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Determination of Valdecoxib From Pharmaceutical Formulations and In-Vitro Comparison of Dissolution Profiles

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1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) mainly express their effects by the inhibition of cyclooxygenase enzymes (COX), which are the rate limiting enzymes in the arachidonic acid pathway (1) (Figure 1).COX inhibitors are generally used for the treatment of acute and chronic pain, rheumatoid arthritis and osteoarthiritis. Due to the inhibition of COX activity and related pro-inflammatory mediators like prostaglandins (PG) and tromboxanes (TBX). COX exists in two isoforms, COX-1 and COX-2 (2, 3). While COX-1 is thought to account for homeostatic amounts of eicosanoids, COX-2 is induced during inflammation leading to the formation of pathologic amounts of prostaglandins. The inhibition of prostaglandin synthesis by NSAIDs has been demonstrated to effectively reduce inflammatory symptoms such as oedema and pain (3, 4). However, this does not satisfactorily explain all the NSAIDs' analgesic effects. Other mediators of inflammation such as reactive oxygen products and cytokines have also been shown to considerably contribute to inflammation and inflammatory pain (5, 6). So, concurrently to the transcriptional induction of the COX-2 gene, the expression of the gene encoding inducible nitric oxide synthase (iNOS) is induced, leading to increased levels of nitric oxide (NO) in inflamed tissues (7).

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Valdecoxib (VDX), (4-[5-methyl-3-pheny-4-lisoxazolyl]-benzenesulfonamide), is an anti-inflammatory drug belonging to the class of COX-2 inhibitors of the NSAIDs (8) (Figure 2). VDX is rapidly absorbed after oral ingestion with an absolute bioavailability of 83% in humans (9). It is a poorly soluble and high permeable drug, and listed in class 2 of biopharmaceutical classification of drugs. The protein binding of VDX is about 98% and the elimination half life is 8-11 hours (10). The in-vitro IC_{50} values for valdecoxib has been found as 0.005 µM for recombinant COX-2 enzyme and 140 µM for COX-1 recombinant enzyme, proving its

