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ORIGINAL ARTICLE

Batch-to-batch consistency in the quality attributes of a phyto-pharmaceutical MA001 used to treat typhoid in Ghana

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In pharmaceutical manufacturing, products are made using standard operating procedures which are specific for each product. As a result, it is expected that, any number of batches of the same product selected at random for evaluation should either have the same results for all tested criteria or the difference between them should be within the acceptable limits. Batch-to-batch consistency study of herbal products is an important quality control parameter for assessing their reproducibility, efficacy and safety profile. This study sought to assess the batch-to-batch consistency of MA001, a herbal product using parameters such as colour, odour, taste, pH and total solid residue. Other assays conducted on the products included UV spectrometry and HPLC fingerprinting. Elemental analysis of the three batches of MA001 were selected for the study. The results from this study showed that, the products from the different batches had the same physico-chemical parameters as specified by the manufacturer. Ultimately, the sampled batches passed the batch-to-batch consistency test, hence, the reproducibility, efficacy and safety of MA001 decoction is assured.

Keywords: MA001, batch-to-batch, consistency, elemental content, microbial load, spectroscopic markers, physicochemical test

Introduction

A batch of herbal medicine is the finished quantity or consignment of labelled medicinal products which contain same active ingredient(s) produced at one time under the same conditions (Ahmad et al., 2014). As a result, any samples selected from a batch for testing should have the same attributes for all tested criteria with negligible differences between them (Yadav & Dixit, 2008). Batch variation occurs when there is a crossover of the acceptance limit between batches of the same product undergoing batch-to-batch assay. To ensure consistency in dosage units, each unit in a batch should have their drug content within the acceptable narrow range around the labeled drug content (British Pharmacopoeia, 2016). Unlike the single chemical entities which forms the basis of modern pharmacology and drug development, herbal medicines contain a myriad of compounds in

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complex matrices which often work in synergy in delivering the therapeutic effect. A single herbal ingredient may contain several chemical compounds working together to provide the observed effect. Thus, the herbal material in its totality is normally considered the active ingredient (Serra, 2007). In most instances, the chemical compositions of these herbal preparations are not well characterized and the active compounds producing a particular therapeutic effect may also not be known (Xie et al., 2006). Batch-to-batch consistency assay is a means of analyzing the quality of herbal products. This is done by investigating some common parameters which are likely to be seen in all batches of the same herbal product (Owusu et al., 2021). A batch-to batch consistency evaluation is effective in validating the working procedures during preparation and a means of ensuring that herbal products are of the required specifications.

Typhoid fever is caused by *Salmonella enterica* serovar typhi and *Salmonella enterica* serovar paratyphi A bacteria. It is transmitted mostly by contaminated food, water, and person-to-person contact (Crump et al., 2019). Typhoid fever incidence occurs increasingly due to variation in geographical areas at the same time within the same country. Thus, this disease is most common in poor communities within the same geographical area, with poor sanitation and unhygienic sources of drinking water (Marchello et al., 2019). Typhoid fever symptoms include persistent fever, headache, nausea and vomiting, constipation or diarrhoea, and the appearance of faint-pink spots on the abdomen, chest, and back (Eissa et al., 2018). Clinically, it is treated with antibiotics such as ciprofloxacin, azithromycin, ceftriaxone, ampicillin, chloramphenicol, and fluoroquinolones. However, the reported side effects coupled with the occurrence of antimicrobial resistance with these approved antibiotics have resulted in several treatment failures (Mishra et al., 2017; Yang et al., 2017) . The use of herbal medicines as alternatives (Sarkodie et al., 2016) in the treatment of infectious diseases is becoming increasingly relevant due to the development of such reported microbial resistance to some of these orthodox antibiotics (Newman et al., 2011). This antibiotic resistance has led to people preferring herbal remedies as an alternative in the treatment of typhoid fever (Vadhana et al., 2015). MA001 is a decoction produced by the Centre for Plant Medicine Research, (CPMR), Mampong-Akuapem. The product has been used to treat typhoid fever in Ghana for at least twenty years due to its activity against Salmonella typhi and other gram-negative bacteria (Kumadoh et al., 2015). It is registered with the Food and Drugs Authority of Ghana under the brand name MA001 (FDA/HD.07-7097). The product is manufactured from *Citrus aurantifolia* (leaves), *Spondias mombin* (leaves), *Latana camara* (leaves), *Bidens pilosa* (aerial part), *Trema occidentalis* (leaves), *Psidium guajava* (leaves), *Morinda lucida* (leaves), *Vernonia amygdalina* (leaves), *Persea americana* (leaves), *Paulina pinnata* (leaves), *Momordia charantia* (aerial part) and *Cnestis ferruguinea* (leaves) (Adi-Dako et al., 2021). Currently, a formulation of granules and capsules has been developed from MA001 as alternative to this decoction in an attempt to improve compliance, stability and taste of the product (Adi-Dako et al., 2021; Kumadoh et al., 2015). The consistency of the product is critical in the treatment of typhoid fever. Wide variations in different batches may lead to lack of desired antimicrobial effect due to decreased quantities of phytochemical constituents. Over dosage may also occur with batches that contain more than the specified range of constituents. The dosage regimen for MA001 is 30 mL three times daily for three weeks. The aim of the study was to evaluate the quality and batch-batch consistency of three randomly selected batches of MA001 decoction.

