Varian 320-MS

Qualifier Assessment and Ion Ratio Stability

Advantage Statement: Varian's 320-MS triple quadrupole system meets the quality guidelines for qualifier assessment and ion ratio stability set by the European Union for the detection and analysis of toxic compounds.

The toxicological quality guidelines set by the European Union for the detection of toxins and drugs by LC/MS/MS systems recommends the use of at least one qualifier ion for the confirmation of target analytes. A minimum of at least one ion ratio must be measured and all the measured ion ratios must meet the criteria for the allowable tolerances. The allowable tolerances mainly depend on the relative intensity of the qualifier ion with respect to the base peak (quantifier ion). Table 1 gives the maximum permitted tolerance for relative ion intensities for LC/MS/MS set by European Commission Council Directive 96/23/EC;SANCO/1805/2000(1).

Relative Intensity (% of base peak)	Maximum permitted tolerance for relative ion intensity with LC/MS/MS
>50%	±20%
>20% - 50%	±25%
>10% - 20%	±30%
<10%	±50%

Table 1 Maximum permitted tolerances for relative ion intensities using LC/MS/MS set by European Union (European Commission Council Directive 96/23/EC/SANCO/1805/2000).

The applicability of the above criteria for LC/MS/MS on Varian's 320-MS triple quadrupole system was evaluated using the carcinogenic mycotoxins Aflatoxins B1 and B2. For more information about the analytical methodology see Reference 2.

Qualifier Assessment using the 320-MS Method Editor: The quan ions dialog box of the 320-MS method editor allows the user to specify the quantifier and qualifier ions from a reference spectrum (Figure 1). The product ion with the highest intensity from a Multiple Reaction Monitoring (MRM) experiment is chosen by the user as the quantifier ion and the remaining ions from the reference spectrum are loaded or added as qualifiers to the qualifiers table. The software integrates the peak areas and automatically enters the ratio of the qualifier ion to the quan ion in the ratio field with a default 20% tolerance level. The user can increase or decrease the tolerance level manually if needed.

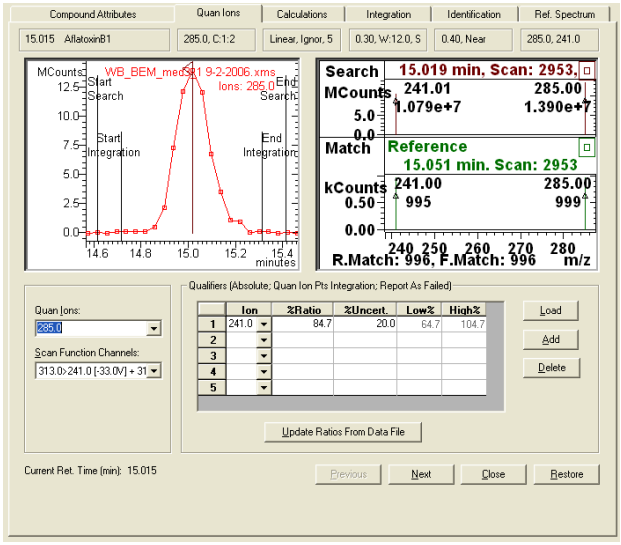

Figure 1 320-MS Method Editor

Summary of Experiment: Aflatoxins B1 and B2 were obtained from Sigma-Aldrich. The LC/MS/MS was done on the 320-MS. The ion ratios were estimated by the software. The ion ratios for the above compounds and their respective tolerance levels as per EU criteria are given in Table 2.

Target analyte	Quan Ion Transition	Qualifier Ion Transitions	Ion ratio	Tolerance (EU Criteria)
Aflatoxin B1	313->285	313->241	84.7%	±20%
Aflatoxin B2	315->287	315->259	18.0%	±30%

Table 2 Ion ratios for Aflatoxins B1 and B2 on the 320-MS and their respective tolerance levels as per EU criteria

The overlaid chromatograms of the qualifier and quantifier ions give an idea of the qualifier assessment (Figure 2).

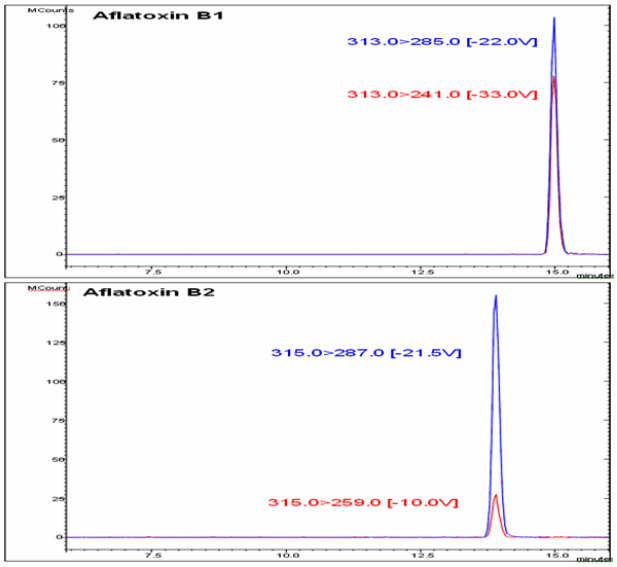

Figure 2 Qualifier ion assessments for Aflatoxins B1 and B2 on the 320-MS

The above compounds were spiked in buffer at concentrations ranging from 0.35 ppb to 350 ppb i.e., over four orders of magnitude. They were run in triplicate on the 320-MS. The ion ratio tolerance was assessed over the entire range. The specified ion ratios, actual ion ratios and the deviation from the specified ion ratios are given in Table 3.

