Toxicity evaluation and nasal mucosal tissue deposition of dexamethasone-infused mucoadhesive in situ nasal gelling systems

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ABSTRACT

To demonstrate safety of a developed intranasal dexamethasone-infused in situ gelling formulation, quantification of a validated clinical biomarker indicative of cytotoxic potential using a human sinonasal explant model was first confirmed. Systematic cytotoxicity studies using the lactate dehydrogenase (LDH) detection assay revealed no elevation from baseline, in LDH levels, with tissue integrity of explanted human nasal mucosa also maintained; this was further corroborated using tissue histopathological examination. Next, with safety confirmed ex vivo, freshly excised human nasal tissue was utilised to quantify dexamethasone release from the lead sol–gel systems; this being achieved through development and validation of a HPLC-UV analytical method, which reliably quantified controlled therapeutic release and deposition into mucosal tissue. Collectively, these findings indicate promise in the safety of each excipient within the concentrations employed in the functional sol–gel system, complemented by successful and reliable drug release and deposition into human nasal mucosal tissue. These findings pave the way for application of the dexamethasone-based sol–gel system to the extended delivery of corticosteroids to nasal mucosa in the management of localised inflammatory conditions of an acute and chronic nature, such as chronic rhinosinusitis, which can be expected to benefit from controlled and extended drug delivery characteristics imparted by appropriately engineered in situ gelling systems.

ARTICLE INFO

Article history:
Received 6 February 2019
Accepted 22 June 2019
Available online 28 June 2019

Keywords:
Intranasal soluble gels
Sinusitis
Explant human nasal mucosa
Tissue toxicity
LDH assay
Drug deposition in human nasal mucosa

1. Introduction

Intranasally-administered agents/formulations play a major role in the medical management of sinonasal disorders. Direct intranasal administration of therapeutics is the preferred approach for the treatment of various localised inflammatory nasal conditions (Romeo et al., 1998). Chronic rhinosinusitis (CRS) is one such condition, characterised by nasal inflammatory symptoms that last beyond 3 months. For management of CRS, a combination of medical and surgical intervention is required. In relation to the latter, functional endoscopic sinus surgery (FESS) is performed to ameliorate CRS-associated symptoms; however, an intranasal formulation with characteristics to have a local and sustained effect, while displaying controlled drug release behaviour, and minimal tissue toxicity is critical for the promotion of wound healing and maintenance of ostium patency.

Conventional methods of nasal drug delivery (e.g. solutions, suspensions, and ointments) suffer from significant drawbacks such as a short residence time in the nasal cavity, highly variable efficacy, low rates of drug release/permeability, and inconvenience caused by leakage (Saindane et al., 2013). In contrast, in situ polymeric gelling systems are an ideal and favoured vehicle given that they are in a soluble (liquid) form for ease of administration, but once administered, they rapidly undergo in situ gelation resulting in controlled drug release and local, sustained therapeutic effect (Miyazaki et al., 2003, 1999).

Dexamethasone is a glucocorticoid used widely in the clinic for treatment of various immune-inflammatory conditions including
severe allergies, asthma and chronic obstructive lung disease. Considering many beneficial roles of dexamethasone for the treatment of rhinitis (Stephens and Boggs, 1968; McAllen and Langman, 1969; Wang et al., 2015; Aaron and Muttitt, 1964), and in order to provide prolonged therapeutic activity and prevention from the recurrence of CRS conditions, we have systematically developed and optimised thermo-responsive sol-gels infused with clinically relevant concentrations of therapeutic, dexamethasone 21-phosphate disodium salt (DXN) displaying extended mucosal contact time and drug release properties (Pande et al., 2017). Preliminary findings through extensive rheological and mechanical evaluation demonstrated that developed poloxamer-based thermostresponsive DXN-sol–gel formulations possess an optimal gelation temperature, leading to liquid like flow behaviour at room temperature, and a rapid increase in viscosity at/around nasal temperature, which is expected to extend nasal residence time (Pande et al., 2017).

