

Degradation of Artemisinin-Based Combination Therapies

under Tropical Conditions

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Supplementary Information

Inventory:

- **Table S1** – shows results for anti-malarial assays on degradation products
- **Figure S1** – shows photographs of samples placed in stability chamber and stored in Ghana
- **Figure S2** – shows MS spectra for artemisinin-derivatives and their partner drugs
- **Figure S3** – shows MS spectra for all degradation products observed
- **Figure S4** – shows chemical structures of artemisinin-derivatives and a degradation product of AS
- **Figure S5-S6** – shows % API change over 3 years in tropical conditions for ACTs
- **Appendix** – shows LC-MS results for degraded field samples purchased in Enugu, Nigeria and their packaging

Anti-plasmodial activity assay

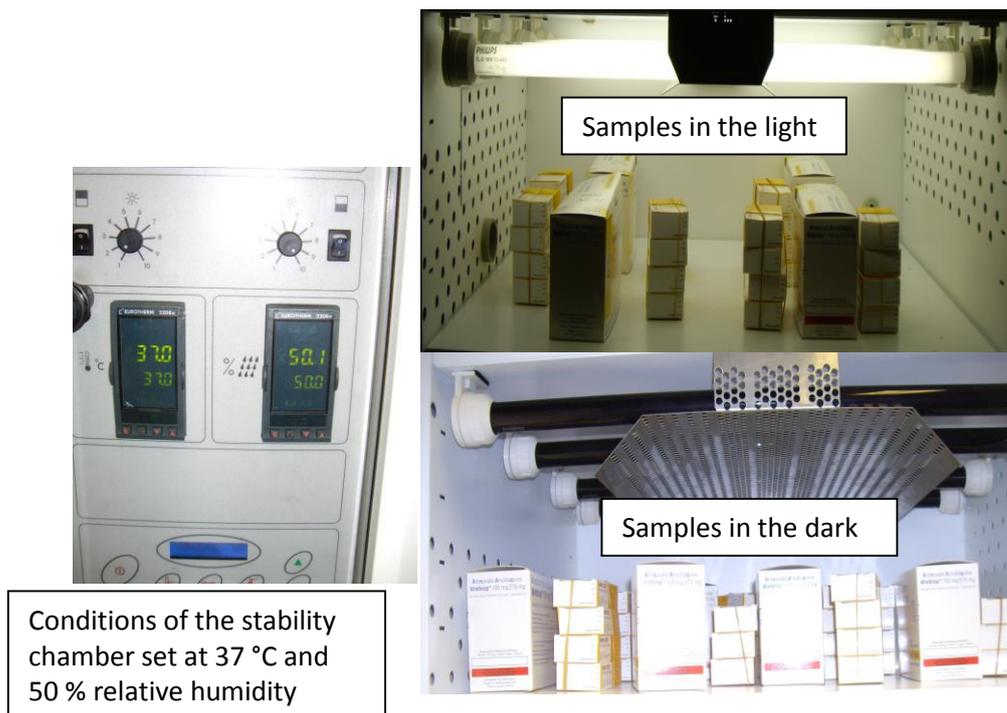
IC₅₀ estimates were obtained for the artemisinin-derivatives and their degradation products for two established parasite lines, HL1210 and HL1204. In addition IC₅₀ values were estimated for AS, AM, and two of the synthesised standards - 9, 10-anhydroartemisinin (epoxy bridge intact) and 2-deoxyartemisinin (epoxy bridge broken; corresponds to D2). Both parasite lines are sensitive to AS and resistant to pyrimethamine. However, HL1204 is sensitive to chloroquine, whereas HL1210 is chloroquine resistant. Therefore AS, chloroquine and pyrimethamine were also tested as internal controls. Each assay was performed in duplicate, using 3-fold dilutions of the test compound to cover a large concentration range. Primary stock solutions for all compounds, with the exception of chloroquine were made by dissolution in dimethyl sulfoxide (DMSO; Sigma Aldrich, St Louis, MO, USA). Subsequent dilutions were carried out in RPMI 1640 growth media (Sigma Aldrich, St Louis, MO, USA). The DMSO present in the test concentration range was of low to negligible toxicity to the parasites. Chloroquine stock solution was prepared using deionized water (Millipore, Watford, UK).

Table S1. IC₅₀ values for anti-plasmodial activity assays on test compounds using two strains of *Plasmodium falciparum*. IC₅₀ values of \approx 10 nM or less are typically required for compounds to be of clinical interest.

Compound	Strain HL1204 (chloroquine sensitive)	Strain HL1210 (chloroquine resistant)
Controls		
Chloroquine	19.8 nM	138 nM
Artesunate	6.6 nM	3.4 nM
Pyrimethamine	12200 nM	9300 nM
Test compounds		
Artesunate	5.2 nM	5.2 nM
Artemether	3.4 nM	3.4 nM
D1*	9036 nM	5103 nM
D2*	2429 nM	1993 nM
D3*	8869 nM	5085 nM
D4*	2759 nM	20900 nM
9,10-anhydroartemisinin	10.6 nM	7.1 nM
2-deoxyartemisinin	Not tested	1099 nM

* used an estimated molecular weight 282 g/mol (artemisinin)

A



B



Figure S1. Representative samples placed in the stability chamber at LSHTM (A) and under typical storage conditions at the Kintampo Research Health Centre, Kintampo, Ghana (B).

Identification of degradation products using 'forced degradation' studies

Mass spectra were examined for the artemisinin-derivatives (Figure S2) and their degradation products (Figure S3), which were consistently observed under conditions of stress testing. Fragile artemisinin-derivative molecules undergo fragmentation in the mass spectrometer. Consequently mass spectra are characterised by complex fragmentation patterns rather than a peak(s) corresponding to the molecular ion (plus adducts). As such the resulting mass spectra can be informative for identification, particularly when compared with known standards.