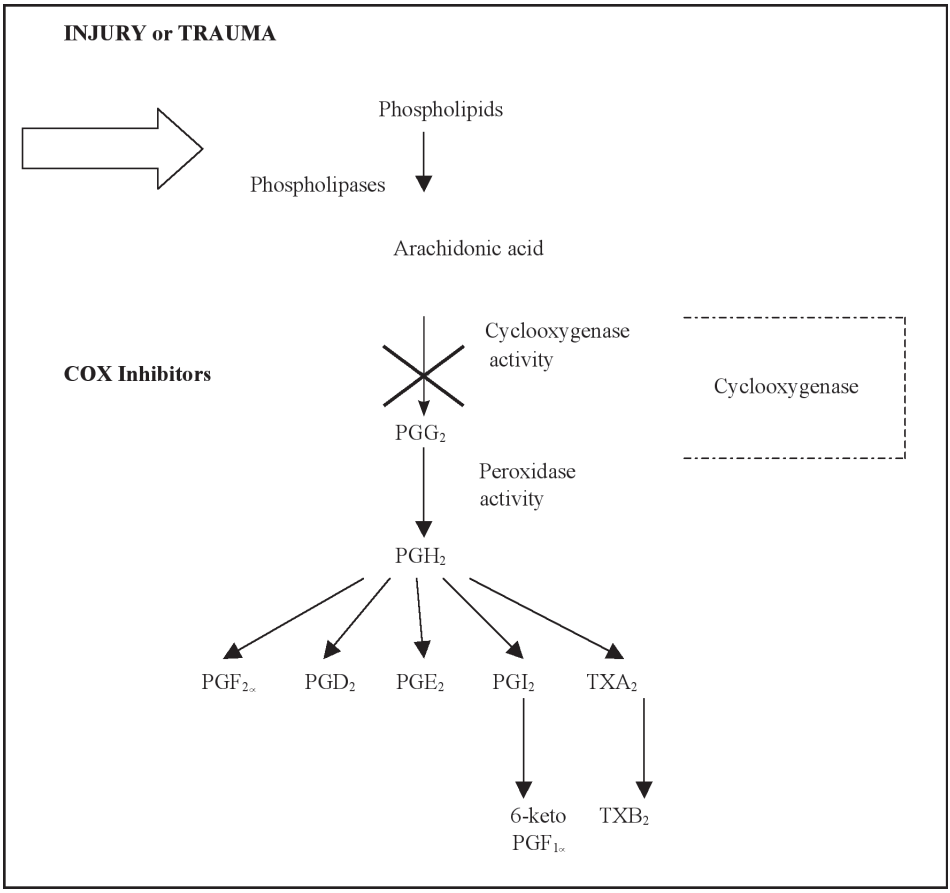


Figure 1

Prostaglandin synthesis pathway after injury or trauma. After an injury or trauma, arachidonic acid is formed by phospholipase activity. Arachidonic acid is converted to PGH_2 via PGG_2, which is the common precursor for the synthesis of PGs. COX inhibitors block the activity of the enzyme cyclooxygenase, thus inhibiting the formation of PGG_2 (1)

selective blockage effect of the prostaglandin E_2 synthesis by the these two enzymes. (IC_{50} =concentration required for the 50% inhibition of the enzyme activity) (11). Thus selective inhibition of COX-2 while preserving COX-1 function maintains analgesic and anti-inflammatory effects without any gastrointestinal side effects.(12-14).

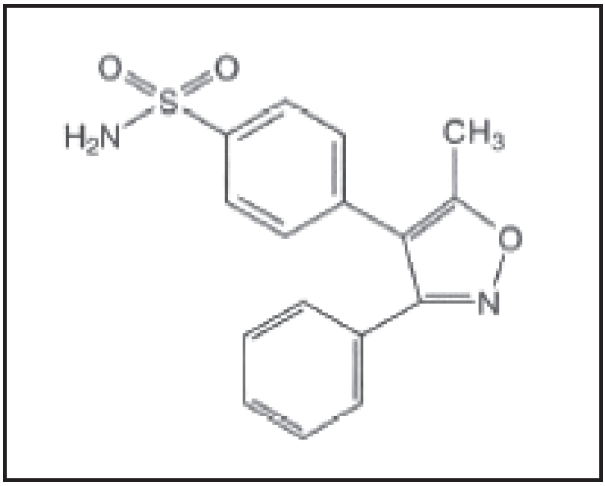


Figure 2

Chemical structure of Valdecoxib (8)

In this study, the determination of valdecoxib from pharmaceutical tablet formulations has been evaluated by using a high pressure liquid chromatography method equipped with an UV detector. The aqueous solubility of VDX in the dissolution media containing sodium lauryl sulphate (SLS) was assessed to prepare a dissolution system which satisfies sink conditions for testing commercially available VDX tablets.

2. Materials and Methods

2.1. Materials

The active ingredient valdecoxib was from Hetero International (Mumbai, India). The other chemicals used were all chemical grade and used without further purification.

2.2. Chromatography

The high performance liquid chromatography system (HP 1050, Hewlett Packard GmBH, Germany) was equipped with a quaternary pump, an auto sampler, an injector with a 100µL loop, a column oven, a UV detector and a HP Chem Station software (version A 08.03). The separation of VDX was made on a Supelco Spherisorb ODS 2 column (5µm; 250x4.6 mm i.d.) at ambient temperature. The mobile phase was a mixture of acetonitrile (ACN): water (50/50-v/v) pumped at a flow rate 1.0 mL/min. The total injection volume of the samples was set as 20 µL. Detection was set at a wavelength of 210 nm.

2.3. Analytical method validation

2.3.1. Calibration and control samples

The working solutions for calibration were prepared from the stock solution of VDX (100 µg/mL) in deionized water containing 1% (w/v) SLS and 2% (w/v) SLS. A calibration curve was constructed from blank sample and 7 non-zero samples covering the total range of 1 µg/mL up to 64 µg/mL. Calibration curves were generated on 6 different batches and linearity was assessed by weighed ($1/x^2$) least squares regression analysis. The acceptance criterion was set as the 2% coefficient of variation at same concentration of the 6 different batches. The other validation parameters were evaluated only over the SLS 1% working curves.

