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Development and validation of a new HPLC method for the analysis of a novel oral suspension formulation of 50 mg/ml ursodeoxycholic acid for newborns

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Abstract

Objectives: Drugs are developed for adults, making it difficult to find suitable treatments for children. Hospital pharmacy has developed alternatives to respond to this medical need. The objective of this study is to present a new liquid formulation of ursodeoxycholic acid (UDCA) at a concentration suitable for treatment of neonatal jaundice, and to introduce a novel high pressure liquid chromatography (HPLC) assay method.

Methods: Four formulations have been developed using suspension vehicles due to the low solubility of the active ingredient, and different concentrations of excipient, xanthan gum, needed to facilitate resuspension. An HPLC method coupled to a diode array detector (DAD) has been developed. This method was used to analyze chemical and microbiologic stabilities, as well as physicochemical properties and palatability.

Results: After formulation was chosen, our new HPLC method assay was developed and validated for the quantification of chemical and microbiological stabilities of our

product. Both parameters were stable over three months. Palatability has been improved thanks to the addition of universal suspension adjuvants. Odor, appearance and taste were judged pleasant despite a bitter aftertaste, with a persistence of the UDCA resuspension after one month.

Conclusions: Three months after informing neonatal department about the availability of the drug, patients and caregivers are satisfied, and production campaigns are routinely planned.

Keywords: chromatography; clinical pharmacy service; high performance liquid; pediatrics; pharmaceutical preparations.

Introduction

Due to financial constraints and trial design challenges, most drugs are developed for adults and are administered to children empirically using fractions of adult dosing. In hospitals, especially pediatric ones, the consequences are major difficulties to find adapted treatment for the pediatric population.

Ursodeoxycholic acid (UDCA) is a natural bile acid present in very small quantities in humans. It is currently used for the treatment of neonatal jaundice. This disease, caused by an immaturity of the biliary production, affects 1 out of 2,500 births [1]. Although it remains generally benign, it can lead to cholestasis, a decrease of biliary secretion, and severe hyperbilirubinemia an increase of the bile acids concentration in blood and tissues that can lead to lipids absorption alteration [1]. UDCA acts on the enterohepatic circulation of endogenous bile acids by increasing their biliary secretion, inhibiting their active reabsorption by the intestine and lowering their concentration in the blood. It also reduces the saturation of bile in cholesterol by reducing its intestinal absorption, increasing its hepatic catabolism, and maintaining it in its soluble form in the bile [2].

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In France, an oral suspension of UDCA called Ursofalk® 250 mg/5 mL is available (Dr Falk Pharma®, Freiburg im Breisgau, Germany) but did not obtained marketing authorization yet. Thus, an approval from the French National Agency of Medicine and Health Products Safety (ANSM) is required to get a Temporary Authorization for Use (ATU) for this unlicensed drug. This approval is given when no appropriate treatment is available on the market and when the effectiveness and safety of the drug are strongly presumed based on the results of therapeutic trials.

However, this oral suspension contains benzoic acid, an antimicrobial agent which is contraindicated in newborns less than 56 days of life due to the risk of developing a kernicterus. Capsules without the excipient were already prepared by our hospital pharmacy to fill this gap but it was complicated to provide all the needs requested by caregivers, in particular a fine adjustment of doses. Given the number of patients who could benefit from this treatment and the repeated medical requests, it has been decided to formulate an oral suspension of UDCA, free of any contraindicated excipient in newborns such as benzoic acid. Besides, as the demand of departments is regular, it is possible to optimize production and expiry dates. As new formulation is developed, a stability indicating diode array detector-high pressure liquid chromatography (DAD-HPLC) method able to quantify UDCA should also be developed.

The objectives of this study were dual. Our first aim was to develop a new oral suspension of UDCA without any contraindicated excipient for newborns, in order to facilitate the adaptation of UDCA doses and its administration to infants. The taste and the appearance of a drug are very important. As UDCA is insoluble in water due to its hydrophobicity and has a tendency to form agglomerates, it was impossible to consider a formulation as an oral solution. Secondly, we aimed to validate its chemical and microbial stability thanks to the development of a new and optimized HPLC assay method.