Given that any developed drug-infused intranasal sol–gel system has the potential to cause toxicity; evaluation of their cytotoxic profile is warranted in the first instance, and this is a fundamental requirement from a regulatory perspective (Lim et al., 2012). The vast majority of topical formulations aim to deliver therapeutic agents locally, and so quantifying drug deposition to target tissue is a reliable preliminary indicator of formulation performance (Nair et al., 2013). A rapid, simple and accurate analytical method is required for reliable determination of drugs concentration in biological matrices, such as nasal mucosa. Various analytical methods (HPLC and LC-MS/MS) have been reported to estimate DXN in human matrices such as whole blood, serum, plasma, cochlear perilymph, urine, and milk (Kumar et al., 2006; Zhang et al., 2011; Patel et al., 2010; Song et al., 2008; Baranowska et al., 2009; Li et al., 2010; Liu et al., 2004). However, at the time of writing and to the best of our knowledge, no analytical method for the determination of DXN in human nasal mucosa was available in the literature.

Therefore, the initial focus was on evaluating the in situ toxicity profile of lead DXN sol–gel formulations, PGHC4 & PGHC7, using a clinically-acceptable biomarker. Next, development and validation of a reliable HPLC-UV method to enable accurate quantification of DXN, when applied to explanted nasal mucosal tissue was undertaken.

2. Materials and methods

2.1. Chemicals

Dexamethasone 21-phosphate disodium salt (DXN) was purchased from Alfa Aesar, USA. Acetic acid, sodium hydroxide (NaOH), formic acid, potassium chloride (KCl), calcium chloride (CaCl2), sodium chloride (NaCl) were of analytical grade and procured from Sigma-Aldrich, Castle Hill, NSW, Australia. HPLC grade acetonitrile (ACN) was from Merck KGaA. Ultrapure water was used as a formulation vehicle.

2.2. Nasal tissue collection

Tissue toxicity and drug deposition studies were carried out using freshly excised human nasal mucosa, which was obtained from patients undergoing surgery for nasal obstruction at the Greenslopes Hospital, Brisbane, Australia. The study was approved by the Greenslopes Hospital, and School of Pharmacy Human Ethics Committees (HREC/14/QPAH/530) and written consent was obtained from all human subjects donating the nasal tissues. Nasal tissue samples were collected from the middle turbinate, inferior turbinate, or superior nasal septum based on the planned operation and used immediately. By means of a scalpel, the human specimens were carefully dissected from undesired connective tissue and carefully removed of debris or blood.

2.3. Human nasal explant culture system and lactate dehydrogenase (LDH) cytotoxicity assay

The biopsied tissues were processed for establishing an organotypic tissue culture system and tissue toxicity predicted using lactate dehydrogenase (LDH) as the biomarker, as previously described with slight modifications (Lim et al., 2012). Briefly, cultured nasal explants from six human subjects were collected, and processed by removal of tissue debris or blood, and divided into 2–4 mm × 2–4 mm × 1–2 mm (L × W × D) pieces with a number 15-blade scalpel. Divided tissue was placed on a 0.4 μm polytetrafluoroethane (PTFE) membrane (Millicell-CM; Millipore, Cork, Ireland) and placed in 24-well culture system (Corning, NY). Following three successive washes with 300 μL of culture medium, tissue was cultured in 300 μL of culture medium/well. The media consisted of 50% Dulbecco’s Modified Eagle Medium (DMEM) high glucose (Invitrogen, Carlsbad, CA), and supplemented with 25% Hank solution (Invitrogen, Carlsbad, CA); 50 μM penicillin G and 40 μg/mL streptomycin (Invitrogen, Carlsbad, CA). Next, tissues were placed on the membrane such that the epithelial side was uppermost and exposed to the air, and then placed in a humidified incubator at 37 °C with 5% CO2. The culture media was replaced every 24 h, and tissues were incubated for three days leading to stabilisation of baseline extracellular LDH levels. Post-three days incubation, tissue was directly incubated with 2 μL of either lead formulations (PGHC4 or PGHC7), 0.9% saline or 1% ZnSO4 (positive control) for a duration of five days. The LDH assay was performed using the CytoTox 96® Non-Radioactive Cytotoxicity Assay Kit (Promega, Madison, WI), and used as per manufacturer protocols. On the day of the assay, 50 μL of culture medium was removed from the treated well and 50 μL of substrate mix was added. Following 30 min incubation at room temperature, the reaction was stopped with 50 μL of stop solution, and absorbance was read at 490 nm using a FLUOstar Omega spectrophotometer (BMG Labtech, Offenburg, Germany). The effects of treatments were calculated as fold changes in extracellular LDH levels of tissues treated with formulation vs. tissues treated with saline.

