DEVELOPMENT AND VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF TRIAMCINOLONE ACETONIDE FROM POLYURETHANEINTRAOCULAR IMPLANTS

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ABSTRACT

An HPLC method was developed and validated aiming to quantify the triamcinolone acetonide incorporated into intraocular implants based on polyurethaneand in direct contact with the degradation products of this biomaterial. The separation was carried out in less than 5 minutes using acetonitrile/water (50:50) as mobile phase, a C_{18} column and ultraviolet detection at 240nm. The method provided selectivity based on resolution among peaks. It was linear over the range of 4.0-28.0μg/mL. The quantitation and detection limits were 1.0 and 2.0μg/mL, respectively. The average accuracies of three concentrations ranged from 98.20 to 101.34% and precision was close to 2%. The proposed HPLC method can be successfully applied for triamcinolone acetonide assay incorporated into implants based on polyurethanes without any interference.

Keywords: Triamcinoloneacetonide, Polyurethane intraocular implant, Validation, HPLC method.

INTRODUCTION

Chronic noninfectious posterior uveitis is estimated to affect 175,000 people in the United States and approximately 800,000 worldwide. The economic cost of blindness resulting from uveitis is similar to that of diabetes-associated blindness[1].The high cost is likely because uveitis afflicts mostly the working age group (20–50 years of age) and causes 10% of the blindness in this population[2].

Although topical therapy is successful in treating inflammation of the anterior uvea, it is not effective in treating posterior segment uveitis. The systemic therapy with immunosuppressive agents and corticosteroids often fails because of the blood-retinal barrier that prevents the diffusion of the drugs into the uveal tissues[3]. The intravitreal injections of the corticosteroids have been shown to produce benefits in eyes with uveitis, but several adverse events have been noted (with elevated intraocular pressure being the most common)[4]. The intraocular implants represent a potential therapeutic alternative to treat posterior uveitis, since they can be implanted into the vitreous cavity, overcoming the blood-retina barrier, allowing drug delivery at therapeutic levels directly into the targeted site, and reducing the side effects frequently observed with intravitreal injections and systemic administration[5-7].

Intraocular implants controlled (sustained)-drug delivery systems based on non-biodegradable or biodegradable polymers are frequently loaded to corticosteroids[8-10] immunosuppressive drugs[11]and/or anti-metabolic substances[12]. Triamcinolone acetonide, incorporated into polymeric intraocular implants, has been widely used in the treatment of various forms of chronic diseases affecting the posterior segment of the eye, including posterior uveitis, because of its anti-inflammatory properties (Figure 1)[13,14].
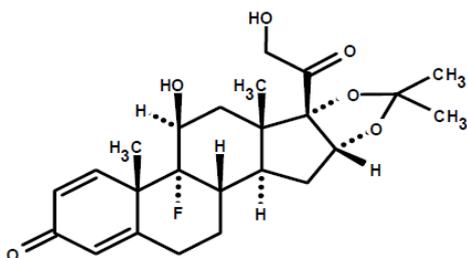

Fig. 1: Chemical structure of triamcinolone acetonide.

Some methods reported in literature for the measurement of triamcinolone concentrations are based on high performance liquid chromatography coupled with mass spectrometry (HPLC-MS).These methods are applied to quantify the triamcinolone in contact with different corticosteroids in the same biological sample. The mass spectrometric detection is a reference technique for the determination of triamcinolone in complex biological samples, because it provides specific measurement of this drug over a wide analytical range[15,16]. Besides the use of HPLC-MS, this technique does not represent viable analytical method to quantify the drug in routine analysis of quality control of triamcinolone in pharmaceutical forms.It is desirable to develop a simple, rapid and reliable HPLC with ultraviolet detection for assaying the anti-inflammatory drug released from pharmaceutical formulations and present in non-complex samples.

