APPLICATION OF EXPERIMENTAL DESIGN AND DESIGN FOR THE SEPARATION OF COMPOUNDS EXTRACTED FROM THE LEAVES OF *STRYCHNOS USAMBARENSIS*

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Malaria is one of the most common parasitic infections in Africa. The resistance of *Plasmodium falciparum* to existing drugs on the market represents a major problem in the field of public health. This is one of the main reasons why the discovery of new antimalarial molecules is a research topic of high importance. The African flora is an immense reservoir of substances with potential therapeutic effect against this tropical parasitosis. These antimalarial properties [1] are present especially in some alkaloids extracted from *Strychnos usambarensis* such as, for example, isostrychnopentamine. In addition, several tertiary alkaloids isolated from the same species also show significant cytotoxic properties. Identification, isolation and quantification of these alkaloids is therefore essential, mostly for their pharmacological exploration. However, the complexity of the alkaloid composition of the leaves of *Strychnos usambarensis* raises some difficulties regarding the separation of all extracted compounds. Considering the similarity of the structures of these compounds, their chromatographic behaviour and their UV-visible spectrum lead to a major analytical challenge in terms of coelution and specific determination. This separation was performed using ion-pair liquid chromatography. The parameters governing the separation of these molecules were optimized by experimental design [2]. The factors belonging to the full factorial experimental design selected were the percentage of organic modifier at the beginning of the gradient, the gradient time to progress from this initial percentage to 70% of organic modifier and the proportion of acetonitrile in mobile phase. Retention times of compounds were modeled by multiple linear equations which allowed the calculation and optimization of the critical separation, i.e. the time between the end and the beginning of two peaks considered as the critical pair. To estimate the robustness, the probability of achieving a separation greater or equal to zero (resolution ~ 1.5) was calculated using Monte Carlo simulations. It was then possible to define the design space of the analytical procedure developed as the area where this probability exceeds a predefined level of quality (eg 80%). The operating conditions included in the design space will provide separations that meet the requirements of separation. This optimization methodology has lead to an optimal experimental condition for the separation of the six compounds of interest, along with a quantification of the robustness of the method.

References

1) M. Frederich et al., Trans. R. Soc. Trop. Med. Hyg., 102 (2008), 11-19.
2) P. Lebrun et al., Chemom. Intell. Lab. Syst., 91 (2008), 4-16.