Research Article

Dual-Acting Subcutaneous Microemulsion Formulation for Improved Migraine Treatment with Zolmitriptan and Diclofenac: Formulation and *In Vitro-In Vivo* Characterization

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Abstract. Subcutaneous triptan provides immediate analgesia in migraine and cluster headache but is limited by high pain recurrence due to rapid drug elimination. A dual-acting subcutaneous formulation providing immediate release of a triptan and slow but sustained release of a nonsteroidal anti-inflammatory drug may provide a longer duration of relief. A microemulsion-based technology has various advantages over other technically complex dosage forms. Oil-in-water microemulsions of zolmitriptan and diclofenac acid using Labrafac Lipophile, Tween 80, Capryol 90 and water were prepared. One formulation was characterised *in vitro* and found to have uniformly dispersed nanosized globules. The formulation provided differential release of zolmitriptan and diclofenac acid both *in vitro* as well as *in vivo* that may be potentially beneficial to migraine patients.

KEY WORDS: diclofenac acid; dual-acting injection; migraine treatment; prolonged release microemulsion; subcutaneous injection; zolmitriptan.

INTRODUCTION

Pain constitutes a major part of the global disease burden. According to the American Academy of Pain Medicine, 26% of Americans aged 20 years or more and 30% of those between 45 and 64 years reported pain that lasted for more than 24 h (1). A significant proportion of the pain patient population comprises those with headache or migraine-related pain, which is also one of the primary reasons for an emergency department visit. As per the latest report (Health, United States, 2012) published by the Centre for Disease Control and Prevention, 16.6% of adults in USA (18 years and older) reported headache or migraine-related pain.

The pathophysiology of migraine includes a combination of neuronal, vascular, and inflammatory components (2,3). Accordingly, various agents used for the treatment of acute episodes and prevention include drugs with diverse mechanisms, such as nonsteroidal anti-inflammatory agents (NSAIDs), vasoconstrictive agents, antiepileptic drugs and beta blockers (4). According to IMS health, a leading

provider of pharmaceutical market intelligence and analytics, recent prescription data for migraine treatment indicates that triptans are the most prescribed active agents followed by NSAIDs. Triptans and NSAIDs given together elicit a synergistic effect by treating both vascular and inflammatory components of pain and result in higher pain relief (5). Oral-fixed dose combinations of sumatriptan and naproxen (Treximet®) have been developed based on this premise (5).

Intramuscular or subcutaneous injectable formulation of triptan is used frequently to treat acute moderate to severe migraine pain and acute cluster headache. The advantage with an injectable formulation is the rapid drug absorption and faster pain relief. The disadvantage of injectable systems is their rapid elimination of drug which can result in the return of moderate to severe pain within 2–24 h of treatment. The recurrence of pain has been reported to be as high as 40% in patients treated with subcutaneous sumatriptan which has an elimination half-life of 115 min (6). Furthermore, patients who experience recurrences are limited to no more than one additional dosing of injectable triptan due to cardiovascular safety concerns (7). This is the key reason for patient dissatisfaction with current treatment regimens; patients prefer treatment which would not only mitigate acute pain component but also provide a sustained pain relief for 2 to 24 h post-initial dosing. For example, in a randomized, open label trial, only 37% of patients who were initially treated with subcutaneous triptan preferred it during the extension phase, whereas 78% of patients preferred to continue oral eletriptan which provided significantly lower recurrence compared to subcutaneous sumatriptan (8).

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A subcutaneously injectable formulation containing fast-acting triptan and NSAID where the triptan component provides rapid relief from acute pain, whilst the NSAID component prevents pain recurrence, is expected to significantly improve treatment outcome without undue safety concern and have better acceptance of patients and physicians. In order to achieve the desired pain relief followed by sustained pain freedom, the formulation should release triptan as an immediate release component, whilst the NSAID should be released slowly, more preferably after the systemic triptan level starts to decline.

