ASSESSMENT OF THE ALTERNARIA MYCOTOXIN TENUAZONIC ACID IN FRUIT JUICE SAMPLES

Vahid Safavizadeh1,2, Ali Shayyamfar3, Masoud Ansarin4, Mahboob Nemati5

Address(es):
1 Food and Drug Safety Research Center, Health Management and Safety Promotion Research Institute, Tabriz University of Medical Sciences, Tabriz, Iran.
2 Department of Pharmaceutical and Food Control, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.
3 Pharmaceutical Analysis Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.
4 Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
5 Halal Research Center of IRI, FDA, Tehran, Iran.

*Corresponding author: nematmn@tbzmed.ac.ir

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ABSTRACT

Tenuazonic acid (TeA) is a secondary toxic metabolite that is produced by some Alternaria species. The aim of this study was to determine the presence of TeA in fruit juice samples. A total of 50 (40 Grape; 5 Apple; 5 Orange) fruit juice samples were collected from Tabriz, Iran local market and were analyzed for TeA contamination via HPLC-UV. Analyte extraction was done by acetone/titrile/water/formic acid (84/16/1 v/v/v). Lower limit of quantitation and upper limit of quantification for the developed method were 10 µg/L and 4000 µg/L respectively. Recovery ranged was between 96 to 108 %. The results showed 42.5% of grape juice samples were contaminated with TeA and the average concentration of TeA was 139.2±115.5 µg/L. However, it was not detected in apple and orange juice samples. This is the first study on the presence of TeA in Iranian food samples and showed that the necessity of more supervision on the production of grape juice.

Keywords: Mycotoxins; Alternaria; Fruit Juices; HPLC; Tenuazonic Acid; Iran

INTRODUCTION

Some microfungi produce toxic secondary metabolites called mycotoxins (Amirkhiz, Arehosseini, Ansarin, & Nemati, 2015; Cunha, Sá, & Fernandes, 2018; Fernández-Cruz, Mansilla, & Tadeo, 2010; Pizzutti et al., 2014; Walravens et al., 2014). Mycotoxins are one of the most important contamination factors in plants and cause the destruction of crops (da Motta & Valente Soares, 2000). Some of the most important types of fungi that produce mycotoxins are Aspergillus spp, Penicillium spp, Fusarium spp and Alternaria spp (Prelle, Spadaro, Garibaldi, & Gullino, 2013; Walravens et al., 2014). Alternaria fungi are pathogens and saprophytic species that have the ability to grow at low temperature (De Berardis et al., 2018; Myresiotis, Testempasis, Vryzas, Karaoglanidis, & Papadopoulou-Vryzsa, 2018). Some of these fungi species can produce more than 70 mycotoxins species. The aim of this study was to determine the presence of TeA in fruit juice samples.

Figure 1 Chemical structures of TeA

TeA (5S)-3-acetyl-5[(2S)-butan-2-yl]-4-hydroxy-1,5-dihydro-1H-pyrryl-2-one, (Fig. 1) is a toxic metabolite, which is produced by Alternaria spp, Phomopsis oryzae and Pyricularia oryzae (Asam et al., 2013; Chen & Qiang, 2017; Liu, Ge, Peng, & Pan, 2017; Oliveira et al., 2017). Having melanize walls in the spores of the fungus is the most important way to control its presence in the foodstuff (Prenedes et al., 2018).
MATERIAL AND METHODS

Chemicals

Acetonitrile and methanol, both HPLC gradient grade were supplied by DUKSAN (Gyeonggi-do, South Korea). Formic acid (≥ 99%) and sodium phosphate monobasic (NaH2PO4) were purchased from Merck (Darmstadt, Germany) and also phosphoric acid was obtained from Kimia Tehran acid co (Tehran, Iran). TeA was procured from Cayman Chemical Company (Ann Arbor, Michigan, United States) and the standard solution was prepared with methanol. Deionized water was prepared using a Mili-Q System (Tehran Absaz co, Iran).

HPLC conditions

The chromatographic system was a KNAUER HPLC instrument (Knauer, Berlin, Germany) consisting of a Detector S2500 Knauer equipped with a Biotech 2003 degasser (United State), K-1000 Knauer controller Quaternary pump and Rheodyne sample valve fitted with a 20 μl loop (United State). The analytical column was SCIX AAA C18 column 150 × 4.6 mm, 5 μm (Foster City, USA). The mobile phase was prepared freshly every day by a mixture of MeOH: 0.1 M NaH2PO4 (2:1 v/v), adjusted to pH 3.2 with phosphoric acid. The eluent flow rate was 1.5 mL/min. The wavelength for recording chromatograms was 279 nm (Fontana et al., 2016).

