



Full Length Research Paper

**CHARACTERIZATION OF SOME IMPURITIES IN TWO BRANDS OF
ARTESUNATE TABLETS**

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ABSTRACT

Artesunate is one of the key components of Artemisinin combination therapies (ACTs) recommended for use as an anti-malaria in malaria endemic areas of the world by World Health Organization (WHO) to ensure high cure rates of *Plasmodium falciparum* malaria and to reduce the spread of drug resistance. During a quality assessment of Artesunate tablets found in Nigerian markets, Thin Layer Chromatography (TLC) revealed an unknown impurity spot which exceeded the identification thresholds of ICH guidelines. This work has detected and identified two degradation products and other impurities that may be present using analytical techniques that are readily available in this region.

Two brands of Artesunate tablets alongside Artesunate and dihydroartemisinin authentic reference standard tablets were extracted with methanol and the extracts subjected to Gas Chromatographic-mass spectrometric (GC-MS) and confirmatory TLC Procedures.

The GC-MS spectra of the samples when compared to that of the reference standard revealed high levels of succinic acid in both brands; this was confirmed by TLC which also revealed Dihydroartemisinin (DHA) to be the other degradation product. Quantitative analysis revealed the level of succinic acid in the degraded samples to be 28.7%w/w and 26.42%w/w.

The results suggest an acid-catalyzed hydrolytic degradation of artesunate which resulted in a chain reaction in the presence of residual moisture in the tablets. The incomplete conversion of dihydroartemisinin to artesunate during the synthesis of the starting raw material is also a possible source of these impurities

Keywords: Artesunate, degradation, hydrolytic, ICH, GC-MS, TLC.

INTRODUCTION

There is an ever increasing interest in impurities present in active pharmaceutical ingredients (API's). According to the international conference on harmonization: technical requirement for registration of pharmaceuticals for human use (ICH guidelines)^[1], organic impurities may arise during the manufacturing process and/or on storage of the drug substance, it may be identified or unidentified, volatile or non-volatile and may include: starting materials, by-product, degradation products.

Many degradation products can originate from an interaction between the API and excipients used to formulate a drug substance; in addition, a drug substance is subjected to a variety of conditions that can cause its degradation during the process of formulation. Functional

groups related typical degradation is exemplified in ester hydrolysis of drugs like ethyl paraben^[2] and photo-oxidation of ergometrine^[3].

It is often necessary to isolate degradation products. Generally, chromatographic and non-chromatographic techniques are used prior to their characterization. Among the chromatographic methods used are TLC, GC, accelerated gradient chromatography (AGC) and high performance liquid chromatography (HPLC)^[4].

Artesunate is a semi-synthetic artemisinin derivative synthesized by esterifying dihydroartemisinin with succinic anhydride under alkaline conditions^[5]. Industrial conversion to tablets involves dry or wet granulation. The latter involves mixing Artesunate drug powder with some quantity of water, drying it at 50-55°C and compressing the resulting granules with other materials in form of binders, disintegrants, lubricants, colorants and preservatives. Due to the high instability of Artesunate, the limits of its

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impurities are higher than those of other artemisinin derivatives, consequently several authors have noted that these limits should be reduced to ensure uniformity^[6].

In another yet to be published study carried out in Nigeria, different brands of Artesunate were analysed for identity and content of active ingredient using TLC. 18 of 42(43%) of these brands were found to have impurities or extra spot on the TLC with the same retention factor (Rf) values. It was also observed that as the intensity of the coloration of the impurity increased, that of Artesunate decreased implying a decomposition of Artesunate to a degradation product.

This study as an extension of the above observation did seek to establish the identity of the impurities. Some pharmacovigilance have reported adverse drug reactions following the administration of ACTs with artesunate (Personal communication with the Head of Pharmacovigilance centre of Jos University Teaching Hospital, Nigeria). The identity of the impurities may assist to explain some of the patients' reactions to the medicines and help to improve on safety of use by consumers.

MATERIALS AND METHODS

Chemicals and Reagents:

Analytical grades of methanol, sulphuric acid 96%, Acetone, Ethyl acetate, glacial acetic acid, toluene, authentic reference standards for Artesunate and dihydroartemisinin were supplied with the MiniLab® from Global Pharma Health Fund (GPHF) of Germany. Succinic acid was purchased from Merck(Germany). Samples were sourced from drug outlets in Nigeria.

