



Monitoring and **MoniQA** Quality Assurance

The use of mycotoxin methodology in practice: a need for harmonization

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EU Regulation on mycotoxins

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COMMISSION REGULATION (EC) No 401/2006

of 23 February 2006

laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules ⁽¹⁾, in particular Article 11(4),

ochratoxin A in foodstuffs ⁽⁴⁾, Commission Directive 2003/78/EC of 11 August 2003 laying down the sampling methods and the methods of analysis for the official control of the levels of patulin in foodstuffs ⁽⁵⁾ and Commission Directive 2005/38/EC of 6 June 2005 laying down the sampling methods and the methods of analysis for the official control of the levels of *Fusarium*-toxins in foodstuffs ⁽⁶⁾ similarly establish sampling methods and performance criteria for ochratoxin A, patulin and *Fusarium*-toxins respectively.

MoniQA

www.moniqa.org



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SIXTH FRAMEWORK PROGRAMME



Official control of the levels of mycotoxins in foodstuffs (EC N. 401/2006)

- **CRITERIA FOR SAMPLE PREPARATION AND FOR METHODS OF ANALYSIS**
- **METHOD PERFORMANCE CRITERIA FOR:
aflatoxins, ochratoxin A, fumonisins,
deoxynivalenol, patulin, zearalenone,
T-2 and HT-2 toxins**

Performance criteria for ochratoxin A (EC N. 401/2006)

Level $\mu\text{g}/\text{kg}$	Ochratoxin A		
	RSD _r %	RSD _R %	Recovery %
< 1	≤ 40	≤ 60	50 to 120
1-10	≤ 20	≤ 30	70 to 110

Measurement uncertainty

(EC No. 401/2006)

$$U_f = \sqrt{(LOD/2)^2 + (a \times C)^2}$$

where:

- U_f is the maximum standard uncertainty ($\mu\text{g}/\text{kg}$)
- LOD is the limit of detection ($\mu\text{g}/\text{kg}$)
- a is a constant, numeric tabulated factor to be used depending on the value of C . For $C < 50 \mu\text{g}/\text{kg}$ $a = 0.2$
- C is the concentration of interest ($\mu\text{g}/\text{kg}$).

With **LOD** = 3 $\mu\text{g}/\text{kg}$ and **C** = 10 $\mu\text{g}/\text{kg}$

$$U_f = 2.5 \mu\text{g}/\text{kg} (=25\%)$$

Objective

To carry out a survey of current practices concerning the use and application of test methods for determination of mycotoxins regulated in the EU.

Mycotoxins considered

(EC No. 1881/2006)

Aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂)

Aflatoxin M₁ (AFM₁)

Ochratoxin A (OTA)

Fumonisin (FB₁, FB₂)

Patulin (PAT)

Deoxynivalenol (DON)

Zearalenone (ZEA)

T-2 and HT-2 toxins (T-2, HT-2)

Survey of current practices on mycotoxin methodology

The questionnaire was sent to 23 laboratories (MoniQA network).

Part I: laboratory accreditation, participation in proficiency testing, use of validated methods, certified reference materials and reference calibrants, laboratory practices.

Part II: food commodities analysed, method accreditation, method performance characteristics, measurement uncertainty, cost.

**Control
laboratories**

ARPA (IT)
ASBP (ES)
CLAP (TK)
VWA (NL)
GCSL (GR)

**Research
laboratories**

DSA (IT)
GAIKER (ES)
TUBITAK (TK)
CCOA (PRC)
SCU (PRC)
JRC-IRMM (BE)
CER (BE)
BUTE (HU)
UFT (BG)
ESR (NZ)

**Commercial
laboratories**

CHELAB (IT)
LAV (ES)
CCFRA (UK)
EUROFINS (D)

Total: 19 laboratories from 12 countries

Percentage of laboratories that fulfill quality control parameters

Quality parameter	%
Routine recovery experiments	84
Laboratory accreditation (ISO 17025:2005)	74
Participation in collaborative studies/proficiency testing	74
Use of certified standard solutions	74
Use of method validated through a collaborative study	58
Use of reference materials	47