Samples

A total of 50 homogenized juice samples (40 Grape; 5 Apple; 5 Orange) that were purchased in March-April 2018 were randomly selected to quantification of TeA from retail stores.

Sample preparation

Sample preparation was preformed based on the reported method by Lopez and coworkers (38). The samples were shaken for homogeneity. Then, 2.5 mL of juice transferred to a 15 mL centrifuge tube and was mixed with 10 mL of acetonitrile/water/formic acid (84/16/1 V/V/V). The mixture was manually shaken for 5 min. After centrifugation at 4000 rpm for 5 min, an aliquot of 0.5 mL of the supernatant was taken and filtered. Subsequently, 20 μL of the solution was injected directly into the HPLC-UV system.

Method validation parameters

The HPLC-UV method for the determination of TeA in juices was validated for linearity, accuracy, precision. Calibration curve was prepared by spiking six concentrations (10, 50, 125, 250, 500,1000 µg/L) of TeA in a blank grape juice. The linearity was calculated using these six concentrations in triplicate also linearity requirements were fulfilled when the correlation coefficient was greater than 0.99. The calibration range included concentrations from the lower limit of quantification (LLOQ) to the upper limit of quantification (ULOQ). The LLOQ is defined as the lowest concentration of TeA that can be determined with acceptable precision and accuracy as well as the highest amount of TeA that can be quantitatively determined with precision and accuracy is ULOQ (Ershadi & Shayanfar, 2018; Kollipara, Bende, Agarwal, Varshney, & Palwil, 2011). Recovery and precision were evaluated over three consecutive days at three nominal TeA concentrations (80, 200 and 400 µg/L) by spiking an uncontaminated matrix.

RESULTS AND DISCUSSION

Method validation

For the developed analysis methods, coefficients of determination (R2) above 0.99 show an acceptable linear relationship between concentration and response. In this research, R2 is obtained 0.999. The sensitivity parameters, LLOQ and ULOQ were 10 µg/L and 1000 µg/L respectively. The validity of method was checked by three different concentrations of TeA in fruit sample. Details of the method validation for the developed analysis method for quantification of TeA in fruit samples have been listed in Table 1. The relative standard deviation (RSD) was from 1.5 to 2.8 % for inter-day (n = 3) and 2.9 to 6.6 % for intra-days (n = 3, three days) analysis. The recovery of the developed method for quantification of TeA was between 96 to 108 %. The acceptable range for RSD is ≤ 20% and accuracy is 70%-120% (Fontana et al., 2016).

Table 1 Recovery and precision (as RSD) of the developed analysis method for quantification of TeA

<table>
<thead>
<tr>
<th>TeA spiked (µg L−1)</th>
<th>TeA found (µg L−1)</th>
<th>Recovery (%)</th>
<th>RSD% Inter-day</th>
<th>RSD% Intra-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>76.8</td>
<td>96%</td>
<td>2.8</td>
<td>6.6</td>
</tr>
<tr>
<td>200</td>
<td>202</td>
<td>101%</td>
<td>1.8</td>
<td>5.1</td>
</tr>
<tr>
<td>400</td>
<td>432</td>
<td>108%</td>
<td>1.5</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Comparison with HPLC-MS

HPLC-UV and HPLC-MS are commonly used systems for TeA analysis. Each of these systems has advantages and disadvantages. However, HPLC-MS has more ability than HPLC-UV for detecting analytes but HPLC-UV was able to detect TeA sufficiently and had shown good efficiency. The reason for choosing HPLC-UV in this study is its low cost and high availability. A few studies have been conducted on the presence of tenuazonic in foodstuffs throughout the world. More studies should be done especially in developing and least developed countries. In this study, we have tried to use methods that allow researchers around the world to analyze TeA in fruit juices by low cost and fast.

Survey of grape juice samples from the Iranian market

The above validated method was finally evaluated on the real fruit juice samples. Fig. 2 shows the HPLC analysis of TeA in fruit juice samples including a standard, spiked sample and a positive grape juice sample.

