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PAPER

High throughput quantitation of artesunate and its degradation products by flow injection gradient ratio standard addition mass spectrometry (FI-GRSA-MS)†

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There is a pressing need to equip post-marketing drug quality surveillance systems with higher throughput and robust analytical tools that can reinforce pharmaceutical quality control to ensure safety of patients and integrity of treatment practices. This manuscript reports the development of a flow injection analysis mass spectrometry-based gradient ratio standard addition (FI-GRSA-MS) method for quantification of artesunate, a common antimalarial active pharmaceutical ingredient in a simple, efficient, accurate, and high throughput manner. Dodecylamine (DDA) was evaluated as an electrospray modifier for improving the sensitivity of the GRSA quantitation method. The linear dynamic range and precision for artesunate quantification were found to be 7 ng to 86 pg artesunate and 2.1%, respectively. When compared to high performance liquid chromatography, results were found to be equivalent at the 95% confidence level. To identify chemical markers of improper drug storage and artesunate degradation, tablets were artificially exposed to heat for time periods ranging between 1 and 12 hours and the degradation products detected were identified *via* accurate mass and isotopic cluster abundance measurements. Moreover, degradation behavior of artesunate tablets from two different manufacturers under identical degradation conditions was investigated, indicating that tablet formulation plays a significant role in the shelf life of these valuable medicines.

Introduction

Combating malaria is one of the top global priorities of the World Health Organization (WHO), and a key activity towards accomplishing the United Nations' Millennium Development Goals.[1,2] The quality of antimalarial drugs not only defines their efficacy and safety, but it also likely contributes to the development of parasitic resistance, together with factors such as poor prescription practice and adherence.[3,4] Although National Medicines Regulatory Authorities (NMRA) periodically review medicine quality before placement on the market, in many developing countries limited resources hamper assurance of acceptable drug quality.[5,6] Occurrence of falsified and substandard drugs in the open market, in addition to the fact that the quality of genuine drugs may easily deteriorate through improper handling and storage, demands robust and high speed monitoring platforms to ensure optimal treatment outcomes.[7] The cause of death for several malarial patients under treatment has been blamed on the consumption of falsified antimalarial drugs, particularly artesunate.[8] Production, distribution, and sale of falsified, substandard, and degraded drugs are considered widespread in some developing countries, and poses not only a serious danger to patients, but also a significant source of financial loss to the pharmaceutical industry.[9] To ensure that loss of confidence in health care and spread of drug resistant pathogens do not occur, rapid but reliable analytical techniques that can survey large numbers of medicine samples in a more timely and cost-effective manner are needed.

Liquid chromatography-mass spectrometry (LC-MS), the most widespread analytical platform applied to routine analysis in pharmaceutical industries, lacks the required sample throughput to analyze large sample numbers in a convenient and cost-effective manner.[10] Gradient optimization, stationary phase conditioning, and detector calibration create the bottlenecks of LC-MS-based analysis for drug quality screening and country-wide survey applications. Despite these disadvantages, LC-MS remains the most preferred method in industry because of its reliability and ability to confirm results through multiple and established online databases.

The flow injection analysis (FIA) approach developed by Ruzicka and Hansen in the early 1970's revolutionized the field of analytical chemistry by providing automation capabilities to routine industrial and clinical chemistry applications.[11] For samples with a limited number of components, FIA-MS can be a

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viable and faster alternative to LC-MS high throughput screening approaches,[12] but carries the risk of uncontrolled interferences due to ionization suppression effects. In FIA, calibrations can be performed not simply by successive injections of standards of increasing concentration, but also by approaches that make use of the dynamic nature of the FIA dispersion process, and the concentration gradients thus created. In the last three decades, a multitude of flow-injection calibration approaches has been developed for quantitation,[13] of which the gradient ratio calibration method (GRCM) has been shown to be time and labor efficient, and applicable in the presence of strong interferences. The GRCM was introduced by Fan and Fang[14] and subsequently implemented as a calibration method for flame atomic absorption spectrometry (FAAS) by Koscielniak[15] In the GRCM, two solutions (a standard and the sample) are sequentially injected into the flow system. When the response peaks for both standard and sample are time-aligned, the time-dependent signals at any given instant after injection represent the local concentration of the analyte and standard after being diluted to the same extent. The initial concentration of the standard and the sample-to-standard signal ratios at finite time intervals are then used to calculate the apparent concentration of the analyte in the sample and the dilution factor. The calibration relationship between the apparent concentration and the dilution factor when extrapolated to infinite dilution provides the actual concentration of the analyte in the sample. The curvature of the relationship indicates the effect of interferents on analyte detection. In the GRCM it is assumed that at infinite dilution the above relationship becomes linear and the interference effect is eliminated. In practice however, interferences are not always eliminated at infinite dilution which results in error in the measured analyte concentration. To mitigate interference effects, standard addition to the sample is implemented in the gradient ratio-standard addition method (GRSA).[16] Response peaks for the sample and the sample with added standard are then processed according to the GRCM approach and the calibration points are extracted from the measurement data stored in the descending portion of the peaks.

