UNIVERSITÀ DI PARMA
ARCHIVIO DELLA RICERCA

University of Parma Research Repository

A rapid microextraction by packed sorbent - liquid chromatography tandem mass spectrometry method for the determination of dexamethasone disodium phosphate and dexamethasone in aqueous humor of patients with uveitis

This is the peer reviewd version of the followng article:

Original
A rapid microextraction by packed sorbent - liquid chromatography tandem mass spectrometry method for the determination of dexamethasone disodium phosphate and dexamethasone in aqueous humor of patients with uveitis / Bianchi, Federica; Mattarozzi, Monica; Riboni, Nicolo'; Mora, Paolo; Gandolfi, Stefano; Careri, Maria. - In: JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS. - ISSN 0731-7085. - 142:(2017), pp. 343-347. [10.1016/j.jpba.2017.05.025]

Availability:
This version is available at: 11381/2825524 since: 2023-03-25T15:15:37Z

Publisher:
Elsevier B.V.

Published
DOI:10.1016/j.jpba.2017.05.025

Terms of use:

Anyone can freely access the full text of works made available as "Open Access". Works made available

Publisher copyright

note finali coverpage

(Article begins on next page)

17 April 2024

A rapid microextraction by packed sorbent - liquid chromatography tandem mass spectrometry method for the determination of dexamethasone disodium phosphate and dexamethasone in aqueous humor of patients with uveitis

Federica Bianchi[1*], Monica Mattarozzi[1], Nicolò Riboni[1], Paolo Mora[2], Stefano A. Gandolfi[2], Maria Careri[1]

[1]Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy

[2] Unità di Oftalmologia - Dipartimento di Medicina e Chirurgia, Via Gramsci 14, Università di Parma, 43121 Parma, Italy

*Corresponding author:

Federica Bianchi: E-mail: federica.bianchi@unipr.it.

Phone: +39 0521 905446 ; Fax: +39 0521 905556

Abstract

A new method based on microextraction by packed sorbent (MEPS) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and validated for the determination of dexamethasone and dexamethasone disodium phosphate in human aqueous humor. A central composite design was applied to investigate the effects of both loading and eluting cycles in the MEPS procedure; subsequently the multicriteria method of the desirability functions allowed to find the best conditions for the simultaneous extraction of both the analytes. Detection was performed on a LTQ XL linear ion trap mass spectrometer operating in the positive electrospray ionization mode applying multiple reaction monitoring mode. The assay was validated in accordance with the guidelines bioanalytical method validation obtaining quantitation limits in the low µg/L range, a precision characterized by RSD ≤ 16% and recovery rates in the 91 – 119% range.

Keywords: Microextraction by packed sorbent**;** Dexamethasone disodium phosphate; Dexamethasone, Aqueous humor; Uveitis; Experimental design

1. Introduction

Inflammatory disorders of the uveal tract of the eye (i.e. uveitis) are present all over the world with an incidence in the developed countries ranging between 15 and 20 cases per 100,000 population/year. They may affect patients of any age, and represent a particular burden of disease during early adulthood and between the age from 50 to 60 years [1]. Cataract formation is a frequent complication of uveitis, as a sequel to inflammation itself or to the required steroidail treatment. Cataract surgery in uveitic eyes has considerable potential for postoperative worsening or relapsing of intraocular inflammation. Thus, perioperative dedicated prophylaxis may be warranted [2]. In patients with a history of noninfectious uveitis (idiopathic or related to a systemic autoimmune disease), the role of absolute control of inflammation continues with a greater focus on perioperative supplementation with corticosteroids [3]. Among this class, dexamethasone –DEX- (Fig. 1 left) is one of the most prescribed worldwide. It is a potent glucocorticoid (about 30 times more than cortisone), used to treat a large variety of ocular diseases such as both anterior and posterior segment inflammations, as well as to reduce inflammation following various ocular surgeries [4]. In topical ophthalmic products, due to the low water solubility, dexamethasone is usually replaced by the more hydrophilic dexamethasone disodium phosphate –DEX-SP- (Fig. 1 right) [5] that can be hydrolyzed to free desamethasone by phosphatase [6]. In order to obtain an effective treatment of infection, dexamethasone has to reach the targeted site, achieving and maintaining the therapeutic concentration of about 1 µg/mL [7]. The major drawback of the topical administration of this drug lies in its poor permeability towards the corneal epithelium: in fact, less than 3% of the instilled dose reaches the aqueous humor [8]. Dexamethasone concentration in the vitreous humor is far lower when it is administered via eye drops rather than via subconjunctival/subtenon injections. Only 0.001% of the drug when administered as an ophthalmic solution is expected to reach the posterior segment, whereas about 0.01–0.1% is achieved through periocular injections [8]. In addition, taking into account that dexamethasone's half-life is about 3-6 h [9], a frequent administration of the drug is required. Although these indisputable limits, topical

