International Journal of Pharmaceutics 557 (2019) 154–161

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Impact on intestinal permeability of pediatric hyperosmolar formulations after dilution: Studies with rat perfusion method

J.M. delMoral-Sanchez[a,b], A. Ruiz-Picazo[a,b], M. Gonzalez-Alvarez[b], A. Navarro[c], I. Gonzalez-Alvarez[b,*], M. Bermejo[b]

[a] Institute of Molecular and Cellular Biology of Miguel Hernandez University, Avda de la Universidad s/n, 03202 Elche (Alicante), Spain
[b] Department of Pharmacokinetics and Pharmaceutical Technology, Miguel Hernandez University, San Juan de Alicante, 03550 Alicante, Spain
[c] Pharmacy Service, General University Hospital of Elche, 03202 Elche (Alicante), Spain

ARTICLE INFO

Keywords:
Oral administration
Pediatrics
Bioavailability
Necrotizing enterocolitis
Security
Osmolarity
Intestinal permeability
Intestinal perfusion

ABSTRACT

Introduction: There is no consensus on administering hyperosmolar formulations by mouth to neonates. In 1976, the Committee on Nutrition of the American Academy of Pediatrics published a recommendation of not administer formulations with an osmolarity higher than 400 mOsm/L due to the possible damage to intestine and relationship with necrotizing enterocolitis. Since this recommendation, exists a general trend of reducing osmolality of oral formulations without considering the pharmacokinetics of absorption of the drugs.

The objective of this study was to characterize the permeability values of drugs formulated at different osmolalities by using a well-established rat intestinal perfusion model and to measure the osmolality of the most used formulations in our neonatology unit.

Methods: For the osmolality measurement study, most common used oral drugs were selected (compounded formulations and drug products). Osmolality of three dilutions (1:1, 1:4 and 1:8) were measured using a cryoscopic descent osmometer.

Atenolol, caffeine, furosemide, hydrocortisone and paracetamol were selected for the permeability study. Three suspensions were elaborated of each drug (150 mOsm/kg, 300 mOsm/kg and 1500 mOsm/kg). Permeability values and absorption rate coefficients were determined in complete small intestine using *in situ* "closed loop" perfusion method.

Results: The formulations that resulted to be hyperosmolar (> 400 mOsm/kg) were 86% (70% of these proved to be above 1500 mOsm/kg).

The permeability study shown that the osmolality is inversely proportional to the apparent permeability of the drug in the studied drugs. The permeability values obtained with hyperosmolar samples were lower compared to 150 mOsm/kg or 300 mOsm/kg.

Conclusions: Osmolality parameter is of particular relevance in oral drug administration in neonate because the risk of damaging the gastrointestinal tract and because of the risk that modifying osmolality also modifies its permeability, resulting in a potential change in bioavailability.

1. Introduction

Pediatric patients need liquid medication for oral administration, which leads the pharmacy services of hospitals to compound many drugs as syrups when there is no availability of a marketed drug product for kids. The osmolality of these formulations is not usually measured, nor their possible effect on absorption and consequently in oral bioavailability. The clinical need of administering the prescribed dose takes over the uncertainty that the reformulation process can modify oral absorption. Consequently, this remains as an unanswered question with potential clinical consequences.

The general trend of reducing osmolality in orally administered formulations in neonates started in 1976 when the Committee on Nutrition of the American Academy of Pediatrics published its recommendations document (American Academy of Pediatrics, 1976). The main recommendation was not to administer formulations with an

Abbreviations: API, active principle ingredients; NEC, necrotizing enterocolitis; WFI, water for injection

* Corresponding author at: Pharmacokinetics and Pharmaceutical Technology Area, Carretera Nacional 332 Alicante-Valencia, Campus de San Juan Edificio 2, 03550 San Juan de Alicante, Alicante, Spain.
E-mail address: isabel.gonzalez@goumh.umh.es (I. Gonzalez-Alvarez).

https://doi.org/10.1016/j.ijpharm.2018.12.047
Received 26 October 2018; Received in revised form 16 December 2018; Accepted 20 December 2018
Available online 27 December 2018
0378-5173/

osmolality higher than 400 mOsm/L due to its potential relationship with necrotizing enterocolitis (NEC) (American Academy of Pediatrics, 1976; Sántulli et al., 1975). NEC is a highly lethal clinical condition with non-established pathogenesis in the neonate, which can lead to intestinal perforation due to ischemic necrosis of the gastrointestinal tract. In developed countries, the incidence of NEC is approximately 5–12% for very low birth weight infants (Shulhan et al., 2017). Although prematurity is the major risk factor (Shulhan et al., 2017; Pearson et al., 2013; Chauhan et al., 2008), oral administration of hyperosmolar formulations has been associated with intestinal mucosa injury and possible development of NEC. The determination of osmolality is not easy in clinical practice for reasons of resources or time. Consequences of using formulations with osmolalities above 400 mOsm/L are unclear (Steele et al., 2013). Pearson et al. (2013) explored the published studies of physiological responses in animal models to hyperosmolar solutions. All studies conclude that there is no relationship between osmolality of formula and NEC but they were carried out in gastrointestinal tract of mature animals. In 2016 Miyake et al. (2016) assessed the effects of osmolarity in an experimental model of NEC in mice pups and their results indicates that hyperosmolarity does not affect incidence of NEC.

However, given that the risk of NEC is multifactorial and that there is controversy about the causal relationship, many studies have emerged to elucidate this question (Shulhan et al., 2017; Samuels et al., 2017). Currently, the risk has been related to the intestinal microbiome (Shulhan et al., 2017; Samuels et al., 2017; Neu and Walker, 2011). Inefficient absorption and digestion allow the growth of microbiota and this, combined with immunological immaturity, can increase intestinal permeability and facilitate the initial inflammatory process (Shulhan et al., 2017).

