



PuriTox Total Myco-MS

Art. No. TC-MT3000

Validation Report



PuriTox Total Myco-MS

Art. No. TC-MT3000

1. General Information

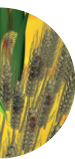
LC-MS/MS Conditions

LC-MS/MS conditions for using PuriTox Total Myco-MS for the detection of aflatoxin B1, B2, G1 and G2, ochratoxin A, deoxynivalenol, fumonisins, zearalenone, T-2 and HT-2.

Mobile Phase A	1 mM ammonium formate and 0.1 % formic acid in 5 % methanol
Mobile Phase B	1 mM ammonium formate and 0.1 % formic acid in 98 % methanol
Flow	0.3 ml/min
Column	Gemini C18, 5 µm, 150 mm x 3 mm
Column Temperature	40 °C
Injection Volume	50 µl

Mass Spectrometry Conditions	
Instrument	Waters® ACQUITY TQ Detector with Electrospray Ionisation
Mode	Multiple Reaction Monitoring (MRM) Mode with positive polarity
Capillary Voltage	+1,500 Volts
Source Temperature	150 °C
Desolvation Gas Temperature	350 °C
Desolvation Gas Flow	600 l/hr (N)
Cone Gas Flow	50 l/hr (N)

Instrument Settings						
Toxin	Time Segment (min)	Precursor Ion (m/z)	Product Ions (m/z)	Dwell Time (s)	Cone Voltage (V)	Collision Voltage (eV)
Deoxynivalenol (DON)	3.0 - 6.0	297.01 [M+H] ⁺	249.10 (Quantifier)	0.661	24	10
			231.08 (Qualifier)		24	12
Aflatoxin G2 (AFT G2)	6.5 - 9.5	331.01 [M+H] ⁺	245.13 (Quantifier)	0.105	48	32
			189.07 (Qualifier)		48	40
Aflatoxin G1 (AFT G1)	6.5 - 9.5	329.01 [M+H] ⁺	243.06 (Quantifier)	0.105	50	28
			199.88 (Qualifier)		50	44
Aflatoxin B2 (AFT B2)	6.5 - 9.5	315.07 [M+H] ⁺	287.12 (Quantifier)	0.105	56	26
			259.15 (Qualifier)		56	30
Aflatoxin B1 (AFT B1)	6.5 - 9.5	313.00 [M+H] ⁺	284.93 (Quantifier)	0.105	52	22
			241.10 (Qualifier)		52	40
Fumonisin B1 (FUM B1)	6.5 - 9.5	722.39 [M+H] ⁺	334.39 (Quantifier)	0.105	52	40
			352.40 (Qualifier)		52	38
Fumonisin B2 (FUM B2)	8.5 - 10.5	706.39 [M+H] ⁺	336.40 (Quantifier)	0.105	56	40
			318.39 (Qualifier)		56	42
HT-2	9.5 - 11	442.21 [M+NH ₄] ⁺	263.16 (Quantifier)	0.272	18	12
			215.10 (Qualifier)		18	14
T-2	9.5 - 11.5	484.21 [M+NH ₄] ⁺	305.14 (Quantifier)	0.272	26	14
			245.12 (Qualifier)		26	14
Ochratoxin A (OTA)	10.5 - 13.0	403.9 [M+H] ⁺	239.0 (Quantifier)	0.428	32	22
			358.1 (Qualifier)		32	14
Zearalenone (ZON)	10.5 - 12.5	319.11 [M+H] ⁺	283.17 (Quantifier)	0.256	22	12
			187.1 (Qualifier)		22	20



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2. Matrix Matched Calibration: Determination of Matrix Effects

Method - Cereals

Sample matrix effects using PuriTox Total Myco-MS with LC-MS/MS were determined by preparing a matrix matched calibration series (prepared using a relevant matrix blank extract, which has been processed using the PuriTox Total Myco-MS procedure) to produce a calibration line. A solvent based calibration series is prepared at the same time for comparison.

