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# Transfer, validation and application of HPLCanalytical method for simultaneous determination of paracetamol and diclofenac in tablets

**Emile Tweni Baruti[1¥], Jérémie Mbinze Kindenge[2], Issa Yakusu Issa[3], Marguerite Borive Amani [1], Elodie Nsasi Bankiantima[1], Justin Ntokamunda Kadima[1,5], Roland Marini Djangeing'a [1,4].**

*1. Department of Pharmacy, Faculty of Medicine and Pharmacy, University of Kisangani, Democratic Republic of Congo;*

*2. Drug Analysis Laboratory, Department of Galenics and Drug Analysis, Faculty of Pharmaceutical Sciences, University of Kinshasa, Democratic Republic of Congo;*

*3. Department of Internal Medicine, Faculty of Medicine and Pharmacy, University of Kisangani*

*4. Laboratory of Pharmaceutical Analytical Chemistry, Faculty of Medicine, University of Liège, Belgium;*

*5. Department of Clinical Pharmacology, School of Medecine and Pharmacy, University of Rwanda, Rwanda.*

[¥]*Corresponding author: emiletweni@gmail.com*

**ABSTRACT**

*Various pharmaceutical preparations containing paracetamol in combination with other nonsteroidal anti-inflammatory drugs are produced for the treatment of postoperative pain management. A lack of selective analytical techniques makes it difficult to fully assay all incorporatedactive ingredients. An original HPLC method developed at the University of Liège for the assay of the paracetamol-diclofenac combination in tabletshas been transferred and validated at the University of Kisangani for its application locally. The use of an Xbridge® column (C18, 100 x 4.6 mm/3.5µm) instead of Xbridge® (C18, 250 x 4.6mm/5µm) allowed to reduce the time of analysis from 60 min to 20 min, as well as the consumption of methanol in the mobile phase, while maintaining the efficiency and selectivity of the original method. After validation the method was applied for the analysis of two generics marked in the city. The tranferedmethod allows an appreciable saving in time and in the solvent economy to be applied in routine for quality control of generics in circulation.*

**Keywords**: *Paracetamol, Diclofenac, Tablets, HPLC, Geometric transfer, Validation, Optimization*

## INTRODUCTION

According to the WHO, 10% of drugs circulating in the world are of substandard quality, and the rate can reach proportions of 25 to 80% in Africa [1]. This requires the development of reliable analytical methods to ensure quality control [2, 3]. Increasingly, paracetamol is being formulated in combination with other nonsteroidal anti-inflammatory drugs (NSAIDs) for the treatment of postoperative pain[4]. The lack of physical methods of separating paracetamol in combination with diclofenac in compressed forms makes it difficult to fully dose these two active ingredients[5]. The United States Pharmacopeia (USP 39, 2016) and other monographs do not provide analytical methods for this combination. In 2012, Mbinze et al. [5] had developed and validated at the University of Liège in Belgium, an analytical method by high-performance liquid chromatography (HPLC)coupled to the UV detector for the simultaneous analysis of several combinations of NSAIDs under the same chromatographic conditions. The method received an Award at the 2012 HPLC International Congress in California, United States [6].

In the Democratic Republic of the Congo(RDC) and perhaps also in other African countries, the control laboratories are limited to dosing only paracetamol by default of separative methods. It is in this context that we tried to transfer, validate and apply the original method to the analysis of generics combining paracetamol and diclofenac with the objectives of reducing the analysis time and the consumption of methanol while maintaining efficiency and selectivity.

## MATERIALS AND METHODS

### *MATERIALS*

We used Diclofenac (SCR) (99.7%) from Sigma-Aldrich (Antwerp, Belgium), and Paracetamol SCR (99.5%) fromFagron N.V. (Waregem, Belgium) as reference standards and two generics DICDOL and VOLPA®containing each paracetamol 325 mg + diclofenac 50 mg. DCDOL presented as white-orange tablets scored inblister pack; batch 08; made on 07/2016; expire on 06/2019; manufactured by Renumed Pharmaceutical labs,Plot N ° 15, DEWAN α Sons Udyog Nagar, Polgar, MH in INDIA. VOLPA® presented as white-orange tabletsin blister packs; batch 00880; made on 07/2016; expire on 06/2019; manufactured by Syncom formulations (I)Ltd. Mumbai- 400093 in INDIA The samples were purchased in a community drug dispensary in the

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municipality of Makiso (Kisangani, DR Congo). The solvents consisted of HPLC grade methanol, hydrochloric acid (37%) from Merck (Darmstadt, Germany); ammonium formate (99%) from Alfa Aesar (Karlsruhe, Germany); ultra-pure quality water (18.2 MΩ.cm) purified with Milli-Q Plus 185 from Millipore (Billerica, Massachusetts-MA, USA) from Arauphar (Kinshasa, DR Congo).