2.3.2. Specificity

The chromatogram of 4 µg/mL VDX sample was compared with the other chemicals' HPLC chromatograms (mobile phase and SLS) for the investigation of possible peak interactions.

2.3.3. Recovery

Three concentrations as low, medium and high (1, 16 and 64 µg/mL) were set as the control points of the analytical method. Six different solutions at these three concentrations were prepared and analyzed with the HPLC method. The recovery of the VDX was then calculated with the calibration curve and the results were evaluated over the coefficient of variation at these concentrations.

2.3.4. Precision

Within batch accuracy and precision evaluations were performed by repeated analysis of VDX solution having concentrations of 1, 16 and 64 µg/mL as low, medium and high concentrations. The reproducibility of the determined concentrations was evaluated over six different batches. The repeatability of the stock solutions was assessed by six replicates over the same batch. The results are expressed in terms of means, standard deviations and coefficient of variation.

2.3.5. Stability

The short term stability of VDX was examined by keeping the stock solutions of VDX at concentrations of low (1 µg/mL) and high (64 µg/mL) at 37±0.5°C and room temperature with or without exposure to light for 24 hours. For each concentration and each storage condition, three replicates were analyzed in one analytical batch. The concentration of VDX after each storage period was related to its initial concentration as determined for the samples that were freshly prepared and possessed immediately.

2.3.6. Sensitivity

The sensitivity of the analytical method has been evaluated by the determination of limit of detection (LOD) and limit of quantification (LOQ) parameters (15,16). The LOD value was recorded as the concentration of VDX with the signal to noise ratio of the HPLC chromatogram was 3:1. Similarly the LOQ value was recorded as the signal to noise ratio was 10:1.

2.4. Dissolution of Bextra ® Tablets

The dissolution of the commercially available VDX tablets was investigated in three different aqueous media. Due to the poor solubility of the VDX, varying concentrations of SLS has been added within the range of 1-2% (w/v). The dissolution profiles of VDX tablets containing 10 mg, 20 mg and 40 mg VDX were carried out in the Sotax A7 Smart Dissolution Test Device with the Apparatus 2 (pedal) at 37±0.5°C over six replicates. The dissolution profiles were determined in two different media as SLS (1%-w/v) solution and SLS (2%-w/v) solution with total volume of 1000

mL. The rotation speed of the apparatus was set as 75 rpm and at certain time intervals 5 mL of samples were withdrawn and replaced with the same volume of fresh medium immediately. The samples were analyzed with the HPLC method which was previously described.

2.5. Dissolution Profile Comparison

The dissolution profiles of VDX tablets containing 10 mg, 20 mg and 40 mg VDX were compared by using model independent approach (17, 18). Determination of the similarity of the profiles, f_2 test has been executed by using the following formula;

$$f_2 = 50\ x \log\left\{\sqrt{1+\frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2}\right\} x 100$$ where

f_2 = similarity factor

n = number of time points

R_t = released amount from reference product at time t

T_t = released amount from the test product at time t

The profiles are of the dosage forms are accepted as similar when the f_2 values are greater than 50 when two profiles are compared with each other.

3. Results and Discussion

3.1. Chromatography

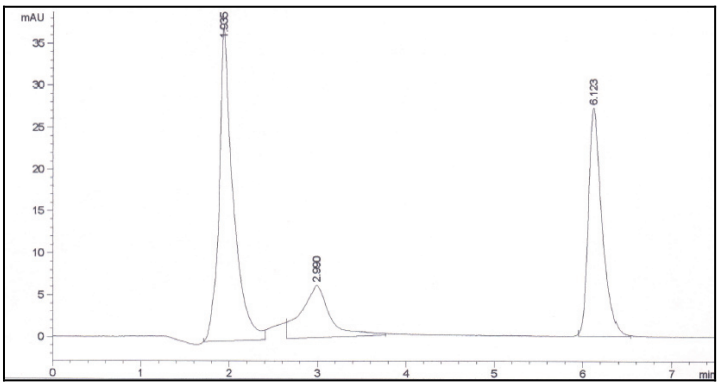
VDX was well separated from the materials existing in the stock solutions at retention time of 6.1 min (Figure 3). The peak was of good shape, completely resolved from another peaks originating from the other chemicals in the samples without any interference.

