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

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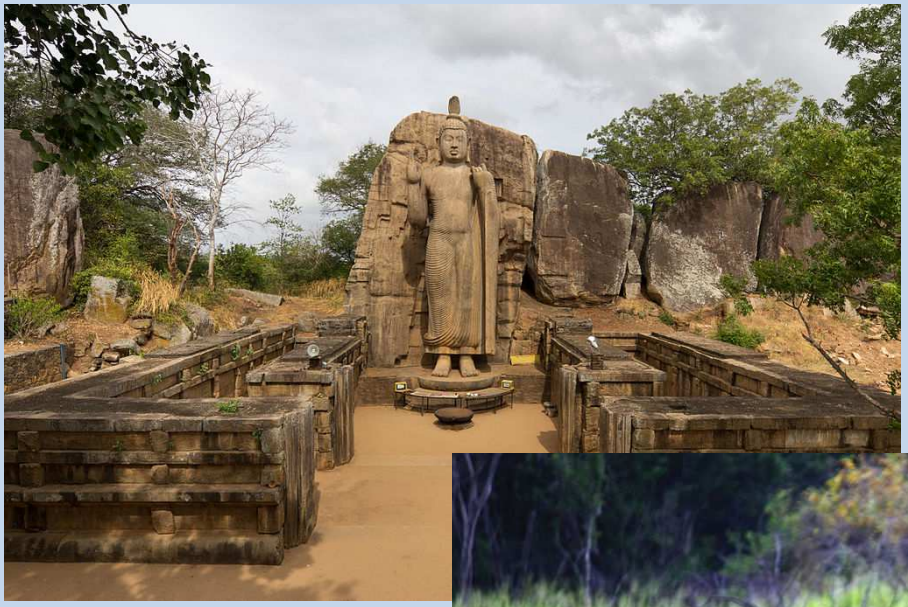
Outline

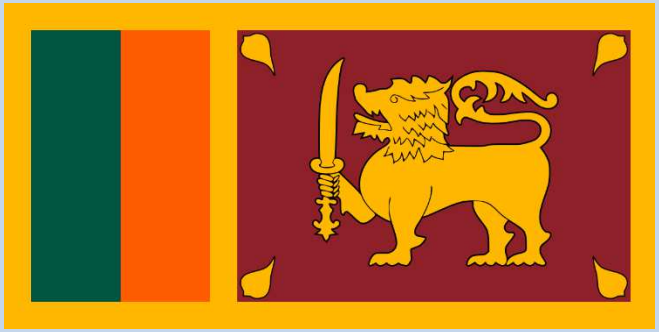
- Sri Lanka
- Shrimp farming
- Nitrofuran issue
- Objectives of the study
- Method development and validation
- Results
- Discussion/conclusions

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

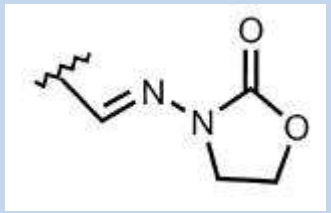
Objectives

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)

