

6.4 Within laboratory repeatability

The repeatability (within days) and reproducibility (between days) was determined by spiking a blank urine sample at 1 ng/ml. The urine sample was analysed on five different days in triplicate. The results given in table 9 are the average values of all measurements.

Table 8, Accuracy (%) repeatability (within days, 5 days, three analyses)

ID	? -zearalanol	ß-zearalanol	? -zearalenol	ß-zearalenol	Zearalenone	Zearalanone
Average	95.3	103.1	153.6	88.3	65.7	87.7
CV (%)	2.1	4.1	2.9	3.0	4.2	2.9
Average	107.3	106.6	113.6	113.6	99.5	80.1
CV (%)	2.7	1.4	2.2	3.9	9.4	9.8
Average	114.5	111.3	120.9	1309	69.5	109.1
CV (%)	3.3	0.9	3.8	2.8	5.5	7.9
Average	101.2	100.5	134.2	119.1	89.2	114.2
CV (%)	4.0	4.1	6.2	2.4	8.6	8.2
Average	118.2	118.1	153.8	130.2	69.9	155.4
CV (%)	5.3	3.0	5.0	2.0	5.7	2.2

Table 9, Accuracy (%) reproducibility (between days, 5 days, three analyses)

ID	? -zearalanol	ß-zearalanol	? -zearalenol	ß-zearalenol	Zearalenone	Zearalanone
Average	107.3	107.9	135.2	116.4	78.8	107.5
CV (%)	8.7	6.5	13.1	14.0	18.5	27.5

The results are in good agreement with those described under 6.1, table 6.

6.5 Specificity

The specificity was determined by analysing different urine materials, which were collected for National control programs. Since all possible metabolites are included in this method, no experiments were performed to check the influence of closely related compounds.

Table 10, Concentration (ng/ml),

ID	? -zearalanol	ß-zearalanol	? -zearalenol	ß-zearalenol	Zearalenone	Zearalanone
99M1474	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
99M1475	<0.5	<0.5	**0.9**	<0.5	**0.5**	<0.5
99M1476	<0.5	<0.5	**1.2**	<0.5	<0.5	<0.5
99M1477	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
99M1478	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
99M1479	<0.5	<0.5	<0.5	<0.5	<0.5	**0.7**
99M1480	<0.5	<0.5	<0.5	<0.5	<0.5	**0.6**
94M3435	<0.5	<0.5	**0.5**	<0.5	<0.5	<0.5
99M0659	<0.5	<0.5	<0.5	**0.9**	<0.5	<0.5
99M0660	<0.5	<0.5	<0.5	**2.5**	<0.5	<0.5
99M1743	<0.5	<0.5	**1.2**	**6.1**	**1.4**	<0.5

From table 10 it is clear that some samples contain ? /ß-zearalenol, zearalenone and zearalanone. The most probable explanation for the observed results is that there are background levels of these compounds, originated from the Fusarium toxins present as natural contaminants. These fungi are present in animal food and produce ? /ß-zearalenol and zearalenone. When animals consume this contaminated feed ? /ß-zearalenol and zearalenone are excreted in the urine. Confirmation analyses are necessary for verification in these cases.

6.6 Ruggedness

The ruggedness was determined by spiking 10 different samples of bovine urine, at 1.0 ng/ml.

Table 10, Accuracy (%) ruggedness (n=10)

ID	? -zearalanol	ß-zearalanol	? -zearalenol	ß-zearalenol	Zearalenone	Zearalanone
Average	119.6	126.4	126.3	239.5	132.3	227.5
CV (%)	7.4	11.1	8.2	48.6	35.5	31.6

The bad accuracy for ß-zearalenol, again is due to background concentrations of ß-zearalenol. There is no guarantee that these samples are completely blank (see 6.5). From these experiments, it is concluded that it is not possible to determine zearalanone quantitative.
To determine the ruggednes of ß-zearalenol and zearalenone quaranteed blank material is needed. Such material is, however, not available.

6.7 Supplementary validation for bile, meat, kidney and liver

The change to another matrix is considered as one mayor change, therefor a supplementary validation (ref. 4) was performed. This validation consisted of the analyses of 10 urine samples and 10 "new" materials, liver, meat, kidney and bile. All materials were fortified with 0.5 ng/ml-g of each compound.
A t-test ($t = |\bar{x}_1 - \bar{x}_2| / s\sqrt{1/n_1 + 1/n_2}$) to compare the means and an F-test ($F = s_1^2 / s_2^2$) to compare the standard deviations, is performed (ref. 6). According to statistics, there is a significant difference between the two matrices when the calculated value for the t-test exceeds 2.10 (18 degrees of freedom) and for the F-test 4.026 (9 degrees of freedom).