2.4. Hematoxylin and eosin (H & E) staining

Pre/post-treatment tissue samples were retained from each well (as explained in Section 2.3 above). For histological evaluation, retained tissue from each test specimen was fixed using 4% formaldehyde, and stored at 4 °C overnight. Followed by dipping in 20% sucrose overnight at 4 °C. Next, specimens were embedded in OCT (Sakura Finetek USA, Inc., Torrance, CA), frozen at −20 °C and cryosectioned in slices. Then, samples were stained in hematoxylin and eosin (H & E) (Surgipath Medical Industries, Inc., Richmond, IL) for 1 min each. Stained tissues were dehydrated in ethanol, defatted in xylene and cover-slipped for histological examination.

2.5. Experimental setup for DXN deposition study to explanted nasal mucosal tissue

Tissue deposition studies were carried out using vertical Franz diffusion apparatus equipped with temperature-controlled water jackets set at 34 ± 1 °C. Prepared nasal mucosa was sandwiched between the donor and receiver compartments such that the dorsal side of the tissue faced the donor compartment and the ventral side faced the receiver compartment. The donor and receiver
compartments were filled with 1.5 mL of the lead sol–gel formulation, containing DXN (0.1% w/w), and 12 mL of simulated nasal fluid (SNF) respectively, equilibrated at 34 ± 1 °C. The compositions of SNF include 1.29 mg/mL KCl, 7.45 mg/mL NaCl and 0.32 mg/mL CaCl2·2H2O, adjusted to pH 5.5 ± 0.2 with 0.1 M HCl/0.1 N NaOH (Callens et al., 2003). Following 0 h, 2 h, 4 h, 6 h and 8 h of exposure, collected tissue samples were rinsed with ultrapure water, dried with filter paper, wrapped with aluminium foil to protect from light and stored at −80 °C for further analysis (Matta et al., 2018).

2.6. Analytical HPLC method development and validation for DXN in nasal tissue

2.6.1. Chromatographic condition

DXN was analysed using an integrated RP-HPLC system (Shimadzu Prominance UFLC, Japan) equipped with a low-pressure quaternary gradient pump along with a dual wavelength UV detector (at 254 nm), auto sampler (10 µL injection volume) and column oven (maintained at 25 °C). The chromatographic separation was performed using analytical Vydac218p C18, 5 µm (250 × 4.6 mm) column under protection of a C18 guard column (Altech, USA). Reservoir A (Mobile phase A) contained 0.1% formic acid in ultrapure water and reservoir B (Mobile phase B) contained 0.1% formic acid in acetonitrile (ACN). A 10 min binary linear gradient was employed: 10% B at 0 min, increasing to 50% B in 4.5 min, followed by isocratic elution with 50% B for a further 1 min. The solvent composition was reverted back to 10% B within 0.6 min, and the column was re-equilibrated with that composition for a further 3.9 min.

2.6.2. Standards and quality control samples

A stock solution of DXN was prepared at a concentration of 1 mg/mL in ultrapure water and diluted to 20, 50 and 100 µg/mL for working calibration curve (CC) standards. Quality control (QC) samples at low (3 µg/mL), medium (10 µg/mL) and high (80 µg/mL) concentrations were prepared in blank nasal tissue homogenate. The CC solutions and QC samples were wrapped in aluminium foil (providing protection from light) and stored at −80 °C until further use.

2.6.3. Tissue sample preparation for HPLC analysis

On the day of analysis, the tissue samples were allowed to thaw and blotted using filter paper, frozen with liquid nitrogen, ground with a mortar and pestle (Burden, 2008). Next, powdered nasal tissues were added to ultrapure water to prepare 100 mg/mL concentration of tissue homogenate. For linearity, blank tissue homogenate (10 µL) was spiked with 10 µL of DXN standard dilutions; while test and QC samples (10 µL) were spiked with 10 µL of water. The samples were vortexed for 10 s, and extraction of tissue sample was performed by addition of ACN (30 µL). The samples were again vortexed for 10 s, centrifuged at 10,000 rpm for 5 min, and 30 µL of supernatant was transferred to an auto injector vial for HPLC analysis.

