UIMP Universidad Internacional Menéndez Pelayo

PIRINEOS 2016

CONTAMINANTES EMERGENTES EN EL SIGLO XXI

Del 28 al 29 de septiembre de 2016

Escuela Politécnica Superior de Huesca

Director:
Juan R. Castillo
Catedrático de Química Analítica
Instituto de Investigación en Ciencias Ambientales de Aragón (IUCA), Universidad de Zaragoza

BIOSENSORES PARA LA DETERMINACIÓN RÁPIDA Y PORTABLE DE MICOTOXINAS

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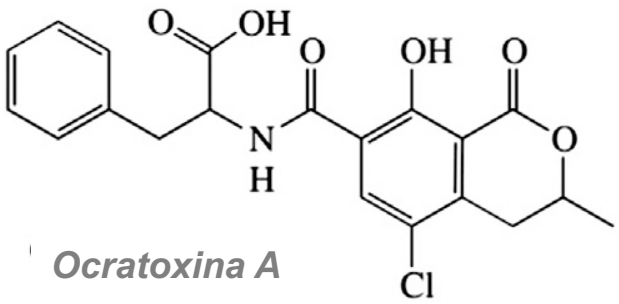
UNIVERSIDAD DE ZARAGOZA
Zaragoza · España

Universidad Zaragoza
1542

29 – Septiembre - 2016

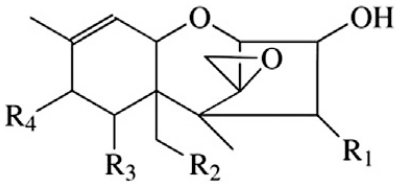
Las **micotoxinas** son un grupo muy amplio y variado de moléculas **tóxicas**, producidas como metabolitos secundarios por hongos filamentosos, y que pueden contaminar a un grupo muy amplio de alimentos.

*(**Aspergillus** & Penicillium)*



Ocratoxina A

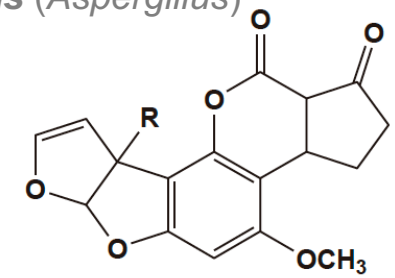
Tricotecenos



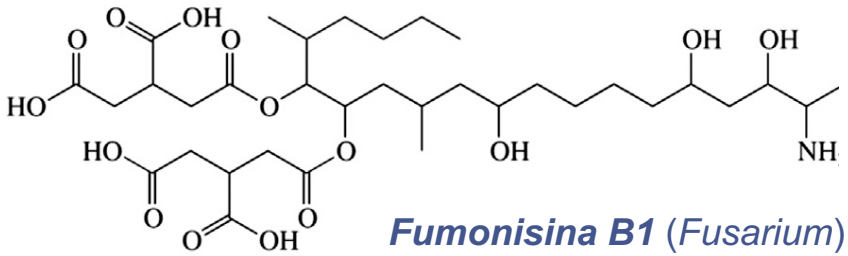
Deoxinivalenol (Fusarium)

	R_1	R_2	R_3	R_4
Deoxynivalenol	H	OH	OH	=O
T-2	OAc	OAc	H	$-OCOCH_2CH(CH_3)_2$
NT-2	OH	OAc	H	$-OCOCH_2CH(CH_3)_2$

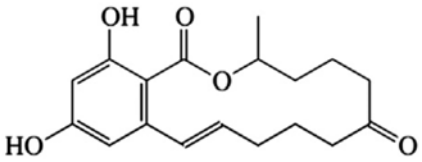
Aflatoxins *(Aspergillus)*



Aflatoxin B1	R = H
Aflatoxin M1	R = OH



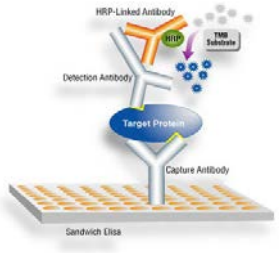
Fumonisina B1 *(Fusarium)*



Zearalenone
(Fusarium)

Castillo, J.R., Vidal-JC , et al., Electrochemical Affinity Biosensors for Detection of Mycotoxins: A Review, Biosens. Bioelectr. 49 (2013) 146-158

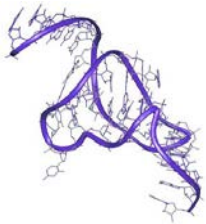
MÉTODOS RÁPIDOS PARA LA DETERMINACIÓN DE MICOTOXINAS



ELISAs

Anticuerpos

Aptámeros



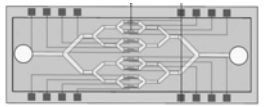
APTASENSORES

INMUNOSENSORES

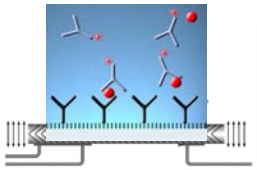
BIOSENSORES Y MÉTODOS RÁPIDOS

Electroquímicos (VOLT, AMP, EIS)

Fluorescencia (FP, IAC-FLD, F.O.)



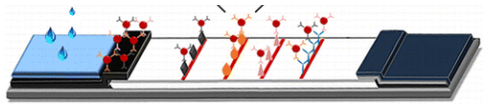
Microfluídica (*Lab-on-a-chip*)



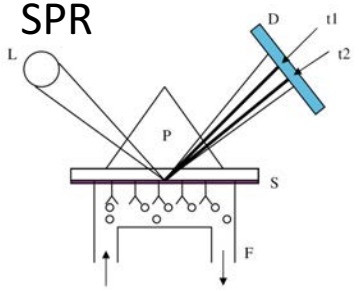
QCM

- **Extracción** (aislamiento micotoxina)
- Tratamiento sencillo de la **muestra**
- Evitar interferencias de **matriz**
- Selectividad del **ligando**
- **Sensibilidad (< ppb´s)**
- Determinación **rápida**
- Bajo **coste** (instrumentación sencilla)
- Portabilidad (***in-situ***)
- Determinación **múltiple**
- *Kits* **comerciales**
- Sistemas de alerta (***screening***)

Flujo Lateral (LFDs)



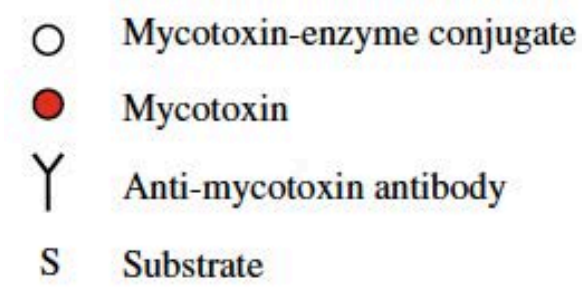
SPR



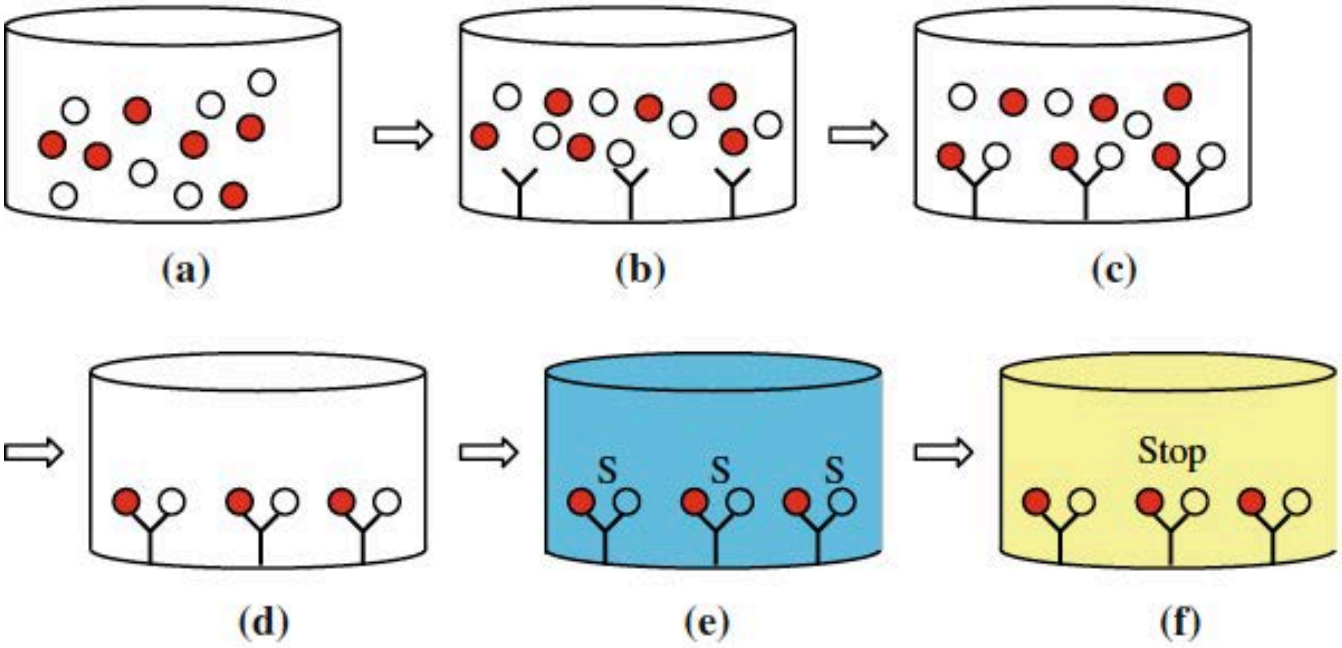
ELISAs

enzyme linked immuno-sorbent assays

Lector ELISA
Bio-Rad - 680



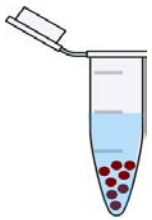
Ensayo competitivo
Amplificación enzimática



- *Kits* ELISAs comerciales

- Portabilidad (in-situ)
- Micotoxinas más importantes
- Tiempos medida: ~ 0,5-4 h.
- Sensibilidad y sencillez
- *Screening* / Cuantitativos.

LODs ≈ 0.1-1 ng mL^{-1}



Muestra
(extracto micotoxina)

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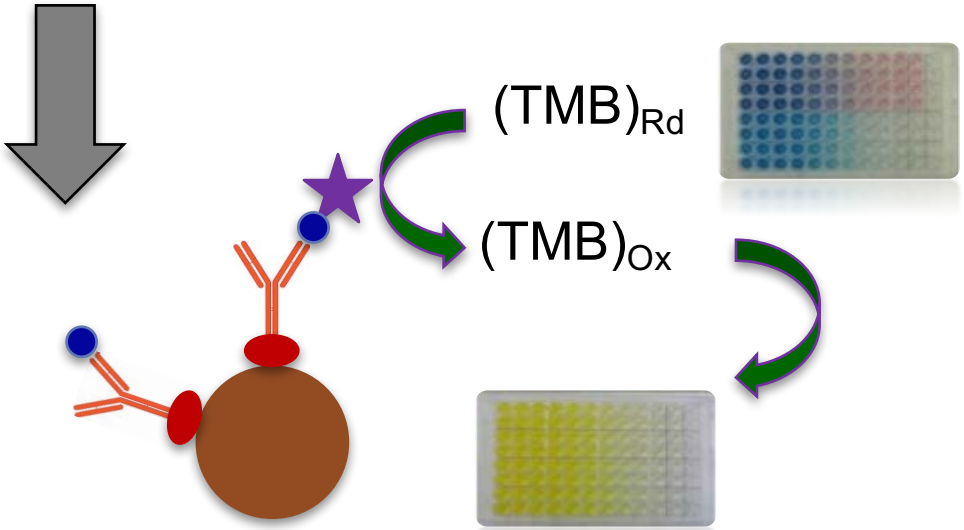
Zaragoza · España

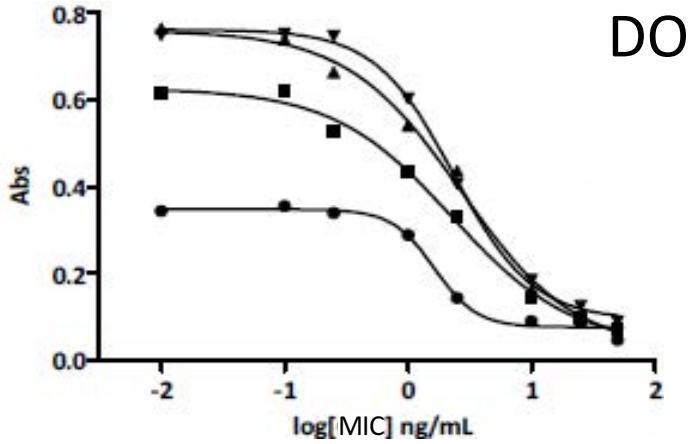
GRUPO DE ESPECTROSCOPÍA ANALÍTICA Y SENSORES
GEAS
UNIVERSIDAD DE ZARAGOZA · ZARAGOZA · ESPAÑA



*mp*ELISAs

OTA
FB1
DON

***mp*EIA**



Castillo, J.R., Vidal, J.C., et al., Rapid dtm. of recent COC use with mpEIA in serum, saliva and urine fluids, J. Pharm. Biomed. Anal. 125 (2016) 54-61

KITS INMUNOSENSORES ELECTROQUÍMICOS Y ESPECTROFOTOMÉTRICOS

KIT FEATURES	FB1	DON	OTA
Allowed levels (European Legislation)*	200-4,000 µg kg^{-1}	200-1,750 µg kg^{-1}	0.5-80 µg kg^{-1}
Total Test Time (ELISA)	120 min.	120 min.	120 min.
UZ-CapherIDI Test Time	110 min.	140 min.	110 min.
Limit of Detection Cereal	6 µg kg^{-1}	5.3 µg kg^{-1}	0.29 µg kg^{-1}
Limit of Detection Wine	-	-	0.18 µg kg^{-1}

GEAS: Grupo de Espectroscopía Analítica y Sensores (UZ)

® Patents

Electrochemical Immunosensors for quantitative detection of Fumonisin B1, Deoxynivalenol and Ochratoxin A Mycotoxins.