Preparation of a parenteral formulation that provides a differential release profile of two active ingredients has not been much investigated. Whilst dosage forms like liposomes and microspheres have been evaluated for sustained release of actives through the parenteral route, they often pose significant development, manufacturing and cost challenges (9,10). Microemulsions are unique in this regard. They are isotropic (and hence transparent) and thermodynamically stable systems containing oil, an aqueous phase and an emulsifier. There are several reviews that describe the pharmaceutical features of microemulsions (11,12), to which the reader is referred. Whilst microemulsions have not been much investigated for a prolonged release application through the parenteral route, intravenously administered emulsions and submicron emulsions have demonstrated prolonged release capability. Koga *et al.* prepared an oil-in-water (o/w) formulation of glycyrrhizin (13) which significantly slowed down the elimination of drug as compared to an aqueous solution when administered by the intravenous route. Constantinides *et al.* developed an o/w submicron emulsion formulation using tocopherol as the oil phase (14). This formulation showed improved efficacy in the form of higher log cell kill values as compared to Taxol®. The improved efficacy was attributed to the preferential uptake of the formulation by tumour cells due to long circulating lipid droplets with globule size smaller than endothelial fenestrae, resulting into an increased cellular/droplet interaction. The prolonged release potential of such formulations is a result of various factors that include the formulation component (15–17), properties of the drug (e.g. lipophilicity, salt form, etc.) (18,19), formulation pH (12) and localization of the drug (20).

The objective of the present work was to develop an o/w microemulsion formulation containing a fast-acting triptan and a NSAID agent. In order to achieve the desired pharmacological effect, it is important that the triptan is released rapidly, whilst the NSAID should be released slowly, such that the two drugs have distinctly separate T_{max} . Such a formulation should (in theory) have the major portion of the triptan in the aqueous phase, whilst the NSAID should be predominantly in the oily phase. Zolmitriptan is a water-soluble fast-acting triptan with lower therapeutic dose as compared to other triptans, whilst diclofenac acid is a commonly used NSAID for treating mild to moderate headache pain and has a substantially lower therapeutic dose as compared to other commonly used NSAIDs such as ibuprofen or naproxen. The acid form of the diclofenac has a high log P (4.75) (21), indicating high lipophilicity. Therefore, an o/w microemulsion was used as the delivery vehicle. The selected human dose of zolmitriptan and diclofenac acid were 1 and 25 mg, respectively, per injection

(i.e. 2 g of formulation). These doses were calculated based on the oral dose and bioavailability of approved formulations.

MATERIALS AND METHOD

Materials

Following materials were purchased: diclofenac acid and soya bean oil (Santa Cruz Biotechnology, Inc., CA, USA), Tween® 80 (Fischer Scientific, NJ, USA) and Brij® 97 (Sigma Labs, CA, USA). The following materials were received as gift samples: zolmitriptan (Dr. Reddy's Laboratories Ltd., Hyderabad, India), Capryol™ 90 and Labrafac™ Lipophile (Gattefosse SAS, France). Deionized water was used throughout, and all other chemicals were of analytical grade.

Method

Solubility Study

The solubility of zolmitriptan and diclofenac acid was determined separately in soya bean oil, deionized water and Labrafac Lipophile. The weighed quantity of drug and solvent was mixed, and the mixture was sonicated for 30 min to break agglomerates and disperse the powder in solvent. The samples were then warmed at 50°C for 30 min. The mixtures were allowed to stand at room temperature for up to 72 h. Each sample was prepared in triplicates. Samples were analysed at 24 and 96 h to determine drug solubility using ultraviolet (UV) spectroscopy (Perkin Elmer UV/VIS Spectrophotometer, Lambda 2). The method comprised centrifuging solubility samples and diluting a weighed quantity of clear supernatant with methanol. The diluted sample was measured for absorbance at 285 and 281 nm λ_{max} for zolmitriptan and diclofenac acid, respectively.

