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IMPROVED CHROMATOGRAPHIC METHOD FOR DETERMINATION OF SODIUM DICLOFENAC IN INJECTABLE SOLUTION AND PREDICTION OF CHEMICAL STABILITY

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Abstract

Two modified methods for assaying sodium diclofenac were developed by GC and HPLC. Diclofenac was converted into methyl ester derivative by methyl iodide in acetone. The ester was extract and subjected to GLC with flame ionization detector. %5 SE-30/chrom W-HP (80-100 mesh) was used as a column in GC. For reversed phase HPLC, the mobile phase was methanol and water (55-45). The separation was performed on an analytical 300×3.9 mm i.d. μ-bondapak phenyl column using UV detector (274 nm). O-(4-chlorobenzoyl)benzoic acid and mefenamic acid were used as internal standard for GC and HPLC method respectively. The stability of diclofenac was examined by results obtained from two chromatographic methods at high temperature. The described HPLC and GC methods were successfully applied for the prediction of the shelf life period of diclofenac.

Keywords: Diclofenac; Determination; Chemical stability

Introduction

The chemical stability of a drug is investigated to predict the shelf life of a drug in marketing stage. It is well known that change in the actual field storage temperature causes the reaction rate constant of some products to change according to the Arrhenius relationship [1]. The use of accelerated temperature stability studies using the Arrhenius equation is routine for estimating the stability of a drug at room temperature [2]. Sodium diclofenac, [2-(2,6-dichlorophenyl) amino phenyl acetic acid sodium salt], is an active anti-inflammatory and anti-rheumatic agent. Several methods [3-13] such as spectrometry, GLC and HPLC have been reported for determining of diclofenac. In the reported GLC procedures derivatization was carried out using reagents such as methanol/H_2SO_4, pentafluropropionic anhydride, 2,2,2-trifluroethanol/H_2SO_4, diazomethane, iodomethane and etc. two methods have used diazomethane as a derivatizating agent that is not

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safe for analyzer [3,12]. Some of them [3,7,8] used an expensive electron capture (ECD), a facility that is not commonly available in all laboratories. There is a report [5] that discus about formation of two compounds via derivatization procedures with methanol/H_2SO_4, indolone (compound C, Fig. 1) and methyl ester of diclofenac. Assay of two products diminish the precision and accuracy of method. Gieger [3] reported a method for the determining of diclofenac, which consisted of derivatizing the compound into indolone and analyzing the derivative by GLC. Ikeda, *et al.* [4] presented a different method for the determination of diclofenac which utilizes derivatization of diclofenac into methyl ester and showed that the method has a three-folder higher sensitivity than the indolone method, but unfortunately in their method they used ECD detector.

Accelerated stability testes of diclofenac were conducted on both raw material and drug formulation product [14]. Sodium diclofenac injectable solution with a 2-year expiration date is currently available in Iranian pharmaceutical market.

The present report describes rapid, simple and accurate method for the determination of the shelf life of diclofenac with GC and HPLC.

Material and Methods

Chemical

HPLC-grade methanol and all other analytical grade reagent were purchased from Merck Company (Darmstadt, Germany). HPLC-grade water was obtained by double distillation in glass and purified through a Mill-Q water purification system (Millipore, Bedford, MA). Water was filtered through 0.45-µm filter and mobile phase was filtered through 0.22-µm filters (Millipore). Injectable solution of sodium diclofenac and standard USP-grade of sodium diclofenac were supplied by Tolid Daru Pharmaceutical Company (Tehran, Iran). ^{1}HNMR spectra were recorded on a Brucker AC-80 spectrometer.

GC Analysis

Two stock solutions of sodium diclofenac (20 mg/ml) in distilled water (stock A) and O-(4-Chlorobenzoyl) benzoic acid, internal standard (i.s.), (20 mg/ml) in 0.01 M phosphate buffer (pH=8.5), (stock B) were prepared. Seven samples containing different volumes (0.5-3.5 ml) of stock A solution and 1 ml of stock B solution were prepared in test tube with a good fit cap. 0.1 ml of hydrochloric acid solution (0.1 M) was added and extraction was done with diethyl ether (2×5 ml). Organic layer was dried on sodium sulphate and evaporate under reduced pressure. Methylation was carried out by adding 0.2 ml methyl iodide in 1 ml acetone in the presence of 20 mg potassium carbonate. The mixture was heated at 60°C with shaking for two hours. The solvent was evaporated under reduced pressure. To the result was added 10 ml of chloroform and 2 ml of distilled water. Organic phase was separated and dried over sodium sulphate. 1-µl of organic phase was injected to GC.

