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Original article

DETERMINATION OF NAPROXEN SODIUM FROM POLY(LACTIDE-CO-GLYCOLIDE) CORNEAL SCAFFOLDS

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Abstract

A simple, very specific, and reliable HPLC method has been developed and validated for the determination of naproxen sodium (NS) in poly(lactide-co-glycolide) (PLGA) (50:50) scaffolds. Efficient chromatographic separation was achieved on a reverse phase column (ODS2 C_{18} 200x4.6mm) with the mobile phase consisted of methanol and acetate buffer (pH 5.1) (55:45 v/v) at a flow rate of 1 mL.min^{-1} by using fluorescence detector at 254 nm ex/ 352 nm em wavelength. The retention time was about 9.3 min. Calibration curve was linear over the concentration range of 0.08-75 µg.mL^{-1}. The intra- and inter-day precision relative standard deviation was 3.71 % or less, and the accuracy was within 2.96 % of the nominal concentration. The developed HPLC method was successfully applied to quantitate NS in PLGA (50:50) scaffolds.

Key words: *Naproxen sodium, PLGA, HPLC, Validation*

Naproksen Sodyumun Poli(laktid-ko-glikolid) Korneal Doku İskelelerinden Miktar Tayini

Naproksen sodyumun poli(laktid-ko-glikolid) (PLGA) (50:50)doku iskelelerinden tayini için basit, özgün ve güvenilir bir HPLC metodu geliştirilmiştir. Çalışmada ters faz kolon (ODS2 C_{18} 200x4.6mm), mobil faz olarak methanol ve asetat tamponu (pH 5.1) (55:45 v/v) kullanılmış, akış hızı 1 mL.dakika^{-1} olarak ayarlanmıştır. Dedektör olarak 254 nm eksitasyon/ 352 nm emisyon dalga boyunda floresans dedektör kullanılmıştır. Naproksen sodyum için alıkonma zamanı 9.3 dakika olarak bulunmuştur. Kalibrasyon doğrusu 0.08-75 µg.mL^{-1}derişim aralığında doğrusal olarak bulunmuştur. Gün içi ve günler arası kesinlik için bağıl standart sapma değeri % 3.71 den, doğruluk için bağıl hata değeri % 2.96 dan düşük bulunmuştur. Geliştirilen HPLC metodu naproksen sodyumun PLGA (50:50) doku iskelelerinden miktar tayini için başarıyla uygulanmıştır.

Anahtar kelimeler: *Naproksen sodyum, PLGA, HPLC, Validasyon*

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INTRODUCTION

The cornea is a transparent tissue in the eye that is responsible for the refraction of incoming light and is a multilayered tissue made up of three major cell layers: the epithelium, the stroma, and the endothelium (1). The epithelium may particularly be damaged by various diseases, traumas, and injuries which brings the loosing of avascular and transparent structure to this tissue. Furthermore, medication to control the damage of the cornea is usually the first line of the treatment, but once medication will not halt or alter the damage, a corneal transplant is considered. This surgical procedure involves the removal of the damaged tissue and replacement with a healthy, donated human cornea (2). Currently, research on corneal treatments are intensified on tissue engineering, and it could be a very important solution to the serious corneal damages since it could overcome the present disadvantages of corneal transplantation, primarily immune reaction and the shortage of the donor corneas (3). In order to eliminate the pathological conditions and to regain natural function and structure of cornea such as transparency and avascularity, corneal cell replacement and the treatment of the inflammation occurred in the damaged area might be necessary. Therefore it is a promising approach to support the formation of tissue by delivering cells and the anti-inflammatory drug to damaged area (4).