Construction of Pseudo-Ternary Phase Diagrams

A pseudo-ternary phase diagram was plotted using water-Labrafac Lipophile-Tween 80-Capryol 90 to identify a region of monophasic fluid microemulsion. Microemulsions were prepared using various proportions of ingredients. Briefly, weighed quantities of ingredients in a sample bottle were subjected to magnetic stirring along with heating at 75±5°C for 10 min, followed by magnetic stirring at room temperature for 10 min. The formulation was set aside for 24 h and was later observed for clarity and flow. Formulations that were clear and fluid were considered as microemulsion formulations. Based on the results, phase diagrams were plotted using SigmaPlot (trial version 12.3) software.

Preparation of Drug-Loaded Microemulsion

Microemulsions containing diclofenac acid and zolmitriptan were prepared using water-Labrafac Lipophile-Tween 80-Capryol 90 system using the compositions as indicated in Table I. Drug loading was carried out by dissolving zolmitriptan in the aqueous phase and diclofenac acid in the mixture of Labrafac Lipophile, Capryol 90 and Tween 80. A microemulsion was then prepared using the method described above.

Table I. Composition of Various Drug-Loaded Microemulsions and Results of Physical Observation

Ingredients	Formulations		
	A	B	C
Labrafac Lipophile (% w/w)	3	3	3
Surfactant mixture ^a (% w/w)	20	25	30
Deionized water q.s. (% w/w)	100	100	100
Diclofenac acid ^b (% w/w)	1.25	1.25	1.25
Zolmitriptan ^b (% w/w)	0.05	0.05	0.05
Appearance of microemulsion			
Clarity	Hazy	Clear	Turbid
Flow	Liquid	Liquid	Viscous

^a A mixture of Tween 80 (five parts) and Capryol 90 (one part)

^b The final dose of diclofenac and zolmitriptan was 25 and 1 mg, respectively, per 2 g of formulation

Globule Size Determination

Globule size was determined by the dynamic light scattering method using a Zetasizer device (Malvern, nanos, ZEN3600). A weighed quantity of microemulsion was diluted 20-fold with deionized water which was previously filtered with a 0.2- μ polyvinylidene fluoride syringe filter. The diluted sample was transferred into a disposable sizing cuvette placed in the sample holder, and the sizes of dispersed globules were expressed as their hydrodynamic diameter.

Transmission Electron Microscopy (TEM)

The morphology of dispersed globules in microemulsion was investigated by conducting TEM on one representative sample. One drop of the formulation was placed on a copper grid. Excess liquid was removed using a filter paper, and a small quantity of a 2% aqueous solution of phosphotungstic acid (PTA, pH 7.4) was placed on a formulation sample on the grid and left for 30–60 s to allow staining. The excess was removed with a filter paper. The grid was then examined under the transmission electron microscope at 200 kV accelerating voltage using a FEI Tecnai 20 transmission electron microscope.

In Vitro Release (IVR) Studies

IVR studies were conducted for zolmitriptan and diclofenac acid separately using release media comprising *n*-saline phosphate buffer (pH 7.4) for zolmitriptan and a mixture of ethanol/water (40:60) for diclofenac acid. The media were selected based on the solubility of each drug in the respective solvents to ensure that the selected media provides a sink condition. The IVR study for each batch of formulation (2 g) was run using dialysis tubes (Mega GeBAflex-tube, Gene Bio-Application Ltd., molecular weight cut-off of 3.5 kDa). The formulation sample bottles were rinsed thrice using 1 mL of deionized water, and the rinsing was added to the tube. A small magnetic bar was placed inside the tube, and the tube was placed in a glass container containing release media (prewarmed to 37 $\pm$ 2°C) and a magnetic bar. The whole assembly was placed in a magnetic stirrer-cum-hot plate to maintain the temperature of release media at 37 $\pm$ 2°C and slow stirring speed (1,000 rpm). Four millilitres of media was

withdrawn at selected time points, and the withdrawn volume was replaced with an equal amount of fresh media. The quantity of drug in each sample was determined using the UV method after appropriate dilution with methanol. Based on the drug quantity in the sample, dilution factor and total volume of release media, percentage drug release was calculated.

