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Development and Validation of LC Method for the Diclofenac Sodium Release Determination Using Accuracy Profile Concept

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The article presents a rapid and sensitive RP-HPLC method with UV detection, using the diclofenac sodium release determination in solid pharmaceutical formulations. A novel validation strategy based on accuracy profiles was used to select the most appropriate regression model with highest accuracy within well defined acceptance limits. Furthermore, the strategy was used to determine the limits of quantification as well as the suitable concentration range. The validation phase was completed by investigating of the risk profiles of various acceptable regression models in order to avoid obtaining measurements outside the acceptance limits fixed *a priori*. On the other hand, the present study shows how the LC method can be used more accurately to assess the kinetic dissolution profiles, instead the UV-visible method required by monographs of the USP. Robustness study was also performed in order to demonstrate the capability of this method to remain unaffected by a small and deliberate variation in method parameters. The LC method was validated using the total error approach, as a decision tool, guarantees that each of the future results that will be within the acceptance limits settled at ± 5 %. The UV spectrophotometric method based on the USP monograph, gives rise of impurities from diclofenac sodium in acidic condition (HCl 0.1 N). These impurities absorb at the same wave length (276 nm) as the active principal ingredient, which will yield some significant error in the per cent release during the dissolution test study. Described analytical method is a simple, sensitive, specific and more accurate indicating that this LC method is useful for manufacturing and quality control assay.

Key Words: Validation, Diclofenac sodium, Quality control, Release, HPLC/UV, Accuracy profiles.

INTRODUCTION

Diclofenac sodium (DS) is a potent non-steroidal antiinflammatory drug (NSAID), extensively used for the treatment of active rheumatoid arthritis and osteoarthritis, ankylosing spondylitis, non-articular rheumatism and sport injuries. Another therapeutic uses of diclofenac sodium are as analgesic and antipyretic. The activity of NSAID is primarily attributed to inhibition of distinct steps in the arachidonic acid cascade, particularly, the cyclo-oxygenase pathway[1].

Several different methods have been reported for the determination of diclofenac sodium matrix, including potentiometry[2-4], high liquid performance chromatography[2,3,5-7], high liquid thin layer chromatography[8,9], capillary electrophoresis[10,11], FT-Raman spectroscopy[12,13], near-infrared spectroscopy[14], chromatographic-densitometric method[15], diffuse reflectance photometry[16], ion probe spectrofluorometry[17] and atomic absorption spectrometric method[18] and UV-visible spectrophotometry method[19].

Drug release from different dosage forms, including matrix tablets, can be evaluated by mean of dissolution testing, which is a very important tool in the pharmaceutical industry. Dissolution test results can lead to approval or rejection of batches in quality control and to allow its use for *in vitro-in vivo* correlation and as surrogates for *in vivo* bioavailability and bioequivalence testing[20].

A key determinant of the reliability of results of dissolution testing is the validity of analytical method that used to determine the percentage of active ingredient release. The USP reports an UV-visible spectrophotometric method to determine the release of diclofenac sodium in solid forms[21]. This method uses HCl 0.1 N as dissolution medium. However, in this acidic condition diclofenac sodium gives rise of impurities which absorb in the same wave length as the active pharmaceutical

ingredient. Thus the results obtain from the dissolution studies should be biased. In order to avoid this disagreement, we have decided firstly, to develop an LC analytical method in our study for determination of diclofenac sodium release in solid pharmaceutical forms. Secondly, an original statistical approach based on the concept of accuracy profile (total error), by means of tolerance intervals in validating this LC analytical method[22] has been applied.

Total error designated the simultaneous combination of systematic (measured by biases, *i.e.*, method trueness) and random errors (measured by relative standard deviation "RSD", *i.e.*, method precision)[23]. The total error approach fulfils completely the validation requirements expressed in the ICH (International Harmonization conference) or FDA (food and drug administration) guidelines[24-26]. It allows conciliating the validation objectives with the main goal of any analytical method that is quantified accurately during routine use. The methodology to build such a profile was more detailed by Hubert *et al*[27]. This accuracy profile methodology uses only one statistical decision tool, namely a β-expectation tolerance interval computed at each concentration level. This interval represents the location where β % (80 90 or 95 %) of the future results is expected to lie. This profile is then compared to a prior settled acceptance limit, which is within ± 5 % limit in the current work. If this tolerance interval is included inside the acceptance limits, the analytical method is declared to be valid. Within the scope of this study, the validation means, this LC method is able to generate enough information to have guarantees that it will provide, in routine, measurements close to the true value, with not more than 5 % of futures measurements will fall outside the predefined acceptance limits.