Materials and methods:

Materials

The quantities of MA001 needed from a batch for this study were purchased from the dispensary of CPMR, within a period of three-months. It was ensured that a different batch was obtained upon each purchase and that the batch was at least more than six months to expiry as the shelf life was indicated as 12 months. They were stored at room temperature, 25^{o}C (the stated condition of storage of the product) for further analyses

All reagents used were of analytical grade.

Methods

Determination of organoleptic properties

The parameters such as colour, odour, taste and turbidity for the 3 batches of MA001 were determined. All the necessary aseptic procedures such as hand hygiene and environmental controls and precautions such as hygienic handling of product were undertaken during the study so as to prevent microbial contamination of the products.

Determination of colour

A volume of 5.0 mL of decoction was measured into a clean dry beaker. It was then diluted with 5.0mL distilled water to obtain a clearer less opaque form of the decoction. An amount of 1.0 g of cobalt chloride was weighed into the diluted sample. The mixture was then stirred to dissolve the cobalt chloride. The mixture gave a dark brown coloration which was recorded. This was repeated for the other sampled MA001 (British Pharmacopoeia, 2016).

Determination of odour

A volume of 10 mL of one of the sampled MA001 products was poured into a clean dry beaker. The odour was determined by slowly and repeatedly inhaling the blown air over the decoction (British Pharmacopoeia, 2016). This was repeated for the other sampled MA001 products from the different batches

Physicochemical properties:

Determination of total solid residue

The weight of an empty clean and dried evaporating dish was determined and recorded as W0. A volume of 10 mL of one of the sampled MA001 products was measured into the weighed evaporating dish. The preparation was dried in an oven (Hongjin RH-35, China) at 105^{o}C and the final weight was recorded as W1. The weight of the dried sample (W1-W0) was then calculated and extrapolated to percentage weight in volume (%w/v). This was repeated for the other sampled MA001 products (British Pharmacopoeia, 2016).

Determination of pH

The pH of 10 mL each of the sampled MA001 prodcuts were determined using the pH meter (PL-700PV from Nickel-Electro Ltd, UK) at 25 °C (Sigel et al., 1991) after the calibration of the pH meter with standard buffer solutions of pH 7, 4 and 10 (IP, 2018).

Qualitative phytochemical screening

Qualitative phytochemical screening of sampled MA001 was undertaken to determine the presence or otherwise of major natural compounds (Evans & Evans, 2009; Sofowora, 1996)

Analysis of UV maximum wavelength of absorption

The absorbance of graded concentrations (0.1- 0.001%v/v) of MA001 samples were determined over a range of UV-Visible wavelengths (200 – 1100 nm) in quartz cuvettes of 1 cm path length (Behera, 2012) using UV-Visible spectrophotometer (Merck industries, Germany) and distilled water as diluent.

HPLC analysis

The methods employed by (Butnariu et al., 2012; Wagner et al., 2015) were harmonized and utilized in analyzing the batches. In this analysis, the HPLC system (Agilent 1200 series HPLC system, USA) with RP18 column (4.6 x 250 nm, 5.0 μm) comprised a solvent system which consisted of acetic acid (Sigma Aldrich, USA) (solution A) and acetonitrile (Sigma Aldrich, USA) (solution B) as the mobile phase. The analysis was carried out at a flow rate of 1 mL/min. Fifty microliters was the injection volume at a maintained column temperature of 40 ° C and a run time of 30 min. A volume of 1 mL of decoction was centrifuged for 5 minutes and filtered using Whatman filter paper. A volume of 500 μL of the resulting supernatant was pipetted into a clean empty HPLC vial with addition of 500 μL of distilled water and the mixture centrifuged again for another 8 minutes. A volume of 1 mL of the resulting supernatant was pipetted into a clean and dry HPLC vial and inserted into the injection chamber. Gradient elution was employed in the chromatographic process. The chromatogram was monitored at 320 nm.

Elemental analysis of products

The heavy metal content of the decoction of MA001, was determined according to the method adopted by (Uddin et al., 2016).

Five (5) mL of 65 % HNO3 (Sigma Aldrich, USA) was added to 5ml of MA001.

The mixture was boiled gently for 30–45 min and cooled. A volume of 2.5 mL of 70 % HClO4 (Merck, USA) was added, and the mixture was gently boiled until dense white fumes appeared and then cooled. Ten milliliters of deionized water was added followed by further boiling until the fumes were totally released. Heavy metals were measured using a Perkin Elmer atomic absorption spectrometer (A Analyst 800).

