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A New Hplc Approach for Determination of *In-Vitro* Solubility of Naproxen Sodium

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Introduction

Naproxen sodium (NAS) is a non-steroidal anti-inflammatory drug used to relieve moderate to severe aches and pains (1-3). Most of its therapeutic activity is probably mediated through prostaglandin synthesis inhibition (1,4,5). Once dissolved in biologic fluids, naproxen and NAS are chemically identical species and have the same biologic properties. Administration of naproxen as the sodium salt, however, permits more rapid absorption from the gastrointestinal tract (1,2,6). Peak plasma concentration is reached in 1 to 2 hours after ingestion of the sodium salt (6-8). Food reduces the rate but not the extent of absorption (4). Naproxen is extensively metabolized in the liver to 6-O-desmethyl naproxen, which is inactive. Both naproxen and 6-O-desmethyl naproxen are further metabolized to their respective acylglucoronide conjugated metabolites. Approximately 95% of naproxen is excreted in the urine with <1% as unchanged naproxen, <1% as 6-O-desmethyl naproxen, and 66-92% as their glucuronides or other conjugates (1,9). Three percent or less of an administrated dose is excreted in the feces. Naproxen is an acidic drug that is highly bound (>99%) to plasma proteins at therapeutic concentrations. Naproxen has a high therapeutic index and a shallow dose-response curve, so the effect of other drugs on its pharmacokinetics is not likely to have a large clinical impact (1). Chemical structure of NAS is presented in Figure I.

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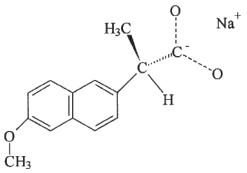


Figure 1
Structural formula of NAS

USP 24 refers a spectrophotometric method (λ_{max}=332 nm) for dissolution studies of naproxen and NAS tablets (10). Several chromatographic methods have been reported for determination of NAS in raw material (11), tablets (10,12-14), plasma (2,7,15,16), urine (17) and intestinal perfusion samples (18). These methods used for analysis of the related compound have been applied in the different chromatographic conditions having various mobile phases, columns and detector systems (UV or fluorescence) (Table I). Therefore, some of the above methods have not only adequate retention (18), but also have not been formally validated (14). On the other hand, although the retention time of naproxen is shorter than 2 min in the HPLC method reported by Monser et al., the column used in that study is not easily commercially available (13). As it is known that the main purpose of the chromatographic analysis or determination is to optimize the retention time to minimize the analysis time or to maximize the separation from other substances. To overcome the above mentioned drawbacks of the separation techniques, new HPLC methods, which are very easy to apply, should be presented for the quantitative analysis and routine analysis of target drugs.

The aim of this study was to develop a simple and rapid HPLC method for quantification of NAS obtained from *in-vitro* solubility studies according to FDA guidance. Applicability of this assay was demonstrated for the *in-vitro* solubility studies.

Material and Methods

Chemicals and reagents

NAS was kindly supplied by Bilim İlaç (purity, 99%; Türkiye). HPLC-grade methanol and acetonitrile were purchased from Sigma-Aldrich (Germany). All other chemicals were of analytical-grade and used without further purification.

TABLE I

Chromatographic conditions of the reported methods for the separation and determination of NAS.

Samples	Column	Detector	Mobile Phase	Flow rate	Extraction	LOD	LOQ	Retention Time	Literature
Internal standard	C_8 (250x2.1mm 5µm)	UV (235 nm)	0.01M Phosphate Buffer : ACN (35:65)	0.3 $mL.min^{-1}$	-	-	-	6.28 min	(14)
Tablet	Porous graphitic carbon (100x4.6mm 7µm)	UV (272 nm)	Tetrahydrofuran: Methanol (80:20)	1 $mL.min^{-1}$	-	0.05 $\mu g.mL^{-1}$	0.2 $\mu g.mL^{-1}$	1.77 min	(13)
Plasma	-	Fluorescense 280 nm (exc.) 350 nm (em.)	-	-	Liquid-Liquid extraction	-	40 $\mu g.mL^{-1}$	-	(6)
Intestinal perfusion	ODS (250x4.6mm 5µm)	UV (270 nm)	Methanol: ACN:Water (20:28:52)	1.5 $mL.min^{-1}$	-	0.05 $ng.mL^{-1}$	0.3 $ng.mL^{-1}$	19 min	(18)
Urine	C_{18} (150x4.6mm 5µm)	Fluorescense 280 nm (exc.) 350 nm (em.)	ACN:Water: Trietanolamine (500:500:1)	1 $mL.min^{-1}$	-	-	-	-	(17)
Pharmaceutical Preparations	L1 (4.5 mm x 15cm, 5 µm)	UV (254 nm)	ACN: Water : GAA (50:49:1)	1.2 $mL.min^{-1}$	-	-	-	-	(10)