EXPERIMENTAL

Diclofenac sodium was obtained from Drugs Quality Control Laboratory of Rabat as certified external secondary standard (98.2 %). Methanol was of HPLC grade from Sigma-Aldrich (Germany). Hydrochloric acid and phosphoric acid were supplied by Merck KGaA (Germany). Sodium phosphate tribasic was obtained from Riedel-de Haeri (Germany). The placebo used in validation of the analytical method was prepared by mixing the same excipients (calcium phosphate tribasic, sodium starch glycollate, magnesium stearate, polyvinylpyrrolidone, microcrystalline cellulose, sucrose, purified talc, disperse red, lactose, selenium dioxide, cellulose acetophthalate, titanium dioxide, ethanol, polyethylene glycol, iron oxide red, iron oxide yellow, maize starch, silica colloidal anhydrous, silicone antifoam, sodium methyl carboxyle and polysorbate 80) as the same proportion of the commercial products without the principal active ingredient.

The chromatographic system consisted of Waters 2695 pump, auto sampler and Waters 2998 photodiode-array detector (PDA). Data acquisition was performed by the Empower Software data registration TM. Dissolution Test of Hanson SR8-Plus™ (USA) and UV-VIS spectrometer (Perkin, USA), pH meter was used from Schott (Germany).

Chromatographic conditions: The separation was made in isocratic mode with a Waters YMC C_{18}-3 µm 150 mm × 4.6 mm column; thermostatised at 30 ºC. The mobile phase is consisted of a mixture of aqueous phosphate buffer (0.5 g/L phosphoric acid 1.4 g/L sodium phosphate monobasic dehydrate, pH 2.5) and methanol (30:70 v/v). The mobile phase was filtered through 0.45-µm Millipore TM Durapore filter and degassed by vacuum. The flow rate was 1mL/min, the injection volume was 20 µL and the wavelength of the detector was set at 276 nm.

Validation of the analytical method

Experimental design and solution used for validation phase: Analytical method was validation based on accuracy profiles which required two solutions of calibration standards (CSs) and validation standards (VSs). Calibration standards were prepared in separate by accurately weighting 0.54, 1.09, 5.48, 13.54, 46.33, 54.81 and 68.38 mg of diclofenac sodium RS and diluting them, respectively in 100 mL of mixture A (water and methanol 30:70 v/v). These solutions were mixed and sonicated for 10 min. Dilution was performed by transferring 5 mL from theses stock solution in 50 mL of the dissolution medium (phosphate buffer pH 6.8) to reach seven levels of concentrations covering the calibration domain (0.54, 1.09, 5.4, 13, 46.33, 54.81 and 68.38 µg/mL). Each level was repeated twice for three series of analysis. Altogether 3 (series) × 7(levels) × 2(replicates) = 42 standards solutions were prepared and independently measured. The validation standards (VSs) were prepared in the placebo (the reconstituted solution described above) to reach seven concentrations levels like the calibration standard. The validation standards were repeated in triplicates and were analyzed during three series. Altogether 3(series) × 7(levels) × 3 (replicates) = 63 calibration standards were prepared and independently measured.

The parameters to be validated for HPLC assay were according to the ICH (International Harmonization conference) guidelines Q2R1[26] and SFSTP (French Society of Pharmaceutical Sciences and Techniques) 2003[27]. The specificity against the pharmaceutical excipients present in the commercial formulation was validated as follows: an amount of 270 mg of the placebo mixture prepared previously was dispersed in 100 mL of mixture A and dilution was performed by transferring 5 mL from theses stock solution in 50 mL of the dissolution medium. The resulting suspensions were ultrasonically agitated for 10 min and filtered prior to injection into the HPLC system.

The validation based on the approach of total error introduced by Hubert *et al*.[27] can be carried out as follows: (i) Selection of the acceptance limits, which depend on different nature of the matrix (in the following of this paper, it's settled to 5 %). (ii) Fitting of a regression model from the calibration standards (response function). (iii) Calculation of the concentrations of all validation standards using the appropriate regression model. (iv) Determination of the mean bias at each concentration level. (v) Calculation of two-side β-expectation tolerance limits of the mean bias at each concentration level considering the variance for intermediate precision.

Plotting of the accuracy profile, representing as a function of concentration, the mean bias, the β-expectation tolerance intervals as well as the acceptance limits.

Robustness study: In this robustness study, five factors were selected: pH of the mobile phase (2.5 ± 0.1), flow rate

(1 ± 0.1 mL/min), detector wavelength (276 ± 2 nm), % of methanol in the mobile phase (70 ± 5 %) and column temperature (30 ± 2 ºC). The mixed standard solution is injected in sex replicates and sample solution of 100 % concentration is prepared and injected in triplicate for every condition. The assay of diclofenac sodium and its % RSD were calculated for each condition[28].