The field of biomaterials developed in a new direction called tissue engineering, involves use of cells and drugs together with biodegradable carriers (5–8). In tissue engineering, polymers are used as temporary carriers for the cells and drugs. Polyesters are among the most widely investigated polymers and poly(lactide-co-glycolide) (PLGA) (50:50) is a synthetic polymer among the polymer family of poly(α-hydroxy esters) (9). It is a widely investigated and used biodegradable and biocompatible polymer in biomaterial applications and known to be suitable as a scaffold material due to its previous use in sutures and fixation devices; it is Food and Drug Administration (FDA) approved and it can be formed into many architectures using a range of fabrication techniques (10).

Naproxen sodium (NS) is a non-steroidal anti-inflammatory drug used to relieve moderate to severe aches and pains (11-14). Most of its therapeutic activity is probably mediated through prostaglandin synthesis inhibition (11,15-17). As an ophthalmic application, Papa et al. (18, 19) reported that at a concentration rate of 0.2%, NS is able to decrease aqueous levels of proteins, polymorphonuclear leukocytes and PGE2 following experimentally induced inflammation in cornea.

Several chromatographic methods have been reported for determination of NS in raw material (20), tablets (21-23), plasma (24-26), urine (27), intestinal perfusion samples (28) and pharmaceutical preparations (29, 30). These methods used for the analysis of related compound have been applied in the different chromatographic conditions including various mobile phases, columns and detector systems (UV or fluorescence). Therefore, some of the above methods have not only adequate retention (27), but also have not been formally validated (23). Furthermore, these methods were not specific for the analysis of released NS from the matrix (PLGA (50:50) scaffolds). Our previous studies showed that relatively close polarities of NS and PLGA(50:50) related compounds made it difficult to separate and analyze NS without any interference coming from the matrix components. Therefore, previous HPLC methods for the determination of NS in tablets and various biological fluids could not be used for the separation and quantification of NS in PLGA(50:50) scaffolds. In order to solve this problem, there was a necessity to develop a new and rapid HPLC method for the quantitative analysis of released drug from PLGA (50:50) scaffolds.

The aim of this study was to develop a new specific HPLC method used for the quantitation of released NS from PLGA (50:50) scaffolds and to validate this analytical method. We intend to formulate PLGA (50:50) scaffolds containing corneal epithelial cells and NS to treat severe corneal injuries by the replacement of epithelial cells with avoiding rejection of tissue by using

NS into the damaged, inflammatory area. The amount of NS in scaffolds will be determined by the developed HPLC method and release of NS from the scaffolds will be quantified by this analytical method.

EXPERIMENTAL

Materials

NS was donated by Abdi İbrahim (Turkey). PLGA (50:50) was commercially available as Resomer RG 503 and was purchased from Boehringer-Ingelheim (Germany). Analytical reagent grade sodium acetate trihydrate, calcium chloride dihydrate, sodium chloride and potassium chloride were purchased from Merck (Germany). HPLC grade methanol and acetic acid were supplied from Sigma Aldrich (Germany). High purity water was prepared by using Millipore Milli-Q plus water purification system.

Methods

Chromatographic system and conditions

The HPLC system consisted of Waters 2690 Separations Module equipped with a Waters 2695 Fluorescence Detector (Waters, USA). The analytical column was a Waters Spherisorb S10 ODS2 column (C18; 200x4.6mm; USA). The mobile phase comprised of methanol and acetate buffer (pH 5.1) (55:45, v/v). Acetate buffer (1 L) consisted of 5.08 g sodium acetate trihydrate and 6.3 mL of acetic acid (2 N), pH adjusted to 5.1. Before using the mobile phase, it was mixed, degassed on an ultrasonic bath and then mobile phase filtered by a Millipore vacuum filter system equipped with a 0.45 μm filter. The flow-rate of mobile phase 1 $mL.min^{-1}$ at 30 °C. The fluorimetric detector was set at an excitation wavelength of 254 nm and emission wavelength of 352 nm. At this wavelength, peak areas were measured and used for the quantitative evaluations.

