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Highlights

- We examined the HPLC-UV relative response factors for degradants of β-artemether.
- A dry heat stress approach under different conditions is proposed.
- A mean relative response factor for β-artemether degradants of 21.2 is obtained.
- Ames testing did not indicate a genotoxic qualification risk for the degradants.

Relative response factor determination of β-artemether degradants by a dry heat stress approach.

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ABSTRACT

During the stability evaluation of β-artemether containing finished drug products, a consistent and disproportional increase in the UV-peak areas of β-artemether degradation products, when compared to the peak area decline of β-artemether itself, was observed. This suggested that the response factors of the formed β-artemether degradants were significantly higher than β-artemether. Dry heat stressing of β-artemether powder, as a single compound, using different temperatures (125 °C – 150 °C), times (10 min – 90 min) and environmental conditions (neutral, $KMnO_4$ and zinc), resulted in the formation of 17 degradants. The vast majority of degradants seen during the long-term and accelerated ICH stability study of the drug product, were also observed here. The obtained stress results allowed the calculation of the overall average relative response factor (RRF) of β-artemether degradants, *i.e.* 21.2, whereas the individual RRF values of the 9 most prominent selected degradants ranged from 4.9 to 42.4. Finally, Ames tests were performed on β-artemether as well as a representative stressed sample mixture, experimentally assessing their mutagenic properties. Both were found to be negative, suggesting no mutagenicity problems of the degradants at high concentrations. Our general approach and specific results solve the developmental quality issue of mass balance during stability studies and the related genotoxicity concerns of the key antimalarial drug β-artemether and its degradants.

38 *Keywords*: β-artemether; relative response factor (RRF); dry heat stress stability; genotoxicity

39 (AMES); stability mass balance

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1. Introduction

In 2009, the World Health Organization (WHO) reported 225 million cases of malaria, caused by the Plasmodium falciparum parasite, leading to approximately 781 000 deaths [1]. Current treatment protocols entail the use of artemisinin-based combination therapy (ACT), in which one of the active components is an artemisinin derivative, mostly β-artemether [2]. This ACT strategy improves treatment outcome and reduces Plasmodium falciparum resistance.

Artemisinin is extracted from the herbal plant *Artemisia annua*, and its derivatives, *e.g.* dihydroartemisinin (DHA) and β-artemether, are obtained using one or two synthetic steps [3]. The structures of β-artemether, DHA and artemisinin can be seen in Figure 1. All three compounds have an endoperoxide moiety, which is essential for the anti-malarial activity [4]. β-artemether can be seen as a prodrug form of DHA, with addition of an O-methyl ether group in beta position. In-vivo, β-artemether is metabolized to DHA, both having similar parasiticidal activity, which is higher than that of the artemisinin parent compound [5]. β-artemether, due to its low water solubility properties, is currently administered as a tablet, oral suspension or as an oily intramuscular injection. As these finished pharmaceutical products (FPP) are used in regions characterized by relatively high temperatures and humidity (climate zones III and IV, as defined by WHO), accurate knowledge regarding short-, mid- and long-term stability of β-artemether is crucial. However, until now, only β-artemether assay methods and results have been reported, with no comprehensive details about its degradants [6-9]. Several of the artemisinin derivates, in particular dihydroartemisinin are prone to temperature induced degradation [10]. Knowledge of degradants is of great importance not, just from a pharmaceutical and regulatory point but also from a clinical and safety point of view.

Therefore, these FPP were stored in ICH-compliant climate controlled storage cabinets and periodically analyzed for β-artemether assay and degradant levels by a stability-indicating ultra-high pressure liquid chromatography method coupled to ultra-violet diode array detection (UPLC-UV/DAD). The stability samples were characterized by a consistent and disproportional increase in the peak areas of β-artemether degradation products, when compared to the peak area decline of β-artemether itself, making peak area balance as currently requested by the pharmaceutical regulatory authorities impossible. Without the proper knowledge of the response factors (RF) of these unknown degradation compounds, this led to a peak area mass balancing problem [11]. In addition, in view of the number and peak areas of degradant peaks, mutagenic risks are to be evaluated [12]. Both aspects are to be solved as quickly and efficiently possible in early development of drugs, *i.e.* without complete isolation, identification and synthesis of each of the observed degradation impurity peaks.

This problem was efficiently approached by short-term stressing β-artemether in its dry form, as a single compound, under various time, temperature and environmental conditions. Since all the degradant peaks formed were directly related to β-artemether, absence of placebo peaks could be ascertained. Using a mathematical approach, the corresponding RF could be calculated, thus solving the peak area mass balance question. Further analysis permitted to obtain evidence that the mutagenic potential of the degradants was absent and hence solved a major issue in the β-artemether impurity qualification.

