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Evaluation of a short stability-indicating HPLC method for diclofenac sodium gels

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This work is dedicated to Univ.-Prof. Dipl.-Ing. Mag. Dr. Christian Noe on the occasion of his 65th birthday.

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A fast and reproducible high performance liquid chromatography method has been developed for the determination of diclofenac sodium and its degradation products in commercial and in in-house produced ointments. The method employs a RP-LiChrospher® select B (C8) column with a mobile phase containing methanol/water (63:37, v/v) and detection at 220 nm. This rapid and simple HPLC assay was used for QA/QC of large scale in-house produced diclofenac gel. The validation protocol was designed following international guidelines, e. g. ICH Q2(R1). Selectivity tests also included the separation of synthesis related by-products like 1-(2,6-dichlorphenyl)indoline-2-one (impurity A) and indoline-2-one (impurity E), and in addition selectivity with regard to several photodegradation products produced by both UV and simulated sunlight irradiation has been shown.

1. Introduction

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) of the phenylacetic acid chemical class. It shows anti-inflammatory, analgesic and antipyretic activity by inhibiting competitively both cyclooxygenase isoenzymes, COX-1 and COX-2. Diclofenac sodium is extensively applied for the relief of pain and inflammation in the management of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. It is also used for other painful conditions such as renal colic, acute gout, dysmenorrhoea and migraine. In addition it is applied in treatments following some surgical procedures (Sweetman 2011; Small 1989). It is used in a variety of pharmaceutical formulations such as injections, tablets, ophthalmic solutions and suppositories. Besides that, diclofenac sodium is also available as a 1% topical gel for the local symptomatic relief of pain and inflammation. A four times daily topically applied diclofenac gel has been shown to effectively relieve the pain of osteoarthritis in a large placebo controlled study by Niethard et al. (2005). There are two different methods published in the USP and the European Pharmacopoiea for the test on related substances of diclofenac sodium (API) and in the USP in addition a method for testing of an oral dosage form is mentioned (European Pharmacopoeia 2011; United States Pharmacopeia 2011). A number of methods for the determination of diclofenac by HPLC in pharmaceutical dosage forms, blood and synovial fluid, have been published previously (Kubala et al. 1993; Roskar and Kmetec 2003). Determination of diclofenac in gel formulations has been published in some studies. Different shortcomings e.g. regarding long runtimes, inadequate peak symmetry, and insufficient selectivity as to photodegradation products, respectively, let the published methods seem unsuitable for our purpose (Adhikari et al. 2011; Hájková et al. 2002; Hanyšová et al. 2005; Mulgund et al. 2009; Shah et al. 1994). Panda et al. (2012) published a HPLC assay for the determination of diclofenac potassium and metaxalone in tablets claiming the method to be stability indicating. Forced photodegradation was carried out by irradiation with UV 365 nm. The authors detected a loss of four percent after 180 min of irradiation. This could not be confirmed by our studies since we found a much greater extend of degradation with UV as well as with simulated sunlight (comp. Fig. 1). Other groups have used ESI-MS, LC/ESI/TOFMS and GC-MS techniques for the elucidation of diclofenac phototransformation products (Galmier et al. 2005; Agüera et al. 2005; Pioger et al. 2001). Here

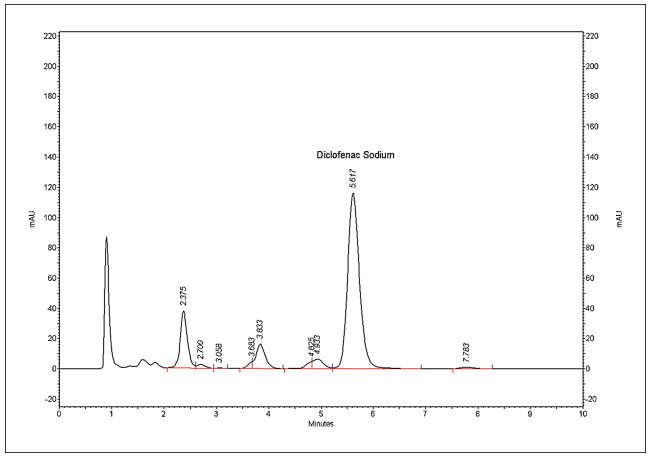


Fig. 1: Chromatogram of diclofenac sodium (t_{ret} 5.6 min) subjected to simulated solar light or to UV 366 nm

we present a short and reliable method for the determination of diclofenac and two of the five potential impurities 1-(2,6-dichlorphenyl)indoline-2-one (impurity A) and indoline-2-one (impurity E), listed by the European Pharmacopoeia. Furthermore, the method is suitable to distinguish between diclofenac and its forced degradation products, received by either sunlight or UV irradiation.

