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Development of simplified HPLC methods for the detection of counterfeit antimalarials in resource-restraint environments

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ABSTRACT

Regular quality control and post-marketing surveillance of pharmaceuticals has been a critical challenge for countries of the developing world ever since. Counterfeit and substandard medicines are widely distributed and the real extent of their prevalence still remains unknown. Compendial protocols and methods utilizing high-performance liquid chromatography (HPLC) which are described in the major pharmacopoeias are widely applied for the quality control of a compound. They often require expensive solvents, delicate reagents and/or sophisticated apparatus, and may not be applicable and affordable for laboratories with limited capabilities. Simple but robust HPLC methods for the determination of five commonly used antimalarial agents, i.e. amodiaquine, mefloquine, proguanil, artemether and lumefantrine, were developed and their suitability for routine use in resource-restraint environments is discussed. They solely require readily available chemicals and solvents and exhibit a high grade of ruggedness.

1. Introduction

The quality of pharmaceutical and medicinal products is critical because it eventually determines effectiveness and safety of modern therapies. Of note, the prevalence of counterfeit and substandard medicines is particularly high in countries of the developing world, e.g. in sub-Saharan Africa, and the demand for simple methods of analysis for their detection has been anticipated e.g. by Glass, Tremblay or Kovacs et al. recently [1–3]. The careless application of antibiotics and anti-infective agents, respectively, which contain less than the declared amount of an active pharmaceutical ingredient (API) or a more or less high percentage of impurities, may not only lead to intoxication or even lethal treatment failures but may also evoke substantial resistances [4,5]. These threats cannot be ignored nowadays as the incidence for deaths and consequently the overall mortality rate caused by the application of such products correlates with a broadened distribution of these APIs on the one hand and the resistances on the other hand.

The *World Health Organization* (WHO) defines counterfeit medicines as follows: "Spurious/falsely-labelled/falsified/counterfeit (SFFC) medicines are medicines that are deliberately and fraudulently mislabelled with respect to identity and/or source. (. . .) Both branded and generic products are subject to counterfeiting (and) (. . .) may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient or too much active ingredient, or with fake packaging. (. . .) They range from random mixtures of harmful toxic substances to inactive, ineffective preparations." [6].

This description is considered the most detailed nowadays, however many mixed forms exist and it is not trivial to allocate counterfeit products to one of the following five categories:

(i) imitation of commercial products either of acceptable or poor quality (plagiarisms);
(ii) products which do not contain the declared API at all or contain a substitute;
(iii) formulations which contain considerably more or less than the declared amount of an API; and
(iv) contamination with known and/or unknown/unexpected impurities.

Recently Almuzaini et al. evaluated data from 15 studies on the distribution of counterfeit medicines and found a considerably high prevalence of adulterated products (approx. 30%) in 25 low- and middle-income countries [7]. Even worse, National Medicine Regulatory Authorities (NMRAs) in the developing world often cannot shoulder the challenges of regular post-marketing surveillance for several reasons, mainly due to the lack of adequately trained

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personnel, limited laboratory facilities as well as a stagnant supply of chemicals and supplies.

Even though these problems are well known no conclusive strategies to control the dissemination of low-quality products exist and the real extent of the situation still remains unknown. The mobile *GPHF Minilab*® (ML) for routine medicine quality testing under field conditions provides simple assays for routine use. It employs basic colorimetric tests for the identification and semi-quantitative thin-layer chromatographic (TLC) determinations of meanwhile 70 APIs which have been chosen in accordance with the *WHO Essential Medicines List* [8]. Besides the fact that the content of a sample can be determined semiquantitatively at the very most, potential impurities or degradation products may not be resolved reliably and compound replacements may not be detected at all. An investigation on the quality of antimalarial medicines which was published by the WHO in 2011 revealed that the ML protocols failed to determine non-compliance of the content in approximately 60% of the cases [9].