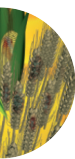
The matrix matched slope is then divided by the slope of the solvent based calibration line and expressed as a percentage. If the values obtained are $\leq 90\%$ or are $\geq 110\%$ for individual toxins, this indicates that some form of signal suppression or enhancement caused by the matrix is occurring. In such instances, a matrix matched calibration series is necessary to correct for these issues.

Extraction Method

1. Weigh 25 g of sample into a blender jar.
2. Add 100 ml of 80 % acetonitrile and blend at high speed for 2 minutes.
3. Filter the sample through Whatman No. 113 (centrifuge at 4000 rpm if needed).
4. Add 200 μ l of acetic acid to 20 ml of sample filtrate and stir.

PuriTox Total Myco-MS column method

1. Apply 1.4 ml of above sample to SPE and collect.
2. 500 μ l of eluate was diluted with 1.5 ml of water containing 1 % acetic acid.
3. 900 μ l of solution was added to 100 μ l of 1 % acetic acid in 20 % acetonitrile.
4. Inject 50 μ l onto LC-MS/MS system.



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Preparation of Matrix Matched Calibration Series

1. A relevant matrix blank is prepared using the extraction procedure and PuriTox Total Myco-MS method, 10 ml of the diluted eluate is collected (1.4 ml of filtrate is passed through 4 SPE columns and pooled, then 2.5 ml is diluted with 7.5 ml water : acetic acid (1:1 v/v)).
2. A working solution of standard (containing a mixture of all analytes at x10 the required final concentration) is prepared.
3. For a 5 point calibration series, a dilution series of the working standard is made and 100 µl of each standard concentration is added separately to 900 µl of the diluted sample eluate.
4. For a solvent based calibration, a working solution of standard (containing a mixture of all analytes at x10 the required final concentration) is prepared.
5. For a 5 point calibration, a dilution series of the working standard is made and 100 µl of each standard concentration is added separately to 900 µl of diluent containing the same solvent composition as the sample solutions.
6. For each slope, plot the peak area versus the standard concentration for both the matrix matched calibration and for the solvent based calibration series.
7. Divide the slope of matrix matched with the slope of solvent based and multiply by 100 to express the matrix effects as a percentage, in order to determine the influence of matrix effects on each analyte.

It should be noted that isotopic internal standards can alternatively be used in a solvent based calibration series and in diluted sample eluate to compensate for matrix effects. The internal standards are added to all of the solutions to be injected in the HPLC vials.

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Results - Recoveries for Cereals

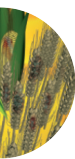
The following recoveries were obtained for different cereal matrices when using matrix matched calibration standards. Samples tested in this study include maize (spiked at the legislative levels and at medium and high levels of contamination), wheat and oats spiked around the EU legislative levels and also shows the % RSD obtained when using PuriTox Total Myco-MS over 6 replicate analyses.

Sample	Maize (Low Level Contamination) - Results Obtained for Matrix Matched Calibration Standard n = 6										
Toxin	B1	B2	G1	G2	OTA	FB1	FB2	DON	ZON	T-2	HT-2
Contamination (Spiked)	2 ppb	2 ppb	2 ppb	2 ppb	4 ppb	400 ppb	200 ppb	500 ppb	100 ppb	50 ppb	50 ppb
Average Recovery (Matrix Matched Calibration)	117 %	110 %	118 %	113 %	88 %	30 %	44 %	126 %	88 %	91 %	86 %
% RSD	8.1 %	8.2 %	4.5 %	15.2 %	5.4 %	12.8 %	10.4 %	3.8 %	6.1 %	8.0 %	7.5 %

Sample	Maize (Mid Level Contamination) - Results Obtained for Matrix Matched Calibration Standard n = 6										
Toxin	B1	B2	G1	G2	OTA	FB1	FB2	DON	ZON	T-2	HT-2
Contamination (Spiked)	4 ppb	4 ppb	4 ppb	4 ppb	8 ppb	800 ppb	400 ppb	1000 ppb	200 ppb	100 ppb	100 ppb
Average Recovery (Matrix Matched Calibration)	107 %	106 %	114 %	111 %	89 %	30 %	43 %	134 %	94 %	96 %	104 %
% RSD	5.7 %	5.9 %	6.3 %	9.5 %	4.3 %	6.4 %	3.6 %	2.4 %	3.7 %	4.5 %	4.7 %