2. Investigations, results and discussion

2.1. HPLC method development

The method development was initiated using a mobile phase consisting of 65% methanol and 35% aqueous phase. The aqueous phase consisted of a mixture of o-phosphoric acid and of 0.05 M triethylamine with an acidic pH of 2.8. A stressed sample (3 h Suntest CPS Accelerated Exposure Machine) of a standard solution containing 50 µg/mL diclofenac sodium was analysed. The method was optimized in order to improve the resolution between diclofenac and the nearby eluting degradation products. Therefore the mobile phase was changed to 63% methanol and 37% aqueous phase. Fig. 1 depicts the chromatogram obtained with the current method. The peaks of all the degradation products and diclofenac sodium were well separated. This method was further validated.

2.2. Method validation

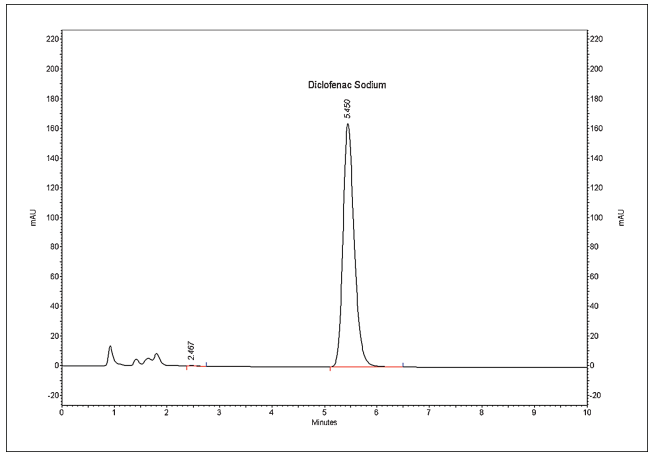
The HPLC method, used to quantify diclofenac sodium, was validated according to current ICH guidelines (ICH Q2R1 2005). The validation included specificity, linearity, precision, LOD/LOQ and accuracy.

2.2.1. Specificity

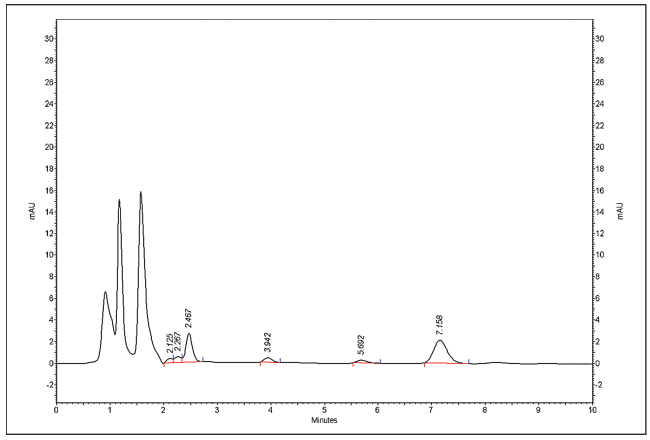
The specificity of the method was tested by injecting a freshly prepared solution of diclofenac sodium (25 µg/mL), a solution of the blank gel base containing Ultrasicc®, Carbopol®, isopropanol and purified water without addition of diclofenac sodium, and a diluted sample of the in-house produced diclofenac gel 1%. In the blank gel base solution no interfering peaks were found at the retention time of diclofenac sodium. Diclofenac sodium raw material as well as diclofenac sodium contained in the in-house produced diclofenac gel 1% elute at identical retention times (t_{ret} 5.5 min). Samples that were stressed with solar light and elevated temperatures showed the formation of several degradation products eluting at t_{ret} 2.4, 3.8 and 4.9 min, respectively. None of the peaks of the degradation products observed in stressed samples interfere with the peak corresponding to diclofenac sodium. Moreover impurity E and impurity A elute at different retention times (t_{ret} 1.5 min for impurity E and 3.4 min for impurity A). Peak purity indices were found to be >0.999 in all chromatograms, proving the specificity of the method and its suitability for routine work. Typical chromatograms for a freshly prepared solution of diclofenac sodium, of a blank gel base containing Ultrasicc®, Carbopol®, Isopropanol and purified water, and a sample in-house produced diclofenac gel 1% are presented in Fig. 2a–c.