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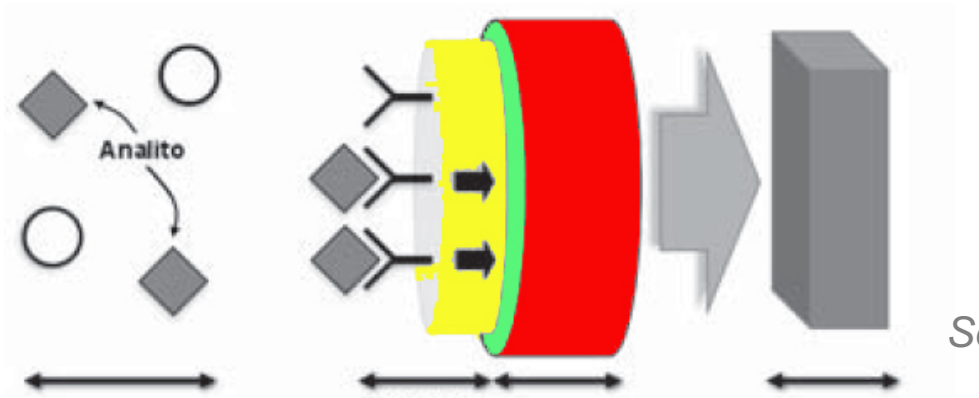
GEAS

Universidad Zaragoza

capher I+D+i

Biosensores para micotoxinas

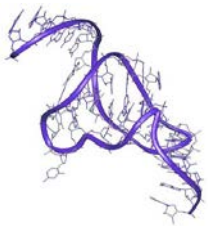


Biorreconocimiento

Transducción

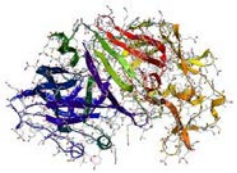
Afinidad

Anticuerpos



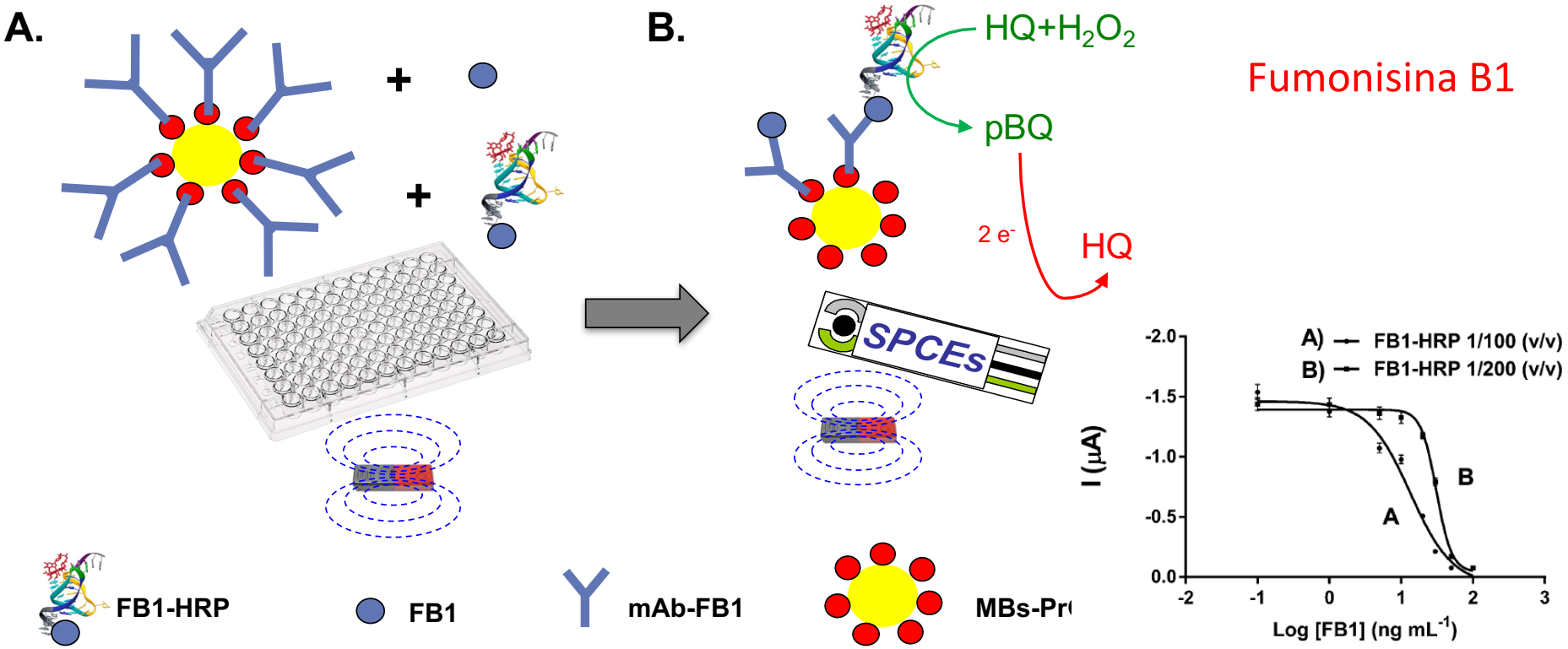
Aptámeros

Catalíticos



Enzimas (inhibición)

- Óptica (FM)
- Electroquímica
- QCM
- SPR



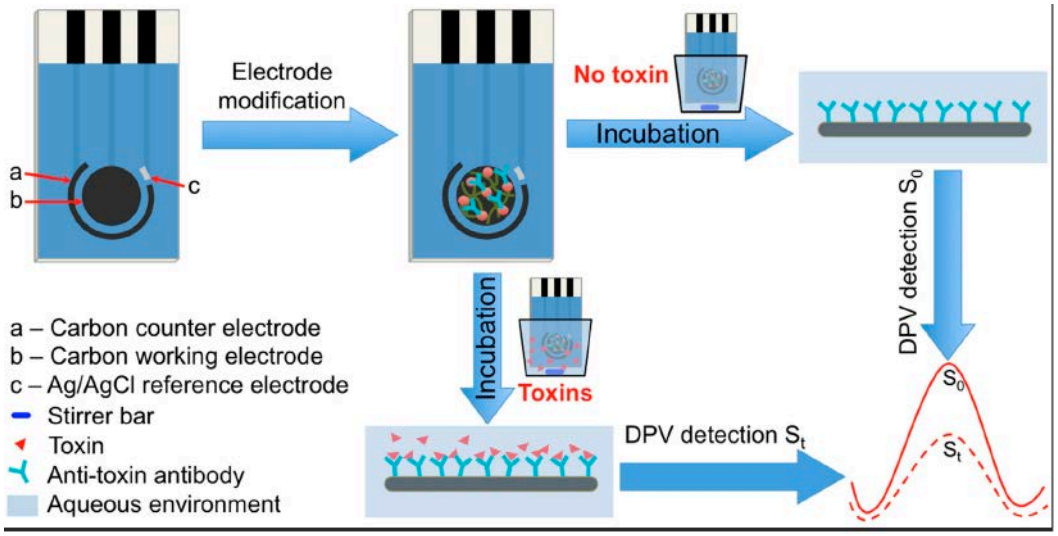
- LOD: 0,58±0,05 ng mL^{-1} FB1
- EC_{50}: 4,34±0,15 ng mL^{-1} FB1
- *Range*: 0,70-54 ng mL^{-1} FB1
- 7-13% RSD

Analytical Methods

A validated multi-channel electrochemical immunoassay for rapid fumonisin B1 determination in cereal samples†

Castillo, J.R., Vidal, J.C., et al., Anal. Meth. 7 (2015) 3742-3749

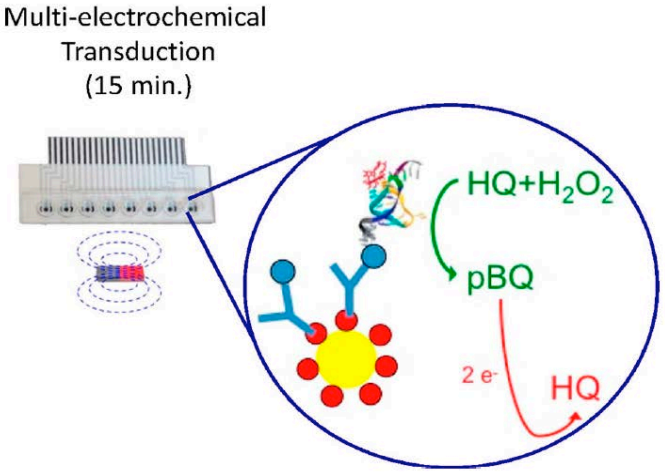
TRANSDUCCIÓN MULTI-ELECTROQUÍMICA

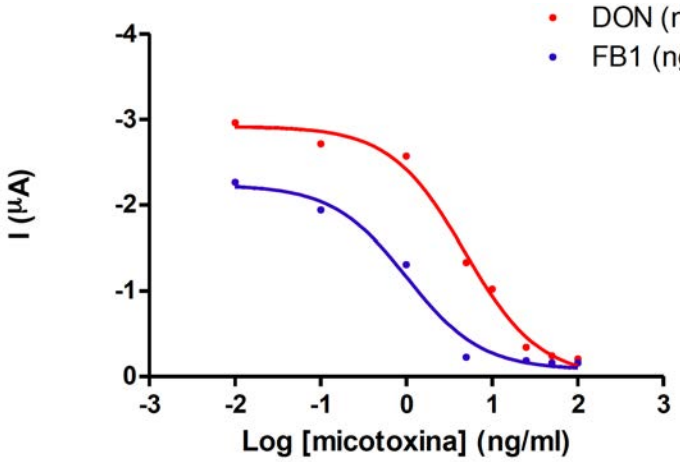


SPCEs

screen-printed carbon electrodes

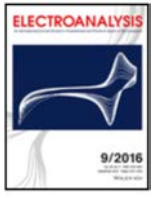
- Multi-potenciostato
- Multi-micotoxinas
- Nanoestructuración
- *Arrays* serigrafiados (8X)

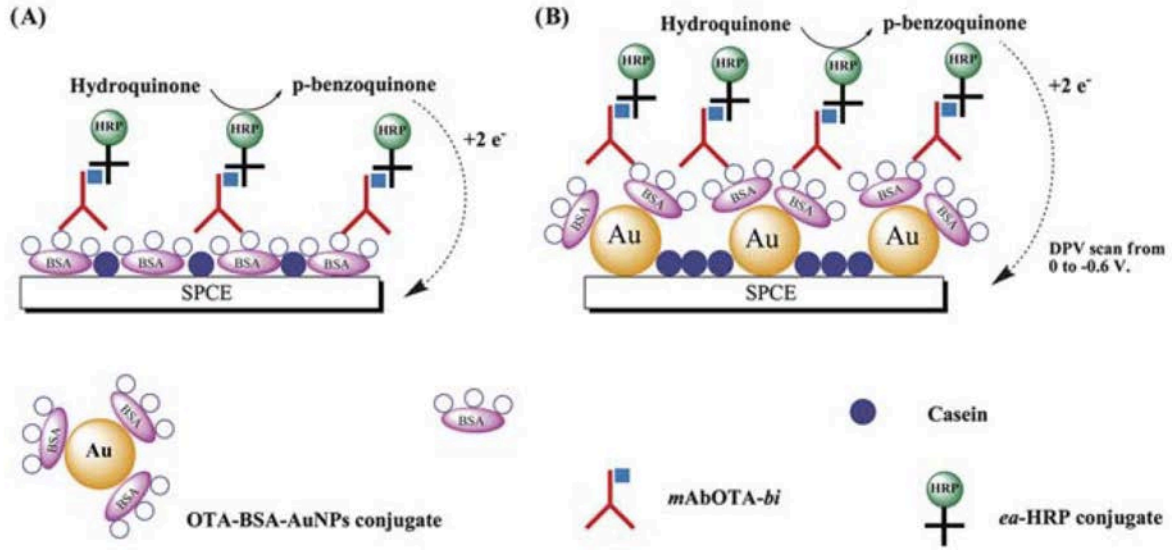




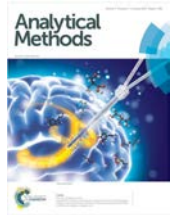
Castillo, J.R., Vidal, J.C., et al., A multi-electrochemical competitive immunosensor for sensitive COC dtm. in biological samples, Electroanal. 28 (2016) 685-694