Preparation of standard solutions

Stock solution of NS (500 $\mu g.mL^{-1}$) was prepared in simulated lacrimal fluid (SLF). SLF was selected as the solvent because of its characteristics appropriate to a release medium for ophthalmic formulations and was prepared consisting of 84 mg calcium chloride dihydrate, 1.4 g potassium chloride and 8.3 g sodium chloride for one liter, pH adjusted to 7.4 (31). Stock solution was then diluted with SLF for the preparation of calibration standards. The calibration solutions of NS were prepared in the concentration between the range of 0.08-75 $\mu g.mL^{-1}$.

Preparation of scaffold samples for analyses

In order to determine the amount of NS in PLGA(50:50) scaffolds, the drug loaded scaffolds were dissolved in 5 mL dichloromethane and 10 mL SLF was added to each scaffold. The samples were then put on an ultrasonic bath for 30 minutes and centrifuged at 14000 rpm for 10 minutes. After centrifugation, the supernatants were separated and filtered through 0.45 μm membrane filters and then injected to the HPLC column. The amount of NS in PLGA(50:50) scaffolds was calculated through the peak area values by the calibration curve.

Analytical method validation

According to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines, the method was validated in terms of following parameters (32,33).

Specificity

Specificity was examined by analyzing the release medium and blank PLGA (50:50) scaffold in the release medium.

Linearity range

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample (32). The calibration curves for NS were constructed under optimum conditions and the linearity of the method was determined by performing injections at seven different concentrations in the linear range over 3 different days.

Sensitivity

The sensitivity of the analytical method was evaluated by determining limits of detection (LOD) and quantitation (LOQ). The signal-to-noise ratios of 3:1 and 10:1 were taken as LOD and LOQ, respectively (32).

Precision and accuracy

Three different concentrations of standard NS (within the linear range) were analyzed three consecutive days (inter-day precision) and within the same day (intra-day precision). The relative standard deviation (RSD) and the bias of intra- and inter-day studies were calculated.

Stability

The standard stock solutions of NS were stored at +4 °C for 24 hours and protected from daylight. During this period, it was analyzed periodically at 6, 12, 24 h.

RESULTS AND DISCUSSION

Method development and optimization

In order to prepare standard NS solution, NS was dissolved in SLF medium (1 $\mu g.mL^{-1}$). Blank or NS loaded PLGA (50:50) scaffolds (50 % w/v per scaffold) were suspended in tubes containing 5 mL of SLF medium and incubated for 24 h at 37 °C in horizontal shaker in order to obtain release samples. Afterwards, the tubes were centrifuged at 13500 rpm for 20 minutes and the supernatants were filtered through 0,45 μm membrane filter to obtain release samples in a clear solution form. Standard solution and the release samples were scanned in a UV-visible spectrometer and all of them had UV maxima at around 230 nm. Hence detection at 230 nm was selected for method development purposes. Several mobile phases (Table 1) were tried at this wavelength to abstain the interference between PLGA (50:50) and NS peaks, but none of them was achieved. In order to avoid this interference and reach to very low detection limit for NS to implement in vitro release studies accurately, UV detector was changed with fluorescence detector assuming that the polymer PLGA (50:50) did not have the fluorescence property. Various mobile phase combinations indicated in the literature were tested with fluorescence detector using an excitation wavelength of 230 nm and emission wavelength of 352 nm (Table 1). However, repeatedly none of the mobile phases at this wavelength had the ability to separate the NS and PLGA(50:50) peaks. Finally, one mobile phase system consisting of acetate buffer (pH 5.1) and methanol (45:55) were tried with fluorescence detector at a wavelength of 254 nm ex/352 nm em and this method had been successful to separate the NS and PLGA(50:50) peaks. In this method NS peak were obtained at a retention time of 9.3 min and there was no interference of PLGA (50:50) with NS peak (Figure 1) and the tailing factor of the NS peak was found 1.16.

Table 1. Chromatographic conditions examined in our preliminary studies for the separation and determination of NS.