Materials and methods

Chemicals and reagents

Ursodeoxycholic acid powder, InOrpha®, Ora-blend® and xanthan gum were provided by Inresa® (Bartenheim, France) as a raw material for pharmaceutical use. InOrpha® and Ora-blend® were used as universal admixture suspension, and xanthan gum as excipient to promote the stability of emulsions by increasing their viscosity.

Capsules of Delursan 200 mg, supplied by Aptalis Pharma® (Bridgewater Township, New Jersey) were used as external quality controls.

All reagents and chemicals were analytical grade and included methanol, potassium dihydrogenophosphate and orthophosphoric acid. They are provided by VWR (Radnor, Pennsylvania, États-Unis).

Formulation of the new UDCA drug

A single strength of UDCA at 50 mg/mL was prepared to match the former formulation of Ursofalk® 250 mg/5 mL. This concentration is in accordance with the limit of fluid intake that is of 5 mL for a child under 5 years old and 10 mL beyond [3].

Two suspension vehicles for oral preparation (InOrpha® and Ora-blend®) and two concentrations of xanthan gum (0.25%, 0.50%) were investigated during the development of the formulation (Table 1).

Sedimentation tests were performed by the centrifugation of 3 mL of the formulation at 3,000 rpm during 3 min. A floating sample was taken before resuspension, which was standardized by ten inversions. Then three samples per tube were taken (one at the surface, one in the middle and one at the bottom) and analyzed using HPLC after dilution of the sample to 1/4. This method was previously validated on a spirinolactone suspension [4].

Quantification using HPLC

HPLC instrumentation and chromatographic conditions: The HPLC system consisted of an Ultimate 3000 (Thermo Scientific™, Waltham, USA), with a diode array detector (DAD) operating between 190 and 800 nm. The results were analyzed using Chromeleon software (ThermoFisher®).

Method validation: The HPLC method was validated according to the guidelines of the International Conference on Harmonization (ICH Q2, R1) [5] in terms of:

- *Linearity of the response function*, defined as the ability (within a range from 50 to 500 µg/mL) of the method to obtain test results directly proportional to the amount of UDCA in the sample. The demonstration of its linearity was performed with triplicates for six concentrations (1, 2, 2.5, 3 and 4 mg/mL) and considered admissible if the variance was constant based on Cochran test (p -value < 0.05).
- *Matrix effect*: The same conditions than above were applied to a range of pure active ingredient using a range of active ingredient and reconstituted solution of excipient in order to assess matrix effect. At this step, it is important to use a diluted solution because the xanthan gum may clog the chromatography column. The slopes or y -intercepts were compared using statistical student tests (p value p < 0.05).

Table 1: Composition of the four formulations of ursodeoxycholic acid (UDCA) tested in the study. Bold values stand for three chosen formulation after the 4 tests.

	Ora-blend®	InOrpha®	Xanthan gum	UDCA
Formulation 1	X		0.25%	X
Formulation 2	X		0.5%	X
Formulation 3		X	0.25%	X
Formulation 4		X	0.5%	X

- *Accuracy*, defined as:
- *Trueness*: the closeness agreement between the value found and the value accepted as true. The procedure would be declared adequate if 95% confidence interval of the average recovery includes the value of 100%. Each day, a new quality control was performed at the beginning and at the end of the sequence.
- *Precision*, also defined as:
- *Repeatability*: evaluated by preparing and analyzing three replicates of low (100 µg/mL), three intermediate (300 µg/mL) and three high (400 µg/mL) concentration within a day.
- *Intermediate fidelity*: during three days, a new quality control was performed daily. Samples were analyzed three times.

The levels of the control sample were selected to reflect low, medium and high concentration levels. Analytical concentration variations were expressed as a relative bias compared to the theoretical concentration. The method was considered acceptable if the coefficient of variation of all concentrations were in a range of $\pm 15\%$.

- *Specificity*, defined as the ability of method that indicates stability to discriminate between compounds of close related structures, such as the degradation products. Hence, UDCA was exposed to different degrading conditions (acidic, basic, oxidative, UV and heat), in order to reach a degradation of more than 20% of the active ingredient.

