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A SPECIFIC STABILITY INDICATING HPLC METHOD TO DETERMINE DICLOFENAC SODIUM IN RAW MATERIALS AND PHARMACEUTICAL SOLID DOSAGE FORMS

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ABSTRACT

A high performance liquid chromatography method is described for the determination of diclofenac sodium, its related compounds and degradation products in commercial sources of raw materials and solid dosage forms. This method is specific, accurate and stability indicating. The method employs a reverse-phase octylsilane (C18) column with a mobile phase composed of acetonitrile/methanol/pic B-6 (25:25:50) and detection at 229 nm. The method resolves six principal related compounds with quantitation in the range 0.3 - 1.5%. Assay recoveries by spiking commercial formulations with diclofenac sodium were 99.64 ± 1.30%. Drug content in several commercial formulations are reported. Accelerated stability tests were conducted on raw materials and drug products and 1-(2,6-dichlorophenyl)-2-indolin-2-one was identified for the first time as a degradation product in solid dosage forms which are stressed under humidity and heat.

INTRODUCTION

Diclofenac sodium is a widely prescribed potent nonsteroidal anti-inflammatory drug. Although, this drug is now available from numerous commercial sources, the drug standards for diclofenac sodium have not been published in any compendial reference. This has necessitated the need to develop specific assay procedures for diclofenac in the presence of its related compounds and potential degradation products.

During the course of this work a study was published on the determination by HPLC of diclofenac sodium and related compounds in raw materials and formulations (1). However, the study does not report extensively on any impurities or potential degradation products that may be present in solid dosage forms. An earlier method was also published (2) for diclofenac sodium, but no specific assay validation data was reported. Other analytical methods have been reported (3) for the determination of diclofenac and its metabolites in biological fluids by HPLC.

In acidic aqueous solutions the cyclisation of diclofenac, by hydronium ion catalysis, to an indolinone derivative has been reported (4). The kinetics of cyclisation is a pseudo first order rate process dependent on the diclofenac concentration and pH, providing the solution possesses a hydrochloric acid molarity within the range 0.005 to 0.5 M. Similar cyclisation reactions have not been reported for diclofenac occurring in the solid state.

This paper reports on a specific HPLC procedure to determine diclofenac sodium and its related compounds, as well as degradation products that may be present in raw materials and pharmaceutical solid dosage forms. In the light of the analyses, the stability of diclofenac raw materials and commercial solid dosage forms of the drug are discussed.

EXPERIMENTAL

Reagents

Diclofenac Sodium and Related Compounds - Related compounds N-Chloroacetyl-N-phenyl-2,6-dichloroaniline (1), [2-(2-Bromo-6-chloroanilino)phenyl] acetic acid, sodium salt (11), N-Phenyl-2,6-dicloroaniline (111), N-1-(2,6-Dichlorophenyl) -2-indolin-2-one (1V), [o-(2,6-Dichlorophenylamino) phenyl] glyoxalic acid (V), and [o-(2,6-Dichoroanilino) phenyl] acetic acid methyl ester (V1) were obtained from Novopharm Ltd., Toronto, Ontario, Canada. Related compound (1V) was also prepared according to the method by Larson etal.(5). Diclofenac sodium raw materials and drug products were obtained from three manufacturers.

Solvents and buffers were obtained from the following: Methanol and Acetoniltrile HPLC grades Fisher Scientific, Ontario, Canada; Water HPLC grade Burdick and Jackson, Illinois, USA; and Pic B-6 buffer, Waters Scientific, Mississauga, Ontario, Canada.

Apparatus

A chromatographic pump (Waters 6000A) with a U6K injector and equipped with a variable wavelength detector (Waters Model #441) set at 229 nm. All data was collected from peak areas using an electronic integrator (Spectro-Physics, SP4290).

High Pressure Liquid Chromatogaphy (HPLC) Analyses.