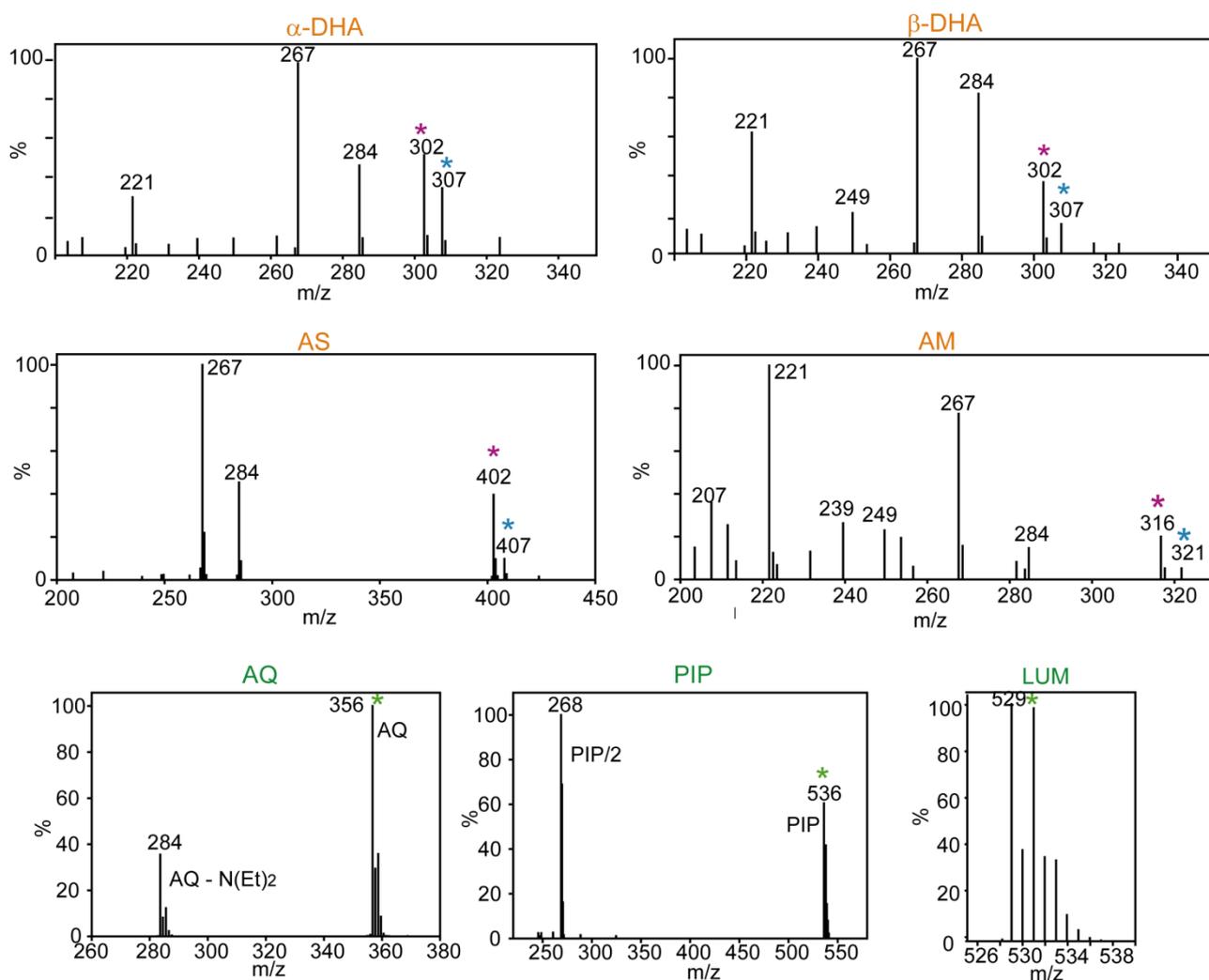


Figure S2. Mass spectra for artemisinin-derivatives (orange text) and their partner drugs (green text). Starred peaks indicate the molecular ion + H⁺ (green), + NH₄⁺ (pink) and + Na⁺ (blue).

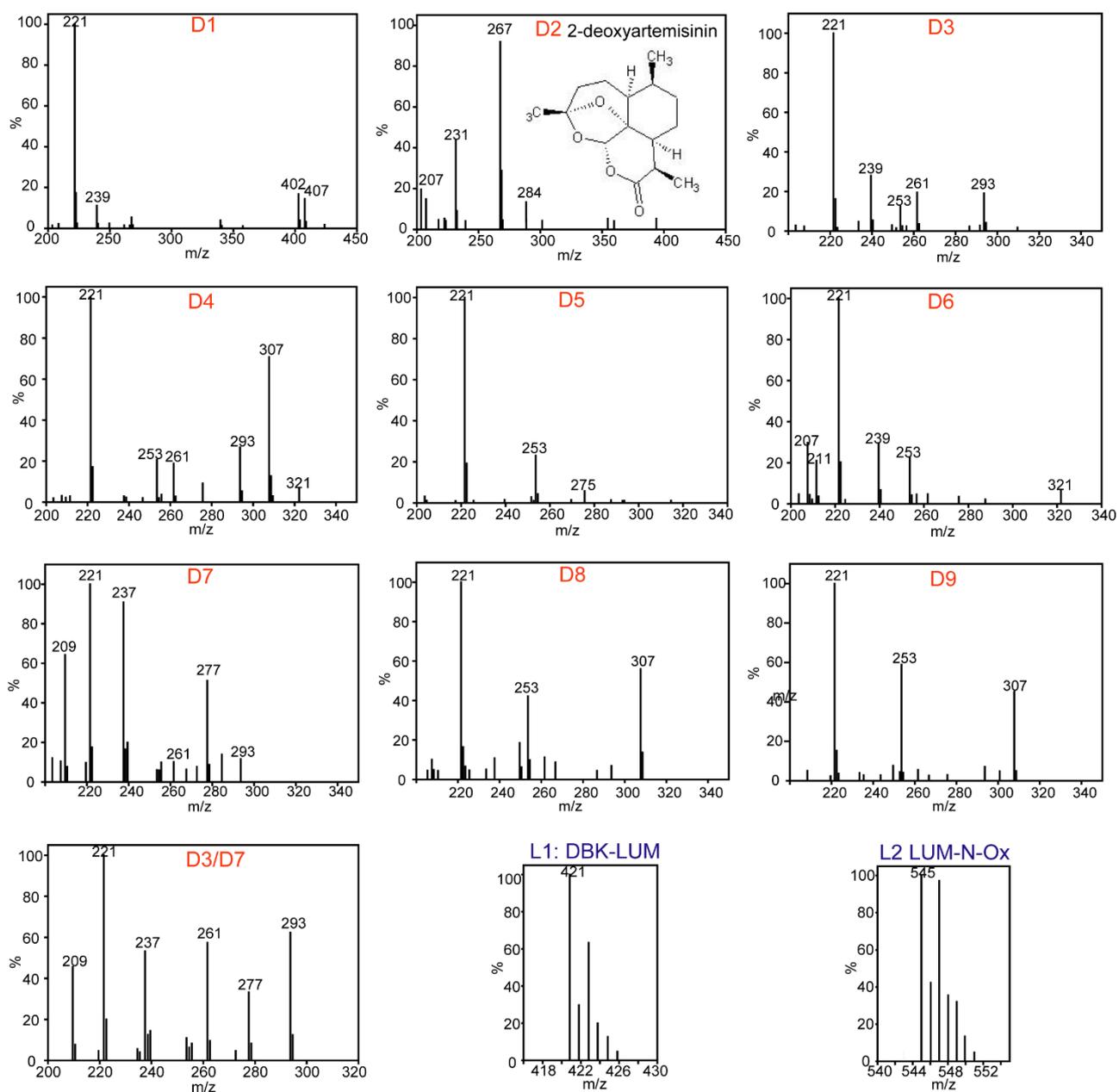
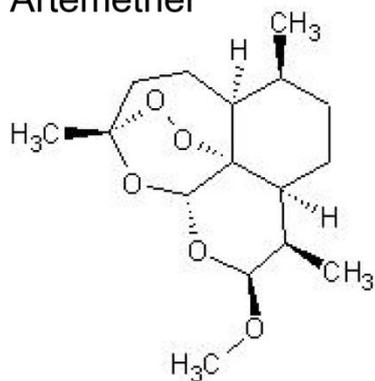
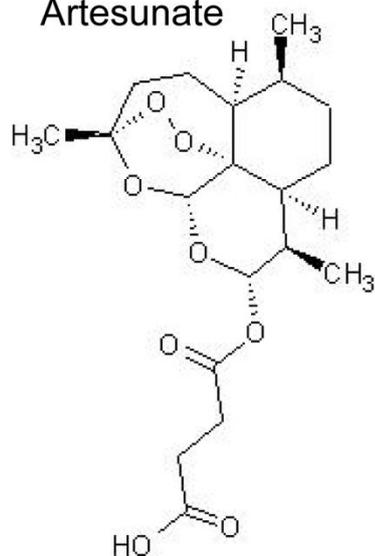