Statistical analysis: The e. Noval software v2.0 (Arlenda, Liège, Belgium) was used to compute the validation results of the LC analytical method as well as to obtain the accuracy profiles.

Routine applications

Real content in tablets: Prepare a suitable degassed mixture of methanol and water (70:30, v:v mL) which are used as diluent. Dissolve an accurately weighed quantity of diclofenac sodium RS in mobile to obtain a solution having a known concentration about 0.054 mg per mL. Weight and finely powder not fewer than 20 tablets (product A, B and C). Transfer an accurately weighed portion of powder, equivalent to about 50 mg of diclofenac sodium, to a 100 mL volumetric flask, add about 100 mL of diluent, shake by mechanical means for 10 min, sonicate for about 10 min and mix. Transfer 5.0 mL of this solution to a 50 mL volumetric flask, dilute with the same diluent to volume and mix. Pass a portion of this solution through a filter having a 0.45 µm.

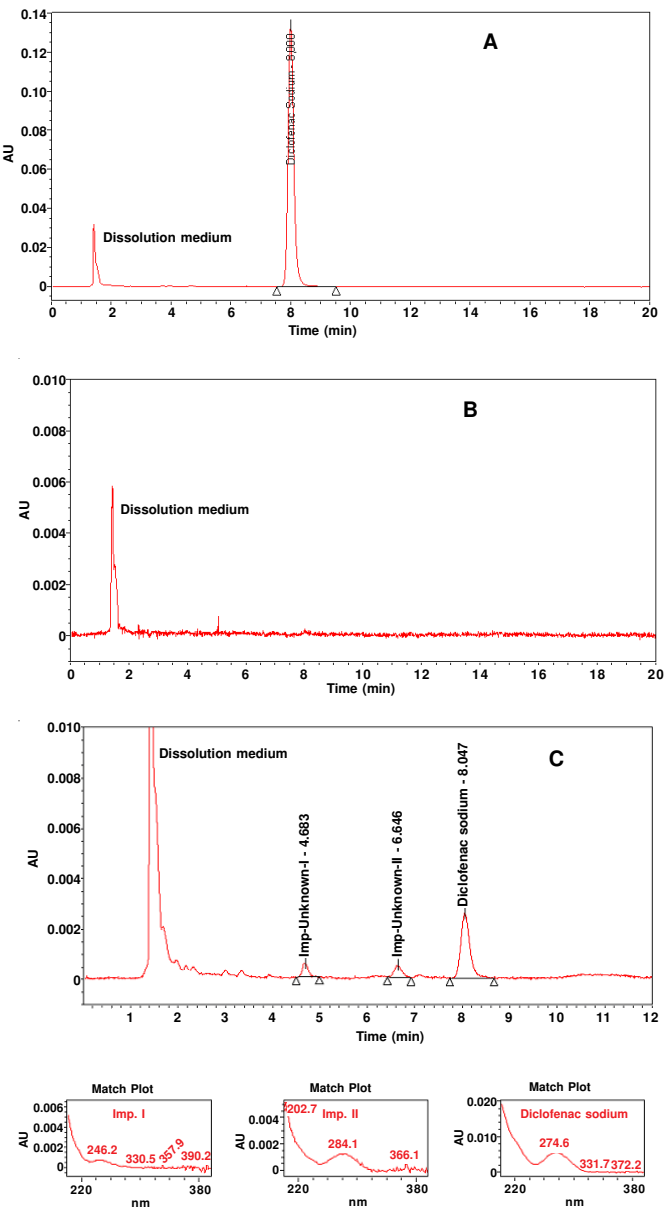
Dissolution test: All dissolution studies were performed using USP paddle method (Apparatus 2). Two tests were performed, the first, was carried out in 900 mL of HCl 0.1 N at 37 ± 0.05 ºC at 50 rpm for 2 h. The second test was carried out in 900 mL of phosphate buffer at pH 6.8, with a speed of rotation at 50 rpm for 45 min. The phosphate dissolution medium was prepared by adding 250 mL of sodium phosphate tribasic (76 g/L) to 750 mL of 0.1 N HCl. The final pH was adjusted by 2 N HCl.