The acidic and basic degradation were performed by the addition of solutions of HCl or NaOH at a concentration of 0.25 M in the UDCA suspension (0.5 mg/mL) and the degradation was verified by a dosage 24 h later. The oxidative degradation was carried out by the addition of a solution of H_2O_2 at a concentration of 0.25 M in the UDCA suspension (0.5 mg/mL) and the degradation was also verified by a dosage 24 h later. The light induced degradation was performed with an exposure to UV for 24 h ($\lambda = 253.7$ nm, 4.9 W, Sankyo denki G15T8). The heat degradation was performed at 50° and 100 °C for 15 min, using a hot bath.

- *Quantification limit*: considering that the dosage is not performed in a biological fluid but in a defined matrix, and that the concentration to be determined is known, it is not necessary to have a large range of amplitude. Therefore, we stayed focused around the expected dosage.

HPLC development

Calibration range: A 10 mg/mL stock solution of UDCA was prepared by dissolving 100 mg of UDCA powder in 10 mL of methanol.

Calibration standards of 1, 2, 2.5, 3 and 4 mg/mL were prepared by diluting UDCA working stock solution and 50 µL of a solution miming matrix effect (20%), into the mobile phase. After a centrifugation at 3,500 rpm for 10 min, 1 mL of each of these supernatants were taken and kept into separated vials.

Quality controls: Quality control samples were prepared by mixing a capsule of Delursan 200 mg with the solution miming matrix effect (20%) into methanol. After centrifugation, they were diluted at 1/40,

1/13 and 1/10 into the mobile phase to reach a concentration respectively of 100, 300 and 400 µg/mL.

Sample preparation: Five hundred microliter from each sample was taken and dissolved into methanol in a calibrated flask of 5 mL to obtain an UDCA solution of 5 mg/mL. Thereafter, the flask was placed in an ultrasonic bath for 10 min. Two milliliter of the solution was then decanted into a glass tube and centrifugated for 10 min at 3,500 rpm. 500 µL of the supernatant diluted with 500 µL of mobile phase, aliquoted into a vial, and vortex.

Oral suspension of UDCA stability

The variations of the analyzed concentration were expressed as a relative bias compared to the initial concentration measured at time zero (T0). Flasks were stored in the control lab at two different temperatures: 22 ± 2 °C and 4 ± 2 °C. Analyses were performed in triplicate. The oral suspension was considered to be stable if less than 10% loss of the initial concentration occurs after 1, 2 and 3 months. This limit was chosen according to a validated process in our unit.

Microbiology quality testing of non-sterile products

This preparation is not produced by aseptic processes and, therefore, is not expected to be totally free from microbial contaminations. As it is specified in European Pharmacopoeia for multidose aqueous oral formulation, enumeration of total aerobic counts (ETAC) was established and had to be fewer than 10^2 CFU/g. Furthermore, the preparation must also be free from *Escherichia coli* in 1 g of product. The fertility applicability assays have not been developed. Indeed, the hospital microbiology laboratory which performed the tests had validated this assay upstream.

The microbiological quality of the samples was tested daily on the day of fabrication as well as two weeks and one-month post fabrication, with a daily opening to reflect the “real life use of the product”. Moreover, a microbiological test was performed one month post-fabrication without opening as a control [6]. All tests have been done in triplicate originated from three different vials.

Physicochemical, organoleptic characters and palatability

Visual examination and organoleptic characters: A visual examination was performed daily on the first two weeks, then each week during the first month, on three separated batches. To describe the obtained oral suspension, each bottle was fully opened to detect any modification. The stability of the following parameters was tested at room temperature and 4 °C [7]: the product odor, color, and appearance as well as the resuspension of the active ingredient and the pH. The pH determination was performed using a pH paper, since a small variation is not considered dangerous when the drug is taken orally. A variation of one unit in the pH value was considered significant enough to indicate a modification compared to the initial pH of the initially prepared solution [8].

Palatability: The taste of the product was evaluated because the suspension can be taken orally or administered using a nasogastric tube. The formula has been tasted by 10 volunteers working at the pharmacy. Each of them tasted a teaspoon from a bottle of the final product, stored at room temperature or in the refrigerator. Anonymously, they completed a table using the proposed evaluation scales with the following items, product taste and aftertaste between (very good, good, bad or very bad); appearance and odor between (pleasant or unpleasant).