Column	Detector (wavelength)	Mobile phase	Flow rate	Retention time	PLGA (50:50) - NS separation
ODS2 C18 column (200x4.6mm)	UV (230 nm)	0.05M Phosphate Buffer (pH4.0): Methanol: Acetonitrile (50:20:30)	1 mL min^{-1}	7.41 min (NS) 7.36 min (PLGA)	Not possible
ODS2 C18 column (200x4.6mm)	UV (254 nm)	0.05M Phosphate Buffer (pH4.0): Methanol: Acetonitrile (50:20:30)	1 mL min^{-1}	7.39 min (NS) 7.34 min (PLGA)	Not possible
ODS2 C18 column (200x4.6mm)	UV (270 nm)	0.05M Phosphate Buffer (pH4.0): Methanol: Acetonitrile (50:20:30)	1 mL min^{-1}	7.45 min (NS) 7.39 min (PLGA)	Not possible
ODS2 C18 column (200x4.6mm)	UV (230 nm)	Acetate buffer (pH 5.1): Methanol (45:55)	1 mL min^{-1}	9.58 min (NS) 9.63 min (PLGA)	Not possible
ODS2 C18 column (200x4.6mm)	UV (254 nm)	Acetate buffer (pH 5.1): Methanol (45:55)	1 mL min^{-1}	9.57 min (NS) 9.66 min (PLGA)	Not possible
ODS2 C18 column (200x4.6mm)	UV (270 nm)	Acetate buffer (pH 5.1): Methanol (45:55)	1 mL min^{-1}	9.58 min (NS) 9.64 min (PLGA)	Not possible

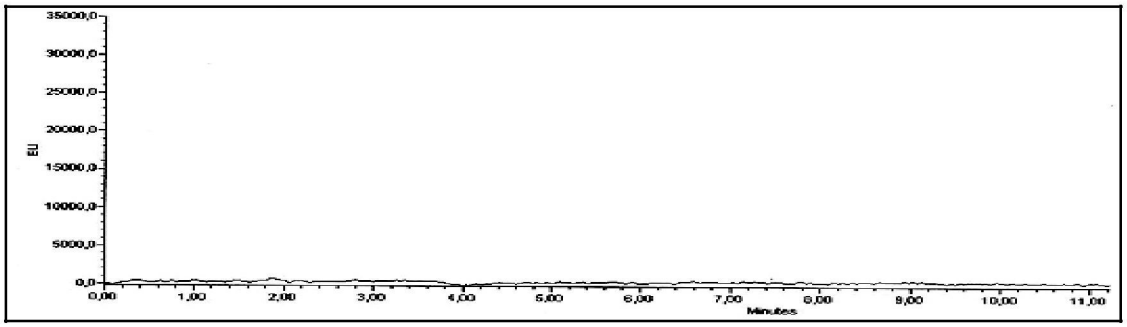
ODS2 C18 column (200x4.6mm)	Fluorescence (230 nm) (ex) (352 nm) (em)	Water (pH 3.2): Methanol: Acetonitrile (35:15:50)	1 mL min^{-1}	4.12 min (NS) 4.18 min (PLGA)	Not possible
ODS2 C18 column (200x4.6mm)	Fluorescence (230 nm) (ex) (352 nm) (em)	Water (pH 3.2): Acetonitrile	1 mL.min^{-1}	6.44 min (NS) 6.42 min (PLGA)	Not possible
ODS2 C18 column (200x4.6mm)	Fluorescence (230 nm) (ex) (352 nm) (em)	Acetic acid (1%): Methanol: Acetonitrile (40:20:40)	1 mL min^{-1}	5.63 min (NS) 5.59 min (PLGA)	Not possible
ODS2 C18 column (200x4.6mm)	Fluorescence (270 nm) (ex) (352 nm) (em)	Acetate buffer (pH 5.1): Methanol (45:55)	1 mL min^{-1}	9.6 min (NS) 9.56 min (PLGA)	Not possible
ODS2 C18 column (200x4.6mm)	Fluorescence (230 nm) (ex) (352 nm) (em)	Acetate buffer (pH 5.1): Methanol (45:55)	1 mL min^{-1}	9.6 min (NS) 9.65 min (PLGA)	Not possible
ODS2 C18 column (200x4.6mm)	Fluorescence (254 nm) (ex) (352 nm) (em)	Acetate buffer (pH 5.1): Methanol (45:55)	1 mL min^{-1}	9.3 min (NS)	Possible (Only NS peak)