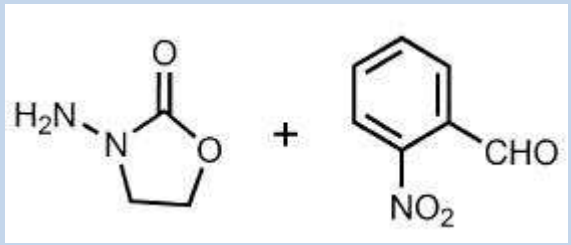
Method: Derivatization and Extraction

→ Homogenize → 5g → 50%, 75% and 100% methanol and water



37°C

H^+, H_2O



2-NBA

→ Neutralized the acidic medium

↓

Extracted with ethyl acetate

← Evaporated to dryness

← Redissolved

← NBA removed by an extraction with hexane

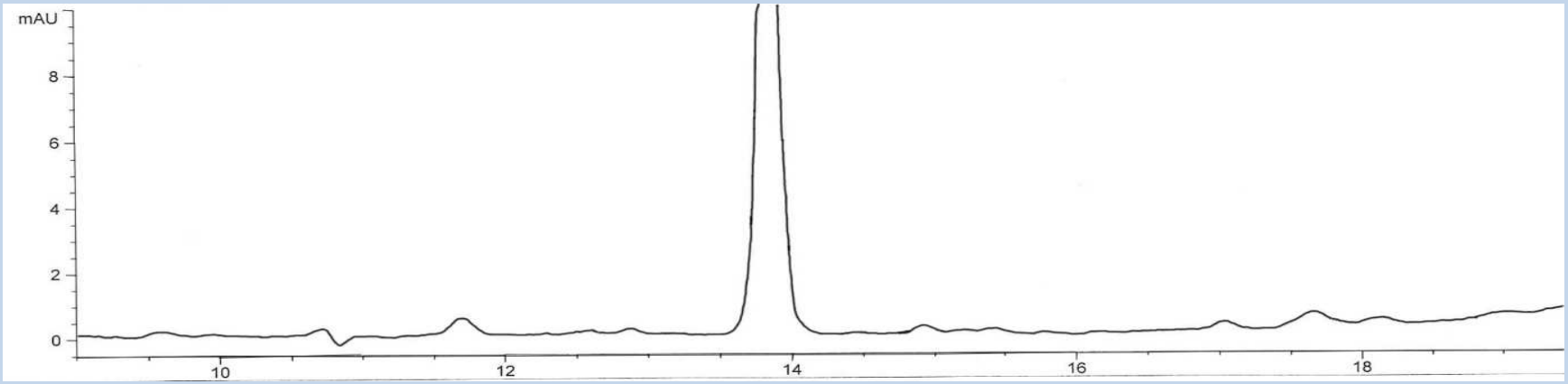
HPLC-DAD Analysis

- 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

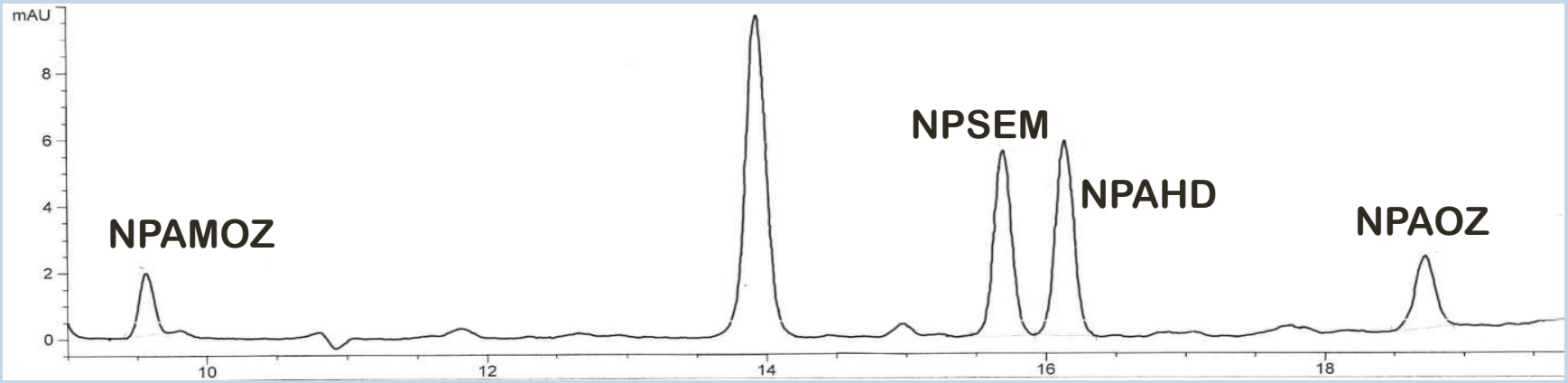
Validation

- Guidelines of EU Commission Decision 2002/657/EC
- 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

