The analysis of mycotoxins in samples with IMMUNOPREP® ONLINE AFLATOXIN cartridges

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Content

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- ✓ Product description;
- ✓ Working principle;
- ✓ Results from validation studies thus far
- ✓ Performance characteristics
- ✓ Summary of Technical benefits
- ✓ Summary of Financial benefits

Background

- ✓ Time pressure on laboratories is increasing;
- ✓ Staff costs are increasing;
- ✓ Improvement in quality is a NEN/ISO17025 demand;
- ✓ Regulations relating to food and laboratory safety are increasing

Greater automation presents an opportunity to better manage these factors

IMMUNOPREP® ONLINE platform technology

A true on-line immunoaffinity column based upon a patented technology involving an antibody bound to a rigid polymer able to withstand the high pressures of HPLC or LC-MS/MS

Combining the benefits of an off-line immunoaffinity column:

1. High selectivity
2. High concentration of dilute sample extracts
3. Removal of interfering components

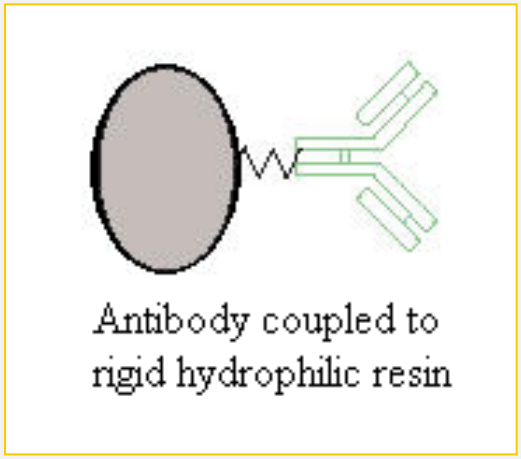
With the benefits of automation:

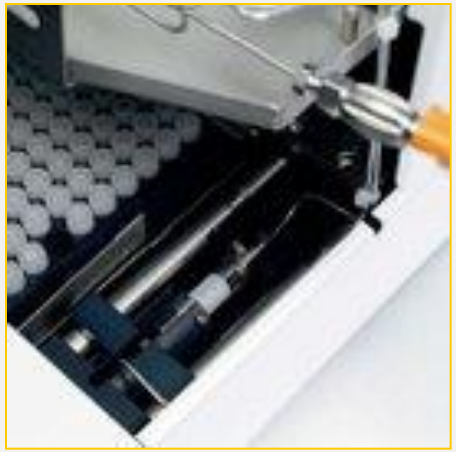
1. Time and Labour saving in the process
2. Increased capacity and reproducibility through automation
3. Improved traceability of samples
4. Improved quality control

Product description

The monoclonal antibody is bound to a rigid hydrophilic polymer, which is packed into a 20 mg cartridge.

Automation and use of these IMMUNOPREP® ONLINE cartridges is performed with the Symbiosis™ Pico HPLC System.