Method Validation

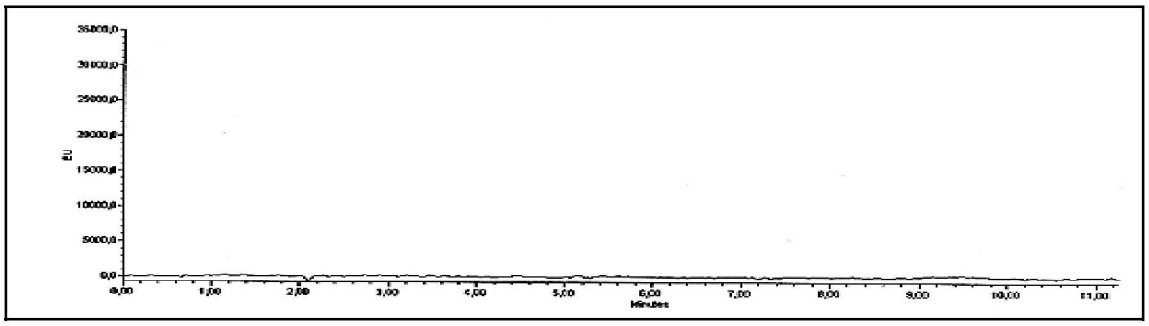
Specificity

The chromatogram obtained from NS containing PLGA(50:50) scaffold was identical with that obtained from the blank scaffold containing PLGA(50:50). The representative chromatograms (Fig 1) show no other peaks on the retention time of NS. Accordingly, the proposed method could be considered selective.

a)



b)



c)

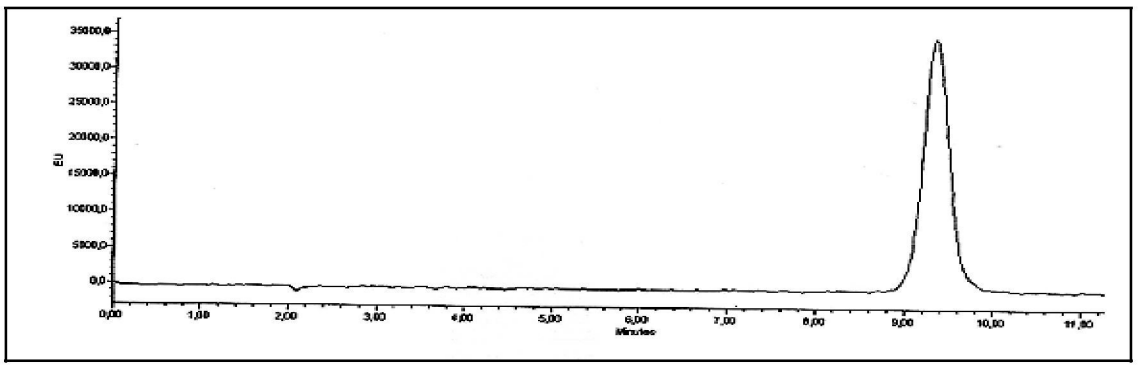


Figure 1. The chromatogram obtained in optimum conditions (a: release medium; b: PLGA(50:50) containing blank scaffold in release medium; c: NS containing PLGA(50:50) scaffold in release medium.

Linearity Range

The peak areas of NS were plotted against the corresponding nominal concentration to obtain calibration graph. The peak shapes and symmetries were worsened after injection of 75 μg mL^{-1} concentration of the compound. Thus, the method was evaluated linear in the range of 0.08 to 75 μg mL^{-1} for NS. The regression equation data are given in Table 2.