Results

UDCA formulation

The active substance of our formulation is UDCA, at a concentration of 50 mg/ml. This concentration was chosen to match the former formula, namely Ursofalk®, that is contraindicated for newborns and to avoid administration mistakes. The choice of this concentration also avoids the administration of excessive volumes to infants.

Universal suspension vehicles, such as InOrpha® or Ora-blend®, allow faster preparation of drugs in liquid forms and promote the suspension of active ingredients. They also enable a precise dosage, a pleasant taste and easy administration by enteral tube. However, precaution must be taken with excipients as they may cause adverse reactions, and increase suspension osmolality that should stay below 1,000 mOsm/kg if administered orally and 300 mOsm/kg if administered by enteral tube in newborn although it remains possible to dilute the preparation [9].

Two complex excipients admixtures were tested for the compounding of the oral liquid. These excipients are InOrpha® and Ora-blend® which are both suitable for pediatric applications and which are currently used in our hospital unit. InOrpha® was chosen because the suspension with Ora-blend® is less homogeneous with the presence of many lumps in the formulation. The non-homogeneity of the suspension was visible with naked eyes. Thus, we did not measure the concentration in several places in the vial to quantify homogeneity of the Ora-blend containing products. However, for the formulation containing InOrpha, a previous study conducted in the same unit had already justified this homogeneity [4].

Xanthan gum is known to increase the viscosity of aqueous liquids as it was proven in a previous study conducted in our unit [4]. Different concentrations (0.25% m/V, 0.5% m/V) of Xanthan gum were tested to slow down the sedimentation rate of the solid matter contained in the suspension and facilitate the resuspension of UDCA. The

influence of Xanthan concentration on these two parameters was studied using HPLC dosages. No significant difference has been observed between the two formulations containing 0.25 and 0.50% of xanthan gum. The smallest concentration has been chosen to reduce the excipient exposure, corresponding to the formulation 3 (Table 1).

A bottle made of glass type I with and amber colored has been chosen to stock our product in order to prevent any reaction with container or light.

Quantification method: HPLC-DAD parameters

During our research, it was found that the analytical method described in the IXth edition of the European Pharmacopeia [10] is not suitable with the equipment available in our control laboratory. Indeed, it suggests to use an infrared absorbent spectrophotometer. Two alternative methods are quoted: the use of a thin layer chromatography and the use of colorimetric identification. However, these two methods are not precised enough to be used for the quantitative analysis of UDCA [10].

Thus, it was decided to use a method developed by Hôtel-Dieu (Paris, France). This method is adapted to our laboratory equipment and served as a basis for our study. In this method, Pierrey et al. used HPLC with inverted phase polarity, coupled with an UV detection at 200 nm. Separation is carried out on a C18 column, 5 µm, 4.6 × 250 mm, heated at 60 °C. The mobile phase is an aqueous buffer at pH 2.5 composed of potassium dihydrogen phosphate at 10 mmol/L and methanol (70:30 v/v). Flow rate is fixed to 0.8 mL/min for a total analysis time of 15 min. Injection volume is 30 µL [11].

Other articles have helped us to optimize our analytical procedure [12–14]. Ballet-Guffroy et al. used a C18 column, 3.9 × 150 mm and a mobile phase made of potassium dihydrogen phosphate at 30 mmol/L and methanol (24:76 v/v). In this study, the chosen flow rate is 1.2 mL/min and the Injection volume is 20 µL [12]. In 2011, Peepliwal et al. used a C18 column, 4.6 × 150 mm, 5 µm, heated at 40 °C. The mobile phase is a mixture of 0.1% acetic acid and methanol (30:70 v/v) and the flow rate is 0.8 mL/min for an analysis time of 22 min [13].

Finally, in the last study, the authors used a C18 column, 3.9 × 150 mm, 4 µm, kept at 40 °C. The mobile phase is a mixture of 0.1% acetic acid and methanol (30:70 v/v). Flow rate is 0.8 mL/min [14].

The method developed by Pierrey et al. *In Hotel Dieu* was the starting point of our study since the characteristic

Table 2: Validation of the quantification method (with n representing the number of replicates for each point).