administration still remains ideally preferable. Systemic or intraocular steroids, indeed, pay a higher availability with a greater risk for general and local side effects [10,11]. The analysis of dexamethasone and dexamethasone sodium phosphate in aqueous humor requires high sensitivity and selectivity due to the presence of these compounds at low concentration levels. Another drawback lies in the low amount of biological sample that can be collected during surgery.

Gas chromatography-mass spectrometry (GC-MS) [12,13], liquid chromatography-mass spectrometry (LC-MS) [13,14], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [13,15-17] and liquid chromatography-diode array detection (LC-DAD) [13,18] have been used to analyze dexamethasone in different biological matrices, such as blood, urine and humor aqueous. Compared to the GC-MS analysis, LC has the advantage of direct analysis of samples without a derivatization step. In addition, LC-MS and LC-MS/MS were reported to provide lower detection limits and better selectivity compared to LC-DAD analysis [13].

Various analytical methodologies, such as microextraction techniques, that reduce environmental pollution, have been proposed for sample clean-up and analyte enrichment [13]. Among them, microextration by packed sorbent (MEPS) proposed by Abdel-Rehi et al. in 2004, may represent an interesting analytical option, since it combines sample processing, concentration and clean-up into a fully-automated online sampling/injecting device [19, 20]. MEPS can be considered as a miniaturized form of conventional solid phase extraction (SPE), by combining SPE phases inside a special needle assembly that is then attached to the MEPS syringe. Compared to traditional SPE, MEPS uses lower amount of sorbent. A noticeable advantage of this technique is the possibility to process a wide range of sample volumes (from few µL to several mL) with high selectivity, thus guaranteeing high-throughput analyses [21-23].As in the SPE, the core of the extraction is the sorbent material: several commercial sorbents are available, being able to retain the analytes via reversible interactions (hydrophilic, van der Waals, ionic, hydrogen bond, etc). The extraction procedure consists of four main steps: i) sorbent conditioning; ii) sample collection; iii) sorbent material washing; iv) analyte elution.

The present research study reports, for the first time, to the best of our knowledge, the development and validation of a fast, efficient, sensitive, reliable and high throughput MEPS-based methodology combined with LC-MS^2 for the simultaneous determination of dexamethasone sodium phosphate and dexamethasone in human aqueous humor. Owing to the limited amount of sample available, MEPS proved to be the technique of choice to perform extraction, clean-up and pre-concentration of the investigated analytes in one step.

2. Materials and methods

2.1. Chemicals and materials

Dexamethasone-21 disodium phosphate salt (> 98% purity), dexamethasone (≥ 98% purity), acetic acid (99% purity), ammonium acetate (98% purity) and methanol (>99.9 % purity) were purchased from Sigma-Aldrich (Milan, Italy).

C2, C8 and C18 MEPS BIN were from SGE Analytical Science (Milton Keynes, UK).