Table 12, correlation between of urine (n=10) and meat (n=10), given values are the accuracy and CV (%)

ID	? -zearalanol		ß-zearalanol		? -zearalenol		ß-zearalenol		Zearalenone		Zearalanone	
	Urine	Meat	Urine	Meat	Urine	Meat	Urine	meat	Urine	meat	Urine	meat
Average	111.8	53.5	129.9	57.0	399.9	148.3	1995	135.1	521.4	338.1	166.8	378.5
CV (%)	8.2	5.0	8.2	5.7	12.4	16.8	9.6	5.9	15.8	38.8	14.3	41.5
t-test	18.2		19.5		13.6		29.0		3.5		4.0	
F-test	11.6		10.5		3.9		591.3		2.5		43.3	

The blank urine used in this experiment contained very high amounts of Fusarium toxins. Because of these high levels there is a bad correlation between these two compounds. Because of this contaminated urine, an F-test and a t-test can not be used.

Table 13, Correlation between urine (n=10) and kidney (n=10), given values are the accuracy and CV (%)

ID	a-zearalanol		ß-zearalanol		a-zearalenol		ß-zearalenol		Zearalenone		Zearalanone	
	Urine	Kidn	Urine	Kidn	Urine	Kidn	Urine	Kidn	Urine	Kidn	Urine	Kidn
Average	53.0	55.0	62.9	65.5	67.6	66.3	85.3	57.3	158.2	178.8	248.3	234.1
CV (%)	4.1	10.5	6.0	9.2	17.0	14.1	9.1	12.5	50.2	35.7	35.7	23.5
t-test	1.0		1.1		0.3		7.9		0.6		0.4	
F-test	6.9		2.5		1.5		1.2		1.5		2.6	

The t- and F-test indicate that the results for ? /ß-zearalanol, ? -zearalenol, zearalenone and zearalanone do not differ significantly. The results for ß-zearalenol however do differ significant. This due to background values for ß-zearalenol in urine.

Table 14, Accuracy (%) of urine (n=10) and liver (n=10), given values are the accuracy and CV (%)

ID	? -zearalanol		ß-zearalanol		? -zearalenol		ß-zearalenol		Zearalenone		Zearalanone	
	Urine	Liver	Urine	Liver	Urine	Liver	Urine	Liver	Urine	Liver	Urine	Liver
Average	90.4	86.6	94.4	91.1	160.8	164.9	100.9	134.5	75.7	91.5	63.5	95.7
CV (%)	2.9	3.1	2.2	2.9	20.2	16.9	2.8	3.9	63.9	64.6	66.2	57.5
t-test	3.0		2.9		0.3		16.8		0.6		1.4	
F-test	0.9		0.6		1.4		0.3		0.7		0.6	

The t- and F-test indicate that the results for, ? -zearalenol, zearalenone and zearalanone do not differ significant. The results for ? /ß-zearalanol ß-zearalenol however do differ significant. The "bad" performance for the t-test is because of the very small standard deviation. Despite the significant difference according to the t-test it can be concluded that the results for a/ß-zearalenol are comparable.

Table 15, Accuracy (%) of urine (n=10) and bile (n=10), given values are the accuracy and CV (%)

ID	? -zearalanol		ß-zearalanol		? -zearalenol		ß-zearalenol		Zearalenone		Zearalanone	
	Urine	Bile	Urine	Bile	Urine	Bile	Urine	Bile	Urine	Bile	Urine	Bile
Average	116.6	111.5	127.7	120.0	118.5	187.0	111.7	189.4	373.9	120.7	355.8	131.7
CV (%)	6.2	5.4	5.0	6.2	13.3	3.8	4.4	4.5	36.5	38.5	36.4	45.3
t-test	1.6		2.4		11.9		23.6		5.6		4.7	
F-test	1.4		0.7		4.9		0.3		8.6		4.7	

The t- and F-test indicate that the results for ? /ß-zearalanol do not differ significant. The results for the other compounds however do differ significant. This due to high background values of ? /ß-zearalenol in bile.

From table 12 – 15 it can be concluded that there is no significant difference for ? /ß-zearalanol between urine and kidney, liver and bile. There can not be a conclusion drawn from the meat-urine experiment because of high background values of all analytes. Because of the Fusarium toxins present from natural contaminants it is not possible to compare the quantitative results for the other compounds in different matrices.

6.8 Conclusion and discussion

From the validation it is clear that there is a difference in performance between [? /ß-zearalenol, ? /ß-zearalanol] and [zearalenone, zearalanone]. For the first group a quantitative analysis is possible in contrast with the second group in which there is a big variation in the quantitative results. It is concluded that the method a quantitative screening method is for ? /ß-zearalenol, ? /ß-zearalanol and a qualitative screening method for zearalenone, zearalanone.
The difference in performance between the different matrices is for ? /ß-zearalanol in most cases not significant. For the other compounds the difference is significant. However, the difference is caused by background values of the Fusarium toxins present from natural contaminants. This makes full validation for all compound / matrix combinations impossible. Despite this fact, it is concluded that the method is suitable for bovine urine, bile, meat, liver and kidney.