Preclinical Pharmacokinetic Studies

A subcutaneous pharmacokinetic study was conducted with one formulation in male Wistar rats. Three rats (mean weight $\pm$ S.D. = 210 $\pm$ 2 g) were administered 1 mL (equivalent to 0.5 mg zolmitriptan, 12.5 mg diclofenac) of formulation by the subcutaneous route in the lateral abdominal region. Blood samples were collected from each animal by retro-orbital plexus puncture under isoflurane anaesthesia at time points 0 min (pre-dose); 5, 10, 15, 30 and 60 min; and 2, 4, 8, 24, 48 and 72 h. Blood samples were placed on wet ice until centrifugation at 10,000 rpm (4°C) for 5 min. Plasma was transferred into labelled Eppendorf tubes and stored at –20°C until analysis using a validated high-performance liquid chromatography with mass spectrometric (HPLC-MS) method.

At the time of analysis, plasma was extracted with 1 mL ethyl acetate and vortexed for 5 min. The sample was centrifuged at 13,000 rpm (4°C) for 5 min, the supernatant was transferred to RIA vials, and the ethyl acetate was evaporated to dryness using a TurboVap LV Evaporator (Zymark, Hopkinton, MA, USA) at 40°C under a stream of nitrogen. The extracts were reconstituted in 100 μ L acetonitrile, and 5 μ L sample/calibration standards were separated by HPLC (Phenomenex Luna C18 device; using a 2.5- μ m, 3 $\times$ 100-mm column; 5 μ L injection volume; 0.250 mL/min flow rate; and 0.08-min run time, at a temperature of 40°C) followed by MS analysis (Thermo Scientific™ TSQ Quantum Access MAX triple stage quadrupole mass spectrometer; run time of 8 min.). The lower limit of quantification (LLOQ) for both drugs was 5 ng/mL. Animal experiments were conducted in full compliance with local, national, ethical and regulatory principles and local licensing regulations, per the spirit of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International's expectations for animal care and use/ethics committees.

RESULTS AND DISCUSSION

Labrafac Lipophile (also known as caprylic/capric triglyceride) is a commonly used medium-chain triglyceride and is a good solvent for lipophilic compounds. Soya bean oil is another vehicle containing medium-chain triglyceride which has been used in parenteral formulations such as total parenteral nutrition. Figure 1 shows the solubility of diclofenac and zolmitriptan in deionized water, soya bean oil, and Labrafac Lipophile. Zolmitriptan has similar solubility in water and soya bean oil, but relatively lower solubility in Labrafac Lipophile. Diclofenac acid has higher solubility in Labrafac Lipophile compared to soya bean oil, whilst its solubility is lowest in water. Labrafac Lipophile was selected as the oil phase based on this result, as the higher oil solubility of diclofenac would result into its slower release in

Fig. 1. Equilibrium solubility of zolmitriptan (a) and diclofenac acid (b) in various solvents at 24 and 96 h time points. *Zolmi* zolmitriptan, *Diclo* diclofenac acid, *soya* soya bean oil, *labra* Labrafac Lipophile. Data are presented as the mean ($\pm$ SD) of three experiments

aqueous media, whilst more hydrophilic zolmitriptan was expected to release faster.