2. Materials and methods

2.1 Materials

Acetonitrile (LC-MS grade) was purchased from Fisher Scientific (Aalst, Belgium). Water was purified using an Arium 611 purification system (Sartorius, Gottingen, Germany) yielding ≥ 18.2 MΩ.cm quality water. Monobasic potassium phosphate was bought from Sigma Aldrich (Bornem, Belgium), potassium permanganate and zinc powder were obtained from Flandria (Ghent, Belgium) and Janssen Chimica (Beerse, Belgium), respectively. β-artemether, drug substance and FPP, as well as related compounds, *i.e.* dihydroartemisinin (DHA), artemisinin, 9,10-anhydroartemisinin (LEI, Late Eluting Impurity) and β-artemether, were gifts from Dafra Pharma (Turnhout, Belgium). Synthesis impurities 9-epi-artemisinin and artemisitene were purchased from SensaPharm (Wearfield, UK). The degradation compound 2-[4-methyl-2-oxo-3-(3-oxobutyl)cyclohexyl]propanal (DKA) was obtained from Sigma Tau (Rome, Italy).

2.2 Liquid chromatography

The UPLC apparatus, implemented for quantification of β-artemether and its degradants, consisted of a Waters Acquity H UPLC Class Quaternary Solvent Manager, a Waters Acquity Sample Manager, combined with a Flow Through Needle, and a Waters Acquity Ultra Performance LC PDA detector with Empower 2 software for data acquisition (all Waters, Milford, MA, USA). An Acquity UPLC BEH Shield RP 18 (100 mm × 2.1 mm I.D., 1.7 µm particle size) column (Waters, Zellik, Belgium), thermostated in an oven set at 30 °C, was used. The flow rate was set at 0.6 ml/min and a linear gradient was applied (where A = 25 mM phosphate buffer, adjusted to pH 2.5 using diluted sodium hydroxide, and B = acetonitrile), running from 30 to 75% B from 0 to 12.5 min, followed by returning to the initial conditions and re-equilibration. The sample compartment was thermostated at 5 °C and UV detection was performed at 210 nm. The injection volume used was 1.7 µl. The reporting

threshold for the dry heat stressed samples (see section 2.3) was set at 5% peak area relative to unstressed β-artemether.

2.3 β-artemether dry heat stress samples

β-artemether, in its native dry powder form, was exposed to various high temperatures. In addition, the influence of an oxidizing and reductive environment on its dry heat-induced degradation profile was also evaluated by mixing β-artemether (3 parts) with potassium permanganate and zinc, respectively (1 part), using mortar and pestle, thus obtaining two different powder mixtures [13].

The powders were accurately weighed (40 mg β-artemether) and transferred into separate glass vials (12×32 mm, Borosilicate, Type 1, Class A glass), which were then incubated in a preheated heating block (Stuart, Stone, United Kingdom). Temperature settings varied from 125 to 150 °C, with incubation times ranging from 10 to 90 minutes, depending on the β-artemether powder composition. After incubation, the stressed samples were immediately placed on ice, in order to prevent further degradation. Unstressed β-artemether samples, *i.e.* not incubated in the heating block, were also stored in ice to guarantee identical treatment.

The contents of the unstressed and stressed vials were solubilized with acetonitrile. The resulting solution was transferred quantitatively into a 10 ml volumetric flask and diluted to volume using acetonitrile. An aliquot was transferred into a HPLC vial and analyzed (see Section 2.2).

2.4 UPLC profiling of degradation impurities

The degree of β-artemether degradation was calculated from the residual β-artemether peak area after heat stress (see section 2.3). Experiments yielding between 10 to 90% β-artemether degradation were withheld for further calculations, *i.e.* for the calculation of average and individual relative response factors (RRF) of the β-artemether degradants. Those stress conditions with extreme (*i.e.* > 90%) or very limited (*i.e.* <10%) degradation were not withheld due to their irrelevance for our problem. Due to the huge number of relatively small and/or infrequently observed peaks in the withheld chromatograms, the selection of the β-artemether degradants for the calculation of their individual RRF was limited to the most important peaks (see Supplementary Information).