Sensitivity

The values of LOD and LOQ for NS were 0.03 μg mL^{-1} and 0.08 μg mL^{-1}, respectively, and are given in Table 2.

Table 2. Linearity data of the developed HPLC method (n=6)

Regression equation[a]	$y = 5.3640x10^{9}x + 5.7410\ x10^{9}$
Standard error of intercept	$2.3029\ x10^{8}$
Standard error of slope	$2.4239\ x10^{7}$
Correlation coefficient	0.9996
Linearity range (μg mL^{-1})	0.08 – 75
Number of data points	7
LOD (μg mL^{-1})	0.03
LOQ (μg mL^{-1})	0.08

[a] where y is peak area and x is concentration in μg mL^{-1} of NS

Precision and Accuracy

The relative standard deviation (RSD) and the bias of intra- and inter-day studies were within acceptable range indicating that the precision and the accuracy of the method were satisfactory. The results are given in Table 3.

Table 3. Precision and accuracy of the developed method.

	Intra-day			**Inter-day**		
Added (μg mL^{-1})	**Found[a] (μg mL^{-1})**	**Precision[b] (RSD %)**	**Accuracy[c] (Bias %)**	**Found[a] (μg mL^{-1})**	**Precision[b] (RSD %)**	**Accuracy[c] (Bias %)**
2.5	2.44±0.06	1.24	-2.21	2.42±0.06	1.38	-2.96
10	10.11±0.28	3.05	1.16	10.22±0.46	3.71	2.20
50	50.05±0.14	2.14	0.10	50.26±0.28	3.18	0.53

[a] mean ± standard error (n=6)

[b] Relative standard deviation

[c] Bias % = ((Found-Added)/Added)x100

Stability

During 24 h, no unexpected peak observed which might indicate the degradation. In addition, the peak areas did not changed significantly ($p > 0.05$) for the compound when it was compared with fresh prepared standard.

Application of the developed method to scaffold sample

The proposed HPLC method with fluorescence detection was applied to the analysis of 3 mg mL^{-1} NS containing PLGA (50:50) scaffolds (n=5) to determine the amount of NS in PLGA(50:50) scaffolds. There was no interference from the matrix and the NS concentration found in the sample was 2.86 ± 0.12 mg mL^{-1}.

CONCLUSION

Nowadays, research on the treatment of serious corneal damages are intensified on tissue engineering in order to deal with present handicaps of corneal transplantation. To overcome with these drawbacks, more sophisticated drug and cell delivery system combining different properties and allowing for multiple functions need to be developed. Reasonably, it is crucial that the development of advanced determination methods with HPLC for the analysis of drug from the sophisticated delivery system.

In this study, a simple, very specific, and reliable HPLC method using fluorimetric detection has been developed and validated for the assay of NS from PLGA (50:50) scaffolds. The developed method was successfully applied for the analyses of NS while in vitro release studies from PLGA(50:50) scaffolds. The method shows a good performance with respect to linearity, sensitivity, accuracy, precision, specificity. By this way, it provides the necessaries in order to determine the released amount of NS from PLGA (50:50) scaffolds.

