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Method for quantitative determination of diclofenac in chitosan microspheres and chitosan films by HPLC–UV and UV spectroscopic methods

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ABSTRACT

Rapid and simple methods for determination diclofenac (DF) in both chitosan films and microsphere formulations using HPLC and UV-visible spectrophotometer were developed and validated. The HPLC assay was performed using a reversed phase ACE® C8 column (150 mm x 4.6 mm) with a mobile phase system consisting of methanol : phosphate buffer pH = 2.5 (66: 34 by volume). The flow rate was set at 1 mL/min and the UV detection at 254 nm. The Hewlett Packard 8452A, Diode Array Spectrophotometer set at 278 nm was used for method comparison. The accuracies reported as % recovery were found to be 98.84-100.91% and 92.61-99.64% for the UV-vis spectrophotometer and HPLC, respectively. Inter-day precisions (reproducibility) were 0.23–0.55%RSD (UV-vis) and 1.91-2.66–0.55%RSD (HPLC), while intra-day precisions (repeatability) were 0.23–0.55%RSD (UV-vis) and 0.34–1.62%RSD (HPLC). The calibration curve was found to be linear with the equations of y = 0.0445x + 0.006 and y = 57694x - 10767 with correlation coefficients of 0.9996 and 0.9994 (r^2) over a concentration range of 5–25 µg/mL for the UV-vis and HPLC, respectively with a low LOD and LOQ. The method can be applied for the quantitative analysis of diclofenac in both chitosan film and microsphere formulations without any complexity for sample preparations.

Key words: Quantitative, Determination, Diclofenac, Chitosan microspheres, Chitosan films.

INTRODUCTION

Diclofenac is 2-(2-(2,6-dichlorophenyl amino) phenyl) acetic acid (Figure 1). It is a non-steroidal antiinflammatory drug (NSAID) with anti-inflammatory, antipyretic, and analgesic action as a result of its ability to block prostaglandin synthesis by inhibition of the cyclooxygenase (COX) enzyme [1-4]. Many publications have described the use of bio-materials to produce effective anti-inflammatory formulations of this therapeutic agent, e.g. in beads or microspheres [1-3, 5-10] and films [11-16]. Several analytical methods have been reported in the literature for the determination of diclofenac in pharmaceutical and biological fluids, including spectrophotometric [17-19] and chromatographic [20, 21] methods. High Performance Liquid Chromatography (HPLC) normally provides more accurate determination of the drug than spectrophotometric methods especially in the presence of interfering matrices that result from the different formulations. However, most of the available HPLC methods may not be the best analytical methods of choice when it is required to increase the throughput and reduce analysis costs compared to the UV visible spectrophotometric methods. For routine quality control work, hundreds of samples need to be analyzed on a daily basis and HPLC methods are very costly in terms of time and resources. A UV visible spectroscopic (UV-vis) method is known to be a simpler, faster and more economical method for the detection and quantification compared to HPLC. Both HPLC and UV-vis methods have been employed for the determination of diclofenac in microspheres and film formulations [15, 16, 22-24]. However, no literature has reported a validated method for the quantitative determination of diclofenac in films and microsphere formulations. Method validation is a process of establishing that the performance characteristics of the analytical method are suitable for the intended

application. The validation method process for analytical procedures begins with the planned and systemic collection by the applicant of the validation data to support analytical procedures [25]. Moreover, the analytical methods of pharmaceutical products should be validated according to the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures [26]. This work therefore aimed to validate and compare the effectiveness between two analytical procedures, UV-vis and HPLC methods for the determination of diclofenac in chitosan films and chitosan microspheres.

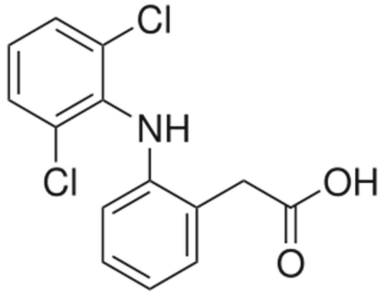


Figure 1 Structural formula of diclofenac

MATERIALS AND METHODS

2.1 Chemicals and reagents

Diclofenac raw material was from China. Methanol (AR grade) was from Labscan Ltd., Bangkok, Thailand. Sodium dihydrogen orthophosphate (AR grade) was from Finechem Pty Ltd, Australia. All other chemicals and solvents were reagent grade.