Microbial quality of three (3) batches of MA001

All determinations were carried out under aseptic conditions. The presence of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp in MA001 were determined (USP, 2013) as well as the level of aerobic bacteria and fungi which may be present in 1 mL of sampled MA001 were quantified (BP, 2021). The pour plate method via the use of malt extract agar (HKM, Guangdong-China) was employed to evaluate the viability of fungi whiles the plate count agar (Oxoid, England) was also employed for bacterial enumeration at an incubation period of 22.5 ± 2.5 °C for 3-7 days and 32.5 ± 2.5 °C for 24-72 h respectively for fungal and bacterial enumeration after which the colony-forming units per gram (CFU/g) was recorded (BP 2021).

Escherichia coli: One milliliter of MA001 was transferred under aseptic conditions into 10 ml nutrient broth (Oxiod, England). The inoculated broth was incubated at 37 °C for 24 h. A loopful of the inoculated broth was taken and streaked along the S plane of MacConkey agar (Oxoid England) plate, for the investigation of the presence or otherwise of E. coli. The streaked plate was incubated at 37 °C for 48 h. The growth of colonies on the MacConkey Agar plates indicated the possible presence of *E. coli*. Isolates were identified using biochemical tests such as oxidase test, ureases test, citrate utilization test indole test and Gram stain (Feglo, & Opoku, 2014).

Staphylococcus aureus: The same procedure was used to determine the growth or otherwise of *Staphylococcus aureus*. A volume of 1 mL of MA001 was poured into 10mL of peptone water and incubated at 37°C for 24 h. After incubation, a loopful was streaked on a plate of Mannitol Salt Agar (Oxoid, England) using a sterilized inoculating loop. Incubation was done at 37°C for 24 h. The identification of the organism was observed using catalase test and Gram stain. The development of white colonies engulfed by a yellow zone shows the presence of *S. aureus* (Kerr, 2004).

Salmonella spp.: In this procedure, 1mL of MA001 was poured into 10mL of peptone water (Oxoid, England) and incubated at 37 °C for 24 h. After incubation, a loopful was streaked on a plate of Bismuth sulphate agar (Oxoid England), using a sterilized inoculating loop. Incubation was done at 37 °C for 24 h. The identification of the Salmonella was done using oxidase test, indole test, citrate utilization test and Gram stain. The growth of red colonies with either black or no black centres revealed the presence of *Salmonella spp.*(Feglo & Opoku, 2014).

Statistical analyses

Statistical analysis of obtained data was carried out using Excel and GraphPad Prism for windows version 5 (Graph Pad software Inc., San Diego, CA, USA). The results were measured in triplicate and were expressed as mean ± standard deviation

Results and discussion

Organoleptic and physicochemical properties

Organoleptic evaluations are conducted to ensure the appropriate identification, authenticity and homogeneity of polyherbal preparations. Plant medicine characteristics are affected by the geographical locations, time of harvesting and processing. This could lead to variability in their constituents which ultimately affects their efficacy (Bezerra Carvalho et al., 2014). There were no signs of abnormal odour or discolouration or change in turbidity which could be indicative of the presence of foreign matter, degradation, and instability which has implications on the safety of the herbal medicine (Hussain et al., 2009). The reported strong phenolic odour of the sampled batches of MA001 could be due to the presence of tannins found in *Paullina pinnata* present in the preparation (Imade et al., 2015) and the foliage smell could be because of the leaves used in the

preparation. Also, the anise smell could be as a result of the presence of *Persea americana* in the preparation (Wolstenholme & Whiley, 1999). The decoction from the different batches were all found to be dark brown in colour (Table 1) and batch B was the darkest. This obtained decoction colour could be due to the colour of the dried leaves used, thus brown. The bitter taste of the preparation could be due to the presence of bitter gourd (*Mormodica charantia*) and biter leaf (*Vernonia amygalina*) which are known to be very bitter (Ahmad et al., 2016; Farombi & Owoeye, 2011).

The total solid residue (TSR) obtained for the samples were between 0. 2-0.4 %w/v which is accepted because a difference of ± 0.2 %w/v in weights taken is appropriate (The United States Pharmacopeial Convention, 2013). This implies that, the TSR analyzed were consistent amongst the nine selected MA001 samples. Though statistical analysis demonstrated no significant difference ($p < 0.05$) in the total solid residue between the batches (Figure 1), batch A recorded the highest TSR (0.126 ± 0.01 %w/v) with batch B recording the lowest value, 0.119 ± 0.002 %w/v.

The pH of herbal medicines is essential in establishing the quality, safety and efficacy of the product (Adi-Dako et al., 2018, 2021; Eshun Oppong et al., 2021). The pH of the three batches fell consistently within the range of 4.2-6.2 (Table 1) with batch A recording the lowest

Table 1. Organoleptic and physicochemical properties of samples from 3 batches of MA001 decoction

Organoleptic properties	Batches								
	A			B			C		
	1	2	3	1	2	3	1	2	3
Odour	Strong phe-nolic, foliage and anise smell	Strong phe-nolic, foliage and anise smell	Strong phe-nolic, foliage and anise smell	Strong phe-nolic, foliage and honey-like	Strong phe-nolic, foliage and anise smell	Strong phe-nolic, foliage and anise smell	Strong phe-nolic, foliage and anise smell	Strong phe-nolic, foliage and anise smell	Strong phe-nolic, foliage and anise smell
Colour	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Taste	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter
Turbidity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Physicochemical									
Total solid residue ($\%w/_v$)	0.135	0.119	0.125	0.118	0.121	0.119	0.123	0.121	0.120
pH at 25 ºC	4.2	5.5	5.6	5.8	5.9	5.5	5.7	6.0	6.2