2.6.4. Method validation

The HPLC-UV method for quantification of DXN in human nasal tissue was validated as per US-FDA (September 2013) guidelines in terms of system sensitivity, specificity, linearity, recovery, accuracy and precision (U.S. Dept. of Health and Human Services, 2013; Singh et al., 2008). The lower limit of quantification (LLOQ) was defined as the concentration of the sample that can be quantified with suitable precision and accuracy (<20%). The nasal mucosal tissues were procured from six different patients (having no previous treatment with DXN) to test for matrix interference with retention times of DXN. LLOQ sensitivity was determined by injecting six LLOQ samples into the HPLC, and signal of the analyte was compared with those of blank samples. Peak areas of blank interference should not exceed 20% of mean peak area of LLOQ. The linearity was established in the range of 1–100 µg/mL concentrations (1, 2, 5, 10, 20, 50 and 100 µg/mL) of DXN. The linearity was verified using least-square linear regression analysis (r² = 0.998) with estimates of correlation coefficient. For precision and accuracy, three batches including CC standards and six replicates of QC samples (low, medium and high relative concentration levels) were performed in nasal tissue homogenate. Intra-day and inter-day precision was measured as a relative standard deviation (RSD) of the replicate measurements, and the data was analysed statistically using analysis of variance (ANOVA). Accuracy was measured as percentage bias from the theoretical concentration. Stability of DXN was determined in terms of freeze thaw cycle (i.e. 3 cycles - thawed at room temperature and stored at −80 °C, bench top (4 h at room temperature) and long term stability at three concentration levels (20, 50 and 100 µg/mL spiked in nasal tissue homogenate).

2.7. Statistical analysis

LDH assay data were expressed as mean ± standard error mean (SEM, n = 3). Statistical differences compared between multiple groups of the formulation treated groups and appropriate negative controls were analysed by one-way ANOVA and followed by Bonferroni multiple comparison test. A p value < 0.05 was considered to indicate statistical significance. GraphPad Prism software (version 6.07) was used for all analyses. The chromatographic data was processed as an external standard method using LC solution 1.24 SP1 software.

3. Results and discussion

3.1. Tissue toxicity and histology of applied DXN sol–gel formulations

The use of tissue culture in laboratory settings is a valuable tool for screening safety of therapeutic agents within formulations. Given that LDH is released by stressed/dying cells, toxic responses associated with DXN-containing in situ gelling formulations can be reliably and directly measured using LDH as a biomarker, over exoplanted nasal mucosal tissue. Here, a stable tissue culture system was first established to understand the ‘baseline’ profile of LDH in untreated tissue, prior to treatment with test formulations (Lim et al., 2012; Bermúdez et al., 2008). To achieve this the LDH level of nasal tissue cultured in growth media alone was monitored. Initially, the LDH levels were found to decrease, stabilising after 3 days of incubation with tissue (Fig. 1A). Therefore, a lead time of 3 days was used before tissues were exposed to selected lead sol–gel formulations (PGHC4 & PGHC7) in the tissue toxicity assay. Considering zinc sulphate’s (ZnSO4) well-reported toxic effects on olfactory epithelium (McBride et al., 2003), it was used as a positive control, eliciting potent cytotoxic effects at 1% w/v ZnSO4 on nasal mucosal tissue, as expected. As depicted in Fig. 1B, there was no significant difference in cytotoxic responses (indicated by consistent, baseline LDH levels) after 5 consecutive days of treatment with either lead sol–gel formulation, which was in close agreement with the negative control, saline solution.

In order to further corroborate the LDH assay results, we next turned to histology where any pathological effects could be visualised. To confirm the safety of developed lead formulations with respect to tissue integrity, histology of mucosal tissue after 5 days of treatment with all formulations was performed via H & E staining. As depicted in Fig. 2A and B, the olfactory epithelium lining and pseudostratified epithelium layer remained intact and
unaffected after treatment with either lead sol–gel formulation, PGHC4 and PGHC7, relative to negative control (Fig. 2C). However, post-treatment with 1% ZnSO₄ (positive control), histology analysis revealed clear and widespread signs of toxicity characterised by degeneration of basal cells and severe inflammatory cell infiltration, as seen in Fig. 2D.