The developed and validated method was applied for dissolution profiles comparison and were realized on three different final products namely A (reference) and B, C (generics). The procedure used to assay the release of active pharmaceutical ingredient from these three products was identical as described above. In all experiments, 5 mL sample aliquots were withdrawn at 10, 20, 30 and 45 min using a glass syringe. If necessary, immediately replaced with equal volumes of fresh medium at the same temperature to maintain constant total volume during the test. All samples were filtered through 0.45 µm filters. The per cent drug release was assayed using the USP spectrophotometric method at 276 nm and using our in-house LC validated method based on the accuracy profile described below. Eight teen tablets of each pharmaceutical formulation were studied to obtain statistically significant results. The results obtained were compared with those obtained by the USP method using UV spectrophotometer detection at 45 min.

RESULTS AND DISCUSSION

Method development

Selection of the chromatographic conditions: Described in USP, the dissolution kinetic of diclofenac sodium was assessed using spectrophotometric method with λ = 276 nm and 0.1 N HCl as a dissolution medium. First attempt was to apply the RP-HPLC developed method to determine diclofenac sodium in the acidic condition as required by the USP guideline. As shown in the Fig. 1, beyond the principal peak (retention time "R_T" = 8.00 min) which correspond to the diclofenac sodium (product B), two another peaks appear at R_T = 4.68 and R_T = 6.64 min which correspond to two degradation impurities in acidic medium (0.1 N HCl). Unknown impurity II was absorbed in the same wave length as the active ingredient. So, using 0.1 N HCl as dissolution medium associated with an UV visible detection to perform *in vitro* dissolution, found to be biased in the quantification of the percent release of the active ingredient from pharmaceutical product. In order to enhance the accuracy in quantifying the release of diclofenac sodium from solid forms, a RP-HPLC method was developed and validated according to the ICH guidelines and using accuracy profile as decision tool to prove that accurate and reliable results will be obtained by this method during the future routine application of the assay.



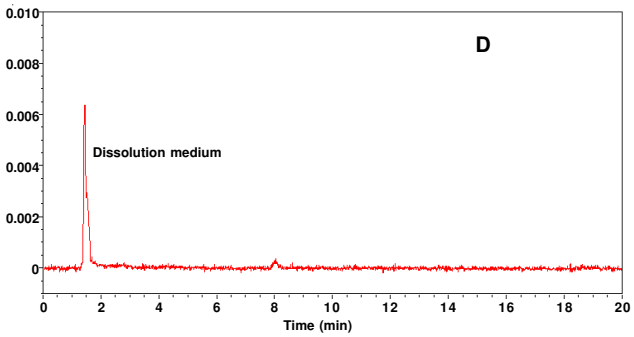


Fig. 1. (A) Standard of diclofenac sodium at 100 % (B) Not release of diclofenac sodium product A in acid stage medium (C) Release of diclofenac sodium from product B tablet in acid stage HCl 0.1 N < 10 % (D) Not release of diclofenac sodium product C in acid stage medium

Selectivity: The absence of matrix interferences (excipients present in the final product) at the retention time of diclofenac sodium was demonstrated in Fig. 1, which illustrates chromatograms obtained after analysis of blank, placebo and standard solution of the active ingredient. In addition, the specificity of the developed RP-HPLC method for diclofenac sodium was investigated in order to obtain an indication of the possible interferences from the degradation product of the drug under release of API in acidic medium at routine analysis. The results in Fig. 1 show that there is no interference with diclofenac sodium peak. Diclofenac sodium (product B) is observed to be well resolved from the degradation peaks (Imp I and II). Resolution value between first degradation product (Imp I) and second degradation product (Imp II) was 8.24 and between diclofenac sodium and second degradation product (Imp II) product was 4.65.

System suitability: The results of system suitability of analytical method for determination of diclofenac sodium release was estimated namely % RSD 1.2, R_T = 8.00 min, asymmetric factor 1.34 and theoretical plate 6800. They are used to verify that the reproducibility of the chromatographic system is adequate for the analysis to be done.

Analysis of the response functions: In order to find the most suitable regression model, several response functions (standard curves) were fitted, namely the weighted ($1/X^2$) quadratic regression, weighted ($1/X^2$) linear regression, weighted (1/X) linear regression, linear regression after square-root transformed data, weighted (1/X) quadratic regression, linear regression after log transformed data, quadratic regression, linear regression through 0 fitted using level 1.0 only and linear regression through 0 fitted using the highest level only. From each regression curve obtained, the concentrations of the validation standards were back-calculated, which allowed obtaining at each concentration level relative mean bias, the upper and the lower β-expectation tolerance limits at 95 % level by considering the standard deviation for the intermediate precision. From these data, different accuracy profiles were plotted to select the most appropriate regression model for the indented use of the analytical LC method.