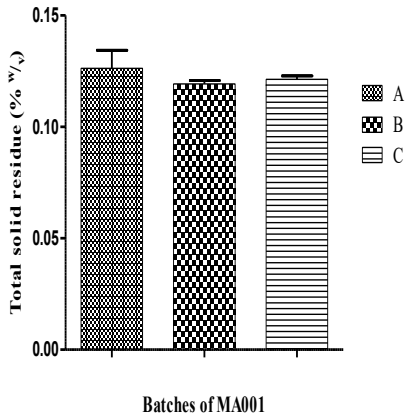

Figure 1. Comparison of the Total Solid Residue (TSR) of batches A, B and C (n=3) of MA001 using one-way ANOVA followed by Bonferroni's multiple comparison test showing no significant difference ($p < 0.05$) in the TSR of the 3 batches of MA001.

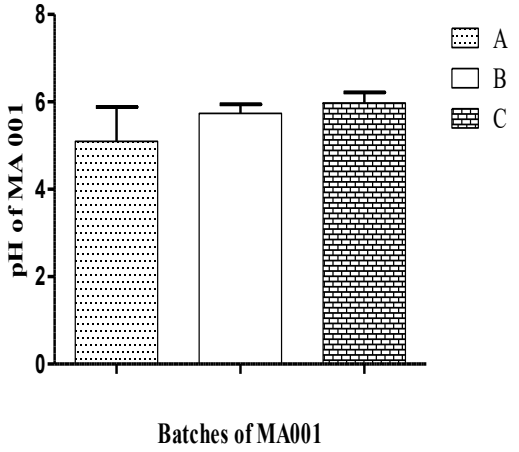

Figure 2. Comparison of the pH of batches A, B and C (n=3) of MA001 using one-way ANOVA followed by Bonferroni's multiple comparison test showing no significant different ($p < 0.05$) in the pH of the MA001 batches

Phytochemical composition of MA001

Qualitative analysis of phytochemicals on MA001 showed the presence of phytochemical constituents as summarized in Table 2. Reducing sugars, flavonoids, cardiac glycosides, anthraquinones, tannins, saponins, flavonoids, terpenoids and alkaloids were present in all the three batches of MA001. Natural plants contain various groups of phytochemicals which are beneficial in the development of new drugs and are also produced by the plant for defensive purposes against microorganisms, prey, and stress (Chew et al., 2009). Also, it ensures predictability of the pharmacological and therapeutic activity of their formulated herbal remedies (Agbafor & Nwachukwu, 2011; Nethathe & Ndip, 2011). The medicinal plants used in the formulation of MA001 have been reported to contain similar phytochemical compounds as those reported for the treatment of typhoid fever (Okigbo et al., 2009). Flavonoids are the main group of compounds found in various medicinal plants (Górniak et al., 2019). According to (Yuan et al., 2021), flavonoids play a role in the cell membrane by disrupting gram-positive bacteria and thus inhibiting phospholipid bilayers and ATP synthesis. Alkaloids have also been reported to possess antimicrobial activity and inhibit cell division (Nas et al., 2018).

Table 2: Phytochemical Analysis for 3 samples from Batch A, B and C after six-month storage

Phytochemical constituents	Batch A/ after six months of storage	Batch B/ after six months of storage	Batch C/ after six months of storage
Terpenoids	+	+	+
Reducing sugars	+	+	+
Flavonoids	+	+	+
Cardiac glycosides	+	+	+
Anthraquinones	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Alkaloids	+	+	+

KEY: (+) Presence of phytochemical; (-) Absence of Phytochemical

Spectroscopic Evaluation of MA001 decoction

This analysis was done for the purpose of setting spectroscopic standards for the evaluation and identification of the decoction

UV-Vis analysis.

The UV spectrum of the 3 batches of MA001 as shown in figure 3 indicates two major peaks at λmax 230 nm and 292 nm. Although it is ideal to use the maximum wavelength of absorption from UV spectrum (in this case 290 nm) in HPLC analysis, it is also possible during HPLC fingerprinting of plant extracts to use a wavelength on the shoulder of the UV peak (in this case 320 nm) particularly when, in combination with other resolution factors, there is better chromatogram for analysis.

The observed peak at 230 nm was also reported for all herbal medicines investigated by (Kumadoh et al., 2020) in their investigation of the stability of some herbal preparations. The emergence of this common peak could be due to the existence of a plant constituent (s) (Kumadoh et al., 2020). This peak (230 nm) is also suggested to be relative to carboxyl groups of organic acids (Martelo-Vidal & Vázquez, 2014). However, this obtained maximum wavelength of absorption (λmax 292 nm) is different from the λmax 356 nm reported (Adi-Dako et al., 2021). This difference could be as a result of the difference in the geographical sites of plant parts collected for the preparation of MA001 which led to variation in phytochemical constituents (Ghasemzadeh et al., 2018).