3.2. Analytical HPLC-UV method development and validation

A gradient reverse phase chromatography method was successfully developed for proper separation (i.e. with no interference from nasal tissue) of DXN with a retention of 6.1 min recorded (Fig. 3). The calibration curve was established and found to be linear across the concentration range of 1.0–100 µg/mL. Linear regression, “weighting factor of 1/x²” mean linear correlation coefficient (r) were calculated to be 0.9982. A regression equation was obtained: 

\[ y = 7,409.53x + 1700.90 \]

where y represents the peak area of DXN, x represents the concentration of DXN and the slope of the line is expressed as m (7,409.53). The LLOQ (1 µg/mL) sensitivity of DXN was determined as percent coefficient of variation (CV, 10.6% – 14.2%), which was within acceptable US-FDA prescribed limits (i.e. ±20%). Precision and accuracy batches were determined by multiple analyses (n = 3) of QC samples at three concentrations (3, 10 and 80 µg/mL). As illustrated in Table 1, inter- and intra-day accuracy and precision for DXN quantification was within acceptable limits of ±15%. The mean recoveries of DXN

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**Fig. 1.** Cytotoxicity of formulations applied to human nasal mucosal tissue. (A) LDH levels stabilised after 3 days of incubation with growth media; (B) Over 5 days of exposure, tissues treated with lead sol–gel DXN formulations (PGHC4 & PGHC7) and 0.9% saline solution maintained LDH at baseline levels, while 1% w/w ZnSO₄ (positive control) elicited a significant cytotoxic and severe inflammatory response.

**Fig. 2.** Formulations PGHC4 and PGHC7 did not adversely impact tissue integrity (A) & (B), respectively; healthy untreated mucosal tissue (negative control) (C); 1% w/w ZnSO₄ (positive control) altered tissue morphology (D) (n = 3). Both the intact morphology and disrupted morphology were shown at 10X magnification.
in low, medium and high QC concentrations were in the range of 79–85% with a percentage CV < 6% at all concentration levels measured.

Stability of DXN was examined by comparing area under curve of stored CC standards (20 μg/mL, 50 μg/mL, and 100 μg/mL) at −80 °C with fresh stock of DXN. To confirm bench top stability, CC standards (20 μg/mL, 50 μg/mL, 100 μg/mL) wrapped in aluminium foil were kept at room temperature for 4 h, and chromatograms were compared with DXN stock solution. The DXN stock solution stored at −80 °C for 2 months was found to be stable across all concentrations tested (98.6% ± 5.8). Similar to an earlier report (Chen et al., 2008), DXN stock solution was found to be stable at room temperature for 4 h, and for 2 months when stored at −80 °C. Freeze-thaw stability was also evaluated to understand effect of freezing-thawing cycling on stability of DXN in nasal tissue. Following three freeze-thaw cycles, DXN (across all concentrations tested) was found to be stable in nasal tissue yielding a non-significant deviation from control (2.2% ± 1.9%).

### 3.3. Ex vivo nasal tissue deposition of DXN

In our previous work (Pandey et al., 2017), we optimised and developed DXN-containing sol–gel formulations (PGHC4 and PGHC7), which possessed longer residence time and extended drug release, locally. Both lead sol–gel formulations displayed desired rheological/mechanical properties, paralleled by favourable drug release and permeation characteristics. Given the close correlation in data from tissue toxicity and histology and from our earlier work, we expected similar findings for both PGHC4 & PGHC7, and so only one lead formulation, PGHC4 was selected for ex vivo evaluation in nasal explant tissue.

The use of freshly excised, cultured human tissue, as an ex vivo model to study nasal drug absorption, deposition and metabolism was adopted (Agu et al., 2008). The earlier developed and validated HPLC method was used for quantification of DXN in nasal tissue. The retention of DXN (mean ± SEM, μg) per gram nasal tissue was found to be 73.34 ± 8.55, 118.19 ± 4.08, 132.05 ± 2.05, 135.32 ± 2.87 at 2 h, 4 h, 6 h and 8 h post-application, respectively (Fig. 4). Interestingly, there was a statistically significant difference in amount of DXN deposited in tissue after 2 h of treatment in comparison to 4 h, 6 h, and 8 h, and this could be attributed to controlled deposition of DXN through the gelled formulation to nasal tissue. In addition, there was no significant differences observed among treatment group for 4 h, 6 h, and 8 h, suggesting controlled release and adherence of DXN to nasal tissue over the three latter time points of the study period.