Castillo, J.R., Vidal, J.C., et al., An electrochemical immunosensor for OTA determination in wines based on a mAb and paramagnetic microbeads, Anal. Bioanal. Chem. 403 (2012) 1585-1593





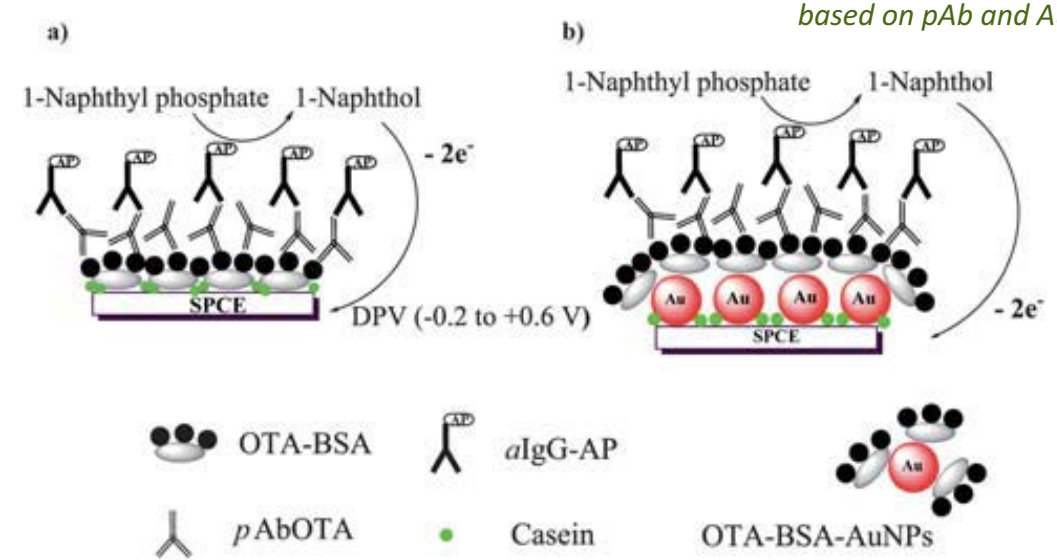
- **mAb-OTA-bi**
- OTA-BSA-AuNPs
- OTA: 0,15 – 10 ng mL^{-1}
- LOD: **0,10** ng mL^{-1} OTA
- %RSD: 10%



Castillo, J.R., Vidal, J.C., et al., Improved electrochemical immunosensor for OTA with a biotinilated mAb capture probe and AuNPs nanotructuring, Anal. Meth. 3 (2011) 977-984

La nanoestructuración mejora las propiedades del biosensor (inmunosensor)

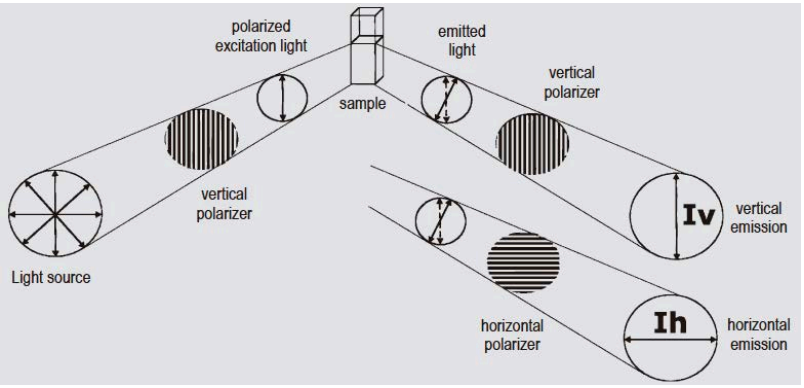
Castillo, J.R., Vidal, J.C., et al., OTA nanostructured electrochemical immunosensors based on pAb and AuNPs coupled to the antigen, Anal. Meth. 2 (2010) 335-341



- **pAb-OTA**
- OTA-BSA-AuNPs
- OTA: 0,9 – 9 ng mL-1
- LOD (sin AuNPs): **0,86** ng mL^{-1} OTA
- LOD (AuNPs): **0,20** ng mL^{-1} OTA
- %RSD: 10,6% y 8,0%
- Recuperaciones: 105%

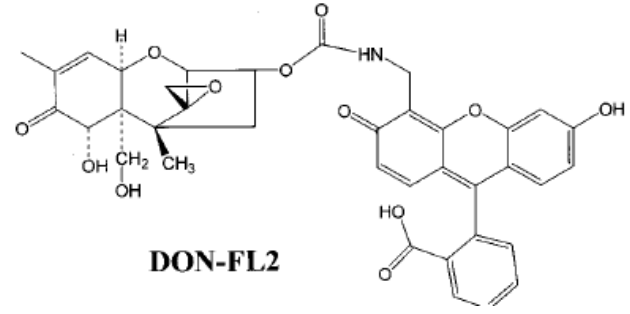
MÉTODOS RÁPIDOS: FLUORESCENCIA DE POLARIZACIÓN

FPIA



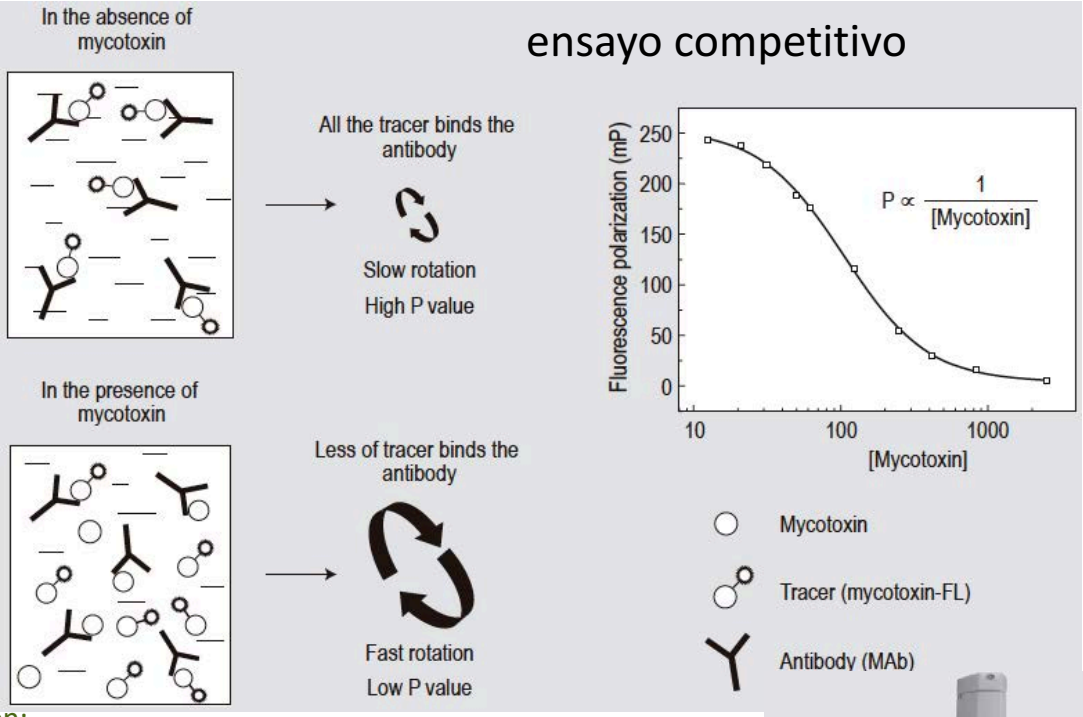
- Fase **homogénea** (rapidez)
- No es preciso separación (lavado)
- Velocidad de rotación
- Instrumentos portables (1 sóla micotoxina, 1 λ_{em})
- Tiempo de vida fluorescencia: t desde *exc*. a *emis*. (∼4 ms.)
- Alineamiento dipolos con la luz polarizada, tiempo emis.
- 1ª aplicación FPIA (myctx): 2001 (FB1 en maíz)
- t incubación: 2-10 min. (fase homogénea)

MYCTX	LODs ó EC_{50}
Aflatoxinas	EC_{50}: 28 ng mL^{-1}
DON	*LOD*: 100 μg kg^{-1}; EC_{50} ∼ 20 ng mL^{-1}
FB1	*LOD*: 500 μg kg^{-1}
OTA	*LODs* ∼ 0,5 - 10 ng mL^{-1}
ZEA	*LODs* ∼ 70 - 130 μg kg^{-1}
T-2 + HT-2	*LODs* ∼ 8 μg kg^{-1}



3 min. (extract.) + 2 min. (FPIA)

ensayo competitivo



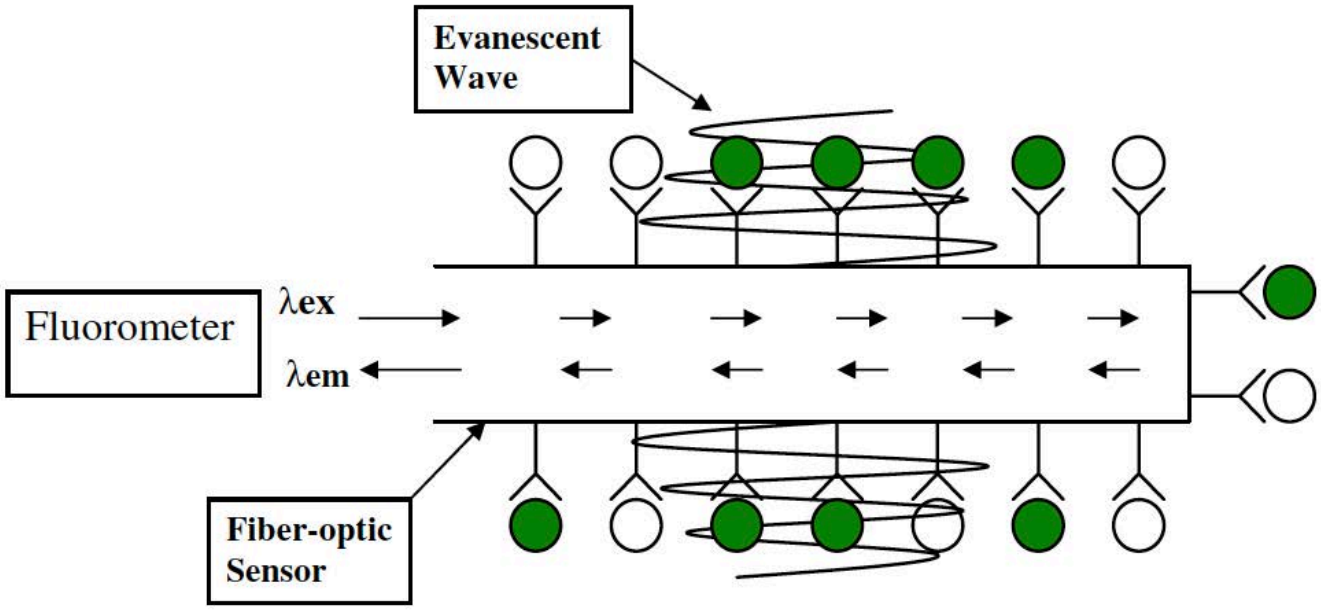
Más información:

Maragos, C. *et al.*, *Fluorescence polarization immunoassays for rapid, accurate and sensitive determination of mycotoxins*, World Myc. J. 7 (2014) 479-489

$$P = \frac{I_v - I_h}{I_v + I_h}$$

MÉTODOS RÁPIDOS: Inmunosensores de fibra óptica

Onda evanescente (interfase)
($\boldsymbol{\eta}$ ext.menor)



Ejemplo:

FB1: EC_{50}=25 μg g^{-1} (LOD=3,2 μg g^{-1})

⇩ IAC

EC_{50}=5 μg g^{-1} (LOD=0,4 μg g^{-1})

AFB1: < 2 ng mL^{-1}

- Alta especifidad
- Portabilidad (miniaturización)
- Medidas en tiempo real
- Falta de sensibilidad
- Influencia $\boldsymbol{\eta}$ disolventes

Más información:

Maragos, C. *et al.*, *Fiber-optic immunosensor for mycotoxins, Natural Toxins 7 (1999) 371-376*

Leung, A. et al., A review of fiber-optic biosensors, Sens. Act. B 125 (2007) 688-703

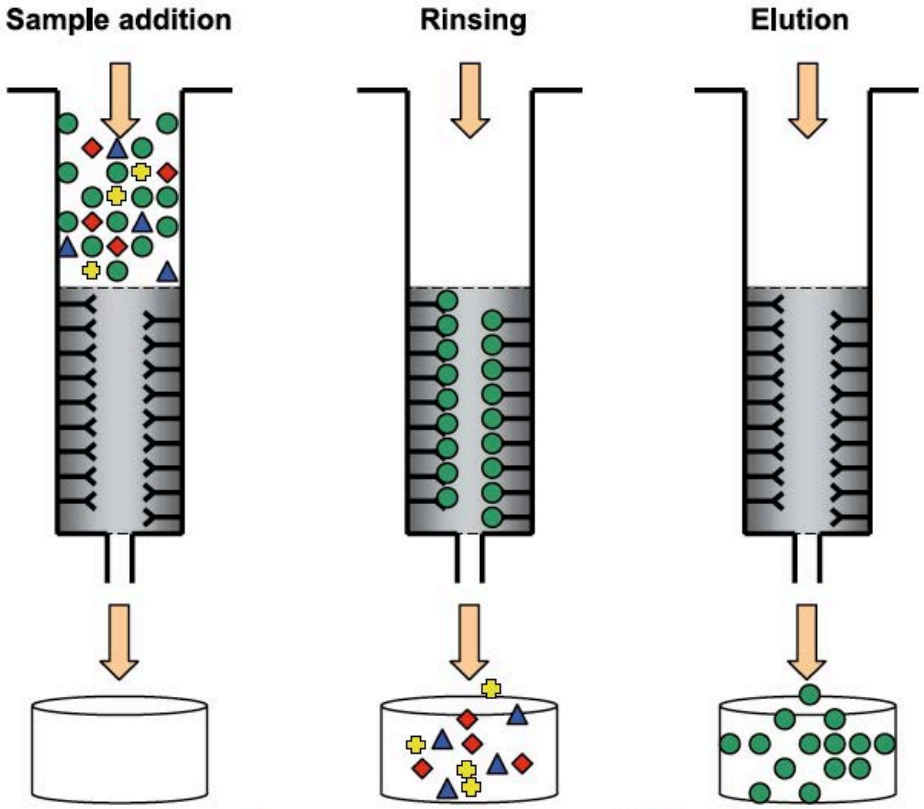


Figure 4. Principle of immunoaffinity columns (mycotoxin: ●; imprities: ◆ ▲ ✚).