The acceptance limits were settled to ± 5 % and by considering the first order risk of 5 %, all the response functions allowed demonstrating the capability of the method to quantify diclofenac sodium over the whole concentration range chosed, since the tolerance intervals were totally included inside the acceptance limits. Even though these regression models seem to be the most appropriate to describe the best relationship between concentration and analytical signal, some of (quadratic, squared and weighted ones), their application in routine analysis can be dedicating and time consuming. On the other hand, as it currently practiced in the pharmacopoeia's monographs, only one concentration level is used for calibration to determine the sample. Consequently, the simplest regression model fitted using only the high concentration level was selected. This model was then used to evaluate the different validation criteria and can be used in routine analysis.

Trueness: The results of trueness were expressed in terms of relative bias or absolute bias and were assessed from the validation standards at seven concentration levels ranging from 0.0005411-0.06838 mg/mL. The proposed method was accurate enough since the bias did not exceed the values of 5 % irrespective to the concentration level (Table-1).

Recovery: The recovery of diclofenac sodium was determined at the seven concentration levels used to construct the accuracy profile in Table-1. All recoveries are within acceptable limits, indicating that the method is suited for the analysis of diclofenac sodium release in tablets matrix.

Precision: The precision of the analytical method was estimated by calculating repeatability and intermediate precision at each concentration level of the validation standards. The RSD % values presented in Table-1 were relatively low. The relative standard deviation values for repeatability and intermediate precision were between 0.03666 and 0.5058 %, illustrating the good precision of the proposed method.

Accuracy: Accuracy refers to the closeness of agreement between the test result and the accepted reference value expressed as the conventionally true value. The accuracy takes into account the total error including systematic and random errors that are related to the test result. It is represented from the accuracy profile illustrated in Fig. 2. The proposed method was accurate over the concentration range investigated, since the upper and the lower β-expectance limits did not exceed the acceptance limits settled at 5 % for each concentration level. Consequently, the method is able to provide accurate results over the concentration range studied.

Linearity: The linearity of an analytical method represents the ability within a definite range to obtain results directly proportional to the concentration (quantity) of the analyte in the sample. Consequently, for all series, a regression lines was fitted. This was done by using back-calculated concentrations *versus* the introduced concentration by applying the linear regression model namely Y = 0.00001100 + 1.001 X for which the determination coefficient, the slope and the intercept are given in Table-1. Moreover, in order to demonstrate method linearity, the approach based on the absolute β-expectation confidence limits was applied as shown in Fig. 3. The absolute β-expectation tolerance limit were within the acceptance limits and the value of correlation coefficient R^2 = 1 demonstrating the linearity of the method.

Detection and quantitation limits: The limit of detection is the smallest quantity of the targeted substance that can be detected, but not accurately quantified in the sample. The lower limit of quantitation (LOQ) is the smallest quantity of the

TABLE-1
RESULTS OF THE VALIDATION OF THE METHOD DEDICATED TO THE DETERMINATION OF DS PERCENT RELEASE IN TABLETS FORM USING THE LINEAR REGRESSION MODEL THROUGH 0 FITTED WITH HIGH CONCENTRATION

Response functions	Day 1	Day 2	Day 3
Slope	4.3743E + 07	4.3776E + 07	4.3763E + 07
R^2	ND	ND	ND
RSS	5.2488E + 04	3.2527E + 06	2.4398E + 06
Trueness	Absolute bias (mg/mL)	Relative bias (RSD %)	Recovery (%)
0.5411×10^{-3}	0.00788×10^{-3}	1.457	101.5
1.090×10^{-3}	0.00774×10^{-3}	0.7097	100.7
5.486×10^{-3}	0.00372×10^{-3}	0.06773	100.1
13.54×10^{-3}	0.02287×10^{-3}	0.1688	100.2
46.33×10^{-3}	0.08698×10^{-3}	0.1877	100.2
54.81×10^{-3}	0.05112×10^{-3}	0.09326	100.1
68.38×10^{-3}	0.02388×10^{-3}	0.03492	100.0
Accuracy	Beta-expectation confidence limits (mg/mL) (%)	Risk (%)	
$0.5411x\ 10^{-3}$	$[\ 0.5423, 0.5557] \times 10^{-3}$	[0.2195, 2.694]	0.009647
1.090×10^{-3}	$[\ 1.096, 1.099] \times 10^{-3}$	[0.5771, 0.8423]	0
5.486×10^{-3}	$[\ 5.477, 5.502] \times 10^{-3}$	[-0.1641, 0.2996]	0.0005163
13.54×10^{-3}	$[\ 13.52, 13.62] \times 10^{-3}$	[-0.1984, 0.5360]	0.00000020
46.33×10^{-3}	$[\ 46.01, 46.83] \times 10^{-3}$	[-0.7017, 1.077]	0.0001634
54.81×10^{-3}	$[\ 54.81, 54.91] \times 10^{-3}$	[-0.002924, 0.1894]	0
68.38×10^{-3}	$[\ 68.33, 68.48] \times 10^{-3}$	[-0.07248, 0.1423]	0
Precision	Repeatability (RSD %)	Intermediate precision (RSD %)	
$0.5411x\ 10^{-3}$	0.5058	0.5058	
1.090×10^{-3}	0.05420	0.05420	
5.486×10^{-3}	0.03666	0.06598	
13.54×10^{-3}	0.1501	0.1501	
46.33×10^{-3}	0.3635	0.3635	
54.81×10^{-3}	0.03879	0.03916	
68.38×10^{-3}	0.03836	0.04197	
Linerity			
Range (mg/mL)		0.5411×10^{-3} - 68.38×10^{-3}	
Slope		1.001	
Intercept		0.00001100	
R^2		1.00	
Regression equation		Y = 0.00001100 + 1.001 X	
RSS		0.00000027	
LOD and LOQ		(mg/mL)	
LOD		0.082×10^{-3}	
Lower LOQ		0.5411×10^{-3}	
Upper LOQ		68.38×10^{-3}	