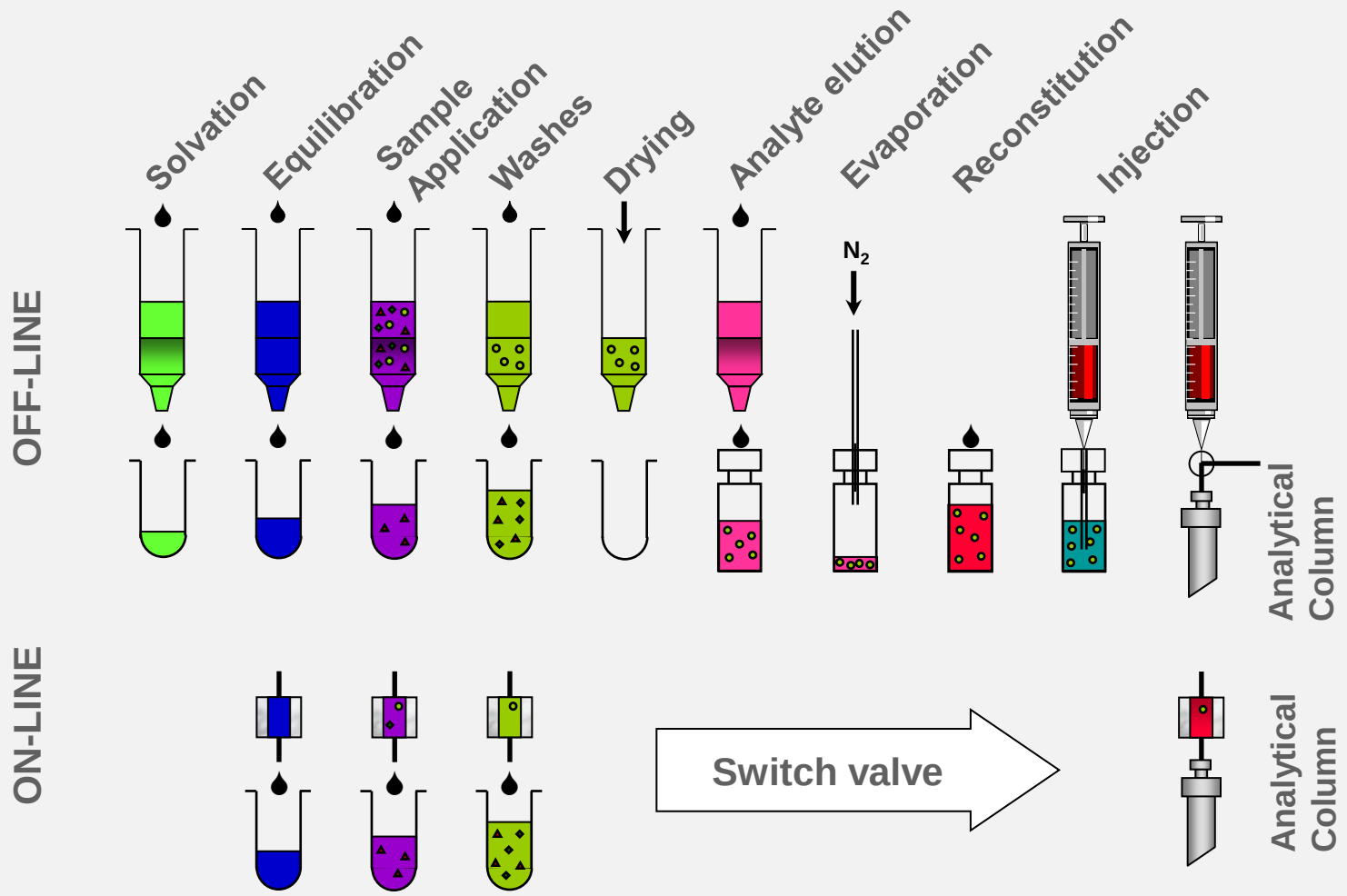




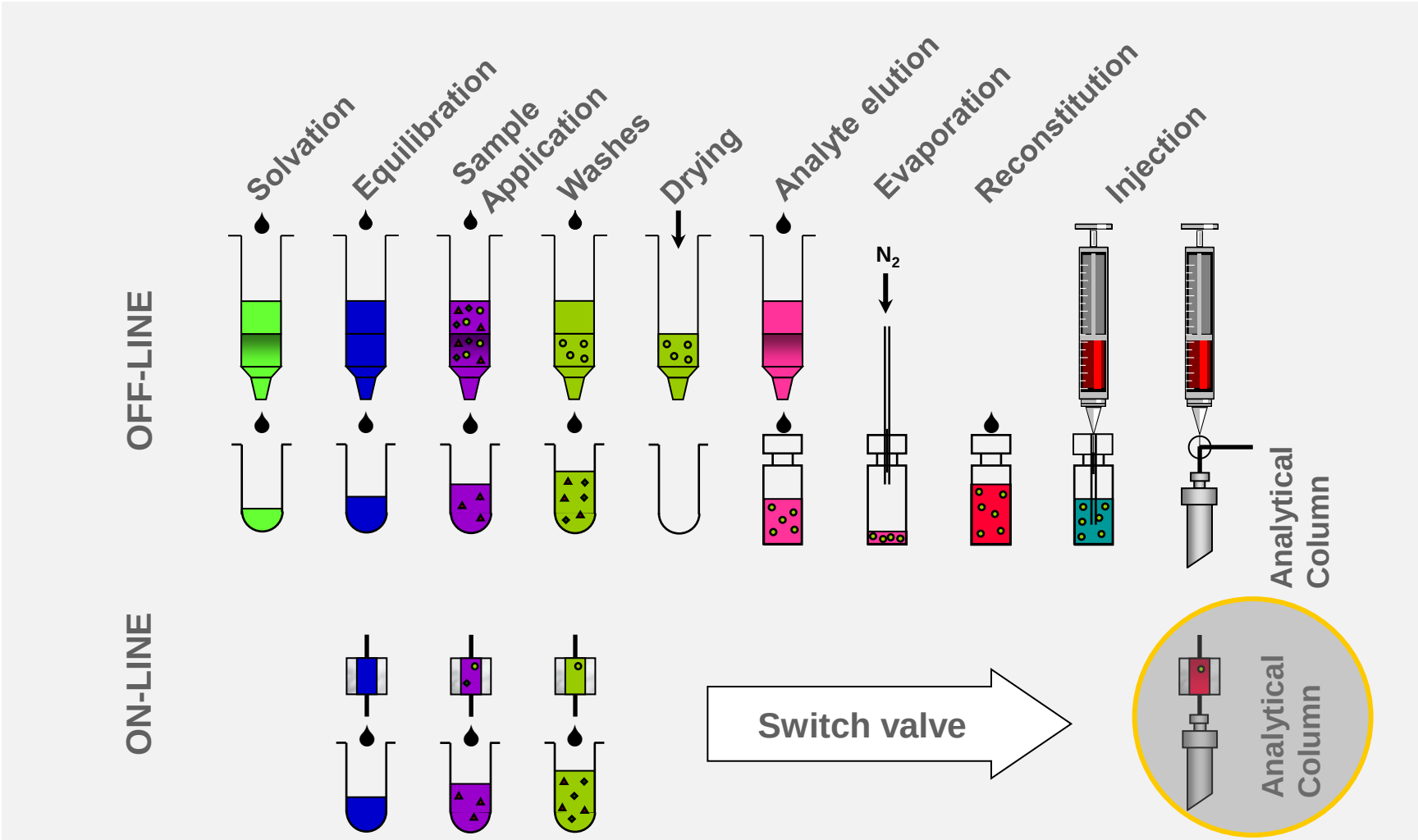
Procedure

1. Extract sample or slurry sample and filter
2. Dilute sample, transfer to autosampler vial and insert vial into autosampler
3. Clean up and analysis of sample is fully automated
4. Clean up is performed during analysis of prior sample: no HPLC time is wasted on clean up;
5. A series of standard, QC sample, method blank and a maximum of 12 samples are analysed with each cartridge