2.2 Instrumentation

The HPLC analyses were carried out on an Agilent 1100, and diode array detector with Chemstation software. The chromatographic system including a reversed phase ACE® C8, 5 µm, 4.6 x 150 mm HPLC column (ACE, Scotland) as the stationary phase, a mixture of methanol: phosphate buffer pH = 2.5 (66: 34 by volume) was the mobile phase at a flow rate of 1.0 mL/min. Detection of diclofenac was performed by measuring the absorption at 254 nm and the injection volume was 20 µg/mL.

The UV-vis spectrophotometer was a Hewlett Packard 8452A, Diode Array Spectrophotometer, USA with a 1 cm path-length, a quartz cuvette was used for the measurement of the absorption of diclofenac at 278 nm. The spectral bandwidth was set at 2 nm. All weights were measured on an analytical balance (model BSA 2248, Scientific Promotion co., Ltd., Thailand)**.**

2.3 Preparation of standard solutions

A stock solution of diclofenac was prepared by dissolving 100 mg of the diclofenac reference standard in methanol in a 100 mL volumetric flask to give a 1 mg/mL solution of diclofenac. This solution was stored at 4˚C until used. The stock solution was then serially diluted with the mobile phase to provide calibration standard solutions of 5, 10, 15, 20 and 25 µg/mL. These standard solutions were used for both the HPLC and UV-vis methods.

2.4 Sample Preparation

The chitosan films or chitosan microspheres (100 mg) were placed in 50 mL of methanol in a 100 mL erlenmeyer flask and sonicated for 60 min then left standing at room temperature for 60 min. The clear supernatant was taken and filtered through a filtering membrane (0.45 µm) before being used for the determination process by HPLC and UV visible spectrophotometry.

2.5 Validation Methods

Validation parameters of the analytical method for diclofenac entrapped in films or microsphere formulations were optimized including: linearity and range, precision, selectivity, accuracy, limit of detection (LOD) and limit of quantitation (LOQ). The optimized method was validated according to ICH guidelines for the validation of analytical methods [26].

The linearity and range of the method was determined at five concentration levels from 5-25 µg/mL of diclofenac in methanol.

The precision of the method was based on intraday variability that was determined by replicate analysis of the calibration standards in the same day. The reproducibility was taken as the inter-day variability and was determined by replicate analysis of the calibration standards in different days with one replicate being analyzed each day. The relative standard deviation values (RSD) were calculated from the ratios of the standard deviation (SD) to the mean and expressed as a percentage.

The specificity and selectivity of the diclofenac samples was analyzed by comparing the samples with blanks obtained from chitosan films and chitosan microspheres. The UV-visible spectrum of diclofenac was recorded over a range of 190- 390 nm. Selectivity of the HPLC method was performed using extraction solutions from the blank samples and film and microsphere samples containing diclofenac.

The accuracy of the method was determined using three concentrations in triplicate at 1.84 (80%), 2.30 (100%) and 2.76 (120%) µg/mL of diclofenac. The percentage recovery within 80-120% illustrated the accuracy of the method.

The limit of detection (LOD) is the lowest concentration of analyst that was detectable at the most sensitive instrument settings, but not necessarily quantitated, under the stated experimental conditions. The limit of quantification (LOQ) is the lowest concentration of analyst that can be determined with acceptable precision and accuracy, under the stated experimental conditions [25]. Standard solutions of diclofenac were analyzed in the range of 5, 10, 15, 20 and 25 µg/mL. The LOD and LOQ were determined on the basis of the response and slope of the regression equation from the calibration curve and calculated according to the following equations.

$$\text{LOD} = \frac{3.3\sigma}{S}$$

$$\text{LOQ} = \frac{10\sigma}{S}$$

Where, σ is the standard deviation of the response and S is the slope of the calibration curve.

RESULT AND DISCUSSION

3.1 Detection linearity and calibration curve

The linearity of the HPLC and UV-vis methods were performed using standard solutions of diclofenac in methanol over a range of 5-25 µg/mL and the results are listed in Table 1. The UV-vis spectrophotometer was set to measure the absorbance of diclofenac at 278 nm., which was found to be the λ-max of diclofenac in methanol whereas HPLC was set to detect at λ = 254 nm according to the USP34 monograph of a diclofenac tablet [27]. The linearity's of calibration curves for standard diclofenac solutions by both methods were obtained by plotting the absorbance values versus concentrations with the correlation coefficients (R^2) > 0.999. The relative standard deviation (%RSD) values of repeated analytical experiments were < 2% and indicated that both methods were sufficiently precise. It should be noted that at the time of the sample determination, a new standard curve was constructed for each experimental interpretation.

