INTRODUCTION

Red wine is a rich source of phenolic compounds, antioxidants that have health-protective and antioxidant benefits [1]. The analysis of pesticides, such as fungicides and insecticides, to improve grape yields, is common practice in vineyards. Pesticide residues may remain in the grapes after harvest and in the wine that are made from them. Therefore, it is important to analyze for the presence of pesticide residues in red wines. The analysis of pesticide residues in red wine is challenging due to the complexity of the matrix, which contains alcohol, organic acids, sugars, phenols and pigments, such as anthocyanins. QuEChERS introduction for quick, easy, cheap, effective, rugged, and safe is a concern analytical approach that was first publised in 2003 by Anastassiades et al. for the analysis of pesticide residues in vegetables and fruits [2]. The QuEChERS procedures involve the extraction of pesticides into acetone (MeCN) with the aid of salts and buffers, followed by dispersive solid phase extraction (dSPE) to clean up on-sorbents. The aim of this study was to use a QuEChERS extraction, but develop a clean-up approach that is easier and faster than the dSPE used in QuEChERS. This sample clean-up method is based on the filter and clean concept: the red wine extract is passed through a push thru cartridge containing magnesium sulfate (MgSO₄) and primary and secondary amine (PSA), and then through a C₁₈ (carbon) cartridge. Calculation steps include: (1) extraction of red wine and fortification with pesticide mixture; (2) cleanup of the fortified red wine extract with MeCN and MgSO₄; (3) suppression of organic acids with sodium chloride (NaCl); and (4) extraction of the cleanup procedure with ethyl acetate (EtOAc) and filtration.

EXPERIMENTAL

Materials

50 mL polypropylene centrifuge tube (UCT cat#: RFV0050CT)
Mylar Pouch containing 4000 mg MgSO₄ and 2000 mg NaCl (UCT cat#: ECQUUS2-MP)
Quick QuEChERS mini-cartridge containing 110 mg MgSO₄ and 180 mg PSA (UCT cat#: CPQ9510/3500MS)

Procedures

QuEChERS extraction

- A. Add 10 mL red wine to 50 mL polypropylene centrifuge tube.
- B. Add with appropriate amounts of target analytes for fortified samples, vortex 30 sec and equilibrates for 15 min.
- C. Add 10 mL MeCN, vortex 30 sec.
- D. Add 40 mL of Mylar pouch (MeCN and NaCl), shake vigorously for 1 min.
- E. Centrifuge at 80 rpm for 5 min, the extract is ready for cleanup.

Quick QuEChERS clean-up

- A. Load 1 mL red wine extract with polyethylene syringe.
- B. Pass the extract slowly through a Quick QuEChERS mini-cartridge (MgSO₄ and PSA).
- C. Collect 65 mL cleaned extract into 2 mL auto-sampler vial.
- D. Add 100 µL of primary and secondary amine (PSA) as internal standard, the extract is ready for LC/MS/MS analysis.

RESULTS AND DISCUSSIONS

Matrix matched calibration, limit of detection (LOD), and limit of quantification (LOQ)

Calibration curves were obtained by analysis of matrix matched standards, which were prepared by spiking appropriate amounts of 2 ppm pesticide mixture to blank red wine extract after Quick QuEChERS cleanup. Six matrix matched standards at 5, 10, 50, 100, 200, and 400 ng/mL levels were analyzed. The response was linear over the calibration range. LOD and LOQ were the concentrations that give signal-to-noise ratio (S/N) of 3 and 10, respectively. In this study they were tested over the calibration range. LOD and LOQ are the concentrations that give signal-to-noise ratio (S/N) of 3 and 10 respectively. In this study they were tested over the calibration range. LOD and LOQ are the concentrations that give signal-to-noise ratio (S/N) of 3 and 10 respectively. In this study they were tested over the calibration range.

Matrix matched calibration, LOD and LOQ

<table>
<thead>
<tr>
<th>Component</th>
<th>LOD (ng/ml)</th>
<th>LOQ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbendazim</td>
<td>0.86</td>
<td>3.4</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.86</td>
<td>3.4</td>
</tr>
<tr>
<td>Diazinone</td>
<td>0.86</td>
<td>3.4</td>
</tr>
<tr>
<td>Methamidophos</td>
<td>0.86</td>
<td>3.4</td>
</tr>
<tr>
<td>Pyrimethanil</td>
<td>0.86</td>
<td>3.4</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>0.86</td>
<td>3.4</td>
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Calibration Curves

Graphs showing the calibration curves for each pesticide, demonstrating linearity and sensitivity.

CONCLUSIONS

An easy, fast, novel, and efficient clean-up method for red wine samples was successfully developed in this study. Pesticide residues in red wine samples were extracted into acetone by using Quick QuEChERS. Cleanup was accomplished by passing 1 mL of red wine extract through a push thru cartridge containing MgSO₄ and NaCl, adsorbed water remaining in the matrix, while PSA treated organic acids, sugars, and pigments. This clean-up method, based on the filter and clean concept, takes less than one minute per sample. Combined with the QuEChERS extraction, this method is an excellent choice for high throughput laboratories.

APPENDIX

Accuracy and Precision Data

Red wine samples fortified with 10, 50 and 100 ng/mL target pesticides were extracted with QuEChERS and cleaned up with Quick QuEChERS mini-cartridges. Recoveries ranged from 60% to 120% with overall recovery of 80%. Relative standard deviations (RSD) based on four replicates for three spiking levels were less than 15%. The recovery and RSD data indicated that method is robust, accurate and precise for the determination of pesticide residues in red wine samples.

Chromatograms of red wine sample 1 fortified with 10 ng/mL pesticides

Photos showing the cleanup procedure:

[Insert photos or diagrams as applicable]