2.2. Aqueous humor sampling

Following respective ethics committee approval, patients with a history of uveitis requiring cataract surgery were divided into two groups of perioperative steroidal supplementation. Group A (topical prophylaxis alone): from 4 days before surgery: disodium dexamethasone 0.15% eye drops (4 instillations/day). Group B (topical + oral prophylaxis): from 4 days before surgery: oral dexamethasone (0.05 mg/Kg/day, in a single dose in the morning), and adjunctive topical treatment as in the group A, as detailed in a previous report [24]. A total of 15 patients per group were involved. The day of surgery the patients were asked to put one drop of dexamethasone 0.15% in the operating eye just before moving to the Hospital (2 to 4 hours before surgery). Cataract extraction was performed on all patients through standard pharmacoemulsification technique with foldable hydrophobic acrylic lens implantation, under topical or local anaesthesia (peribulbar

injection). After cutting the corneal limbus, before the filling of the anterior chamber of the eye with viscoelastic matrix, an aliquot part of the aqueous humour (at least 50 µL, depending on the anatomy and the clinical characteristics of the eye) was drawn with a suitable sterile syringe with flat needle. This withdrawal did not change the modalities and the prognosis of surgery, being the aqueous humor normally dispersed on the surgical field. The liquid was quickly transferred in a microcentrifuge tube and stored at -80°C. From subjects who agreed to enter the study, written informed consent was obtained according to the tenets of the Declaration of Helsinki.

2.3. Experimental design and optimization of the MEPS procedure

The experiments were carried out on blank aqueous humor samples spiked with DEX-SP and DEX both at 3 µg/L.

A 2^2 two-levels full factorial design (FFD) [25] was carried out by investigating the effects of both loading and eluting cycles. In both cases low and high levels were 5 and 25 cycles, respectively. A *F*-test comparing the experimental and calculated responses at the centre of the experimental domain was performed to evaluate the existence of relevant quadratic effects and a star design was added to the factorial design experiments [26]. The final regression models were then calculated using the Central Composite Design (CCD) experiments, obtained both from the FFD and the star design and used to find the optimal extraction conditions by using the multicriteria method of the desirability functions [27-29]. All statistical analyses were carried out by using the statistical package SPSS Statistics 24.0 (IBM, Milano, Italy).

2.4. MEPS procedure

A commercial e-Vol® equipped with a C18 Barrel Insert and Needle (BIN) was used for MEPS extraction. Prior to extraction, each BIN was activated by using 4x50 µL of methanol and 2x50 µL of water. Fifty µL of aqueous humor were drawn up and down through the BIN 19 times. Then, the analytes were eluted with 10x26 µL of methanol and analyzed by LC–MS^2. After extraction, 10

wash cycles each with 10x50 µL of methanol were used to clean the sorbent material and to avoid carryover effects. Both a fill and injection speed of 1 arbitrary unit were used.

2.5 LC-MS^2 analysis

Chromatographic separation was performed on a HPLC system (Thermo Electron Corporation, San Josè, CA, USA) coupled with a LTQ XL linear ion trap mass spectrometer (Thermo Electron Corporation) equipped with a pneumatically assisted electrospray (ESI) interface. The system was controlled by the Xcalibur software (Thermo Electron Corporation). The mobile phase was delivered by the Surveyor chromatographic system (Thermo Electron Corporation) equipped with a 200-vial capacity sample tray. A volume of 20 µL of each extract was injected into a GEMINI C18 100 mm x 2.0 mm, 3 µm 110 Å column (Phenomenex, Torrance, California, USA), equipped with a C18 security guard cartridge and thermostated at 25 °C, at a flow rate of 250 µL/min. The mobile phase consisted of solvent A (methanol) and solvent B (5 mM ammonium acetate) (pH=4.2). The initial condition was 40% solvent A and 60% solvent B. A linear gradient was performed with mobile phase A increasing from 40 to 90% within 1 min. After 7 min the mobile phase was returned to the initial conditions and re-equilibrated for 2 minutes. The sheath gas (nitrogen, 99.99% purity), the auxiliary gas (nitrogen, 99.99% purity) and the sweep gas (nitrogen, 99.99% purity) were delivered at flow rates of 30, 10 and 5 arbitrary units, respectively. Optimized conditions of the source were set as follows: ESI voltage, 4.5 kV; capillary voltage, 30 V; capillary temperature, 300°C; tube lens, 100 V. DEX-SP and DEX were analysed in multiple reaction monitoring (MRM) mode using an electrospray probe in the positive ionization mode. The following transitions were monitored: *m/z* 517 → *m/z* 499 (used for quantitation), 479 and 395 for DEX-SP and m/z 393 → 373 (used for quantitation), 355 and 337 for DEX. A collision energy of 30 V was used.