Figure S3. AS/AQ, AM/LUM and DHA/PIP tablets were artificially degraded at 60 °C. The mass spectra for the major degradation products consistently identified are shown. **D1-D2** and **D3-D6** are the major AS and AM degradation products respectively. **D2**, **D3** and **D7-D9** are degradation products of DHA. **L1** and **L2** are desbenzyketo derivative of LUM and LUM-N-oxide respectively, degradation products of LUM. A mass spectrum is also shown for a mixture of **D3/ D7**.

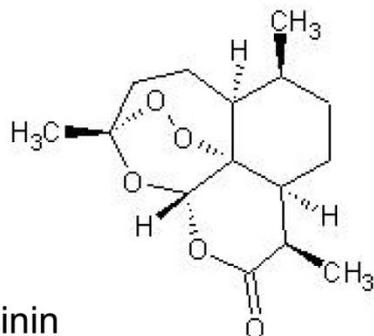
Artemether



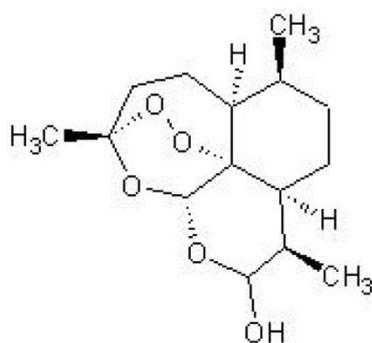
Artesunate



Artemisinin



Dihydroartemisinin



2-deoxyartemisinin

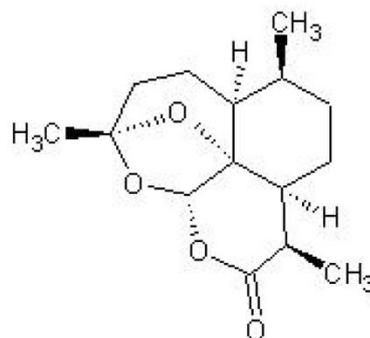


Figure S4. Structures of artemisinin, its therapeutic derivatives including β -artemether, α -artesunate and dihydroartemisinin, and 2-deoxyartemisinin - a degradation product of artesunate and dihydroartemisinin.

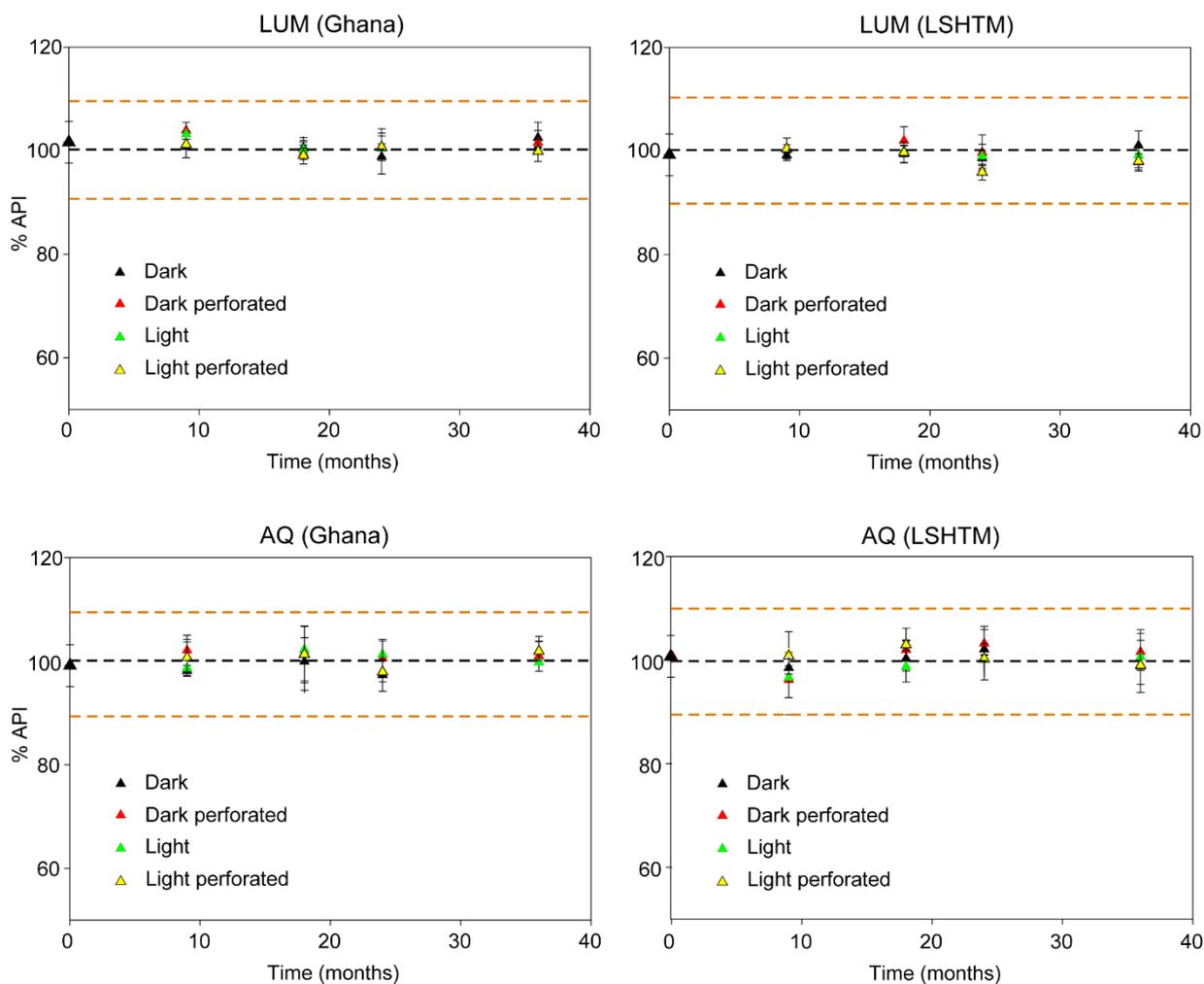


Figure S5. Coartem® AM/LUM and Winthrop® AS/AQ samples were aged for 36 months in a stability chamber (LSHTM) with temperature and humidity settings corresponding to concurrent conditions of a field-site in Kintampo Ghana (Ghana). Samples were periodically removed and % API (LUM and AQ) quantified using HPLC-PDA. Results are shown for tablets stored in the dark or light and with intact or perforated packaging. Error bars show standard deviation (N = 36). Orange dashed lines show acceptable range (90 – 110 % API) according to the International Pharmacopoeia.