3.2. Analytical Method Validation

3.2.1 Linearity and Specificity of the Assay

Linear least-square regression analysis of the calibration graph on six different batches demonstrated linearity between the response and nominal concentration of VDX over the range of 1-64 µg/mL. The equations of the standard curves of VDX in 6 different batches are shown in Table 1 and 2. The results of linear regression analysis revealed determination coefficient (r^2) values better than 0.9999.

a.



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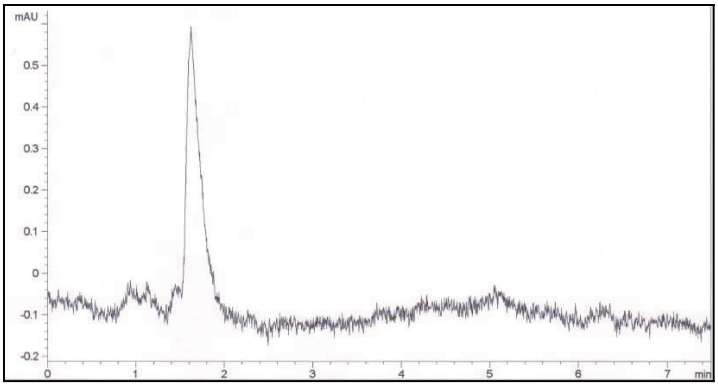

c.

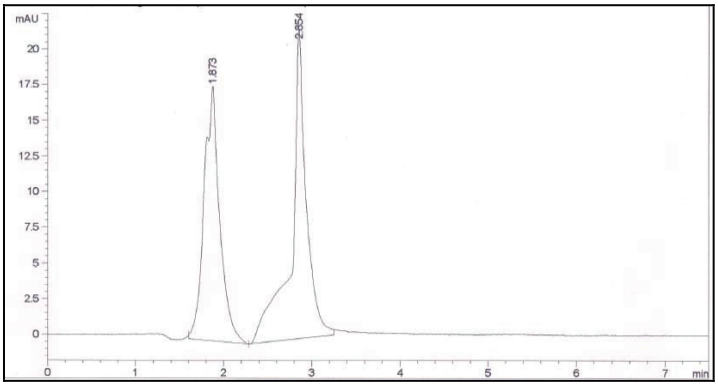

Figure 3
HPLC choromatogram of a. VDX (4 µg/mL), b. Mobile phase c. SLS (1%-w/v) solution in deionized water.

TABLE I

The coefficients of the standard curves for HPLC assay of VDX (SLS 1%-w/v)

	Slope	Intercept	r^2
1	72.983	3.759	0.9998
2	72.491	5.461	0.9996
3	72.387	8.863	0.9997
4	72.572	7.397	0.9999
5	72.564	6.758	0.9999
6	72.345	9.712	0.9995
Mean	72.557	6.992	0.9999
S.D.	0.230	2.187	0.0002

The confidence intervals were calculated as 72.557±0.182 (slope) and 6.992±1.735 (for the intercept) at the significance level = 0.05

S.D.= Standard Deviation; r^2 = Determination Coefficient

TABLE II

The coefficients of the standard curves for HPLC assay of VDX (SLS 2%-w/v)

	Slope	Intercept	r^2
1	72.983	3.759	0.9993
2	72.483	5.838	0.9996
3	72.387	8.863	0.9999
4	72.572	6.085	0.9999
5	72.682	5.208	0.9998
6	72.718	6.982	0.9999
Mean	72.638	6.123	0.9997
S.D.	0.209	1.718	0.0002

The confidence intervals were calculated as 72.638±0.166 (for the slope) and 6.123±1.362 (for the intercept) at the significance level = 0.05

S.D.= Standard Deviation; r^2 = Determination Coefficient

3.2.2. Precision

The precision of the analytical method was investigated over the sub parameters of repeatability and reproducibility. The results of the precision parameters are given in Table 3 and Table 4 with the mean±standard deviations. The data showed excellent reproducibility of the sample analysis and perfect recovery with coefficient of variations all smaller than 2%.