Equipment:

Shimadzu GCMS-QP2010 PLUS of Japan was available for use from National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria. Pestle, Aluminum foil, laboratory glass bottles with a filling capacity of 25 to 100ml, funnel, set of straight pipettes (1 to 25ml), 10ml vials, Merck TLC aluminum plates pre-coated with silica gel 60F254, size 5 X 10 cm, glass microcapillaries of 2µl

filing capacity, hot plate, TLC developing chamber, filter paper, pair of scissors, pair of tweezers, TLC dipping chamber (Petri-dish) were available at the Departmental laboratories.

Gas chromatography – mass spectrometric analysis

Two different Artesunate tablet samples labeled samples B and G, and the reference Artesunate standard labeled A, were extracted with methanol and prepared for GC-MS analysis using the guidelines modified by A. Danell^[7]. GC-MS analysis was carried out at the National Research Institute for Chemical Technology (NARICT) Zaria, Nigeria.

Thin layer chromatographic analysis

A modification of the TLC test developed by USP/USAID for the analysis of Artesunate tablets in Africa^[8] was used. One fixed dose tablet of samples A, B, G and dihydroartemisinin (D) were crushed in aluminum foil using a pestle. Each was extracted with 10ml methanol and filtered. 2µl of each were spotted on the chromatoplate alongside succinic acid using microcapillaries. The plates were developed in a chamber containing 4ml of acetone, 18ml of ethyl acetate, 18ml of toluene and precisely 0.1ml of glacial acetic acid. The spots were detected by dipping the plates into methanolic sulphuric acid solution using a pair of tweezers, drying and heating on a hot plate for a few seconds.

Results

The gas chromatograms of the degraded samples B and G (Fig. 1) and that of the Artesunate reference standard (Fig. 2) comprise two sets of peaks; a peak at retention time (RT) 11.70 min and another set of peaks very close to each other at the right side of the chromatograms. The first was revealed by the coupled mass spectrometry to be dimethylsuccinate based on the fragmentation pattern (Fig. 3). Quantitative analysis using the peak intensities showed the percentage concentrations of dimethylsuccinate in samples B and G was estimated to be 26.42% w/w and

28.37%w/w, respectively, while that of the reference standard was 3.80% (table 1). Thin layer chromatography of methanolic extracts of samples B, G and A alongside dihydroartemisinin and succinic acid confirmed the presence of succinic acid as a degradation product of samples B and G and dihydroartemisinin as the other degradation product (Fig. 4).

Discussion

The set of peaks found at the right side of the chromatograms could have been products of Artesunate decomposition in the gas chromatographic chamber with column oven temperature of 60⁰C confirming the high instability of Artesunate under mild conditions ^[9]. Some of these products revealed by mass spectrometry include 2-deoxy dihydroartemisinin, 2-deoxyartesunate, artemether, dideoxy dihydroartemisinin, dideoxyartesunate and other products that arise via thermal decomposition, these products could also be formed on the shelf if these medicines are not properly stored. This explains the small amount of dimethylsuccinate found in the Artesunate reference standard.

The outrageously high levels of dimethylsuccinate in samples B and G suggest a previous degradation that had taken place on the shelf prior to analysis, this was revealed by TLC which also confirmed the other degradation product to be dihydroartemisinin.

The results suggest an acid-catalysed hydrolytic degradation (Fig. 5). Water from residual moisture of wet granulation and/or moisture sorbed into excipients such as starch and lactose ^[10] hydrolysed Artesunate into

dihydroartemisinin which was detected by TLC and succinic acid detected by GC-MS as dimethylsuccinate after esterification by methanol used as solvent for extraction.

The consequences of this degradation are serious and cannot be left unattended to. The first being the risk of development of resistance to Artesunate and dihydroartemisinin over time causing treatment failure, this is because both drugs will be taken in the same tablet at sub-inhibitory concentrations. Secondly, is the risk of toxicity due to salts of succinic acid in humans. Ammonium succinate has been known to be acutely toxic in humans, carcinogenic, neurotoxic and also toxic to the human reproductive and developmental systems ^[11].