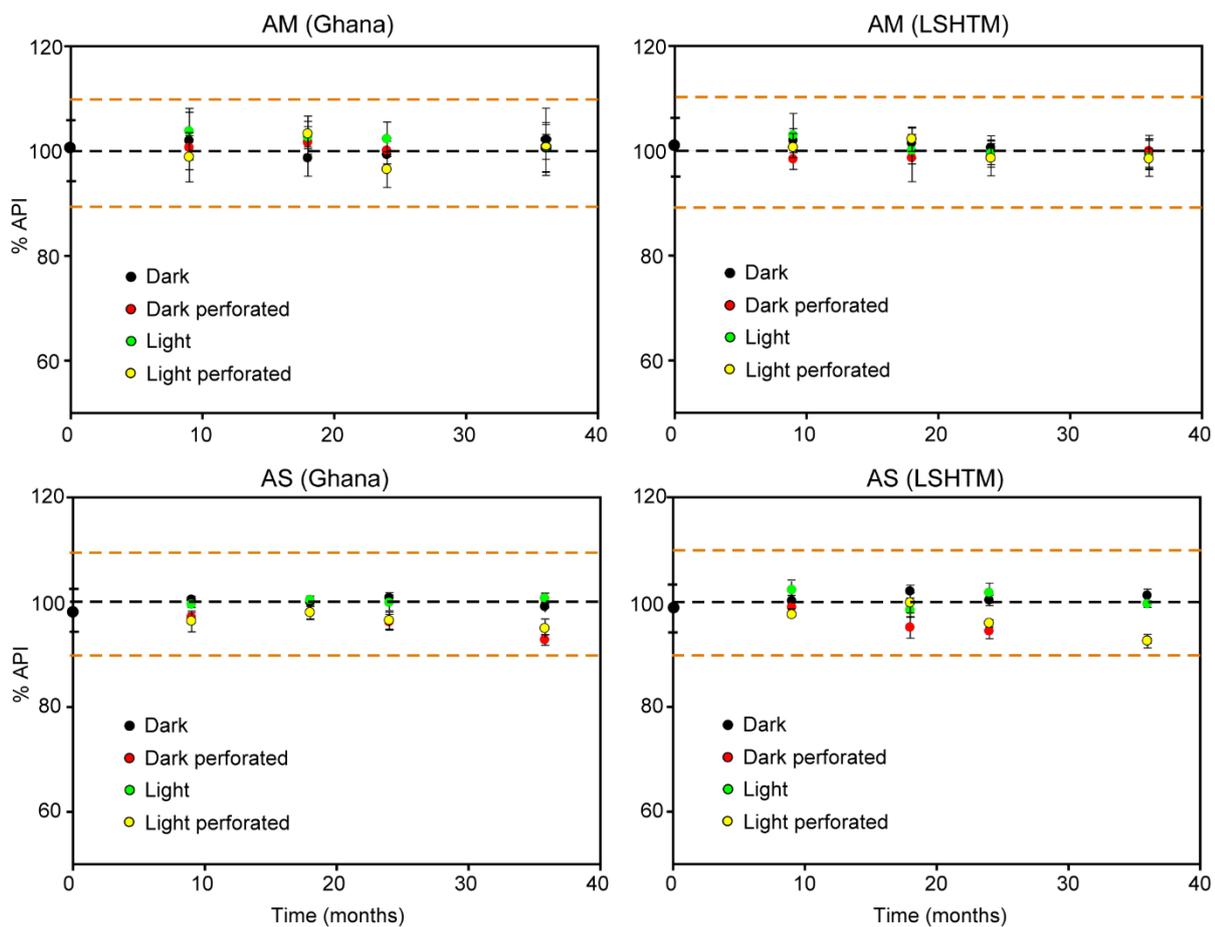
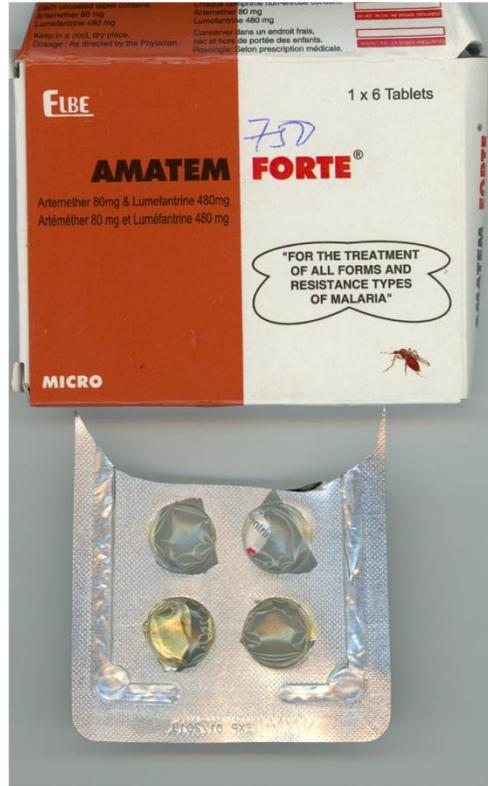
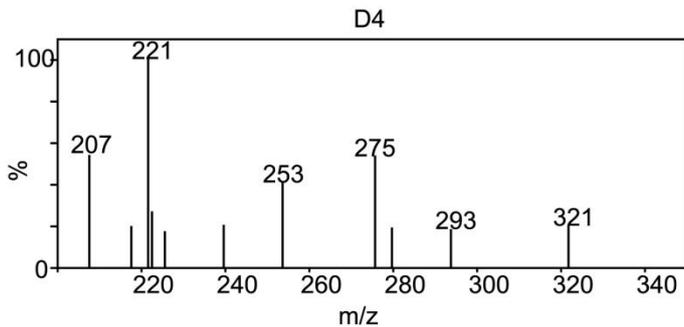
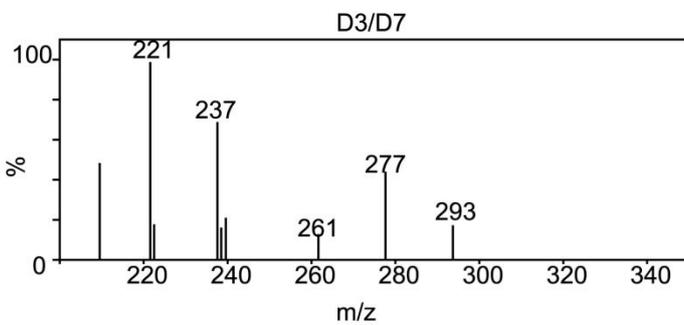
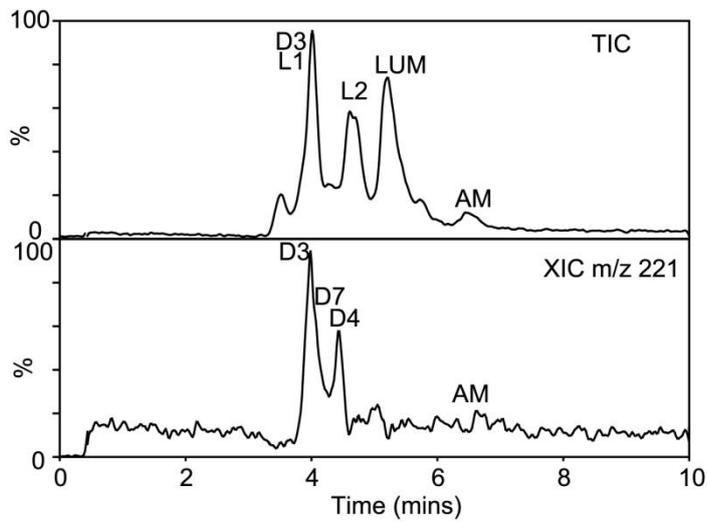
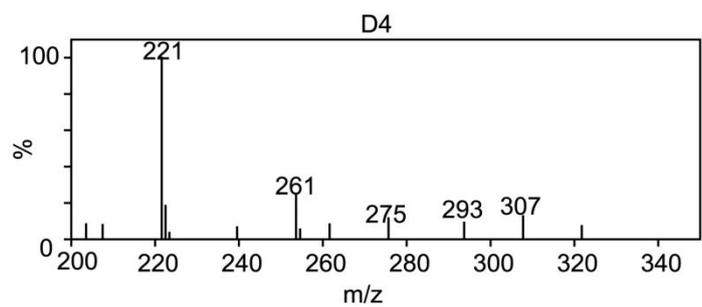
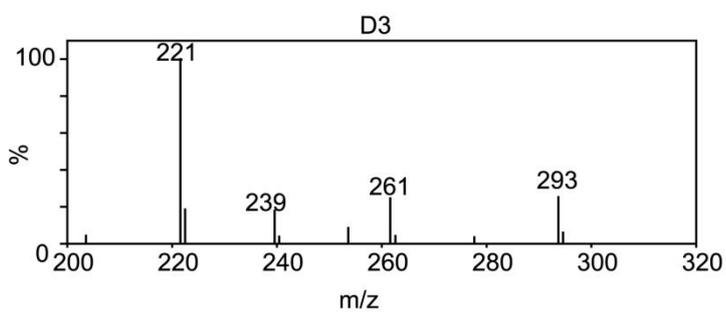
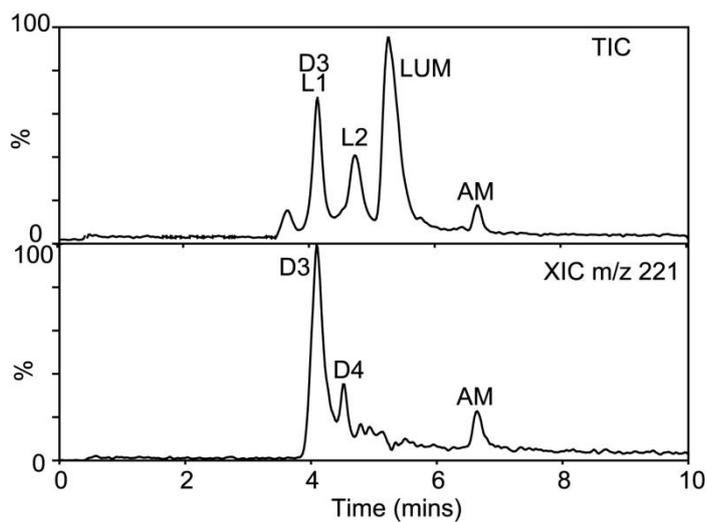