ACN: Acetonitrile GAA: Glacial acetic acid

Chromatographic system and conditions

The HPLC system consisted of Waters 2690 Separations Module equipped with a Waters 2996 Photodiode Array Detector (Waters, USA). The analytical column was a Waters Spherisorb S10 ODS2 column (C_{18}; 200x4.6mm; USA). The mobile phase comprised of 0.05M phosphate buffer (pH 4.0), methanol and acetonitrile (50:20:30 v/v). After mixing, the mobile phase was filtered through a 0.45 µm filter and degassed before use. Analyses were run at a flow-rate of 1 $mL.min^{-1}$ at an ambient temperature. The detector responses were set at 230 nm. At this wavelength, peak areas were measured and used for the quantitative evaluations.

Standard solution

Stock solution of NAS (1 $mg.mL^{-1}$) was prepared in distilled water. This stock solution was then diluted with mobile phase for preparation of calibration standards and calibration solution of NAS in the concentration range of 5-150 $\mu g.mL^{-1}$.

Calibration and validation

The stock solution of NAS was diluted with mobile phase to give the calibration standards at concentrations of 5, 10, 30, 40, 50, 75, 100, 150 $\mu g.mL^{-1}$ NAS. The peak area was plotted against the corresponding concentration to obtain the calibration graph. Calibration curve and corresponding determination coefficient (r^2) were then calculated by least-squares linear regression analysis. The method was validated according to the International Conference on Harmonization (ICH) Guideline and Text on Validation of Analytical Procedures: Text and Methodology Q2R1 (19). The USP tailing factor was also calculated for all conditions.

The proposed method was validated as to specificity, linearity, precision (repeatability and intermediate precision), and accuracy. Assay specificity was examined in relation to interference from matrix components in drug-free buffers used for determination of solubility of NAS. Linearity was evaluated by the calibration equation which is characterized by determination coefficient, slope and intercept. Precision was determined by analyzing drug-free mobile phase spiked with known concentrations of NAS (10, 50 and 100 $\mu g.mL^{-1}$). Injection repeatability was determined by

six subsequent injections from the same standard solution prepared at three different concentration levels (10, 50, 100 µg.mL^{-1}), whereas, method repeatability was determined by single injections from six independent standard solutions (10, 50 and 100 µg.mL^{-1}). Repeatability was assessed by calculating the relative standard deviation (RSD %) of the measurements. Intermediate precision of the method were performed at three different concentrations by using six independent series in the same day (intra-day precision) and six consecutive days (inter-day precision) within each series, and calculated as the RSD (%) between the nominal (10, 50 and 100 µg.mL^{-1}) and measured concentrations. The inter-day and intra-day accuracy of the method was determined at three test concentrations and calculated as percentage recovery. The sensitivity of the analytical method was evaluated by determining the limits of detection (LOD) and quantitation (LOQ). The detection limit is defined as the lowest amount of analyte in a sample which can be detected, and the quantitation limit as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Based on FDA guidance, the signal-to-noise ratios of 3:1 and 10:1 were taken as LOD and LOQ, respectively. The values of LOD and LOQ were calculated using Empower software and confirmed by taking dilutions from the stock solution.

Solubility studies

The assay was applied to the *in vitro* solubility studies of NAS. The saturation solubility of the compound was determined at five different pH values (1.0, 3.2, 4.2, 5.2, 7.5) according to FDA guidance (20). Solubility studies were repeated six times at each pH conditions. Five different pH mediums (Table II) were prepared according to USP 24. Access amount of NAS was added in a suitable buffer solution and agitated overnight in a horizontal shaker (100 rpm; 37 ± 0.5ºC). One milliliter of samples were withdrawn at the end of the experiment and filtered through a La-Pha-Pack HPLC syringe PTFE filter (0.45 µm) which has no absorption for NAS. An aliquot (200 µL) of filtered samples was diluted with mobile phase to a final volume of 1 mL, and then 50 µL injected into the HPLC system.