Some methods described in literature for quantitation oftriamcinolone acetonide are based on the high performance liquid chromatography with ultraviolet detection (HPLC-UV). The *in vitro* and *in vivo* triamcinolone release from poly(D,L-lactide) implants were measured by an HPLC method using a C18 reversed phase column, at a wavelength detection of 252 nm, and running time of 15 minutes[17]. Sudsakorn et al(2006)[18]developed and validated an HPLC-UV method for simultaneous determination of the corticosteroid as well as oxymetazoline hydrochloride in nasal spray formulation. Matysováet al (2003)[19]reported the development and validation of an HPLC-UV method for concomitant measurement of triamcinolone acetonide, its degradation product (occurring in formulation after long-term stability tests) and two preservatives presented in the cream-methylparaben and propylparaben.Moreover, the United States Pharmacopeia (2008)[20] described an isocratic HPLC-UV method for assaying triamcinolone in topical aerosol using a mixture of acetonitrile and water (30:70) as mobile phase, at wavelength of 254 nm, a C18 reversed phase column and 14.5 min of retention time. All the HPCL-UV methods indicated could be successfully applied to assay triamcinolone in different pharmaceutical preparations, manufactured likely by different processes and excipients; but, the chromatographic conditions of the described methods were not suitable to quantify unequivocally triamcinolone acetonide released from biodegradable intraocular implants derived from polyurethane.

In this study, our main goal was the development and validation, through specificity, limits of detection and quantitation, linearity,

precision and accuracy, of a simple and reliable HPLC-UV method to quantify the triamcinolone acetonide incorporated into intraocular implants synthesized by polyurethane. Additionally, the analytical method was applied to assay the triamcinolone acetonide from these polymeric devices in direct contact with the degradation products of the polyurethane.

MATERIAL AND METHODS

Triamcinolone acetonide reference standard was purchased from Sigma-Aldrich (99% of purity). Polycaprolactone-diol (Tone Polyol 2221, $\overline{M_n}$ = 1000 g.mol⁻¹) and polycaprolactone-diol(Tone Polyol 0249, $\overline{M_n}$ = 2000 g.mol⁻¹) were provided by Dow (USA). Isophoronediisocyanate was obtained from Bayer (Brazil). Dibutyl tin dilaurate and hydrazine (solution 64%) were obtained from MiracemaNuodex (Brazil) and Arch Química (Brazil) respectively. All these chemicals were employed throughout this work without any treatment. Triethylamine (98%, Vetec) and 2, 2- bis(hydroxymethyl) propionic acid (98.3%, Fluka) were purchased and used as received. Acetonitrile HPLC grade was purchased from Fluka. Methanol analytical grade was obtained from Vetec (Brazil). Water was distilled, deionized and filtered through a 0.22 μm filter (Millipore, USA).

Preparation of the polyurethane intraocular implants

Synthesis of the aqueous polyurethane dispersion (PUD)

Aqueous polyurethane dispersion was prepared by a prepolymer mixing process, using a 250 mL three-neck glass flask equipped with a heating mantel, a mechanical stirrer and a thermometer. The macrodiol components polycaprolactone-diol (PCL 1000), polycaprolactone-diol (PCL 2000) and poly(ethylene glycol) (PEG 1500), isophoronediisocyanate (IPDI) (NCO/OH ratio of 2.3) and 2, 2- bis(hydroxymethyl) propionic acid (DMPA) were added to the reactor in the presence ofdibutyl tin dilaurate(DBDLT) and the

reaction was carried out at 70-75ºC under nitrogen atmosphere for 4 h. The amount of free NCO groups on a percentage basis was determined by the standard di-butyl amine back titration method. After titration, the prepolymer temperature was allowed to drop to 40 ºC. The carboxylic acid groups were neutralized by the addition of triethylamine (TEA). The mixture was stirred for further 40 min to ensure the reaction was completed.All samples were dispersed by adding deionized water to the neutralized prepolymer which was stirred vigorously. After the dispersion, the amount of hydrazine (HZ) enough to react with free NCO groups was added to the reactor with a small amount of water, and stirring was continued for further 30 min. This chemical procedure was successful in producing polyurethanedispersion with solid content about 25%. The composition of the polyurethane dispersion (PUD) is shown in Table 1. Films were produced by casting the dispersion in a Teflon mould and allowing them to dry at room temperature for one week. Afterwards the films were placed in an oven at 60 ºC for 24 h[21].A schematic representation of the chemical reactions and processing steps used to produce the polyurethane dispersion is shown in Figure 2.