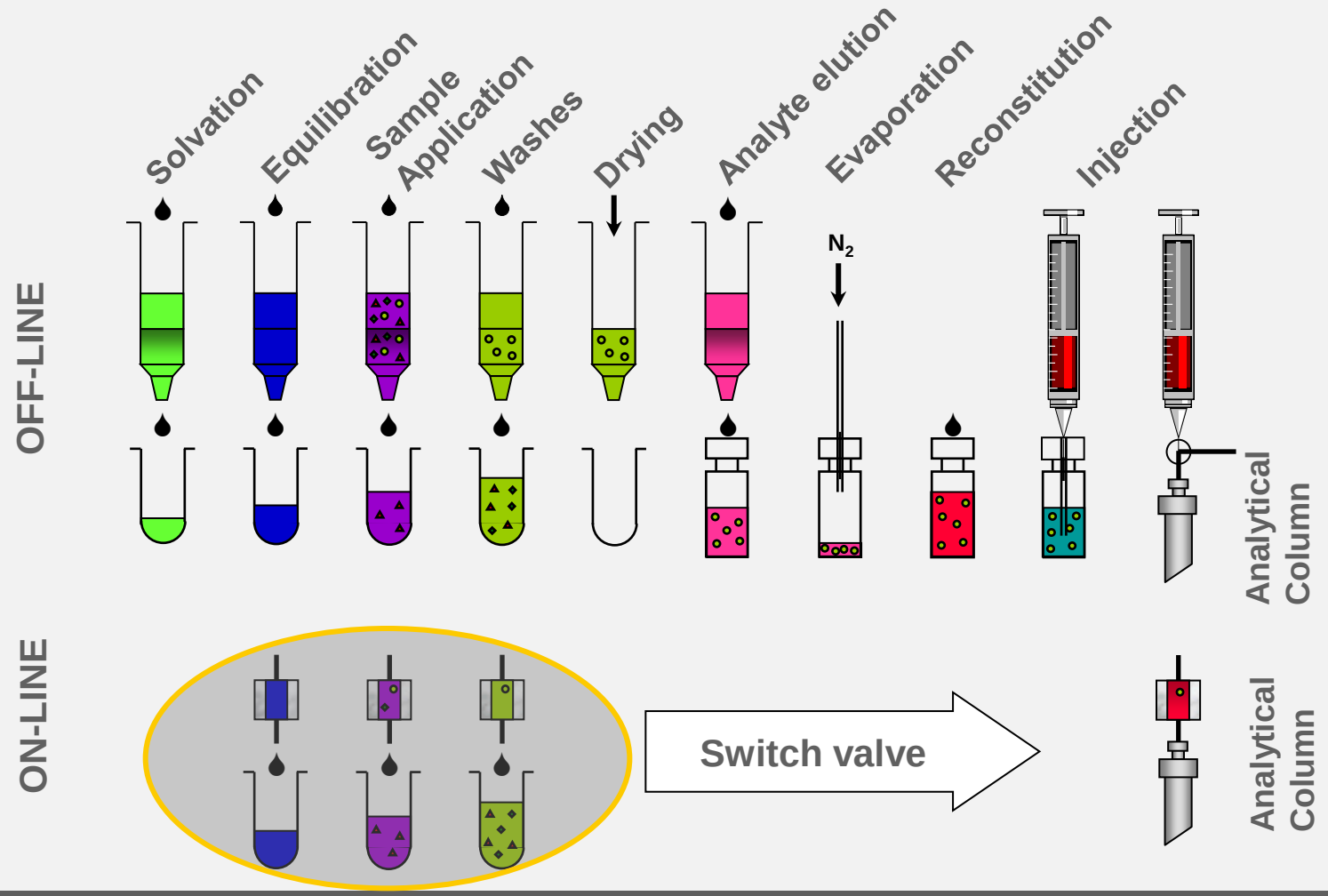
Comparison of off-line and on-line immunoaffinity clean up



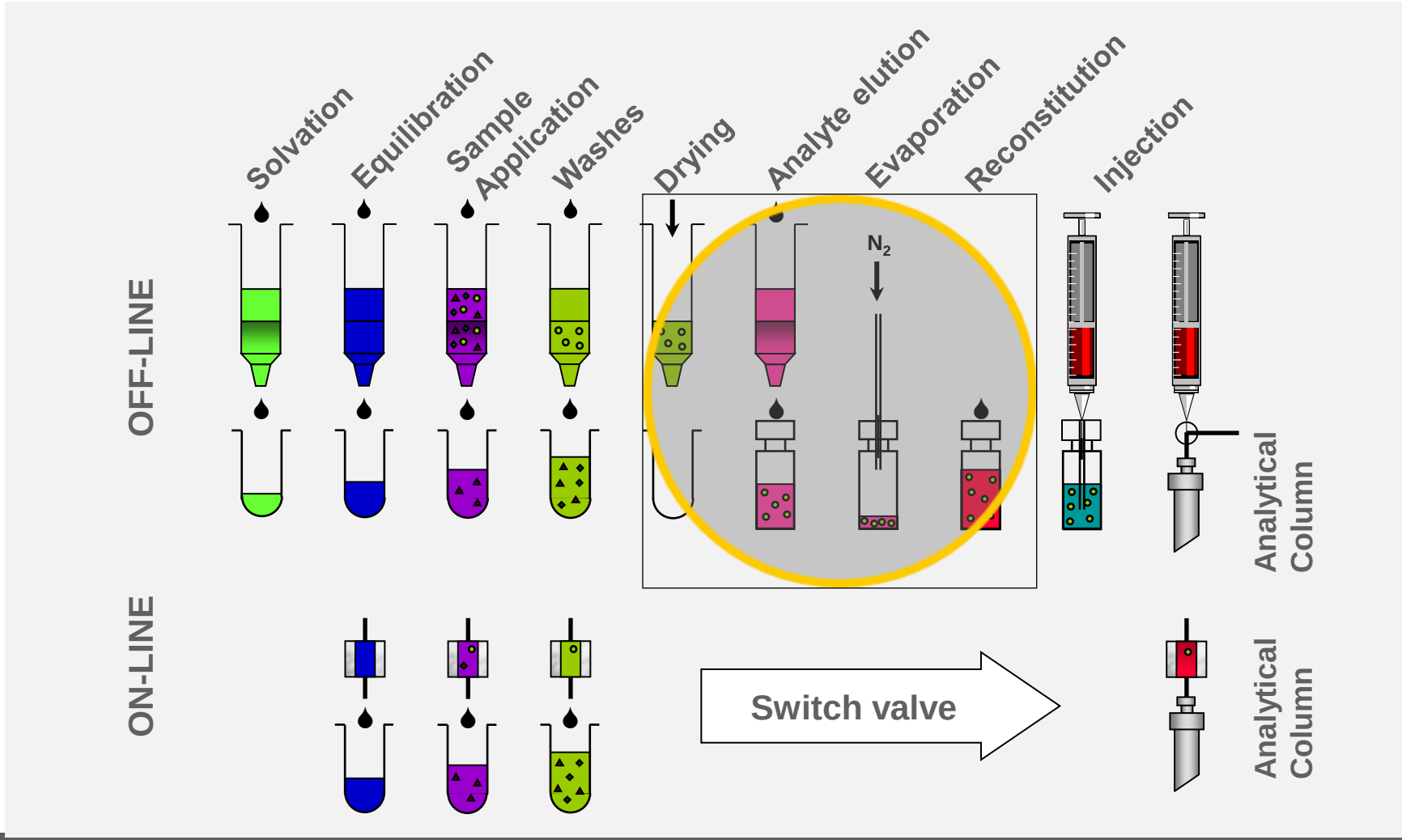
Benefits of IMMUNOPREP® ONLINE: 100% sample injection