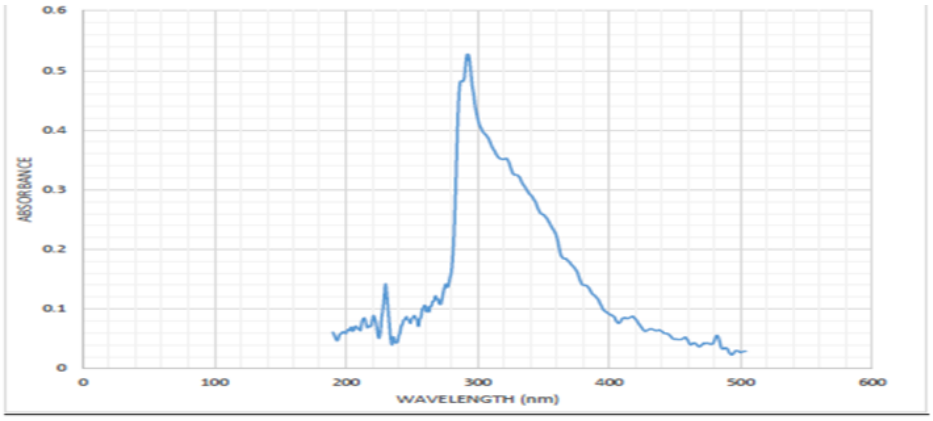

Figure 3 A. UV spectrum for batch A

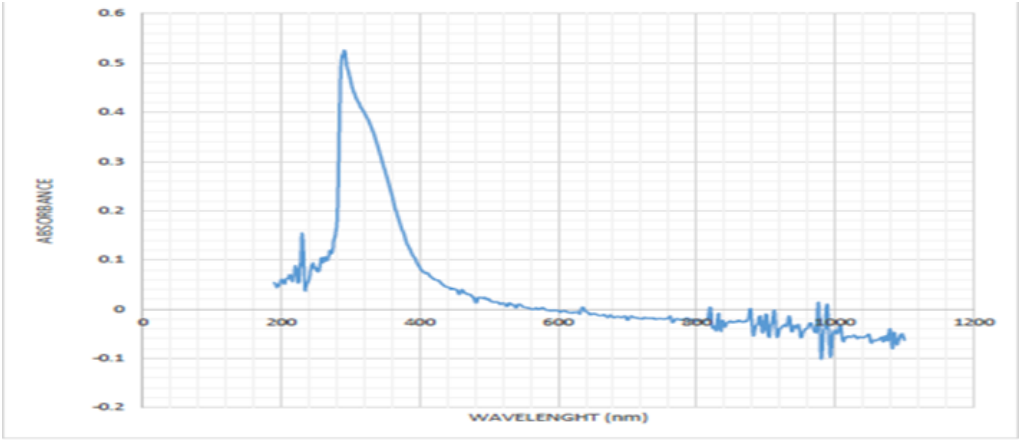

Figure 3 B. UV Spectrum for batch B

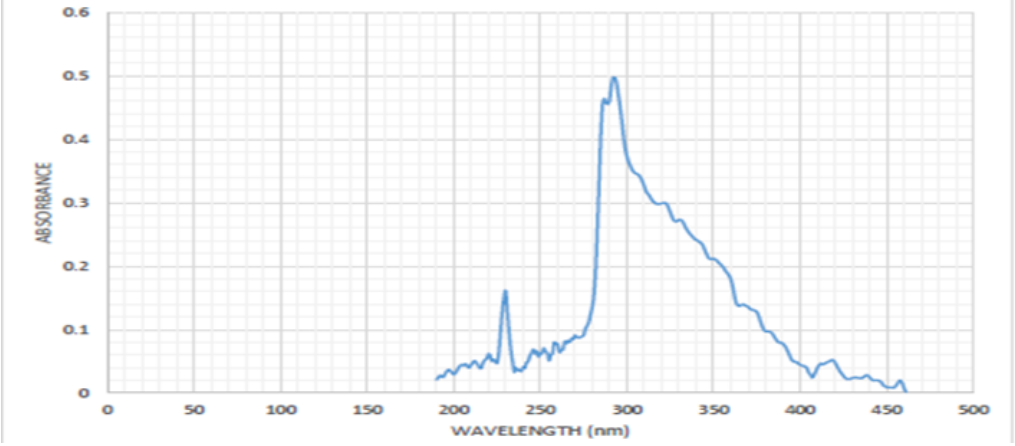

Figure 3 C. Spectrum for batch C

HPLC Fingerprints for MA001 decoction

In the ever-increasing market of herbal medicinal products, HPLC chromatogram fingerprinting has become a necessary tool for the qualitative but also quantitative analysis of plant medicines. For qualitative identification and verification of unknown plant extracts, principal component analysis (PCA) and hierarchical clustering analysis (HCA) are usually employed using specific bi-omarkers or reference standards (Wei et al., 2020). However, in the absence of a suitable reference standard, a simple and standard chromatographic fingerprint of the herbal product MA001 will suffice to pass regulatory requirements by the Ghana Food and Drug Authority and the World Health Organization (WHO 2017). Here, for determination of batch-to-batch consistency analysis, the retention times (RT) of resolved peaks common to the three sampled batches of MA001 are employed. A gradient HPLC method with increasing concentrations of acetonitrile in the mobile phase was used.