Table 1 Quantitative parameters for diclofenac determination using HPLC and UV-vis (Solvent = methanol) (n = 3)

Parameters	Regression analysis results (UV-visible)	Regression analysis results (HPLC)
λ max (nm)	278	254
Correlation coefficient (R^2)	0.9996	0.9994
Slope± SD	0.0445 ± 0.0001	57694 ± 85
Intercept ± SD	0.006 ± 0.005	10767 ± 41
RSD (%)	0.67	1.10
Concentration range (µg/mL)	5.0 - 25.0	2.0 - 15.0

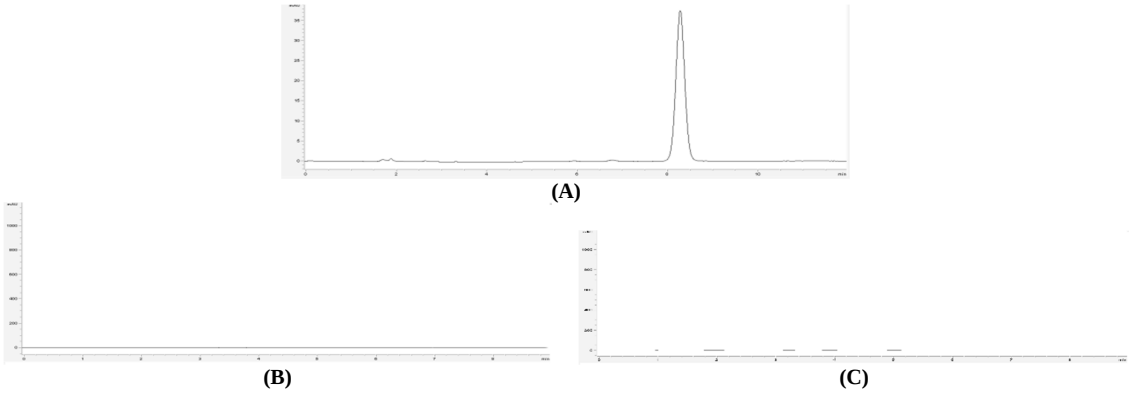
3.2 Precision

The precision of the analytical procedures for both repeatability and reproducibility were determined. The repeatability or intra-day precision (n = 3) and reproducibility or inter-day precision (n = 3) of various concentrations of diclofenac (5-25 µg/mL) were reported as RSD (%) values and found to be 0.67% and 0.77% for the UV-vis and 1.10% and 2.18% for the HPLC methods, respectively. The result demonstrated that both repeatability and reproducibility did not exceed 5%, indicating for the method precision.

3.3 Specificity and selectivity

The specificity and selectivity of both methods were performed using extracted solutions from blank chitosan films and microspheres compared to the extract solutions from the films and microspheres containing diclofenac. The HPLC chromatogram of the extract solutions from the blank chitosan films and chitosan microspheres showed no interference peak, at the retention time of 8.277 min for diclofenac, were detected in the chromatogram of the

diclofenac standard solution (Figure 2). The UV-Visible spectra of the extract solutions prepared from the blank chitosan films or microspheres also showed no absorption band at 278 nm (Figure 3). The results demonstrated that both HPLC and UV-vis methods were specific for the assay and can be used selectively for the quantification of diclofenac from both chitosan films and microspheres.