Table 1: Composition (wt. %) of the aqueous PU dispersion[*].

Reagents	Concentration (wt. %)
Isophoronediisocyanate (IPDI)	8.58
Polycaprolactone-diol1000 (PCL1000)	4.85
Polycaprolactone-diol2000 (PCL2000)	9.09
Polyethylene glycol 1500 (PEG 1500)	0.73
2, 2- bis(hydroxymethyl) propionic acid (DMPA)	0.97
Triethylamine (TEA)	0.73
Water	74.70
Hydrazine (HZ)	1.08

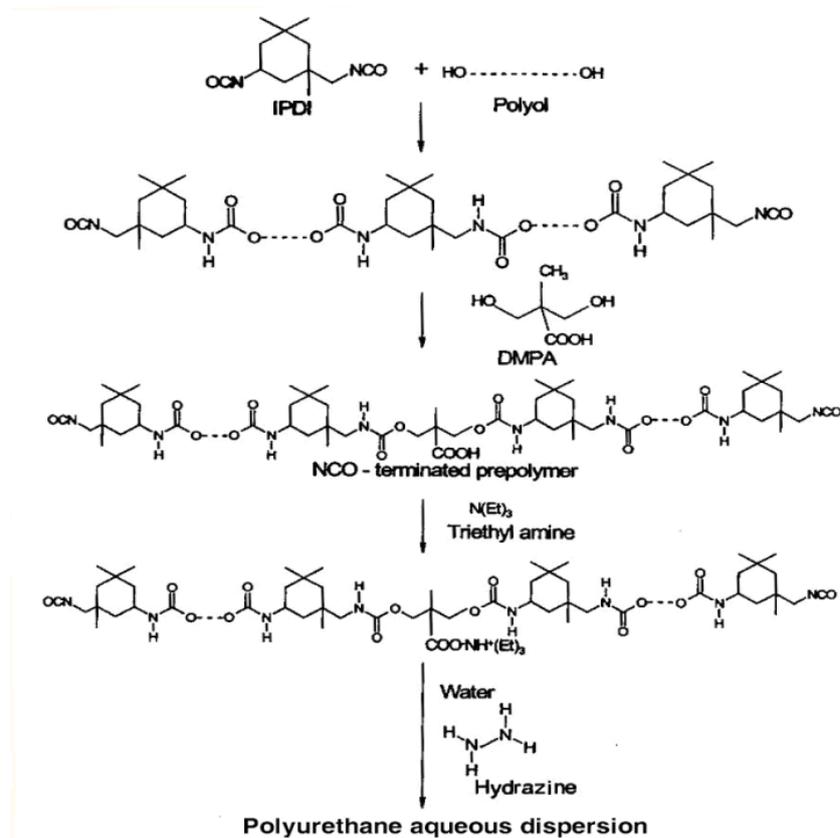[*]0.01 % of dibutyl tin dilaurate based on the amounts of IPDI, PCL and DMPA

Fig. 2: Schematic representation of the chemical reactions and processing steps used to produce the polyurethane dispersion.

Incorporation of triamcinolone acetonide and preparation of the implants

The triamcinolone acetonide was incorporated into the polyurethane by dispersing it into the aqueous polyurethane dispersion prior from casting the film to yield materials having 20.4 wt.% of the drug. The dried film was cut into squares (5 mm in ray). The implants were white in color and presented 100 μm in thickness. The mean weight of the implants containing polyurethane and triamcinolone acetonidewas 3.72 ± 0.43 mg, corresponding to approximately 0.76 mg of the drug for each implant.