The ratio of the surfactant (Tween 80) and cosurfactant (Capryol 90) was decided based on a separate study, which is not reported here, where mixtures containing different levels of the two ingredients were evaluated to formulate microemulsions. The mixture at 5:1 ratio (Tween 80/Capryol 90) was found to be the most optimal. Having selected the oil phase and the surfactant, the cosurfactant ratio, the region of microemulsion formation was determined by constructing a phase diagram using the Labrafac Lipophile, water and surfactant mixture for formulations prepared at different levels of surfactant, oil and aqueous phase. Clear and fluid microemulsions were obtained in the region as shown in Fig. 2. Our main interest was the system with the highest proportion of oil that would enable incorporation of the target dose (25 mg) of diclofenac acid, whilst it would still be sufficiently fluid and have adequate stability against phase

separation. Based on the phase diagram, we selected three systems for drug loading (Table I). Replicates of all the systems were formulated to check reproducibility, and all the compositions consistently produced microemulsions. Subsequently, drug loading was conducted with the three compositions, and the compositions of these systems are shown in Table I. Clear, fluid drug-loaded microemulsions were obtained only with the composition comprising 25% surfactant mixture and 3% oil, with water making up the remaining quantity. Drug-loaded microemulsions with 4% oil were clear but viscous and, hence, were not used for further studies. The drug-loaded microemulsion formulation with 3% oil was checked for reproducibility and was found to consistently form clear, fluid microemulsions. The average (of triplicate) globule size (based on volume) of the formulation was found to be 12.0 ± 3.3 nm, and the mean polydispersity index (PDI) was 0.123 ± 0.08 . As shown in Fig. 3, size distribution plots of all the samples had only one peak indicating monomodal

Fig. 2. Pseudo-ternary phase diagram. X-axis represents surfactant mixture, Y-axis represents Labrafac Lipophile, whilst Z-axis represents deionized water level

Fig. 3. Globule size distribution of three replicates of 25%:3%:72% surfactant/oil/water microemulsion formulation containing diclofenac acid and zolmitriptan

globule size distribution. Low PDI indicated uniform globules with a narrow size distribution. TEM pictures (Fig. 4) confirmed that the microemulsion had spherical, uniform globules with a similar size range as that determined by Zetasizer measurement. This formulation was used for subsequent studies *in vitro* and *in vivo*.

Release of diclofenac acid and zolmitriptan from the drug loaded 25%:3%:72% surfactant/oil/water microemulsion *in vitro* is shown in Fig. 5. The release of zolmitriptan was significantly faster with 25% of total drug releasing in 1st hour. By comparison, diclofenac release was relatively slow. As zolmitriptan is predominantly present in the external

aqueous phase, the drug should have been released faster than observed; the slower release may be due to a proportion of the drug partitioning into the oil and surfactant phase, which then would release slowly. This slower release of water-soluble drug in the external phase was also observed by Koga *et al.* (13) who reported that the inner oily phase slows down the release of glycyrrhizin, a water-soluble moiety incorporated in o/w emulsion. On the other hand, diclofenac showed significantly slower release *in vitro* as this highly lipophilic drug remains located within the oily phase and undergoes slow partitioning into the aqueous phase. Physical separation of media and the formulation by dialyzing membrane may be

Fig. 4. Representative transmission electron microscopy image of 25%:3%:72% surfactant/oil/water microemulsion formulation containing diclofenac acid and zolmitriptan. The black spheres represent dispersed globules

Fig. 5. *In vitro* release profile of zolmitriptan and diclofenac acid from 25%:3%:72% surfactant/oil/water microemulsion formulation. Data are presented as the mean ($\pm$ SD) of three experiments

an additional factor which retards the release of both the drugs. It has been reported earlier (22) that dialysis tube-based release often results into slower release profile vis-à-vis *in vivo* release. As our main intent was to study the relative release rates of two of the drugs from the formulation to ensure that they would be released separately, this release profile confirmed their differential release rate and prompted further investigation *in vivo*.