	Conditions	Studied value	Expected value	Results	Conclusion
Linearity	n=3 for each of the 6 range points	Variance	Homogeneous variances	Cochran test proved homoscedasticity	Linear
Matrix effect	n=3 for each of the 6 range points (2 ranges: with and without matrix)	Equation of line (slope and intercept)	If $p < 0.05$: difference If $p > 0.05$: no difference	Slope: $p = 0.016$ so slope is different Intercept: $p = 0.183$ so intercept is not different	Matrix effect with a constant bias
Accuracy	n=3 for each of the 6 range points	Percentage of error and recovery rate	Confidence interval containing 100	Confidence interval = [99.00, 102.1]	Yes
Repeatability	n=6 for each quality control (low, intermediate, high)	Coefficient of variation	Coefficient of variation <15%	Concentration (µg/mL) Low (100 µg/mL) Intermediate (300 µg/mL) High (400 µg/mL)	% of error -1.10% -7.89% -1.43% Coefficient of variation 2.00% 1.20% 1.30%
Intermediate precision	n=6 for each quality control (low, intermediate, high). Experiment repeated over 3 days	Coefficient of variation	Coefficient of variation <15%	Concentration (µg/mL) Low (100 µg/mL) Intermediate (300 µg/mL) High (400 µg/mL)	% of error -1.98% -0.47% 0.23% Coefficient of variation 3.70% 4.20% 2.60%

used were, the most adapted to our equipment [11]. However, several modifications were made.

Firstly, several chromatographic conditions were investigated such as the column length (150, 250 mm), the temperature (25, 30, 40, 45, 60 °C), the injection volume (20, 30 µL), the run duration (5–60 min), the conditions of the pre-treatment (with or without ethanol) and the mobile phase composition (acetic acid/methanol, acetonitrile/methanol with different percentages, buffered water/methanol with different pH and different percentages).

Secondly, tests were done in order to simplify the method: shorter for a routine use, without heating to avoid heating up time and with a solvent easier to prepare.

The best conditions were similar to the one used in the method developed in the Hotel-Dieu. The detailed protocol is the following: an aliquot of 30 µL of the sample solutions was injected on Siligel OD1 C18 reverse phase column (5 µm, 4.6 × 250 mm, Interchim®, Montluçon, France) at 60 °C. The mobile phase is a mixture of 10 mM potassium dihydrogen phosphate buffer solution (pH=2.5) and methanol in the ratio 70:30 (v/v). Analysis was carried out at a flow rate of 0.8 mL/min and a detection wavelength of

$\lambda = 200$ nm, with an isocratic program. The run time is 15 min.

Due to the UDCA chemical structure with poor chromophores, the molecule absorbance is low (Figure 1). The maximum absorption observed is around 200 nm (Figure 2).

Using the optimized HPLC method just described, UDCA 10 mg/mL solution was detected in the form of a 50 mAu peak that tends to spread to the right. Its retention time is 10 min. The background noise remains very close to zero, and two peaks identified as excipients are detected at retention time of 3.5 and 4.5 min (Figure 3).

Every measure has been done in triplicate (Table 2).

Linearity: response variance was a constant ($C_{exp} = 0.423 < C_{th} = 0.445$) with a mean coefficient correlation of 0.9994. Thus, the function response was linear from 1 to 4 mg/mL.

Matrix effect: The equation line obtained is $y = 0.0061x - 0.0530$ without excipient and $y = 0.0050x - 0.0186$ with excipients. These slopes are not significantly different ($p = 0.183$, Student's *t*-test). However, *y*-intercepts are

Figure 1: Chemical formula of ursodeoxycholic acid.

significantly different ($p=0.016$, Student's t -test) which means that there was a constant bias which could not be neglected. Thus, I was decided that every dosage has to be performed in the matrix (InOrpha+xanthan gum).

Accuracy: value of 100% recovery was included in the 95% confidence interval, so accuracy of the analytical method was then verified.

Precision: coefficient of variation of repeatability and intermediate fidelity are lower than 15%. Thus, precision of the HPLC method was proven.