In this work a rapid, non-chromatographic FI-GRSA method for the analysis of antimalarial tablet extracts by MS was developed and its figures of merit were evaluated. The FI-GRSA-MS method was validated against High Performance Liquid Chromatography (HPLC) with good results, and applied to determine the concentration of active pharmaceutical ingredients (API) present in tablets after being thermally degraded. The FI-GRSA-MS method presented here enables the analysis of the API content without the need for lengthy chromatographic separations or stable isotope labeled internal standards, its high throughput making it appealing for large-scale drug quality studies.

Experimental

Solvents, standards, instrumentation

Artesunate tablets were obtained from Mediplantex National Pharmaceutical Co. (Vietnam), and Guilin Pharmaceutical Co. (China). The tablets were first powdered and homogenized using a mortar and pestle, mixed with 10 mL of HPLC grade methanol (Sigma, St Louis, MO, US), and shaken for 40 minutes in an ice-cooled glass vial to extract artesunate. The methanolic artesunate extract was then filtered through a 0.45 μm PTFE membrane (MicroLiter Analytical Supplies, Inc., Suwanee, GA, US). For FI analysis, the methanolic artesunate extracts were first diluted to a theoretical concentration of 1 μM. One aliquot of the extract was diluted with methanol in a 50 : 50 ratio. A second aliquot of the methanolic sample extract was combined with a 1 μM artesunate standard in a 50 : 50 ratio. A 50 μM solution of dodecylamine (DDA) in methanol was added in a 50 : 50 ratio to these aliquots immediately prior to analysis. All solutions were kept on ice until injected onto the FI manifold.

Flow injection setup

The FI-GRSA-MS workflow used for quantification of artesunate in antimalarial tablets is shown in Fig. 1. An Agilent HPLC pump (Fig. 1(i), 1100 Series, Agilent, Santa Clara, CA, US)

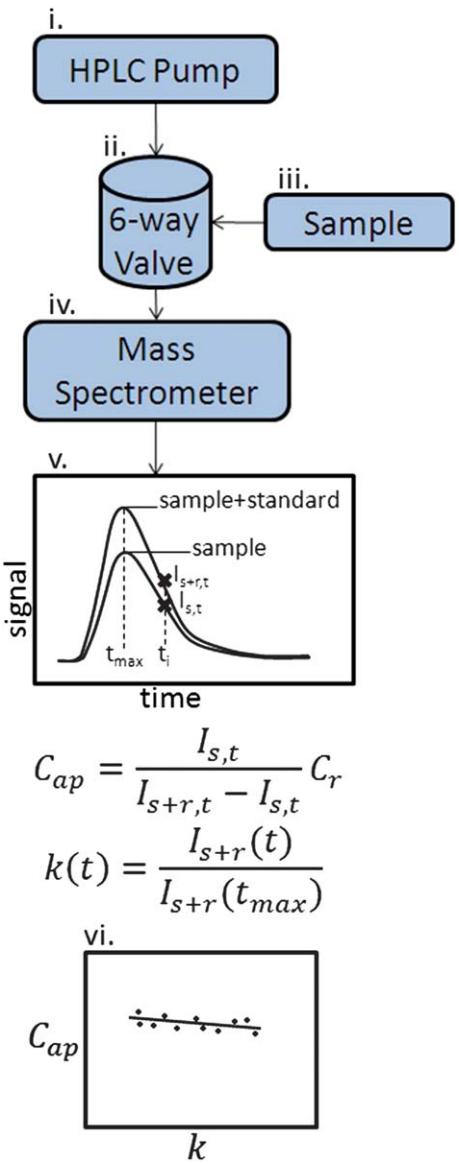


Fig. 1 Workflow for flow injection gradient ratio standard addition mass spectral analysis. Additional details provided under experimental.