TABLE III

Reproducibility Results

	Concentration (µg/mL)		
N	1	16	64
1	0.949	16.049	64.329
2	0.932	16.088	63.781
3	0.940	16.052	63.778
4	0.978	16.084	63.922
5	0.954	16.049	63.862
6	0.965	16.055	63.721
Mean ± S.D.	0.954 ± 0.016	16.063 ± 0.017	63.899 ± 0.347
C.V.	1.748	0.112	0.347

TABLE IV

Repeatability parameters

	Concentration (µg/mL)		
N	1	16	64
1	0.960	16.060	63.198
2	0.956	16.035	63.977
3	0.959	16.119	63.863
4	0.978	16.056	63.958
5	0.946	16.103	64.034
6	0.952	16.171	64.092
Mean ± S.D.	0.958 ± 0.010	16.091 ± 0.051	63.974 ± 0.081
C.V.	1.109	0.313	0.127

3.2.3. Stability

The stability of the representative concentrations of 1 µg/mL and high 64 µg/mL were performed at conditions that samples might experience during experimental flow. The stability results revealed that both of the VDX solutions were stable at the investigated experimental conditions as summarized in Table 5 and Table 6.

TABLE V

Stability results of VDX (1µg/mL)

	Sample Concentration (1 µg/mL)	Bias (%)
Ambient Tempereture (n=3)		
Light Exposure (-)	0.9471 ± 0.016	0.305
Light Exposure (+)	0.9604 ± 0.008	1.009
37±0.5°C (n=3)		
Light Exposure (-)	0.9587 ± 0.013	-0.464
Light Exposure (+)	0.9873 ± 0.026	-1.164

TABLE VI

Stability results of VDX (64µg/mL)

	Sample Concentration (64 µg/mL)	Bias (%)
Ambient Tempereture (n=3)		
Light Exposure (-)	64.244 ± 0.332	0.064
Light Exposure (+)	64.093 ± 0.124	0.046
37 ± 0.5°C (n=3)		
Light Exposure (-)	64.036 ± 0.019	0.030
Light Exposure (+)	63.962 ± 0.076	0.043

3.2.4. Sensitivity

The sensitivity of the HPLC method for invitro determination of VDX has been investigated by determining the LOD and LOQ. The minimum detected concentration was 0.175 µg/mL with the signal:noise ratio (3:1) and the limit of quantification was 0.350 µg/mL with the signal:noise ration of 10:1.

3.3. Dissolution of VDX Tablets

The dissolution medium volume for VDX tablets was determined in order to maintain the sink conditions in the dissolution experiments. There is a growing evidence in the literature that SLS the most common surfactant which is used to maintain sink conditions in dissolution experiments, especially for the poorly soluble drugs (19-25). Subramanian et al. have investigated the maximum solubility of VDX in aqueous media containing SLS at different concentrations in which they have evaluated suitable dissolution media for VDX tablets. According to the solubility results, the maximum concentrations of VDX have been found as 798 ± 6.1 µg/mL and 1471 ± 6.2 µg/mL for 1% SLS and 2% SLS respectively (26). Depending on the saturation solubility concentrations, the sink conditions will be maintained for all media when the total volume of dissolution medium is set as 1000 mL. Thus, when all of the VDX in 40 mg Bextra® tablets is dissolved, the total maximum concentration will be 40 µg/mL and the saturation solubility in all media is at least more than quarter fold of this concentration. So, 1000 mL volume will be enough for the dissolution of VDX from tablet formulations regardless of the saturation solubility of VDX.

The dissolution profiles of 3 different strengths of VDX tablets (10-20-40 mg) were investigated as described in section 2.4. (Figure 4-5). The dissolution experiments were finalized when the concentration difference between two consecutive points is statistically insignificant (ANOVA; $p>0.05$). The dissolved percentages for VDX with respect to time for two different dissolution media are summarized in Table 7 and 8.