Columnas de inmunoafinidad (IACs)

Ochratest
DONTest
Fumonitest

- Limpieza / preconcentración en **HPLC-FLD (MS)**
- Determinación posterior (**FM**, VOLT, Biosensores)
- Mejora de la selectividad y sensibilidad.
- Menores LODs.
- Evitamos el efecto de la **matriz** en la técnica instrumental.

LODs ≈ 0.01-5 ng/mL
(Fluorescencia intrínseca de la micotoxina)

Más información:

Zheng, M.Z. et al., A review of rapid methods for the analysis of mycotoxins, Mycopathologia 161 (2006) 261-273

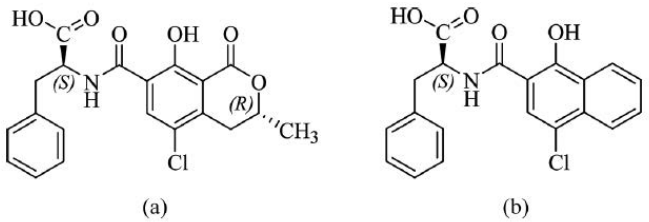
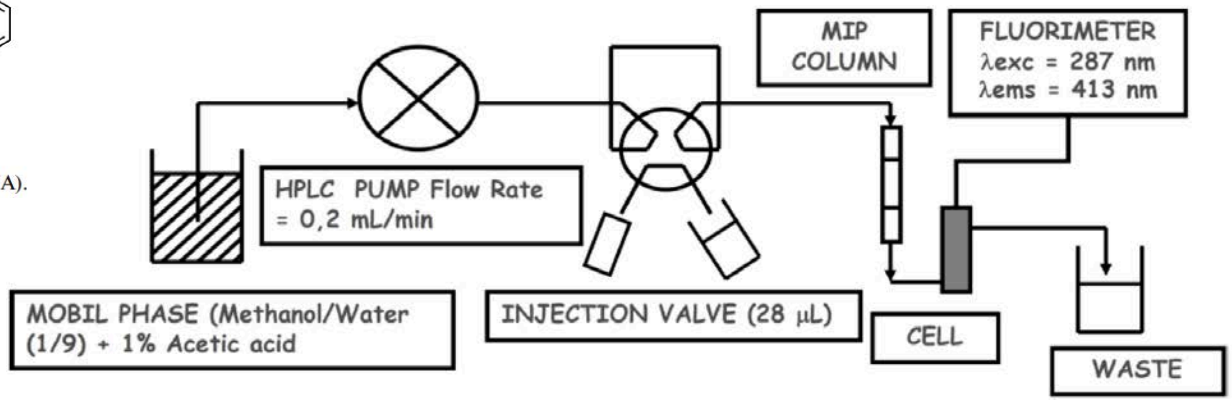
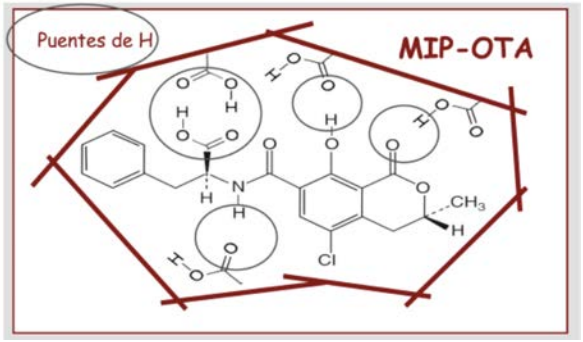


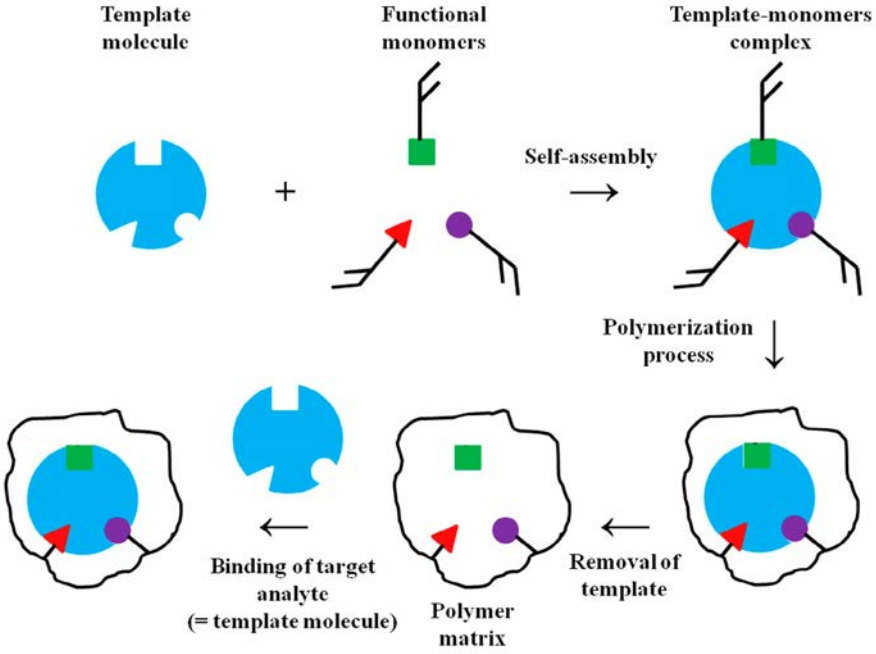
Figure 1. Molecular structures of: (a) OTA and (b) OTA*m* (L-Phen-CHNA).

MIPs



- OTA: 3-18 ng mL^{-1} OTA
- LOD: 1,2 ng mL^{-1} OTA
- *Recovery*: 93±9 % (cereal)
- *Dynamic binding capacity*: 118±9 ng OTA/45 mg MIP

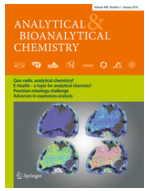




Molecularly Imprinted On-Line Solid-Phase Extraction Coupled with Fluorescence Detection for the Determination of Ochratoxin A in Wheat Samples

Castillo, J.R., Vidal, J.C., et al., Anal. Lett. 45 (2012) 51-62

QCM



Use of polyclonal antibodies to ochratoxin A with a quartz–crystal microbalance for developing real-time mycotoxin piezoelectric immunosensors

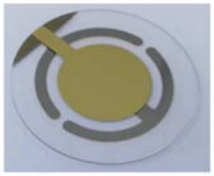
Castillo, J.R., Vidal, J.C., et al., Anal. Bioanal. Chem. 394 (2009) 575-582

Modelización:

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_q \mu_q}}\Delta m$$

Ecuación Sauerbray

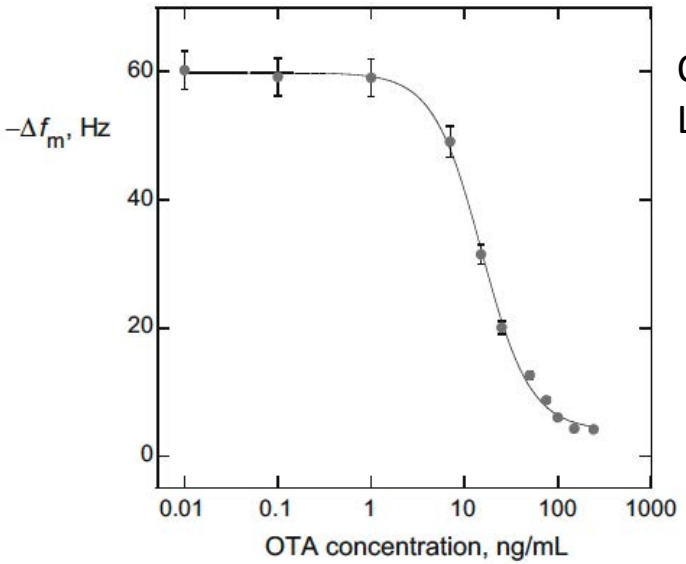
- Viscoelasticidad
- FIA
- No etiquetado



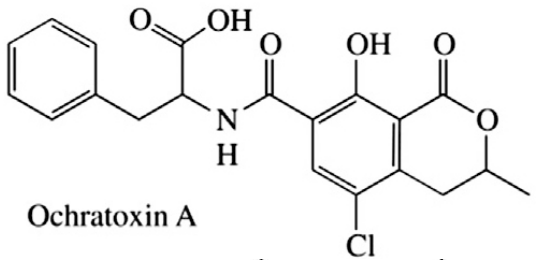
f_0=5 MHz

$$\Delta f[\mathrm{Hz}] = -0.0565[\mathrm{Hz/ng}] \times \Delta m[\mathrm{ng}] \quad \text{or}$$
$$\Delta m[\mathrm{ng}] = -17.7[\mathrm{ng/Hz}] \times \Delta f[\mathrm{Hz}]$$

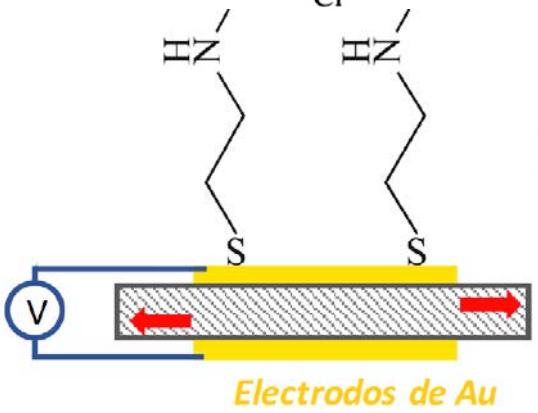
5 MHz crystals ($f_0 = 5 \times 10^6$ Hz) with an active area of 0.4 cm^2.