Instrumentation and analytical conditions

A Hewlett Packard 1100 HPLC system equipped with quaternary pump, automatic injector and diode array detector (DAD) module was used in conjunction with Eppendorf CH-500 column oven. ChemStation for LC software Rev. 07.01 (Hewlett Packard, USA) was used for data acquisition. A Chromolith Performance® 100 RP-18e column, 250 mm × 4.6 i.d. and 5 μm particle size (Merck, Germany), was used and maintained at 30°C. The wavelength of 240 nm and the automatic injector fitted at 10 μL were set. The mobile phase consisted of acetonitrile and ultrafiltrated water (50:50, v/v) was used at the flow rate of 1.2 mL/min.

Preparation of solutions

Triamcinolone acetonide standard solution: approximately 5 mg of triamcinolone acetonide reference compound were dissolved in a 25 mL volumetric flask. Methanol (18 mL) was added, the solution was sonicated for 3 minutes and the volume was adjusted to 25 mL with ultrafiltrated water. An aliquot (1 mL) was transferred to volumetric flask (10 mL) and the volume was completed with methanol, to obtain a solution at 20 μg/mL.

Triamcinolone acetonide sample solution: the polyurethane intraocular implant was transferred to volumetric flask (10 mL) and the methanol (5 mL) was added. The solution was sonicated for 5minutes and the volume was adjusted to 10 mL with ultrafiltrated water. An aliquot (3 mL) was transferred to volumetric flask (10 mL) and the volume was completed with methanol to obtain a solution of approximately 22.8 μg/mL.

Degradation products from polyurethane solution: the polyurethane intraocular implant (without triamcinolone acetonide) was transferred to volumetric flask (10 mL) and the methanol (5 mL) was added. The solution was sonicated for 5 minutes and the volume was adjusted to 10 mL with ultrafiltrated water. An aliquot (3 mL) was transferred to volumetric flask (10 mL) and the volume was completed with methanol.All the prepared solutions were filtered through a 0.45 μm filter (Sartorius, Germany).

Method validation

The method was validated according to the International Conference on Harmonization (2005)[22]guidelines for validation of analytical procedures.

Specificity

The chromatographic peaks of triamcinolone acetonide reference standard, at 20 μg/mL (test concentration), and of degradation products from polyurethane were obtained. To achieve the specificity of the method, no peak, with the same retention time of triamcinolone acetonide, was allowed.

Linearity

The standard calibration curves were obtained with seventriamcinolone acetonide reference standard solutions. The concentrations used were 4.0, 8.0, 12.0, 16.0, 20.0, 24.0 and 28.0 μg/mL.Each solution was inject three times in the chromatographic system (n= 3 for each concentration). The linearity was estimated by linear regression analysis by the least square regression method. The correlation coefficient was calculated.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were determined based on the signal to noise method. Triamcinolone acetonide reference standard solutions at 2.0, 1.5, and 1.0 μg/mL were prepared and evaluated. Each solution was inject three times in the chromatographic system (n= 3 for each concentration).

Precision

The intra-day precision was evaluated by analyzing eight replicates of triamcinolone acetonide sample solutions (n = 8), at test concentration (20 μg/mL). Similarly, the inter-day precision was evaluated in two consecutive days (n= 16). The concentration of triamcinolone acetonide was determined and the relative standard deviation (R.S.D.) was calculated.

Accuracy

The accuracy was evaluated assaying, in triplicate, samples of known concentration with the addition of different concentrations of the chemical substance of reference (2, 3, and 4 μg/mL).The recovery (%) of the pure drug added was calculated as recovery (%) = [(Ct – Ca)/Cs] × 100, where Ct is the total drug concentration measured after standard addition; Cs, drug concentration in the formulation sample; Ca, drug concentration added to the formulation[23].The mean percentage recovery of triamcinolone acetonide at each level between 98 and 102% indicated the accuracy of the UV method[24].