*PREPARATION OF SOLUTIONS*

The standard stock solutions were prepared by accurately weighing 80 mg of SCR PC and 12.3mg of DICLO into a 20 ml volumetric flask. The drugs were dissolved in methanol, and the solution was diluted to volume with methanol-water milli-Q (50/50, v / v) to have 4000µg / ml. Successive dilutions were made from this stock solution with methanol-water milli-Q to prepare three solutions for calibration 240µg/ml, 160µg/mL), 80µg/mL.Also, successive dilutions were made from the stock solution with methanol-water milli-Q to have five standards solutions for validation 240µg/ml, 200µg/ml, 160µg/mL), 120µg/mL, 80µg/mL.The solution of 10 mM ammonium formate buffer was prepared by dissolving 0.16 g of ammonium formatein 200.0 mL water Milli–Q and adjust to pH 3.00 with 1N HCl.

*INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS*

The protocol [5] required an HPLC chain Hitachi coupled to a UV-DAD 5430 detector (Antwerp, Belgium), using Chromaster software and Dell-type computer (Hangzhou, China), XBridge C18 column, 100 x 4.6 mm, (3.5 µm)Waters (Milford, Massachusetts - MA, USA). The adjustment of the pH values of the different buffer solutions was carried out using a HI 2211 PH / ORP pH meter (Hangzhou, China). We used the FV-220C GRAM analytical balance (Hangzhou, China), an ultrasonic bath CPX1800H-E VWR Hitachi. We used e-noval® software to process the validation results, and the HPLC calculator v3.0 software to perform the geometric transfer and fix the elution gradient.All solutions were filtered using PALL ACRODISC PSF GHP 0.45 µm brand filters before injection into HPLC.

**METHOD VALIDATION[7]**

*SPECIFICITY*

The specificity of this HPLC method was determined by complete separation of Paracetamol and Diclofenac without any interference of the excipient peak.

*LINEARITY AND RANGE*

For linearity, calibration curves were plotted over a concentration range of 80 to 240µg/mL for paracetamol and 12.3 to 37µg/mL for diclofenac. All measurements were repeated three times for each concentration, and the standard curve was constructed by plotting the peak areas of the analyte against the corresponding drug concentration. R-value ≥ 0.99 confirmed the good linearity of the method.

*DETECTION AND QUANTIFICATION LIMITS*

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the linearity parameters. The values were calculated from the standard deviation (SD) of the response and the slope of the curve (S) using the equations: LOD = 3.3(SD/S) and LOQ = 10(SD/S).

*PRECISION*

The precision defined by repeatability and intermediare precision was evaluated by preparing three different sample solutions at low, medium, and high concentrations, which were freshly prepared and analyzed three times in 3 daysThe precision of the method was expressed in RSD% and in Horwitz value.The relative standard deviation was evaluated by analyzing standard drug solutions within the calibration range.

*ACCURACY*

The accuracy was calculated as the difference between the theoretical amount added and the amount practically recovered. Recovery assays were performed in triplicate by the standard addition method at 50%, 100%, and 150%. The known amount of paracetamol and diclofenac standard was added to pre-analyzed samples and was subjected to the proposed method.

*ANALYSIS OF THE COMMERCIAL FORMULATION*

Aliquot of DCDOL and VOLPA powder exactly weighted close to 10 mg of paracetamol were introduced in a 10.0 mL and gauged with methanol. After filtration, dilutions were made to obtain 200 µg/mL of paracetamol.

Solutions of the generic samples on the market were analyzed accordingly to the developed method. The sampleswere extracted with methanol using ultrasound for 15 minutes, then filtered using a 0.45 µm PTFE filter.

## RESULTS AND DISCUSSION

### *MODIFICATION OF THE ORIGINAL METHOD*

Geometric transfer consisted of changing the geometry of the chromatography column in an analytical method. Table 1 compares the operating conditions for modified and original methods. All conditions but column and mobile phase gradient were maintained. We replaced XBridge C18 (250 x 4,6 mm, 5µm) with XBridge C18 (100 x 4,6 mm, 3,5 µm).