![Image](1163)
In this study, samples, which TeA was not detected in, were analyzed and TeA was presented in concentrations was 212 µg/L. The results of TeA concentration in positive samples were reported in Table 2. TeA was detected in 14 grape juice samples, respectively. In this study, samples from 4 countries were used and the concentration range from 212 to 702 µg/L was reported. As a result, TeA was present in over 32.5% of the grape juice samples. On the other hand one sample of grape juice, which expired 9 months ago was tested and the highest amount of TeA was observed in it. The maximum and minimum TeA concentrations was 212, 702 µg/L, respectively. However, TeA was not found in orange juice and apple juice samples.

Comparison with results of other studies

There are not many studies about the presence of TeA acid in foodstuff. In Table 3, the most relevant reported studies for TeA level in other research studies have been compared. In a study in Italy (Prelle et al., 2013), TeA was detected in apple juice. Ten apple juice samples were analyzed and TeA was present in two samples, but it was not found in Beers, Tomato products, Olive and Dried basil samples. In another study in Argentina (Fontana et al., 2016) on wine grapes, the presence of TeA in 57% of the samples was showed and the maximum concentration level of samples was 595 µg/g. A survey in the Netherlands (López et al., 2016) showed that all Dried figs, sunflower seeds and tomato sauces were contaminated with TeA, and also in three samples of wine and one olive sample, TeA was detected. There was no TeA in the fresh citrus or apple juice samples.

Table 3 Presence of TeA presences in various samples with other studies

<table>
<thead>
<tr>
<th>Sample</th>
<th>NO. of samples</th>
<th>Occurrence</th>
<th>Range</th>
<th>Country</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple Juice</td>
<td>10</td>
<td>20%</td>
<td>45.3 - 24.3 (ng g⁻¹)</td>
<td>Italy</td>
<td>(Prelle et al. 2013)</td>
</tr>
<tr>
<td>Wine grape</td>
<td>14</td>
<td>57%</td>
<td>0.595 - 0.057 (µg g⁻¹)</td>
<td>Argentina</td>
<td>(Fontana et al. 2016)</td>
</tr>
<tr>
<td>Wine</td>
<td>5</td>
<td>60%</td>
<td>5.0 - 46 (mg kg⁻¹)</td>
<td>Netherlands</td>
<td>(López et al. 2016b)</td>
</tr>
<tr>
<td>Beer</td>
<td>43</td>
<td>88%</td>
<td>174.6 - 8.7 (µg kg⁻¹)</td>
<td>Germany</td>
<td>(Siegel et al. 2010b)</td>
</tr>
<tr>
<td>Citrus juice and wine</td>
<td>103</td>
<td>62% juice</td>
<td>1.10 - 60.0 (µg L⁻¹)</td>
<td>Germany</td>
<td>(Zwickel et al. 2016)</td>
</tr>
<tr>
<td>Ice wine</td>
<td>26</td>
<td>Not quantified</td>
<td>Not quantified</td>
<td>Canada</td>
<td>(Abramson et al. 2007)</td>
</tr>
<tr>
<td>Wine and apple juice</td>
<td>27</td>
<td>8.3% wine</td>
<td>1.75-49.61 (µg L⁻¹)</td>
<td>China</td>
<td>(Fan et al. 2016)</td>
</tr>
<tr>
<td>Grape juices</td>
<td>40</td>
<td>32.5% grape juices</td>
<td>212, 702 (µg L⁻¹)</td>
<td>Iran</td>
<td>This work</td>
</tr>
</tbody>
</table>

The results of this study showed HPLC–UV method could applied successfully for the quantification of the TeA in fruit juices. The data from this survey illustrated that TeA occurs at high levels, up to a maximum of 702 µg/L in grape juices. The method features a LLOQ of 10 µg/L, good selectivity and a rapid sample preparation and analysis procedure.

Acknowledgments: This is a paper of a database from the thesis entitled “Determination of TeA in some fruit juice samples using HPLC method” registered in the Tabriz University of Medical Sciences. We also gratefully acknowledge their help and financial assistance as grant.

CONCLUSION

The results of this study showed HPLC–UV method could applied successfully for the quantification of the TeA in grape juices. This data from this survey illustrated that TeA occurs at high levels, up to a maximum of 702 µg/L in grape juices. The method features a LLOQ of 10 µg/L, good selectivity and a rapid sample preparation and analysis procedure.

REFERENCES