Benefits of IMMUNOPREP® ONLINE: continuous Flow

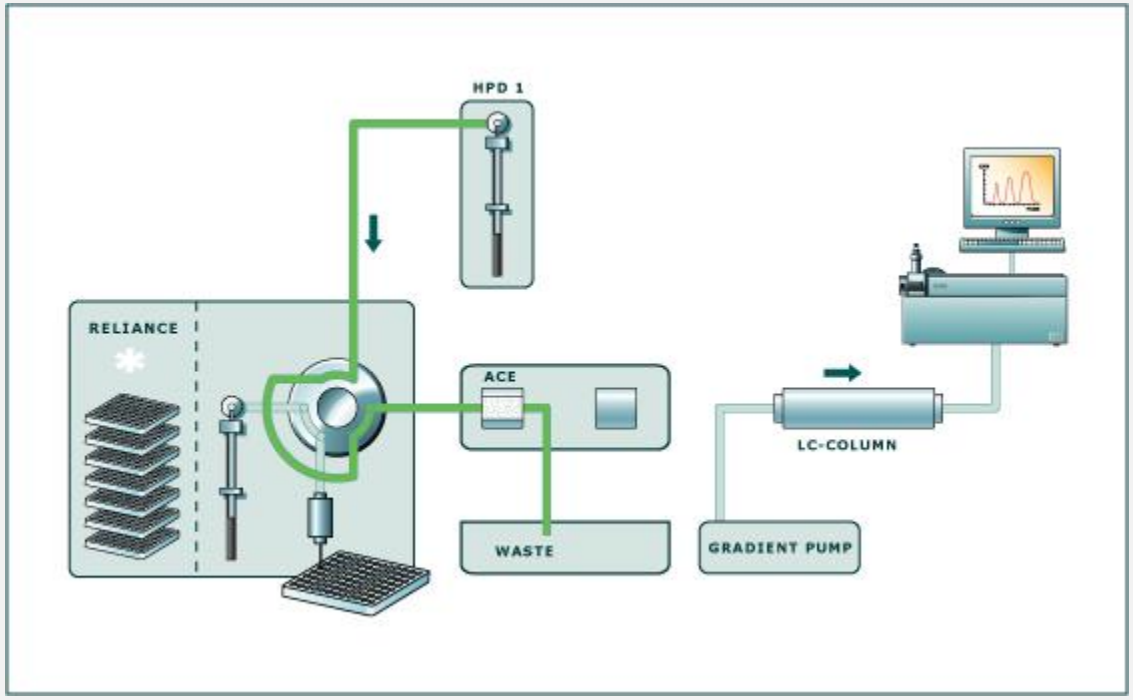


Benefits of IMMUNOPREP® ONLINE: fewer steps



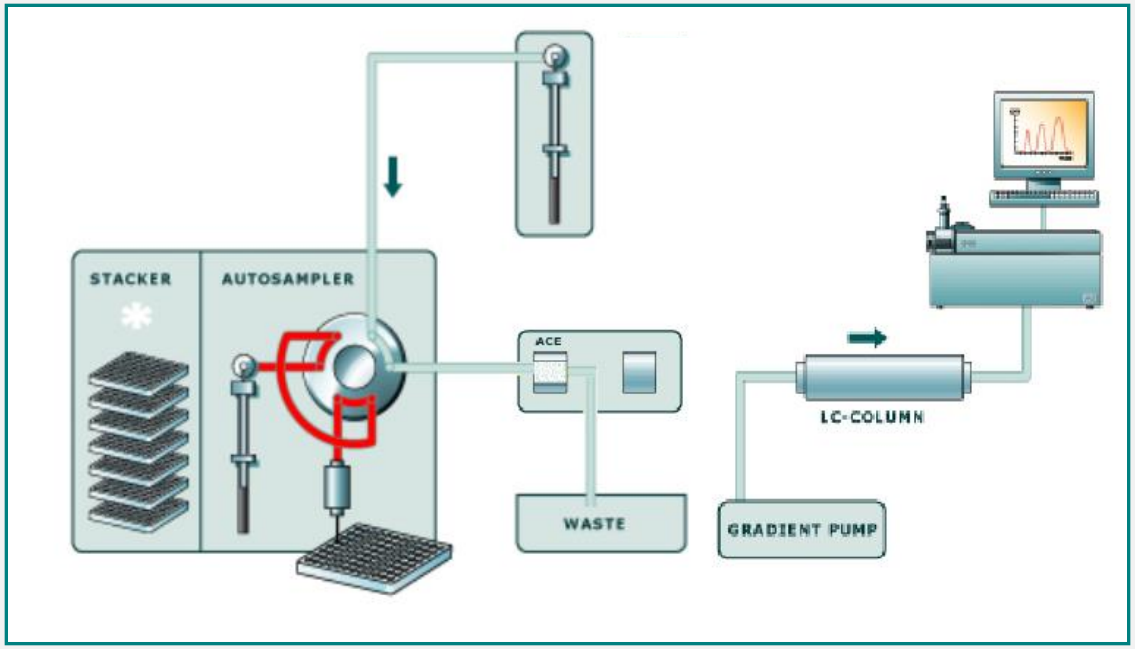
Symbiosis™ Pico system

What's happening in the Symbiosis?
Step 1: Activate cartridge



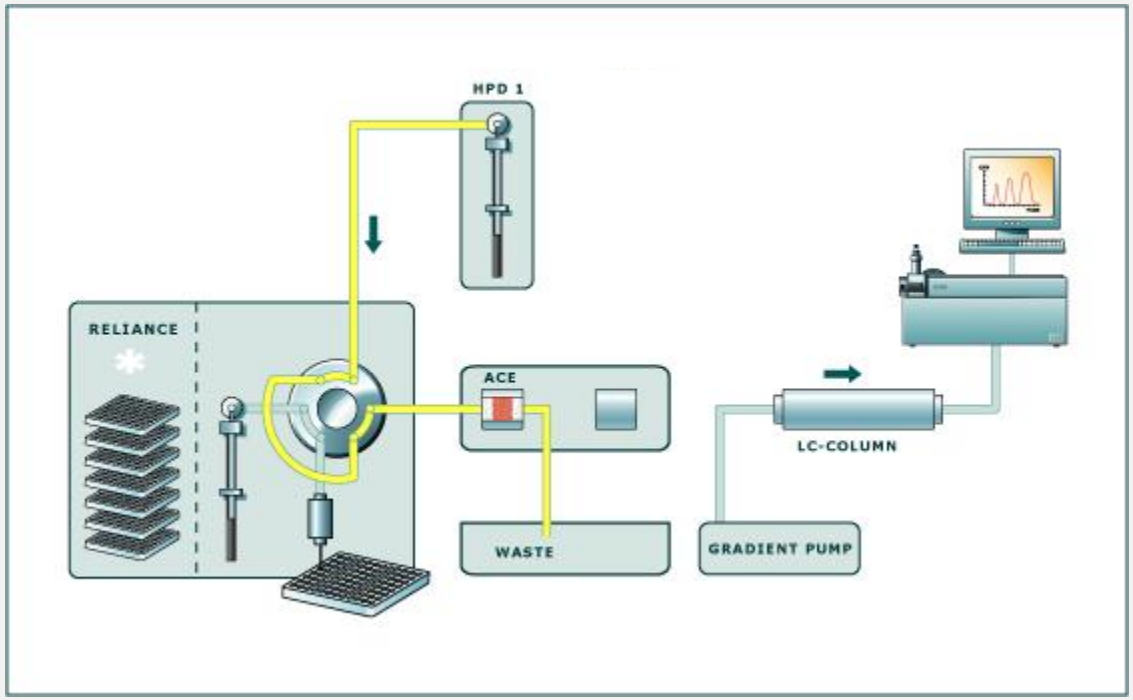