2.2.2. Linearity

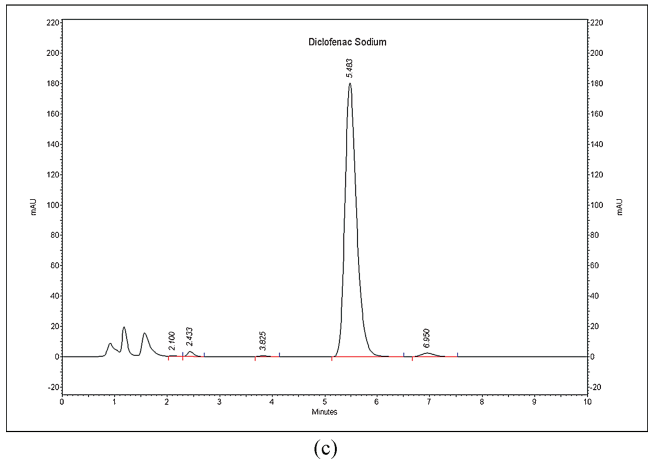
Five levels of concentration within the range of 5 and 50 µg/mL diclofenac sodium were prepared. Linear correlation was obtained between the peak area ratio of diclofenac sodium and the corresponding concentration. The regression line was y = 97432.440x + 2771.589 and had a coefficient of determination (R^2) of 1.000. This data indicates that the method is linear.



(a)



(b)



(c)

Fig. 2: Chromatogram of (a) a freshly prepared solution of diclofenac sodium, (b) a blank gel base, and (c) an in-house produced diclofenac gel 1%

2.2.3. Precision

Repeatability was determined by analyzing three replicate injections of the standard solutions of diclofenac sodium (5–50 µg/mL). This procedure was repeated on three different days for calculating intermediate precision (see Table 1).

2.2.4. Limit of detection/Limit of quantification (LOD/LOQ)

The LOD was defined as a S/N ratio of 3:1, the LOQ was determined to be S/N ratio of 10:1. The values were 0.31 µg/mL and 0.83 µg/mL. for LOD and LOQ for diclofenac sodium, respectively.

Table 1: Results obtained for the precision study

Day	Mean peak area ± R.S.D. (%)				
	Diclofenac sodium concentration (μg/mL)				
	5	10	18	25	50
1	491623.00 ± 1.76	1005355.00 ± 1.02	1813141.56 ± 2.28	2555936.67 ± 1.46	5107192.00 ± 0.33
2	498819.00 ± 0.22	1000047.00 ± 0.30	1834226.36 ± 0.21	2589734.00 ± 0.45	5046577.67 ± 0.49
3	497369.00 ± 1.66	1000410.33 ± 0.44	1831798.46 ± 0.54	2571964.67 ± 0.43	5124312.33 ± 1.41
Intermediate precision	495937.00 ± 1.38	1001937.44 ± 0.63	1826388.80 ± 1.29	2572545.11 ± 0.98	5092694.00 ± 1.04

Table 2: Accuracy results of diclofenac sodium

Defined value (μg/mL)	Found value (μg/mL)	Recovery (%)
5	5.13	102.53
10	10.25	102.53
18	18.72	103.81
25	26.17	104.69
50	51.73	103.46

2.2.5. Accuracy

The accuracy of the method was determined by comparing the predefined and the found value. The values were measured at each standard solution level using nine replicates. The difference of the predefined and the found values was between 102.53% and 104.69% for diclofenac sodium, depicted in Table 2. In order to test the method on the in house gel preparation, recoveries of diclofenac sodium were investigated. The diclofenac sodium concentrations of the spiked sample and the non-spiked sample were compared. The results indicated excellent recovery of 102% of diclofenac sodium.

2.3. Method application: assay of the in-house diclofenac sodium gel preparation and of commercial cream preparations

Once validated, the developed method was applied to assay diclofenac sodium concentrations in commercial cream preparations as well as in-house gel preparations. The assay results obtained by analysing the marketed formulations containing diclofenac sodium (1%, w/w) were in good agreement with the labelled amounts. The average contents of diclofenac sodium were assessed for two in-house batches. The results of the in-house batch analyses based on the validated system confirmed the quality of the tested products. The found drug concentration deviated about ± 5% from the declared values, thus being within the limit of tolerance. All tested batches met the quality requirements. The obtained results are shown in Table 3.