TABLE II

Composition of buffers used for the determination of NAS's solubility.

Buffer Solutions	Added amount (mL)							
	pH 1.0	pH 2.9	pH 3.2	pH 3.9	pH 4.2	pH 4.9	pH 5.2	pH 7.5
$C_8H_5O_4K$ (0.2M) (Phthalic acid)	-	50	50	50	50	50	50	-
KH_2PO_4 (0.2M)	-	-	-	-	-	-	-	50
NaOH (0.2M)	-	-	-	-	3.7	16.5	29.95	39.1
KCl (0.2M)	25	-	-	-	-	-	-	-
HCl (0.2N)	54.2	28.9	14.7	2.9	-	-	-	-
Distilled Water (q.s.)	100	200	200	200	200	200	200	200

Results and Discussion

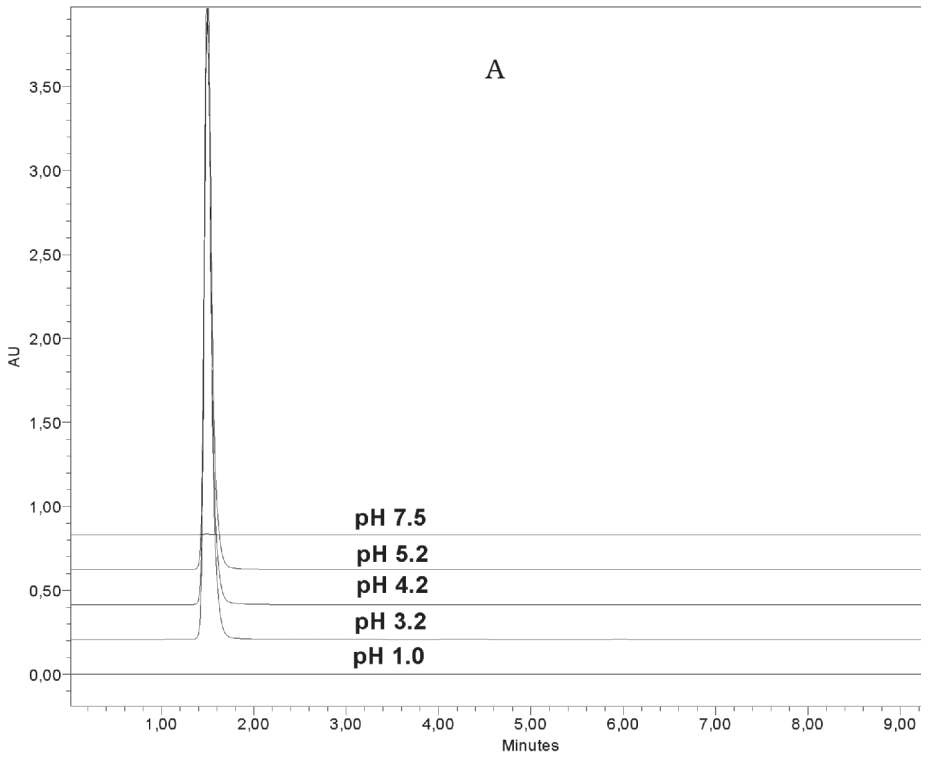
Method development

In the present study, the composition of the mobile phase to obtain the optimal chromatographic separation and determination of NAS was determined by monitoring USP tailing factor estimation after injection of NAS into a Waters Spherisorb S10 ODS2 (200x4.6 mm) column system. As it known perfectly that symmetric peak has a tailing factor of 1.0. This depends on the symmetry of a chromatographic peak. For this reason USP tailing factor was considered for the optimization of the chromatographic conditions. In our case, a mobile phase consisting of 0.05M phosphate buffer (pH=4.0), methanol and acetonitrile (50:20:30 v/v) was found to be suitable for the chromatographic determination of NAS. As the tailing factor of the NAS peak was unacceptable over pH value of 4.0, pH of the phosphate buffer (in mobile phase) was adjusted to 4.0 by the addition of orthophosphoric acid. When phosphate buffer (pH 4.0) and methanol mixture was used as the mobile phase, the peak of NAS was observed in 2 min with a high tailing factor. With the addition of acetonitrile to this mobile phase, tailing problem was resolved and NAS eluted around 7 min. Therefore, the final composition of the mobile phase was chosen as 0.05M phosphate buffer (pH 4.0), methanol and acetonitrile (50:20:30