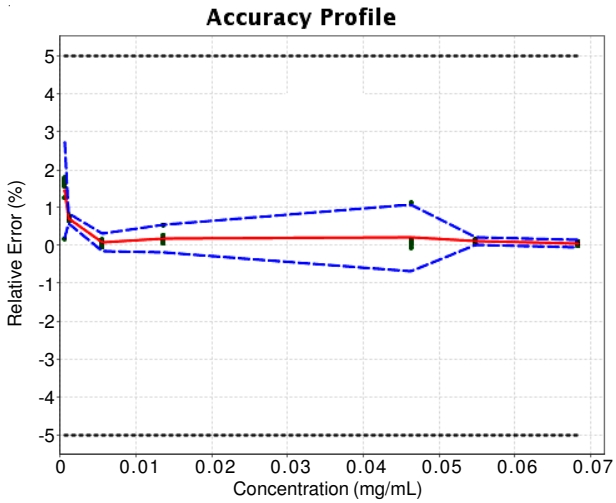


Fig. 2. Accuracy profile obtained for the validation of the HPLC-UV analytical method for the quantification of diclofenac sodium release by considering: linear regression through 0 fitted using the highest level only; plain line: relative bias; dashed lines: β-expectation tolerance limits; dotted curves: acceptance limits (%) and dots: relative back-calculated concentrations of the validation standards

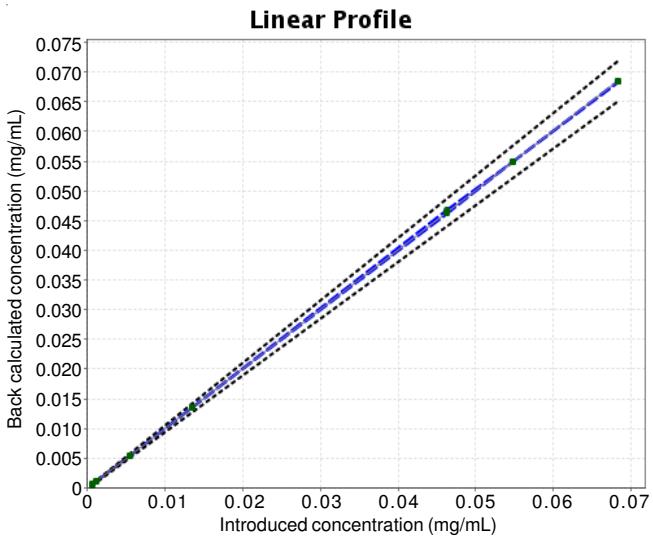


Fig. 3. Linear profile of diclofenac sodium. The dashed limits on this graph correspond to the accuracy profile, *i.e.*, the β-expectation confidence limits expressed in absolute values. The dotted curves represent the acceptance limit at 5 % expressed in the concentration unit

targeted substance in the sample that can be assayed under experimental conditions with well defined accuracy. The definition can also be applicable to the upper limit of quantitation which is the highest quantity of the targeted substance in the sample that can be assayed under experimental conditions with well defined accuracy. The limits of quantitation are obtained by calculating the smallest and highest concentration beyond which the accuracy limits or β-expectation limits go outside the acceptance limits. In the present study, the limit of detection (LOD) was estimated using the mean intercept of the calibration model and the residual variance of the regression. By applying this computation method, the LOD of the developed method was equal to 0.08200×10^{-3} mg/mL, while the lower LOQ was equal to 0.5411×10^{-3} mg/mL and the upper LOQ was equal to 68.38×10^{-3} mg/mL (Table-1).