Determination oftriamcinolone acetonide content in the polyurethane intraocular implants

The polyurethane intraocular implant (n = 10) was transferred to volumetric flask (10 mL) and the methanol (5 mL) was added. The solution was sonicated for 5 minutes and the volume was adjusted to 10 mL with ultrafiltrated water. An aliquot (3 mL) was transferred to volumetric flask (10 mL) and the volume was completed with methanol. The amount of triamcinolone acetonide was determined by high performance liquid chromatographic method described above. The obtained amount of the drug was expressed as the percentage content of the pre-indicated value 20.4 wt.%. The relative standard deviation was calculated.

***In vitro* release of triamcinolone acetonide from polyurethane intraocular implants**

The United States Pharmacopeia (2008)[20] states in the general chapter <1092> the dissolution procedure: "sink conditions are defined as the volume of medium at least three times that required to form a saturated solution of drug substance. When sink conditions are present, it is more likely that dissolution results will reflect the properties of the dosage form". The *in vitro* release of triamcinolone acetonide was carried out under sink conditions during 240 days. As the aqueous solubility of triamcinolone acetonide is 21.0 μg/mL at 28°C[25], sink conditions were achieved with at least 181 mL for the evaluated polymeric implants. The triamcinolone acetonide-loaded polyurethane implants were placed in different erlenmeyers containing 181 mL of phosphate buffer solution (PBS pH = 7.4) (n = 6). These erlenmeyers were placed inside an incubator set at 37°C and 30 rpm. At predetermined intervals, 181 mL of the PBS was sampled and the same volume of fresh PBS was added to each tube. The amount of triamcinolone acetonide released from each implant was assayed by the validated high performance liquid chromatographic method previously described, and expressed as the cumulative percentage of the drug released in the medium. The average of the obtained measurements was calculated and used to plot the release profile curve.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

In this work, the HPLC-UV method was developed and validated for quantifying the triamcinolone acetonide incorporated into polyurethane intraocular implants and in direct contact with the degradation products of polyurethane. Our main objective was to develop an HPLC-UV method using isocratic conditions, furnishing a symmetrical peak of the drug in a short time. The optimal absorption wavelength for detection of triamcinolone acetonide was chosen based on the higher detector response for this drug. Therefore, the absorption wavelength for detection was 240 nm.Different proportions of acetonitrile and ultrafiltrated water, as mobile phase,

were tested. The same proportion of acetonitrile and water (50:50) provided the resolution among peaks of triamcinolone acetonide and degradation products of the polyurethane, and the formation of a symmetrical peak of the drug. It was observed that the use of this proportion of mobile phase contributed to the attainment of a favorable retention time of the triamcionoloneacetonide.

The higher proportion of the organic solvent induced the formation of an asymmetrical chromatographic peak of the drug; and the lower proportion of acetonitrile increased the retention time of the triamcinolone acetonide, a highly hydrophobic drug.Additionally, the flow of the mobile phase was adjusted in order to provide the best

retention time of the drug (4.2 min), without sacrificing chromatographic peak shape.