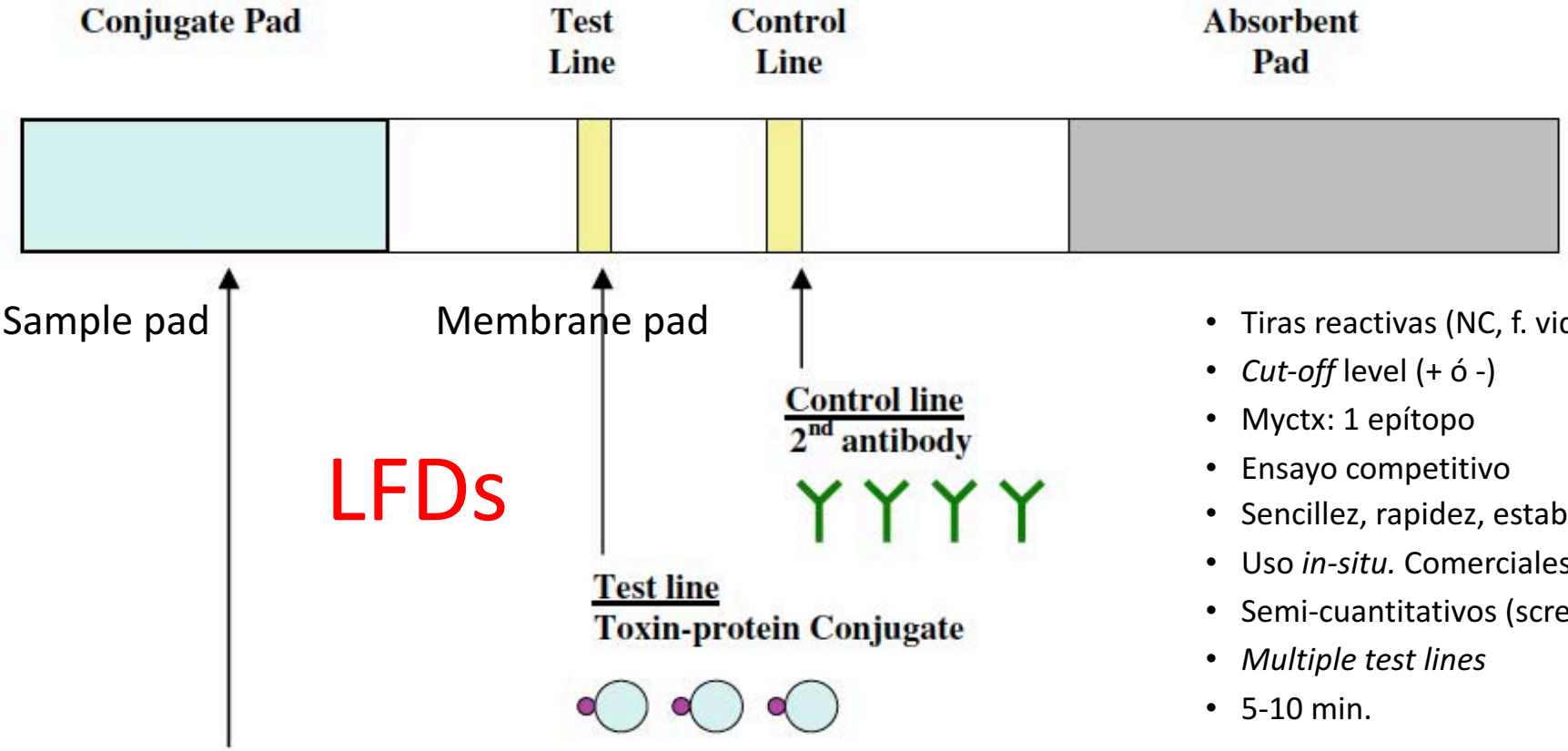
OTA: 10-128 ng mL-1
LOD: 8 ng mL-1



Ochratoxin A



Electrodos de Au



- Tiras reactivas (NC, f. vidrio)
- *Cut-off* level (+ ó -)
- Myctx: 1 epítopo
- Ensayo competitivo
- Sencillez, rapidez, estabilidad
- Uso *in-situ.* Comerciales.
- Semi-cuantitativos (screening)
- *Multiple test lines*
- 5-10 min.

Dispositivos Inmunocromatográficos

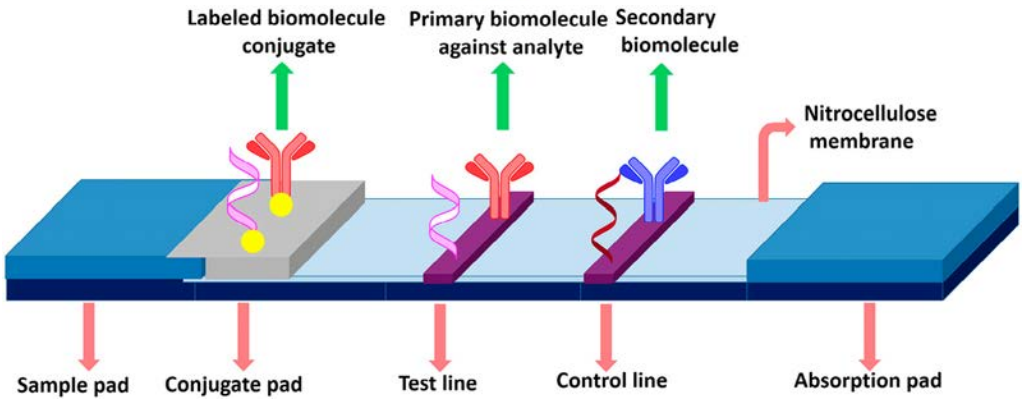
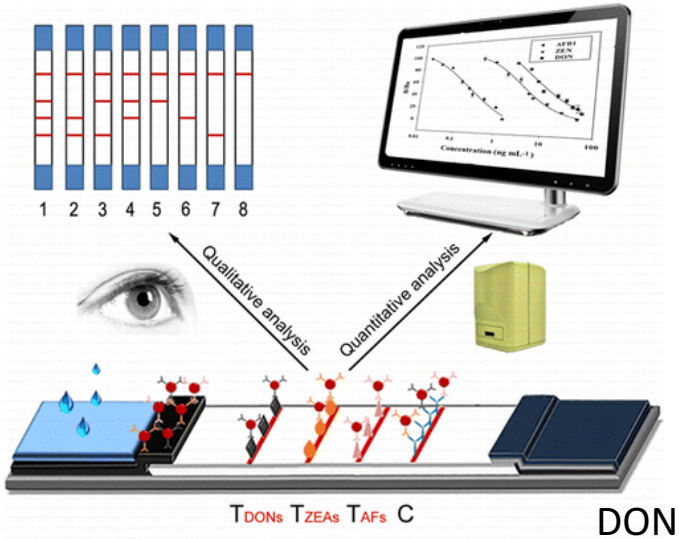


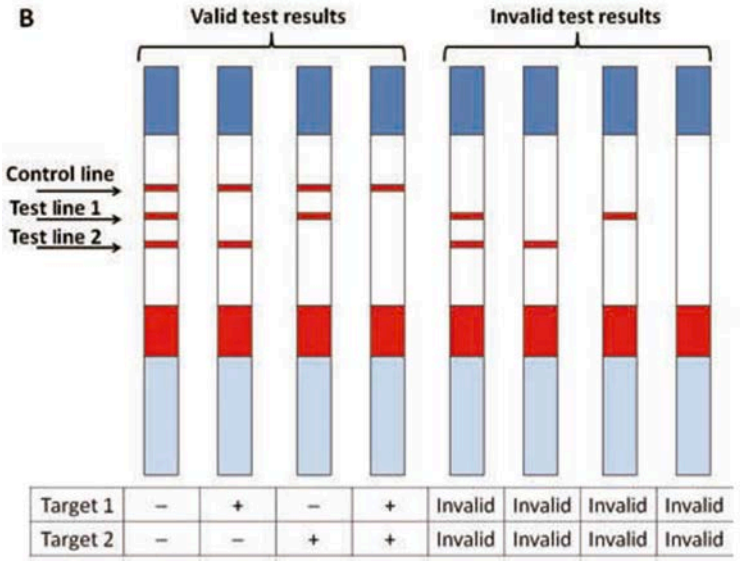
Table 1 Rapid test strips developed for the detection of microbial toxins during the last five years

Analyte[1)]	Conjugated probe[2)]	Visual LOD[3)] (ng mL^{-1})	Cutoff value (μg kg^{-1})	Assay time (min)	Tested food types or strains	Reference
Ochratoxin A	OTA-mAb-gold	2.5	5	15	Corn	Shim *et al.* 2009
Zearalenone	ZEA-mAb-gold	5	10	15	Corn	Shim *et al.* 2009
T2-mycotoxin	T2-mAb-gold	100	100	4	Wheat and oat	Beloglazova *et al.* 2014
Aflatoxin B1	B1-mAb-gold	0.06	0.6	15	Peanut	Zhang *et al.* 2012
Cholera toxin	CT-pAb-gold	10	10	15	Clinical *V. cholerae*	Yamasaki *et al.* 2013
BoNT A	BoNT-mAb-gold	5 and 25	5 and 25	10–15	Milk and apple juice	Ching *et al.* 2012
BoNT B	BoNT-mAb-gold	10	10	10–15	Milk and apple juice	Ching *et al.* 2012

Fuente: Nimal, J. et al., Mycotoxin detection – Recent trends at global level, J. of Integrative Agriculture 14 (2015) 2243-2264



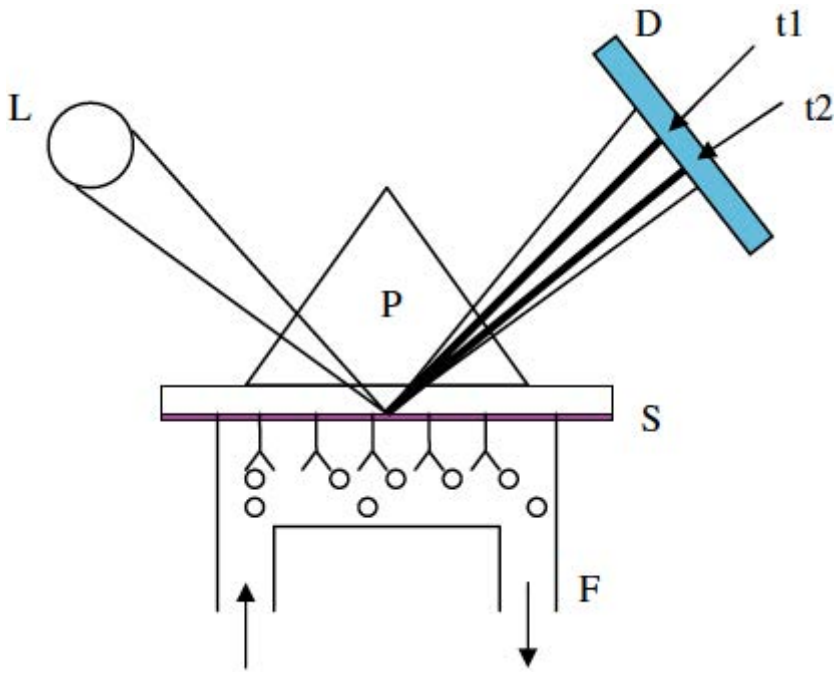
DON+ZEA+AFB1



Fuente: Song, S. et al., Multiplex LFD for mycotoxin determination, Anal. Chem. 66 (2014) 4995

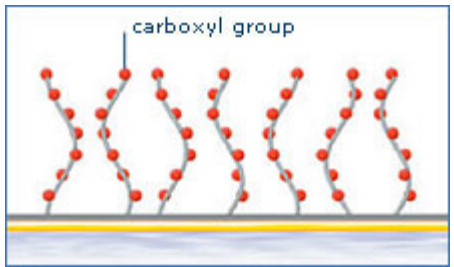
LFDs

Resonancia de Plasmón superficial (Inmunosensores)



- Inmunosensores para (múltiples) micotoxinas
- Se requiere muy poco volumen ($\sim\mu$L)
- Reutilización *sensor chip*
- Cinética micotoxina-anticuerpo
- No se requiere etiquetado (inmunosensor)
- Miniaturización de la instrumentación SPR (*in-situ*).
- Myctx (PM < 700 Da << 10 kDa): *sandwich*, *indirect. competitive*.

Micotoxinas	LODs (ng g^{-1})
AFB1 + ZEA + FB1 + DON	0,2 / 0,01 / 50 / 0,5
OTA	0,01
T-2, HT-2	25



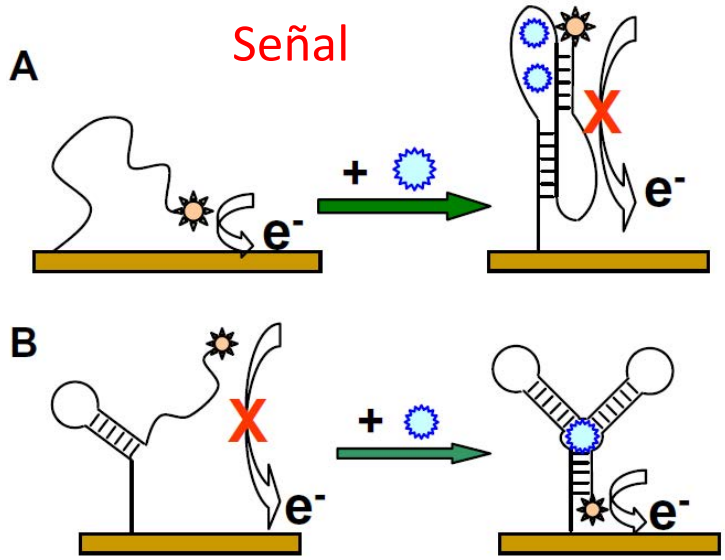
Sensor Chip

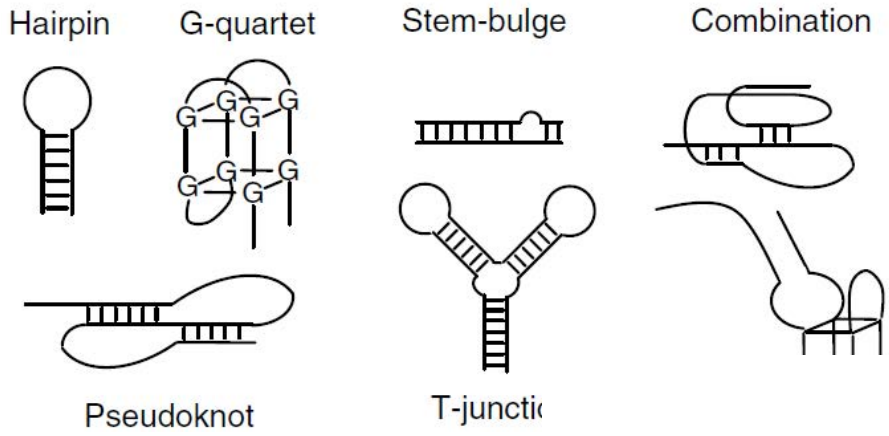
Más Información:

Ying, L. *et al.*, *Review: Recent developments and applications of SPR biosensors for the detection of mycotoxins in foodstuffs*, Food Chem. 133 (2012) 1548-1554

Aptámeros:

- Monocadenas oligonucleótidos (ssDNA, RNA)
- Método SELEX (~1995): Selectividad
- Mayor estabilidad vs. anticuerpos
- Síntesis más económica
- Sensibles a enzimas nucleasas
- OTA, FB1, AFB1 (aptámeros).