- During the analysis of sample 1, the cartridge is placed in the left clamp of the ACE
- Prior to loading sample 2, the cartridge is conditioned with loading buffer

What's happening in the Symbiosis?
Step 2: Fill loop of autosampler with sample



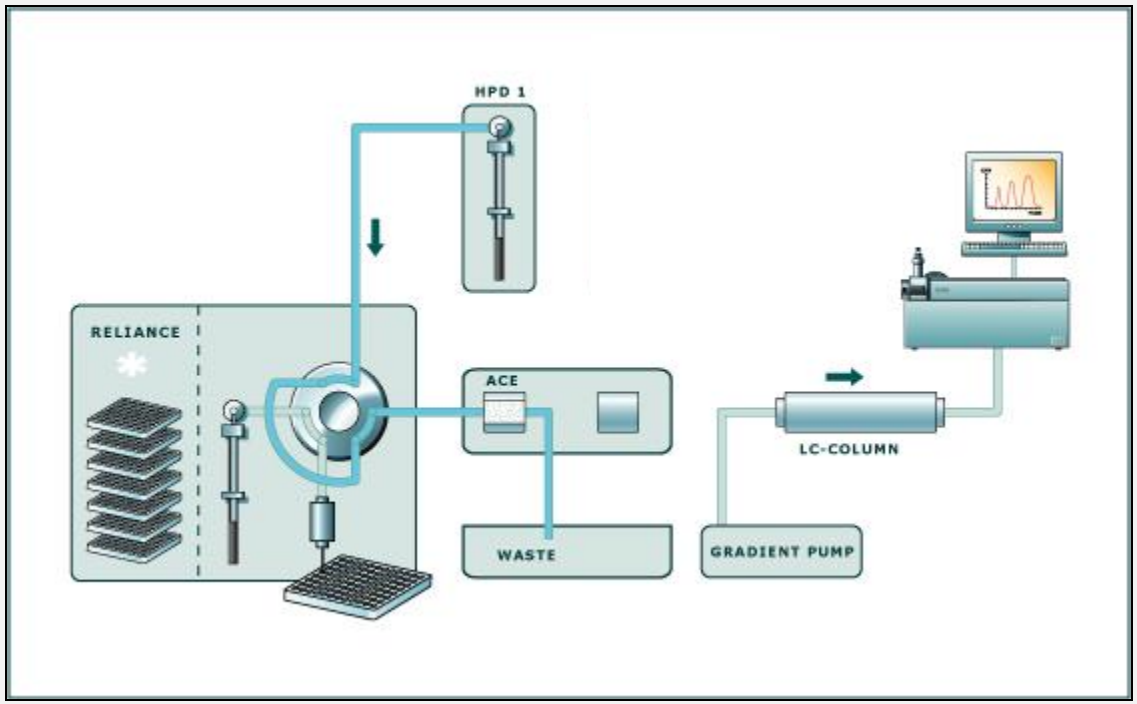
- After conditioning, the sample loop of the autosampler is filled with sample 2