Method validation

The degradation products of the polyurethane and triamcinolone acetonide eluted at approximately 1.6 min and 4.2 min, respectively (Figure 3A and 3B). The chromatographic peaks were completely resolved and any substance presented the same retention time of the corticosteroid, allowing the unequivocal determination of the drug. According the obtained result, the method demonstrated specificity for triamcinolone acetonide in direct contact with the by-products of the polyurethane.
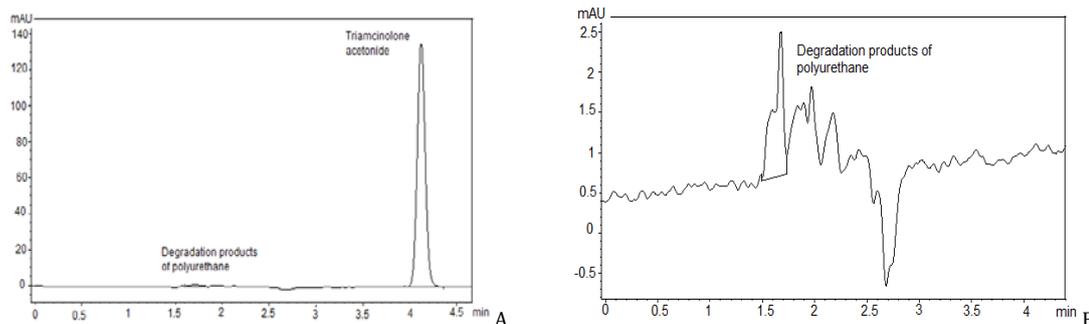

Fig. 3: (A)Chromatogram of the degradation products of polyurethane and triamcinolone acetonide. (B) Chromatogram of the degradation products of polyurethane. Chromatographic conditions: C_{18} column 250 mm × 4.6 mm at 30ºC; acetonitrile and water (50:50, v/v); 1.2 mL/min of flow rate; wavelength of 240 nm.

The linearity is determined by the ability of the method to obtain test results, which are directly proportional to the concentration of the compounds of interest in the sample[26].The linearity of the method was tested by plotting a calibration curve over the range of 4.0 to 28.0 µg/mL of triamcinolone acetonide, which was subjected to regression analysis by the least square method. The representative linear equation was $y = 0.0044x + 0.0025$, where y and x were area and concentration (µg/mL), respectively. The correlation coefficient (r) was 0.9999, showing highly significant correlation between concentration and peak area. The significance of the intercept obtained in the calibration curve was tested and this parameter was not statistically significant ($p > 0.05$), consequently, it can be considered that the curve passes through the origin.

The LOD was 1.0 µg/mL and this concentration displayed a signal-to-noise ratio of 3:1. The LOQ was 2.0 µg/mL and this concentration displayed a signal-to-noise ratio of 10:1 and RSD of 1.54% and 1.78%, respectively (n= 3 for each concentration).

The system precision is indicated by the repeatability of multiple injections and indicates the performance of the HPLC instrument under the prescribed chromatographic conditions[26].In the intra-day and inter-day precision analyses (n = 8 for each day), the mean content of triamcinolone acetonide incorporated into the polyurethane implants was 97.89% (RSD = 1.74%) and 98.56% (RSD = 1.98%), respectively. RSD values lower than 2% attested the precision of the HPLC method.

The accuracy of the chromatographic method, expressed as percent recovery of triamcinolone acetonide, ranged from 98.20% to 101.34%.

Determination oftriamcinolone acetonide content in the polyurethane intraocular implants

The validated HPLC method described was used to quantify triamcinolone acetonide incorporated into polyurethane intraocular implants. The results of content uniformity showed that triamcinolone acetonide presents a uniform distribution in the polyurethane implants. No unit was outside the range of 85.0–115.0% of the pre-indicated amount of triamcinolone acetonide (approximately 0.76 mg of the drug per implant). The relative standard deviation for replicates was 3.87%.