REFERENCES

1. **Reichl, S., Muller-Goymann, C.C.,** "The use of a porcine organotypic cornea construct for permeation studies from formulations containing befunolol hydrochloride" *Int. J. Pharm.*, 250(1), 191–201, **2003.**
2. **Alaminos, M., Sanchez-Quevedo, M., Munoz-Avila, J.I., Serrano, D., Medialdea, S., Carreras, I., Campos, A.,** "Construction of a complete rabbit cornea substitute using a fibrin-agarose scaffold" *Invest. Ophthalmol. Vis. Sci.*, 47(8), 3311–3317, **2006.**
3. **Nishida, K.,** "Tissue engineering of the cornea" Cornea, 22(7), 28-34, **2003.**
4. **Duarte, A.R.C., Mano, J.F., Reis, R.L.,** "Dexamethasone-loaded scaffolds prepared by supercritical-assisted phase inversion" *European Polymer Journal*, 45(1), 141-148, **2009.**
5. **Griffith, M., Malik, H., Shimmura, S., Watsky, M.A., Li, F., Carlsson, D., Doillon, C.J., Nakamura, M., Suuronen, E., Shinozaki, N., Nakata, K., Sheardown, H.,** "Artificial human corneas: scaffolds for transplantation and host regeneration" *Cornea*, 2l(2), 54-61, **2002.**
6. **Minami, Y., Sugihara, H., Oono, S.,** "Reconstruction of cornea in three-dimensional collagen gel matrix culture" *Invest. Ophthalmol. Vis. Sci.*, 34(7), 2316–2324, **1993.**
7. **Germain, L., Carrier, P., Auger, F.A., Salesse, C., Guerin, S.L.,** "Can we produce a human corneal equivalent by tissue engineering?" *Prog. Retin. Eye Res.*, 19(5), 497–527, **2000.**
8. **Griffith, M., Osborne, R., Munger, R., Xiong, X., Doillon, C.J., Laycock, N.L., Hakim, M., Song, Y., Watsky, M.A.,** "Functional human corneal equivalents constructed from cell lines" *Science*, 286(5447), 2169–2172, **1999.**

9. **Tsai, R.J.F, Ho, Y.S., Chen, J.K.,** "The effects of fibroblasts on the growth and differentiation of human bulbar conjunctival epithelial cells in an in vitro conjunctival equivalent" *Invest. Ophthalmol. Vis. Sci.*, 35(6), 2865–2875, **1994.**
10. **Middleton, J.C., Tipton, A.J.,** "Synthetic biodegradable polymers as orthopedic devices" *Biomaterials,* 21(23), 2335–2346, **2000.**
11. **Todd, P.A., Clissold, S.P.,** "Naproxen. A reappraisal of its pharmacology, and therapeutic use in rheumatic diseases and pain states" *Drugs*, 40(1), 91–137, **1990.**
12. **Segre, E.J.,** "Naproxen sodium (Anaprox): pharmacology, pharmacokinetics and drug interactions" *Reprod. Med.*, 25(4), 222-225, **1980.**
13. **Bansal, V., Dex, T., Proskin, H., Garreffa, S.J.,** "A look at the safety profile of over-the-counter naproxen sodium: a meta-analysis" *Clin. Pharmacol.*, 41(2), 127-138, **2001.**
14. **Di Martino, P., Barthelemy, C., Joiris, E., Capsoni, D., Masic, A., Massarotti, V., Gobetto, R., Bini, M., Martelli, S.,** "A new tetrahydrated form of sodium naproxen" *J. Pharm. Sci.*, 96(1), 156-167, **2007.**
15. **Moyer, S.,** "Pharmacokinetics of naproxen sodium" *Cephalalgia,* 6(4), 77-80, **1986.**
16. **Li, J.J., Norton, M.B., Reinhard, E.J., Anderson, G.D., Gregory, S.A., Isakson, P.C., Koboldt, C.M., Masferrer, J.L., Perkins, W.E., Seibert, K., Zhang, Y., Zweifel, B.S., Reitz, D.B.,** "Novel terphenyls as selective cyclooxygenase-2 inhibitors and orally active anti-inflammatory agents" *J. Med. Chem.*, 39(9), 1846-1856, **1996.**
17. **Kaynak, M.S., Şahin, S.,** "A new hplc approach for determination of in-vitro solubility of naproxen sodium" *Hacettepe University J. Faculty Pharm.*, 28(1), 49-62, **2008.**
18. **Spampinato, S., Marino, A., Bucolo, C., Canossa, M., Bachetti, T., Mangiafico, S.** "Effects of sodium naproxen eye drops on rabbit ocular inflammation induced by sodium arachidonate" *J. Ocular Pharmacol.*, 7(2), 125–133, **1991.**
19. **Bucolo, C., Spadaro, A.,** "Effect of sodium naproxen on inflammatory response induced by anterior chamber paracentesis in the rabbit" *J. Pharm. Pharmacol.*, 47(9), 708–712, **1995.**
20. **Ekpe, A., Tong, J.H., Rodriguez, L.,** "High-performance liquid chromatographic method development and validation for the simultaneous quantitation of naproxen sodium and pseudoephedrine hydrochloride impurities" *J. Chromatogr. Sci.*, 39(3), 81-86, **2001.**
21. **Dinc, E., Ozdemir, A., Aksoy, H., Ustundag, O., Baleanu, D.,** "Chemometric determination of naproxen sodium and pseudoephedrine hydrochloride in tablets by HPLC" *Chem. Pharm. Bull.*, 54(4), 415-421, **2006.**
22. **Monser, L., Darghouth, F.,** "Simultaneous determination of naproxen and related compounds by HPLC using porous graphitic carbon column" *J. Pharm. Biomed. Anal.*, 32(4-5), 1087-1092, **2003.**
23. **Mitakos, A., Panderi, I.,** "A validated LC method for the determination of clopidogrel in pharmaceutical preparations" *J. Pharm. Biomed. Anal.*, 28(3-4), 431-438, **2002.**
24. **Tashtoush, B.M., Al-Taani, B.M.,** "HPLC determination of naproxen in plasma" *Pharmazie,* 58(9), 614-615, **2003.**
25. **Nielsen-Kudsk, F.,** "HPLC-determination of some antiinflammatory, weak analgesic and uricosuric drugs in human blood plasma and its application to pharmacokinetics" *Acta Pharmacol. Toxicol.*, 47(4), 267-273, **1980.**
26. **Phillips, T.M., Wellner, E.F.,** "Measurement of naproxen in human plasma by chip-based immunoaffinity capillary electrophoresis" *Biomed. Chromatogr.*, 20(6-7), 662–667, **2006.**
27. **Mikami, E., Goto, T., Ohno, T., Matsumoto, H., Nishida, M.,** "Simultaneous analysis of naproxen, nabumetone and its major metabolite 6-methoxy-2-naphthylacetic acid in pharmaceuticals and human urine by high-performance liquid chromatography" *J. Pharm. Biomed. Anal.*, 23(5), 917-925, **2000.**