The plasma profile of zolmitriptan and diclofenac after subcutaneous administration in a 25%:3%:72% surfactant/oil/water microemulsion is shown in Fig. 6. The T_{max} for zolmitriptan and diclofenac acid was found to be 0.08–0.5 and ~4 h, with a corresponding C_{max} of 68 ± 64 ng/mL and 4264 ± 954 μ g/mL, respectively. By the time diclofenac reached its C_{max} (at 4 h), zolmitriptan level had declined to about 1/6th of its C_{max} . The release of diclofenac was more sustained and continued up to 24 h post-dose. The area under the curve from 0 to 24 h ($AUC_{(0-24)}$) for zolmitriptan and diclofenac acid, calculated using the trapezoid rule, was found to be 0.12 ± 0.02 and 41.6 ± 6.7 μ g/h/mL, respectively. Whilst this proof-of-concept study was not intended to calculate the relative bioavailability from the 25%:3%:72% surfactant/oil/water microemulsion, comparison with published AUC values in the rat for zolmitriptan (22) and diclofenac (23) indicates approximately 40–50% bioavailability from this formulation. *In vivo* release was faster, and for diclofenac, it was more complete, as compared to *in vitro* release. This may be

due to the method used in our case, as the dialysis membrane method used results in slower release due to the physical separation of formulation and release media (24) which is not the case during the *in vivo* release when the formulation is diluted by the interstitial fluids. Unlike the formulations like microspheres that provide a physical barrier against rapid drug diffusion, microemulsion would also be more prone to the degradation of oil globules by enzymatic lipolysis in subcutaneous adipose tissue (25), resulting in faster and more complete *in vivo* drug release compared to *in vitro* release. In our case, the more complete release of diclofenac from the formulation resulted in a higher than expected exposure, with adverse events (diarrhoea, darkened stools) at 24 h post-dose that were consistent with the known gastrointestinal irritant properties of this drug (26). Some local effects (skin discoloration) at the injection site were also apparent at 24 h.

CONCLUSION

Our main aim was to evaluate the feasibility of developing a dual-acting microemulsion-based formulation that provides a differential release of two drugs in a manner that potentially provides fast onset of action followed by sustained drug release. There are several pain conditions where such formulations may provide improvements in patient outcome, and the approval of formulations like DepoDur®, and Exparel® that provide pain relief for 2–3 days indicates a significant demand. However, lack of viable technology often proves to be a major obstacle. Microemulsion, being simple to manufacture, filter sterilizable, stable and containing an aqueous as well as an oil domain, provides a significant opportunity to design such formulations and to refine their properties to match particular drug pairs. The results in the current study indicate that a subcutaneous microemulsion can be designed to provide differential release of zolmitriptan and diclofenac acid. The formulation is simple to manufacture and has particle size which makes it to have filter-sterilizable features which resolve some of the key challenges in manufacturing parenterally modified release formulations. Such a formulation may be appropriate in the treatment of diseases such as migraine, where the immediate onset therapeutic action of one drug needs to be followed by the sustained release of another.

Fig. 6. Plasma concentration-time curve for zolmitriptan (a) and diclofenac acid (b) after subcutaneous administration of the 25%:3%:72% surfactant/oil/water microemulsion formulation in Wistar rats. Data are presented as the mean ($\pm$ SD) of three animals