(A)

(B) **(C)**

Figure 2 Chromatograms of (A) a standard diclofenac solution at 20 µg/mL, (B) Extract solution from blank chitosan film and (C) Extract solution from blank chitosan microspheres

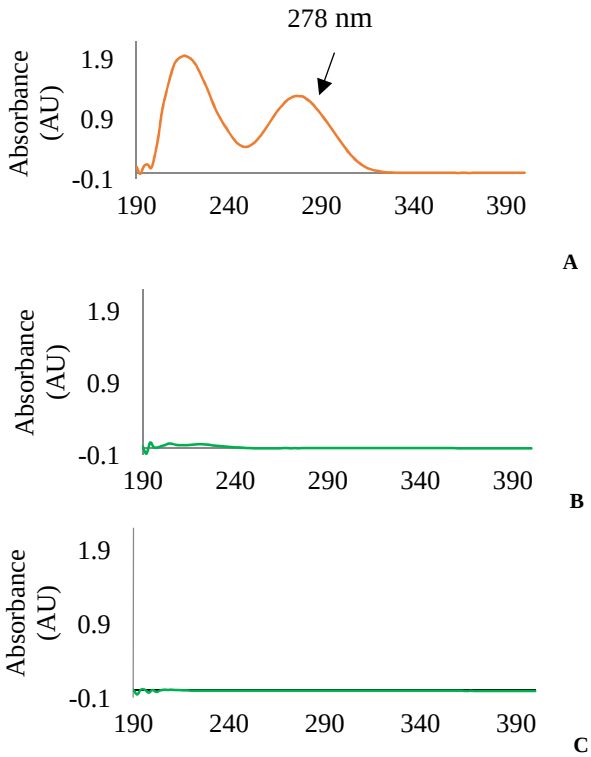


Figure 3 Determination of the specificity for diclofenac by the UV-vis spectroscopic method

(A) standard diclofenac solution at 20 µg/mL,
(B) Extracted solution from a blank chitosan film
(C) Extracted solution from blank chitosan microspheres

3.4 Accuracy

The accuracy of the test methods were assessed by determination of the percentage recovery of spiked diclofenac at three-level of concentrations from both the blank chitosan films and the microspheres. The extraction was performed by simply addition of methanol and sonication for 60 min. The clear supernatant after it settled was used for analysis. The mean absorption values (UV-vis) and mean peak areas (HPLC) obtained from triplicate measurements were calculated for the amount found by using standard solutions of diclofenac (5-25 µg/mL) and reported as % recoveries and the results are summarized in Tables 2-3. The mean recoveries for 1.84, 2.30, and 2.76 µg/mL for UV-vis and HPLC techniques were in a range of 98.84-100.91% and 92.61-99.64%, respectively. High percentage recoveries were observed for both formulations. It was therefore confirmed that the methods were highly accurate. Moreover, the major advantage of these analytical methods is the short time taken with only a simple preliminary sample treatment.

Table 2 Accuracy of measurement of diclofenac in chitosan films with UV vis and HPLC

Spiked concentration (µg/mL)	UV-vis, (n =3)		HPLC (n =3)	
	Amount found (µg/mL)	Recovery (%)	Amount found (µg/mL)	Recovery (%)
1.84	1.86±0.04	100.91±2.20	1.83±0.12	99.64±6.44
2.30	2.27±0.03	98.84±1.26	2.19±0.04	95.36±1.65
2.76	2.75±0.04	99.52±1.27	2.57±0.05	93.24±1.67

Table 3 Accuracy of measurement of diclofenac in chitosan microspheres with UV vis and HPLC

Spiked concentration (µg/mL)	UV-vis, (n =3)		HPLC, (n =3)	
	Amount found (µg/mL)	Recovery (%)	Amount found (µg/mL)	Recovery (%)
1.84	1.83±0.03	99.28±1.57	1.79±0.02	97.46±1.26
2.30	2.30±0.02	100.14±0.91	2.13±0.04	92.61±1.74
2.76	2.77±0.03	100.36±0.96	2.68±0.04	96.98±1.51

3.5 The limit of detection (LOD) and the limit of quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) parameters were established based on the signal-to-noise (3:1 for LOD and 10:1 for LOQ), according to ICH recommendations [26]. The LOD and LOQ of the UV-vis method were calculated using parameters from the calibration determination by measuring the absorption at 278 nm and gave 0.12 µg/mL and 0.36 µg/mL, respectively. The LOD and LOQ of the HPLC analysis was optimized using the above described chromatographic system using the UV detector at 254 nm and resulted in 0.054 µg/mL and 0.164 µg/mL, respectively. The lower LOD and LOQ values from the HPLC method indicated a higher sensitivity to detect diclofenc in both formulations. Moreover, the described analytical methods demonstrated that the analyses being performed were in a region above the quantitation limit values.

