

1. Introduction

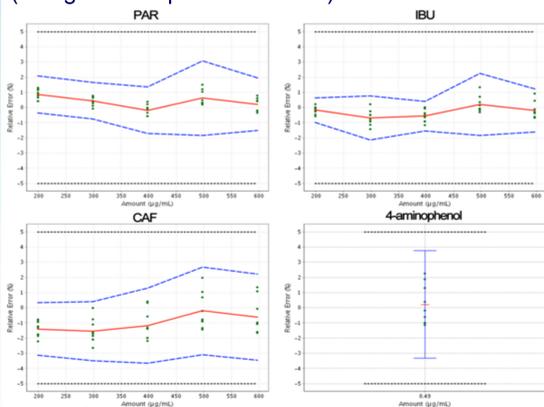
Non-steroidal anti-inflammatory drugs (NSAIDs) are used against pain, fevers of various origins and inflammation. Although they are widely prescribed throughout the world, many of them are associated with side effects. These drugs are often used in self-medication as their purchase is also unrestricted over the Internet. The risk of administration of uncontrolled medicines is thus naturally greater.

Furthermore, NSAIDs are often subject to the practice of counterfeit medicines, which is gaining increasing momentum in the world and particularly in emerging countries where unimaginable proportions rising up to 80 % of counterfeit have been reported. This has adverse consequences for public health.

5. Validation

The developed methods were validated according to the total error strategy using the accuracy profile as decision tool [Ph Hubert et al., *J. Pharm. Biomed. Anal.* 45 (2007) 82]. An illustration is given for group 4 (Figures 3).

Figures 3 : Accuracy profiles for the molecules of group 4 and for 4-aminophenol (a degradation product of PAR).



For 4-aminophenol, a one-level calibration was used: 0.1% (0.5 µg/mL) in 100% of PAR (500 µg/mL).

All validation criteria recommended by ICH Q2(R1) (trueness, precision, accuracy) were simultaneously tested by means of the applied strategy.

Since the Beta-expectation tolerance intervals or limits (for 4-aminophenol) are included within the acceptance limits (Figures 3), one can conclude that the method is suitable for the quantification purpose.

Legend
 (—): Relative bias (%).
 (---): Acceptance limits (5%).
 (---): Beta-expectation tolerance interval (95%).
 (*): Relative error of the back-calculated individual measurement.

6. Application

The validated method was applied to the identification and the assay of PAR, CAF and IBU. As a real case, five different brands of pharmaceutical drugs were tested. They were purchased in capsule form in D.R. Congo (DRC) and were mainly from Indian origin. The five drugs contained the three active ingredients but as shown in Table 3, most of the products were in one way or another non-compliant to the specifications of EMA (95.0 – 105.0%).

Table 3 : Assay results of five pharmaceuticals brands marketed in DRC

Coded specialties (Brands)	Declared amount Content obtained (mean % ± SD; n = 3)		
	PAR	CAF	IBU
A	325 mg ; 98.4 ± 0.41 %	30 mg 90.7 ± 1.49 %	200 mg 103.7 ± 0.74 %
	B	325 mg 100.0 ± 0.35 %	40 mg 94.7 ± 0.63 %
C		200 mg 90.4 ± 0.22 %	40 mg 85.2 ± 0.79 %
	D	325 mg 78.2 ± 0.39 %	40 mg 74.5 ± 0.44 %
E		325 mg 78.9 ± 0.28 %	40 mg 75.9 ± 0.31 %

2. Objectives

To develop a generic analytical method by means of an experimental design combined to a novel Design Space (DS) optimization strategy using Liquid Chromatography with UV detection.

To demonstrate the robustness of the developed method (transfer HPLC to UHPLC).

To validate the developed methods and apply them in analysis of real samples.

27 molecules were selected, including 18 NSAIDs, 3 molecules often associated with NSAIDs, 1 degradation product and 5 preservatives. These molecules were divided into 5 groups on basis of the pharmaceutical form of the NSAIDs.

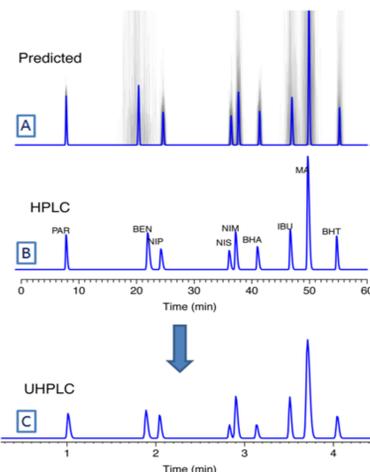
Group 2 includes the compounds presented in syrup and suspension (paracetamol (PAR), Na-benzoate (BEN), nipagine (NIP), nipasol (NIS), nimesulide (NIM), butylated hydroxyanisole (BHA), ibuprofen (IBU), mefenamic acid (MA) and butylated hydroxytoluene (BHT). Group 4 includes the pharmaceutical combinations presented in tablet or capsule (paracetamol, ibuprofen and caffeine (CAF), etc.).