Table 3: Results obtained when analysing in-house gel preparations and commercial samples

Mean recovery rates of diclofenac sodium (%) ± R.S.D. (%) (n = 3)	
Diclofenac Gel 1% 130711	100.04 ± 0.91
Diclofenac Gel 1% 060911	99.26 ± 1.35
Diclobene®-Gel	99.74 ± 1.49
Diclostad® 1% Gel	98.60 ± 1.68
Diclofenac Genericon® 1% Gel	99.75 ± 1.24
Voltaren® Emulgel-Gel	100.03 ± 1.77

2.4. Irradiation enhanced stability studies

Degradation was observed after light exposure. Diclofenac sodium showed a mean initial concentration of 25.61 mg/mL ± 0.89% (R.S.D.). The mean ± R.S.D. diclofenac sodium concentration measured after irradiation in the suntest was: for 1 h: 23.98 mg/mL ± 0.94%, for 2 h: 21.99 mg/mL ± 1.90% and for 3 h: 19.87 mg/mL ± 5.29%. No "dark reaction" occurred, as the diclofenac sodium amounts from solution B did not differ after the 24 h storage in the dark to the ones measured immediately after irradation. After 3 h (corresponding to 45 h of natural sunlight exposure) of forced irradiation 80% of diclofenac sodium remained. Qualitatively similar results were observed for UV light irradiation, although the greater degree of degradation of diclofenac was observed after UV light exposure. After 3 h approximately 63% of diclofenac sodium remained.

2.5. Conclusion

A rapid stability indicating HPLC assay for selective determination of diclofenac is described and validated according to international standards (ICH). The selectivity of the system was confirmed with special emphasis to degradation products resulting from forced irradiation in a sunlight simulator, synthesis related impurities mentioned in compendial monographs had been considered as well. For semisolid products such as gels, a sample preparation procedure was developed to assure complete extraction of diclofenac. Thus, the proposed method is suitable for quantitation of diclofenac in various types of commercial formulations as well as for quality control of hospital in-house preparations and will facilitate preformulation and formulation studies of topical diclofenac preparations. The proposed assay is faster than pharmacopeal methods and can readily be implemented for routine analysis.

3. Experimental

3.1. Materials

Ultrasicc® (Lot no. 11395A) was purchased from Intendis Austria Handels GesmbH. Carbopol® 940 (Lot no. 0283/0210) was purchased from Gatt-Koller GmbH, Austria. Isopropanol (Lot no. KN-0859/10) was purchased from ACM, Herba Chemosan, Austria. Diclofenac sodium (Lot no. 06J23-N23) was purchased from Fagron GmbH, Germany. Methanol (HPLC grade, Fisher Scientific, Lot no. 1015381) and water (HPLC grade, Fisher Scientific, Lot no. 1086627) were purchased from Fisher Scientific. Triethylamin (Lot no. BCBF3275 V) was purchased from Sigma-Aldrich, Austria. o-Phosphoric acid 85% (Merck, Lot no. K27185373) was purchased from Merck, Germany. 0.45 μm syringe filters (Minisart RC25, Lot no. 17765) were purchased from Sartorius, Austria. Indoline-2-one (diclofenac impurity E, Lot no. S11367 V) was purchased from Sigma-Aldrich, Austria. 1-(2,6-dichlorphenyl)indoline-2-one (diclofenac impurity A, current batch number 7) was purchased from EDQM, France. Diclobene®-Gel (Lot no. L09782), Diclostad® 1% Gel (Lot no. KH5195), Diclofenac Genericon® 1% Gel (Lot no. N10423), Voltaren® Emulgel-Gel (Lot no. W7461) were purchased in a pharmacy.

3.2. Instrumentation

A SHIMADZU (Shimadzu Corporation, Japan) HPLC system equipped with 2 pumps (LC-10AS), autosampler (SIL-10AD), diode array detector (SPD-M10A), communications module (CBM-10A) and computer with software (LC-10) was used for the method development. Data acquisition, validation results and analysis were performed by SHIMADZU LC-2010AHT with software (Class-VP, Version 6.14 SP2A). The analytical column was a RP-select B column, 5 μm particle size (EcoCART® 125-3, LiChrospher® 60, Lot no. 813855, Merck KGaA, Germany). The column temperature was 30 °C. The aqueous phase consisted of a mixture of o-phosphoric acid and of 0.05 M triethylamine with an acidic pH of 2.8. The mobile phase consisted of methanol and aqueous phase in the ratio of 63:37 (v/v). The flow rate was set at 0.8 mL/min. The detection wave length was set at 220 nm. Sample injection volume was 20 μL.