Table 1 Operating conditions

| Specifications | Original method | Transferred method |
|---|---|---|
| HPLC brand | VWR Hitachi | VWR Hitachi |
| Detector | UV-DAD 5430 | UV-DAD 5430 |
| Wave | 220 nm | 220 nm |
| Processor computer | Dell™ (Hangzhou) | Dell™ (Hangzhou) |
| Software | Chromaster de VWR Hitachi | Chromaster de VWR Hitachi |
| **Column** | XBridge C18 (250 x 4,6 mm, 5µm) | XBridge C18 (100 x 4,6 mm, 3,5 µm) |
| Temperature | 30ºC | 30ºC |
| Injection | 20 µL | 20 µL |
| Flow | 1 mL/min | 1 mL/min |
| Mobile phase | Methanol/Buffer pH 3 | Methanol/Buffer pH 3 |
| **Gradient** | 0min : 15/85 | 0min : 15/85 |
| | 20min :95/5 | 7.22 min :95/5 |
| | 30min :95/5 | 11.22 min :95/5 |
| | 31min :15/85 | 11.64 min :15/85 |
| | 60min :15/85 | 20min :15/85 |

### *VALIDATION OF THE METHOD*

The transferred method was validated using the same total error strategy that uses the accuracy profile as a decision tool. As shown in Table 2 and Figure 1, the fixed optimal conditions reduced the retention time of analysis from 60 min to 20 min, as well as the consumption of methanol used as eluent while maintaining efficiency and selectivity. The validation criteria concerned in this study are, selectivity, trueness, repeatability (CV% = 1.10-2.62) and intermediate precision (CV % = 1.10-2.46), accuracy, linearity (paracetamol = 2,984 + 0.980t; r2 = 0.996 and diclofenac = 0.37 + 0.989t, r2 = 0.994). Detection limits (0-240) and 12.3-37), limit of quantification (LOQ), dosing interval.The acceptance limit was set at ± 10% (i.e. 90 to 110%.

Table 2 System Suitability Parameters

| System SuitabilityParameters | Transferredmethod | | Original method | |
|---|---|---|---|---|
| | PC | DC | PC | DC |
| Retention time | 3.127 | 10.473 | 6.182 | 19.536 |
| Relative Resolution time | 0.299 | 0 | 0.316 | 1 |
| LOQ range µg/ml | 80- 240 | 12.3-37 | 80- 240 | 12.3-37 |
| Target concentration µg/ml | 160 | 24.64 | 160 | 24.64 |
| Absolu biais µg/ml/ | 2.626 | 1.35 | | |
| Relatif biais %) | 1.402 | 0.792 | | |
| Repeatability% RSD | 1.774 | 1.466 | | |
| Intermediaryprecision% RSD | 1.828 | 1.488 | | |
| LinearityVxo | 2.984 | 0.370 | | |
| LinearitySlope | 0.980 | 0.989 | | |
| Linearity R | 0.999 | 0.994 | | |
| Accuracy% | 99.05–100.9. | 99.05–100.9 | 99.05–100.9 | 99.05–100.9 |

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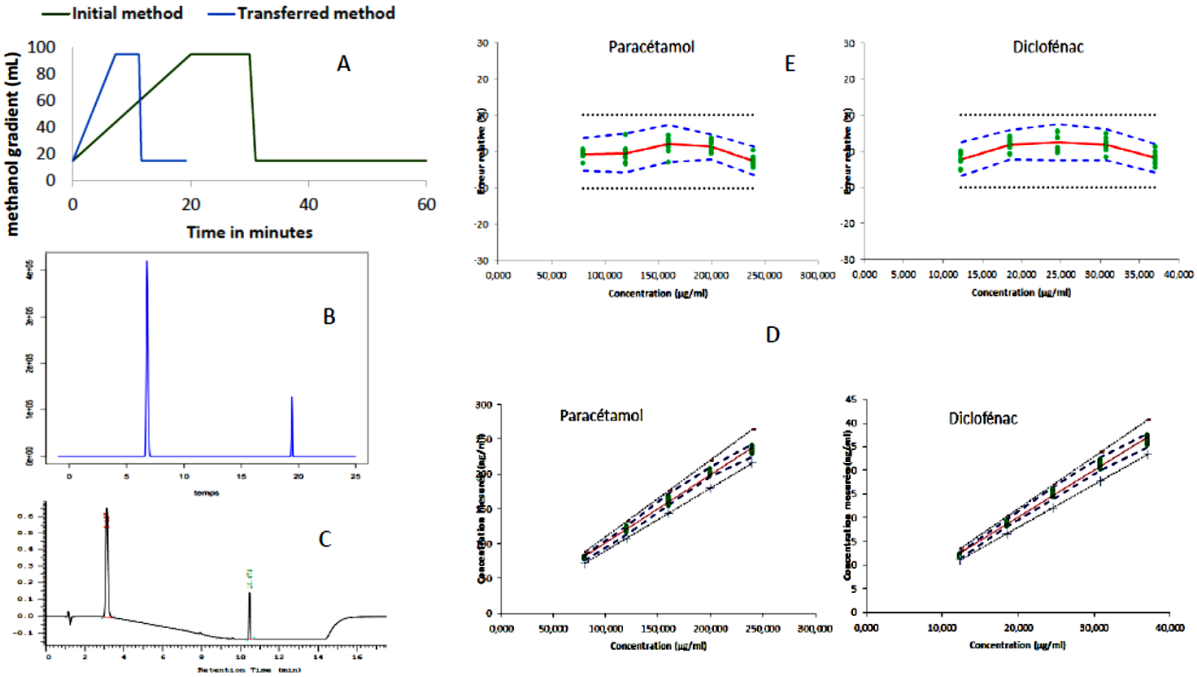