185 *2.6 Validation*

186 Validation was carried out under the optimized conditions, to meet the acceptance criteria for
187 bioanalytical method validation [30].

188 Aqueous humor samples extracted from patients submitted to cataract surgery not treated with
189 DEX-SP were used as blank matrix. For both the analytes, the lower limit of quantification (LLOQ)
190 was calculated as signal-to-noise ratio, S/N = 5, using five independent samples and tested for
191 accuracy and precision to meet the previously cited international criteria. The calibration curves
192 were evaluated on six concentration levels in the LLOQ-150 µg/L range, performing two replicated
193 measurements for each level. Lack-of-fit and Mandel's fitting test were performed to check the
194 goodness of fit and linearity. The significance of the intercept (significance level 5%) was
195 established by running a Student *t*-test. Precision in terms of both within-run and between-run
196 precision was calculated in terms of RSD % on three concentration levels (LLOQ, 3 and 70 µg/L),
197 performing five replicates at each level. Between-run precision was estimated over three days
198 testing for homoscedasticity among the data and performing the analysis of variance (ANOVA) at
199 the confidence level of 95%. Accuracy was calculated in terms of recovery rate (RR%) as follows:

200 $RR\% = c_1/c_2 \times 100$

201 where c_1 is the measured concentration and c_2 is the concentration calculated from the quantity
202 spiked into the sample. Three different concentration levels (LLOQ, 3 and 70 µg/L) with five
203 replicated measurements were analyzed. The extraction yields in terms of percent recovery was
204 calculated by comparing the results obtained from the MEPS-LC-MS^2 analysis of the biological
205 matrix spiked both with DEX-SP and DEX at three concentration levels (LLOQ, 3 and 70 µg/L)
206 with those obtained for the true concentration of the analyte in solvent (n=5).

207 Method selectivity was assessed by testing for interference 10 blank aqueous humor samples.

208 Finally, stability was evaluated in terms of both long-term and bench-top storage, and processed
209 sample stability. Three replicates at the LLOQ and at 70 µg/L were always performed.

210

3. Results and discussion

3.1.MEPS optimization

Taking into account that the aim of the study was the quantitation of DEX-SP and DEX in the aqueous humor of patients affected by uveitis requiring cataract surgery in order to evaluate the effectiveness of the topic administration of the corticosteroids,the microextraction by packed sorbent approach is very attractive in bioanalysis to promote both the purification of the matrix and the concentration of the analytes, since reduced size of sample is required. According to previous published studies [18,31], preliminary investigations carried out by using C2, C8 and C18 BINs, showed that the highest extraction yields could be obtained by using C18 and methanol as packed sorbent and eluting solvent, respectively (Figg. 2,3). In order to optimize the MEPS conditions, a 2^2 FFD was performed by investigating the effects of both loading and eluting cycles. The experimental domain was defined taking into account that in order to favor a good interaction among the analytes and the sorbent material, a minimum number of loading cycles have to be performed. In our study this value was set as n=5. The same was for the elution process. As for the fill/injection speed, a value of 1 arbitrary unit was used to avoid the presence of bubbles in both the filled and in the eluted solutions. Repeatability of measurements was assessed by performing 4 replicates at the centre of the experimental domain. For each compound, main and interaction effects were calculated. The presence of curvature was assessed for both the analytes, thus requiring to perform the experiments corresponding to a star design. Table 1 list the regression models used to search for the highest MEPS-LC-MS^2 response by means of the multicriteria method of desirability functions. The optimal experimental conditions were found in correspondence to a number of loading cycles=19. Taking into account that a global desirability D=0.90 and that very good single desirability values were obtained, the developed procedure proved to be suitable for the simultaneous extraction of the investigated corticosteroids. As for the eluting cycles, taking into account that samples containing higher amounts of corticosteroids compared to the 3 µg/L used in