What's happening in the Symbiosis?
Step 3: Apply sample onto the cartridge



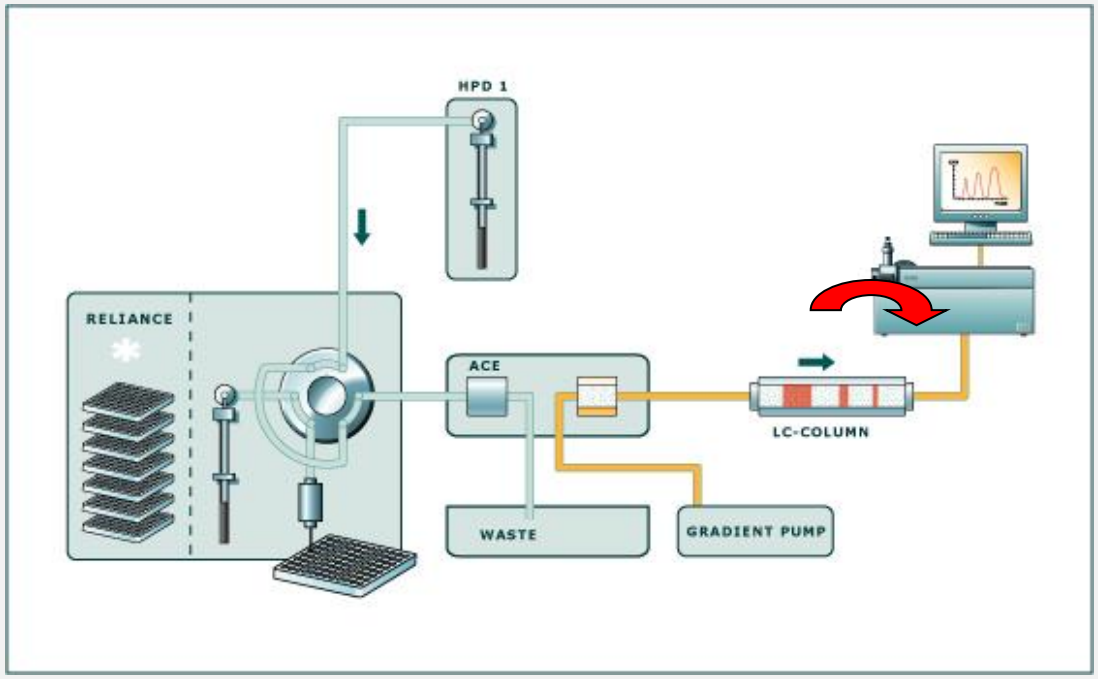
- The cartridge is switched online with the sample loop;
- The immunoaffinity cartridge is loaded with the sample by transferring the sample from the loop to the cartridge with the HPD syringe at a defined flow rate

What's happening in the Symbiosis?
Step 4: Wash cartridge



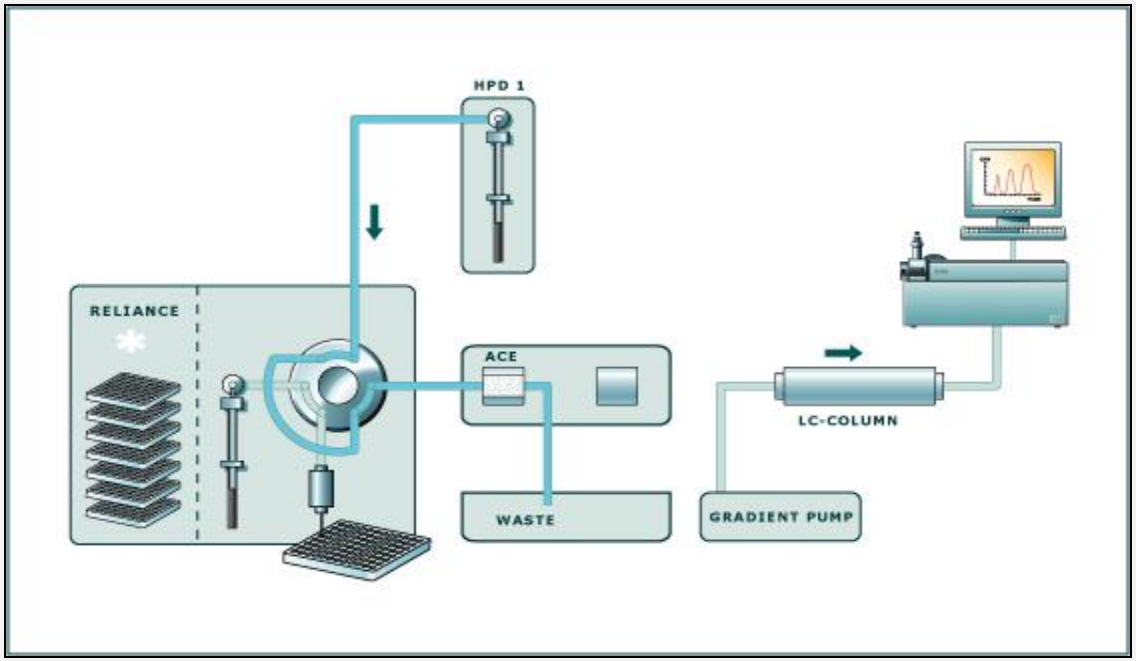
- The immunoaffinity cartridge is washed at a defined flow rate and volume, using the HPD syringe, and unbound material (matrix components) is removed from the cartridge.

What's happening in the Symbiosis?
Step 5: Elute the cartridge and start analysis



- The immunoaffinity cartridge is moved from the left to the right clamp and is switched online with the analytical column;
- The captured toxins are eluted and analysis and data acquisition starts automatically

What's happening in the Symbiosis?
Step 6: Wash the cartridge



- After elution the cartridge is moved from the right clamp to the left clamp and washed with loading buffer in order to allow cartridge reuse
- Thanks to optimized elution conditions, there is no carry over between samples
- During analysis of sample 2, clean-up of the next sample will start automatically

Multi purpose instrument

CARTRIDGES

IMMUNOPREP® ONLINE can be regarded as platform technology offering solutions for many target analytes.