Conclusion

Artesunate drug substance degrades easily in the presence of moisture and an acidic environment to dihydroartemisinin and succinic acid. This degradation which has grievous short and long term consequences occurs often in Artesunate tablets marketed for consumption. A review of the monographs for artemisinin antimalarials which should include a reduction in the limits of the impurities in Artesunate (active ingredients and tablets) will go a long way to salvage the situation. Also, the use of dry granulation instead of wet granulation before tableting will help minimize this degradation. GC-MS and confirmatory TLC techniques were seen to be relatively easy and readily available means of detecting and quantifying the level of succinic acid and dihydroartemisinin in Artesunate tablets.

Table I: QUANTITATION OF SUCCINATES IN SAMPLES

SAMPLES	PEAK INTENSITY OF DIMETHYLSUCCINATE (X10 ⁶)	PEAK INTENSITY OF OTHER PEAKS (X10 ⁶)	TOTAL PEAK INTENSITY (X10 ⁶)	% PEAK INTENSITY OF DIMETHYLSUCCINATE	%CONCENTRATION DIMETHYLSUCCINATE IN SAMPLE
A	0.4	10.12	10.52	3.80	3.80
B	7.27	20.25	27.52	26.42	26.42
G	6.14	15.50	21.64	28.37	28.37

Fig. 1: CHROMATOGRAM OF DEGRADED ARTESUNATE TABLET

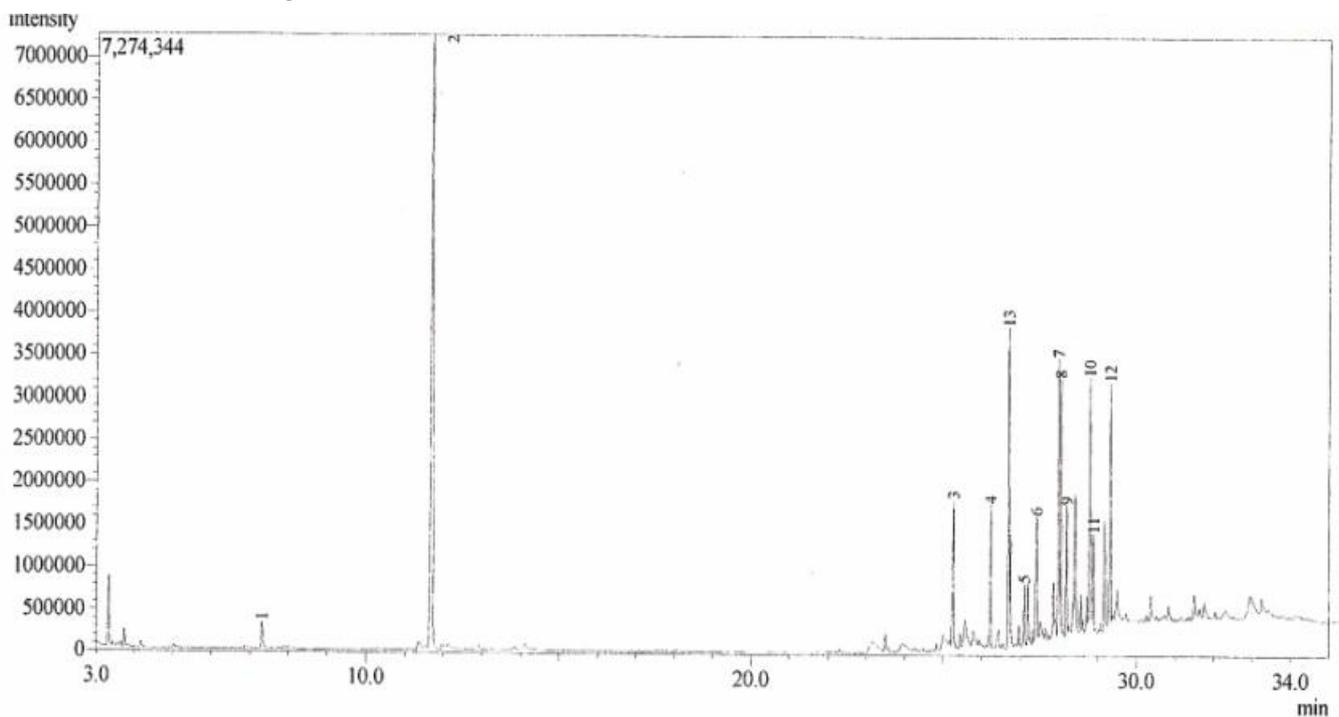


Fig 2: CHROMATOGRAM OF STANDARD ARTESUNATE TABLET

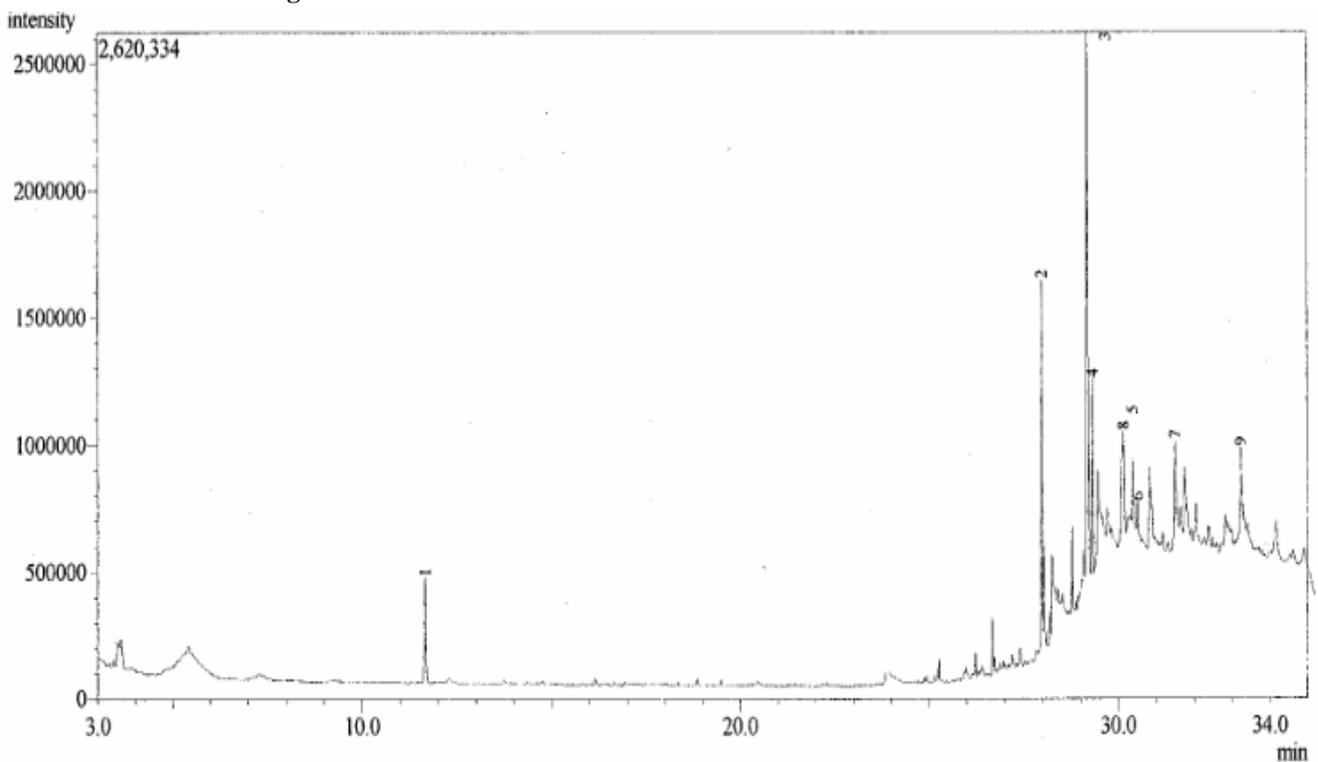


Fig 3: FRAGMENTATION PATTERN OF DIMETHYLSUCCINATE

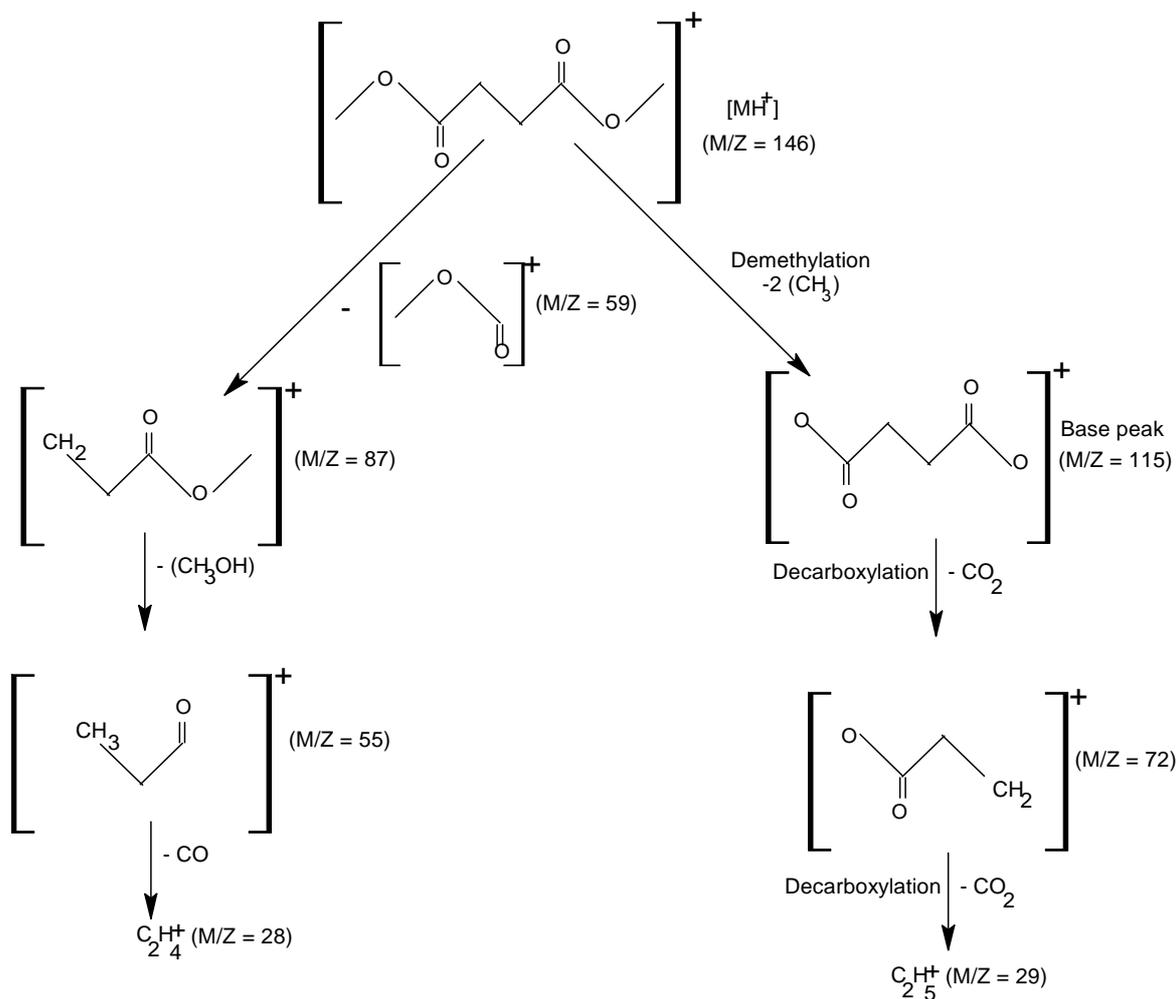
