CONTRIBUTION TO FIGHT AGAINST COUNTERFEIT MEDICINES APPLYING SEVERAL ANALYTICAL TOOLS

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1. INTRODUCTION

Counterfeiting has been dramatically increasing this last decade throughout the world and particularly in developing countries, where unimaginable proportions rising up to 80 % of counterfeit have been reported, meaning that a patient has only 1 chance over 5 to have a medicine that can really be useful and helpful. This negative situation presents many consequences such as adverse impacts on public health, economics and negative reputation for the pharmaceutical industry. Recognizing the impact of this situation, Health Authorities at national, regional and international levels are trying to fight against this scourge. For example, several strategies are discussed among which the setting-up of effective quality control that need to be reinforced through generic, fast and specific detection methods.

2. OBJECTIVES

❑ Contribution to the reinforcement of the strategies elaborated by Health Authorities in order to provide scientific support of decisions from Regulatory Agencies.

❑ Presentation of several analytical tools applied to the detection and quantitation of counterfeit drugs :

1.- Liquid Chromatography (LC) tool for antimalarial drugs;

2.- Capillary electrophoresis (CE) tool for antiHIV drugs;

3.- Near Infrared (NIR) spectroscopy tool for paracetamol in syrup.

3. RESULTS AND DISCUSSION

3.1. LC tool

Our contribution was focused on monitoring of 20 compounds, including 16 antimalarial drugs and 4 conservatives most commonly used.

❑ **Optimisation of the LC method**

Three factors (Table 1) previously selected among others were tested and studied with regards to the chromatographic behaviour of analytes (with the logarithm of the retention factors being selected as chromatographic responses).

Table 1. Selected factors and their levels for the optimisation of the LC conditions

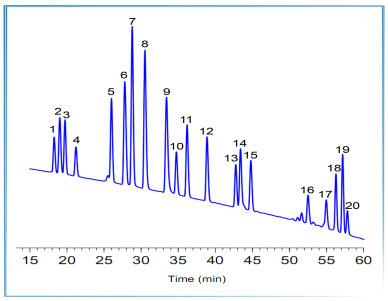
Factors	Level of the selected factors				
Column temperature (**T°**)	25°C		30°C		35°C
Aqueous fraction of the mobile phase pH (**pH**)	2.5	4.0	6.0	8.0	10.0
Gradient time from 5% to 95% of methanol (T_G)	20 min		40 min		60 min

An experimental full factorial design was applied while modelling all peak responses by means of the equation 1.

$$\text{Log}(k) = \beta_0 + \beta_1.pH + \beta_2.pH^2 + \beta_3.pH^3 + \beta_4.pH^4 + \beta_5.T_G + \beta_6.T_G^2 + \beta_7.T° + \beta_8.T°^2 + \beta_9.pH.T_G + \beta_{10}.pH.T° + \beta_{11}.T°.T_G + \beta_{12}.pH.T°.T_G + \varepsilon \quad \text{(Equation 1)}$$

with k the retention factor ($k = (t_R - t_0)/t_0$). Logarithms of peak half-widths were also modelled by equation 1. Each analyte's chromatographic behaviour was independently modelled by a set of 3 equations, for the retention factor and for left and right half-widths. Models were adjusted through a stepwise regression and were found excellent ($R^2_{adjusted}$ > 0.99) meaning that the responses were correctly modelled. Residuals were normally distributed, mostly between -1 and 1 min. The Design Space (DS), which defines the multidimensional sub-region in which the probability that a criterion reaches the preset threshold is greater than a selected quality level, was calculated using the equation 2.

$$DS = \left\{ x_0 \in \chi \middle| E_\theta\left[P(S > \lambda) \middle| \theta \right] \geq \pi \right\} \quad \text{(Equation 2)}$$



Legend:
Chloroquine (**1**), Sulfalene (**2**), Amodiaquine (**3**), Sulfadoxine (**4**), Cinchonine (**5**), Methylparaben (**6**), Quinine (**7**), Pyrimethamine (**8**), Piperaquine (**9**), Primaquine (**10**), Proguanil (**11**), Propylparaben (**12**), Mefloquine (**13**), Butylhydroxyanisole (**14**), Artesunate (**15**), Artemether (**16**), Arteether (**17**), Butylhydroxytoluene (**18**), Lumefantrine (**19**), Atovaquone (**20**).