***In vitro* release of triamcinolone acetonide from polyurethane intraocular implants**

Figure 4 shows the cumulative triamcinolone acetonide release profile from polyurethaneimplants. The drug leached from the implantable devices was quantified by the HPLC method. Neither any peak for a degradation product nor any alteration of the chromatographic pattern of triamcinolone acetonide was observed, indicating that the specificity for the drug and reliability of the validated HPLC method.The corticosteroid was released constantly from polymeric implants during the approximately 240 days period of evaluation. The almost linear relationship between time and drug release can suggest that a combination of diffusion and polymer degradation (instead of pure a square-root time drug diffusion) is the main mechanism of drug delivery.The presence of poly(ethylene glycol) in the soft segments of the polyurethane increased hydrophilicity and aqueous permeability in the polymer backbone, promoting hydrolytic cleavage of ester bonds of the poly(caprolactone) also present in the soft segments of the polyurethane[27]. The hydrolysis of the soft segment of the polyurethane contributes to the biodegradation of the polymeric chains, and consequentlyprovides a controlled release of the drug.
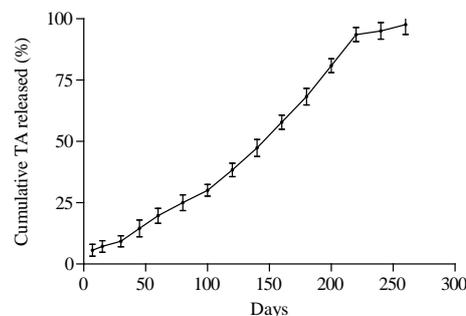

Fig. 4: *In vitro* triamcinolone acetonide release from polyurethane implants. Results represent mean ± standard deviation (*n* = 6).

CONCLUSION

In this study, the developed HPLC-UV method provided the determination of triamcinolone acetonide content in the polyurethane intraocular implants. The method also provided unequivocal determination of the drug in direct contact with the degradation products of the polyurethane. The validated HPLC-UV method demonstrated to be specific, linear, precise and accurate, as well as simple and reliable.