The chromatograms of formulated batches A, B and C under the same chromatographic condition showed a similar profile (Figure 4) with a cluster of peaks between retention times 11 and 20 min, and a few isolated peaks. Seven well resolved peaks common to the three batches of MA001 were selected as internal markers for quality control as presented in Table 3 below. The shifting retention times for the selected peaks from batch to batch are a common occurrence in HPLC and may be due to slight differences in factors including mobile phase composition, pH and flow rates (Bārzdiņa, 2022). The mean RT values and their corresponding standard deviations therefore allow for such minor variations in batch-to-batch handling. The % RSD between the RT values for the three batches for each marker peaks was less than 2.5 %. As there are no internationally accepted standards for herbal products, an inference from the ICH standards for inter-batch precision of orthodox drugs where % RSD values 0.5 % to 2.5 % are acceptable (ICH, 2022). Therefore, the HPLC chromatographic conditions gave precise and repeatable retention times with acceptable % RSD. The results indicates that, there is qualitative batch to batch consistency for the sampled batches of MA001.

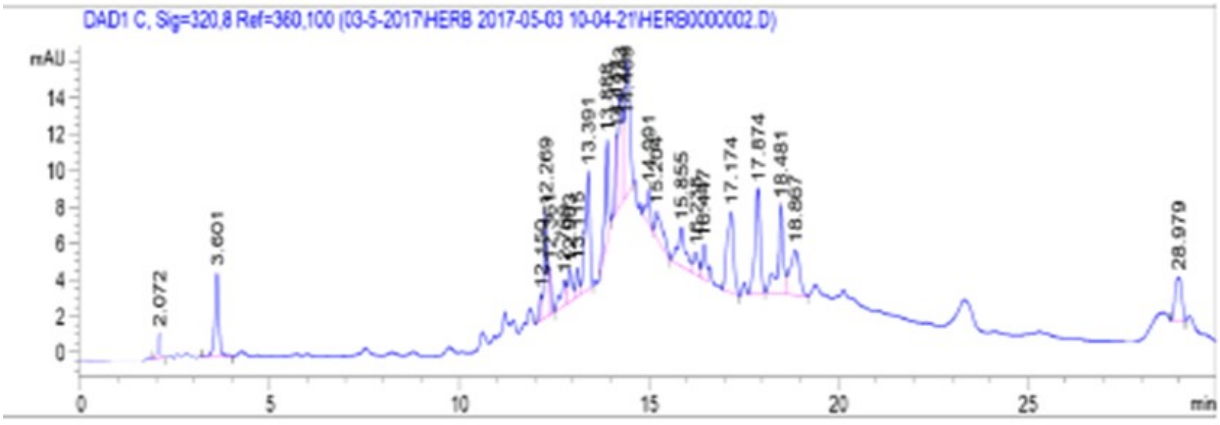

Figure 4 A. HPLC fingerprinting of batch A

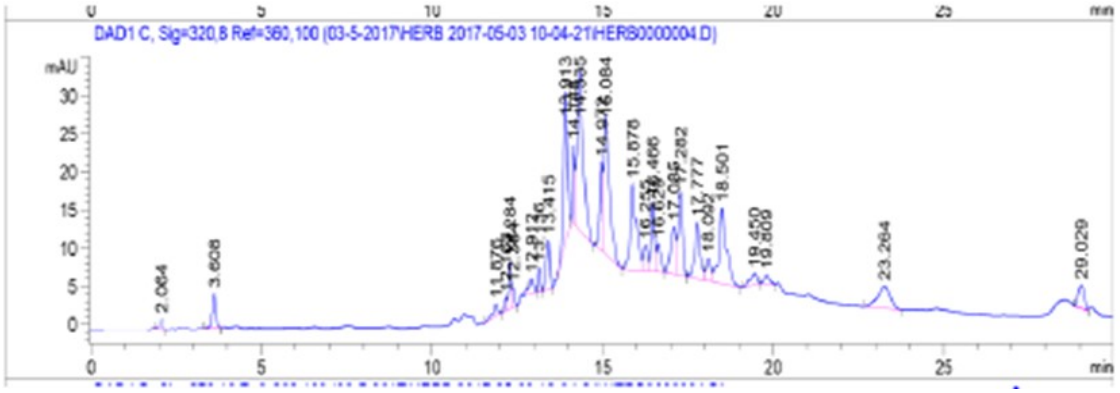

Figure 4 B. HPLC Fingerprinting of batch B

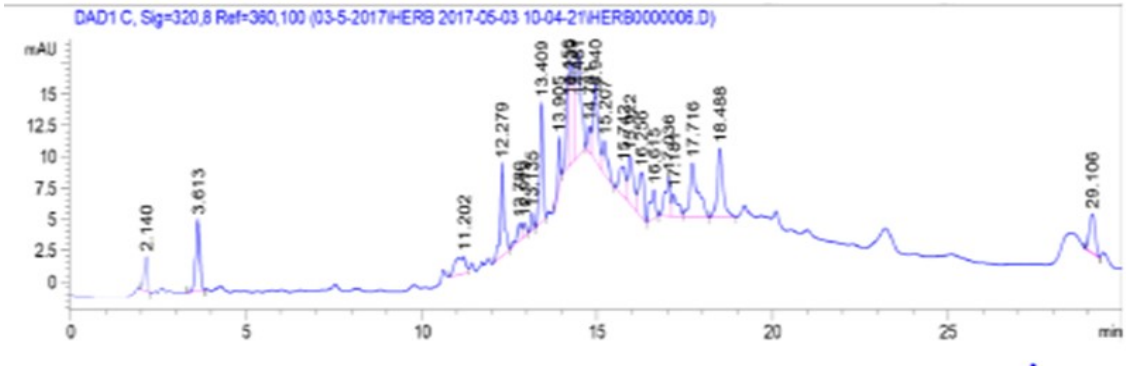

Figure 4 C. HPLC Fingerprinting of batch C

Table 3. HPLC retention times of selected peaks as markers for batch to batch consistency analysis

	Batch A	Batch B	Batch C	Mean R_T ± SD (min)	% Relative standard deviation (% RSD)
Retention times (R_T) of common peaks (min)	2.072	2.064	2.140	2.092 ± 0.0418	1.9962
	3.601	3.608	3.613	3.607 ± 0.0061	0.1671
	13.391	13.415	13.409	13.405 ± 0.0125	0.0932
	17.874	17.777	17.716	17.789 ± 0.0797	0.4479
	18.481	18.501	18.488	18.490 ± 0.0101	0.0549
	23.218	23.264	23.259	23.247 ± 0.0252	0.1086
	28.978	29.029	29.106	29.038 ± 0.0644	0.2219

Elemental contents in MA001 decoction

Elemental analysis on the test samples showed the presence and concentration of specific elements. The results indicated that the elemental contents in the sampled batches were within the permissible limits (Table 4). Also, statistical analysis of this result employing one-way ANOVA using Newman-Keul's multiple comparison test showed