Tray

96 cartridges per tray

Tray is SBS format

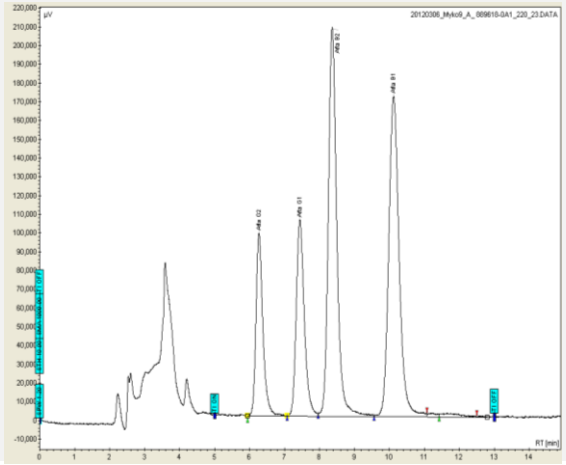
XLC-MS TECHNOLOGY

AUTOMATED FROM SAMPLE LIST TO MS-RESULTS

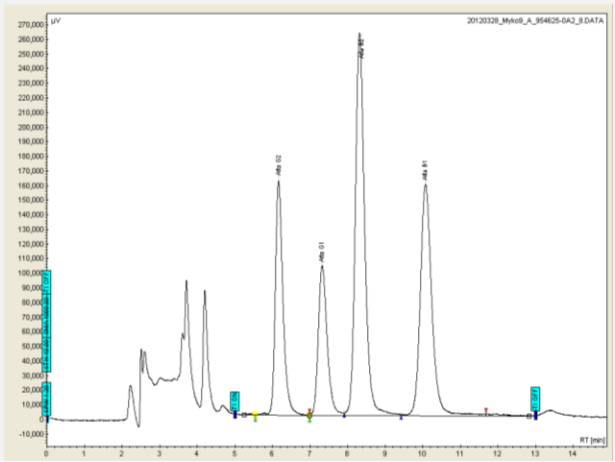
Some results

Analysis of Aflatoxins in various matrices by IMMUNOPREP® ONLINE AFLATOXIN Cartridges with Symbiosis™ Pico system

Determination of aflatoxins in maize (0.5 EU Level)

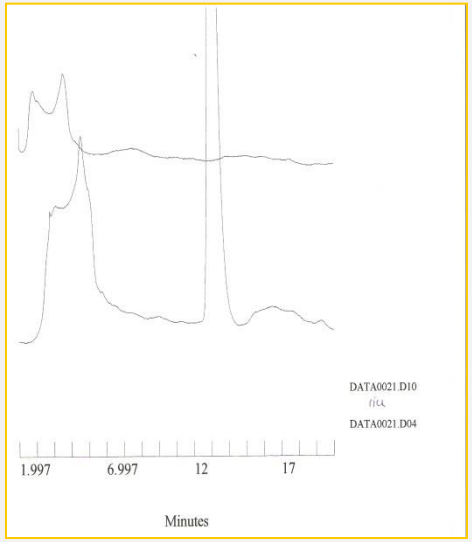


Determination of aflatoxins in dried figs (0.5 EU level)

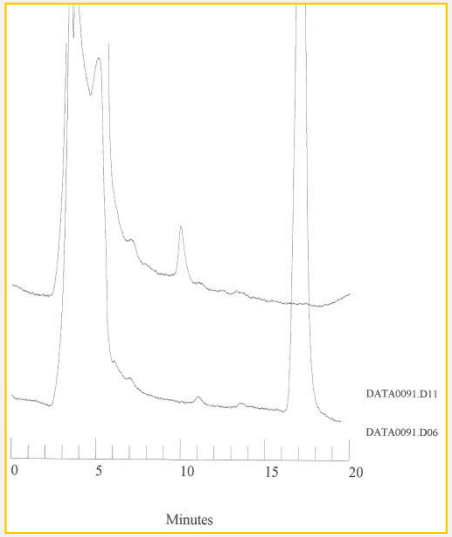


Analysis of Ochratoxin A in various matrices by IMMUNOPREP® ONLINE OCHRATOXIN A cartridges with Symbiosis™ Pico system

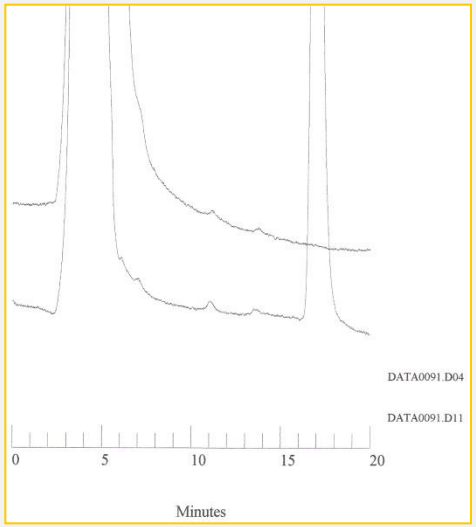
Rice



Nutmeg



Green coffee



(Upper chromatogram: blank matrix; lower chromatogram: standard OTA)

Validation Aflatoxins in Nuts conform NEN7777 (entitled: Performance characteristics of measurement methods)

Parameter	Requirements	AFB1	AFG1	AFB2	AFG2
LOD	AFB1 ≤ 0.1µg/kg	0.1µg/kg			
Blank matrix	<LOD for all AFT	Yes	Yes	Yes	Yes
Linearity	Linear within DBC	Yes	Yes	Yes	Yes
Within-day repeatability ([AFT]: < 1 µg/kg)	RSD ≤ 10%	4%	6%	2%	5%
Between-day repeatability ([AFT]: < 1 µg/kg)	RSD ≤ 20%	10%	7%	5%	11%
Accuracy (recovery)					
[AFT]: < 1 µg/kg	50 – 120%	83%	88%	107%	102%
[AFT: 1 – 10 µg/kg	70 -110%	90%	84%	104%	98%
[AFT]: > 10 µg/kg	80 -110%	98%	80%	101%	98%
Selectivity	Selective	Selective	Selective	Selective	Selective

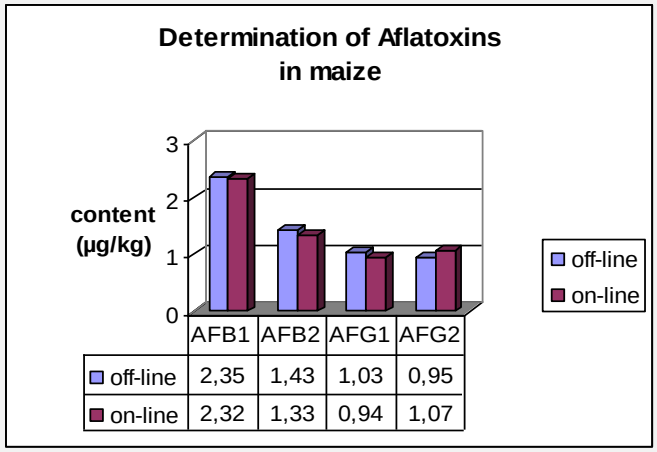
Validation Aflatoxins in Cereals conform NEN7777 (entitled: Performance characteristics of measurement methods)