delivered methanol at a rate of 150 μL min^{-1} into the inlet of a software-controlled 6-way divert valve (Fig. 1(ii), Rheodyne, Oak Harbor, WA, US), which was installed in the front panel of the mass spectrometer. The HPLC pump provided a continuous solvent flow and the software-controlled 6-way divert valve injected a defined volume of a sample solution as a pulse into the mass spectrometer (Fig. 1(iii and iv)). The exit port of the divert valve was connected to the ESI ion source. A syringe pump (Cole Parmer, Vernon Hills, IL, US) was used to load the sample into the PEEK tubing sample loop (7.5 μL) of the divert valve. Sample injections were alternated with flushing of the sample injection loop with methanol (~250 μL) to avoid carryover from previous samples. Six seconds into each FI run, the 6-way valve was switched to inject the contents of the sample loop as a plug into the constant stream of methanol flowing into the ion source. Each FI-GRSA-MS run was acquired for a total of 30 seconds.

Mass spectral analysis

Mass spectrometric detection was performed using a Bruker MicroTOF Q I mass spectrometer (Billerica, MA) operated in positive ion mode. The mass range acquired was 100–1000 Da, which was calibrated for obtaining accurate mass measurements using a 5 μM solution of polyethylene glycol 400 in methanol. The instrumental parameters were optimized for highest sensitivity and resolving power for the protonated artesunate–dodecylamine proton-bound non-covalent complex at $m/z = 570.4$.

Data analysis

Acquired data were analyzed using the GRSA method using eqn (1) and (2), adapted from the work by Koscielniak:[15]

$$C_{ap} = \frac{I_{s,t}}{I_{s+r,t} - I_{s,t}} C_r \quad (1)$$

$$k(t) = \frac{I_{s+r}(t)}{I_{s+r}(t_{max})} \quad (2)$$

C_{ap} is the apparent concentration of the sample without artesunate standard added. $I_{s,t}$ is the intensity obtained for the sample injection at time t, and $I_{s+r,t}$ is the intensity of the sample plus standard at time t. C_r is the concentration of the standard that was added to the sample. The relative dilution ($k(t)$) is obtained from the ratio of the sample plus standard intensity at a particular time point, $I_{s+r}(t)$, and the intensity of the sample plus standard at the maximum $I_{s+r}(t_{max})$. The maximum of the sample plus standard trace is chosen as the first point for data analysis, and $k(t)$ and C_{ap} are then calculated point by point down to the 10% relative dilution (Fig. 1(v)). The apparent concentration is then plotted against the relative dilution (Fig. 1(vi)). The y-intercept of a linear trendline fitted to the data points in this plot represents the best estimate for C_{ap}.

HPLC conditions

A reverse phase C18 column (Supelco, St Louis, MO, US) was used as the stationary phase. A potassium phosphate buffer was prepared using potassium dihydrogen phosphate (Sigma, St Louis, MO, US) and deionized water. The buffer was adjusted to pH 3 using phosphoric acid (Sigma, St Louis, MO, US) and used as the mobile phase at a flow rate of 0.6 mL min^{-1} by mixing with acetonitrile (50 : 50 v/v solution). The UV detector was set to a wavelength of 216 nm and each HPLC run was acquired for 15 minutes.

Sample degradation procedure

Genuine, unexpired artesunate tablets were artificially degraded outside of their blisterpacks in an oven heated to approximately 105 °C for periods of 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours. Humidity was not controlled.

Results and discussion

ESI-MS artesunate chronograms

Due to relatively large API concentration in the pharmaceutical tablet extracts along with the absence of chromatographic separation and the presence of concomitant excipient compounds, ionization suppression is often observed during electrospray ionization.[17] To improve artesunate detection by ESI, DDA a primary amine, was used as an ESI modifier to quantify artesunate in tablets by FI-GRSA-MS. The reasons for using DDA as an ESI modifier for improving artesunate analysis sensitivity were several-fold, including: (1) primary amines such as DDA have been shown to preferentially form a stable proton-bound non-covalent complex with artesunate and other artemisinins while inhibiting the formation of unwanted species that split the ionic signal into various channels,[18] (2) the formation of the more stable DDA–artesunate complex inhibits collision-induced fragmentation during transport through the ion optics, resulting in a higher ESI intensity, (3) the aliphatic carbon chain in DDA increases the hydrophobicity of the complex with artesunate, increasing its overall fugacity, ion evaporation ability and ion yields,[19] and (4) the localization of the positive charge at the DDA nitrogen atom results in an additional gain in ESI sensitivity.[20]

