Supplemental Information

Rapid determination of *Alternaria* mycotoxins in tomato samples by pressurized liquid extraction coupled to liquid chromatography with fluorescence detection

Alberto Rico-Yuste,*$* Lidia N. Gómez-Arribas,*$* María Concepción Pérez-Conde, Javier L. Urraca,\* María Cruz Moreno-Bondi.\*

$ These authors contributed equally to this work.

Department of Analytical Chemistry, Faculty of Chemistry, Universidad Complutense de Madrid, Avenida Complutense, s/n, 28040 Madrid, Spain.

\* Corresponding authors: J.L Urraca, Fax: (+) 34 913944329, E-mail: [jurracar@ucm.es](file:///C%3A%5CUsers%5CQuimica%20Analitica%207%5CDownloads%5Cjurracar%40ucm.es); M.C. Moreno-Bondi, Fax: (+) 34 913944329, E-mail: [mcmbondi@ucm.es](file:///C%3A%5CUsers%5CQuimica%20Analitica%207%5CDownloads%5Cmcmbondi%40ucm.es).

ORCID ID: [0000-0001-7072-3894](https://www.scopus.com/redirect.uri?url=http://www.orcid.org/0000-0001-7072-3894&authorId=56682080800&origin=AuthorProfile&orcId=0000-0001-7072-3894&category=orcidLink) (A.Rico-Yuste), 0000-0003-2843-0865 (J.L. Urraca); 0000-0002-3612-0675 (M.C. Moreno-Bondi)

## UPLC–MS/MS equipment and chromatographic conditions

UPLC–MS/MS confirmatory analyses were done as described elsewhere (Walravens et al. 2014), using an Acquity UPLC system from Waters (Milford, MA, USA) coupled to a Xevo TQ-S triple quadrupole mass spectrometer, also from Waters, that was equipped with an electrospray interface (ESI) and operated in negative ESI mode. Separation was done on an Acquity UPLC HSS (100mm × 2.1mm, 1.8 μm) column from Waters (Milford, MA, USA) at 35 °C. The chromatographic gradient involved using 1% acetic acid (solvent A) and ACN containing 1% acetic acid (solvent B) as follows: 5% A (0.5 min, 0.4 mL·min−1), 31–38% B (3.75 min, 0.4 mL·min−1), 90% B (0.85 min, 0.4 mL·min−1), 5% B (0.5 min, 0.3 mL·min−1) and 5% B (3 min, 0.3 mL·min−1). Then, the column was re-equilibrated prior to the next injection. The injected volume was 5 µL and the total run time 7 min. The ESI source was operated in the negative ionization mode under the following conditions: capillary voltage, 2.95 kV; source block and desolvation temperature, 125 and 350 °C, respectively; desolvation and nebulizer gas flow-rate, 800 and 50 L·h−1, respectively; and argon pressure in the collision cell, 9.1×10−3 mbar. Data acquisition for quantification was performed by using the multiple reaction monitoring (MRM) mode to follow two transitions for AOH (257.1 > 213.1 and 257.1 > 215.1, corresponding to the fragment ions [MH–CO2]− and [MH–CH2OH]−, respectively) and another two for AME (271.2 > 256.2 and 271.2 > 228.2, corresponding to the fragment ions [MH–CH3]− and [MH–CO2]−, respectively).

Table S1. Extraction recoveries (R, %, n = 3) for AOH and AME (2000 ng g-1) obtained after PLE process as a function of the MeOH content, temperature, pressure and the number of cycles. In all the cases some parameters were fixed: heat-up time 5 min, extraction time 5 min, flush volume 60 %, purge time 1 min, cell volume 22 mL, total extraction time 13 min and total solvent used 33 mL.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| MeOH (%) | T (○C) | Pressure (psi) | Number of cycles | RAOH (%) | RSDAOH (%) | RAME (%) | RSDAME (%) |
| 100 | 50 | 1000 | 1 | 97.6 | 3 | --- | --- |
| 75 | 50 | 1000 | 1 | 91.1 | 5 | --- | --- |
| 50 | 50 | 1000 | 1 | 87.8 | 4 | --- | --- |
| 25 | 50 | 1000 | 1 | 83.8 | 9 | --- | --- |
| 0 | 50 | 1000 | 1 | 68.4 | 3 | --- | --- |
| 100 | 70 | 1000 | 1 | 94.8 | 6 | 95.3 | 3 |
| 75 | 70 | 1000 | 1 | 92.2 | 8 | 93.1 | 4 |
| 50 | 70 | 1000 | 1 | 93.5 | 6 | 90.9 | 7 |
| 25 | 70 | 1000 | 1 | 96.9 | 4 | 90.2 | 3 |
| 10 | 70 | 1000 | 1 | 90.3 | 3 | 66.6 | 6 |
| 0 | 70 | 1000 | 1 | 84.1 | 11 | 39.4 | 14 |
| 25 | 70 | 2000 | 1 | 90.8 | 1 | 86.7 | 2 |
| 25 | 70 | 3000 | 1 | 91.8 | 8 | 88.6 | 5 |
| 25 | 70 | 1000 | 2 | 82.7 | 3 | 42.7 | 12 |
| 25 | 70 | 1000 | 3 | 88.2 | 2 | 55.2 | 3 |