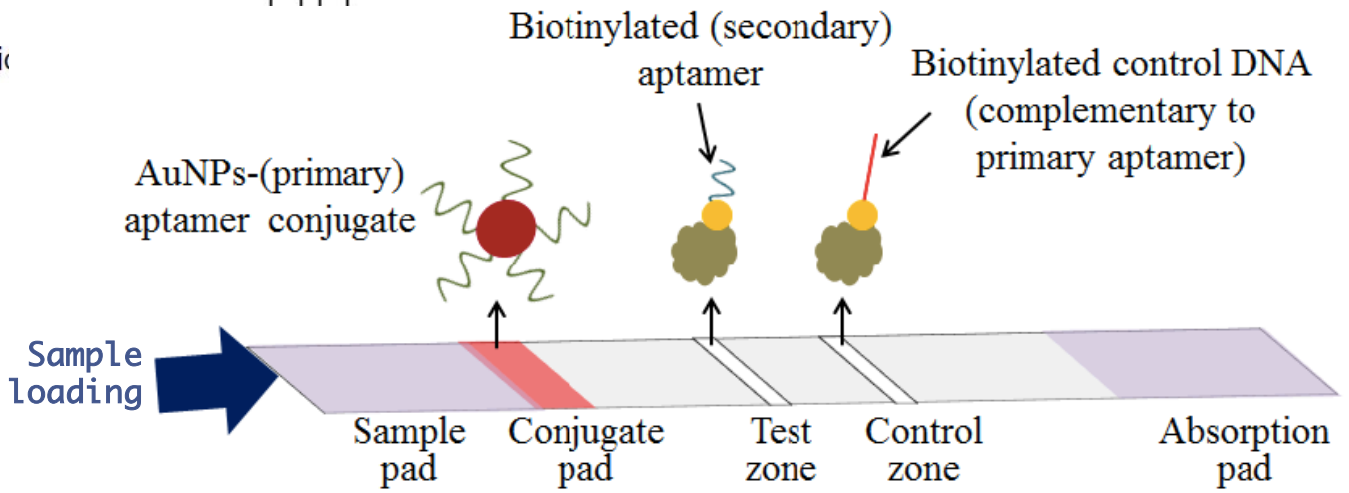




-C-G-
-A-T-

ELIMAs

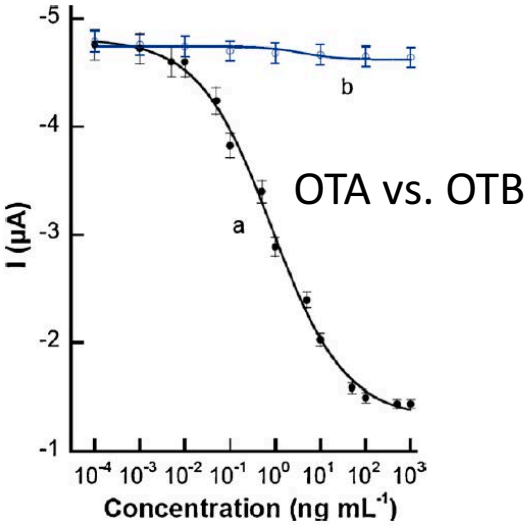
LFDs



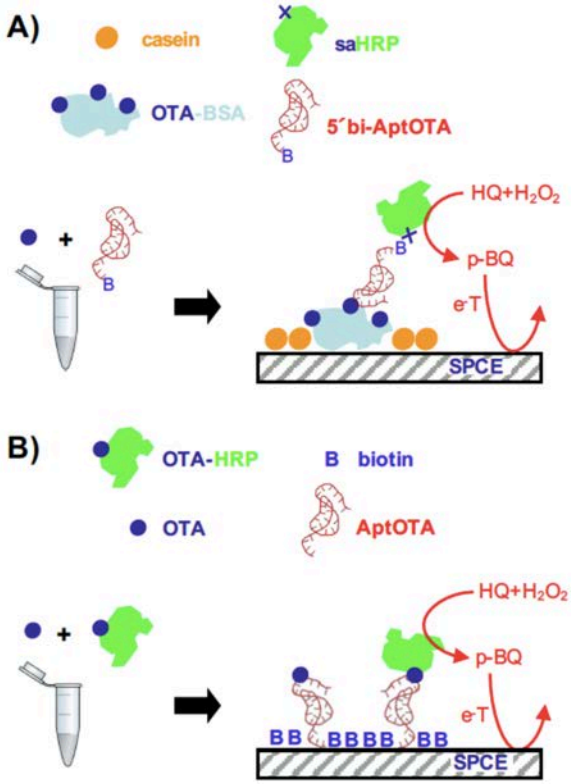
Más información:

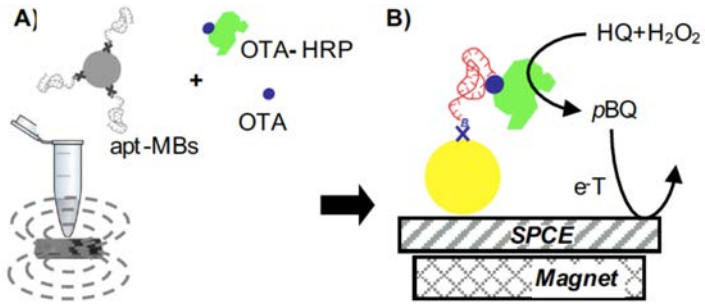
Yang, X. et al., *Review: Application of Aptamer Identification Technology in Rapid Analysis of Mycotoxins*, Chin. J. Anal. Chem 41 (2013) 297-306.

5´ Bi-GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA



- OTA: 0,7-8,8 ng mL^{-1}
- LOD: 0,07±0,01 ng mL^{-1}
- RSD: 8%

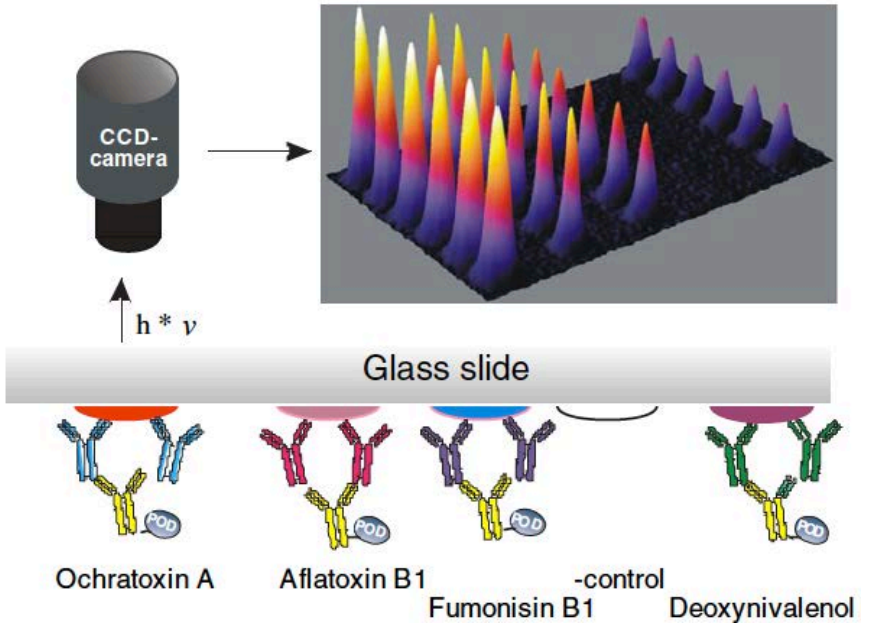




An electrochemical competitive biosensor for ochratoxin A based on a DNA biotinylated aptamer

Castillo, J.R., Vidal, J.C., et al., Biosens. Bioelectr. 26 (2011) 3254-3259

Array biosensor optical assays



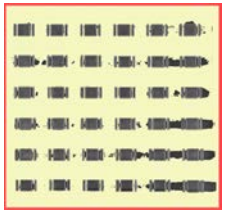
- Inmunoensayo múltiple (indirecto)
- *Spotting over a defined surface.*
- *Chip*: di-amino-PEG sobre superficie vidrio
- Quimiluminiscencia.
- Aflatoxinas (B1, B2, G1, G2), OTA, DON, FB1
- Extracción múltiple (MeOH:Agua, 80:20, V:V)
- a-IgG-HRP (luminol, H_2O_2).
- **Tiempo total: 19 min.**
- Re-utilización *biochip*: 50 veces.

Micotoxina	LODs (µg kg^{-1})	Linear range (µg kg^{-1})
OTA	1,1	1,7 – 15,4
AFB1	0,9	2,6 – 11,6
FB1	159	168 - 2216
DON	40,5	76 - 1173

Más información:

Oswald, S. et al., *Automated regenerable microarray-based immunoassay for rapid parallel quantification of mycotoxins in cereals*, Anal. Bioanal. Chem. 405 (2013) 6405-6415.

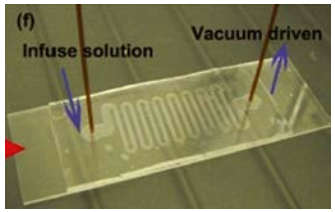
Avidin (silanized) coated glass slide (PDMS template) biosensor array. Each pattern: 21 x 1 x 2.5 mm. (L x W x H)



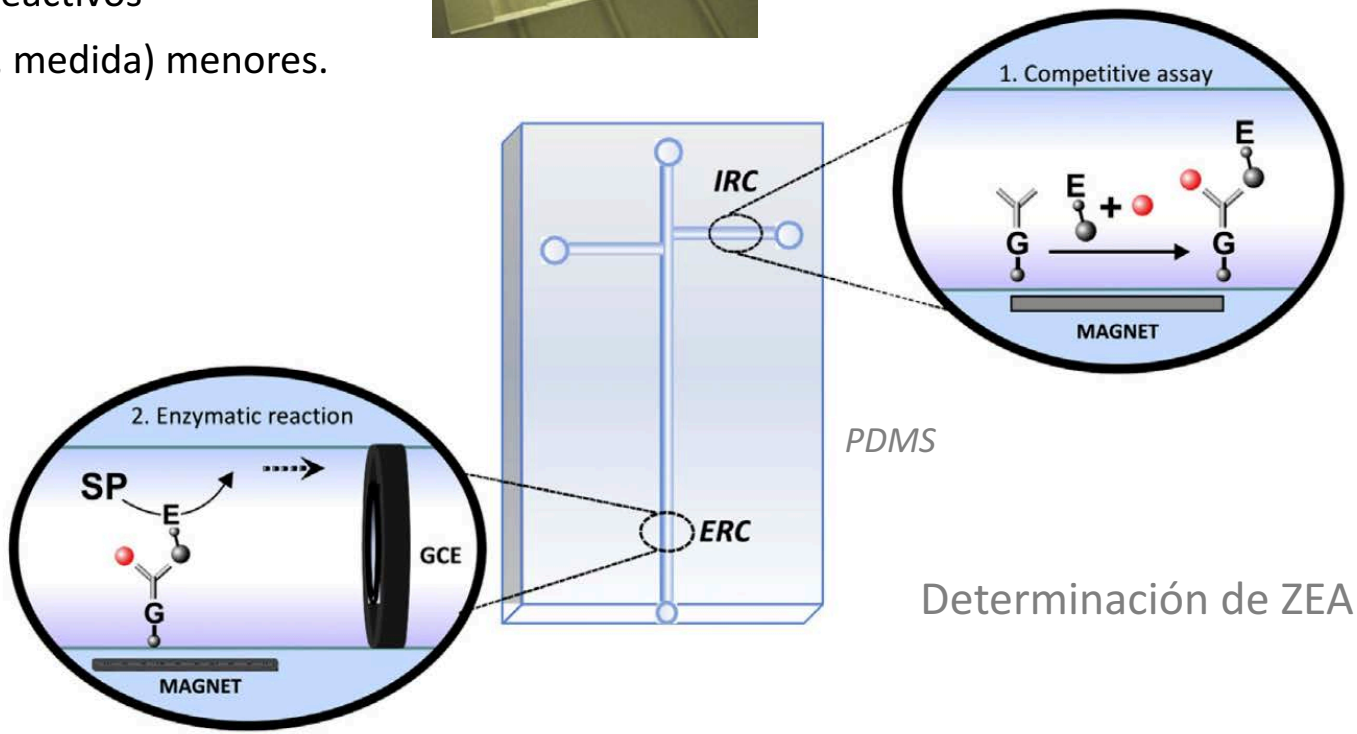
- μTAS (1980´s)
- *Lab-on-a-chip*
- Preparación muestra, filtración, reacción, detección.
- Canales microfluídicos: 10-200 μm
- Consumo menor de reactivos
- Tiempos (incubación, medida) menores.