Sample	Maize (High Level Contamination) - Results Obtained for Matrix Matched Calibration Standard n = 6										
Toxin	B1	B2	G1	G2	OTA	FB1	FB2	DON	ZON	T-2	HT-2
Contamination (Spiked)	8 ppb	8 ppb	8 ppb	8 ppb	16 ppb	1600 ppb	800 ppb	2000 ppb	400 ppb	200 ppb	200 ppb
Average Recovery (Matrix Matched Calibration)	109 %	110 %	114 %	108 %	82 %	25 %	37 %	128 %	94 %	84 %	93 %
% RSD	5.3 %	4.2 %	2.7 %	5.3 %	5.7 %	6.2 %	1.5 %	7.2 %	2.0 %	3.5 %	4.7 %

Sample	Wheat - Results Obtained for Matrix Matched Calibration Standard n = 6										
Toxin	B1	B2	G1	G2	OTA	FB1	FB2	DON	ZON	T-2	HT-2
Contamination (Spiked)	2 ppb	2 ppb	2 ppb	2 ppb	4 ppb	888 ppb	218 ppb	1300 ppb	200 ppb	100 ppb	100 ppb
Average Recovery (Matrix Matched Calibration)	121 %	98 %	123 %	113 %	96 %	16 %	28 %	109 %	96 %	104 %	109 %
% RSD	4.5 %	3.9 %	14.5 %	5.0 %	5.8 %	3.7 %	3.7 %	3.0 %	1.9 %	3.7 %	1.0 %



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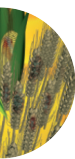
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Sample	Oat - Results Obtained for Matrix Matched Calibration Standard n = 6										
	B1	B2	G1	G2	OTA	FB1	FB2	DON	ZON	T-2	HT-2
Toxin											
Contamination (Spiked)	2 ppb	2 ppb	2 ppb	2 ppb	4 ppb	888 ppb	218 ppb	1300 ppb	200 ppb	100 ppb	100 ppb
Average Recovery (Matrix Matched Calibration)	111 %	112 %	115 %	123 %	92 %	24 %	36 %	106 %	90 %	92 %	94 %
% RSD	4.8 %	6.4 %	6.1 %	5.0 %	6.6 %	4.7 %	1.9 %	3.9 %	1.1 %	2.7 %	8.6 %

Comments - Recoveries for Cereals

In all cases, using matrix matched standards alongside PuriTox Total Myco-MS with the prescribed method and LC-MS/MS, all mycotoxins, with the exception of fumonisins B1 and B2 gave recoveries between 80 % and 120 %. This is in line with the EU Method Performance Criteria and indicates that matrix effects for cereals were compensated for using PuriTox Total Myco-MS with the matrix matched calibration standards.

Fumonisin B1 and B2 recoveries however were low due to a compromise in the extraction required for the extraction of multiple mycotoxins. % RSD values however showed that the method was consistent over a number of analysis for all toxins and that an appropriate correction factor for the recovery could be used to correct for fumonisins. This is confirmed by the following analysis where FAPAS samples were analysed and corrected for recovery using PuriTox Total Myco-MS columns using with the prescribed method and LC-MS/MS, and were found to give results within the acceptable range for all toxins including fumonisins.



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Method - FAPAS Samples (correction factor applied)

A maize blank was used to prepare the matrix matched calibration series. The same maize blank was spiked at appropriate levels to determine the recovery of each analyte and these values were used to apply a correction factor to the measured values of each FAPAS sample.

FAPAS QC Samples T04246 and T04260 of maize were analysed.

Extraction Method

1. Weigh 25 g of sample into a blender jar.
2. Add 100 ml of 80 % acetonitrile and blend at high speed for 2 minutes.
3. Filter the sample through Whatman No. 113 (centrifuge at 4000 rpm if needed).
4. Add 200 µl of acetic acid to 20 ml of sample filtrate and stir.