Figure S6. Coartem® AM/LUM and Winthrop® AS/AQ samples were aged for 36 months in a stability chamber (LSHTM) with temperature and humidity settings corresponding to concurrent conditions of a field-site in Kintampo Ghana (Ghana). Samples were periodically removed and % API (AM or AS) quantified using HPLC-PDA. Results are shown for tablets stored in the dark or light and with intact or perforated packaging. Error bars show standard deviation (N = 36). Orange dashed lines show acceptable range (90 – 110 % API) according to the International Pharmacopoeia.

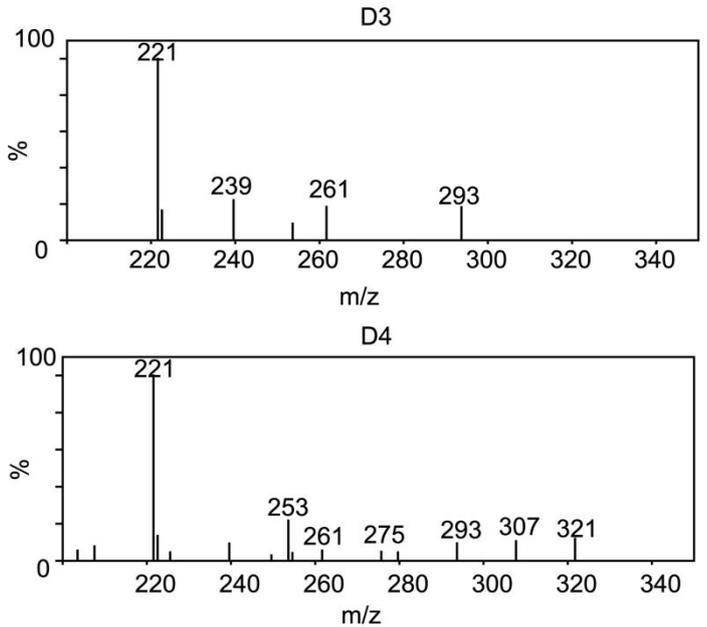
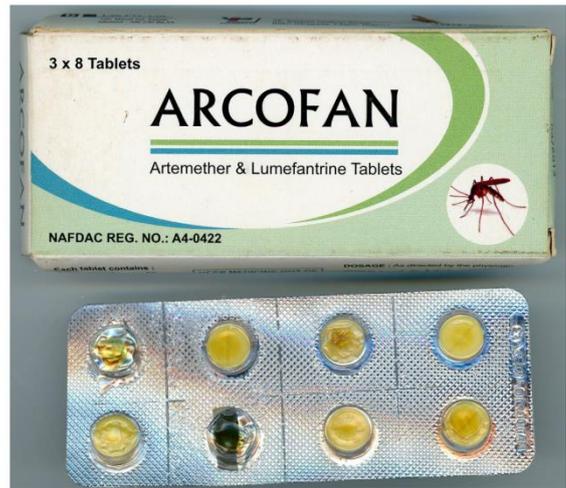
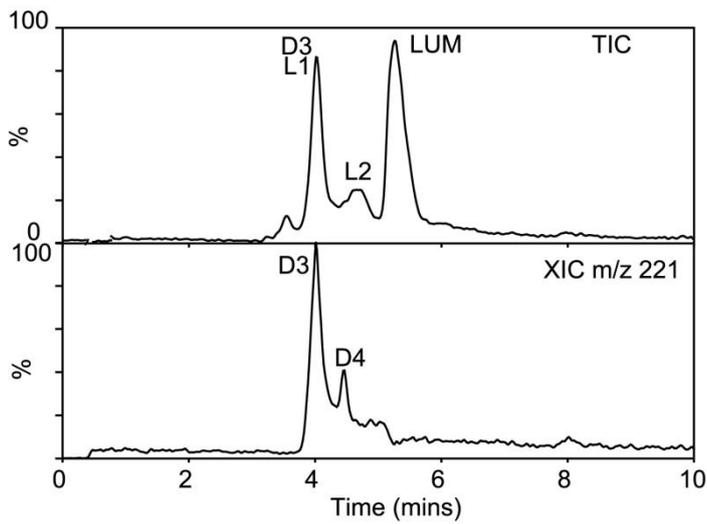
Appendix