To date, the assumption that hyperosmolarity and NEC are associated has concluded in the administration of diluted drug formulations by some practitioners, with the potential implications in safety and absorption pharmacokinetics that this encompasses. The fluid volume in the gastrointestinal tract is influenced by osmolality, which can modify intestinal concentration and drug absorption (Dahlgren et al., 2018), especially for low-permeability drugs (Ichijo et al., 2017). Recently, Tanaka et al. (2015) described this relationship between bioavailability after oral administration and the osmolality of the formulation administered. They reported a significant increase in absorption of drugs formulated in hyposmotic vehicles compared to isosmotic administration.

The general recommendation of diluting the oral liquid formulations to reduce osmolality and reduce aggression to the intestinal mucosa as much as possible requires the knowledge of the effects of this practice on the drug oral absorption. The objective of the present study was to measure the osmolality of the most used formulations in our neonatology unit and to characterize the permeability values of drugs formulated at different osmolalities by using a well-established rat intestinal perfusion model. The secondary objective would be establishing a relationship between osmolality and drug permeability if possible in order to predict the effects over other drugs.

2. Materials and Methods

2.1. Formulation osmolality measurement study

Most common used oral drugs at our reference neonatology unit were included in the formulation osmolality measurement study, whether they were compounded formulation prepared at Hospital Pharmacy Service or marketed drug formulations.

Osmolality was determined by freezing point depression using a cryoscopic descent osmometer (Osmomat® 030, Gonotec GmbH). Three dilutions were prepared for each drug product (1:1, 1:4 and 1:8) with water for injection (WFI) (0.0 Osm/L). The samples were diluted with WFI for two reasons: (American Academy of Pediatrics, 1976) validating the theoretical calculation of osmolality by dilution as explained below and (Sántulli et al., 1975) put then within the osmometer range of detection. Samples were measured in duplicate and the mean osmolality was calculated. The upper limit of measurement of the device is 2000 mOsm/kg, so osmolalities above this limit were calculated by the results of 1:4 and 1:8 dilutions using the following equation:

$$Osm_F = [(Osm_D \cdot V_D) - (Osm_{WFI} \cdot V_{WFI})]/V_F \quad (1)$$

where Osm_F is osmolality of the formulation, Osm_D is osmolality of the dilution, Osm_{WFI} is osmolality of WFI (0.0 mOsm/kg), V_D is volume of dilution, V_{WFI} is volume of WFI added for obtain the dilution, and V_F is the volume of formulation ($V_F + V_{WFI} = V_D$).

2.2. Intestinal permeability studies

Atenolol, caffeine, furosemide, hydrocortisone and paracetamol were selected for the absorption experiments because the variability in the osmolality of their formulations and because of their common use in our reference unit.

It was necessary to elaborate suspensions of each selected drug at different osmolalities for de subsequent absorption study. All drugs were formulated into suspensions at three values of osmolality, namely 150 mOsm/kg (hyposmolar), 300 mOsm/kg (isosmolar) and 1500 mOsm/kg (hyperosmolar).

Atenolol 0.5 mg/mL, caffeine 2 mg/mL, furosemide 0.1 mg/mL, hydrocortisone 1 mg/mL and paracetamol 10 mg/mL were prepared by dissolving active principle ingredients (API) in water for injection (WFI). The osmolality of each formulation was reached by adjusting the final volume with simple syrup (2780 mOsm/kg) without varying the final concentration of drug.

All drugs were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Methanol, acetonitrile and water were HPLC grade. All other chemicals were of analytical reagent grade.

Absorption rate coefficients and permeability values of the studied drugs were determined in complete small intestine (n = 6–7) using *in situ* "closed loop" perfusion method based on Doluisio Technique (Doluisio et al., 1969) which was approved by the Scientific Committee of the Faculty of Pharmacy, Miguel Hernandez University, and followed ARRIVE guidelines, the guidelines described in the EU Directive 2010/63/EU, the U.K. Animals (Scientific Procedures) Act (1986), the Council of the Europe Convention ETS 123 and Spanish national laws governing the use of animals in research. Four male Wistar rats for each drug and each osmolality formulation were tested. Rats were anesthetized using a mixture of pentobarbital (40 mg/kg) and butorphanol (0.5 mg/kg). Isolated segments in small intestine (≈100 cm) were obtained and the *in situ* rat intestinal closed loop experiment was executed as described previously (Doluisio et al., 1969; Lozoya-Agullo et al., 2015a). The formulation (at different predefined osmolalities) was introduced inside the compartment and samples were collected every 5 min up to a period of 30 min.

In order to separate solid components from the samples, they were centrifuged 5 min at 5000 r.p.m. and the supernatant was analyzed by High Performance Liquid Chromatography (HPLC) as described in Table 1.

At the end of the experiments there is a reduction in the volume of the perfused solutions due to water reabsorption. Consequently, a correction is necessary to calculate the absorption rate constants accurately. Water reabsorption was characterized as an apparent zero order process. A method based on direct measurement of the remaining volume of the test solution was employed to calculate the water reabsorption zero order constant (k_o). The volume at the beginning of the experiment (V_0) is composed of the volume of the drug solution (10 mL for complete small intestine) plus the residual volume generated by flushing the intestinal segment. This residual volume was previously determined to be, on average, 0.3–0.5 mL. The volume at the end of the experiment (V_{end}) was measured for each animal by carefully extracting

Table 1
Validated procedures for HPLC analysis. Note: MeCN: acetonitrile, MeOH: methanol, UV: drug analyzed with an ultraviolet detector. [a]Water (pH 3) with 1% trifluoroacetic acid.