2.5 Calculation of average relative response factor of β-artemether degradation products

The residual β-artemether amount present in the stress samples, expressed in mass units, was calculated from the peak area in the chromatogram. The total mass-amount of formed degradants (= mass amount degradation) was then deduced by subtracting the calculated residual β-artemether amount from the originally weighed β-artemether amount present in the stress samples. The average RRF value for each stress condition, *i.e.* temperature, incubation time and environment, is calculated using following formula:

$$\text{Average RRF per experiment} = \frac{\text{Residual } \beta\text{-artemether (mg)} \times \text{degradation peak areas}}{\text{Residual } \beta\text{-artemether peak area} \times \text{mass amount degradants (mg)}}$$

2.6 Calculation of individual relative response factor of selected β-artemether degradation products

Selection of experiments and degradant peaks, of which the individual RRF values were calculated, was performed as described in Supplementary Information. The following model was used to calculate the individual response values of the selected degradants in Matlab:

Mass amount degradants (mg) = $\sum \frac{AU_{degradant\ i}}{RF_{degradant\ i}}$

with $AU_{degradant\ i}$ the experimentally obtained peak area units and $RF_{degradant\ i}$ the unknown RF expressed in area units per mg.

The mass amount degradants (in mg) is calculated as described in section 2.5. As the individual RRF values of the most important unidentified degradation peaks are calculated, some small degradation peaks and previously identified peaks are ignored. In order to obtain a more precise estimate of the unknown RRF values, a correction is made for these ignored peaks. Moreover, additional correction is made for minor mass losses, due to formation of volatile components, *e.g.* CO_2, by comparing the weight before and after dry heat stress (see Supplementary Information for more details about these corrections). The calculated individual RF of the degradants are transformed to their respective RRF values by dividing with the β-artemether RF, obtained from the unstressed samples.

2.7 Real life β-artemether stability samples

The β-artemether degradant profiles, obtained during the various dry heat stress conditions, are compared with the profiles seen in accelerated and long-term ICH stability samples of β-artemether finished pharmaceutical drug products (FPP). For this, a 80 mg/ml arachis oil stability sample, stored at 40°C / 75 % R.H. for 1 year, was analyzed using the described

UPLC method. The oily formulation for intramuscular injection first underwent a sample preparation step in which β-artemether and degradants were quantitatively extracted to acetonitrile. This acetonitrile phase is subsequently injected into the UPLC system.

2.8 Mutagenicity evaluation of the β-artemether degradation products

The mutagenic potential of β-artemether and its degradation products were evaluated *in-vitro*, using the Ames bacterial reverse mutation test [14-19]. This Ames test evaluates the ability of test compounds to induce reverse mutations in the histidine gene-deficient *Salmonella typhimurium* strains TA98, TA100, TA102, TA1537 and TA1535. The tester strains were obtained from Molecular Toxicology Inc. (Moltox, USA). The test was performed both in the presence and absence of rat liver post-mitochondrial fraction (S9 homogenate), thus taking into account metabolic activation pathways. A number of positive, mutagenic control compounds, *i.e.* 2-nitrofluorene, 9-aminoacridine, sodium azide, 2-aminoanthracene and 4-nitroquinoline-N-oxide, were included to assure appropriate responsiveness of the test system.

Prior to testing, the unstressed and dry heat stressed (145°C – 30 min, converting approximately 70% β-artemether to degradants) β-artemether samples were dissolved in DMSO. Seven different concentrations, ranging from 78.13 to 5000 µg/plate, vehicle controls and positive controls were plated in triplicate using the plate incorporation method. The following solutions were successively added to 2 ml histidine-biotin supplemented top agar: 0.1 ml of an overnight bacterial culture of the tester strain, 0.1 ml of a dilution of the test item, vehicle control or positive control and either 0.5 ml of S9-mix containing 100 µl S9/ml (50 µl S9/plate) for the activation portion, or 0.5 ml phosphate buffer for the non-activation portion. The content of the tube was then mixed and poured onto minimal glucose agar plates. The

plates were incubated in the dark at 37°C for 48 to 72 hours, after which they were counted automatically using a colony counter (Sorcerer 2.2., Perceptive Instruments, UK). A test item was considered positive (mutagenic) if the test item produced a twofold increase in the mean number of revertants with one of the strains TA98, TA102 or TA100, or a threefold increase in the mean number of revertants with one of the strains TA1535 or TA1537 at one or more concentration levels in comparison to the mean concurrent vehicle control value and a concentration-related effect was observed.