that, there was no significant difference ($p < 0.05$) in the contents of Ni, Co, Mn, Cr, Mg and Ca among the 3 batches as well as in the contents of Al, Cu and Zn in batches A and B. However, there was a significant difference ($p < 0.0001$) in the Al contents between batches A versus B and A versus C; Fe contents between batches A versus C, B versus C and A versus B; Cu contents between batches A versus C and B versus C and finally, Zn contents between batches B versus C. Also, there was a significant difference ($p < 0.0011$) in the contents of Zn between the batches A versus C. This may be due to the use of plant parts sourced from different geographical area in the preparation of these 3 batches. Furthermore, the three batches of MA001 had some macro and micronutrients which are beneficial in the prevention and treatment of disease (Gasser et al., 2009; Shaban et al., 2016; Street, 2012) and were within accepted amounts (World Health Organization, 2003).

Table 4. Elemental contents in the 3 batches of MA001 decoction

Type of element	Batches						RDA (µg/day)
	A (µg/mL)	Max. qty. Per daily dose (µg/day)	B (µg/mL)	Max. qty. Per daily dose (µg/day)	C (µg/mL)	Max. qty. Per daily dose (µg/day)	
Toxic minerals							
Ni	0.006 ± 0.20	0.108 ± 0.20	0.006 ± 0.01	0.108 ± 0.01	0.005 ± 0.05	0.09 ± 0.05	100 (Das and Dasgupta, 2002)
Al	2.66 ± 0.00	47.88 ± 0.00	2.75 ± 0.08	49.5 ± 0.08	1.52 ± 0.04	27.36 ± 0.04	10×10^3 (Greger, 1992)
Micronutrients							
Co	0.002 ± 0.64	0.036 ± 0.64	0.004 ± 0.21	0.072 ± 0.21	0.003 ± 0.52	0.054 ± 0.52	
Fe	9.20 ± 0.15	165.6 ± 0.15	10.26 ± 0.11	184.68 ± 0.11	8.56 ± 0.25	154.08 ± 0.25	$18^f / 8^m \times 10^3$ (Koubova *et* al., 2018)
Cu	2.19 ± 0.01	39.42 ± 0.01	2.23 ± 0.23	40.14 ± 0.23	1.15 ± 0.18	20.7 ± 0.18	1.20×10^4 (N.R Council, 1989)
Zn	0.012 ± 0.09	0.216 ± 0.09	0.014 ± 0.33	0.252 ± 0.33	0.007 ± 0.08	0.126 ± 0.08	$1\text{-}2 \times 10^4$ (N.R Council, 1989)
Mn	0.141 ± 0.07	2.538 ± 0.07	0.137 ± 0.19	2.466 ± 0.19	0.084 ± 0.13	1.512 ± 0.13	1.10×10^4 (Russell, 2001)
Cr	0.008 ± 0.12	0.144 ± 0.12	0.01 ± 0.26	0.18 ± 0.26	0.005 ± 0.11	0.09 ± 0.11	20 (WHO, 2007)
Macronutrients							
Mg	1.0 ± 0.5	18 ± 0.5	1.02 ± 0.67	18.36 ± 0.67	0.06 ± 0.43	1.08 ± 0.43	$3.20^f / 4.20^m \times 10^6$ (Koubova *et* al., 2018)
Ca	0.16 ± 0.47	2.88 ± 0.47	0.11 ± 0.25	1.98 ± 0.25	0.10 ± 0.09	1.8 ± 0.09	1.00×10^6 (Koubova *et* al., 2018)