28. Zakeri-Milani, P., Barzegar-Jalali, M., Tajerzadeh, H., Azarmi, Y., Valizadeh, H., "Simultaneous determination of naproxen, ketoprofen and phenol red in samples from rat intestinal permeability studies: HPLC method development and validation" *J. Pharm. Biomed. Anal.*, 39(3-4), 624-630, **2005.**

29. Hsu, Y., Liou, Y., Lee, J., Chen, C., Wu, A., "Assay of naproxen by high-performance liquid chromatography and identification of its photoproducts by LC-ESI MS" *Biomed. Chromatogr.*, 20(8), 787-793, **2006.**

30. USP, The United States Pharmacopeia, 24th edn. The United States Pharmacopeial Convention Inc., Rockville, MD, naproxen sodium monograph, **2006.**

31. Weyenberg, W., Vermeire, A., Vandervoort, J., Remon, J.P., Ludwig, A., "Effects of roller compaction settings on the preparation of bioadhesive granules and ocular minitablets" *Eur. J. Pharm. Biopharm.*, 59(3), 527–536, **2005.**

32.ICH Steering Committee. Validation of analytical procedures: Text and Methodology Q2(R1). Harmonized Tripartite Guideline, **2005.**

33. Ünal, K., Palabıyık, İ.M., Karacan, E., Onur, F. "Spectrophotometric determination of amoxicillin in pharmaceutical formulations" *Turk. J. Pharm. Sci.*, 5(1), 1-16, **2008**.

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