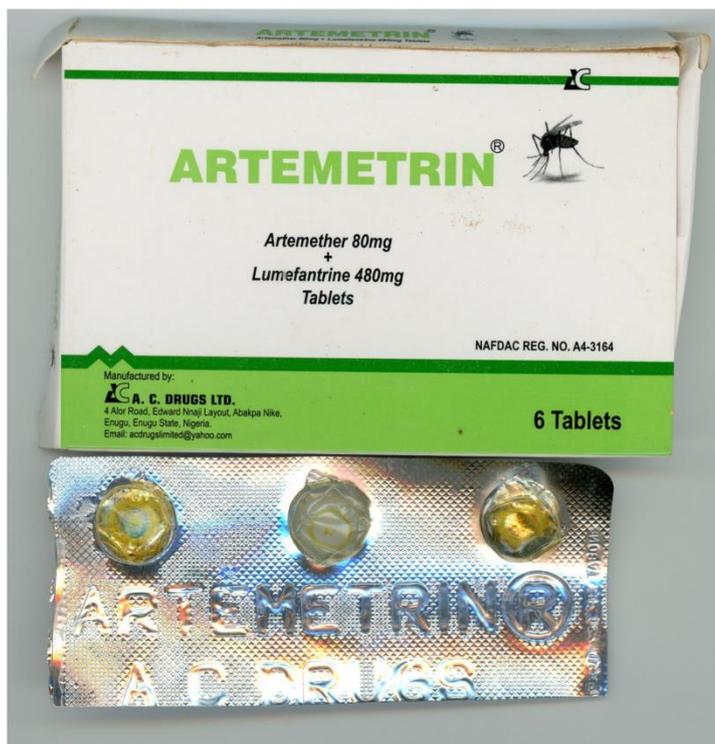
LC-MS analysis of AM/LUM Amatem Forte® tablet reveals similar degradation products to Coartem® AM/LUM tablets artificially degraded. Extracted ion chromatograms for m/z 221 (signature fragment ion) are shown below the total ion chromatogram. Interestingly, a mixture of D3 and D7 (seen previously in DHA forced degradation) was observed, in addition to D4. Examination of the Amatem Forte® packaging revealed a sticky residue on the inside of the blister pack.



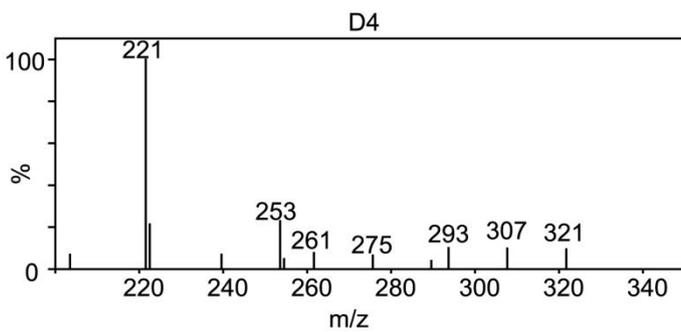
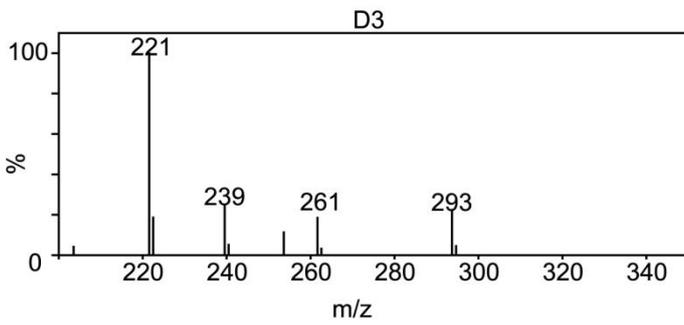
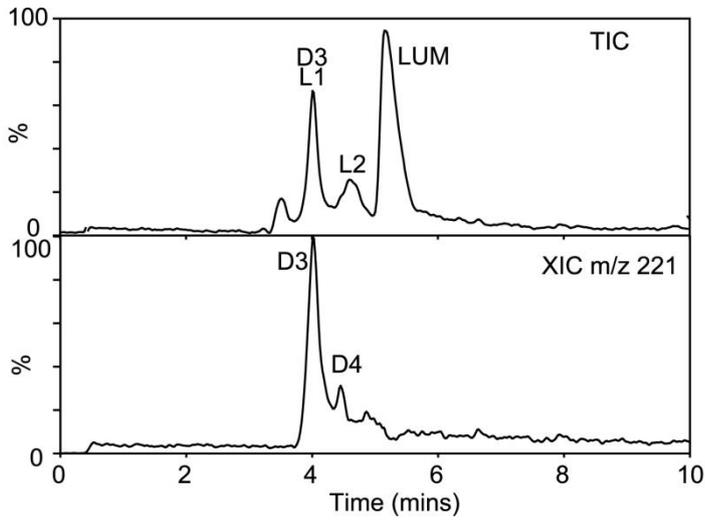
LC-MS analysis of AM/LUM Amatem Tab® reveals similar degradation products to Coartem® AM/LUM tablets artificially degraded. Examination of the packaging revealed a sticky residue on the inside of the blister pack.



