LC-MS/MS METHOD TO DETERMINE OCHRATOXIN A IN WINES AND GRAPE JUICES

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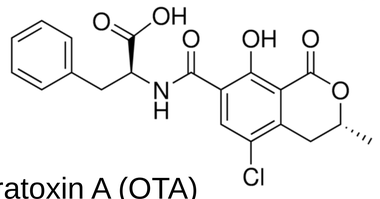
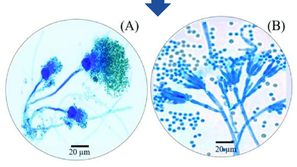
INTRODUCTION



Ochratoxin A (OTA)

Secondary metabolite



Aspergillus *Penicillium*

Aspergillus carbonarius
Aspergillus niger

Terada-Nascimento, J. et al. ***Toxics***, *11*(9), 762, 2023.
GIL-SERNA, J et al. **Beverages**, v.4, n. 6, jan 2018.
ORTIZ-VILLEDA, B et al. **Toxins**, v.13, n. 7, p.478, jul 2021.

OTA CONTAMINATION: vine, transportation, storage and handling.

- Winemaking process is one of the factors that can influence the OTA content in the final product.

Higher concentrations of OTA are reported in red wines than in rosé and white wines.

GIL-SERNA, J et al. **Beverages**, v.4, n. 6, jan 2018.
ORTIZ-VILLEDA, B et al. **Toxins**, v.13, n. 7, p.478, jul 2021.

TOXICITY

CUMULATIVE EFFECT: toxic effects.

nephrotoxicity, hepatotoxicity, teratogenesis and mutagenicity.

Classify as HUMAN CARCINOGEN by the International Agency for Research on Cancer (IARC).

MAXIMUM TOLERATED LIMIT in wine is 2 µg L^{-1}

BRASIL. **Instrução normativa - IN nº 160, de 1º de julho de 2022**. DOU, Brasília, n.126, 6 jul, 2022.
BUI-KLIMKE, T.R.; WU, F. **Crit. Rev. Food Sci. Nutr**., v. 55, n. 13, p, 2015.
GIL-SERNA, J et al. **Beverages**, v.4, n. 6, jan 2018.
ORTIZ-VILLEDA, B et al. **Toxins**, v.13, n. 7, p.478, jul 2021

The main foods contaminated by OTA:

cereals, starchy foods, coffee, spices, dried fruits, beer and **WINE**.

Due to the importance of ochratoxin A and the concern about the food safety, a new method of identification and quantification, by mass spectrometry liquid chromatography, was developed and validated.

METHODOLOGY

Identification and Quantification

High performance liquid chromatography coupled to mass spectrometry (LCMS/MS). Electrospray ionization source (ESI)

Shimadzu LCMS-8045

COLUMN: Shim-pack GIST C18 (2.1 mm x 75 mm, 3 µm).

PHASE A: water with 0.15 mM ammonium fluoride.

PHASE B: methanol with 0.15 mM ammonium fluoride and 0.002% (v/v) acetic acid.

0,3 mL min^{-1} gradient (0 min, 10% B; 2,00 min, 100% B; 4,00 min, 100% B; 4,01 min, 10% B), 7 min, 40°C.

Multiple Reaction Monitoring (MRM)

Precursor ion: *m/z* 404.

Fragmentation: *m/z* 404>239; 404>358; 404>221; 404>193; 404>341.

Sample preparation

DIGESTED EXTRACTS (DE): dry red wine, white moscatel sparkling wine, white brut sparkling wine, sweet white wine and whole red grape juice.

EXTRACTION

3 mL matrix
3 mL acetonitrile
1.2 g of $MgSO_4$
0.3 g of NaCl

DISPERSION

2 mL of SUPERNATANT
0.067 g of DSC-C_{18}
0.02 g of $MgSO_4$

SUPERNATANT: digested extract (DE)

DILUITION: acetonitrile 1:1
FILTRATION: PTFE 0,22 µm

WEI, D. et al. **Scientific Reports**, v.8, n. 2051, 2018.

Analytical curve

MATRICES: H_2O and fortified white matrix DE (dry red wine, white moscatel sparkling wine and whole red grape juice).

- **Levels**: 0,1, 0,5, 1,0, 1,5, 2,0, 2,5 and 3,0 µg L^{-1}.
- **Linear curve fitting:** weighted least squares method, $1/A^2$.
- **Instrumental responses**: heteroscedastic.