v/v). Total run time for the assay using this mobile phase was approximately 10 min and the interval between two consecutive injections was 1 min. The USP tailing factor was decreased from 1.078 to 1.005 when the pH value of the NAS containing samples was changed from 1.0 to 7.5. In addition, because of minimal sample manipulation and the high reproducibility (RSD < 0.42%) of the HPLC method, an internal standard was not incorporated into the solubility samples.

Validation of the assay

The representative chromatograms obtained in the absence and presence of NAS at different pH values (1.0, 3.2, 4.2, 5.2, 7.5) are depicted in Figure II. NAS was well separated from the matrix components with no interfering peaks in the relevant portion of the trace, and the retention time was not influenced by the pH value of the sample (6.96-6.99 min).



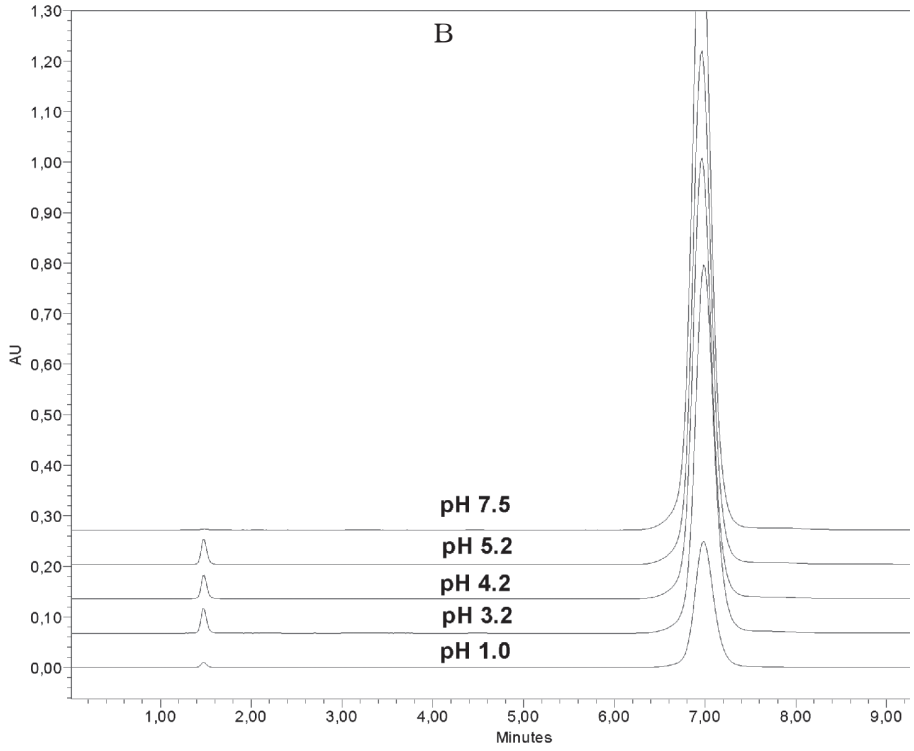


Figure 2

Chromatograms obtained in the absence (A) and presence of NAS (B) at different pH values.

The proposed method was linear in the range of 5-150 µg.mL^{-1} (y=167858 . x – 145262; r^2= 0.9990 where y is peak area and x is concentration of NAS in µg.mL^{-1}). Limits of detection (LOD) and quantitation (LOQ) were estimated from signal-to-noise ratio. The values of LOD and LOQ for NAS were 19 ng.mL^{-1} and 78 ng.mL^{-1}, respectively.

Precision of the proposed method was evaluated by means of repeatability and intermediate precision. Repeatability expresses the precision under the same operating conditions over a short interval of time, whereas intermediate precision expresses within-laboratories variations; different days, different analysts, different equipment. The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. The relative standard deviations (%) calculated for the injection- and method-repeatabilities were lower than 1.48% for all conditions (Table III).