CONCLUSION

Both HPLC and UV visible methods have been investigated for the quantitative determination of diclofenac in chitosan films and microsphere formulations. The analytical procedures were found to be rapid, sensitive, and specific. The accuracy and precision of the method were within the acceptable range according to ICH recommendations. The simplicity of the techniques and the high sensitivity make these techniques particularly attractive for the quantification of diclofenac in both chitosan films and microspheres. These method are recommended for routine and quality-control analysis of the investigated drug in these chitosan formulations.

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REFERENCES

[1] Basarkar G D., Shirsath G N and Patil S B, **2013**, *Bull. Pharm. Res*, *3*: 14-22.
[2] Basavaraj B V., Deveswaran R., Bharath S., Abraham S., Furtado S and Madhavan V, **2008**, *Pak J Pharm Sci., 21*: 451-454.
[3] Bv B., Abraham S and Furtado S, **2008**, *Pak J Pharm Sci. 21*: 451-454.
[4] Chowdhury J A., Jahan S T., Morshed M M., Mallick J., Nath A K., Uddin M Z., Dutta M., Islam M K and Kawsar M H, **2011**, *Bengladesh Pharm J, 14*: 41-48.
[5] Al-Kahtani A A and Sherigara B S, **2014**, *Colloids Surfaces B., 115*: 132-138.
[6] Al-Kahtani Ahmed A., Bhojya Naik H S and Sherigara B S, **2009**, *Carbohydrate Res., 344*: 699-706.
[7] Çalış S., Bozdağ S., Kaş H S., Tunçay M and Hıncal A A, **2002**, *Il Farmaco, 57*: 55-62.
[8] Cooper D L and Harirforoosh S, **2014**, *PloS one, 9*: e87326.
[9] Fernández-Hervás M J., Holgado M A., Fini A and Fell J T, **1998**, *Int J Pharm., 163*: 23-34.
[10] González-Rodríguez M L., Holgado M A., Sánchez-Lafuente C., Rabasco A M and Fini A, **2002**, *Int J Pharm., 232*: 225-234.
[11] Ahmed M G., Harish N M., Charyulu R N and Prabhu P, **2009**, *Tropical J Pharm Res., 8*: 33-41.
[12] El-Sousi S., Nácher A., Mura C., Catalán-Latorre A., Merino V., Merino-Sanjuán M and Díez-Sales O, **2013**, *J Pharm Pharmacol., 65*: 193-200.
[13] Jadhav R T., Kasture P V., Gattani S G and Surana S J, **2009**, *Int J of PharmTech Res, 1*: 1507-1511.
[14] Kramar A., Turk S and Vrečer F, **2003**, *Int J Pharm., 256*: 43-52.
[15] Liu D., Ge Y., Tang Y., Yuan Y., Zhang Q., Li R and Xu Q, **2010**, *J Microencapsul., 27*: 726-734.

[16] Singh U V., Pandey S and Udupa N, **1993**, *Indian J Pharm Sci., 55*.
[17] Pandya E J., Kapupara P and Shah K V, **2014**, *J Chem Res, 6*: 912-924.
[18] Mehta S K., Bhasin K K and Dham S, **2008**, *J Colloid Interf Sci., 326*: 374-381.
[19] Ioele G., De Luca M., Tavano L and Ragno G, **2014**, *Int J Pharm., 465*: 284-290.
[20] Davarani S S H., Pourahadi A., Nojavan S., Banitaba M H and Nasiri-Aghdam M, **2012**, *Anal Chim Acta, 722*: 55-62.
[21] Song X-Y., Shi Y-P and Chen J, **2012**, *Talanta, 100*: 153-161.
[22] De Souza R L and Tubino M, **2005**, *J Brazil Chem Soc., 16*: 1068-1073.
[23] Narayana Charyulu R., Ahmed M G., Nayak P and Dixit M, **2014**, *Int J Pharm., Review and Research, 28*: 207-213.
[24] Oliveira M C., Bindewald E H., Marcolino L H, Jr. and Bergamini M F, **2014**, *J Electroanal Chem., 732*: 11-16.
[25] Ravichandran V., Shalini S., Sundram K M and Rajak H, **2010**, *Int J Pharm Pharm Sci., 2*.
[26] I. C. H. Harmonized Tripartite Guideline, **1997**, *Fed. Regist, 62*.
[27] U S Pharmacopeia, **2011**, *General Chapter on Validation of Compendial Procedures, 1225*.