### 4. Conclusions

The developed DXN-infused sol–gel formulations intended for intranasal administration were found to be safe in terms of cytotoxicity, through maintenance of baseline LDH levels and tissue integrity when assessed in explanted human nasal mucosa. A HPLC method for estimation of DXN in human nasal tissue was developed, validated and applied for ex vivo local deposition of DXN from a lead sol–gel formulation (PGHC4). The HPLC method was found to be specific, accurate and precise, with results from subsequent tissue deposition studies indicating controlled release and
local deposition of DXN in nasal mucosa. With the lead sol–gel formulation demonstrating promise in healthy explanted nasal mucosal tissue, clinical trials are now warranted to demonstrate therapeutic efficacy in patients with disease, such as CRS.

Acknowledgement

The authors thank The University of Queensland Graduate School & School of Pharmacy for a UQ International PhD Scholarship to Preeti Pandey. The authors thank the Princess Alexandra Hospital Foundation for a small research grant. We also thank Ms. Preeti Pandey. The authors thank the Princess Alexandra School & School of Pharmacy for a UQ International PhD Scholarship.

Declarations of competing interest

The authors declare that they have no competing interests.

Author contributions

HSP, PP, SP, PJC, BW and BP conceived and designed the experiments. PP and SP performed the experiments. PP wrote the article and discussed the results. HSP, PJC, BW and BP contributed reagents/materials/analysis tools. HSP, SP, PJC, BW and BP critically reviewed the manuscript.

References

Aaron, T.H., Muttit, L.C., 1964. Intranasal dexamethasone phosphate in perennial allergic rhinitis. Ann. Allergy 22, 155–160.

Agu, R.U., Ugwoke, M.I., 2008. In situ and ex vivo nasal models for preclinical drug delivery studies. In: Ehrhardt, C., Kim, K.-J. (Eds.), Drug Absorption Studies: In S itu, In Vitro and In Silico Models. Springer US, Boston, MA, pp. 112–134.

Baranowska, I., Markowski, P., Baranowski, J., 2009. Development and validation of an hplc method for the simultaneous analysis of 23 selected drugs belonging to different therapeutic groups in human urine samples. Anal. Sci.: Int. J. Jap an Soc. Anal. Chem. 25, 1307–1313.

Bermúdez, J.M., Jimenez-Kairuz, A.F., Olivera, M.E., Allemandi, D.A., Manzo, R.H., 2008. A ciprofloxacin extended release tablet based on swellable drug polyelectrolyte matrices. AAPS PharmSciTech 9, 924–930.

Burden, D.W., 2008. Guide to the homogenization of biological samples. Random Primers, 1–14.

Callens, C., Ceulemans, J., Ludwig, A., Foreman, P., Remon, J.P., 2003. Rheological study on mucooadhesivity of some nasal powder formulations. Eur. J. Pharm. Biopharm. 55, 323–328.

Chen, Q., Zielinski, D., Chen, J., Koski, A., West, D., Nowak, S., 2008. A validated, stability-indicating hplc method for the determination of dexamethasone related substances on dexamethasone-coated drug-eluting stents. J. Pharm. Biomed. Anal. 48, 732–738.

Danonente, G., Salis, A., Rossi, L., Magnani, M., Benatti, U., 2007. High throughput hplc-esi-ms method for the quantification of dexamethasone in blood plasma. J. Pharm. Biomed. Anal. 43, 376–380.

Kumar, V., Mostafa, S., Kayo, M.W., Goldberg, E.P., Derendorf, H., 2006. Hplc determination of dexamethasone in human plasma and its application to an in vitro release study from endovascular stents. Pharmazie 61, 908–911.

Li, C., Wu, Y., Yang, T., Zhang, Y., 2010. Rapid simultaneous determination of dexamethasone and betamethasone in milk by liquid chromatography tandem mass spectrometry with isotope dilution. J. Chromatogr. A 1217, 411–414.