Más información:

Fang, K., *Application of microfluidic "lab-on-a-chip" for the detection of mycotoxins in foods, Trends in Food Sci. Tech. 46 (2015) 252-263*



Un chip microfluídico típico



Determinación de ZEA

UIMP Universidad Internacional Menéndez Pelayo

Universidad Zaragoza 1542

Instituto Universitario de Investigación en Ciencias Ambientales de Aragón Universidad Zaragoza

Gracias por su atención

GRUPO DE ESPECTROSCOPÍA ANALÍTICA Y SENSORES GEAS UNIVERSIDAD DE ZARAGOZA · ZARAGOZA · ESPAÑA

Juan Carlos Vidal Ibáñez

BIOSENSORES PARA LA DETERMINACIÓN RÁPIDA Y PORTABLE DE MICOTOXINAS

29 de Septiembre de 2016

Universidad Zaragoza
1542

UIMP Universidad Internacional Menéndez Pelayo

PIRINEOS 2016

CONTAMINANTES EMERGENTES EN EL SIGLO XXI

Del 28 al 29 de septiembre de 2016

Escuela Politécnica Superior de Huesca

Director:
Juan R. Castillo
Catedrático de Química Analítica
Instituto de Investigación en Ciencias Ambientales de Aragón (IUCA), Universidad de Zaragoza

BIOSENSORES PARA LA DETERMINACIÓN RÁPIDA Y PORTABLE DE MICOTOXINAS

Juan Carlos Vidal Ibáñez

Información adicional para el Curso

29 de Septiembre de 2016

Dominated by commercial test kits.....

ELISAs

Analyte	Sample	Format	Extraction	Antibody Cross-reactivity	Limit of Detection	Brand Name	Manufacturer
T-2	Cereals, silage	EIA	Acetonitrile/water 84:16 % v/v	T-2 100% Acetyl T-2 12	30 – 55 µg/kg	T-2 Toxin EIA	Eurodiagnostica B.V., The Netherlands
T-2	Cereals, feed	EIA	Methanol/water 70/30 % v/v	T-2 100% Acetyl T-2 114%	< 5 µg/kg	RIDASCREEN® T-2 Toxin	R-Biopharm AG, Germany
T-2	Grain, cereals	ELISA	Not reported	Not reported	35 µg/kg	AgraQuant® T-2 Toxin	Romer Labs Diagnostica GMbH, Austria
T-2	Maize and derived products	ELISA	Methanol/water	T-2 100% HT-2 38%	25 µg/kg	ELISA kit for T2 Toxin	Tecna S.r.l. Italy
T-2/ HT-2	Barley, maize, oats, rye, soy, wheat	ELISA	Methanol/water 70/30 % v/v	T-2 100% HT-2 100%	Not reported. Limit of Quantification 25 µg/kg	Veratox® for T-2/HT-2 Toxins	Neogen Corporation, U.S.A.
DON	Cereal, food, feed, beer	ELISA	Distilled water	DON 100% 3AcDON 96%	10 - 30 µg/kg	DON EIA	Eurodiagnostica B.V. The Netherlands
DON	Grain, cereals	ELISA	Not reported	Not reported	0.2 mg/kg	AgraQuant® DON	Romer Labs Diagnostica GMbH, Austria
DON	Barley, oats, wheat, wheat bran, flour, midds	LFD	Distilled water	DON 100% & 3AcDON	Semi-quantitative	Reveal® for DON SQ	Neogen Corporation, U.S.A.
DON	Wheat, wheat flour, bran, barley, maize, m	ELISA	Not reported	DON 100% & 3AcDON (not reported)	1 mg/kg	Agri-Screen® for DON	Neogen Corporation, U.S.A.
DON	Cereals, malt, feed, beer, wort	EIA	Distilled water	DON 100% 3AcDON > 100	Cereals, malt feed 18.5 µg/kg	RIDASCREEN® DON	R-Biopharm AG, Germany
DON	Cereals, malt, feed	EIA	Distilled water	Not reported	< 0.2 mg/kg	RIDASCREEN® FAST DON	R-Biopharm AG, Germany
DON	Grain	LFD	Distilled water	Not reported	0.5 – 1.25 mg/kg	RIDA® QUICK DON	R-Biopharm AG, Germany

Existe un gran número de kits ELISAS comerciales para todo tipo de micotoxinas

ELISAS: ALGUNOS OTROS EJEMPLOS

ELISAs

Dominated by antibody based methods.....

Analyte	Sample	Format	Extraction	Clean up	Antibody Cross-reactivity (%)	Limit of Detection	% Recovery	Reference
DON	Wheat	ELISA	Phosphate buffered saline	None	DON 100 3-AcDON 632 15-AcDON 3.3	0.2 µg/L	89	Maragos et al 2000
DON	Wheat	ELISA	Acetonitrile/water 10.5/89.5 % v/v	None	DON 100 3-AcDON 15 15-AcDON 4	2 µg/L	80-125	Schneider et al, 2000
DON	Maize, wheat	ELISA	Methanol/water 10/90 % v/v	None	DON High 3-AcDON Low 15-AcDON High	50 µg/L	Not reported	Sinha et al, 1995
T-2/ HT-2	Wheat kernels	ELISA	Acetonitrile/water 85/15 % v/v	None	T-2 100 HT-2 100 AcT-2 79	30 µg/kg	94-112	Yoshizawa et al, 2004
DON	Wheat kernels	ELISA	Acetonitrile/water 85/15 % v/v	None	3,15-diacetylDON 100 DON > 10000 15AcDON 3 Nivalenol >10000	80 µg/kg	94-112	Yoshizawa et al, 2004
DON	Wheat	LFD	Methanol/water 80/20 % v/v	None	DON 100 15-AcDON 429	Ranges of 250-500 µg/kg 1000-2000 µg/kg	N/A	Kolosova et al, 2008
T-2	Wheat, oat	LFD	Methanol/water 70/30 % v/v	None	T-2 100 HT-2 5.4	Not reported	N/A	Molinelli et al, 2008
DON	Wheat	FPIA	Phosphate buffered saline	None	DON 100 3-AcDON 339 15-AcDON 8	Not reported	71	Maragos et al, 2002

Algunos tipos de biosensores para la determinación de micotoxinas

Biosensor systems that utilise the antibody–antigen immunochemical relationship for toxin-specific molecular recognition.

Toxin	Matrix	Recognition method	Detection method	LOD
Total AF	Peanut	Immunochromatographic strip (ICS)	Colorimetric	0.03 ng mL^{-1}
Total AF and OTA	Model samples	Direct binding	Microcantilever	3–6 ng mL^{-1}
AF B1	Olive Oil	Direct binding	Electrochemical impedance spectroscopy	0.03 ng mL^{-1}
AF B1	Model samples	Direct binding onto antibody modified carbon nanotube	Electrochemical	0.08 ng mL^{-1}
AF B1	Maize	Direct binding onto immunochromatographic chip	FD	0.42 pg mL^{-1}
AF B1	Various foods	Direct binding	Electrochemical enhanced by ionic liquids	1 fM
AF B1	Groundnut	Direct binding	Piezoelectric	0.1 ng mL^{-1}
AF B1	Model samples	Direct binding	FD (intrinsic quenching)	0.35 ng mL^{-1}
AF B1	Model samples	Antibody coated nanoparticles and magnetic beads	Surface enhanced Raman spectroscopy	0.1 ng mL^{-1}
AF B1	Various	Phage-probe linked immunoassay	Colorimetric	0.117 ng mL^{-1}
AF B1	Wheat	Indirect competitive immunoassay	Colorimetric	15 pg g^{-1}
AF B1	Model samples	Indirect competitive immunoassay	Piezoelectric	0.01 ng mL^{-1}
AF B1	Various	lateral-flow immunodipstick assay	Colorimetric	0.1 ng mL^{-1}
AF B1	Model samples	Lateral-flow assay	Colorimetric	10 μg mL^{-1}
AF B1	Rice	Indirect competitive ELISA	Electrochemical	0.06 ng mL^{-1}
AF B1	Model samples	Antibody coated RnNi nanoparticles immobilised to an indium-tin oxide surface	Electrochemical	32.7 ng dL^{-1}
AF B1	Model samples	antigen-modified magnetic nanoparticles	Antibody functionalized upconversion nanoparticle Signal probes.	0.01 ng mL^{-1}
AF B2	Nuts	lateral-flow immunodipstick assay	Colorimetric	0.9 ng mL^{-1}
AF M1	Milk	Impedimetric assay	Electrochemical	1 pg mL^{-1}
AF M1	Milk	Direct competitive ELISA	Electrochemical	39 ng L^{-1}
AF M1	Milk	Direct competitive ELISA	Electrochemical	5 pg mL^{-1}
AF M1	Milk	Direct binding	Surface Plasmon enhanced fluorescence	0.6 pg mL^{-1}
AF M1	Milk	Two-step lateral flow immunoassay	Colorimetric	0.02 μg mL^{-1}
AF M1	Powder Milk	Indirect competitive ELISA	Electrochemical	15 ng L^{-1}
DON	Wheat and Maize	Direct binding to polymer coated quantum dots	FD	220–500 μg kg^{-1}
DON	Wheat	Competitive immunoassay	Fluorescence polarisation	120 μg kg^{-1}
DON	Wheat	Direct binding	Electrochemical	6.25 ng mL^{-1}
DON	Wheat and maize	Immunochromatographic strip (ICS)	Colorimetric	50 ng mL^{-1}
DON/ZON	Maize and wheat	Competitive inhibition immunoassay	Surface Plasmon resonance	10–17 ng mL^{-1}
Fumonisins	Corn	Indirect competitive ELISA	Electrochemical	5 μg L^{-1}
Fumonisins	Maize	Direct competitive ELISA	Chemi-luminescence	2.5 μg L^{-1}
Fumonisins	Maize	Immunodipstick assay	Colorimetric	2.5 ng mL^{-1}
Fumonisins	Maize	Lateral flow immunoassay	Colorimetric	199 μg kg^{-1}
Fumonisins	Beer	Direct competitive magnetoimmunoassay	Electrochemical	0.33 μg L^{-1}
Fumonisin B1 and OTA	Grains	Microsphere linked indirect competitive fluid array	Fluorescent flow cytometry	10–100 ng g^{-1}
HT-2	Model Sample	signal transduction by ion nano-gating (STING)	Electrochemical	100 fg mL^{-1}
Nivalenol and DON	Wheat	Indirect competitive immunoassay	Surface plasmon resonance	0.05 mg kg^{-1}
OTA	Model samples	Label-free immunosensor	Electrochemical impedance spectroscopy SPR	0.01 ng mL^{-1} 1 ng mL^{-1}
OTA	Model samples	Direct immobilisation	Piezoelectric	10 ng mL^{-1}
OTA	Model Samples	Mimotope peptide antibody based lateral flow strip	Colorimetric	~10 ng mL^{-1}
OTA	Wheat	Competitive immunoassay	Fluorescent polarization	0.8 μg kg^{-1}
OTA	Cereal and beverages	Competitive immunoassay linked to gold nanoparticles	SPR	0.042 ng mL^{-1}
OTA	Wine	Indirect competitive immunoassay	Electrochemical	0.5 μg L^{-1}
OTA	Model samples	Colloidal gold- antibody conjugate	SPR	60 pg mL^{-1}
OTA	Model samples	Direct immunoassay	Electrochemical impedance spectroscopy	N/A
OTA	Wine and cereals	Indirect competitive immunoassay	Fluorescence	0.2 μg L^{-1}
OTA	Wine and cereals	Flow immunoassay	Fluorescence	0.01 μg L^{-1}
OTA	Wheat	Direct competitive ELISA	Electrochemical	0.86 ng mL^{-1}
OTA	Model Sample	Nanostructured ZnO supporting antibodies	Electrochemical	0.006 nM L^{-1}
OTA	Model Sample	Cerium oxide nanoparticles/ITO coated glass slide	Electrochemical	0.25 ng dL^{-1}
OTA	Red wine	Flow-through gel and membrane based direct competitive assays	Colorimetric	2 μg L^{-1}
Patulin	Model samples	Competitive immunoassay	SPR	0.1 nM
Patulin and parathion	Apple puree	Sandwich assay	Piezoelectric	50 -140 nM
Various	Barley	Direct inhibition assay	Colorimetric via targeted reporter microspheres	2–1000 μg kg^{-1}

Fuente: Anal. Chim Acta 902 (2015) 12-33

Ejemplos del uso de Columnas de Inmunoafinidad

Toxin	Matrix	Recognition method	Detection method	LOD
Various	Cereals grains and silage	Flow-through membrane based direct competitive assays	Colorimetric	2.5 μg L^{-1}
Various	Model samples	Lateral flow immunoassay	Colorimetric	0.05–3 μg kg^{-1}
Various	Maize	Indirect competitive lateral-flow immunoassay	Chemiluminescence via CCD	1.5–6 μg kg^{-1}
Various	Model samples	Direct binding	Magnetoresistivity	50 pg mL^{-1}
Various	Cereals	Multiplex photonic crystal microsphere suspension array	Optical	0.5 pg mL^{-1}
Various	Corn and peanut	Indirect competitive immunoassay	Colorimetric	0.22 pg g^{-1}
Various	Cereals	Indirect competitive immunoassay	Colorimetric	80% required cut off levels EU directive
Various	Drinking water	Indirect competitive immunoassay	Colorimetric	0.04–35.6 mg mL^{-1}
Various	Cereals	polyvinylidene fluoride (PVDF) membrane-based dot immunoassay	Colorimetric	20–1000 μg kg^{-1}
Various	Various	Flow-through gel and membrane based assays	Colorimetric	3 μg kg^{-1}
Zeranol	Bovine tissue	Chemiluminescent enzyme immunoassay	Optical	0.05 μg kg^{-1}
ZON	Feed	Competitive immunoassay llinked to gold nanoparticles	Surface enhanced Raman Spectroscopy	1 pg mL^{-1}
ZON	Wheat	Non-competitive immunoassay	Fluorescence resonance energy transfer (FRET)	0.8 ng mL^{-1}
ZON	Corn	Immunochromatographic test strip	Colorimetric	3.4–20 μg kg^{-1}
ZON	Cereal	Phage-probe linked rapid-dot immunoassay	Colorimetric/SPR	50 μg kg^{-1}
ZON	Corn	fluorescence polarization immunoassay	FD	137 μg kg^{-1}
ZON	Feed	Direct competitive immunoassay via paramagnetic beads	Electrochemical	0.41 μg kg^{-1}
ZON	Corn	Indirect competitive immunoassay	Colorimetric	2.5 ng mL^{-1}
ZON	Model samples	Metal-oxide semiconductor field effect transistor	Electrochemical/SPR	0.1 μg mL^{-1}
ZON	Wheat	Flow-through gel and membrane based assays	Colorimetric	100 μg kg^{-1}

Fuente: Turner, N.W. et al., *Review – Analytical methods for determination of mycotoxins: An update (2009-2014)*, Anal. Chim. Acta 901 (2015) 12-33

Aptasensores para micotoxinas

Aptamer based biosensor for mycotoxins.