TABLE III

Repeatabilities of injection and method for determination of NAS.

Added NAS ($\mu g.mL^{-1}$)	Injection Repeatability		Method Repeatability	
	Measured* ($\mu g.mL^{-1}$)	RSD (%)	Measured* ($\mu g.mL^{-1}$)	RSD (%)
10	10.26 ± 0.04	0.39	10.54 ± 0.16	1.48
50	49.83 ± 0.61	1.22	49.60 ± 0.60	1.20
100	98.02 ± 0.68	0.68	99.21 ± 0.23	0.23

*Mean ± SD, n=6.

Intermediate precision and accuracy of the assay method were determined for both intra-day and inter-day variations using the replicate analysis of the quality control samples of 10, 50 and 100 $\mu g.mL^{-1}$. The intra-day accuracy ranged from -0.795 to 5.42% and precision from 0.231 to 1.48%. The inter-day accuracy ranged from -3.24 to 4.47% and precision from 1.48 to 3.66% (Table IV). All these results indicate that precision and accuracy of the assay are satisfactory.

TABLE IV

Intra- and inter-day accuracy and precision of the HPLC method.

Added $\mu g.mL^{-1}$	Intra-Day			Inter-Day		
	Measured* ($\mu g.mL^{-1}$)	Bias** (%)	RSD (%)	Measured* ($\mu g.mL^{-1}$)	Bias** (%)	RSD (%)
10	10.54 ± 0.16	5.42	1.48	10.45 ± 0.32	4.47	3.06
50	49.60 ± 0.60	-0.80	1.20	49.70 ± 1.82	-0.60	3.66
100	99.21 ± 0.23	-0.79	0.23	96.76 ± 1.43	-3.24	1.48

*Mean ± SD; n=6

**Bias (%) = (Measured-Added) x100/Added

Application to solubility studies

Biopharmaceutics Classification System (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. An objective of the BCS approach is to determine equilibrium solubility of a drug substance under physiological

conditions. It is recommended that the pH-solubility profile of the test drug substance should be determined at 37 ±1°C in aqueous media with a pH in the range of 1.0-7.5. According to the FDA Guidance (20), the number of pH conditions for a solubility determination can be based on the ionization characteristics of the test drug substance. When the pKa value of drug is in the range of 3-5, the solubility should be determined at pH=pKa, pH=pKa+1, pH=pKa-1 and at pH = 1.0 and 7.5. NAS has a pKa of 4.2 (21). Therefore, we determined the solubility of NAS, in aqueous media at pH values of 1.0, 3.2, 4.2, 5.2 and 7.5. Our experimental results showed that NAS has a pH dependent solubility. Solubility of NAS increases from 0.068 g.mL^{-1} to 0.254 g.mL^{-1} when the pH of the medium increased from 1.0 to 7.5 (Figure III). Our results agree well with those reported by Bhise et al. (22) all these results clearly show that the proposed method can be successfully applied to the determination of the equilibrium solubility of NAS.