PuriTox Total Myco-MS column method

1. Apply 1.4 ml of above sample to SPE and collect.
2. 1 ml of eluate was diluted with 1 ml of water containing 1 % acetic acid.
3. 900 µl of solution was added to 100 µl of 1 % acetic acid in 40 % acetonitrile.
4. Inject 20 µl onto LC-MS/MS system.

Results - FAPAS Samples (correction factor applied)

PuriTox Total Myco-MS - Results Corrected for Recovery Using Matrix Matched Standards

Sample	FAPAS 04246 n = 6										
	B1	B2	G1	G2	OTA	FB1	FB2	DON	ZON	T-2	HT-2
Contamination (Spiked)	2 ppb	2 ppb	2 ppb	2 ppb	3 ppb	1176 ppb	288 ppb	1000 ppb	100 ppb	100 ppb	100 ppb
Average Recovery (Matrix Matched Calibration)	120 %	118 %	122 %	116 %	89 %	48 %	45 %	103 %	93 %	92 %	104 %
% RSD	15.9 %	13.8 %	8.1 %	17.9 %	21.9 %	5.5 %	5.4 %	4.1 %	4.3 %	1.3 %	8.2 %
Corrected Result* (Matrix Matched Calibration)	4.54 ppb	-	-	-	3.22 ppb	434 ppb	570 ppb	673 ppb	246 ppb	148 ppb	95 ppb
FAPAS 04246 Assigned Value	4.91 ppb	-	-	-	2.87 ppb	467 ppb	464 ppb	1058 ppb	212 ppb	151 ppb	106 ppb
FAPAS 04246 Range	2.75 - 7.08 ppb	-	-	-	1.61 - 4.13 ppb	230 - 704 ppb	228 - 699 ppb	722 - 1394 ppb	126 - 298 ppb	87 - 215 ppb	59 - 152 ppb

* Matrix effects are calculated as slope of the matrix matched calibration divided by slope of the solvent based calibration line and expressed as a percentage. If values are ≤ 90 or ≥ 110 % then this would indicate that a matrix matched calibration series is essential.

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PuriTox Total Myco-MS - Results Corrected for Recovery Using Matrix Matched Standards

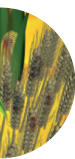
Sample	FAPAS 04260 n = 6										
	B1	B2	G1	G2	OTA	FB1	FB2	DON	ZON	T-2	HT-2
Toxin											
Contamination (Spiked)	2 ppb	2 ppb	2 ppb	2 ppb	3 ppb	1176 ppb	288 ppb	1000 ppb	100 ppb	100 ppb	100 ppb
Average Recovery (Matrix Matched Calibration)	120 %	118 %	122 %	116 %	89 %	48 %	45 %	103 %	93 %	92 %	104 %
% RSD	15.9 %	13.8 %	8.1 %	17.9 %	21.9 %	5.5 %	5.4 %	4.1 %	4.3 %	1.3 %	8.2 %
Corrected Result* (Matrix Matched Calibration)	5.54 ppb	-	-	-	3.02 ppb	1155 ppb	340 ppb	793 ppb	208.6 ppb	2.23 ppb	-
FAPAS 04260 Assigned Value	5.09 ppb	-	-	-	3.29 ppb	-	-	1191 ppb	200.4 ppb	-	-
FAPAS 04260 Range	2.85-7.32 ppb	-	-	-	1.84-4.74 ppb	-	-	820-1563 ppb	1187-2821 ppb	-	-

* Matrix effects are calculated as slope of the matrix matched calibration divided by slope of the solvent based calibration line and expressed as a percentage. If values are ≤ 90 or ≥ 110 % then this would indicate that a matrix matched calibration series is essential.

Comments - FAPAS Samples (correction factor applied)

The PuriTox Total Myco-MS columns offer an improved clean-up when compared to direct injection however they are considered as a suitable screening test for a range of mycotoxins as ion suppression / enhancement can still occur. It is therefore essential to use suitable matrix matched or isotopic labelled standards in order to correct for matrix effects.

For all mycotoxins including fumonisins, when matrix matched standards are used to compensate for matrix effects the values corrected for recovery lie within the specified FAPAS range for the two samples analysed indicating that the PuriTox Total Myco-MS columns are suitable for the analysis of all legislated mycotoxins including fumonisins.