Risk profiles: The risk to obtain future measurements out of acceptance limits is known before starting routine applications of the method. In our study this risk was practically null over the concentration ranges investigated, by considering the linear regression model through 0 fitted using the highest level only where the risk were between 0.00 and 0.009647 % (Fig. 4 and Table-1).

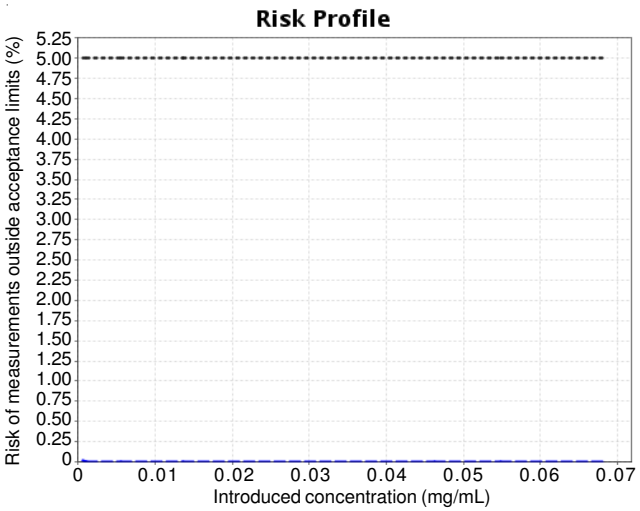


Fig. 4. Dotted line represents the maximum risk level chosen: 5.0 %

Robustness: The experimental results of the robustness study are summarized in Table-2. Robustness of validated method was investigated under a variety of conditions including changes of pH of the mobile phase, flow rate, percentage of methanol in the mobile phase, UV wavelength and column oven temperature. It was observed that there were no marked changes in assay of diclofenac sodium and its % RSD (≤ 2 %), which demonstrate the degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

TABLE-2
RESULTS OF ROBUSTNESS STUDY

Factors	Level	Assay (%)	RSD (%)
pH of mobile phase	2.4, 2.6	96.67, 97.23	1.7, 1.8
λ	274, 278	96.53, 96.37	1.2, 1.3
T (ºC)	28, 32	96.93, 97.24	0.8, 1.7
Methanol (%)	65, 75	98.15, 97.21	1.5, 2.0
Flow rate (mL/min)	0.9, 1.1	96.98, 95.62	2.0, 1.8

Application of validated method

Routine analysis: The developed method was used for routine quality control analysis to determine the percent release of diclofenac sodium in different solid pharmaceuticals forms.

Real content of API in tablet: The results of dosage of diclofenac sodium content in tablets were reported in Table-3. The content of diclofenac sodium tablets was found to be within normal range of USP in three products A, B and C namely 50.43, 50.30 and 50.20 mg, respectively.

TABLE-3
RESULTS OF REAL CONTENT OF API IN TABLET

Products	Real content of API in tablet form
Product A	
Assay (mg)	50.43
Assay (%)	100.84
RSD (%)	0.93
Product B	
Assay (mg)	50.30
Assay (%)	100.62
RSD (%)	0.97
Product C	
Assay (mg)	50.20
Assay (%)	100.4
RSD (%)	0.36

Dissolution profile: According to this method, the results of the gastro resistance test of coated diclofenac sodium presented in Table-5 show that the reference products A and the generic C resist in 0.1 M HCl medium (percent of drug release = 0.0 %), while per cent release of diclofenac sodium of generic (product B) is 2.9 %. Nevertheless, whole results fall within the range per cent release of diclofenac sodium at 2 h less than 10 % required by the USP. On other hand the percent release of diclofenac sodium product B was determined by RP-HPLC and UV methods and the results proved the existence of a significant difference between two methods (n:18) and $p < 0.01$ due to the UV spectrophotometric method based on the USP monograph, gives rise of impurities from diclofenac sodium in acidic condition (0.1 N HCl). These impurities absorb at the same wavelength as the active principal ingredient, which will yield some significant error in the per cent release during the dissolution test study (Fig. 1 and Table-5).

The results of diclofenac sodium release per cent (reference A and B, C as generic marketed in Morocco, tablet 50 mg) in buffer stage 6.8 based on validated method have shown in Table-4. It is appear that generic 2 (product B) dissolute faster than the reference (product A), while the dissolution of the generic 2 is slower than the reference A and the result of generic 2 is not accepted based on USP rules (% drug release ≤ 75 %) at 45 min (Fig. 5). It's may be due to the different of excipient types as well as their physical properties (particles size) or the type of the manufacturing processes using to produce the final product.