HPLC Analysis

Two stock solutions were prepared from sodium diclofenac (1 mg/ml) and mefenamic acid (91 mg/ml). Six different dilutions of standard solution were prepared at a range of 8-35 µg/ml for getting the calibration curve. The concentration of internal standard in all solutions was constant and equal to 1-µg/ml. 25 µl of each standard solution was injected to HPLC.

Chromatographic System and Condition

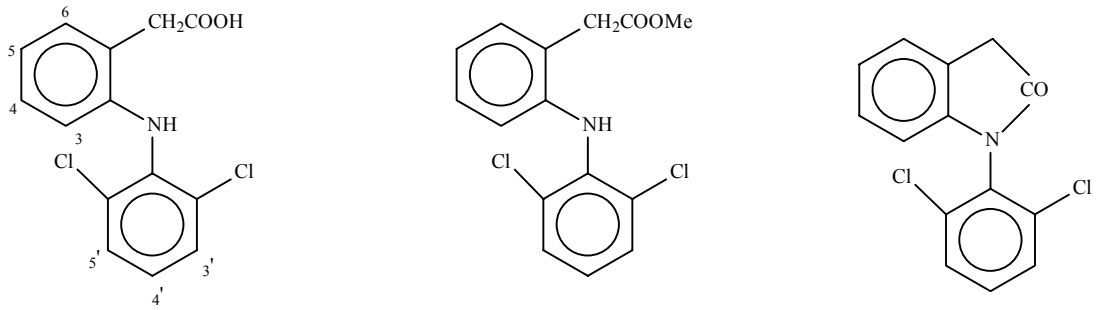
A Varian model 3600 gas chromatograph equipped with flame ionization detector (FID) was used for GC analysis. The column was 5%SE-30/chrom/WHP 80/100 2 m. Each injection was performed in triplicate with an interval of 15 min between two injections to ensure complete elution of the last injection. The temperature of injection port and detector oven was set at 250°C and 300°C respectively.

The column was initially held at 200°C and then the temperature was increased with a rate of 5°C/min to 245°C. the carrier gas was nitrogen at a flow rate of 40 ml/min. the peak area ratio was used for quantitative evaluation.

The high-pressure liquid chromatographies consist of a Waters 510-flow pump, a Waters UK-6 manual injector, a Waters 481 UV detector setting on 274 nm and a Millipore 476 integrator recorder. The column was µ-bondapak phenyl (waters, 300×3.9 mm). The mobile phase was methanol/water (55/45) with a pH=3.3 regulated by acetic acid. The flow rate was 2 ml/min and prior to the first injection was conditioned for 1 h.

Calibration Curve and Determination of Yield

A calibration graph was constructed by plotting the peak area ratio of sodium diclofenac to internal standard against the concentrations of sodium diclofenac. The linearity of the method was demonstrated by analysis of



A **B** **C**

Figure 1. Structure of diclofenac (A), diclofenac methyl ester (B) and degradation product (C).

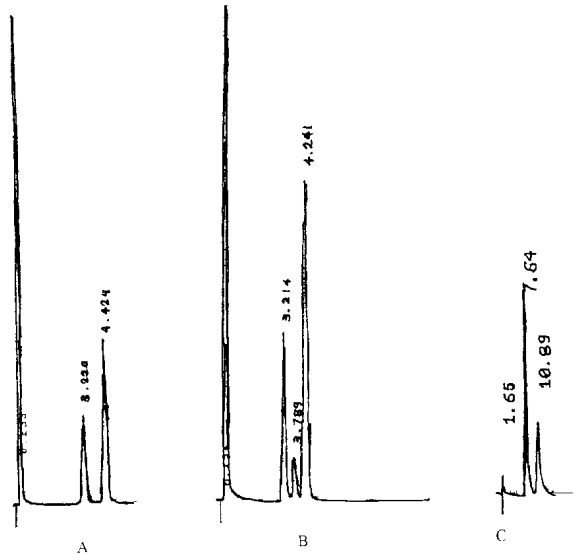


Figure 2. A) GC chromatogram of diclofenac methyl ester, Rt = 3.22 and i.s., Rt = 4.24; B) GC chromatogram of A and compound C (degradation product), Rt = 3.70; C) HPLC chromatogram of sodium diclofenac, Rt = 7.64 and i.s. (mefenamic acid), Rt = 10.89.

seven different concentrations of sodium diclofenac with constant concentration of i.s.

In order to determine yield of methylation in GC sampling, diclofenac methyl ester (Fig. 1-B) was synthesis according to the following procedure:

To 1 g sodium diclofenac in 150 ml distilled water was added 0.3 ml HCl (37%). The free acid (Fig. 1-A) extracted with 3×25 ml diethyl ether and purified by crystallization in $CHCl_3$: Hexane (1:2).