Fig. 2 shows representative mass spectra of artesunate (a) with and (b) without DDA added to the sample solution. The m/z of the [artesunate + Na]$^+$ ion is 407.2 (panel b), while the m/z of the [artesunate + DDA + H]$^+$ complex is 570.4 (panel a). Though the mass spectrum in panel a appears more complex than that in panel b, the overall signal for the [artesunate + DDA + H]$^+$ species was much more intense than that for [artesunate + Na]$^+$ in panel b. Therefore, DDA was added to the solution when the highest sensitivity for artesunate detection was desirable. However, FI operation without DDA addition was employed when new species stemming from artesunate degradation were being investigated, as simpler spectra enabled more straightforward species identification.

Quantitation of artesunate by the FI-GRSA-MS method

The FI-GRSA-MS method consisted of two consecutive injections continuously monitored using the mass spectrometer *via* ESI. The first extract injected contained the analyte (artesunate) in concentration C_s, and the second was composed of the analyte in the original sample extract plus an internal standard (artesunate), with the total analyte concentration $C_{s+r} = C_s + C_r$, where C_r is the analyte concentration from the added standard alone.

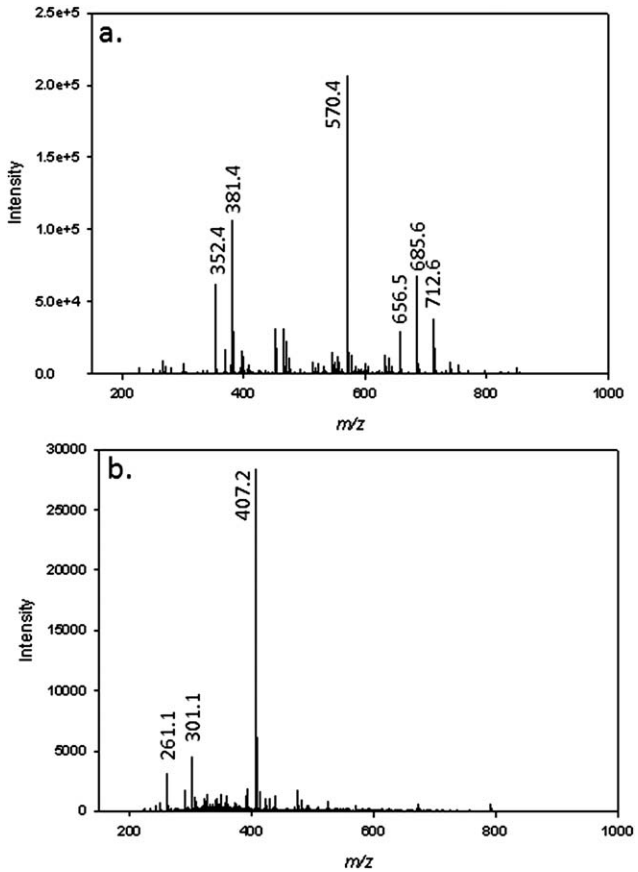


Fig. 2 Representative mass spectra of artesunate with (a) and without (b) DDA added to the solution. The *m*/*z* of [artesunate + Na]$^+$ is 407.2, as can be seen in panel b, while the *m*/*z* of the [artesunate + DDA + H]$^+$ complex is 570.4, as seen in panel a. Note the more complicated spectrum, but significantly more intense signal with the addition of DDA.

Two FI mass chronograms were recorded: the sample extracted ion chronogram with intensity I_s *vs.* time, and the standard plus sample chronogram, with intensity I_{s+r}. These chronograms were overlaid and aligned so that the initial injection times match up. As the injection plug was progressively diluted due to dispersion in the FI manifold, the trailing part of the mass chronograms was treated as an infinite series of progressive sample dilutions (for the first injection) and decreasing multiple standard additions (for the second injection), with only a single physical addition of the standard.