237 the CCD could be analyzed, a number of cycles n=10 was used to favor the complete elution of the
238 analytes.
239
240 *3.2 Method validation*
241 The method was validated using the experimental setting providing the optimized conditions (Table
242 2). LLOQ values of 0.7 and 0.5 µg/L for DEX-SP and DEX, respectively were calculated, thus
243 attesting the capability of the developed method of quantifying the analytes at trace levels.
244 Good linearity was proved by applying Mandel's fitting test in the LLOQ-150 µg/L range for both
245 the analytes. Satisfactory precision was proved both in terms of within-run and between-run
246 precision with RSD always lower than 16% also at the LLOQ levels, thus meeting the criteria as
247 described in the guidelines for the validation of bioanalytical methods [30].
248 Recoveries in the 91(±6)–119(±25)% (n=5) range proved the accuracy of the developed method,
249 whereas extraction yields higher than 85% were always obtained.
250 The method has shown good selectivity, since there was no interference from other endogenous
251 compounds . As for stability, no problems related to the long-term stability of the stock solutions
252 were observed: in fact, ANOVA performed on data obtained by the analysis of standard solutions
253 daily prepared from the stock solutions did not show significant differences (p>0.05) up to 7
254 months when the stock solutions were stored at -20°C. Bench-top stability was evaluated by
255 analyzing standard solutions maintained at room temperature up to 12 h at two concentration levels.
256 By applying the student t-test, no significant differences (p>0.05) were observed between the mean
257 responses. Freeze and thaw stability was not assessed, since it was not possible to perform freeze
258 and thaw cycles due to the low volume of available sample.
259 Finally, the processed sample stability proved that no significant differences between the responses
260 obtained from the same sample analyzed just after the MEPS extraction and after 10 h. This time
261 was the longest period until completion of the analysis.
262

3.3 Real sample analysis

In order to investigate the validity of the devised method, aqueous humor real samples were analyzed as described in 2.2. Section. Neither DEX-SP nor DEX were detected in samples from group A and B, respectively. The obtained results can be explained taking into account both the low number of patients and the time elapsed from the drop instillation. Although one study reported that DEX could be detected 12 h after instillation [12], it is known that along the time a drastic decrease in the concentration of the analytes occurs [5, 31-33]. These evidences reinforce the interest in newly developed ophthalmic drug carries for topical administration, as cyclodextrin-poloxamer aggregates; they also offer to such nanocarrier topical administration a suitable method to assess intraocular concentrations [34-36].

4. Conclusion

A rapid and reliable MEPS-LC-MS^2 method for the determination of dexamethasone disodium phosphate and dexamethasone in humor aqueous was developed and validated. Multi-criteria optimization method based on the desirability function approach allowed for the simultaneous determination of both the analytes at trace levels. The proposed method allowed for rapid analysis time, low volume consumption, good selectivity and high extraction yield, thus being a valuable tool for clinical studies to assess reliability of dexamethasone administration in patients affected by uveitis.

5. References

[1] E. Miserocchi, G. Fogliato, G. Modorati, F. Bandello, Review on the worldwide epidemiology of uveitis, Eur. J. Ophthalmol. 23 (2013) 705-717.

[2] R.N. Van Gelder, T.K. Leveque, Cataract surgery in the setting of uveitis, Curr. Opin. Ophthalmol. 20 (2009) 42-45.

[3] M. Jancevski, C.S. Foster, Cataract and uveitis. Curr. Opin. Ophthalmol. 21 (2010) 10-14.

[4] M. Zhang, G.A. Moore, B.P. Jensen, E.J. Begg, P.A. Bird, Determination of dexamethasone and dexamethasone sodium phosphate in human plasma and cochlear perilymph by liquid chromatography/tandem mass spectrometry, J. Chromatogr. B 879 (2011) 17-24.

[5] C. Cagini, F. Cometa, G. Torroni, A. Pellegrino, R. Pellegrino, G.M. Cavallini, Dexamethasone Disodium Phosphate Penetration Into the Human Aqueous Humor After Topical Application, Curr Eye Res. 41 (2016) 897-899.

[6] P. Rohdenwald, H. Mollmann, J. Barth, J. Rehder, H. Derendorf, Pharmacokinetics of dexamethasone and its phosphate ester, Biopharm. Drug Dispos. 8 (1987) 205-212.