^{1}HNMR ($CDCl_3$) δ: 3.82 (s, 2H, CH_2), 6.55 (dd, J=8Hz and J=1.8Hz, 1H, H_3), 6.81 (bs, 1H, NH), 6.92-6.93 (m, 2H, H_5 and $H_{4'}$), 7.11-7.15 (m, 1H, H_4), 7.23 (dd, J=8.2Hz and J=1.7Hz, 1H, H_6), 7.34 (d, J=8Hz, 2H, $H_{3'}$ and $H_{5'}$), 11.12 (bs, 1H, COOH).

To 0.2 g free acid in 20 ml acetone was added 0.21 g methyl iodide and 1 g potassium carbonate in a round bottom flask. The mixture was refluxed for 2 h at 60°C. the solvent was evaporated under reduced pressure and 50 ml of saturated k_2CO_3 solution was added. Methyl ester was extracted with 3×25 ml chloroform and was purified by column chromatography with $CHCl_3$: Hexane (1:1).

^{1}HNMR ($CDCl_3$) δ: 3.75 (s, 3H, OCH_3), 3.81 (s, 2H, CH_2), 6.54 (dd, J=8.1Hz and J=1.7Hz, 1H, H_3), 6.93-6.97 (m, 3H, NH, H_5 and $H_{4'}$), 7.11-7.15 (m, 1H, H_4), 7.23 (dd, J=8.1Hz and J=1.7Hz, 1H, H_6), 7.35 (d, J=8Hz, 2H, $H_{3'}$ and $H_{5'}$).

Seven samples containing the standard diclofenac methyl ester over range of 1-7 mg/ml were prepared and a second calibration graph was constructed by these concentrations. Comparing the results of this calibration equation and calibration graph of sodium diclofenac in pervious stage showed that the mean of methylation of diclofenac via described method was 93.6%.

Result and Discussion

Typical chromatograms of diclofenac illustrated in Figure 2-A obtained from GC analysis. Under the described chromatographic condition, diclofenac methyl ester and i.s. peaks were well-resolved with retention time of 3.22 and 4.24 respectively. No interfering peak was observed at a retention time similar to diclofenac methyl ester or i.s. Retention time of degradation product was 3.7 min. There are some reports about the structure of degradation product of diclofenac in some dosage forms. Kubala [14] reported 1-(2,6-dichlorophenyl)-2-indoline-2-one (compound C, Fig. 1) was formed in the presence of heat or humidity in solid

dosage forms as a degradation product via a dehydration reaction. In order to assign the structure of peak with 3.7 min retention time, compound C was synthesized according to the literature [3]. Injection of C to GC according to the method described above showed that peak with retention time of 3.7 min in Figure 2-B is 1-(2,6-dichlorophenyl)-2-indoline-2-one.

The calibration curve for determination of diclofenac was linear over the range of 1-7 mg/ml and the corresponding regression equation was $Y = 0.428\ X + 0.0199$ ($r = 0.9996$), where Y is the peak area ratio of sodium diclofenac to i.s. and X is sodium diclofenac concentration.

The precision of the assay was presented by coefficient of variation (Table 1). Within-day coefficient of variation, C.V., (n=3) in all used concentrations was less than 6.77. In Figure 2-C typical chromatogram of sodium diclofenac and i.s. (mefenamic acid) which was obtained by HPLC analysis is illustrated. Retention time for diclofenac and i.s. was 7.64 and 10.84 respectively. The calibration curve was linear over the range of 5-35 µg/ml with a regression equation $Y = 0.135\ X - 0.0227$, where Y is the peak area ratio of sodium diclofenac to i.s. and X is the sodium diclofenac concentration. Table 2 clearly indicates that the assay is precise, with an inter assay coefficient of variation of ≤ 7.27% (n=3) at used concentration. Table 3 shows the results of stability study at 80°C that the concentration of sodium diclofenac was determined by GC. 2-day interval was selected for the determination of remaining concentration of sodium diclofenac. In this table, A is area ratio of diclofenac to i.s. that was kept at room temperature and a-f are remaining of sodium diclofenac that kept at 80°C with 2-day interval (2-12 days for a-f respectively). All determinations were triplicate (1,2 and 3) and assigned as described method for standard solutions. The results clearly indicate that there is no significant reduction in the concentration of sodium diclofenac. The same results have been acquired at 70°C and 90°C. Table 4 shows the results of stability study at 90°C. The concentration of sodium diclofenac was determined by HPLC. "A" is area ratio of diclofenac to i.s. that was kept at room temperature and a-f are remainder of sodium diclofenac at 90°C with 2-day intervals. The same results were obtained at 70°C and 80°C. Here again no significant reduction in the concentration of diclofenac is observed. The statistical test (ANOVA test) showed no significant different between concentrations of all cases compared to control (A term in Table 3 and Table 4) with a $F(5,12) = 0.34$, $P = 0.879 > 0.5$ for Table 3 and a $F(5,12) = 2.421$, $P = 0.097 > 0.05$ for Table 4.