Typical FI-extracted ion chronograms for the [artesunate + DDA + H]$^+$ ion and subsequent data analysis for the FI-GRSA-MS method are shown in Fig. S-1 (ESI†). The traces shown on the top panel of Fig. S-1† correspond to a genuine tablet that has not been degraded, while the panels in the bottom show typical results for a degraded tablet. The green trace represents the chronogram for the sample without standard added to the solution, with a lower intensity following artesunate degradation, while the blue trace represents the injection of the samples plus the standard addition. The *y*-intercept of the relative dilution *vs.* apparent concentration linear fits is used to obtain the artesunate concentration in each case. In this example, the % API in each case was determined to be 98.9% (49.4 mg per tablet) and 28.2% (14.1 mg per tablet), respectively. In both cases, the slope of the linear fit was significantly different from 0, suggesting the presence of matrix effects suppressing the signal of the analyte. It is because of this difference that an external calibration approach to analysis would be insufficient and the use of multi-point analysis is necessary to account for any matrix effects that may be present.

Evaluation of figures of merit of the FI-GRSA-MS method

Several figures of merit for the proposed FI-GRSA-MS method were evaluated to investigate its applicability to antimalarial drug quality analysis. First, the linear dynamic range was investigated. This figure of merit describes the concentration interval within which quantitation can be performed with constant sensitivity and without incurring any bias. As shown in Fig. 3, the upper limit of the linear range for these experiments using the [artesunate + DDA + H]$^+$ ionic signal as the response was approximately 2.5 μM (equivalent to 7.2 ng of artesunate injected), with a lower detection limit in the 30 nM (86.4 pg) range. To match this linear response range, unknown solutions or solutions from degraded samples were diluted to a theoretical concentration of 1 μM for all experiments to ensure that the upper linear limit was not exceeded, thus causing the quantity of analyte determined to be erroneous. The precision of the method, determined by the relative standard deviation of repeat experiments, was measured to be 2.1% (n = 5), while maintaining a sample throughput of 48 FI experiments (24 samples) per hour. If an autosampler were to be utilized for injection, the throughput could increase to up to 50 samples per hour.

Accuracy in quantitation by the FI-GRSA-MS method was first investigated using standards with concentrations within the determined linear range of the method (30 nM to 2.5 μM). The results are presented in Fig. 4. The *x*-axis represents the theoretical concentration of artesunate in the prepared unknown solutions; while the *y*-axis represents the concentration of the sample solution measured using the FI-GRSA-MS method. The confidence intervals for the regression parameters were calculated at the 95% confidence level, with the slope = 1 and intercept = 0 values being included inside those limits,

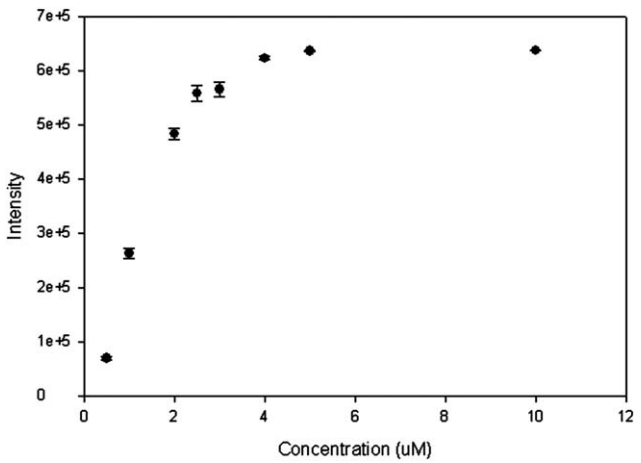


Fig. 3 Positive mode electrospray response for the [artesunate + DDA + H]$^+$ complex. All experiments were run in triplicate. Error bars represent one standard deviation of the replicates performed.

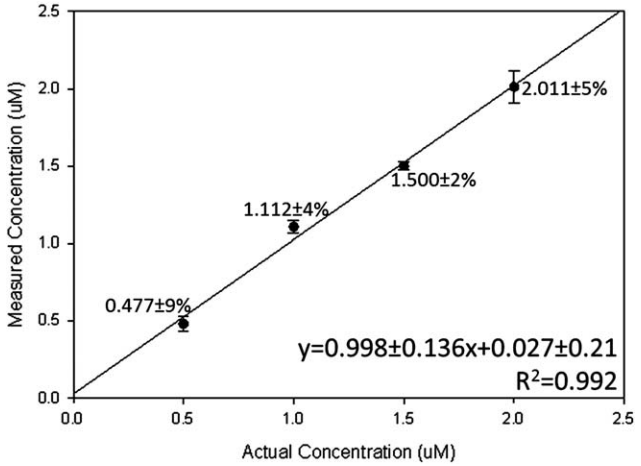


Fig. 4 Accuracy of FI-GRSA-MS analysis estimated by comparing measured concentrations with concentrations of synthetic unknown standards. Experiments were run in triplicate. Error bars represent one standard deviation of the data collected. The mean value and % relative standard deviation for each determination are shown above each data point.