[7] E. Barcia, R. Herrero-Vanrell, A. Dìez, C. Alvarez-Santiago, I. Lòpez, M. Calonge, Downregulation of endotoxin-induced uveitis by intravitreal injection of polylactic-glycolic acid (PLGA) microspheres loaded with dexamethasone, Exp. Eye Res. 89 (2009) 238-245.

[8] J.R. Villanueva, L.R. Villanueva, M.G. Navarro, Pharmaceutical technology can turn a traditional drug, dexamethasone into a first-line ocular medicine. A global perspective and future trends, Int. J. Pharm. 516 (2017) 342–351.

[9] C.N. McGhee, Pharmacokinetics of ophthalmic corticosteroids, Br. J. Ophthalmol. 76 (1992) 681–684.

[10] I.P. Kaur, S., Kakkar, Nanotherapy for posterior eye diseases, J. Control. Release 193 (2014) 100–112.

313 [11] H.Y. Tan, A. Agarwal, C.S. Lee, J. Chhablani, V. Gupta, M. Khatri, J. Nirmal, C. Pavesio, R.
314 Agrawal, Management of noninfectious posterior uveitis with intravitreal drug therapy, Clin.
315 Ophthalmol. 10 (2016) 1983-2020.
316 [12] D. Watson, M.J. Noble, G.N. Dutton, J.M. Midgley, T.M. Healey, Penetration of topically
317 applied dexamethasone alcohol into human aqueous humor, Arch. Ophthalmol. 106 (1988) 686-
318 687.
319 [13] J. Aufartova, C. Mahugo-Santana, Z. Sosa-Ferrera, J.J. Santana-Rodriguez, L. Novakova, P.
320 Solich, Determination of steroid hormones in biological and environmental samples using green
321 microextraction techniques: An overview, Anal. Chim. Acta 704 (2011) 33-46.
322 [14] L. Li, P. Ma, J. Wei, K. Qian, L. Tao, LC-ESI-MS method for the determination of
323 dexamethasone acetate in skin of nude mouse, J. Chromatogr. B 933 (2013) 44-49.
324 [15] M. Zhang, G.A. Moore, B.P. Jensen, E.J. Begg, P.A. Bird, Determination of dexamethasone
325 and dexamethasone sodium phosphate in human plasma and cochlear perilymph by liquid
326 chromatography/tandem mass spectrometry, J Chromatogr. B 879 (2011) 17-24.
327 [16] C. Shu, T. Zeng, S. Gao, T. Xia, L. Huang, F. Zhang, W. Chen, LC-MS/MS method for
328 simultaneous determination of thalidomide, lenalidomide, cyclophosphamide, bortezomib,
329 dexamethasone and adriamycin in serum of multiple myeloma patients, J. Chromatogr. B 1028
330 (2016) 111-119.
331 [17] R. Earla, S.H.S. Boddu, K. Cholkar, S. Hariharan, J. Jwala, A.K. Mitra, Development and
332 validation of a fast and sensitive bioanalytical method for the quantitative determination of
333 glucocorticoids - Quantitative measurement of dexamethasone in rabbit ocular matrices by liquid
334 chromatography tandem mass spectrometry, J. Pharmaceut. Biomed. 52 (2010) 525-533.
335 [18] M.A. Saracino, C. Iacono, L. Somaini, G. Gerra, N. Ghedini, M.A. Raggi, Multi-matrix assay
336 of cortisol, cortisone and corticosterone using a combined MEPS-HPLC procedure, J. Pharmaceut.
337 Biomed. Anal. 88 (2014) 643-648.

[19] M. Abdel-Rehim Z. Altun, L. Blomberg, Microextraction in packed syringe (MEPS) for liquid and gas chromatographic application. Part I – determination of local anesthetics in human plasma samples using gas chromatography-mass spectrometry, J. Chromatogr. B 801 (2004) 317-321.

[20] M. Abdel-Rehim Z. Altun, L. Blomberg, Microextraction in packed syringe (MEPS) for liquid and gas chromatographic application. Part II – determination of ropivacaine and its metabolites in human plasma samples using MEPS with liquid chromatography/tandem mass spectrometry, J. Mass Spectrom. 39 (2004) 1488-1493.