TABLE VII

Dissolved percentage for VDX with respect to time for 1%-w/v SLS solution

	Average Amount Dissolved % of VDX from Tablets (n=6)					
Time (min)	10 mg VDX Tablets		20 mg VDX Tablets		40 mg VDX Tablets	
		C.V. (%)		*C.V. (%)*		*C.V. (%)*
0	0	0	0	0	0	0
5	54.965	5.47	42.401	7.49	36.200	7.84
10	68.925	2.54	57.948	2.28	53.424	1.12
15	77.700	4.38	67.029	1.96	62.233	1.14
20	81.638	1.63	72.862	1.18	67.83	0.50
25	84.366	1.81	75.877	1.05	71.616	0.30
30	86.692	1.76	78.646	1.28	75.617	0.33
45	89.528	1.92	83.973	0.93	80.794	0.79
60	90.690	1.91	86.703	0.82	84.294	0.91
90	91.247	2.39	89.475	0.62	87.932	0.45

C.V. = Coefficient of Variation

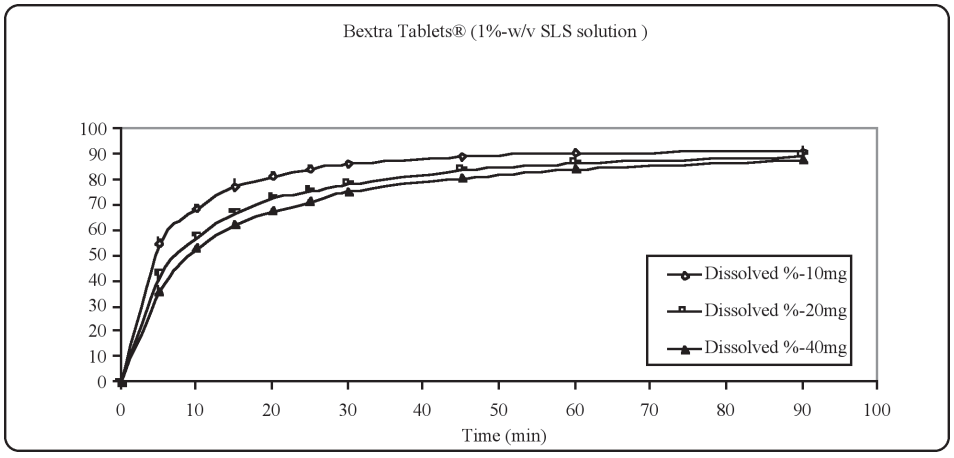

Figure 4
Dissolution profiles of Bextra® tablets (10-20-40mg) in SLS (1%-w/v) aqueous solution media (n=6)

TABLE VIII

Dissolved percentage for VDX with respect to time for 2%-w/v SLS solution

	Average Amount Dissolved % of VDX from Tablets (n=6)					
Time (min)	10 mg VDX Tablets		20 mg VDX Tablets		40 mg VDX Tablets	
		C.V. (%)		*C.V. (%)*		*C.V. (%)*
0	0	0	0	0	0	0
5	65.889	3.74	48.785	5.30	38.713	8.92
10	78.769	2.52	64.157	1.27	59.333	3.14
15	85.747	0.54	73.177	1.40	67.561	0.61
20	90.251	1.13	78.895	0.16	73.486	1.53
25	92.021	0.6	82.766	0.27	77.613	0.66
30	94.388	0.75	85.700	0.60	80.512	0.76
45	97.040	0.97	90.729	0.59	86.068	0.54