REFERENCES

1. Durrani OM, Tehrani NN, Marr JE, Moradi P, Stavrou P, Murray PI.Degree, duration, and causes of visual loss in uveitis.Br J Ophthalmol2004;88 :1154-1158.
2. Pavesio C, Zierhut M, Bairi K, Comstock TL, Usner DW. Evaluation of an intravitrealfluocinoloneacetonide implant versus standard systemic therapy in noninfectious posterior uveitis.Ophthalmology 2010;117(3) :567-575.
3. Sansoretti A, Safran A. Evaluation of an IntravitrealFluocinoloneAcetonide Implant versus Standard Systemic Therapy in Noninfectious Posterior Uveitis. Med Hyg1995;53 :144-149.
4. Haller JA, Bandello F, Belfort RJ, Blumenkranz MS, Gillies M, Heier J. Randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with macular edema due to retinal vein occlusion. Ophthalmology 2010;117(6) :1134-1146.
5. Amo EM, Urtti A. Current and future ophthalmic drug delivery systems: A shift to the posterior segment. Drug Discov Today 2008;13 :135-143.
6. Hughes PM, Olejnik OCLJ, Wilson CG. Topical and systemic drug delivery to the posterior segments. Adv Drug Delivery Rev2005;57 :2010-2032.
7. Yasukawa MD, Kimura H, Tabata Y, Ogura Y. Biodegradable sclera plugs for vitreoretinal drug delivery. Adv Drug Delivery Rev2001;52 :25-36.
8. Fialho SL, Behan-Cohen F, Silva-Cunha AJ. Dexamethasone-loaded poly(ε-caprolactone) intravitreal implants: A pilot study. Eur J PharmBiopharm2008;68 :637-646.
9. Kuppermann BD, Blumenkranz MS, Haller JA, Williams GA, Weinberg DV, Chou C, et al. Randomized Controlled Study of an Intravitreous Dexamethasone Drug Delivery System in Patients With Persistent Macular Edema. ArchOphthalmol2007;125 :309-317.
10. Da Silva GR, Silva-Cunha AJ, Ayres E, Oréfice RL. Effect of the macromolecular architecture of biodegradable polyurethanes on the controlled delivered of ocular drugs. J MaterSciMaterMed2009;20 :481-487.
11. Saliba JB, Faraco AG, Yoshida MI, Vasconcelos WL, Silva-Cunha AJ, Mansur HS. Development and characterization of an intraocular biodegradable polymer system containing cyclosporine-A for the treatment of posterior uveitis. Mater Res2008;11 :207-211.
12. Bourges JL, Bloquel C, Thomas A, Froussart F, Bochot A, Azan F, et al. Intraocular implants for extended drug delivery: Therapeutic applications. Adv Drug Delivery Rev 2006;58 :1182-1202.
13. Ciulla TA, Criswell MH, Danis RP, Fronheiser M, Yuan P, Cox TA, et al. Choroidalneovascular membrane inhibition in a laser treated rat model with intraocular sustained release triamcinolone acetonidemicroimplants.Br J Ophthalmol 2003;87(8) :1032-1037.
14. Beeley NR, Stewart JM, Tano R, Lawin LR, Chappa RA, Qiu G, Anderson AB, et al. Development, implantation, in vivo elution, and retrieval of a biocompatible, sustained release subretinal drug delivery system. J Biomed Mater Res A2006;76(4) :690-698.
15. Croes K, Goeyens L, Baeyens W, Van Loco J, Impens S. Optimization and validation of a liquid chromatography tandem mass spectrometry (LC/MSn) method for analysis of corticosteroids in bovine liver: evaluation of Keyhole Limpet beta-glucuronidase/sulfatase enzyme extract. J Chromatogr B AnalytTechnol Biomed Life Sci 2009;877(7) :635-644.
16. Keeffe O, Mandy J, Martin S, Regan L. Validation of a multiresidue liquid chromatography–tandem mass spectrometric method for the quantitation and confirmation of corticosteroid residues in urine, according to the proposed SANCO 1085 criteria for banned substances.Anal ChimActa2003;483(1-2) :341-350.
17. 17.Kim YM, Lim JO, Kim HK, Kim SY, Shin JP. A novel design of one-side coated biodegradable intrascleral implant for the sustained release of triamcinolone acetonide.Eur J Pharm Biopharm2008;70(1) :179-186.
18. Sudsakorn S, Kaplan L, Williams DA.Simultaneous determination of triamcinolone acetonide and oxymetazoline hydrochloride in nasal spray formulations by HPLC.J Pharm Biomed Anal2006;40(5) :1273-1280.
19. MatysováL, HájkováR, Sícha J, Solich P. Determination of methylparaben, propylparaben, triamcinolone acetonide and its degradation product in a topical cream by RP-HPLC.Anal BioanalChem2003;376(4) :440-443.
20. The United States Pharmacopeia. 32th ed. Rockville: United States Pharmacopeial Convention; 2008.
21. Ayres E, Oréfice RL, Yoshida MI. Phase morphology of hydrolysable polyurethanes derived from aqueous dispersions.EurPolym J2007;43 :3510-3521.
22. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology; 2005.
23. Friedrich RB, Ravanello A, Cichota LC, Rolim CMB, Beck RCR. Validation of a simple and rapid UV spectrophotometric method for dexamethasone assay in tablets. Quim Nova2009;32 :1052-1054.
24. Balan P, Nimila IC, Chundi S, Movva VR, Rajasekar S. A validated RP-HPLC method for estimation of drotaverineHCl in pharmaceutical tablet dosage form.Int J Pharm PharmSci 2011;4(3) :77-80.
25. Ishii H, Todo H, Sugibayashi K. Effect of sebum and ointment rubbing on the skin permeation of triamcinolone acetonide from white petrolatum ointment.BiolPharmaceut Bull2010;33(5) :876-880.
26. Ahamed A, Krishnamurthy G, Bhojya NHS, Ramesha S. Development and validation of stability indicating ultra performance liquid chromatographic method for etravirine. Int J PharmPharmSci2012;4(1) :255-261.
27. Pinto FCH, Silva-Cunha AJ, Pianetti GA, Ayres E, Oréfice RL, Da Silva GR. Montmorillonite clay-based polyurethane nanocomposite as local triamcinolone acetonide delivery system. J Nanomat2011 :1-11.