Drug	Detection	Mobile phase (aqueous:organic)	λ (nm)	Retention time (min)
Atenolol	UV	Water pH 3: MeCN (65:35)[a]	235	2.3
Caffeine	UV	Water pH 3: MeOH (65:35)[a]	273	3.6
Furosemide	UV	25 mM Na_2HPO_4 pH 3: MeCN (45:55)	254	2.5
Hydrocortisone	UV	Water pH 3: MeCN (60:40)[a]	245	3.0
Paracetamol	UV	Water pH 3: MeCN (70:30)[a]	254	4.0

Table 2
1:1, 1:4 and 1:8 osmolality measurements of drug products and compounded formulations in (mOsm/kg). Assigned osmolalities in italics are those that were calculated because they exceeded the upper limit of the osmometer. Underlined osmolality measurements are those that were calculated since the viscosity of the formulation was too high to be analyzed by the osmometer.

Drug	Compounded formulation (yes/no)	Formulation	Osmolality 1:1 dilution	Osmolality 1:4 dilution	Osmolality 1:8 dilution
Acetazolamide	Y	Acetazolamide 25 mg/mL oral suspension	*3516*	879	442
Acetylsalicylate (lysine)	N	Inyesprin 900 mg vial	1437	363	179
Acyclovir	N	Zovirax 80 mg/mL oral solution	*2788*	697	352
Amiodarone	Y	Amiodarone 5 mg/mL oral suspension	1768	439	222
Amoxicillin	N	Clamoxyl 250 mg oral packet	108	30	18
Amoxicillin/clavulanic acid	N	Augmentine 100/12.5mg/mL oral suspension	229	53	28
Bicarbonate (sodium)	N	Sodium bicarbonate 1 M amp	1555	434	225
Caffeine	Y	Caffeine citrate oral solution	201	51	24
Captopril	Y	Captopril 1 mg/mL oral suspension	63	15	9
Carboxymethylcellulose	Y	Carboxymethylcellulose 1% oral excipient	700	175	89
Chloral hydrate	Y	Chloral hydrate 50 mg/mL oral suspension	*2156*	539	273
Chloral hydrate	Y	Chloral hydrate 200 mg/mL oral suspension	*3524*	881	444
Dexamethasone	Y	Dexamethasone 1 mg/mL oral suspension	*2420*	605	272
Digoxin	N	Lanacordin 0.05 mg/mL oral solution	*3952*	988	479
Domperidone	N	Motilium 1 mg/mL oral suspension	*1964*	491	248
Enalapril	Y	Enalapril 1 mg/mL oral suspension	1927	483	244
Erythromycin	N	Pantomicina 100 mg/mL oral suspension	*2152*	538	260
Esomeprazole	N	Nexium 10 mg oral packet	1104	276	144
Ferroglycin sulfate	N	Glutaferro 170 mg/mL oral solution	*2960*	740	
Ferrous sulfate	N	Fer-in-sol 25 mg/mL oral solution	*4128*	1032	519
Flecainide	Y	Flecainide 5 mg/mL oral suspension	*2444*	611	303
Fluconazole	N	Diflucan 50 mg/5mL oral suspension	*2992*	748	371
Folic acid	Y	Folic acid 0.05 mg/mL oral suspension	60	15	7
Folinate (calcium)	Y	Folinate (calcium) 5 mg/mL oral suspension	*2176*	544	269
Furosemide	Y	Furosemide 2 mg/mL oral suspension	1791	448	219
Hydralazine	Y	Hydralazine 2 mg/mL oral suspension	1728	417	213
Hydrochlorothiazide	Y	Hydrochlorothiazide 2 mg/mL oral suspension	*1864*	466	239
Hydrocortisone	Y	Hydrocortisone 1 mg/mL oral suspension	1236	312	159
Levetiracetam	N	Keppra 100 mg/mL oral solution	*4004*	1001	462
Levofloxacin	Y	Levofloxacin 50 mg/mL oral suspension	*2544*	636	287
Midazolam	Y	Midazolam 2.5 mg/mL oral solution	77	17	9
Morphine	Y	Morphine 0.4mg/mL oral solution	285	71	38
Multivitamin complex	N	Supradyn protovit oral solution	*8432*	*2108*	1054
Nifedipine	Y	Nifedipine 1 mg/mL oral suspension	*2400*	600	302
Omeprazole	Y	Omeprazole 2 mg/mL oral suspension	1589	429	222
Paracetamol	N	Paracetamol 100 mg/mL oral solution	*8232*	*2058*	1029
Phenobarbital	Y	Phenobarbital 10 mg/mL oral suspension	*2232*	558	242
Phenytoin	N	Epanutin 30 mg/5mL oral suspension	*1888*	472	239
Phosphates	Y	Joulie's solution 1 mmol/mL oral solution	1578	396	202
Pirimetamine	Y	Pirimetamine 2 mg/mL oral suspension	*3036*	759	374
Potassium glucoheptonate	N	Potasion 264 mg/mL oral solution	*2012*	503	248
Prednisolone	N	Estilsona 7 mg/mL oral solution	693	171	86
Propranolol	Y	Propranolol 1 mg/mL oral suspension	*2248*	562	276
Ranitidine	Y	Ranitidine 15 mg/mL oral suspension	1411	352	174
Simple syrup	Y	Simple syrup oral excipient	*2872*	718	374
Spironolactone	Y	Spironolactone 10 mg/mL oral suspension	1717	428	208
Ursodeoxycolic acid	Y	Ursodeoxycolic acid 15 mg/mL oral suspension	*2828*	707	351
Valganciclovir	N	Valcyte 50 mg/mL oral solution	609	143	79
Valproic acid	N	Depakine 200 mg/mL oral solution	*2836*	709	351
Zidovudine	N	Retrovir 10 mg/mL oral solution	*3020*	755	358

and squeezing the intestinal segment. An individual value of k_o was estimated for each animal as:

$$k_o = (V_0 - V_{end})/t_{end} \quad (2)$$

where V_{end} is the measured volume at the end of the experiment (t_{end} = 30 min) in each animal. k_o value was used to estimate the remaining water volume in the different segments at each time point (V_t). Finally, the experimental analyzed drug concentrations (C_e) were corrected at each time point to obtain the actual C_t by the following equation:

$$C_t = C_e \cdot (V_t/V_0) \quad (3)$$

where C_t represents the drug gut concentration in the absence of any water reabsorption at time *t*, and C_e represents the actual experimental value. The C_t values (corrected concentrations) were used to calculate the actual absorption rate coefficients (Tuğcu-Demiröz et al., 2014).