3. Results

3.1 Average relative response factor of β-artemether degradation products

An overlay chromatogram, displaying various experimental conditions is given in Supplementary Information Fig. S-I. In total, 21 experiments yielded between 10% and 90% β-artemether degradation, in which 17 different peaks were observed at least once above the reporting threshold of 5%. Figure 2 depicts typical UV (210 nm) chromatograms obtained from a dry heat stressed sample (145 °C – 30 min) and the unstressed reference sample, both at identical mass concentrations. From visual inspection, it is already clear that the average RRF values of the β-artemether degradants will be significantly higher than 1. The average RRFs of the β-artemether degradants in the $KMnO_4$, dry heat and zinc stressed samples were calculated to be 12.7, 26.4 and 23.6, respectively, with an overall RRF of 21.2.

3.2 Calculation of individual relative response factor of β-artemether degradation products

When applying the peak selection model (see Supplementary Information), the 9 most prominent peaks are withheld (see degradant 1 to 9 in Figure 2), whereas the data matrix used for the individual RRF calculations consisted of 21 experimental results, obtained under different temperature-time-environmental stress conditions. This number of degradants correlates well with the typical number given by Baertschi, *i.e.* an average of 8.2 major degradation compounds per drug [20]. The DAD-UV spectra of the 9 degradant peaks considered, as well as of β-artemether, are given in Supplementary Information Fig. S-II.

Within two sets of degradation peaks, *i.e.* set 1: degradants 2, 4, 6 and 7; set 2: degradants 5 and 8, a very high peak area correlation over the 21 experiments ($R>0.90$) was seen, and were therefore combined (degradants 2, 4, 6, 7; degradants 5 and 8), acknowledging that the resulting individual RRF value will be an average for each of the two sets of degradants. Using Matlab software, and applying RF boundaries of 15'000 to 10'000'000 area units/mg, the model was evaluated by minimizing the sum of residual squares. To include the experimental variability, numerical sampling was performed, taking n=1000 randomized samples using Gaussian distribution with sigma 1% and within 5% boundaries around the obtained experimental peak-area values. The model estimated (± std dev) RF values are given in Table 1: they range between 4.4×10^4 to 3.8×10^5 area units/mg, which is much higher than the RF of β-artemether (8.9×10^3 area units/mg), leading to RRF-values as high as 42.4. The results obtained by this approach provided good model fitness with a correlation of 0.81 between the calculated and experimental residual mass expressed as β-artemether (Figure 3).

Traditional determination of the RRF of unknown impurities, *i.e.* degradation products from a known parent compound, encompasses several time- and cost-intensive steps. First, the structural identity of the impurity must be determined, which can be accomplished using different strategies, *e.g.* HPLC coupled to MS is most often used to propose a tentative structure. Alternatively and/or subsequently, preparative impurity isolation is used for further

structural elucidation and/or confirmation by spectral techniques, *e.g.* NMR and IR. Finally, the impurity must be manufactured before being co-injected with the parent compound into the chromatographic system. The RRF of the impurity is then calculated by comparing the chromatographic responses, corrected for any concentration differences, of both impurity and parent compound. Alternatively, the RRF of the impurity can be derived from the slopes of the peak area – concentration regression curves of impurity and parent compound, after injecting a dilution series of both [21-24]. The use of alternative, universal detection systems such as refractive index detection (RID), chemiluminescent nitrogen detection (CLND), nebulize-based detectors like evaporative light scattering detection (ELSD) or charged aerosol detection (CAD) also allow in principle direct calculation of the RRF values, without the need for isolation steps. However, these detectors are not always available and each of these universal detectors has its own limitations and disadvantages compared to UV. RID exhibits a rather low sensitivity and is not only sensitive to temperature and flow change, but is also incompatible with gradient elution. The absence of nitrogen atoms in the structure of β-artemether renders the CLND technique useless, as the response is directly proportional to the nitrogen content [25-27]. The ELSD and CAD techniques record only non- or semi-volatile compounds after evaporation of column eluent, often with inherent sensor noise and drift, being sensitive to the mobile phase composition [28-30].

However, when using our stress approach, an estimation of the RRF of β-artemether degradants can be obtained, without having to perform elaborate, expensive and time consuming experiments. Moreover, the β-artemether related degradation impurities are unstable during the MS ionization process, leading to characteristic in-source fragments of m/z 163.2, 221.1 and 238.9, without structural differentiation, so that identification by LC-MS is far from trivial. The construction of the matrix model in which a series of equations, *i.e.* stress experiments, is used to calculate a series of unknowns, *i.e.* the response factors of the

different degradants. The calculated RF values are subsequently transformed into corresponding RRF values by dividing by the RF value of β-artemether.