Figure 1. Typical chromatogram of the 20 compounds obtained under the optimal LC conditions : *XBrigde column (250 x 4.6 mm; i.d.) packed with C18 (5 µm dp). Mobile phase: mixture of methanol and 20mM ammonium formate buffer at pH 4.05. TG : 56 minutes. Flow rate: 1.0 mL.min^{-1}, T°: 25°C. UV- detection: 230 nm.*

with x_0 an experimental point of the domain, χ. λ is the acceptance limit for the selected criterion (*S* for separation) and π is the level of quality. *P* represents the estimator of probability and E, the estimator of mathematical expectation. The highest probability value to attain *S*>0 (i.e. baseline resolved peaks) within *DS* was 42 % that corresponded to LC conditions allowing a suitable separation of all the compounds of interest within an acceptable analysis time (Figure 1).

The method was applied for the assay of Artesunate and Amodiaquine in Coarsucam® tablets. Both active ingredients were conform with contents of 99.3 % and 104.4 %, respectively.

3.2. CE tool

We have focused our contribution on the consideration of a low cost analytical CE device "CE Budget Device Prototype 2" (ECB2), equipped with an original detection system based on Light-Emitting Diodes (LEDs) (Figure 2). The CE technique was Micro Emulsion Electro Kinetic Chromatography (MEEKC). Several typical factors were tested (table 2). The best separation of the peak analytes within a suitable analysis time was obtained using the CE conditions described in the Figure 3.

Table 2. Selected factors and their levels for the optimisation of the CE conditions

Factors	Background buffer and concentration		pH value		Surfactant	Co-surfactant	Organic solvent	
	NH_4-Acetate	Na-Borate			Na dodecyl sulphate	Butanol	Heptane	Octane
Values	20 mM	50 mM	9	10	3.3 %	6.7 %	0.8 %	0.8 %

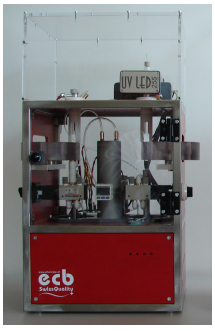


Figure 2. A CE Budget Device Prototype

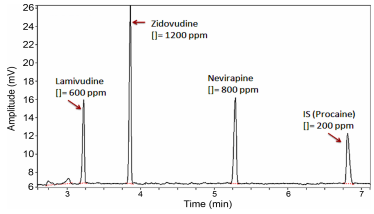


Figure 3. Typical electropherogram of lamivudine, zidovudine and nevirapine in presence of procaine (internal standard).
Separation buffer: Na-borate 50 mM pH 9.0, SDS 3.3 %, Butanol 6.7 %, Heptane 0.8 %.
***CE conditions**: Capillary: 51 cm (total length), 39.2 cm (length to detector), 50 µm (internal diameter). Applied voltage: 20 kV. Injection: 7 s at 50 mbar, UV detection: 254 nm.*

3.3. NIR tool

A NIR method based on Principal Component Analysis (PCA) was developed to distinguish a genuine and counterfeit low-dose pharmaceutical syrup of paracetamol (2% (w/w)). PCA allows exploring data analysis and building predictive models by reducing the multivariate spectra data to only a few important variables called principal components. Three cases were investigated: syrups with inadequate concentration of paracetamol, without paracetamol and where glycerol was replaced by diethylene glycol (a toxic excipient). From figure 4, it can be seen that PCA clearly discriminates genuine from counterfeit syrups. All genuine samples used to test the predictive model fall in the 95% confidence interval while all counterfeit samples fall outside this delimited area even in the case where only slight differences in the concentration of paracetamol exist. Moreover, syrups containing the targeted concentration of paracetamol and diethylene glycol instead of glycerol were also unequivocally identified as a counterfeit product.

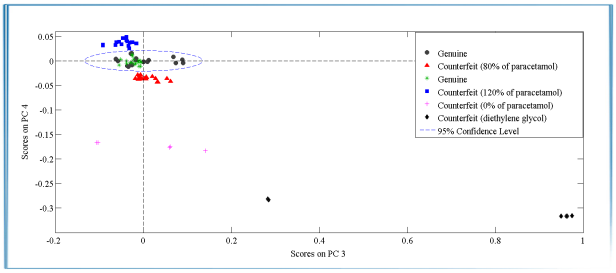


Figure 4 : *PCA score plot of genuine and counterfeit low-dose syrups of paracetamol from NIR data.*
***Legend**: (●) genuine syrups used to build the predictive model, (*) genuine syrups used to test the predictive model (■) counterfeit syrups (120% of targeted concentration), (▲) counterfeit syrups (80% of targeted concentration), (+) counterfeit syrups without paracetamol, (◆) counterfeit syrups containing the targeted concentration and diethylene glycol instead of glycerol and the dotted blue line represents the 95% of confidence interval of the genuine syrups.*

4. CONCLUSION

Very interesting and promising results were obtained with LC, CE and NIR tools in several pharmaceutical dosage forms, thus allowing these tools to strengthen their application in the fight against counterfeiting.

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