Validation

- Limit of quantification and detection.
- Linearity.
- Precision (repeatability and intermediary precision).
- Recovery (%).
- Matrix effect.

Manual de garantia da qualidade analítica de resíduos e contaminantes em alimentos do Ministério de agricultura e pecuária, 2011.
Documento de orientação sobre validação de métodos analíticos do Instituto nacional de metrologia, qualidade e tecnologia, 2020.

RESULTS

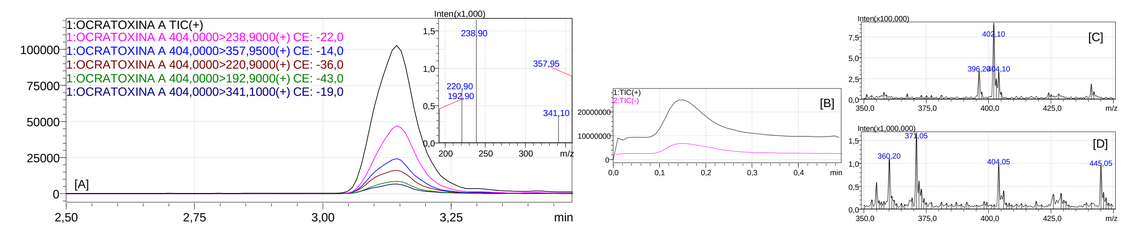


Figure 1: LCMS/MS chromatogram and mass spectrum of Ochratoxin A in ultrapure water [A]. Total ion chromatogram (TIC) [B]. Mass spectrum negative [C] and positive [D].

Table 1: Results of analytical curve in H_2O solvent and in digested extracts (DE).

Matrix	Red wine DE	Moscatel DE	Grape juice DE	H_2O
Intercept	6267	4444	2486	773
Slope	106329	95033	110681	113409
Determination coefficient (r^2)	0,9996	0,9990	0,9997	0,9997
Detection limit µg L^{-1}	0,03	0,03	0,02	0,02
Quantification Limit µg L^{-1}	0,08	0,08	0,07	0,07

Table 2: Results of the matrix effect of the digested extracts (DE) and H_2O solvent.

Matrix	H_2O	Red wine DE	Moscatel DE	Grape juice DE
Red wine DE	matrix effect	-	-	-
Moscatel DE	matrix effect	matrix effect	-	-
Grape juice DE	matrix effect	matrix effect	matrix effect	-
White brut DE	matrix effect	matrix effect	no	matrix effect
Sweet withe wine DE	matrix effect	matrix effect	no	matrix effect

Table 3: Results of repeatability, intermediary precision (IP) and recovery of digested extracts (DE).

Matrix	Red wine DE			Moscatel DE			Grape juice DE		
Fortification µg L^{-1}	**0,5**	**1,5**	**2,5**	**0,5**	**1,5**	**2,5**	**0,5**	**1,5**	**2,5**
Repeatability Day 1 n=6 CV %	4,0	5,5	4,7	10,2	8,5	7,2	3,4	6,1	3,0
IP Day 1 and 2 n=12 CV %	11,9	12,5	10,2	9,1	7,9	6,8	13,7	13,7	14,0
Repeatability different operator n=6 CV %	7,9	9,8	8,3	8,9	7,6	10,6	12,1	7,7	9,0
IP different operator n=12 CV %	13,7	15,7	13,8	9,2	8,4	8,2	13,3	12,3	12,9
Recovery %	96±5	96±6	97±6	96±9	104±8	104±7	88±10	87±7	86±6

CONCLUSIONS

In the present study, an LCMS/MS method for identification and quantification of OTA in wines, sparkling wines and whole red grape juice was developed and validated. The optimized MRM method is specific for identification of OTA in the matrices studied and is accurate for its quantification. Matrix effect was identified for the solvent and digested extracts matrices of dry red wine, sparkling white moscatel and whole red grape juice. Therefore, the quantification of OTA in each of the matrices should be performed with the curve of the respective matrix. However, there is no matrix effect between the matrices digested extracts of white moscatel sparkling wine, white brut sparkling wine and sweet white wine, and the same curve is used for the quantification of OTA. All validation parameters agree to the values established by analytical validation guides. This new method comes as a tool of quality control and food safety.

ACKNOWLEDGEMENTS