Figure 1. Performances of the method

*Legend: A= Gradient of methanol in the mobile phase; B=specificity of the original method; C= specificity of the transferred method; D= linearity profile; E= trueness profile;*

To reduce this time we changed the column and using the HPLC calculator_version 2.1 software, we obtained a new elution gradient. Switching from one column to another with different characteristics sometimes influences retention time and chromatogram quality.This has been exploited in several scientific works, in particular when the modifications of the analytical conditions have affected the chromatographic behaviors, namely the retention time [6-9].To compare the quality of the chromatograms, we used the relative retention time which is calculated from the last peak. Relative retention time = retention time of each peak / retention time of the last chromatographic peak.The results obtained show that the error or the difference between the relative retention times before and after the transfer is negligible, ie 0.017 minutes. The bias is slightly greater than that observed during the validation of the initial method, which is 1.01%. In all cases, that is to say before and after the geometric transfer, the accuracy remains good compared to the acceptance limits.The method has good reliability with a coefficient of variation of less than 2.46%. This CV is slightly higher than that of the previous method which is 1.21%.Accuracy takes into account total error, that is, systematic error and random error associated with the result.The transferred method is correct throughout the dosing range for both active substances. The same observation was made for the initial method [5].We note in Table VI that the R2 of these two molecules are close to unit (1) which explains a good agreement between the concentrations introduced and the concentrations calculated in return.

**ANALYSIS OF MARKETED FORMULATION**

After demonstrating the reliability of the transferred method, we applied it to verify the quality of twobrands marked locally. Beforehand, we performed visual inspection as well as pharmacotechnicalpharmaceutical technology requirements by assessing the disintegration time, friability, and mass uniformity, according to the procedures of the britsh pharmacopeia volume II 1993.The disintegration test was carried out at the laboratory departments of the Office Congolais de Contrôle de Kinshasa with Antou Paar Type DMA35 version 3 P/N 94138 Made in Austria and Crumbling with Friabilimetre Type TAR ERWEKA.The identification of the active ingredients in the sample was carried out by comparing the retention times of controls and those of the samples. Table 2 gives the system suitability parametrs of the two generics tested.

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Table 2 System Suitability Parameters

| System SuitabilityParameters | Dicdol® | | Volpa® | |
|---|---|---|---|---|
| | Paracetamol | Diclofenac | Paracetamol | Diclofenac |
| Retention time | 3.145 | 10.412 | 3.207 | 10.498 |
| RRT | 0.307 | 1 | 0.306 | 1 |
| Gap between RT | 0.034 | 0.164 | 0.096 | 0.086 |
| Label content mg | 325 | 50 | 325 | 50 |
| Assay recovery % | 102±0.26 | 102.2±1.68 | 100.6±0.85 | 102.0±1.17 |
| Friability (n=10) % lost | 2.805 | 2.684 | 2.805 | 2.684 |
| Mean weight (n=10) mg | 684.8 | 684.8 | 615.9 | 684.8 |

## CONCLUSION

The use of a Xbridge® column (C18, 100 x 4.6 mm, 3.5µm dp) instead of Xbridge® (C18, 250 x 4.6mm, 5µm dp) improved the system suitability of the original method .Thistranferedmethod allows an appreciable saving in time and in the solvent economy to be applied in routine for quality control of generics in circulation.

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