Pharmacopoeia Monograph on Artemisinin

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Kamtech Technologies Ltd

WHO/MMV artemisinin conference 2009
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Scope of Presentation

• Overview of artemisinin (ART) monograph methods

• Consider limitations and possible improvements based on a recent study funded by MMV/FSC
Monograph protocols for Artemisinin

Assay:
• HPLC-UV
• Chemical transformation of artemisinin-UV

Related substances:
• HPLC-UV
• TLC-Densitometer

ART quantification limits given for ASSAY methods only

http://apps.who.int/phint/en/p/docf/
Monograph protocols for Artemisinin

<table>
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<th>Assay method</th>
<th>ART content limits</th>
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## Monograph protocols for Artemisinin

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HPLC-UV method

Column: 3μm C18, 10cm x 4.3mm
Detection: 216nm
Solvent: aqACN at 0.6ml per min

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A % v/v of ACN</th>
<th>Mobile phase B % v/v of water</th>
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<tr>
<td>0 – 17</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>17 – 30</td>
<td>60 – 100</td>
<td>40 – 0</td>
</tr>
<tr>
<td>30 – 35</td>
<td>100 – 60</td>
<td>0 – 40</td>
</tr>
<tr>
<td>35 – 45</td>
<td>60</td>
<td>40</td>
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Nature of stationary phase not defined
Quantification by HPLC-UV method

• System suitability checked by ensuring minimum resolution and relative retention times against artenimol.

• Use peak area from reference artemisinin sample to calculate relative purity of test sample and also of “related substances.”
Standards used in the Monograph

Artemisinin

Artenimol
Monograph HPLC-UV method - LIMITATIONS

- Is UV the best detector for quantifying artemisinin?
• Quantification using UV detector requires reference compounds
HPLC of hexane extract of *A. annua*

- For mixtures ELSD better than UV
- Estimation of impurities from combined UV peak areas is misleading
Mass spectroscopy

LC-MS requires HPLC separation

LC-MSMS – does not need HPLC separation
Comparison UV and MS detectors
Synergy Luna C18 (250mm) 30%aqMeOH

MS identified previously undetected compounds
Monograph HPLC-UV method – LIMITATIONS

• Is UV the best detector for quantifying artemisinin?  NO
Monograph HPLC-UV method - LIMITATIONS

• Is UV the best detector for quantifying artemisinin? NO
• Can separation of interfering compounds be improved?
• Column dimensions and type of stationary phase influence resolution
HPLC of *A. annua* extract – comparison of solvents

25%aq MeOH

30%aq ACN

Betasil C18 (250mm) column

- Major differences between ACN and MeOH
Stationary phases in HPLC columns

\[
\text{Si} - \text{O} - \text{Si} - \text{R}
\]

- $\text{R} = (\text{CH}_2)_{17}\text{CH}_3$  \hspace{1cm} \text{C18 reverse phase}
- $\text{R} = (\text{CH}_2)_6\text{Ph}$  \hspace{1cm} \text{Phenylhexyl phase}
- $\text{R} = (\text{CH}_2)_n\text{CN}$  \hspace{1cm} \text{Nitrile phase}

- > 15 columns evaluated
- 8 different phases tested
Comparison of C18 and aromatic bonded columns (ELSD detection)

C18 Betasil (250mm) 25%aqMeOH

Polar RP (150mm) 25%aqMeOH

Phenylhexyl (150mm) 30%aqMeOH

Diphenyl (250mm) 20%aqMeOH

• Clear differences between C18 and aromatic phases
Monograph HPLC-UV method - LIMITATIONS

• Is UV the best detector for quantifying artemisinin? NO
• Can separation of interfering compounds be improved?

YES:
Stationary phase, solvent and column dimensions can result in improved separations
Monograph HPLC-UV method - LIMITATIONS

HPLC-UV method needs to be updated
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Chemical transformation – UV method

UV transparent

UV active than ART
Chemical transformation-UV method

• Method relies on products formed by chemical reaction – complete reaction?
• Very sensitive to reaction conditions
• Dependent upon experimental skill

More reliable methods now available
# Monograph protocols for Artemisinin

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TLC-Densitometer by CAMAG

- Mixture applied to TLC plate and developed
- Plate sprayed with reagent and heated
- ART produces coloured spots and measured with densitometer

MS interface available - 2009
TLC-densitometer method

- Simple and economical method
- Amenable to high throughput
- Widely used

However

- Over-estimation of ART reported
- Need for proper validation with reference samples
### Comparison of analytical methods

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<th>Technique</th>
<th>Precision (RSD)</th>
<th>Additional comments</th>
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<tr>
<td>HPLC-ELSD</td>
<td>&lt;3%</td>
<td>• RSD can be less than ±2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Intra-day precision needs to be monitored</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Careful control of operating parameters necessary</td>
</tr>
<tr>
<td>TLC-Densitometer</td>
<td>?</td>
<td>• Transformation yield &lt;85%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Major advantage of high throughput</td>
</tr>
<tr>
<td>HPLC-UV</td>
<td>2%</td>
<td>• Poor sensitivity but good for ART crystals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Not acceptable for extracts</td>
</tr>
<tr>
<td>HPLC-MSMS</td>
<td>&lt;2%</td>
<td>• 0.4-2.4ng mL⁻¹ has been reported</td>
</tr>
<tr>
<td>HPLC-RI</td>
<td>6%</td>
<td>• Low sensitivity - potential impact on HPLC separation</td>
</tr>
<tr>
<td>TLC-FID [Iatroscan]</td>
<td>8%</td>
<td>• Separation of compounds questionable</td>
</tr>
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Several factors influence choice of analytical technique

Examples:
- HPLC-UV for analysis of crystalline ART
- ELSD and TLC-densitometer for extracts
- HPLC-MSMS for standardising protocols
Summary

• Separation of minor interfering impurities cannot be guaranteed by current Monograph HPLC-UV method.
• Potential for improving current methods has been demonstrated.
• New methods (e.g LCMSMS) should be considered for inclusion.
• Need to:
  – reconsider choice of internal reference standards
  – identify interfering compounds and their effect on quantification of ART
  – ensure availability of reference standards
Acknowledgments

- MMV/FSC for funding
- Rothamsted Research for facilities and general support
THANK FOR YOUR ATTENTION
Title: Validation of the monograph HPLC analytical protocol for artemisinin quantification in biomass and extracts

Alexei Lapkin, Belindha Mlambo and Smain Chemat – University of Bath
Adam Walker – Bioniqs Ltd
Neil Sullivan – SensaPharm Ltd
Bhupinder PS Khambay – Kamtech Technologies Ltd