The absorption rate coefficient (k_a) was determined by non-linear regression analysis of the remaining concentrations in lumen (C_t) versus time.

$$Ct = C_0 \cdot e^{-ka \cdot t} \quad (4)$$

This k_a value was then transformed into permeability value with the following relationship:

$$P_{aap} = k_a \cdot (R/2) \quad (5)$$

where P_{app} is permeability value and *R* is the effective radius of the intestinal segment. *R* was calculated considering the intestinal segment as a cylinder with the relationship:

$$Volume = \pi \cdot R^2 \cdot L \quad (6)$$

Estimation was done using a 10 mL perfusion volume for complete small intestine. The intestinal length (*L*) was 100 cm for complete small intestine.

The statistical analyses were done with the statistical package SPSS (SPSS version 22 (IBM United States) licensed to Universidad Miguel Hernandez). Permeability values were compared using ANOVA to detect the existence of significant differences at the 0.05 probability level. The Levene's statistic was calculated to test the homogeneity of variances and, depending on the result Post Hoc test were applied to determine statistical significant difference between groups.

Permeability values Papp (cm/s) in small intestine in Wistar rat had been collected from literature and have been used to establish a correlation with the *in situ* Papp obtained by Doluisio techniques and oral fraction absorbed in humans (Lozoya-Agullo et al., 2017a,b, 2015b).

That equation was used to calculate the predicted Fabs:

$$F_{abs} = 1 - e^{(-Papp\ (2/R) \cdot T)} \quad (7)$$

where *R* represents the effective radius of the colon that was fixed at a value of 0.3989 cm, and *T* is the effective absorption time.

3. Results

3.1. Osmolality measurement study of oral liquid formulations

From the selected drugs, 58% do not have available any pediatric formulation, thus they were compounded to obtain formulations of a wide range of osmolarities. Table 2 shows the measurement results of 50 oral medicines. 86% of the formulations turned out to be hyperosmolar (above 400 mOsm/kg) and 70% above 1500 mOsm/kg. 82.8% of the 29 compounding formulations measured were shown to be hyperosmolar.

3.2. Intestinal permeability studies

The mean permeability value (n = 5) and final volumes obtained in mL are shown in Fig. 1.

4. Discussion

Due to specific requirements regarding swallowing, palatability and dosage, pediatrics patients need appropriate oral medicines because, until recently, it has been considered an orphan population (Del Moral Sanchez et al., 2018; Preis and Breitkreutz, 2017). The American Academy for Pediatrics published its recommendation (American Academy of Pediatrics, 1976) to not exceed 400 mOsm/L for orally administered formulation and since then, some practitioners dilute the oral formulations to reduce the potential risk.

Nowadays, a marketed formulation is not available in a great percentage of cases and compounding formulations proved to be mostly hyperosmolar but the final osmolalities are not generally due to the drugs themselves, but to the use of hyperosmolar vehicles such as simple syrup, cellulose gels and other excipients.

The marketed drug products were also proved to be hyperosmolar in their majority. This should be taken into account for oral formulations of digoxin, levetiracetam, paracetamol and zidovudine (which exceed 3000 mOsm/kg), even digoxin, paracetamol and zidovudine formulations are specifics to neonates.

Following the general recommendations of the American Academy for Pediatrics, drug manufacturers should modify the composition of their medicines to adapt them to a pediatric isosmolar administration.

There is controversy if osmolarity changes may affect gastric emptying rate. Ramirez et al. (2006) concluded that osmolarity by itself does not affect emptying, other authors (Mogard et al., 1986; Leiper, 2015) advocate for an inhibitory mechanism produced by hyperosmolar administration. This aspect has not been evaluated in the present study but warrant further research as a change in gastric emptying rate could affect also absorption rate (and thus Cmax) of high permeability drugs.

In the present study, the effect on the changes of osmolality in oral fraction absorbed of drugs due to diluting oral formulations for pediatrics population was evaluated

According to the results in Fig. 1, the osmolality change is inversely proportional to the apparent permeability change of the drug. i.e. an increase in osmolarity is reflected in a reduction of the apparent permeability value. For all samples with 1500 mOsm/kg solutions, the permeability values obtained were lower compared to hyposmolar or isosmolar administration. This reduction proved to be more pronounced in the case of atenolol, caffeine and paracetamol. On the other hand, in hypo-osmolar conditions the permeability value is increased.

The volume of fluid contained in the gastrointestinal tract is a determining factor in the absorption of a drug since it influences its luminal concentration (Tanaka et al., 2015). Consequently, the net change in luminal fluid due to changes in osmolarity could affect drug transport at two different levels. In first place, it affects the drug concentration and thus the gradient driving the diffusion/permeation process and in second place, the water flux main direction will impact the diffusion of molecules using the same pathway than the water molecules in its transfer across the cell membranes.

The absorption or secretion of water through the gastrointestinal membrane occurs via paracellular (tight junctions) or transcellular ways through aquaporins (Ichijo et al., 2017; Agre, 2004). Osmotic gradients across this membrane directly lead to water movement, so the osmolarity of the fluid will be an important factor for absorption and secretion (Ichijo et al., 2017; Tanaka et al., 2015).