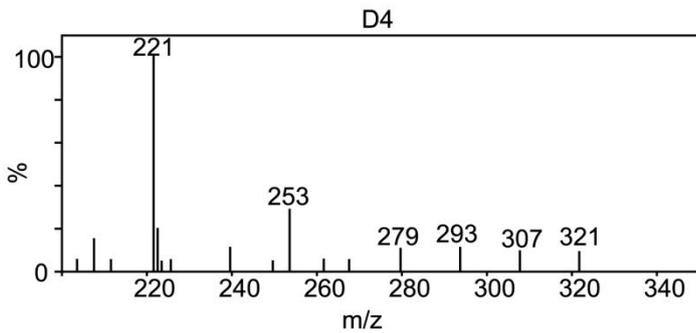
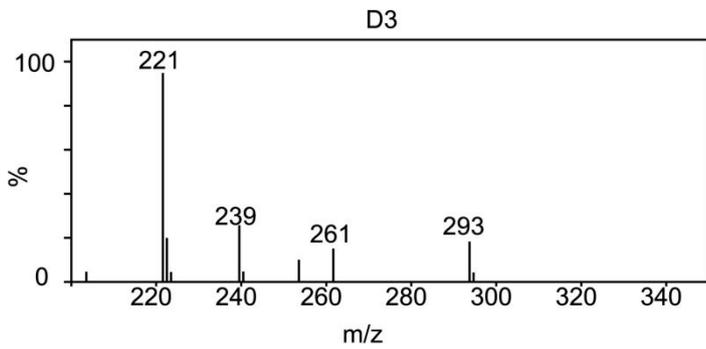
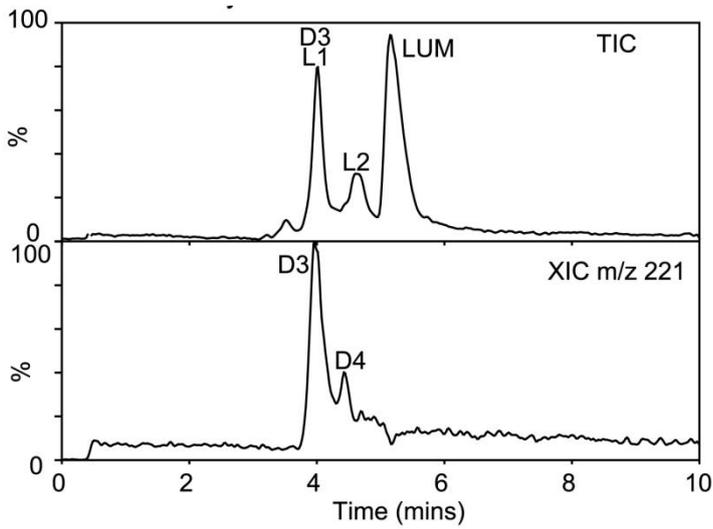
LC-MS analysis of AM/LUM Arcofan tablet reveals similar degradation products to Coartem® AM/LUM tablets artificially degraded. Examination of the packaging revealed a sticky residue on the inside of the blister pack, and tablets were discoloured.



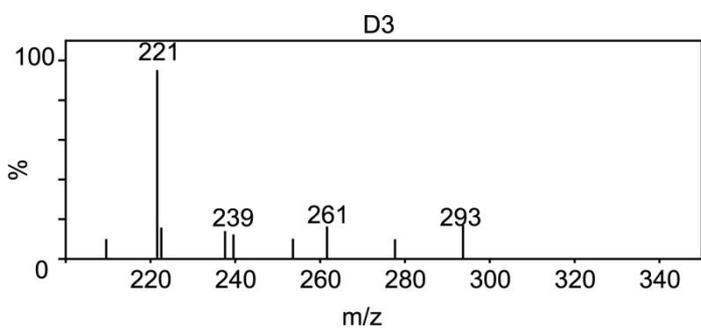
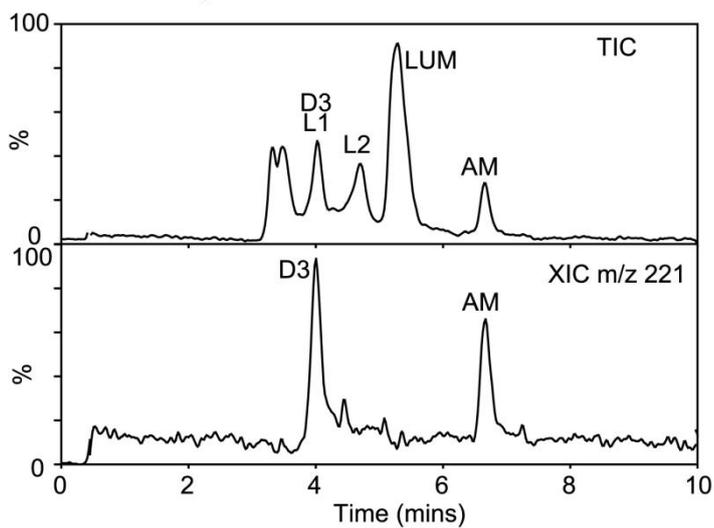
No tablets remained for Artemetrin® and therefore LC-MS analysis was not performed. However the packaging revealed a sticky residue on the inside of the blister pack.



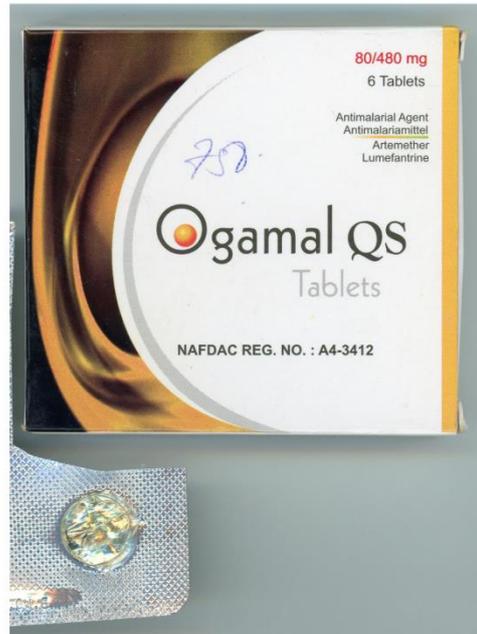
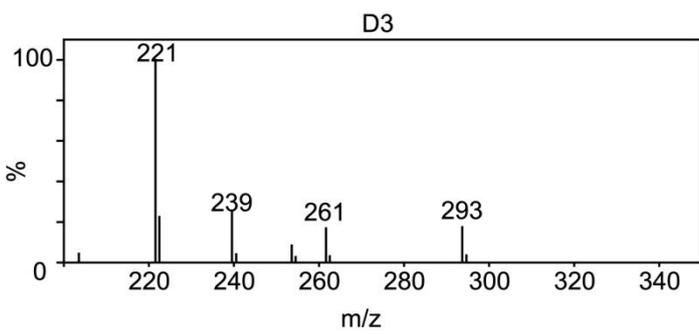
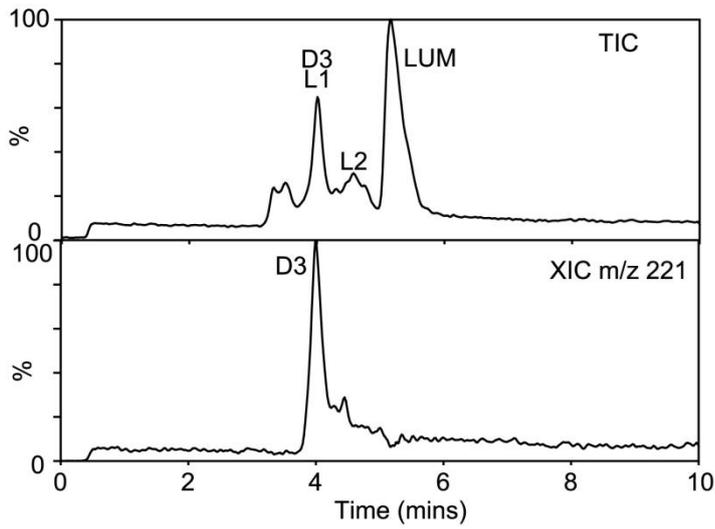
LC-MS analysis of AM/LUM Artrin® tablet reveals similar degradation products to Coartem® AM/LUM tablets artificially degraded. Examination of the packaging revealed a sticky residue on the inside of the blister pack.