A five micron C-18 octadecylsilane bonded phase column, 4.6 X 150 mm (Ultrasphere IP, Beckman, Canada) was used at ambient temperature. The mobile phase was acetonitrile/methanol/pic B-6(25: 25:50). The mobile phase was filtered using a 0.45 um membrane filter and then used to equilibrate the HPLC at 1.2 ml/min. During the analyses run the initial flow rate was 2.0 mL/min. After 28 minutes the flow rate was increased to 3.0 mL/min for the remainder of the run.

Accelerated Storage Testing.

These tests were conducted using a Temperature - Humidity Chamber, Hotpoint, model # 435304, Hotpack Corp., Philadelphia, USA.

Preparation of Solutions used in HPLC Analyses:

Sodium Diclofenac Standard Solution (A) - was prepared by accurately weighing and transferring about 87.70 mg of diclofenac sodium into a 100 mL glass volumetric flask, dissolved and made to volume with methanol and acetonitrile mixture (50:50).

Internal Standard - diflorasone diacetate was dissolved in methanol/acetonitrile (1:1) to give a concentration of about 0.587 mg/mL.

Working Standard Solutions - were prepared by pipetting 2, 3 and 4 mL of (A) and 3 mL of internal standard solution into a 25 mL volumetric flask and diluting to volume with acetonitrile/methanol (50:50) solution. 10 uL was injected into the HPLC.

Sample Solutions - were prepared by grinding 20 tablets to a powder and transferring a weighed amount equivalent to 50 mg of diclofenac sodium to a mixture of methanol/acetonitrile (50:50), sonicating, and diluting to 100 mL with more solvent. The solution was filtered and 3 mL of the filtrate transferred to a 25 mL volumetric flask with 3 mL of internal standard solution and then made to volume with more solvent. 10 uL was injected into the HPLC.

Raw Material Sample Solutions - 50 mg of the material was weighed accurately and dissolved in 100 mL of acetonitrile/methanol mixture (50:50) and appropriately diluted to 160 ug/mL. When precursors and degradation products are being determined the internal standard was omitted. 10 uL was injected into the HPLC.

Related Compound Solutions - were prepared according to the linearity concentration ranges reported in Table 1 at three concentration levels relative to diclofenac sodium present in acetonitrile/methanol (50:50) mixture. 10 uL was injected into the HPLC.

Spiked Solutions - Tablet powder was weighed equivalent to 50 mg of the drug and transferred to a 50 mL volumetric flask with 25 mL of acetonitrile/methanol (50:50) mixture, shaken for 15 minutes, and made to volume with same solvent mixture. 5.0 mL of the solution was transferred to a 50 mL volumetric flask with 6.0 mL of internal standard solution and made to volume with acetonitrile/methanol. 25 mL of this solution was spiked with 0.8779 mg of the drug and 10 uL was withdrawn for injection to the HPLC. Similarly, 10 uL of the unspiked solution was injected into the HPLC.

Accelerated Storage Testing Sample Solutions - were prepared according to sample solutions as described above.

Calculation - the percent label content for diclofenac sodium was calculated separately by the formula (100) (Ru/Rr) (qr/L) (Dref./Dsam.), where Ru and Rr are the analyte and standard ratio of areas to internal standard, respectively, qr is the reference in mg. Dref. and Dsam. are the dilution factors for the reference and sample solutions, respectively. L is the labelled strength for the drug product. When determining related compounds, peak area ratios were used in the formula without the internal standard.

RESULTS AND DISCUSSION

Chromatograhic separations (Fig.1) were achieved for diclofenac (V11) and its related compounds (1), (11), (111), (1V), (V), (V1) using an ion pair solvent system with a reverse phase 5-micron particle size column. This chromatographic system can be used for both raw materials and commercial formulations of the drug as no excipient interferences were