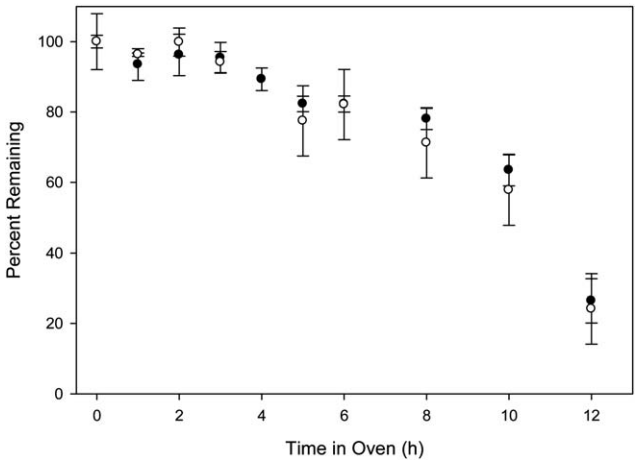


Fig. 5 Percent of API remaining after artesunate tablets manufactured by Mediplantex Pharmaceuticals were degraded in the oven at ~105 °C for 0–12 hours. Solid circles represent the values obtained using FI-GRSA-MS analysis, while the open circles represent data obtained using HPLC. Error bars represent one standard deviation ($n = 3$).

illustrating that this technique is accurate within the observed experimental variance, and suggesting that the concentration of artesunate in solutions of varying concentrations can be accurately detected by this approach.

Validation of the FI-GRSA-MS method by HPLC

The FI-GRSA-MS method was additionally validated by comparing with HPLC. Artificially degraded Mediplantex tablets were extracted in methanol and diluted to a theoretical concentration of 2 mg mL^{-1} using the same solvent. These extracts were filtered through a 0.45 μm nylon filter, and then stored in HPLC vials. The HPLC method described for artesunate analysis in the International Pharmacopoeia was used for validation purposes.[21] Fig. 5 shows the percent of artesunate remaining in each tablet *versus* the amount of time the tablet was left to degrade in the oven. Solid circles represent the quantity of artesunate obtained by FI-GRSA-MS, while the open circles represent the quantity of artesunate obtained by using HPLC. Each sample was run in triplicate for each method. As can be seen in Fig. 5, the results of these two analytical techniques overlapped well in all cases, demonstrating that comparable results can be achieved using both FI-GRSA-MS and HPLC, a widely accepted quantitation method. Fig. S-2† further compares the HPLC and FI results. A confidence level of 95% was used in calculations of the confidence interval for the line fitted to the HPLC data *versus* the FI-GRSA-MS data. These confidence intervals encompass a slope of one and a *y*-intercept of zero, verifying that these data statistically overlap.

Applications of FI-GRSA-MS to simulated degradation studies

Artesunate is prone to degradation upon exposure to even mild temperatures and/or humid conditions for extended periods of time.[22] To chemically identify degradation products in artesunate tablets which could serve as degradation markers and help distinguish poorly manufactured samples from those initially prepared with the correct API content but poorly stored, simple experiments were designed to progressively degrade artesunate tablets and subsequently profile these degraded samples by MS. Genuine, unexpired artesunate tablet samples were artificially degraded in an oven heated to approximately 105 °C for periods of 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours without an artificial atmosphere. These degraded tablets were then examined by FI-GRSA-MS for the artesunate content. Signals whose intensity increased with increased tablet degradation time were designated as possible artesunate degradation products. These species were then tentatively identified using accurate mass measurements provided by the TOF mass analyzer. Fig. 6 shows a typical electrospray mass spectrum resulting from an artesunate tablet extract that had been forcibly degraded for six hours. No DDA was added to the extract in this case to simplify the mass spectrum and allow for simpler identification of any degradation