3.3 Real life β-artemether stability samples

Figure 4 depicts an overlay chromatogram of a dry heat stress β-artemether sample (145°C during 30 min) and the chromatogram obtained from the 80 mg/ml arachis oil stability sample. From this, it is clear that the vast majority of degradants seen in this stability sample were also seen in the dry heat stress samples. An overview of all previously identified β-artemether related degradants and synthesis impurities, together with their RRF, seen in stability samples of different oily FPP, is given in Table S-I. This overview clearly shows that certain β-artemether degradants or synthesis impurities are characterized by RRF significantly larger than 1, and none with a RRF lower than 1.

Accurate knowledge regarding the response factors of formed degradants is vital for peak area mass-balancing during stability studies. In general, related degradants have response factors similar to the native molecule, *i.e.* between 0.8 and 1.2 and hence no RRF correction is required [31]. However, this is clearly not the case for the β-artemether degradants. Instead of the elaborate isolation of each degradant, or the simultaneous use of universal detectors with their uncertainty of variable response [11, 25] we have solved this question of negative mass balance deficit using a new stress approach, performed in different time (minutes) and temperature (125 °C – 150 °C) space compared to current pharmaceutical stress conditions [32-34].

3.4 Mutagenicity evaluation of the formed β-artemether degradation products

The β-artemether and dry heat stressed (145 °C for 30 min) samples did not induce a concentration-related and biologically significant increase (≥ 2- or 3-fold) in the number of revertant colonies above the concurrent vehicle control with the Salmonella typhimurium strains TA98, TA1537, TA100, TA1535 and TA102 in the absence and in the presence of S9-mix (50 µl S9/plate) up to the maximum test concentration of 5000 µg/plate (see Supplementary Table S-I).

4. Discussion

In this study, it was demonstrated for the first time that β–artemether related degradation products have a significantly larger RF, when compared to β–artemether itself. Recently, Stringham et al., reported an RRF range for the synthesis impurity artemisitene, *i.e.* 37 to 43 [8], which was confirmed under our experimental conditions (RRF 40 ± 4). Artemisitene is a synthesis impurity found in artemisinin, which is a starting material for β–artemether. Artemisitene is structurally very similar to artemisinin (see Table S-II), differing in one extra double bond resulting in a conjugation with the keto function, giving a hyperchromic shift. Our findings indicate that also degradation products can result in significant changes in UV-response factors of the formed degradants, causing regulatory mass-balance issues, especially in early development phases. Moreover, the RRF-values are also important in designing fractionation and isolation experiments by semi-preparative chromatography to estimate the masses of the impurities that will be obtained for further spectroscopic characterization. In our approach, two assumptions were made: (i) peaks below the stress reporting threshold do not significantly contribute to the total mass of degradants; (ii) all β-artemether degradation products are separated, eluted and UV-absorbing at 210 nm. Furthermore, retention of degradation compound on the UPLC system and column is considered to be negligible: (i)

semi-quantitative normal-phase TLC of representative dry heat stress samples, according to International Pharmacopoeia and United States Pharmacopeia Salmous [35,36], did not result in observable spots in the frontline of the TLC; (ii) initial UPLC experiments with extensive gradient followed by isocratic elution at high solvent strength, *i.e.* 95% acetonitrile for 5 minutes, did not reveal any strongly retained components; (iii) direct UV comparison at 210 nm of three representative stress samples (ranging from 30 to 70% β-artemether degradation) with the β-artemether unstressed sample revealed similar ratios as obtained by the UPLC method, herein described. The average value when comparing the ratios obtained by UPLC *vs.* the direct UV measurement ratios was calculated to be 96.53%.

The ICH guidelines, legally incorporated in e.g. the European Pharmacopoeia, demand qualification of degradants when above the qualification threshold (QT) of 0.15% or 1.0 mg, whichever is the lowest (maximum daily dose < 2 g), after correction with their corresponding RRF [37]. The In-vitro AMES test is mutagenic discriminatory when degradants are present at minimum 250 µg/plate concentration [38]. Our results demonstrate the lack of mutagenic properties in both β-artemether and its most prominent degradants, *i.e.* degradants 1, 4, 6, 7, 9 and DKA (see Supplementary Information for calculation). It should be pointed out that current EMA guidance for formal qualification of impurities in new drug products requires additional tests, preferably with the isolated specified impurities: (i) determination of reporting, identification and qualification limits, based on the maximum daily dose, (ii) *in-vitro* testing for genotoxicty, (iii) in-vitro toxicity study on a rodent model, lasting 14 to 90 days [39]. Nevertheless, our initial AMES screening results do not immediately raise major mutagenicity concerns for the degradants.