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3. Product Comparison

Method

The same FAPAS QC Samples T04246 and T04260 of maize were analysed using both the PuriTox Total Myco-MS columns and compared against results obtained using a competitor SPE column.

Extraction Method

1. Weigh 25 g of sample into a blender jar.
2. Add 100 ml of 80 % acetonitrile and blend at high speed for 2 minutes.
3. Filter the sample through Whatman No. 113 (centrifuge at 4000 rpm if needed).
4. Add 200 µl of acetic acid to 20 ml of sample filtrate and stir.

PuriTox Total Myco-MS column method

1. Apply 1.4 ml of above sample to SPE and collect.
2. 1 ml of eluate was diluted with 1 ml of water containing 1 % acetic acid.
3. 900 µl of solution was added to 100 µl of 1 % acetic acid in 40 % acetonitrile.
4. Inject 20 µl onto LC-MS/MS system.

For the competitor product the method used was as per instructions provided in test kit. The maize blank was used to prepare a matrix matched calibration series and also spiked to obtain recovery values for each analyte.

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Results

PuriTox Total Myco-MS - Results Corrected for Recovery Using Matrix Matched Standards

Sample	FAPAS 04246 n = 6											
	B1	B2	G1	G2	OTA	FB1	FB2	DON	ZON	T-2	HT-2	
Toxin												
Contamination (Spiked)	2 ppb	2 ppb	2 ppb	2 ppb	3 ppb	1176 ppb	288 ppb	1000 ppb	100 ppb	100 ppb	100 ppb	
Average Recovery (Matrix Matched Calibration)	120 %	118 %	122 %	116 %	89 %	48 %	45 %	103 %	93 %	92 %	104 %	
% RSD	15.9 %	13.8 %	8.1 %	17.9 %	21.9 %	5.5 %	5.4 %	4.1 %	4.3 %	1.3 %	8.2 %	
Corrected Result* (Matrix Matched Calibration)	4.54 ppb	-	-	-	3.22 ppb	434 ppb	570 ppb	673 ppb	246 ppb	148 ppb	95 ppb	
FAPAS 04246 Assigned Value	4.91 ppb	-	-	-	2.87 ppb	467 ppb	464 ppb	1058 ppb	212 ppb	151 ppb	106 ppb	
FAPAS 04246 Range	2.75 - 7.08 ppb	-	-	-	1.61 - 4.13 ppb	230 - 704 ppb	228 - 699 ppb	722 - 1394 ppb	126 - 298 ppb	87 - 215 ppb	59 - 152 ppb	

* Matrix effects are calculated as slope of the matrix matched calibration divided by slope of the solvent based calibration line and expressed as a percentage. If values are ≤ 90 or ≥ 110 % then this would indicate that a matrix matched calibration series is essential.

Competitor Brand 1 - Results Corrected for Recovery Using Matrix Matched Standards

Sample	FAPAS 04246 n = 6											
	B1	B2	G1	G2	OTA	FB1	FB2	DON	ZON	T-2	HT-2	
Toxin												
Contamination (Spiked)	2 ppb	2 ppb	2 ppb	2 ppb	3 ppb	1176 ppb	288 ppb	1000 ppb	100 ppb	100 ppb	100 ppb	
Average Recovery (Matrix Matched Calibration)	124 %	129 %	117 %	139 %	65 %	3 %	1 %	104 %	117 %	109 %	113 %	
% RSD	3.2 %	14.2 %	21.8 %	25.0 %	34.4 %	173.2 %	173.2 %	1.2 %	7.2 %	4.3 %	7.2 %	
Corrected Result* (Matrix Matched Calibration)	3.63 ppb	-	-	-	2.76 ppb	not detected	not detected	651 ppb	192 ppb	127 ppb	91 ppb	
FAPAS 04246 Assigned Value	4.91 ppb	-	-	-	2.87 ppb	467 ppb	464 ppb	1058 ppb	212 ppb	151 ppb	106 ppb	
FAPAS 04246 Range	2.75 - 7.08 ppb	-	-	-	1.61 - 4.13 ppb	230 - 704 ppb	228 - 699 ppb	722 - 1394 ppb	126 - 298 ppb	87 - 215 ppb	59 - 152 ppb	

* Matrix effects are calculated as slope of the matrix matched calibration divided by slope of the solvent based calibration line and expressed as a percentage. If values are ≤ 90 or ≥ 110 % then this would indicate that a matrix matched calibration series is essential.