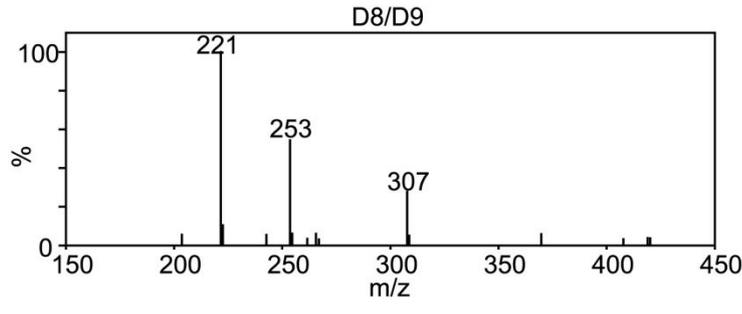
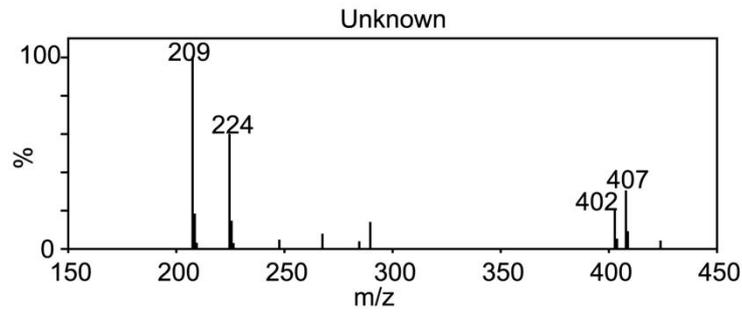
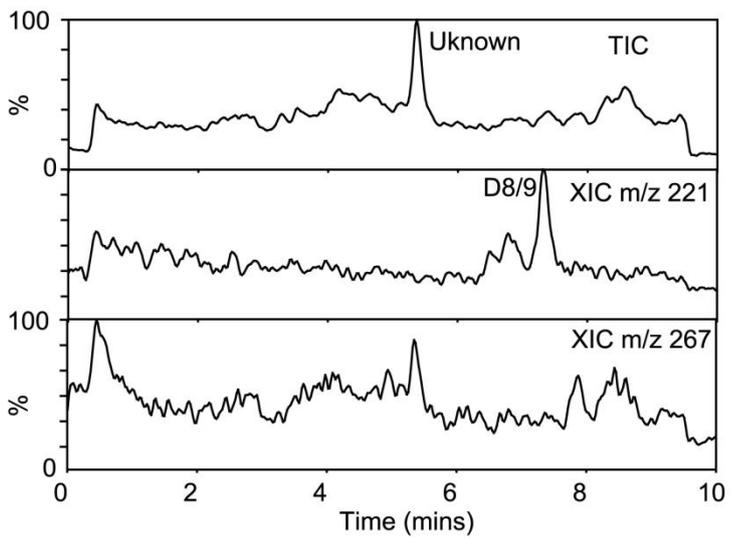
LC-MS analysis of AM/LUM Fynale tablet reveals similar degradation products to Coartem® AM/LUM tablets artificially degraded. Examination of the packaging revealed a sticky residue on the inside of the blister pack.



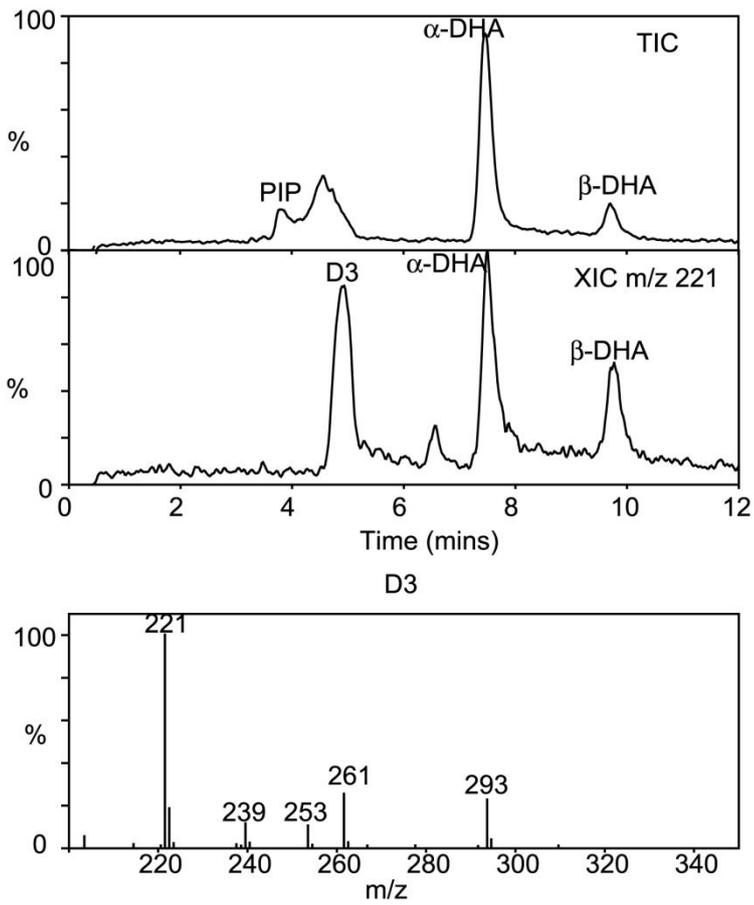
LC-MS analysis of AM/LUM Ogamal tablet reveals similar degradation products to Coartem® AM/LUM tablets artificially degraded. Examination of the packaging revealed a sticky residue on the inside of the blister pack, and tablets were discoloured.



LC-MS analysis of AM/LUM Ogamal QS tablet reveals similar degradation products to Coartem® AM/LUM tablets artificially degraded. Examination of the packaging revealed a sticky residue on the inside of the blister pack, and tablets were discoloured.



LC-MS analysis of AS Maltarka tablet reveals similar degradation products to DHA/PIP tablets (Waipa) artificially degraded (D8/D9) and a previously unseen degradation product. Examination of the packaging revealed a sticky residue on the inside of the blister pack, and tablets were highly discoloured, and brittle.



LC-MS analysis of Droa-Quine® DHA/PIP tablet reveals similar degradation products to DHA/PIP tablets (Waipa) artificially degraded.