As seen from Table 3, the observed ion ratio tolerance for Aflatoxin B1 ranged from 5.1% to 11.6%. The average tolerance observed was 7.15%. This is far less than the tolerance level of ± 20% set by the European Union. For Aflatoxin B2, the observed ion ratio tolerance was found to be around 0.6%, far below the required ±30%. Spike concentration had very little effect on the ion ratio tolerance indicating the exceptional stability and linearity of the 320-MS for the analysis of toxic compounds.

The ion ratio stability was also tested in a complex matrix. Wheat beer was spiked with 40 ppb each of Aflatoxins B1 and B2 and purified using a solid phase extraction cartridge. Nine replicates were run on the 320-MS. The ion ratio stability was assessed. (Table 4).

	AflatoxinB1				Aflatoxin B2			
Concentration in ppb	Specified Ion ratio	Tolerance levels set by EU	Actual Ion ratio	Observed Ion ratio Tolerance	Specified Ion ratio	Tolerance levels set by EU	Actual Ion ratio	Observed Ion ratio Tolerance
350	84.70%	±20%	79.10%	5.6%	18.00%	±30 %	17.50%	0.5%
350	84.70%	±20%	79.10%	5.6%	18.00%	±30 %	17.70%	0.3%
350	84.70%	±20%	78.20%	6.5%	18.00%	±30 %	17.50%	0.5%
35	84.70%	±20%	79.20%	5.5%	18.00%	±30 %	17.30%	0.7%
35	84.70%	±20%	79.40%	5.3%	18.00%	±30 %	17.90%	0.1%
35	84.70%	±20%	79.00%	5.7%	18.00%	±30 %	17.30%	0.7%
3.5	84.70%	±20%	79.60%	5.1%	18.00%	±30 %	17.80%	0.2%
3.5	84.70%	±20%	79.60%	5.1%	18.00%	±30 %	17.40%	0.6%
3.5	84.70%	±20%	78.90%	5.7%	18.00%	±30 %	17.50%	0.5%
0.35	84.70%	±20%	77.00%	7.7%	18.00%	±30 %	18.60%	0.6%
0.35	84.70%	±20%	73.10%	11.6%	18.00%	±30 %	19.60%	1.6%
0.35	84.70%	±20%	74.40%	10.3%	18.00%	±30 %	17.00%	1.0%
Mean				7.15%				0.61%
SD				2.9				0.3

Table 3 Observed ion ratio tolerances for Aflatoxins B1 and B2 over four orders of magnitude on the 320-MS triple quadrupole system

	Aflatoxin B1			Aflatoxin B2	
Specified Ion ratio	Actual Ion ratio	Observed Ion ratio Tolerance	Specified Ion ratio	Actual Ion ratio	Observed Ion ratio Tolerance
84.70%	77.10%	7.60%	18.00%	18.30%	0.30%
84.70%	77.70%	7.00%	18.00%	18.00%	0%
84.70%	78.30%	6.40%	18.00%	18.10%	0.10%
84.70%	77.70%	7.00%	18.00%	18.10%	0.10%
84.70%	78.10%	6.60%	18.00%	18.20%	0.20%
84.70%	77.40%	7.30%	18.00%	18.10%	0.10%
84.70%	77.10%	7.60%	18.00%	18.50%	0.50%
84.70%	77.30%	7.30%	18.00%	18.00%	0%
84.70%	77.20%	7.50%	18.00%	18.30%	0.30%
Mean		7.14%			0.2%
SD		0.43			0.2

Table 4 Ion ratio stability in wheat beer

As seen from the table, the observed ion ratio tolerances for both the compounds in wheat beer were well below the permitted tolerance levels set by European Union.

The 320-MS provides:

- Excellent ion ratio stability across a wide calibration range
- The solution to meet and/or exceed the demanding EU ion ratio tolerance guidelines
- Highly reproducible analytical results in heavy matrices
- A sensitive, robust and rugged LC/MS/MS methodology for the determination of mycotoxins

1. European Commission Decision laying down performance criteria for the analytical methods to be used for detecting certain substances and residues thereof in live animals and animal products according to Council Directive 96/23/EC; SANCO/1805/2000.

2. Rapid Quantitative Analysis of Multiple Mycotoxins by Liquid Chromatography Tandem Mass Spectrometry. Rudrabhatla et al., American Lab, April 2007 (22-25)

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