Passive diffusion through the intestinal barrier is the composite of the diffusion across the lipid bilayer (transcellular route) and the diffusion across the water pores (aquaporins) or tight junctions between adjacent cells (paracellular route) (Lozoya-Agullo et al., 2017a; Bermejo et al., 1999; Sánchez-Castaño et al., 2000). Osmolarity changes and water fluxes would affect mainly to the permeation process of compounds for which the paracellular route and the water pores way is the main component of the overall permeation process. Paracellular diffusion and diffusion through aquaporins is usually restricted to

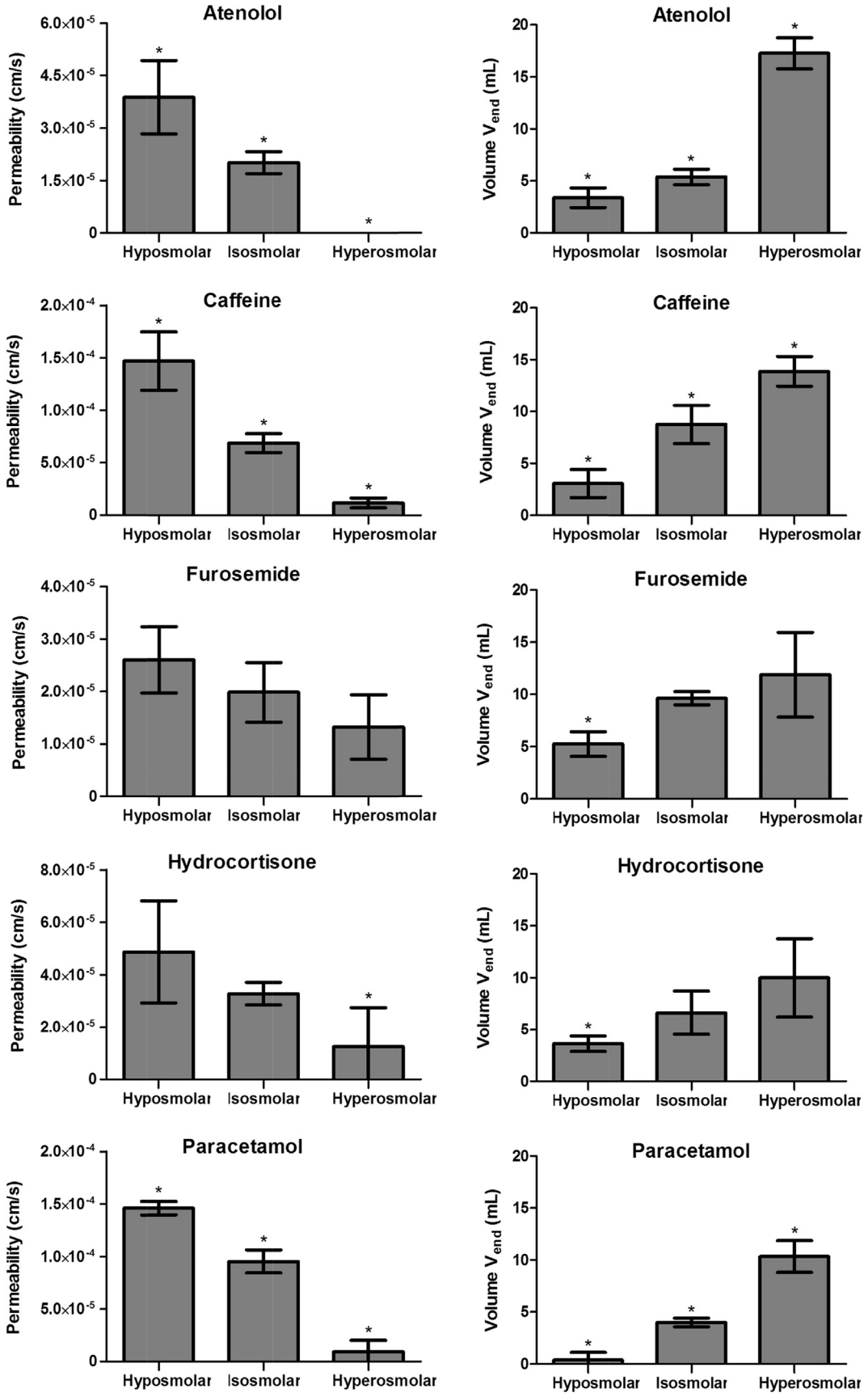

Fig. 1. Mean permeability values in cm/s and mean volumes in mL at the end of experiment for selected drugs in Wistar rat intestine for the three tested conditions. *Statistically significant differences ($p < 0.05$) between groups.