3.3. Preparation of stock- and standard solutions

All solutions were prepared with methanol-0.05 M triethylamine pH 2.8 with o-phosphoric acid (63:37, v/v) as solvent. Three stock standard solutions of diclofenac sodium were prepared by accurately weighing individually 10 mg and two times 5 mg of diclofenac sodium, respectively. Each diclofenac sodium amount was transferred into a 100 mL volumetric flask and was diluted to volume in order to get concentrations of 100 μg/mL (1), 50 μg/mL (2) and 50 μg/mL (3) of diclofenac sodium. Standard solutions of diclofenac sodium were prepared by diluting stock standard solutions: 1. A solution of equal parts (by weight) stock standard solution 100 μg/mL (1) and solvent, obtaining a concentration of 50 μ/mL. 2. A solution of equal parts (by weight) stock standard solution 50 μg/mL (2) and solvent, obtaining a concentration of 25 μg/mL. 3. A solution of one part stock standard solution 50 μg/mL (3) and 4 parts solvent (by weight) obtaining a concentration of 10 μg/mL. 5. A solution of one part standard solution 25 μg/mL and 4 parts solvent (by weight) obtaining a concentration of 5 μg/mL. 5. A solution of one part standard solution 50 μg/mL (2) and 4 parts standard solution 10 μg/mL (by weight) obtaining a concentration of 18 μg/mL.

3.4. In-house preparation of diclofenac sodium gel 1%

120 g diclofenac sodium was weighed and dissolved in 1240 g purified water. 8000 g Ultrasicc® was added to this solution and mixed. 2560 g isopropanolum and 80 g Carbopol® 940 were separately and uniformly mixed using a RW16 basic IKA laboratory stirrer. The previously prepared solution of diclofenac sodium and the gel base were mixed homogeneously using a Fryma vacuum mixer model VME-12/C.

3.5. Assay of in-house formulation samples

An accurately weighed portion (ca. 1 g of diclofenac gel formulation 1%) was dissolved in 100.0 mL of methanol/water (63:37, v/v). The mixture was stirred for 10 min and protected from light. 500 μL of this solution was diluted with 1500 μL of eluent and filtered (0.45 μm microfilter). Spiked samples for recovery studies were obtained by mixing 20 mg diclofenac sodium with 2 g diclofenac gel 1% homogenously. 500 mg of this mixture were transferred into a 200 mL beaker, 100.0 mL of methanol/water (63:37, v/v) were added, 100 μL of this solution was diluted with 300 μL of eluent and filtered (0.45 μm microfilter).

3.6. Assay of marketed formulations

An assay of the marketed formulation containing diclofenac sodium (1%, w/w) such as Diclobene®-Gel, Diclostad® 1% Gel, Diclofenac Genericon® 1% Gel, Voltaren® Emulgel-Gel was performed by preparing the sample solutions as described in the previous section.

3.7. Stability evaluation of diclofenac gel 1% to simulated solar light/UV light 366 nm

The assessment of photostability was based on forced irradiation using a Suntest CPS Accelerated Exposure Machine (Heraeus, Hanau, Germany; Art. no. 55007014): xenon burner NXE 1500, black panel temperature: 49 °C, radiation intensity (1300 W/m2); windowglass filter (Art. no. 56009562); time factor: 15 (1 min Suntest *15 min bright sunlight). Distance of source to specimen table 22 cm. Stability tests were performed with the solution containing 25 μg/mL diclofenac sodium. This solution was transferred into three white glass vials (solution A, B and C), each containing 10 mL. Before applying the sun test the diclofenac sodium concentration was measured in the solutions A, B and C, respectively (t_0). Two solutions (solutions A and B) were exposed to forced irradiation for 1 (t_1), 2 (t_2) and 3 (t_3) h (corresponding to 15, 30 and 45 h of natural sunlight, respectively). 1 mL samples were taken after each irradiation step. To test whether the degradation continued after storage in the dark two samples were taken from solution B after each irradiation step. One was measured immediately and the next one being stored in the dark, was measured after 24 h. One solution was not exposed to solar light and was stored under light protection, thus being the reference solution (solution C). Another sample of 25 μg/mL (solution D) has been exposed to UV light (366 nm) for 3 h.

Parts of this project were presented as a poster at the annual EAHP Congress (European Association of Hospital Pharmacists), February 27–29, 2008, in Maastricht, Netherlands.

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