Method accreditation

Mycotoxin	n. laboratories	accredited methods (%)
Ochratoxin A	18	67
Aflatoxins	17	75
Zearalenone	14	57
Patulin	14	50
Deoxynivalenol	12	58
T-2 and HT-2 toxins	11	58
Fumonisin	11	64
Aflatoxin M₁	10	60

Number of accredited laboratories in each Country

Mycotoxin	Germany	Italy	Turkey	Holland	Hungary	Belgium	Greece	China	UK	Bulgaria
Aflatoxins	>100	56	25	10	-	3	6	>100	18-45 ²	8
Aflatoxin M1	- ¹	45	8	1	-	-	3	1	18-45	-
Ochratoxin A	>100	53	15	6	7	3	7	-	18-45	-
Patulin	>100	7	4	2	3	2	-	-	18-45	8
Deoxynivalenol	-	9	1	4	4	4	1	4	18-45	-
Zearalenone	-	20	1	3	5	2	1	-	18-45	-
Fumonisin	>100	6	1	1	-	1	1	-	18-45	-
T-2/HT-2 toxin	-	2	-	-	2	-	-	-	18-45	-

¹not reported; ²the number varies depending of particular commodities

Number of food matrices analysed for each toxin

Mycotoxin	Range	Mean
Ochratoxin A	3-26	7
Aflatoxins	1-21	7
Deoxynivalenol	1-11	4
Zearalenone	1-11	3
T-2/HT-2 toxins	1-10	2
Fumonisin	1-9	3
Aflatoxin M₁	1-8	3
Patulin	1-6	2

Limit of detection (LOD)

Definition	n. laboratories
Signal/noise 3	7
Blank noise + 3SD	2
Signal/noise 0.6-3	1
Signal/noise 3-10	1
The lowest detected level	1
The lowest detected level associated with a deviation >40%	1
ISO 5725:1987	1
$(3xS)/b \times f$ (conc.)	1
Confidence interval of calibration curve	1

Measurement uncertainty

(EC No. 401/2006)

$$U_f = \sqrt{(\text{LOD}/2)^2 + (a \times C)^2}$$

where:

- U_f is the maximum standard uncertainty ($\mu\text{g}/\text{kg}$)
- LOD is the limit of detection ($\mu\text{g}/\text{kg}$)
- a is a constant, numeric tabulated factor to be used depending on the value of C . For $C < 50 \mu\text{g}/\text{kg}$ $a = 0.2$
- C is the concentration of interest ($\mu\text{g}/\text{kg}$).

Limit of quantification (LOQ)

Definition	n. laboratories
Signal/noise 10	4
The lowest level accurately measured	3
Signal/noise 6	1
Signal/noise 6-100	1
Signal/noise 500	1
Blank noise + 10SD	1
The lowest detected level associated with a deviation >35%	1
Confidence interval of calibration curve	1

“Laboratory sample”

Weight	n. of labs.	no answer
EC n. 401/2006 (1-10 kg)	3	
5-50 g	5	5
50-250 g	2	
250-1000 g	4	

Methods used

Mycotoxin	HPLC	LC-MS/MS	Test kits	TLC or GC-MS
	n. of laboratories			
Aflatoxins	11	4	1	2
Aflatoxin M ₁	4	4	-	2
Ochratoxin A	12	3	2	2
Patulin	9	5	-	-
Deoxynivalenol	3	7	2	1
Zearalenone	6	6	3	1
Fumonisin	5	6	1	-
T-2/HT-2 toxins	-	7	3	1 1

Methods and performance characteristics

Mycotoxin	Method	N. of Labs	% Recovery	LOD (µg/kg)	LOQ (µg/kg)	% Uncertainty
Aflatoxins (B ₁ , G ₁ , B ₂ , G ₂) (Nuts, dried fruits, cereals and derived products, spices, baby foods) EU limits: AFB₁ 0.1-8 µg/kg Total 4-10 µg/kg	HPLC-FLD	11	70-110	0.01-0.5	0.02-0.5	5-30
	LC-MS/MS	4	57-120	0.01-2	0.01-5	10-50
	TLC	2	>75	2.5-5	2.5-5	n.r. ¹
	ELISA	1	80-110	0.01	0.01	10

¹not reported

Methods and performance characteristics

Mycotoxin	Method	N. of Labs	% Recovery	LOD (ng/kg)	LOQ (ng/kg)	% Uncertainty
Aflatoxin M₁ (Milk and derived products) EU limits: 25-50 ng/kg	LC-MS/MS	4	78-98	1-8	10-100	11-50
	HPLC-FLD	4	40-101	0.5-8	1-10	10-54
	TLC	2	92-105	n.r. ¹ -500	n.r.- 500	n.r.