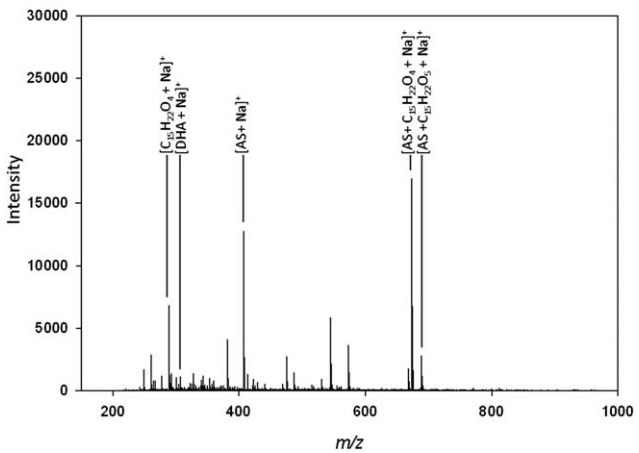


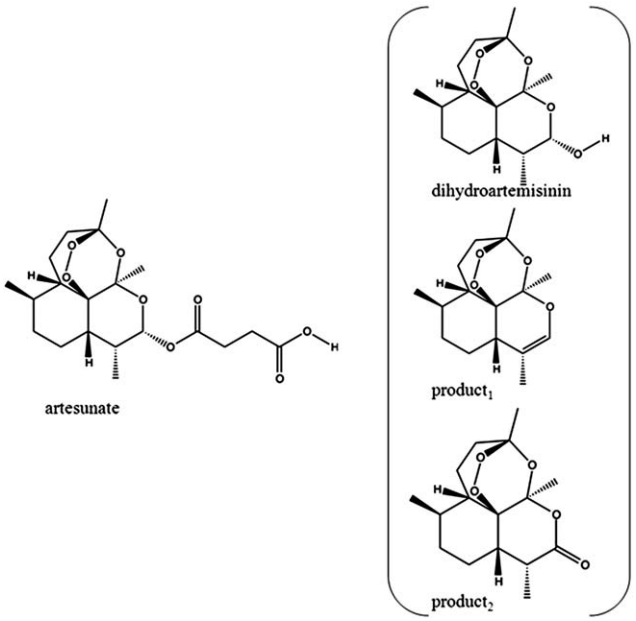
Fig. 6 Mass spectrum of an extract from a degraded artesunate tablet (Mediplantex) without DDA addition. The tablet was artificially degraded for 6 hours.

products that may be present. As compared to Fig. 2(b), the most intense peaks in Fig. 6 represent degradation product adducts, as opposed to the previously observed base peak of artesunate.

While effort was put forth to examine every peak in the mass spectra of degraded samples, it was not possible to identify all species present. Because manufacturers do not fully disclose excipients used in their formulations and because these excipients are most likely also being degraded when exposed to heat for extended periods of time, the number of possible compounds present is virtually endless. The species that were identified in the mass spectrum of degraded artesunate by their accurate mass measurements and verified by their isotopic ratios are shown in Table 1. All experimental accurate mass values and isotopic abundances corresponded well to the proposed elemental formulae, suggesting correct identification of these compounds. The [artesunate + product 2 + Na]$^+$ ion had a significantly higher error than the other components identified in Table 1, however, this discrepancy can be attributed to the fact that this signal was nearing the upper limit of the calibrated *m/z* region. Interestingly, product 1 was detected in the mass spectrum shown in Fig. 6 both individually and as a complex with artesunate, but product 2 was present only as an artesunate complex. It is possible that product 2 did not ionize well by itself, or its affinity to bind with artesunate was very strong.

It is logical to believe that there are several ongoing, perhaps parallel, reactions occurring during the degradation of the API and multiple excipients used to formulate the antimalarial drug. Some evidence for these complex degradation processes is observed in Fig. S-3 (ESI†). The intensity of degradation product 1 appeared to increase gradually over the degradation experiment, but after ~9 hours it began to preferentially appear as bound to artesunate molecules. At this point the [artesunate + product 1 + Na]$^+$ ion (green trace) started to increase drastically in intensity. As the artesunate tablet was further degraded, a plethora of new ionic species was observed in the mass spectrum, but could not be easily identified.