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PuriTox Total Myco-MS - Results Corrected for Recovery Using Matrix Matched Standards

Sample	FAPAS 04260 n = 6											
	B1	B2	G1	G2	OTA	FB1	FB2	DON	ZON	T-2	HT-2	
Toxin												
Contamination (Spiked)	2 ppb	2 ppb	2 ppb	2 ppb	3 ppb	1176 ppb	288 ppb	1000 ppb	100 ppb	100 ppb	100 ppb	
Average Recovery (Matrix Matched Calibration)	120 %	118 %	122 %	116 %	89 %	48 %	45 %	103 %	93 %	92 %	104 %	
% RSD	15.9 %	13.8 %	8.1 %	17.9 %	21.9 %	5.5 %	5.4 %	4.1 %	4.3 %	1.3 %	8.2 %	
Corrected Result* (Matrix Matched Calibration)	5.54 ppb	-	-	-	3.02 ppb	1155 ppb	340 ppb	793 ppb	208.6 ppb	2.23 ppb	-	
FAPAS 04260 Assigned Value	5.09 ppb	-	-	-	3.29 ppb	-	-	1191 ppb	200.4 ppb	-	-	
FAPAS 04260 Range	2.85 - 7.32 ppb	-	-	-	1.84 - 4.74 ppb	-	-	820 - 1563 ppb	1187 - 2821 ppb	-	-	

* Matrix effects are calculated as slope of the matrix matched calibration divided by slope of the solvent based calibration line and expressed as a percentage. If values are ≤ 90 or ≥ 110 % then this would indicate that a matrix matched calibration series is essential.

Competitor Brand 1 - Results Corrected for Recovery Using Matrix Matched Standards

Sample	FAPAS 04260 n = 6											
	B1	B2	G1	G2	OTA	FB1	FB2	DON	ZON	T-2	HT-2	
Toxin												
Contamination (Spiked)	2 ppb	2 ppb	2 ppb	2 ppb	3 ppb	1176 ppb	288 ppb	1000 ppb	100 ppb	100 ppb	100 ppb	
Average Recovery (Matrix Matched Calibration)	124 %	129 %	117 %	139 %	65 %	3 %	1 %	104 %	117 %	109 %	113 %	
% RSD	3.2 %	14.2 %	21.8 %	25.0 %	34.4 %	173.2 %	173.2 %	1.2 %	7.2 %	4.3 %	7.2 %	
Corrected Result* (Matrix Matched Calibration)	5.46 ppb	-	-	-	2.72 ppb	661 ppb	-	799 ppb	220.3 ppb	2.46 ppb	-	
FAPAS 04260 Assigned Value	5.09 ppb	-	-	-	3.29 ppb	-	-	1191 ppb	200.4 ppb	-	-	
FAPAS 04260 Range	2.85 - 7.32 ppb	-	-	-	1.84 - 4.74 ppb	-	-	820 - 1563 ppb	1187 - 2821 ppb	-	-	

* Matrix effects are calculated as slope of the matrix matched calibration divided by slope of the solvent based calibration line and expressed as a percentage. If values are ≤ 90 or ≥ 110 % then this would indicate that a matrix matched calibration series is essential.

Comments

The spiked recoveries in maize were satisfactory for both SPE types and the values corrected for recovery for the FAPAS samples were within the acceptable ranges when using matrix matched standards. Using PuriTox Total Myco-MS the values corrected for recovery for fumonisins were within the acceptable range specified by FAPAS. It should however be noted that the competitor columns did not detect fumonisins confirming that the product is not suitable for the analysis of this mycotoxin. In addition, these columns were considerably more variable than the PuriTox Total Myco-MS. The competitor product involved a number of steps and required additional laboratory equipment. This made it more cumbersome for routine use compared to PuriTox Total Myco-MS which offers a simple, one step syringe format clean-up.