Parameter	Requirements	AFB1	AFG1	AFB2	AFG2
LOD	AFB1 ≤ 0.1µg/kg	0.07µg/kg			
Blank matrix	<LOD for all AFT	Yes	Yes	Yes	Yes
Linearity	Linear within DBC	Yes	Yes	Yes	Yes
Within-day repeatability ([AFT]: < 1 µg/kg)	RSD ≤ 10%	2%	2%	1%	5%
Between-day repeatability ([AFT]: < 1 µg/kg)	RSD ≤ 20%	5%	4%	2%	4%
Accuracy (recovery)					
[AFT]: < 1 µg/kg	50 – 120%	91%	69%	107%	99%
[AFT: 1 – 10 µg/kg	70 -110%	105%	88%	107%	109%
[AFT]: > 10 µg/kg	80 -110%	106%	88%	105%	107%
Selectivity	Selective	Selective	Selective	Selective	Selective

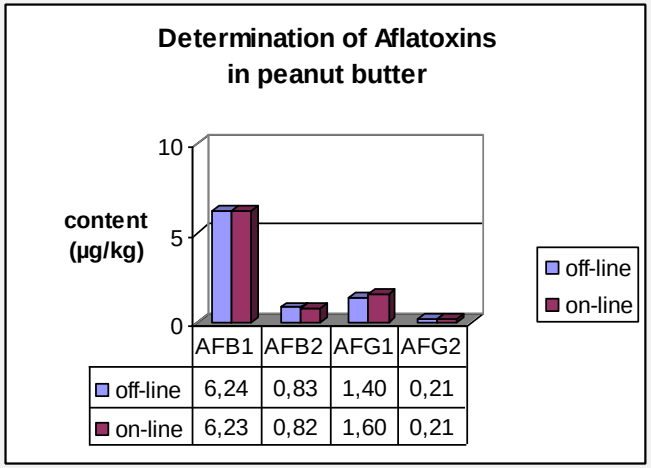
Validation Aflatoxins in Figs conform NEN7777 (entitled: Performance characteristics of measurement methods)

Parameter	Requirements	AFB1	AFG1	AFB2	AFG2
LOD	AFB1 ≤ 0.1µg/kg	0.07µg/kg			
Blank matrix	<LOD for all AFT	Yes	Yes	Yes	Yes
Linearity	Linear within DBC	Yes	Yes	Yes	Yes
Within-day repeatability ([AFT]: < 1 µg/kg)	RSD ≤ 10%	3%	4%	4%	4%
Between-day repeatability ([AFT]: < 1 µg/kg)	RSD ≤ 20%	7%	9%	5%	8%
Accuracy (recovery)					
[AFT]: < 1 µg/kg	50 – 120%	74%	92%	94%	100%
[AFT: 1 – 10 µg/kg	70 -110%	84%	85%	92%	110%
[AFT]: > 10 µg/kg	80 -110%	90%	77%	84%	104%
Selectivity	Selective	Selective	Selective	Selective	Selective

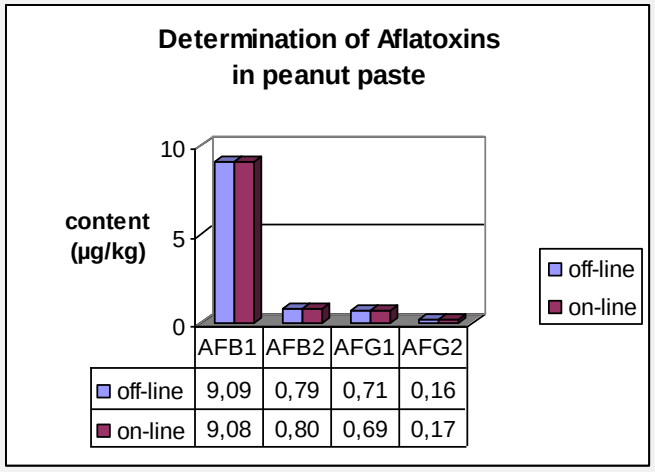
Proficiency studies (real samples) with IMMUNOPREP® ONLINE AFLATOXIN cartridges



	AFB1	AFB2	AFG1	AFG2
off-line	2,35	1,43	1,03	0,95
on-line	2,32	1,33	0,94	1,07



	AFB1	AFB2	AFG1	AFG2
off-line	6,24	0,83	1,40	0,21
on-line	6,23	0,82	1,60	0,21



	AFB1	AFB2	AFG1	AFG2
off-line	9,09	0,79	0,71	0,16
on-line	9,08	0,80	0,69	0,17

Summary Performance characteristics

- ✓ Method exceeds requirements of EU-regulation 401/2006
- ✓ No carry-over from a highly contaminated sample to a blank sample was observed
- ✓ Achieved LOQ of <0.1 µg/kg compares well with current off-line IAC method (LOQ ≤ 0.1 µg/kg)

Summary Technical benefits

- ✓ **Fewer steps during sample clean-up**:
  - ✓ Time saving of 23 minutes per sample in the analysis (ref: private lab)
  - ✓ Increased lab capacity and sample throughput
- ✓ **Improved reproducibility through automation:**
  - ✓ Accurate loaded sample volume;
  - ✓ Reproducible dynamic binding capacity of each cartridge due to defined flow rate and loading time;
  - ✓ Accurate elution at defined flow rate and elution time
- ✓ **Improvement in quality control**:
  - ✓ Less re-analysis of samples
  - ✓ every cartridge is quality controlled
- ✓ **Improved traceability of samples; easier to match results and samples**

Summary Financial performance and benefits

- ✓ Time and labour saving in the process, and as a result, increased lab capacity, sample throughput and better customer service
- ✓ Cost saving of 20-30% on overall consumable, overhead, waste disposal, freight and storage costs and re-analysis time
- ✓ Being a platform technology, a quick return on investment.

Thank you

for your attention!