Toxin	Matrix	Method	Detection method	LOD unless stated otherwise
AF B1	Peanuts/corn	Dendrimer –linked aptameric capture	Electrochemical	0.40 nM
AF B1	Hay/rice	Competitive DNA interaction	PCR	25 fg mL^{-1}
AF B1	Corn	Direct binding	SPR	1 nM
AF B1	Corn	Competitive DNA interaction	Fluorescence	0.1 ng mL^{-1}
AF B1	Corn	Competitive assay	Chemiluminescence	0.1 ng mL^{-1}
AF M1	Milk	Direct binding	Electrochemical	1 ng mL^{-1}
OTA	Wine/peanut oil	Direct binding	SPR	0.094 ng mL^{-1}
OTA	Model samples	Enzyme mimic formation	Colorimetric	~1 nM
OTA	Corn	Structure switching	FD	0.8 ng mL^{-1}
OTA	Wheat	Structure switching	FD	2 pg mL^{-1}
OTA	Wine	Indirect competitive/direct competitive magnetic beads	Colorimetric	0.11 ng mL^{-1}
OTA	Beer	Indirect competitive/direct competitive magnetic beads	Colorimetric	0.05 μg L^{-1}
OTA	Model samples	Direct binding to quantum dot labelled magnetic beads	FD	5.4 pg mL^{-1}
OTA	Wheat	Competitive removal of DNA	Electrochemiluminescence	0.007 ng mL^{-1}
OTA	Wine	Competitive removal of DNA leading to exonuclease action	Electrochemiluminescence	0.64 pg mL^{-1}
OTA	Model samples	Binding leads to protection from endonuclease action	Colorimetric	0.4 pg mL^{-1}
OTA/Fumonisin B1	Model samples	Direct binding to fluorescent nanoparticles	Fluorescence resonance energy transfer (FRET)	0.05 ng mL^{-1}
OTA/Fumonisin B1	Cereals	Competitive removal of DNA from photonic crystal array	FD	0.25 pg mL^{-1}

Fuente: Anal. Chim. Acta 901 (2015) 12-33

Aptámeros seleccionados: **OTA / FB1 / AFB1**

Biosensores para micotoxinas *Fusarium*

Table 1. Recent biosensors and assays for fumonisins determination.

Reference	Technique	Analyte	Element	Sample (Extraction)	LOD	Working Range
[7], 1996	Optical: fiber-optic	FB1	antibody	buffer and corn (80% methanol)	10 ng/mL	10–1000 ng/mL
[8], 1998	Optical: SPR	FB1	antibody	NA	50 ng/mL	NA
[9], 1999	Optical: fiber-optic	FB1	antibody	maize (75% methanol)	0.4–3.2 µg/g	NA
[10], 2001	Optical: FPIA	FB1, FB2, FB3	antibody	maize (PBS)	0.5 µg/g	0.5–100 µg/g
[11], 2010	EC: amperometric	FB1, FB2	antibody	corn (70% methanol)	5 ng/mL	1–1000 ng/mL
[12], 2012	Optical: CL	FB1, FB2	antibody	maize flour (PBS)	2.5 ng/mL	2.5–500 ng/mL
[13], 2013	Optical: FRET	FB1	aptamer	maize (70% methanol)	0.01 ng/mL	0.01–100 ng/mL
[14], 2013	Optical	FB1	aptamer	beer	125 pg/mL	125–1500 pg/mL
[15], 2014	Optical: ECL	FB1	aptamer	NA	0.29 ng/mL	NA
[16], 2015	Optical: FPIA	FB1, FB2	antibody	maize (40% methanol)	53.6–290.6 ng/g	108.0–13166 ng/g
[17], 2015	EC: amperometric	FB1, FB2, FB3	antibody	maize-based foodstuffs (acetonitrile:PBS (50:50)), beer	0.33 ng/mL	0–1000 ng/mL
[18], 2015	EC: impedimetric	FB1, FB2, FB3	antibody	corn (70% methanol)	0.46 pg/L	7–49 pg/mL
[19], 2015	microcantilever array	FB1	aptamer	NA	33 ng/mL	0.1–40 µg/mL
[20], 2015	EC: impedimetric	FB1	aptamer	maize (20% methanol)	2 pM	0.1 nM–100 µM
[21], 2015	EC: amperometric	FB1	antibody	cereal samples (70% methanol)	0.58 ng/mL	0.6–54 ng/mL
[22], 2015	EC: amperometric	FB1	aptamer	wheat	1 pg/mL	1–106 pg/mL
[23], 2015	EC: amperometric	FB1	antibody	corn (50% acetonitrile)	2 pg/mL	0.01–1000 ng/mL

Fuente: Lin, X. et al., *Review - Advances in Biosensors, Chemosensors and Assays for the Determination of Fusarium Mycotoxins*, Toxins 8 (2016) 161-181

Más información: Xu, L. et al., Review: Mycotoxin determination in foods using advanced sensors based on antibodies or aptamers, Toxins 8 (2016) 239-255

Polímeros modelados molecularmente (MIPs)

MIPs for mycotoxins.

Toxin	Matrix	Method	Detection method	LOD unless stated otherwise
OTA	Wine	SPE	HPLC-FD	0.075 ng mL^{-1}
OTA	Cereals	SPE	HPLC-FD	2.5 μg kg^{-1}
OTA	Ginger	SPE	UHPLC-FD	0.09 ng mL^{-1}
ZON	Corn, Rice, Wheat	Optosensing material based on ionic liquid (stabilized CdSe/ZnS quantum dots	SEM/FTIR	0.002 μmol L^{-1}
ZON	Corn	Electropolymerisation onto surface	SPR	0.3 ng mL^{-1}

Biosensores de inhibición enzimática

Biosensor systems to incorporate enzyme or peptides as the recognition element.

Toxin	Matrix	Method	Detection method	LOD unless stated otherwise
AF B1	Model samples	Aflatoxin oxidase bound to carbon nanotube-gold electrode	Amperometry	1.6 nmol L^{-1}
OTA	Wine	Peptide Based ELISA	Colorimetric	2 μg L^{-1}
OTA	Beer and Coffee	Immobilised peroxidase on a screen printed electrode	Amperometry	0.1 ng mL^{-1}
OTA	Red wine	Synthetic peptide bound to chitosan support	Chemiluminescence	0.5 μg L^{-1}
Sterigmatocystin	Model samples	Aflatoxin oxidase bound to carbon nanotube-gold electrode	Amperometry	3 ng mL^{-1}

Fuente: Anal. Chim. Acta 901 (2015) 12-33

Table 2. Performance characteristics of different rapid methods for the detection of aflatoxin in corn

Performance characteristics	ELISA	Flow-through immunoassay	Lateral flow test	Fluorometric assay with IAC clean-up	Fluorometric assay with SPE clean-up
Quantitative or semi-quantitative	Quantitative	Semi-quantitative	Semi-quantitative	Quantitative	Quantitative
Detection limits	2.5 ppb	20 ppb	4, 10 or 20 ppb	1 ppb	5 ppb
Recovery (%)	93.7–122.6%	NA	NA	105–123%	92–102%
Relative Standard Deviation for Repeatability (%)	4.8–15.9%	NA	NA	11.75–16.57%	8.8–19.6%
Correct response for positive test samples spiked at the detection level	NA	97%	100%	NA	NA
Assay time[a]	<25 min	<5 min	5 min	<15 min	<5 min
Equipment	ELISA reader	NA	NA	Fluorometer	Fluorometer
Reference	[15]	[17]	[19]	[21]	[22]

[a] Assay time is the time needed to detect mycotoxins in a single, pre-ground sample after extraction.

Fuente: Mycopathologia 161 (2006) 211

Fuente: Food Sci. Tech. 46 (2015) 252-263

Representative application of microfluidics lab-on-a-chip devices in the detection of mycotoxins.

Mycotoxin types	Target analyte/food matrix	Sample pretreatment	Capture agents	Detection devices	Characteristics of device and analysis	Reference
Aflatoxins (including AFB_1, AFB_2, AFG_1, and AFG_2)	Peanuts, peanut powder, peanut butter	Solvent extraction (MeOH) and immunoaffinity solid-phase extraction (SPE)	Chip-based nano liquid chromatograph (LC)	Triple quadrupole MS system	Linear range: 0.048–16 ng/g; LOD: 0.004–0.008 ng/g; Recovery: 90.8%–100.4%.	(Liu et al., 2013)
Zearalenone (ZEN)	Infant foods	Solvent extraction in an ultrasonic bath	A competitive enzyme-linked immunosorbent assay (ELISA)	Electrochemical detection	LOD: 0.4 μg/L; Recovery: 103% for solid samples and 101% for liquid samples; Immunoassay time: <15 min.	(Hervás et al., 2011b)
Citrininn (CIT)	Rice	Solvent extraction (ACN, aqueous solution of KCl)	A competitive ELISA	Microfluidics electrochemical detection (using amperometric measurements)	LOD: 0.1 ng/mL; LOQ: 0.5 ng/mL; Detection time:<2 min; Total assay time: <45 min.	(Arévalo et al., 2011)
Ochratoxin A (OTA)	Red wine; beer	Liquid–liquid extraction	A competitive ELISA	Chemiluminescence detection	LOD (ng/mL): 0.85 in pure PBS, 0.1 in beer and 2 in red wine.	(Novo et al., 2013)
Ochratoxin A (OTA)	Red wine; beer	No sample pretreatment	An indirect ELISA	Chemiluminescence detection	LOD (ng/mL): 0.5 in wine and beer	(Novo et al., 2012)
Ochratoxin A (OTA)	White wine	No sample pretreatment	An indirect competitive ELISA	Chemiluminescence detection	LOD (ng/mL): 0.5 in pure solutions and 1 in white wine	(Novo et al., 2011)
Ochratoxin A (OTA)	Green coffee	Solvent extraction (methanol/aqueous sodium bicarbonate solution).	An indirect competitive ELISA	Chemiluminescence detection integrated in a regenerable glass microfluidics immunosensor	LOQ: 7 μg/kg in green coffee extract; Analysis time: 12 min; allow for at least 20 assay-regeneration cycles of the biochip surface	(Sauceda-Friebe et al., 2011)
Fumonisin B (includes FB_1 and FB_2)	Maize	Aqueous extraction buffer	Lateral flow immunoassay	Optical method (color intensity)	LOD: 120 μg/L	(Anfossi et al., 2010)
Fumonisin B (includes FB_1, FB_2 and FB_3)	Maize	Solvent extraction (methanol/water)	A paper-based competitive ELISA test strip	Optical method (color intensity)	LOD: 2.5 ng/mL; Total immunoassay analytical time: <15 min.	(Li, Zhang, et al., 2012; Li, Zhou, et al., 2012)
Zearalenone (ZEN)	Corn	Solvent extraction (PBS)	A monoclonal antibody based gold nanoparticle immune-chromatographic assay	Optical method (color intensity)	LOD: 2.5 ng/mL and 30 μg/kg for the standard solution and spike samples; Immunoassay analytical time: <15 min.	(Shim et al., 2009)
Aflatoxin M_1 (AFM_1)	Milk	Centrifugation of milk samples (no extraction)	An indirect competitive ELISA	Electrochemical detection	LOD: 8 ng/L	(Parker et al., 2009)
Ochratoxin A (OTA)	/	No sample pretreatment	Aptamers	Surface enhanced Raman spectroscopy (SERS)	Successfully detected 2.5 μM OTA	(Galarreta et al., 2013)