¹not reported

Methods and performance characteristics

Mycotoxin	Method	N. of Labs	% Recovery	LOD (µg/kg)	LOQ (µg/kg)	% Uncertainty
Ochratoxin A (cereals and derived products, dried vine fruits, coffee, wine, grape juice) EU limits: 0.5-10 µg/kg	HPLC-FLD	13	50 ² -100	0.02-5	0.01-50	8-60
	LC-MS/MS	3	53-105	0.01-8	0.05-16	17-50
	TLC	2	n.r. ³	10	n.r.-10	n.r.-20
	ELISA	1	70-100	1	10	15
	Test kits ¹	1	n.r.	0.6-4	n.r.-2	n.r.

Methods and performance characteristics

Mycotoxin	Method	N. of Labs	% Recovery	LOD (µg/kg)	LOQ (µg/kg)	% Uncertainty
Fumonisin B₁ and B₂ (Maize and derived products, baby food) EU limits: 200-4000 µg/kg	LC-MS/MS	6	36-106	1-100	5-200	8-55
	HPLC-FLD	5	70-110	5-100	20-74	10-30
	ELISA	1	80-100	8 ng/ml	n.r. ¹	n.r.

¹not reported

Methods and performance characteristics

Mycotoxin	Method	N. of Labs	% Recovery	LOD (µg/kg)	LOQ (µg/kg)	% Uncertainty
Patulin (Fruit juices and puree)	HPLC-UV	9	68-99	0.2-100	1-1000	4-29
	LC-MS/MS	5	55-110	1-10	2-50	10-50

**EU limits:
10-50 µg/kg**

¹not reported

Methods and performance characteristics

Mycotoxin	Method	N. of Labs	% Recovery	LOD (µg/kg)	LOQ (µg/kg)	% Uncertainty
Deoxynivalenol (Cereals and derived products) EU limits: 200-1750 µg/kg	LC-MS/MS	7	38-120	0.5-83	1.2-200	5-50
	HPLC-UV	3	83-110	7-100	22-200	5-30
	TLC	1	80-100	100	- ²	-
	ELISA	1	85-110	1	10	15
	Test kits ¹	1	<90	200-250	100	25

Methods and performance characteristics

Mycotoxin	Method	N. of Labs	% Recovery	LOD (µg/kg)	LOQ (µg/kg)	% Uncertainty
T-2 and HT-2 toxins (Cereals and derived products) EU limits: to be defined	LC-MS/MS	7	40-120	0.02-150	0.6-300	7-50
	ELISA	3	n.r.¹	n.r.-1	n.r.-10	20-30
	TLC	1	n.r.	n.r.	n.r.	20
	GC/MS	1	95-105	5	10-15	20-30

¹not reported

Measurement uncertainty (%)

Toxin	HPLC	LC-MS/MS	ELISA	TLC
AFs	5 - 30	10 - 50	10	-
OTA	4 - 60	17 - 58	15	20
AFM ₁	7 - 54	11 - 50	-	-
ZEA	4 - 30	8 - 50	15 - 60	20
DON	5 - 30	5 - 50	15	-
T-2/HT-2	- ¹	7 - 50	20 - 30	20
PAT	4 - 29	10 - 50	-	-
FBs	10 - 31	8 - 55	-	-

¹not reported

Costs

Seventeen out of 19 laboratories reported the cost/analysis

**Chromatographic methods: 50 – 365 euro
(mean 100 euro)**

**Rapid test kits: 5 – 27 euro
(mean 13 euro)**

Issues to be harmonised

- **Accreditation needed**
- **Guidelines on the most “convenient” analytical method for each combination of mycotoxin/matrix**
- **Use of method validated through a collaborative study (if available)**
- **Participation in interlaboratory studies/proficiency testing**
- **Use of reference/certified materials/standard solutions**
- **Use the same definition/calculation for: LOD/LOQ, uncertainty, repeatability**
- **“laboratory sample”; “test portion size”**



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