REFERENCES

- Purdue Pharma L.P. General pain: fact sheet. 2013. http://www.inthefaceofpain.com/content/uploads/2011/12/factsheet_Pain.pdf Accessed 10 Sep 2013.
- Goadsby PJ. Pathophysiology of migraine. *Ann Indian Acad Neurol.* 2012;15 Suppl 1:S15–22.
- Dalkara T, Nozari A, Moskowitz MA. Migraine aura pathophysiology: the role of blood vessels and microembolisation. *Lancet Neurol.* 2010;9:309–17.
- Evers S, Afra J, Frese A, *et al.* EFNS guideline on the drug treatment of migraine—revised report of an EFNS task force. *Eur J Neurol.* 2009;16(Eversa S):968–81.
- Smith TR, Sunshine A, Stark SR, *et al.* Sumatriptan and naproxen sodium for the acute treatment of migraine. *Headache.* 2005;45:983–91.
- Gawel MJ, Worthington I, Maggisano A. A systematic review of the use of triptans in acute migraine. *Can J Neurol Sci.* 2001;28:30–41.
- Gillian C. Triptans in migraine: the risks of stroke, cardiovascular disease, and death in practice. *Neurology.* 2004;62:563–8.
- Schoenen J, Pascual J, Rasmussen S, *et al.* Patient preference for eletriptan 80 mg versus subcutaneous sumatriptan 6 mg: results of a crossover study in patients who have recently used subcutaneous sumatriptan. *Eur J Neurol.* 2005;12:108–17.
- Kikuchi H, Carlsson A, Yachi K, *et al.* Possibility of heat sterilization of liposomes. *Chem Pharm Bull.* 1991;39:1018–22.
- Burgess DJ, Hussain AS, Ingallinera TS, *et al.* Assuring quality and performance of sustained and controlled release parenterals: workshop report. *AAPS Pharm Sci.* 2002;4:13–23.
- Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliv Rev.* 2000;45:89–121.
- Karasulu HY. Microemulsions as novel drug carriers: the formation, stability, applications and toxicity. *Expert Opin Drug Deliv.* 2008;5:119–35.
- Koga K, Nishimon Y, Ueta H, *et al.* Utility of nano-sized, water-in-oil emulsion as a sustained release formulation of glycyrrhizin. *Biol Pharm Bull.* 2011;34:300–5.
- Constantinides PP, Lambert KJ, Tustian AK, *et al.* Formulation development and antitumor activity of a filter-sterilizable emulsion of paclitaxel. *Pharm Res.* 2000;17:175–82.
- Borhade VB, Nair HA, Hegde DD. Development and characterization of self-microemulsifying drug delivery system of tacrolimus for intravenous administration. *Drug Dev Ind Pharm.* 2009;35:619–30.
- Djordjevic L, Primorac M, Stupar M, *et al.* Characterization of caprylocaproyl macroglycerides based microemulsion drug delivery vehicles for an amphiphilic drug. *Int J Pharm.* 2004;271:11–9.
- Djordjevic L, Primorac M, Stupar M. In vitro release of diclofenac diethylamine from caprylocaproyl macroglycerides based microemulsions. *Int J Pharm.* 2005;296:73–9.
- Ke WT, Lin SY, Ho H-O, *et al.* Physical characterization of microemulsion systems using tocopheryl polyethylene glycol 1000 succinate (TPGS) as a surfactant for the oral delivery of protein drugs. *J Control Release.* 2005;102:489–507.
- Washington C, Evans K. Release rate measurements of model hydrophobic solutes from submicron triglyceride emulsion. *J Control Release.* 1995;33:383–90.
- Prince LM. Emulsion and emulsion technology, part I. New York: Marcel Dekker; 1974.
- Fini A, Fazio G, Rapaport I. Diclofenac/*N*-(2-hydroxyethyl)pyrrolidine: a new salt for an old drug. *Drugs Exp Clin Res.* 1993;XIX:81–8.
- Liu J, Zhou X. Determination of zolmitriptan and its primary metabolite, *n*-desmethy-zolmitriptan, in rat plasma by LC-MS-MS. *J Chromatogr Sci.* 2013;51:59–64.
- Peris-Ribera JE, Torres-Molina F, Garcia-Carbonell MC, Aristorena JC, Pla-Delfina JM. Pharmacokinetics and bioavailability of diclofenac in the rat. *J Pharmacokinet Biopharm.* 1991;19:647–65.
- D'Souza SS, DeLuca PP. Methods to assess in vitro drug release from injectable polymeric particulate systems. *Pharm Res.* 2006;23:460–74.
- Etherton TD, Bauman DE, Romans JR. Lipolysis in subcutaneous and perirenal adipose tissue from sheep and dairy steers. *J Anim Sci.* 1977;44:1100–6.
- Wallace JL, Reuter B, Cicala C, McKnight W, Grisham M, Cirino G. A diclofenac derivative without ulcerogenic properties. *Eur J Pharmacol.* 1994;257:249–55.