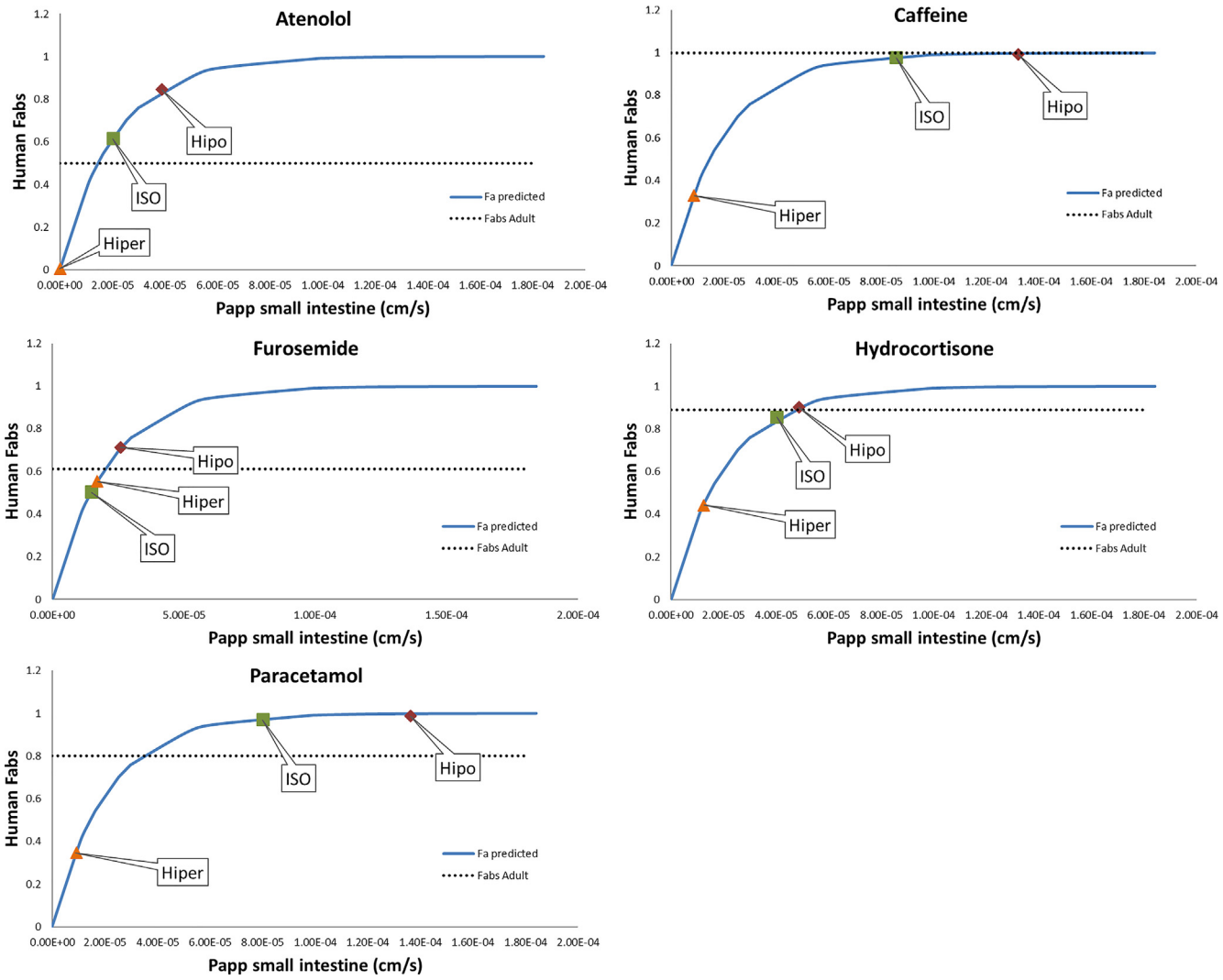

Fig. 2. Correlations between *in situ* method in rat(15–17) and oral human fraction absorbed. (Papp permeability).

compounds with molecular weight lower than 250–275 Da and of hydrophilic nature.

In the assayed compounds, those showing the biggest change in permeability value (and statistical significant differences across osmolarity conditions) were the compounds with molecular weight lower than 275 Da and Log P < 1 i.e more hydrophilic: Atenolol, Caffeine and Paracetamol. Hydrocortisone and Furosemide whose molecular weight is higher than 300 Da and are more liphophilic (Log P > 1) presented smaller changes in permeability due to changes in osmolarity.

From a BCS (Biopharmaceutic Classification System) standpoint (Löbenberg et al., 2000) Paracetamol, Caffeine and Hydrocortisone are classified as high permeability drugs as they have oral fraction absorbed higher than 0.8 and intestinal permeability value higher than Metoprolol, while Atenolol and Furosemide are low permeability. From our results, it seems that the effect of osmolarity is in relation with the main permeation route (transcellular versus paracellular) and not with the magnitude of the permeability value itself. It is expected, that osmolarity changes would affect permeability values of those drugs for which paracellular route is a major component of their permeability.

Luminal presence of excipients and physiological countervailing mechanisms might affect the mucosal barrier (Dahlgren et al., 2018). Administering a drug with an osmolality above the physiological value causes the drug to be confined in the luminal space, increasing the water content to compensate for the hyperosmolality and thus preventing the drug from being absorbed as the water flux into the lumen would reduce the net diffusion from apical to basolateral side of the enterocytes.

In the tested drugs, the hypertonic solutions were those that left the largest residual volume of water in the intestine. As can be noted in Fig. 1, volumes remaining in the assays with hyperosmolar solutions were higher than the volume of administration (10 mL) due to the physiological compensatory mechanism described above.

The volume of breast or formula milk represents mainly the gastric volume in the fed state for neonates (Johnson et al., 2018). The low volume of food intake in this subpopulation and the osmolality of milk (290–299 mOsm/kg) (Guimarães et al., 2018) lead to obtain a hyperosmolar mixture with medicines. Since higher frequencies of food intake are present in neonates, they are most of the time mainly in the postprandial state (Guimarães et al., 2018; Kamstrup et al., 2017). Therefore, medicines are in contact in stomach with milk in most cases.

The repercussion in oral fraction absorbed is high; in fact, it can be reduced by 50% in hyperosmolar formulations (Fig. 2). Nevertheless, the actual consequences in the clinical situation would need further analysis. It is necessary to consider than permeability is only one of the components determining rate an extent of absorption, the luminal drug concentration is the second factor and it depends on the drug solubility and the dissolution rate from the solid particles. For drugs administered as solutions, hyperosmolarity would have a direct impact in permeability as we have shown in this work, but in the case of suspensions the

Table 3
Oral fraction (fa) absorbed calculate for each condition of osmolality assaye.

	fa		
	150 mOsm/kg	300 mOsm/kg	1500 mOsm/kg
Atenolol 0.5 mg/mL	0.80	0.61	0.0042
Caffeine 2 mg/mL	0.99	0.97	0.33
Furosemide 0.1 mg/mL	0.71	0.51	0.55
Hydrocortisone 1 mg/mL	0.90	0.85	0.44
Paracetamol 10 mg/mL	0.98	0.97	0.34

increment in luminal volume would favour dissolution and eventually absorption, so the net effect (i.e better dissolution versus lower permeation) needs to be analyzed case by case.