In stability samples of an oily β-artemether finished drug product (FDP) (80 mg/ml stored at 30°C / 65% R.H. for 2 years), the DKA assay after RRF correction, was above the 0.15% qualification threshold, thus requiring a qualification procedure as stipulated by the ICH

guideline. Other degradation products, *e.g.* LEI, were initially clearly observed above the 0.15% threshold, suggesting the need for similar qualification. However, after correcting the observed peak area with the corresponding RRF value, the LEI assay was calculated to be even below the reporting threshold (RT). These two practical examples also emphasize the importance of this work, in which a fast and efficient method is presented allowing accurate estimation of the RRF values of a set of degradation products combined with an *in-vitro* mutagenicity evaluation of the known and unknown degradants.

5. Conclusions

Until now, peak area mass-balancing during stability studies of β-artemether formulation could not be achieved. Therefore, β-artemether in its native powder form was subjected to a series of dry stress experiments, using different temperature, time and environmental conditions. Subsequently, the average and individual RRF value of the degradants were calculated. A peak selection model was used to ensure that the RRF values of the largest degradation peaks, *i.e.* thus skewing the peak area balance the most, were calculated. The average RRF values per stress condition ranged between 12.7 and 26.4, whereas individual RRF values ranged between 4.9 and 42.4. These values can thus be used in the development of pharmaceutical products to correct the experimentally observed degradation peak areas to mass units, without having to perform time and cost expensive peak isolation steps. Moreover, mutagenic evaluation of β-artemether and its degradants at high concentration by the Ames bacterial reverse mutation test could not demonstrate any mutagenic properties.

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Figure captions

Fig 1. Structure of artemisinin, DHA and β-artemether (left to right)

Fig 2. Overlay chromatogram of a dry heat (145 °C – 30 min) stressed sample (red) and unstressed reference sample (blue)

Fig 3. Correlation between experimental and model-derived amounts of formed impurities

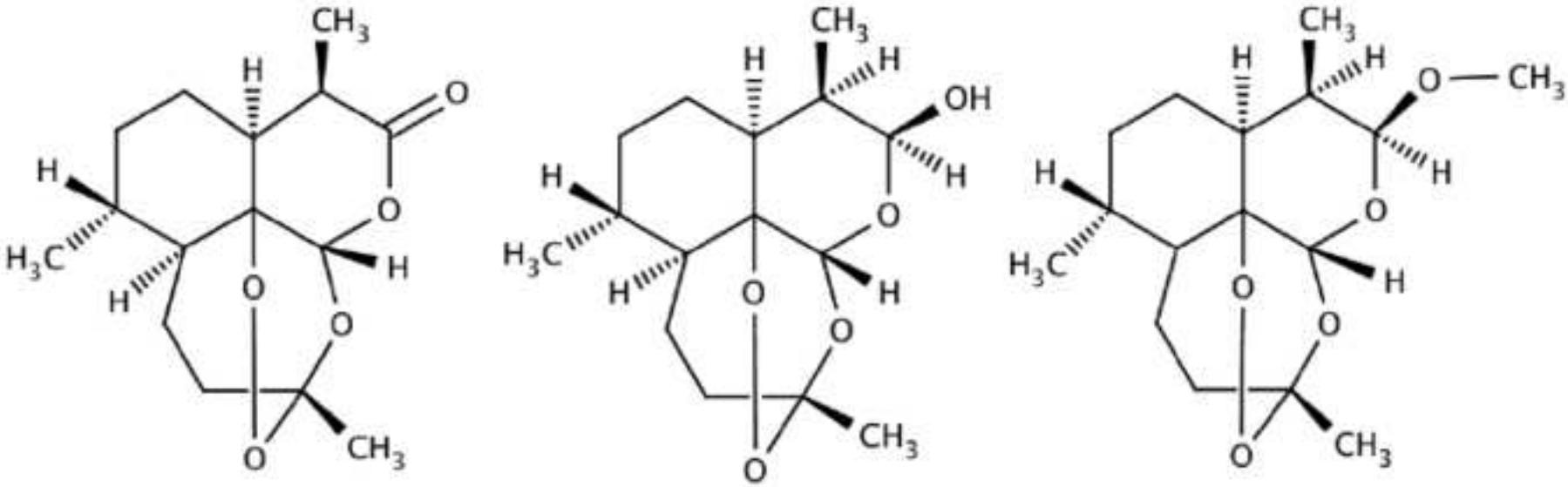
Fig 4. Overlay chromatogram of a dry heat (145 °C – 30 min) stressed sample (red) and a 80 mg/ml arachis oil stability (11 months at 40 °C 75 % R.H.) sample (black)

Table I. Calculated individual RRF values of the major observed β-artemether degradants