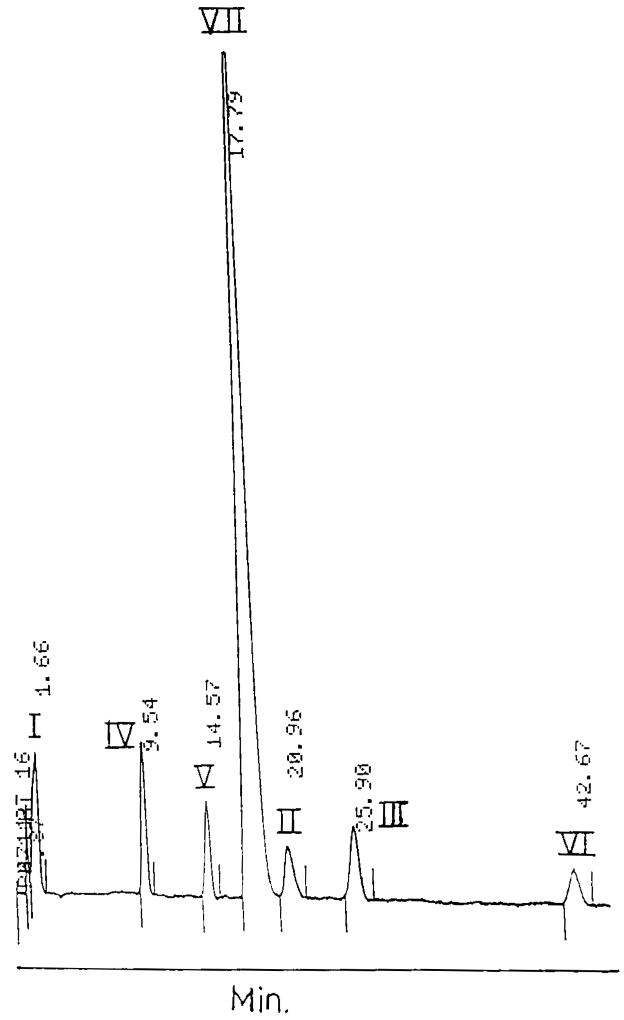

Figure 1

HPLC Chromatogram of Diclofenac Sodium & Related Compounds.

encountered using the HPLC technique. The relative retention times for the related compounds based on the retention time of diclofenac sodium are given in Table 1.

System suitability tests were conducted on solutions of the assay preparations of diclofenac sodium and related compounds according to the USP methods (6), and the tailing and resolution factors are shown in Table 1. Only the peak corresponding to compound (V1) possessed significant tailing, which was due to its long retention time of about 45 minutes.

Table 1 - Analytical Parameters/Retention Times for Diclofenac Sodium and Related Compounds.

Compound	Linearity Range %	Detection Limit %	RRT *	Tailing Factor	Resolution Factor **
I	0.3 - 1.5	0.1	0.83	1.0	0.50
II	0.3 - 1.5	0.1	1.18	2.3	0.49
III	0.3 - 1.5	0.1	1.48	1.0	1.40
IV	0.3 - 1.5	0.1	0.55	2.2	1.48
V	0.3 - 1.5	0.1	0.83	1.0	2.74
VI	--	0.5	2.43	4.5	4.13
VII	***	--	--	1.0	--

*** RRT = retention time relative to diclofenac sodium at about 18 minutes.**

**** Determined with respect to the diclofenac sodium peak.**

***** Calibration range: 0.070 - 0.140 mg/mL**

The linearity of the system was demonstrated by constructing calibration curves for diclofenac sodium and its related compounds. Least squares analyses resulted in coefficient of variations close to unity that confirm the working concentrations used in the assays were linear. This data was obtained by replicate analyses of standard solutions of diclofenac and related compounds at three concentration levels within the linearity ranges specified (Table 1). It was not possible to obtain satisfactory linearity in the range 0.3 - 1.5% for the methyl derivative (V1) due to extensive widening and tailing of the peak.

Assay recoveries for diclofenac sodium were determined by spiking commercial samples with the drug. The average percent recovery (mean ± relative SD) and the precision for four (4) determinations was 99.64 ± 1.30%.

