DETERMINATION OF NITROFURAN METABOLITES IN SHRIMP MUSCLE TISSUE BY LIQUID CHROMATOGRAPHY- PHOTO DIODE ARRAY DETECTION

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Outline

- Sri Lanka
- Shrimp farming
- Nitrofuran issue
- Objectives of the study
- Method development and validation
- Results
- Discussion/conclusions
Sri Lanka
Sri Lanka

- Land area 65,610 km\(^2\)
- Population 20 million
- GDP Per capita US$ 3279
- GDP
  - Agriculture: 12.8%
  - Labor force: 32.7%
Shrimp farming

- Important economic activity (~50% of the export earnings from fisheries)
- High demand in Japan, USA and EU
- >90% of the harvested cultured shrimp are exported
- Deficits in disease control, management, and biosecurity practices
- High disease outbreaks
- Need for antibacterials
Nitrofuran Issue

- Broad-spectrum
- Furazolidone, furaltadone, nitrofurantoin and nitrofurazone
- Rapidly metabolized
- Metabolites reside in animal body for weeks
- Banned due to carcinogenicity
- Abuse due to lack of resources and facilities to test and inadequate legislative framework
- Negatively impacted export and local markets
Objectives

Establish a **financially sustainable** method to screen shrimp muscle tissue for the following nitrofuran metabolites conforming to the EU criteria.

- 3-amino-2-oxazolidinone (AOZ)
- 3-amino-5-morpholino-methyl-1,3-oxazolidinone (AMOZ)
- Semicarbazide (SEM)
- 1-aminohydantoin (AHD)
Method: Derivatization and Extraction

- Homogenize 5g
- Neutralized the acidic medium
- Extracted with ethyl acetate
- Evaporated to dryness
- Redissolved
- NBA removed by an extraction with hexane
- 50%, 75% and 100% methanol and water

NBA removed by an extraction with hexane

Neutralized the acidic medium

Evaporated to dryness

Extracted with ethyl acetate

Redissolved
HPLC-DAD Analysis

- Mobile phase A [water: glacial acetic acid (100:0.08, v/v)]
- Mobile phase B [acetonitrile: water: glacial acetic acid (90:10:0.1, v/v/v)]
- Column: C18 (4.6X150mm)
- Flow rate - 1ml/min
- Total run time - 27min
- Injection volume - 100ul
- HPLC-UV analysis - 275nm
- Peak spectra -190 to 550 nm
Validation

- Analyzed blank samples and fortified shrimp muscle samples at 1, 2 and 5ug/kg
- At four different occasions
Results

Chromatogram of a blank shrimp sample

Chromatogram of a shrimp sample fortified at 5ug/kg with the four metabolites

NPAMOZ  NPSEM  NPAHD  NPAOZ
## Validation Summary

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>$CC_\alpha$ (ug/kg) Decision Limit</th>
<th>$CC_\beta$ (ug/kg) Detection capability</th>
<th>*Within-laboratory CV</th>
<th>% *Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMOZ</td>
<td>0.32</td>
<td>0.61</td>
<td>16.9</td>
<td>107</td>
</tr>
<tr>
<td>SEM</td>
<td>0.65</td>
<td>0.93</td>
<td>12.7</td>
<td>115</td>
</tr>
<tr>
<td>AHD</td>
<td>0.60</td>
<td>0.83</td>
<td>9.3</td>
<td>114</td>
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<tr>
<td>AOZ</td>
<td>0.46</td>
<td>1.69</td>
<td>55.1</td>
<td>107</td>
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</table>
Discussion/Conclusions

- Based on the RIKILT HPLC-UV method developed to detect the tissue-bound furazolidone metabolite AOZ
- Extended to cover AOZ, AMOZ, SEM and AHD
- LOD of the RIKILT method was 2ug/kg.
- Decision limit of the present method is below 1 ug/kg for all four nitrofuran metabolites
- Non-compliant samples can be further verified by comparing the spectral data of the non-compliant samples and positive controls
- Validated under ISO 17025 standards
References

• RHIJN VAN J.A. AND MUDLER, P.P.J., A Standard operating procedure on nitrofuran residue analysis made available to the NRL and CRL network of EU member states at the Meeting of the National Experts April 2002, Brussels.
Acknowledgement

IAEA for the technical and financial support and A. Cannavan of the IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria for his assistance in the HPLC-DAD method development and validation.
Thank you.
## Export Quantity and Value of Shrimp

<table>
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<tr>
<th>Year</th>
<th>Quantity (Mt)</th>
<th>Value (Rs. Mn)</th>
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<tbody>
<tr>
<td>1999</td>
<td>2716</td>
<td>2275</td>
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<tr>
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<td>4855</td>
<td>5041</td>
</tr>
<tr>
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<td>3941</td>
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<tr>
<td>2013</td>
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</table>
Diseases in Shrimp

- Losses due to white spot disease (WSD) were valued at Rs1 billion [6]. Yellow head disease (YHD) was recognized in Sri Lanka in 1998 in infected brood stocks. The dual problems of WSD and YHD caused an approximate 70% drop in exported shrimp products [6].
<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A %</th>
<th>Mobile phase B %</th>
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<tbody>
<tr>
<td>0</td>
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