Many Hospital pharmacy services do not have available an osmometer and they used fixed protocols for all formulations and drugs, such as not diluting or making dilutions. Table 3 shows oral fraction absorbed Fa predicted from the experimental permeability values with Eq. (7) after administration in each osmolarity condition. Each API has certain characteristics and should be administered as appropriate; for example, the osmolarity data obtained for paracetamol in dilution ratio 1:8 would remain hyperosmolar (Table 2).

5. Conclusion

In the administration of drugs in neonates we must pay special attention to the osmolality of the formulations, not only because of the risk of damaging the gastrointestinal tract of the patient, but because of the risk that modifying osmolality also modifies its permeability, lead to an unwanted change in oral fraction absorbed and in consequence in bioavailability. The recommendation to dilute the pharmaceutical forms to iso-osmolality would make more sense for drugs that show a narrow therapeutic range (e.g. digoxin, phenobarbital, phenytoin) or those for which a change in oral fraction absorbed can compromise safety (e.g. amiodarone, levetiracetam, zidovudine).

6. Funding source

Partial support from “Modelos *in vitro* de evaluacion biofarmaceutica” SAF2016-78756 (AEI/FEDER, EU) funded by Agencia Estatal Investigación and European Union, through FEDER (Fondo Europeo de Desarrollo Regional).

7. Conflict of interest statement

The authors have no conflicts of interest relevant to this article to disclose.

Acknowledgements

We acknowledge Iván del Moral for his help and the critical analysis of the manuscript.

Partial support from “Modelos *in vitro* de evaluacion biofarmaceutica” SAF2016-78756 (AEI/FEDER, EU) funded by Agencia Estatal Investigación and European Union, through FEDER (Fondo Europeo de Desarrollo Regional).

References

Agre, P., 2004. Aquaporin water channels (Nobel Lecture). Angew. Chem. Int. Ed. Engl. 43, 4278–4290. https://doi.org/10.1002/anie.200460804.

American Academy of Pediatrics, 1976. Committee on Nutrition, Commentary on breast-feeding and infant formulas, including proposed standards for formulas. Pediatrics 57, 278–285.

Bermejo, M., Merino, V., Garrigues, T.M., Pla Delfina, J.M., Mulet, A., Vizet, P., Trouiller, G., Mercier, C., 1999. Validation of a biophysical drug absorption model by the PATQSAR system. J. Pharm. Sci. 88, 398–405. https://doi.org/10.1021/js980370+.

Chauhan, M., Henderson, G., McGuire, W., 2008. Enteral feeding for very low birth weight infants: reducing the risk of necrotising enterocolitis. Arch. Dis. Child. Fetal Neonatal Ed. 93, F162–F166. https://doi.org/10.1136/adc.2007.115824.

Dahlgren, D., Roos, C., Lundqvist, A., Tannergren, C., Sjöblom, M., Sjögren, E., Lennernäs, H., 2018. Effect of absorption-modifying excipients, hypotonicity, and enteric neural activity in an in vivo model for small intestinal transport. Int. J. Pharm. 549, 239–248. https://doi.org/10.1016/j.ijpharm.2018.07.057.

Del Moral Sanchez, J.M., Gonzalez-Alvarez, I., Cerda-Revert, A., Gonzalez-Alvarez, M., Navarro-Ruiz, A., Amidon, G.L., Bermejo, M., 2018. Biopharmaceutical optimization in neglected diseases for paediatric patients by applying the provisional paediatric biopharmaceutical classification system. Br. J. Clin. Pharmacol. 84, 2231–2241. https://doi.org/10.1111/bcp.13650.

Doluisio, J.T., Billups, N.F., Dittert, L.W., Sugita, E.T., Swintosky, J.V., 1969. Drug absorption. I. An in situ rat gut technique yielding realistic absorption rates. J. Pharm. Sci. 58, 1196–1200.

Guimarães, M., Statelova, M., Holm, R., Reppas, C., Symilllides, M., Vertzoni, M., Fotaki, N., 2018. Biopharmaceutical considerations in paediatrics with a view to the evaluation of orally administered drug products - a PEARRL review. J. Pharm. Pharmacol. https://doi.org/10.1111/jphp.12955.

Ichijo, K., Oda, R., Ishihara, M., Okada, R., Moteki, Y., Funai, Y., Horiuchi, T., Kishimoto, H., Shirasaka, Y., Inoue, K., 2017. Osmolality of orally administered solutions influences luminal water volume and drug absorption in intestine. J. Pharm. Sci. 106, 2889–2894. https://doi.org/10.1016/j.xphs.2017.04.030.

Johnson, T.N., Bonner, J.J., Tucker, G.T., Turner, D.B., Jamei, M., 2018. Development and applications of a physiologically-based model of paediatric oral drug absorption. Eur. J. Pharm. Sci. Off. J. Eur. Fed. Pharm. Sci. 115, 57–67. https://doi.org/10.1016/j.ejps.2018.01.009.

Kamstrup, D., Berthelsen, R., Sassene, P.J., Selen, A., Müllertz, A., 2017. In vitro model simulating gastro-intestinal digestion in the pediatric population (neonates and young infants). AAPS PharmSciTech 18, 317–329. https://doi.org/10.1208/s12249-016-0649-1.

Leiper, J.B., 2015. Fate of ingested fluids: factors affecting gastric emptying and intestinal absorption of beverages in humans. Nutr. Rev. 73 (Suppl. 2), 57–72. https://doi.org/10.1093/nutrit/nuv032.

Löbenberg, R., Amidon, G.L., Modern,, 2000. bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. Eur. J. Pharm. Biopharm. Off. J. Arbeitsgemeinschaft Pharm. Verfahrenstechnik EV 50, 3–12.