Table 1. Reproducibility of the analysis of diclofenac by GC. 1,2 and 3 are peak area ratio of standard to internal standard in a within-day study

	1 mg/ml	**2 mg/ml**	**3 mg/ml**	**4 mg/ml**	**5 mg/ml**	**6 mg/ml**	**7 mg/ml**
1	0.506	0.927	1.250	1.663	2.066	2.580	3.015
2	0.481	0.893	1.252	1.712	2.204	2.548	3.061
3	0.442	0.872	1.293	1.695	2.203	2.553	3.021
Mean ± SD	0.476 ± 0.032	0.897 ± 0.028	1.265 ± 0.024	1.690 ± 0.024	2.157 ± 0.079	2.560 ± 0.017	3.032 ± 0.025
CV%	6.72	3.12	1.89	1.47	3.68	0.66	0.82

Table 2. Reproducibility of the analysis of diclofenac by HPLC. 1,2 and 3 are peak area ratio of standard to internal standard in a within-day study

	5 µg/ml	**10 µg/ml**	**15 µg/ml**	**20 µg/ml**	**25 µg/ml**	**30 µg/ml**	**35 µg/ml**
1	0.639	1.263	1.890	2.671	3.697	4.066	4.781
2	0.722	1.268	2.112	2.533	3.253	3.960	4.614
3	0.660	1.275	1.902	2.784	3.281	4.014	4.568
Mean ± SD	0.670 ± 0.043	1.269 ± 0.006	1.998 ± 0.106	2.663 ± 0.125	3.410 ± 0.248	4.013 ± 0.053	4.654 ± 0.112
CV%	6.44	0.47	5.31	4.72	7.27	1.32	2.41

Table 3. Assay results for diclofenac at 80°C. Analysis was performed by GC. 1,2 and 3 are peak area ratio of standard to internal standard in 3- separated injections. a-f are remaining concentration of sodium diclofenac that kept at 80°C. with 2-day interval (2-12 day for a-f respectively). Data related to a sample that was kept at 25°C is represented by A

	a	b	c	d	e	f	A
1	2.943	2.404	2.328	2.524	2.182	2.535	2.764
2	2.680	2.479	2.852	2.477	2.564	2.174	2.373
3	2.431	2.412	2.390	2.450	2.485	2.487	2.420
Mean ± SD	2.534 ± 0.129	2.432 ± 0.041	2.523 ± 0.286	2.483 ± 0.037	2.410 ± 0.202	2.398 ± 0.196	2.516 ± 0.213
CV%	5.11	1.69	11.35	1.51	8.37	8.17	8.48

Table 4. Assay results for diclofenac at 90°C. Analysis was performed by GC. 1,2 and 3 are peak area ratio of standard to internal standard in 3-separated injections. a-f are remaining concentration of sodium diclofenac that kept at 90°C. with 2-day interval (2-12 day for a-f respectively). Data related to a sample that was kept at 25°C is represented by A

	a	b	c	d	e	f	A
1	2.269	2.351	2.503	2.483	2.534	2.432	2.444
2	2.350	2.143	2.497	2.375	2.254	2.116	2.064
3	2.275	2.213	2.459	2.311	2.404	2.264	2.433
Mean ± SD	2.298 ± 0.045	2.230 ± 0.105	2.486 ± 0.024	2.390 ± 0.087	2.397 ± 0.140	2.271 ± 0.158	2.314 ± 0.216
CV%	1.96	4.75	0.96	3.64	5.84	6.96	9.34

Conclusion

The present technique reports a rapid, simple, accurate and improved method for the determination of diclofenac in aqueous solutions. In GC analysis we used methyl iodide for methylation that is safer than diazomethane. Our method provides adequate sensitivity for routine use with FID detector. Because of short elution time and sampling fluency, analyzing the large number of samples in a short period is possible.

Results obtained from the analysis of sodium diclofenac showed that the rate of decomposition of this compound is so slow even at high temperature that determination of kinetic parameter and Arrhenius equation is impossible. Based on the fact that the chemical stability of compounds at room temperature is generally more than high temperature, the chemical stability of sodium diclofenac injectable solution should be stable for a long period of time at room temperature. For exact determination of the expiration date, the periodical study at room temperature should be done.

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