Validation Summary

Metabolite	CC_α (ug/kg) Decision Limit	CC_β (ug/kg) Detection capability	% *Within-laboratory CV	% *Recovery
AMOZ	0.32	0.61	16.9	107
SEM	0.65	0.93	12.7	115
AHD	0.60	0.83	9.3	114
AOZ	0.46	1.69	55.1	107

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

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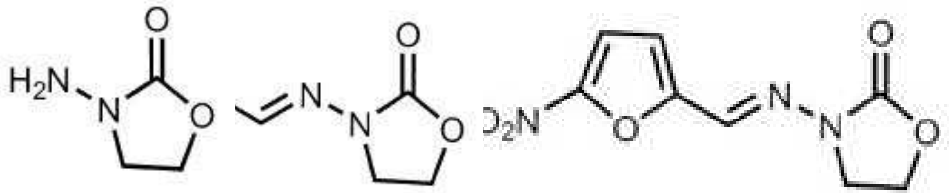
Thank you.

Export Quantity and Value of Shrimp

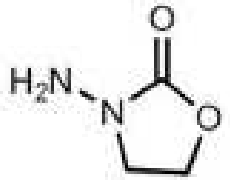
Year	Quantity (Mt)	Value (Rs.Mn)
1999	2716	2275
2000	4855	5041
2001	3941	4300
2002	3368	3286
2003	4467	4165
2004	2462	2359
2005	1800	1769
2006	1837	1987
2007	2023	2487
2008	854	1082
2009	1432	1627
2010	1262	1521
2011	1380	1799
2012	1056	1618
2013	1625	2521

Diseases in Shrimp

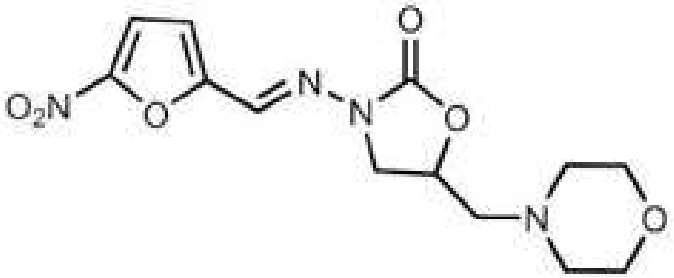
- Losses due to white spot disease (WSD) were valued at Rs1 billion [6]. Yellow head disease (YHD) was recognized in Sri Lanka in 1998 in infected brood stocks. The dual problems of WSD and YHD caused an approximate 70% drop in exported shrimp products [6].



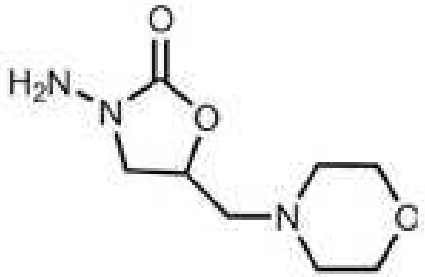
Furazolidone



AOZ

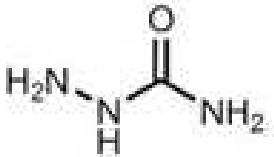


Furaltadone

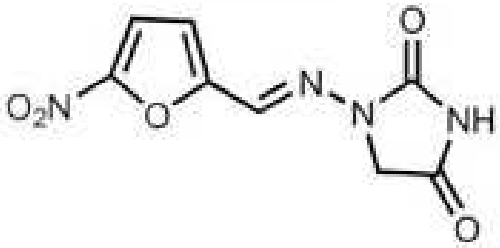


AMOZ

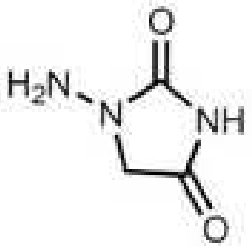
Nitrofurazone



SEM



Nitrofurantoin



AHD

Time (minutes)	Mobile phase A %	Mobile phase B %
0	90	10
1	90	10
20	70	30
21	10	90
23	10	90
24	90	10