Max. – Maximum; qty- quantity; m males; f female; RDA –recommended Daily Allowance

Microbial Quality of the 3 batches of MA001

Microbial contamination of finished herbal products poses a health threat to consumers. Microorganisms that are likely to cause growth in such herbal products and subsequent contamination could result in disease and fatalities. Earlier investigations reported microbial contamination above the stipulated microbial limit in about 50% of the medicinal herbs in a Brazilian market. Usually, the presence of microbial growth could be a problem in aqueous herbal medicines stored at tropical or subtropical temperatures (Bugno et al., 2006). An evaluation of the microbial quality of all the batches of the finished product (MA001) showed the absence of the likely contaminants such as Salmonella, *E. coli*, and Staphylococcus and acceptable limits of fungi and aerobic bacteria (Table 5). However, there was a significant difference ($p < 0.05$) in the total aerobic microbial count for each batch, and no significant difference ($p > 0.05$) in the total yeasts and mold count (TYMC) for batches A and B whiles there was a significant difference ($p < 0.05$) in the TYMC between batch C and the rest of the 2 batches. This is indicative of the required quality of the raw materials and the application of best practices in the handling, processing and storage of the raw materials.

Table 5: Shows the results of the microbial quality test of 3 batches

Batches	Product code	Test conducted				
		TAMC/ 37 ºC/24 h/PCA	TYMC/25 ºC/ 5 days/ MEA	*E.coli*	*Staph. spp.*	*Salmonella spp*
	1	1.0×10^2	0	-	-	-
A	2	1.0×10^2	0	-	-	-
	3	1.0×10^2	0	-	-	-
	1	0	0	-	-	-
B	2	0	0	-	-	-
	3	0	0	-	-	-
	1	1.4×10^3	2.0×10^2	-	-	-
C	2	1.4×10^3	2.0×10^2	-	-	-
	3	1.4×10^3	2.0×10^2		-	-
Acceptance Criterion (BP 2021)		$\leq 5.0 \times 10^7$	$\leq 5.0 \times 10^5$	1000 CFU	Absent	Absent

KEY: (+) Growth observed; (-) No Growth observed; TYMC – Total Yeasts and Mold Count; TAMC – Total Aerobic Microbial Counts

Even though the preparation was aqueous with a susceptibility to microbial growth, and contaminants, the application of good manufacturing practice (GMP) in the preparation and good agricultural and collection practices (GACP) in the collection of the raw herbal materials and processing, played a unique and major role in reducing the risk of such possible contamination. Such measures enhance the microbial quality of potent herbal medicines. The use of water instead of organic solvents for the extraction and preparation of the finished product also has the advantage of reduced contamination due to the presence of residual solvents (Gu et al., 2004). However, no growth observed in the three batches of MA00l might also be due to the presence of phytochemicals. Flavonoids are widely recognized as antibacterial agents against gram-positive and gram-negative bacteria, including *Escherichia coli*, *Pseudomonas*

aeruginosa, and *Staphylococcus aureus*, by preventing cytoplasmic and porin degradation in the cell membrane, blocking the formation of biofilm, and energy metabolism (Liu et al., 2021). Antibacterial activity of tannins has been reported to have the potency to interfere with the cell metabolism and destruction of gram positive and gram-negative bacteria such as *Staphylococcus aureus* and *Escherichia coli* by passing through the cell walls up to the internal membrane and causing blockage. Bacteria cells can die if there is blockage of the bacteria's adherence to the surface (Belhaoues et al., 2020; Vance et al., 2011). Alkaloids show antibacterial activity by preventing ATP-dependent transport of compounds across the cell membrane (Nikaido, 2009) of gram-negative and gram-positive bacterial

Conclusion

All randomly selected products from the three (3) different batches were consistent in all parameters assessed. The three batches of the MA001 showed no microbial growth against the selected strains of bacteria, hence exhibited consistency in their activity against the selected bacteria. This implies that, these batches of MA001 will exhibit consistency in pharmacological activity and safety of the product. These obtained reproducible organoleptic, physicochemical and instrumental characterization of MA001 could be used as a standard for quality evaluation of MA001.

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