Table S2. Comparison of the recoveries of selected analytical extraction methods for AOH and AME in tomato or tomato-based samples.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Extraction technique** | **Extraction Solvent** | **Extraction recovery (%)** | **Preconcentration****and clean-up** | **Analysis** **Technique** | **Total recovery****(%)** | **LOD****(µg·kg-1 / µg·L-1)** | **Reference** |
| LLE | PBS | n.d. | SPE | LC-MS/MS | AOH: 81.3 – 102.2AME: 50.8 – 82.0 | AOH: 1.01AME: 1.21 | (Prelle et al. 2013) |
| SLE | ACNEthyl acetateFormic acid | AOH: 83.0AME: 96.0 | --- | HPLC-MS | AOH: 74.0AME: 90.0 | AOH: 12.2AME: 13.5 | (Van de Perre et al. 2014) |
| SLE | MeOHDNPH | AOH: > 70AME: > 70 | SPE | LC-MS/MS | AOH: 89.0 – 92.6AME: 89.0 – 89.3 | AOH: 2AME: 1 | (Tölgyesi et al. 2015) |
| UAE | ACNWaterMeOH | n.d. | SPE | LC-MS/MS | AOH: 78.4 – 82.1AME: 65.9 – 79.3 | AOH: 5AME: 0.1 | (Zhao et al. 2015) |
| UAE | PB | n.d. | MISPE | HPLC-FLD | AOH: 81.0 – 103.0 | AOH: n.d. | (Abou-Hany et al. 2015) |
| QuEChERS | ACNWaterFormic acid | n.d. | --- | LC-MS/MS | AOH: 112.0AME: 101.0 | AOH: 0.7AME: 0.3 | (López et al. 2016) |
| QuEChERS | ACNWater | 59.0 – 86.0 | --- | LC-MS/MS | AOH: 90.4 – 108.4AME: 90.7 – 105.6 | AOH: 0.3 – 1.1AME: 0.3 – 1.4 | (Walravens et al. 2016) |
| SLE | ACNWaterFormic acid | n.d. | --- | LC-MS/MS | AOH: 83.0AME: 75.0 | AOH: 0.2 – 2.8AME: 0.04 – 0.4 | (Hickert et al. 2016) |
| PLE | PBMeOH | AOH: 96.9AME: 90.2 | MISPE | HPLC-FLD | AOH: 84.0 – 97.0AME: 67.0 – 91.0 | AOH: 7AME: 12 | This work |

n.d.: not determined. LLE: Liquid-liquid extraction; SLE: Solid-liquid extraction; UAE: Ultrasound assisted extraction; PLE: Pressurized liquid extraction; SPE: Solid phase extraction; MISPE: Molecularly imprinted solid phase extraction; HAc: Acetic acid; DNPH: 2,4-Dinitrophenylhydrazine; PBS: Phosphate buffer saline; PB: Phosphate buffer.



Figure S1. Representative HPLC-FLD chromatograms of: (a) an extract of blank tomato; (b) an extract of blank tomato spiked with 125 µg·kg-1 of AOH (1) and 150 µg·kg-1 AME (2). Chromatographic conditions are described in “HPLC–FLD conditions” section.



Figure S2 (a) HPLC–FLD chromatogram for the PLE-MISPE extract from a tomato sample naturally infected with black moulds. Peaks marked with one and two asterisks (\* and \*\*) in chromatogram (a) correspond to the suspected AOH and AME, respectively. The UPLC–MS/MS chromatograms (b) and (c) confirm the presence of AOH and AME, respectively, in the contaminated tomato sample.

**References**

Abou-Hany RAG, Urraca JL, Descalzo AB, Gómez-Arribas LN, Moreno-Bondi MC, Orellana G. 2015. Tailoring molecularly imprinted polymer beads for alternariol recognition and analysis by a screening with mycotoxin surrogates. J. Chromatogr. A. 1425:231-239.

Hickert S, Bergmann M, Ersen S, Cramer B, Humpf H-U. 2016. Survey of Alternaria toxin contamination in food from the German market, using a rapid HPLC-MS/MS approach. Mycotoxin Res. 32:7-18.

López P, Venema D, de Rijk T, de Kok A, Scholten JM, Mol HGJ, de Nijs M. 2016. Occurrence of Alternaria toxins in food products in The Netherlands. Food Control. 60:196-204.

Prelle A, Spadaro D, Garibaldi A, Gullino ML. 2013. A new method for detection of five alternaria toxins in food matrices based on LC–APCI-MS. Food Chem. 140:161-167.

Tölgyesi Á, Stroka J, Tamosiunas V, Zwickel T. 2015. Simultaneous analysis of Alternaria toxins and citrinin in tomato: an optimised method using liquid chromatography-tandem mass spectrometry. Food Addit. Contam., Part A. 32:1512-1522.

Van de Perre E, Deschuyffeleer N, Jacxsens L, Vekeman F, Van Der Hauwaert W, Asam S, Rychlik M, Devlieghere F, De Meulenaer B. 2014. Screening of moulds and mycotoxins in tomatoes, bell peppers, onions, soft red fruits and derived tomato products. Food Control. 37:165-170.

Walravens J, Mikula H, Rychlik M, Asam S, Devos T, Njumbe Ediage E, Diana Di Mavungu J, Jacxsens L, Van Landschoot A, Vanhaecke L, et al. 2016. Validated UPLC-MS/MS Methods To Quantitate Free and Conjugated Alternaria Toxins in Commercially Available Tomato Products and Fruit and Vegetable Juices in Belgium. J. Agric. Food Chem. 64:5101-5109.

Zhao K, Shao B, Yang D, Li F. 2015. Natural Occurrence of Four Alternaria Mycotoxins in Tomato- and Citrus-Based Foods in China. Journal of Agricultural and Food Chemistry. 2015/01/14;63:343-348.