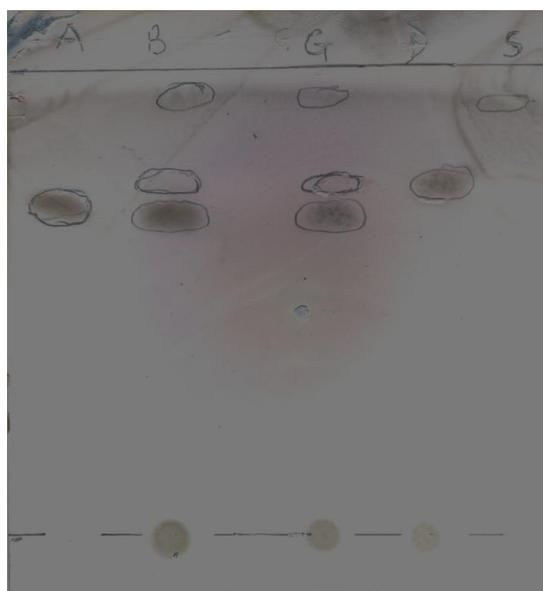
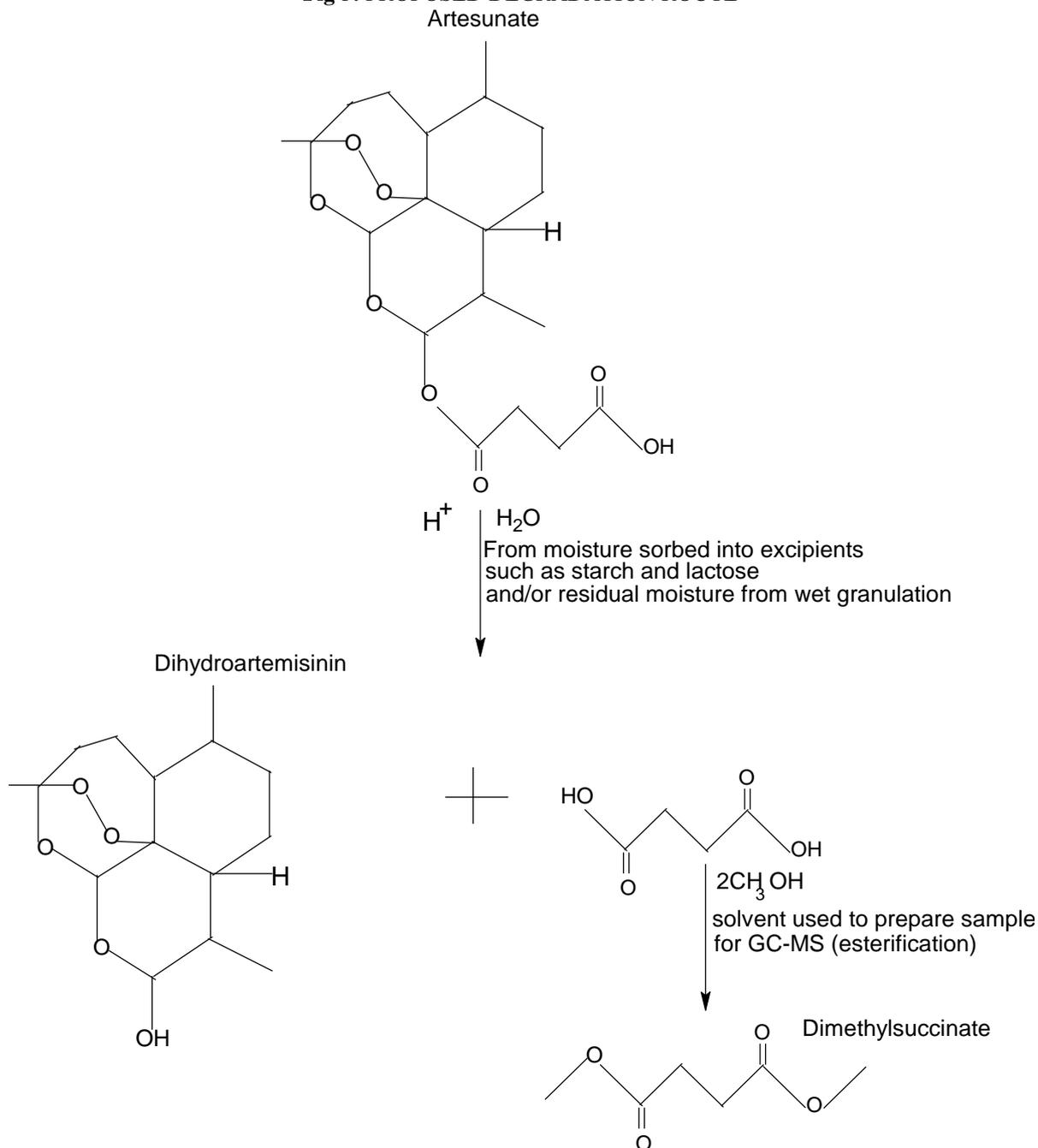


Fig 4: TLC of reference artesunate (A), Degraded artesunate brands (BandG), Reference dihydroartemisinin (D) and reference succinic acid (S)



Spot Identification: A= Artesunate (RF=0.69), B and G=degraded artesunate brands (RF=0.69,0.76,0.94),D=Dihydroartemisinin(RF=0.76), S=Succinic acid (RF=0.94)

Fig 5: PROPOSED DEGRADATION ROUTE



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