C.V. = Coefficient of Variation

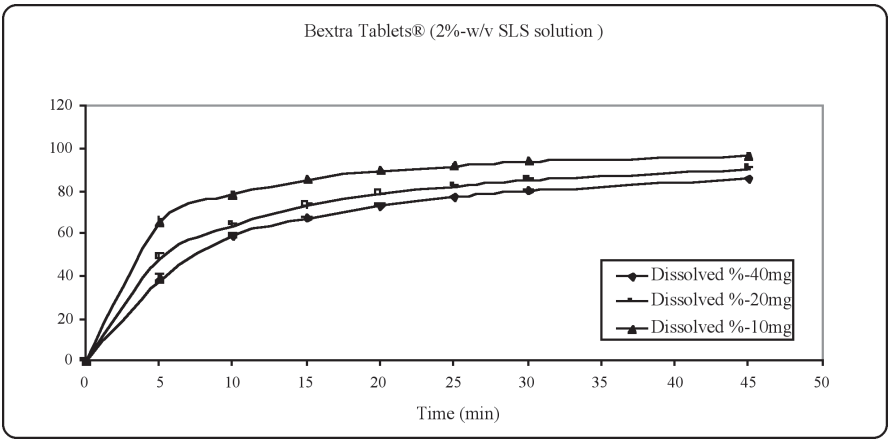

Figure 5
Dissolution profiles of Bextra® tablets (10-20-40mg) in SLS (2%-w/v) aqueous solution media (n=6)

3.4. Dissolution Profile Comparison

The dissolution profiles were compared with the model independent model approach by using f_2 test. The profiles were accepted as similar if f_2 similarity factor value is greater than 50. The comparisons were evaluated both for SLS 1%-w/v and SLS 2%-w/v dissolution media.

TABLE IX

$_2$ values for SLS (1%-w/v) aqueous solution media

1%-w/v SLS		
t (min)	40-20mg	40-10mg
90	69.65	45.95
90 (t =5 min excluded)	71.57	47.89
90 (t =5 and 10 min excluded)	72.37	49.32

TABLE X

f_2 values for SLS (1%-w/v) aqueous solution media

2%-w/v SLS		
t (min)	40-20mg	40-10mg
45	60.38	38.10
45 (t =5 min excluded)	65.60	41.60
45 (t =5 and 10 min excluded)	65.64	43.04

4. Conclusion

In this study, major aim was to partially validate a simple and rapid HPLC analysis method for the determination of VDX from pharmaceutical tablet formulations and to determine a suitable dissolution medium for these formulations. The HPLC method was simple and rapid for the quantification of VDX with a short retention time of 6.1 minute. The investigated parameters were all within the acceptance limits for validation. SLS is generally used for the simulation of bile acids' effects in the gastrointestinal channel, especially for poorly water soluble drugs.

We have used different concentrations of SLS in order to investigate its efficiency on release characteristics of VDX from commercial tablet formulations. As clearly seen from the dissolution profiles, the amount of VDX dissolved from tablet formulations (especially 40 mg tablets) significantly increase in correlation with the increase in SLS concentration.

This is possibly due to the increase in saturation solubility of VDX. As the saturation solubility of VDX increases approximately three fold, the VDX dissolution occurs more independent of the saturation solubility. So, the discriminating effect of increase in SLS concentration has been observed more clearly. This was also approved from the f_2 test results. As the SLS concentration increased, the similarity factor between different strengths decreased. These tablet formulations' profiles were not expected to have similar profiles but as it can be seen from the results of f_2 test, due to the increase in VDX strength, 40 and 20 mg Bextra® tablets seem to have similar profiles. That is possible because of the poor solubility of VDX and poor discriminating capacity of the dissolution medium.

Subramanian et al. have investigated the suitable dissolution medium for in vitro routine testing of VDX tablets by using different concentrations of SLS, Tween 80 as the surfactants and methanol as the co-solvent. They have evaluated the dissolution experiments by using two different generic products at the strength of 20 mg VDX and they have concluded that 0.6% (w/v) SLS concentration may be used as the suitable dissolution medium depending on the release of 85% VDX from tablet formulations in 45 minutes. In contrast with this study, we have investigated the dissolution profiles of the innovator product (Bextra®, Pfizer) at three different strengths as 10-20-40mg. According to our results, the 85% of the VDX is dissolved from tablet formulations only when 2% SLS (w/v) is used with 1000 ml of the medium. As 1% SLS in 1000 ml of dissolution medium is used, only 83.97% (for 20 mg VDX tablets) and 80.79% (for 40 mg VDX tablets) of the VDX is dissolved from tablet formulations in 45 minutes. On the other hand, for all three strengths the 85% release in 45 minutes is achieved only if the dissolution medium of 1000ml contains 2% SLS (w/v) as the surfactant.