4. Demonstration of the robustness

The optimized HPLC methods were transferred to UHPLC with very satisfying results. An illustration is given for group 2 (Figures 2 and Table 2).

Figures 2 : Chromatograms of the developed method under predicted computed conditions (A) and with HPLC (B) and UHPLC (C) equipments.

Table 2 : Results of the transfer from HPLC to UHPLC for group 2 and considering the relative retention times (RRT).



Compound	Predicted RRT	Observed RRT (HPLC)	Observed RRT (UHPLC)
PAR	0.141	0.146	0.150
BEN	0.369	0.406	0.396
NIP	0.446	0.447	0.438
NIS	0.659	0.662	0.657
NIM	0.682	0.682	0.680
BHA	0.749	0.750	0.743
IBU	0.850	0.854	0.849
MA	0.904	0.910	0.908
BHT	1.000	1.000	1.000

RRT for predicted conditions and those obtained with HPLC and UHPLC were close. Similar results were obtained for the remaining groups demonstrating the adequate geometric transfer of the methods. Moreover, the transfer results show that the variability due to the transfer did not decrease the chromatographic separation efficiency.

LC conditions : Analytical column : Xbrigde 250 x 4.6 mm; i.d., dp=5 µm). Mobile phase : mixture of methanol and 20mM NH₄-Formate buffer (pH 4.05). Gradient increased linearly methanol proportion from 15% to 95% in 53.14 minutes.

UHPLC conditions: Analytical column: Acquity BEH (50 mm x 2.1 mm i.d., dp = 1.7 µm). Flow rate: 0.613 µL.min⁻¹.

Flow rate: 1.0mL.min⁻¹, column T°: 23°C. UV-detection: 220 nm

3. Optimisation and prediction

Three factors (Table 1) previously selected after a screening phase were tested and studied with regards to the chromatographic behavior of analytes (with the logarithm of the retention factors being selected as chromatographic responses) [B. Debrus et al., *Anal. Chim. Acta.* 691 (2011) 33].

Table 1 : Selected factors and their levels for the optimization of the LC conditions

Factors	Levels						
	1.85	2.42	3.14	4.42	5.71	6.42	7.00
pH	-	-	-	-	-	-	-
Gradient time (TG, min)	-	20.0	24.5	40.0	55.5	60.0	-
Temperature (T°, °C)	-	20.0	21.7	27.5	33.3	35.0	-

An experimental central composite design custom was applied while modeling all peak responses by means of equation 1.

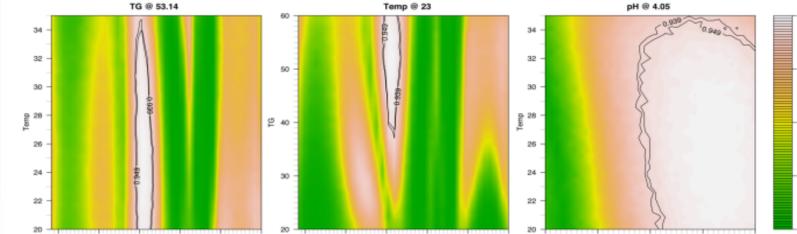
$$\log(k) = \beta_0 + \beta_1 \cdot \text{pH} + \beta_2 \cdot \text{pH}^2 + \beta_3 \cdot \text{pH}^3 + \beta_4 \cdot \text{pH}^4 + \beta_5 \cdot \text{TG} + \beta_6 \cdot \text{TG}^2 + \beta_7 \cdot \text{T}^\circ + \beta_8 \cdot \text{T}^{\circ 2} + \beta_9 \cdot \text{pH} \cdot \text{TG} + \beta_{10} \cdot \text{pH} \cdot \text{T}^\circ + \beta_{11} \cdot \text{T}^\circ \cdot \text{TG} + \beta_{12} \cdot \text{pH} \cdot \text{T}^\circ \cdot \text{TG} + \varepsilon \quad (\text{Eq. 1})$$

with k the retention factor ($k = (t_R - t_0)/t_0$). Logarithms of peak half-widths were also modeled by equation 1. Each analyte's chromatographic behavior was independently modeled 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 modeled. 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 equation 2.

$$DS = \{x_0 \in X : P(CQAs \in \wedge / x_0, data) \geq \pi\} \quad (\text{Eq. 2})$$

Figures 1 : DS for group 2 with the white region including the minimum quality level $\pi = 95\%$



The DS shapes depicted in Figures 1 show broad regions with respect to variations in TG [40, 60] min, in T° [20, 35]°C and in pH [3.90, 4.30].

These broad regions are key results as they represent robustness of the operating conditions if quality level is high, indicating guarantees of quality for future use of the methods.

7. Conclusion

The use of experimental design allowed us to optimize simultaneously three selected factors including T°, pH and TG. This led to the development of HPLC methods for screening NSAIDs.

The DS strategy enabled robust method development, confirmed by a successful method transfer from HPLC to UHPLC. The validated method can easily be used in quality control laboratories in emerging countries.

The results obtained during routine testing confirmed that the phenomenon of poor quality medicines remains a major public health problem.

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