As shown in Table-4, the per cent CV for release of product A and B in USP condition was less than 20 % at 10 min and less than 10 % at other time points. This means that results are valid and homogeneous. While the per cent CV for release of product C in the same condition was more than 20 % at 10 min and more than 10 % at other points time, so the results aren't valid based on FDA norm[29].

TABLE-4
RELEASE OF DICLOFENAC SODIUM IN BUFFER STAGE NAMELY PHOSPHATE BUFFER pH: 6.8. (N=18)

Products	10 min	20 min	30 min	45 min
		Product A		
Drug release(%)	15.09	64.29	93.56	101.06
RSD (%)	19.15	9.98	6.58	1.80
		Products B		
Drug release(%)	50.79	95.04	102.72	103.06
RSD (%)	10.61	2.64	1.65	1.48
		Products B		
Drug release(%)	0.25	9.09	30.3	63.29
RSD (%)	128	69.52	34.78	12.10

TABLE-5
COMPARATIVE BETWEEN VALIDATED AND USP METHODS

Products	Diclofenac sodium release in acid stage based UV spectrophotometer method and RP-HPLC method at 2 h (N= 18)						Diclofenac sodium release in buffer stage based UV spectrophotometer method and HPLC method at 45 min (N = 18)					
	Product A		Product B		Product C		Product A		Product B		Product C	
Methods	HPLC	UV	HPLC	UV	HPLC	UV	HPLC	UV	HPLC	UV	HPLC	UV
Mean(%)	0.00	0.00	2.91	5.2	0.00	0.00	101.06	84.38	103.06	95.30	63.26	61.74
RSD (%)	0.00	0.00	2.74	9.61	0.00	0.00	1.8	5.8	1.48	5.6	12.10	33
p value	–		$p < 0.01$		–		$p < 0.001$		$p < 0.001$		$p > 0.05$	

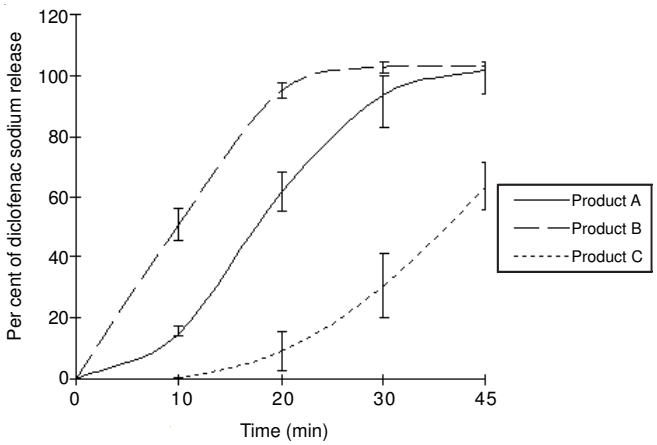


Fig. 5. (A) Dissolution profile of diclofenac sodium product A as reference. (B) Dissolution profile of diclofenac sodium product B as generic 1. (C) Dissolution profile of diclofenac sodium product C as generic 2. (Mean ± SD, n = 18)

Comparative between validated and USP methods: In order to confirm the reliability of the developed method to quantify more accurately the per cent release of the diclofenac sodium from the solid form than the UV spectrophotometric method, the dissolution data obtained by the two methods were compared using student *t*-test. As shown in Table-5, the *p*-value less than 5 % draw the conclusion that exist a difference statistically significant namely in products A and B and the results obtained by HPLC/UV are more accurate than those obtained by UV spectrophotmetric method required by the USP. The HPLC method was found to be adequate and it should therefore be used to obtain accurate stability data for diclofenac sodium in solid form.

Conclusion

RP-HPLC/UV method was developed and validated using a novel approach based on the accuracy profiles for the determination of diclofenac sodium release in solid pharmaceutical forms. This approach gives enough guarantees for the future results that will be generated by this method during routine use will be close enough to the true value. The present study shows that the LC method can be used more accurately to assess the kinetic dissolution profiles, instead the UV visible method required by monographs of the USP. The UV spectrophotometric method based on the USP monograph, gives rise of impurities from diclofenac sodium in acidic stage. These impurities absorb at the same wavelength (276 nm) as the active principal ingredient, which will yield some significant error in the per cent release during the dissolution study. Described analytical method is a simple, sensitive, specific and more accurate indicating that this LC method is useful for manufacturing and quality control assay.

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