Laboratory Analysis for Drug Assurance

Harparkash Kaur,¹ Michael D Green,² Facundo M Fernandez,³

¹London School of Hygiene & Tropical Medicine, ²Centre for Disease Control and Prevention, ³Georgia Institute of Technology
The Yin & Yang of Chemical Detection Technologies

A Chinese concept describing how seemingly opposite concepts interrelate to one another.

Lack of Systematic Comparisons in Terms of Cost/Performance

Chemical content analysis – “Gold Standard”
Laboratory Techniques available at LSHTM

- Colorimetric Screening Test

- HPLC
- Dissolution Testing
- LCMS
**Artemisinin Derivatives Screening Tests**

Tablet of artemether/lumefantrine

Pulverise

Solution in methanol

Presence of artemisinin derivative in tablet results:
- pink colour with the reagent DNP
- a Prussian blue colour with FBS

Test 1

Test 2

Positive tests confirms presence of artemisinin derivative

**Colour Change Reaction on TLC for the Specific Detection of Artemisinin component**

**TWO assays**

1. **DNP**
   - Colour appears after 10 mins
   - Stable for up to 2 weeks
   - **BL** = Methanol
   - **AQ** = Amodiaquine
   - **AS** = Artesunate
   - **AQ/AS** = Combination tablet (50 /150 mg) from Ghana

2. **FBS**
   - Colour appears after 40 mins
   - Stable for up to 2 weeks

**Samples of Coartem 24 (AM/LUM, 20 / 120 mg) – report in Wall St Journal**

Batch no F 2776  F 2605  F 2853  F 2261  F 2261  F 2261
Methods used for the ACTc funded work

Chemical content analysis of >10,000 samples, 142 brands, from 6 countries
Chemical Content Analysis of ACTs

% Active ingredients

HPLC

Degradation studies

Forced ageing at 60°C 0 - 21 days

LC/MS

Artemether – 3.8
Lumefantrine – 4.6
HPLC analysis at LSHTM & CDC

Dihydroartemisinin - 5.2
Artesunate - 4.8
Impurity DHA - 4.3
Artemisinin - 5.5
Artemether - 8.1

Artemether – 3.8
Lumefantrine – 4.6

Fake
Acceptable quality

Fake
Acceptable quality

DHA - 3.097

PIP - 1.98
## Classification of ACTs

<table>
<thead>
<tr>
<th>Drug quality</th>
<th>% Stated API detected</th>
<th>Method used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptable quality</td>
<td>85-115</td>
<td>HPLC &amp; MS &amp; LC/MS</td>
</tr>
<tr>
<td>Falsified</td>
<td>0</td>
<td>HPLC &amp; MS &amp; LC/MS</td>
</tr>
<tr>
<td>Substandard</td>
<td>&lt; 85 - &gt; 115</td>
<td>HPLC &amp; MS &amp; LC/MS</td>
</tr>
<tr>
<td>Degraded</td>
<td>&lt; 85 plus products of degraded API</td>
<td>MS &amp; LC/MS</td>
</tr>
</tbody>
</table>
The Yang: DART-MS AT Georgia Tech

Fragmentation of ion at \( m/z \) 371.31: pattern suggest DEHA or DOA
Nigeria ACT Results: Samples of Acceptable Quality

DART-MS

[Artemether + NH₄]^+  [Lumefantrine + H]^+  
+MS, 4.6-5.5min #(276-328), Background Subtracted

[Dihydroartemisinin + NH₄]^+  [Piperaquine + H]^+  
+MS, 0.8-1.8min #(49-107), Background Subtracted

[Amodiaquine + H]^+  [Artesunate + NH₄]^+  
+MS, 0.5-0.7min #(29-44), Background Subtracted
Nigeria ACT Results: Falsified samples

Ciprofloxacin instead of artemether

DART-MS/MS
Fragmentation pattern indicates ciprofloxacin

NIST MS/MS
Ciprofloxacin

DART-MS
[+MS2(332.1400), 4.4-4.6min #262-272, Background Subtracted]
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**Masters students** - M El Sherbiny, I Fadeyi & I Mamadu

Teams on the ground purchasing and packaging the samples

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More information

www.actconsortium.org/drugquality

http://malaria.lshtm.ac.uk/facilities/analytical-service-measuring-antimalarials-drugs-and-insecticides