[21] L. Yang, R. Said, M. Abdel-Rehim, Sorbent, device, matrix and application in microextraction by packed sorbent (MEPS): A review, J. Chromatogr. B 1043 (2017) 33–43.

[22] M. Abdel-Rehim Microextraction by packed sorbent (MEPS): A tutorial, Anal. Chim. Acta 701 (2011) 119-128.

[23] M.M. Moein, A. Abdel-Rehim, M. Abdel-Rehim, Microextraction by packed sorbent (MEPS), TrAC-Trend. Anal. Chem. 67 (2015) 34-44.

[24] P. Mora, S. Gonzales, S. Ghirardini, P. Rubino, J.G. Orsoni, S.A. Gandolfi, F. Majo, Y. Guex-Crosier, Perioperative prophylaxis to prevent recurrence following cataract surgery in uveitic patients: a two-centre, prospective, randomized trial, Acta Ophthalmol. 94 (2016) 390-394.

[25] E.P. Box, W.G. Hunter, J.S. Hunter, Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building, first ed., John Wiley & Sons, Hoboken, New Jersey, USA, 1978.

[26] A.I. Khuri, J.A. Cornell, Response Surfaces, first ed., Marcel Dekker, New York, USA, 1987.

[27] R. Carlson, Design and optimization in organic synthesis, third ed., Elsevier, Amsterdam, Netherland, 1992.

[28] F. Bianchi, M. Careri, A. Mangia, M. Mattarozzi, M. Musci, Experimental design for the optimization of the extraction conditions of polycyclic aromatic hydrocarbons in milk with a novel diethoxydiphenylsilane solid-phase microextraction fiber, J. Chromatogr. A 1196–1197 (2008) 41–45.

[29] M. Mattarozzi, M. Milioli, F. Bianchi, A. Cavazza, S. Pigozzi, A. Milandri, M. Careri, Optimization of a rapid QuEChERS sample treatment method for HILIC-MS^2 analysis of paralytic shellfish poisoning (PSP) toxins in mussels, Food Control 60 (2016) 138-145.

[30] Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, 2001. https://www.fda.gov/downloads/Drugs/Guidances/ucm368107.pdf (accessed 05.04.2017)

[31] S. Anizan, E. Bichon, F. Monteau, N. Cesbron, J.-P. Antignac, B. Le Bizec, A new reliable sample preparation for high throughput focused steroids profiling by gas chromatography-mass spectrometry, J. Chromatogr. A 1217 (2010) 6652-6660.

[32] K. Araki-Sasaki, O. Katsuta, H. Mano, T. Nagano, M. Nakamura, The effects of oral and topical corticosteroid in rabbit corneas, BMC Ophthalmol. 16 (2016) 160-166.

[33] X. Huang, S. Liu, Y. Yang, Y. Duan, D. Lin, Controllable continuous sub-tenon drug delivery of dexamethasone disodium phosphate to ocular posterior segment in rabbit, Drug Deliv. 24 (2017) 452–458.

[34] P. Jansook, W. Pichayakorn, C. Muankaew T. Loftsson, Cyclodextrin-poloxamer aggregates as nanocarriers in eye drop formulations: dexamethasone and amphotericin B, Drug Dev. Ind. Pharm. 42 (2016) 1446-1454.

[35] S. Shulman, G. Johannesson, E. Stefansson, A. Loewenstein, A. Rosenblatt, Z. Habot-Wilner, Topical dexamethasone-cyclodextrin nanoparticle eye drops for non-infectious uveitic macular oedema and vitiritis – a pilot study, Acta Ophthalmol. Scand. 93 (2015) 411-415.

[36] S. Krag, A. Hessellund, Topical dexamethasone-cyclodextrin microparticle eye drops for uveitic macular oedema, Acta Ophthalmol. Scand. 92 (2014) 689-690.

Figure captions

Fig. 1. Molecular structure of dexamethasone (left) and dexamethasone disodium phosphate (right)

Fig. 2. MEPS sorbent selection. Three replicate measurements for each sorbent

Fig. 3. MEPS eluting solvent. Three replicate measurements for each solvent