Lozoya-Agullo, I., González-Álvarez, I., González-Álvarez, M., Merino-Sanjuán, M., Bermejo, M., 2015a. In situ perfusion model in rat colon for drug absorption studies: comparison with small intestine and caco-2 cell model. J. Pharm. Sci. 104, 3136–3145. https://doi.org/10.1002/jps.24447.

Lozoya-Agullo, I., Zur, M., Wolk, O., Beig, A., González-Álvarez, I., González-Álvarez, M., Merino-Sanjuán, M., Bermejo, M., Dahan, A., 2015b. In-situ intestinal rat perfusions for human Fabs prediction and BCS permeability class determination: investigation of the single-pass vs. the Doluisio experimental approaches. Int. J. Pharm. 480 1–7. https://doi.org/10.1016/j.ijpharm.2015.01.014.

Lozoya-Agullo, I., Gonzalez-Alvarez, I., Zur, M., Fine-Shamir, N., Cohen, Y., Markovic, M., Garrigues, T.M., Dahan, A., Gonzalez-Alvarez, M., Merino-Sanjuán, M., Bermejo, M., Avdeef, A., 2017a. Closed-Loop Doluisio (Colon, Small Intestine) and single-pass intestinal perfusion (Colon, Jejunum) in rat-biophysical model and predictions based on caco-2. Pharm. Res. 35, 2. https://doi.org/10.1007/s11095-017-2331-z.

Lozoya-Agullo, I., Zur, M., Fine-Shamir, N., Markovic, M., Cohen, Y., Porat, D., González-Álvarez, I., González-Álvarez, M., Merino-Sanjuán, M., Bermejo, M., Dahan, A., 2017b. Investigating drug absorption from the colon: single-pass vs. Doluisio approaches to in-situ rat large-intestinal perfusion. Int. J. Pharm. 527, 135–141. https://doi.org/10.1016/j.ijpharm.2017.05.018.

Miyake, H., Chen, Y., Koike, Y., Hock, A., Li, B., Lee, C., Zani, A., Pierro, A., 2016. Osmolality of enteral formula and severity of experimental necrotizing enterocolitis. Pediatr. Surg. Int. 32, 1153–1156. https://doi.org/10.1007/s00383-016-3998-7.

Mogard, M.H., Nylander, G., Flaten, O., Hanssen, L.E., 1986. Gastric emptying and intestinal transit of hyperosmolar solutions in relation to indomethacin and certain gut polypeptides in the rat. Scand. J. Gastroenterol. 21, 348–352.

Neu, J., Walker, W.A., 2011. Necrotizing enterocolitis. N. Engl. J. Med. 364, 255–264. https://doi.org/10.1056/NEJMra1005408.

Pearson, F., Johnson, M.J., Leaf, A.A., 2013. Milk osmolality: does it matter? Arch. Dis. Child. Fetal Neonatal Ed. 98, F166–F169. https://doi.org/10.1136/adc.2011.300492.

Preis, M., Breitkreutz, J., 2017. Pediatric drug development and dosage form design. AAPS PharmSciTech 18, 239–240. https://doi.org/10.1208/s12249-016-0705-x.

Ramirez, A., Wong, W.W., Shulman, R.J., 2006. Factors regulating gastric emptying in preterm infants. J. Pediatr. 149, 475–479. https://doi.org/10.1016/j.jpeds.2006.05.028.

Samuels, N., van de Graaf, R.A., de Jonge, R.C.J., Reiss, I.K.M., Vermeulen, M.J., 2017. Risk factors for necrotizing enterocolitis in neonates: a systematic review of prognostic studies. BMC Pediatr. 17, 105. https://doi.org/10.1186/s12887-017-0847-3.

Sánchez-Castaño, G., Ruíz-García, A., Bañón, N., Bermejo, M., Merino, V., Freixas, J., Garriguesx, T.M., Plá-Delfina, J.M., 2000. Intrinsic absolute bioavailability prediction in rats based on in situ absorption rate constants and/or in vitro partition coefficients: 6-fluoroquinolones. J. Pharm. Sci. 89, 1395–1403.

Sántulli, T.V., Schullinger, J.N., Heird, W.C., Gongaware, R.D., Wigger, J., Barlow, B., Blanc, W.A., Berdon, W.E., 1975. Acute necrotizing enterocolitis in infancy: a review of 64 cases. Pediatrics 55, 376–387.

Shulhan, J., Dicken, B., Hartling, L., Larsen, B.M., 2017. Current knowledge of necrotizing enterocolitis in preterm infants and the impact of different types of enteral nutrition products. Adv. Nutr. Bethesda Md. 8, 80–91. https://doi.org/10.3945/an.116.

013193.
Steele, J.R., Meskell, R.J., Foy, J., Garner, A.E., 2013. Determining the osmolality of over-concentrated and supplemented infant formulas. J. Hum. Nutr. Diet. Off. J. Br. Diet. Assoc. 26, 32–37. https://doi.org/10.1111/j.1365-277X.2012.01286.x.
Tanaka, Y., Goto, T., Kataoka, M., Sakuma, S., Yamashita, S., 2015. Impact of luminal fluid volume on the drug absorption after oral administration: analysis based on in vivo drug concentration-time profile in the gastrointestinal tract. J. Pharm. Sci. 104, 3120–3127. https://doi.org/10.1002/jps.24433.
Tuğcu-Demiröz, F., Gonzalez-Alvarez, I., Gonzalez-Alvarez, M., Bermejo, M., 2014. Validation of phenol red versus gravimetric method for water reabsorption correction and study of gender differences in Doluisio's absorption technique. Eur. J. Pharm. Sci. Off. J. Eur. Fed. Pharm. Sci. 62, 105–110. https://doi.org/10.1016/j.ejps.2014.05.016.