The degradation products that we detected are produced by thermal degradation, as the tablets were not exposed to sunlight, nor were they exposed to any high humidity levels besides the ambient humidity in the laboratory, approximately 9.2 g m^{-3}. The structure of these degradation products is proposed in Scheme 1, partially matching what Haynes *et al.* observed in their investigation of degraded artesunate suppositories.[22–24] In Haynes's study, the most abundant degradation product detected was dihydroartemisinin (DHA). Interestingly, in our study, DHA was present for the entire duration of the artesunate degradation process, in a relatively consistent abundance (Fig. S-4†). Because DHA was detected in tablets even prior to degradation in the oven, the presence of DHA is likely a result of an



Scheme 1 Proposed structures for artesunate degradation products.

incomplete artesunate synthesis. However, degradation product 2 appeared bound to DHA, detected as [DHA + product 2 + Na]$^+$. This could indicate that artesunate must break down to DHA and product 2 (Scheme 1) at roughly the same rate and these two degradation products appear bound to each other in the ESI mass spectra. Until a more comprehensive study of the artesunate degradation mechanism takes place, a firm conclusion cannot be drawn from these data.

Despite these limitations, the knowledge gathered from these experiments could be put to practice for distinguishing degraded from low-API content tablets by subsequently performing the FI-GRSA-MS assay on a tablet extract, followed by a simple FI experiment where extracted ion chronograms for the ions described in Table 1 are created. A degraded sample would be differentiated from a low-API substandard sample if (a) a lower than expected API amount is determined by the first FI experiment and (b) degradation products are detected in the second FI experiment. This second experiment would not require addition of DDA to simplify spectral identification.

The degradation experiment was repeated using 50 mg artesunate oral tablets produced by Guilin Pharmaceuticals (Table S-5, ESI†), a different brand than that reported in Fig. 5 and 6 (Mediplantex). The degradation curves for these two brands of artesunate tablets were very dissimilar, with the tablets from Guilin Pharmaceuticals degrading approximately four times faster than the tablets from Mediplantex. It is possible that

Table 1 Elemental formulae determined for detected degradation products of artesunate

Elemental formula	Calculated mass	Experimental mass	Error (ppm)	Description
$C_{15}H_{22}O_4Na$	289.1416	289.1418	0.8197	[Product 1 + Na]$^+$
$C_{15}H_{24}O_5Na$	307.1521	307.1522	0.2279	[Dihydroartemisinin + Na]$^+$
$C_{19}H_{28}O_8Na$	407.1682	407.1680	0.4593	[Artesunate + Na]$^+$
$C_{34}H_{50}O_{12}Na$	673.3200	673.3205	0.8050	[Artesunate + product 1 + Na]$^+$
$C_{34}H_{50}O_{13}Na$	689.3149	680.3112	5.4330	[Artesunate + product 2 + Na]$^+$

a difference in the excipients used, and therefore their thermal conductivity, contributed to the difference observed in the degradation profiles. It is also possible that the pressure used to press the tablets differed, resulting in a harder or softer tablet, for which the rate of heat exchange would be much different.

Conclusions

In conclusion, the reported study demonstrates that FI-GRSA-MS is a simple, efficient, accurate, and high throughput analytical method for quantification of active pharmaceutical ingredient content as compared to traditional methods used for quantitative analysis of pharmaceuticals. Absence of the need of isotopically labeled standard renders this method more economical than common methods used for quantification by MS. The upper limit of the linear range for artesunate determination by the FI-GRSA-MS technique was approximately 2 μM and the lower limit of detection was ~30 nM. When compared to HPLC, the technique was accurate within the accepted experimental variance, demonstrating its potential to accurately detect the artesunate content and its degradation products in pharmaceutical tablets. Additionally, this reported method combined with MS profiling and accurate mass identification would allow the user to detect and identify degradation products, enabling the distinction between degraded and poorly manufactured pharmaceuticals. The method has also the potential to be applied to the high-throughput analysis of artificially degraded tablets formulated with different excipient combinations and/or using different pressures to better understand which manufacturing conditions cause more rapid degradation and what properties of the excipients may have accelerated or hindered degradation of the active ingredient(s). Identification of different degradation products due to these different processes may also provide useful information allowing for the elucidation of the lifetime of degraded tablets collected in the field. More detailed investigation of the degradation pathways, product structures and slower thermal degradation approaches must be undertaken to help determine the answers to these remaining questions. On a more general context, this method may also be applicable to the determination of one or a few components in biological samples where some degree of matrix effects may be expected.

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