Three brands of enteric coated tablets of diclofenac sodium tablets were assayed by the procedure and the results in percent were as follows: 106.27 ± 1.78 (Formulation A, 25 mg), 100.69 ± 2.01 (Formulation A, 50 mg), 98.81 ± 2.09 (Formulation B) 101.51 ± 2.54 (Formulation

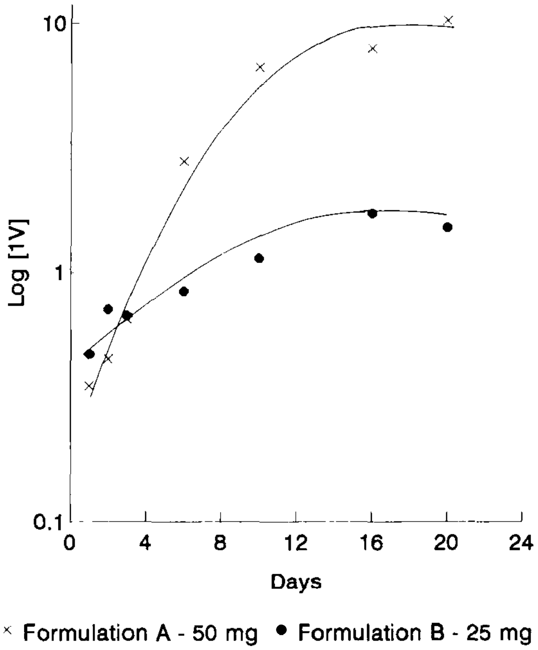

Figure 2
Plot of Rate of Formation of Indolinone Derivative from Diclofenac Sodium Formulations

C, 25 mg) and 94.94 ± 1.98 (Formulation C, 50 mg). The precision of the assays were consistent with the recovery studies previously discussed. None of the related compounds, including the indolinone derivative could be detected in any of the commercial preparations and raw materials studied. Also no excipient interferences were exhibited from the formulations examined.

Accelerated Stability Indicating Studies.

Accelerated stability testing was conducted on commercial samples of raw materials and tablet formulations of sodium diclofenac.

Tablet formulations (powdered) and raw materials samples were kept in a constant temperature/humidity oven at 40 C and 50% humidity over a twentyeight day period. The formulations were powdered to increase the possibility of decomposition. Samples were withdrawn at certain intervals and on the final day of the period. Analyses of the samples by HPLC and TLC did not show the presence of any degradation products. A similar test was repeated at 90 C and 55% humidity over a twenty day period. Samples were withdrawn at noted intervals and analysed by TLC and HPLC. Both TLC and HPLC analyses identified the presence of the related compound, 1 - [2,6 - dicholorophenyl] - 2 -indolin-2-one (1V) as a degradation product in the tablet formulations, which was evidenced by identical rf values and retention times, respectively, to an authentic sample of (1V). However, this compound (1V) could not be detected in any of the samples of the stressed diclofenac raw materials.

The degradation product (1V) found in the tablet formulations that were stressed under accelerated storage conditions was quantitated in percent of labelled strength of diclofenac sodium by HPLC and the results recorded over a twenty day period were plotted as shown in Fig. 2. The log [conc.] - time curves (Fig. 2) for commercial formulations A and B exhibit maxima at 10% and 1% concentrations, respectively, for the formation of (1V). The rate of formation of (1V) did not appear to follow pseudo zero or first order rate kinetics in either formulation. These preliminary results suggest that excipients and tablet potency decrease the stability of the drug, i.e. enhances the rate of formation of the cyclised degradation product.

CONCLUSION

This accurate and precise HPLC method is specific for the determination of diclofenac sodium in the presence of its related compounds and potential degradation products in commercial raw materials and pharmaceutical solid dosage forms. Several commercial dosage forms and raw materials have been screened by the HPLC procedure and found to be stable and contain no principal related compounds or degradation products.

An indolinone derivative was found as a degradation product when formulations were stressed by accelerated storage conditions. The identification of this degradation product proves that the cyclisation of diclofenac to an indolinone derivative is a valid degradation pathway for this drug in solid dosage forms when stressed under humidity and heat. The results suggest that excipients and drug content in formulations may decrease the diclofenac stability.

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