Lim, J.H., Davis, G.E., Rue, T.C., Storm, D.R., 2012. Human sinonasal explant system for testing cytotoxicity of intranasal agents. Int. Forum Allergy Rhinology 2, 63–68.

Liu, H., Chen, X., Zhang, S., Qu, L., Zhao, Y., Liu, H., Dong, M., 2004. Separation and determination of dexamethasone sodium phosphate in cochlear perilymph fluid by liquid chromatography with ultraviolet monitoring and electrospray ionization mass spectrometry characterization. J. Chromatogr. B, Anal. Technol. Biomed. Life Sci. 805, 255–260.

Matra, M.K., Narayanasamy, S., Thomas, C.D., Xu, L., Stewart, S., Chockalingam, A., Patel, V., Rouse, R., 2018. A sensitive uplc-apsi-ms/ms method for the determination of dexamethasone and its application in an ocular tissue distribution study in rabbits following topical administration. Anal. Methods 10, 2307–2316.

McAllen, M.K., Langman, M.J.S., 1969. A controlled trial of dexamethasone snuff in chronic perennial rhinitis. The Lancet 293, 968–971.

McBride, K., Slotnick, B., Margolis, F.L., 2003. Does intranasal application of zinc sulfate produce anosmia in the mouse? An olfactometric and anatomical study. Chem. Senses 28, 659–670.

Miyazaki, S., Aoyama, H., Kawasaki, N., Kubo, W., Artwood, D., 1999. In situ-gelling gellan formulations as vehicles for oral drug delivery. J. Controlled Release: Official J. Controlled Release Soc. 60, 287–295.

Miyazaki, S., Endo, K., Kawasaki, N., Kubo, W., Watanabe, H., Artwood, D., 2003. Oral sustained delivery of paracetamol from in situ gelling xylouglucan formulations. Drug. Dev. Ind. Pharm. 29, 113–119.

Nair, A., Jacob, S., Al-Dhushibi, B., Attimadur, M., Harsha, S., 2013. Basic considerations in the dermotekinetics of topical formulations. Braz. J. Pharm. Sci. 49, 423–434.

Pandey, P., Cabot, P.J., Wallwork, B., Panizza, B.J., Parekh, H.S., 2017. Formulation, functional evaluation and ex vivo performance of thermoresponsive soluble gels - a platform for therapeutic delivery to mucosal sinus tissue. Eur. J. Pharm. Sci. 96, 499–507.

Patel, P., Tanna, S., Mullia, H., Kairamkonda, V., Pandya, H., Lawson, G., 2010. Dexamethasone quantification in dried blood spot samples using ir–ms: The potential for application to neonatal pharmacokinetic studies. J. Chromatogr. B 878, 3277–3282.

Romero, V.D., de Meireles, J., Sileno, A.P., Pimplaskar, H.K., Behl, C.R., 1998. Effects of inhaled dexamethasone on mucus clearance in asthmatic children. Eur. J. Respir. Sci. 49, 423–434.

Saindane, N.S., Pagar, K.P., Vavia, P.R., 2013. Nanosuspension based in situ gelling nasal spray of carvedilol. Development, in vitro and in vivo characterization. AAPS PharmSciTech 14, 189–199.

Singh, U.K., Pandey, S., Pandey, P., Keshri, P.K., Wal, P., 2008. Bioanalytical method development and validation. Express Pharma 2, 1–8.

Song, L., Bai, J., Zhou, W., 2008. Determination of betamethasone and dexamethasone in human urine and serum by mekz after an experimental design. Chromatographia 68, 287–293.

Stephens, A.L., Boggis, P.B., 1968. Intranasal dexamethasone: An adjunct in the treatment of chemical rhinitis. Ann. Allergy 26, 612–613.

U.S. Dept. of Health and Human Services, Food and drug administration, Center for Veterinary Medicine. Bioanalytical Method Validation: Guidance for Industry, 2013.

Wang, W., Jiang, T., Zhu, Z., Cui, J., Zhu, L., Ma, Z., 2015. Dexamethasone suppresses allergic rhinitis and amplifies cd4+foxp3+ regulatory t cells in vitro. Int. Forum Allergy Rhinology 5, 900–906.

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