Degradant #	RRT	RF $\times 10^5$ AU/mg (95% CI)		RRF (95% CI)	
1	0.288	0.4399	(0.4357 – 0.4441)	4.94	(4.90 – 4.99)
2	0.616	3.7771	(3.7652 – 3.7890)	42.44	(42.31 – 42.57)
3	0.694	1.1717	(1.0785 -1.2649)	13.17	(12.12 – 14.21)
4	0.802	3.7771	(3.7652 – 3.7890)	42.44	(42.31 – 42.57)
5	0.826	2.5105	(2.4096 – 2.6114)	28.21	(27.07 – 29.34)
6	0.842	3.7771	(3.7652 – 3.7890)	42.44	(42.31 – 42.57)
7	0.867	3.7771	(3.7652 – 3.7890)	42.44	(42.31 – 42.57)
8	0.935	2.5105	(2.4096 – 2.6114)	28.21	(27.07 – 29.34)
9	1.264	0.7879	(0.7849 – 0.7909)	8.85	(8.82 – 8.89)

(1) RF β-artemether: 0.089 $\times 10^{-5}$ AU/mg

Figure 1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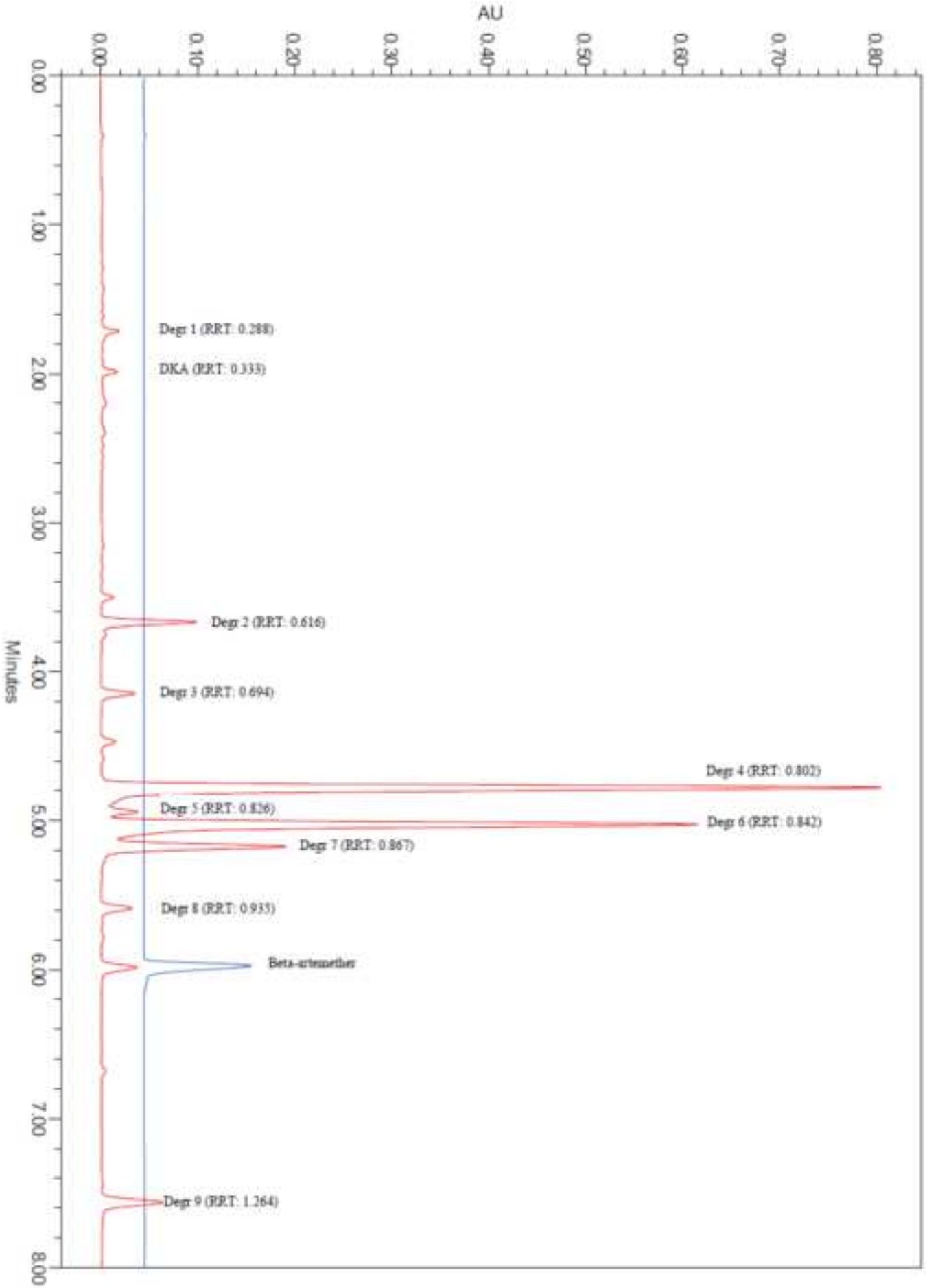
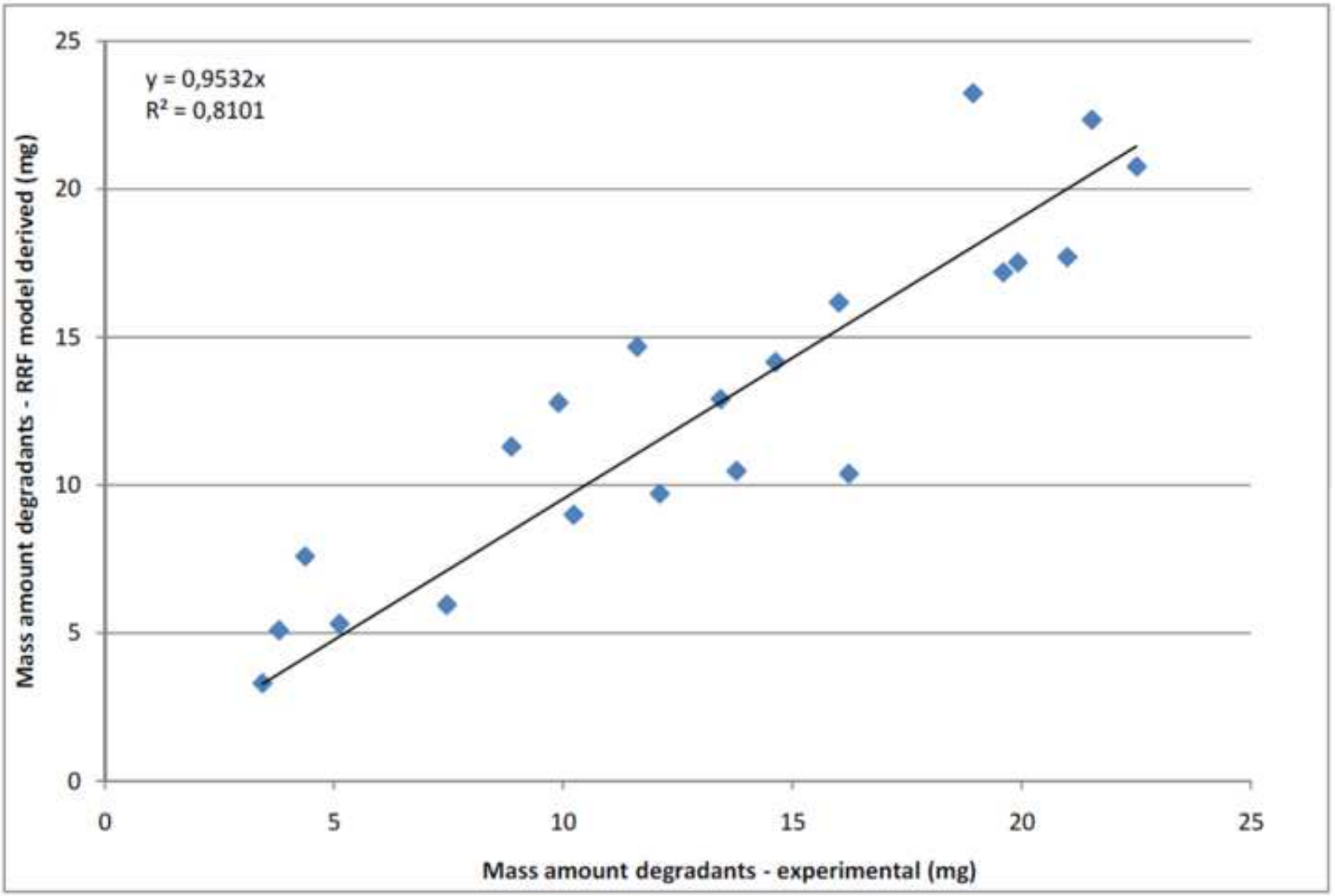


Figure 3


y = 0,9532x
R² = 0,8101
Mass amount degradants - RRF model derived (mg)
Mass amount degradants - experimental (mg)
0
5
10
15
20
25