In contrast, the major pharmacopoeias, e.g. the United States Pharmacopoeia (USP), the British Pharmacopoeia, the Japanese Pharmacopoeia and the European Pharmacopoeia (Ph. Eur.) list highly established compendial methods which are routinely applied in the quality control (QC) of medicines nowadays. Almost all monographs describe the application of sophisticated *High Performance Liquid Chromatography* (HPLC) being the gold standard in pharmaceutical QC [10]. Whereas gradient HPLC and UPLC with a collection of very sensitive detectors is the standard equipment in modern laboratories these facilities are not available in resource-restraint environments (i.e. in low- and middle-income countries of the developing world and especially in rural areas).

On the other hand, the *International Pharmacopoeia* (Ph. Int.) published by the WHO describes individual monographs for the analysis of APIs from the *WHO Essential Medicines List*. The objective was a global unification of quality specifications for selected pharmaceutical products, excipients and dosage forms, but the tests

Fig. 1. Typical setting with limited capabilities of a testing laboratory in Africa.

demand for almost the same methodologies described in the Ph. Eur or the USP [11]. These methods can be used in licensing and surveillance authorities only, which can be very rarely found in low- and middle-income countries and which often lack the power to fight against counterfeits. Laboratories in rural areas often are underequipped and do not have modern instruments or facilities (cf. Fig. 1).

In order to overcome these imponderabilities and to facilitate routine pharmaceutical QC in developing countries we established simplified, individually adapted HPLC testing protocols for the analysis of commonly used antimalarial APIs (i.e. mefloquine, amodiaquine, proguanil, artemether and lumefantrine, cf. Fig. 2). A minimum of readily available and cheap chemicals, solvents and standard C-18 reversed-phase (RP) columns is required, and the methods are designed as simple and robust as possi-

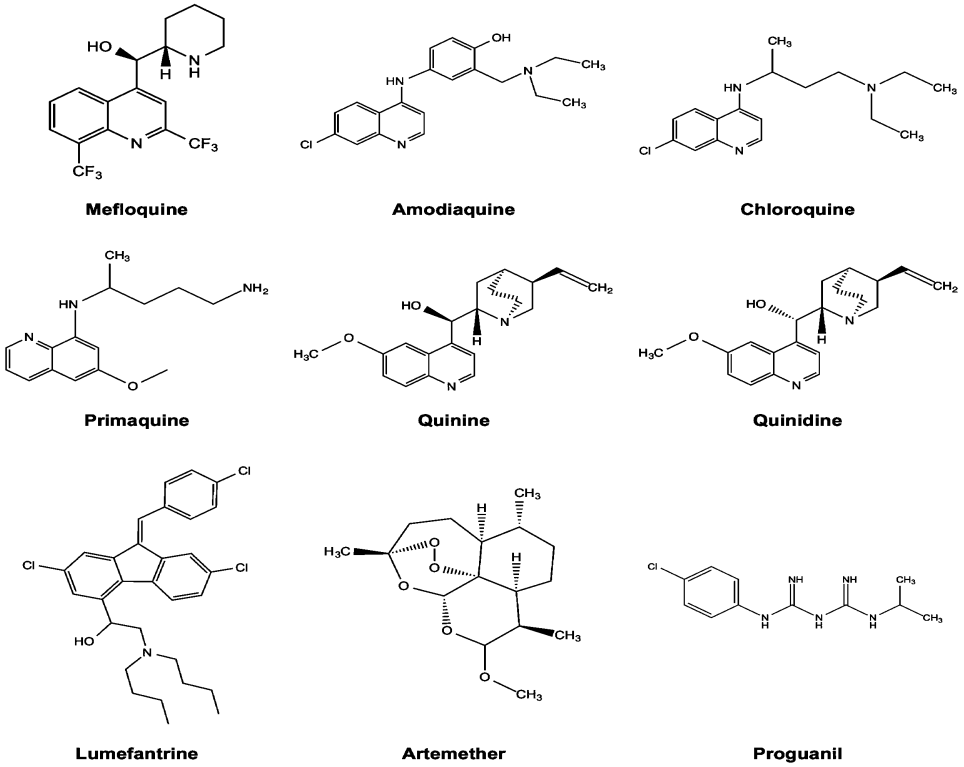

Fig. 2. Structural formulae of mefloquine, amodiaquine, lumefantrine, artemether, proguanil and chloroquine, quinine, quinidine, and primaquine.