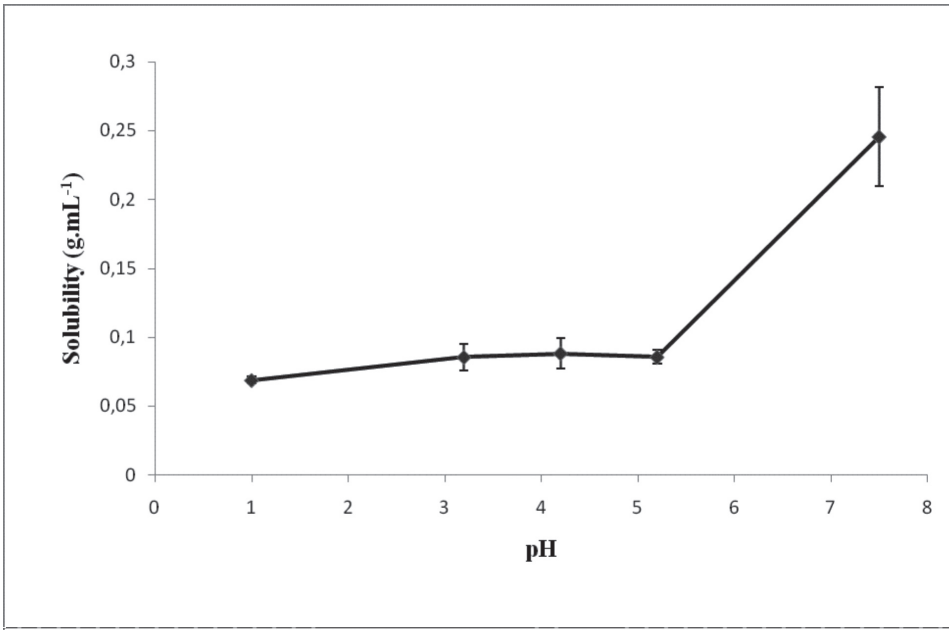


Figure 3
Solubility profile of NAS (mean ±SE, n=6)

Determination of the solubility class of a drug substance according to FDA guidance is very important for the pharmaceutical companies which are taking biowaiver for the immediate release drug products. Although there are various HPLC methods are available for determination of NAS, in this study, a validated HPLC method was developed for quantitative determination of NAS. This method proved to be simple, reliable and also suitable as a single method for studying the solubility of NAS as a function of pH.

Summary

A new HPLC approach for determination of in-vitro solubility of naproxen sodium

Naproxen sodium (NAS) is a non-steroidal anti-inflammatory drug commonly used for the reduction of moderate to severe aches and pains. This study presents a simple and sensitive high-performance liquid chromatographic method for the determination of *in-vitro* solubility of NAS. Chromatographic separation of NAS was achieved on a reversed-phase column (Waters Spherisorb S10 ODS2 C_{18} 200x4.6 mm column) with the mobile phase consisted of 0.05M phosphate buffer (pH 4.0), methanol and acetonitrile (50:20:30 v/v), at a flow rate 1 $mL.min^{-1}$. Chromatographic detection of NAS was performed at 230 nm with UV detector system. The retention time was about 7 min. Calibration curve was linear over the concentration range of 5-150 $\mu g.mL^{-1}$. The limit of quantitation was found to be 78 $ng.mL^{-1}$. The intra- and inter-day precision relative standard deviation was 3.7% or less, and the accuracy was within 5.4% deviation of the nominal concentration. The proposed HPLC method was successfully applied to the analysis of NAS solubility.

Keywords: Naproxen sodium, HPLC, solubility

Özet

Naproksen sodyumun in-vitro çözünürlüğünün tayini için yeni bir HPLC yaklaşımı

Naproksen sodium (NAS) orta ve şiddetli ağrıların giderilmesinde yaygın olarak kullanılan non-steroidal anti-inflamatuar bir ilaçtır. Bu çalışmada NAS'ın *in-vitro* çözünürlüğünün tayini için basit ve duyarlı bir yüksek basınçlı sıvı kromatografisi yöntemi tanımlanmaktadır. NAS'ın ayrımı, ters-faz kolon (Waters Spherisorb S10 ODS2 C_{18} 200x4.6 mm) ile 1 $mL.dk^{-1}$ akış hızında 0.05 M fosfat tamponu (pH 4.0), metanol ve asetonitril (50:20:30 h/h)'den oluşan mobil fazı kullanılarak gerçekleştirilmiştir. NAS'ın kromatografik tayini UV dedektör sistemi ile 230 nm'de gerçekleştirilmiştir. Alıkonma süresi yaklaşık 7 dk'dır. Kalibrasyon doğrusu 5-150 $\mu g.mL^{-1}$ konsantrasyon aralığında doğrusaldır. Kantitatif olarak tayin edilebilen en düşük konsantrasyon 78 $ng.mL^{-1}$ olarak bulunmuştur. Gün-içi ve günler-arası bağıl standart sapması % 3.7 ya da daha az, ve doğruluk ise eklenen konsantrasyondan % 5.4'lük bir sapma göstermiştir. Geliştirilen yöntem NAS'ın çözünürlük tayinlerine başarıyla uygulanmıştır.

Anahtar Kelimeler: Naproksen Sodyum, HPLC, çözünürlük

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