In order to grant a waiver from in vivo bioequivalence studies, the formulation at the highest strength is generally set as the reference. In this study, the highest for VDX tablet formulations is the one containing 40 mg VDX as the active ingredient. This is why, 40 mg VDX tablets are set as the reference and the results of the lower strengths (20 mg and 10 mg VDX tablets) were compared with the 40 mg VDX tablet formulation. The dissolution data shown in Tables 7 and 8 clearly underline the significant difference in the dissolved amount of VDX from tablet formulations in all strengths at initial time points (t = 5 and 10 min). This variation possibly

depends on the differences in disintegration time of the tablets. As stated in the guideline regarding the dissolution testing for immediate release solid oral dosage forms, percent coefficient of variation at earlier time points for dissolved amount (e.g. 15 minutes) should not exceed 20% and for other time points it should not be more than 10% (17). The reason for this acceptance limit is the variances in disintegration time of the tablet formulations. For the elimination of this alterations in earlier time points, dissolution profiles were also compared with f_2 test in which t = 5 and t = 10 minutes have been excluded.

As a result, the dissolution profiles of 40 mg and 20 mg VDX tablets were found as similar (f_2>50), but on the other hand for 40 mg and 10 mg VDX tablets the dissolution profiles were not similar. This may possibly affect to grant a waiver for in vivo bioequivalence studies after scale-up and post approval changes for this VDX product.

Summary

Determination of Valdecoxib from Pharmaceutical Formulations and In-vitro Comparison of Dissolution Profiles

Valdecoxib is an anti-inflammatory drug belonging to the class of COX-2 inhibitors of the non-steroidal anti-inflammatory drugs. It is a poorly soluble and high permeable drug and listed in Class II of biopharmaceutical classification of drugs. In this study, the partial validation of a simple and rapid HPLC method for the determination of valdecoxib from tablet formulations has been evaluated. Also, the dissolution profiles for all strengths of commercially available valdecoxib tablet formulations have been investigated in two different dissolution media and the profiles were compared by using similarity factor, f_2. As the results, the dissolution profiles of tablet formulations containing 40 mg and 20 mg VDX tablets were found as similar in both of the dissolution media, but on the other hand, for 40 mg and 10 mg valdecoxib strengths, the profiles were not found as similar with f_2 values less than 50.

Keywords: Valdecoxib, Dissolution, Profile comparison, HPLC, Validation

Özet

Farmasötik Formülasyonlardan Valdekoksib Miktar Tayini ve Çözünme Profillerinin İn-Vitro Olarak Karşılaştırılması

Valdekoksib, non steroidal anti-inflamatuvar ilaçların COX-2 inhibitörleri sınıfına dahil bir anti-inflamatuvar ilaçtır. Çözünürlüğü düşük ve geçirgenliği yüksek ilaçlar grubunda olmasından dolayı biyofarmasötik sınıflandırma olarak Sınıf II bir etkin maddedir. Bu çalışmada, valdekoksib için kullanılan basit ve hızlı bir HPLC yönteminin kısmi validasyonu yapılmıştır. Aynı zamanda ticari olarak mevcut olan tabletlerin tüm dozlamlarının çözünme testleri iki farklı çözünme ortamında gerçekleştirilmiş ve elde edilen profiler benzerlik faktörü kullanılarak karşılaştırılmıştır. Sonuç olarak her iki çözünme ortamında 40 mg ve 20 mg valdekoksib içeren tablet formülasyonlarının çözünme profilleri benzer bulunurken, 40 mg ve 10 mg valdekoksib tablet formülasyonları için profillerin benzer olmadıkları bulunmuştur.

Anahtar Kelimeler: Valdekoksib, Çözünme, Profil Karşılaştırma, HPLC, Validasyon

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