Forced degradation

No UDCA degradation was detected under basic or oxidative conditions (NaOH 0.25 M and H_2O_2 , respectively) or after UV or heat exposure. Nonetheless, degradation has been demonstrated in acidic condition (HCl

0.25 M) with a decrease of UDCA concentration of 52.40% (Figure 4).

Stability experiments

The concentration of UDCA remains stable during one month with a daily opening of the bottle (Table 3).

Without daily opening, the variation in concentration does not exceed 10% after 3 months. The preparation is therefore considered stable. It was decided not to test the stability for more than 3 months because this duration is sufficient for our needs (Table 3).

It seems consistent with the bibliography. Indeed, a stability of 60 days at 2–6 °C and 22–23 °C for a 25 mg/mL suspension with Ora Plus®, glycerol, orange syrup and simple syrup has previously been proven with UDCA by Mallet et al. [15].

Another study proved that a suspension of UDCA at 50 mg/mL with Ora Plus® and Ora Sweet® SF was stable 90 days at +4 °C and +25 °C [16].

Microbiology quality testing of a non-sterile product

All the tests performed were conformed to European Pharmacopoeia criteria during the month, whether opening or not. No influence of the storage temperature has been observed on the microbiological quality of the suspension (Table 4).

Organoleptic characters and palatability

Organoleptic characters

The different tests performed have showed that scent remains sweet, but less pronounced if cold. Color remains white and opaque. Resuspension persists, just as the smooth and homogeneous appearance, which is thicker if cold. pH is stable between 5 and 6.

Palatability

Taste judged to be between good (70%) and very good (30%). It improves if the product is stored at room temperature; aftertaste was judged bad but improves if the product is stored at room temperature. Appearance and scent were judged pleasant, and no difference was noticed between the flask stored at room temperature and the one stored at 4 °C.

Figure 2: Absorption spectrum of ursodeoxycholic acid. UDCA molecule shows a pick of absorption at 200 nm.

Figure 3: HPLC chromatogram profile of ursodeoxycholic acid at 50 mg/mL in the matrix. UCDA peaks at retention time 10 min. The two others peaks at 3.5 and 4.5 min are identified as excipient components.

Figure 4: HPLC run of ursodeoxycholic acid after degradation with HCl 0.25 M.

Table 3: Evolution of UDCA concentration in the formulation overtime.

Expected concentration		Measured concentration			
		Room temperature		+4 °C	
Day 0 (without opening)	50 mg/mL	47.17 mg/mL ± 0.42	94.33%	49.90 mg/mL ± 2.43	99.80%
Day 7 (without opening)		47.83 mg/mL ± 2.71	101.40%	47.97 mg/mL ± 0.17	96.13%
Month 1 (with daily opening)		48.63 mg/mL ± 1.30	103.09%	49.73 mg/mL ± 1.91	99.66%
Month 1 (without opening)		51.50 mg/mL ± 3.13	109.18%	49.67 mg/mL ± 2.21	99.54%
Month 2 (without opening)		47.67 mg/mL ± 0.49	101.06%	47.67 mg/mL ± 1.32	95.53%
Month 3 (without opening)		54.03 mg/mL ± 3.86	114.54%	48.40 mg/mL ± 4.09	97.00%

The concentration of UDCA was measured 7 days, 1, 2 and 3 months post-production in order to investigate the stability of our product. The products were stored at room temperature or at 4 °C. Results are expressed in mg/mL ± standard deviation. The percentages represent the amount of UDCA remaining in comparison with the day 0 concentration.

Table 4: Quantification of CFU to assess the microbiological stability of our formulation overtime.

Samples		Results	
Day 0	20–25 °C	Flask 1	0 colony forming unit (CFU)
		Flask 2	
		Flask 3	
	4 °C ± 2 °C	Flask 1	0 CFU
		Flask 2	
		Flask 3	
Month 1 (after daily opening)	20–25 °C	Flask 1	0 CFU
		Flask 2	
		Flask 3	
	4 °C ± 2 °C	Flask 1	0 CFU
		Flask 2	
		Flask 3	
Month 1 (without daily opening)	20–25 °C	Flask 1	0 CFU
		Flask 2	
		Flask 3	
	4 °C ± 2 °C	Flask 1	0 CFU
		Flask 2	
		Flask 3	

Four hundred microliters of sample of the product were inoculated and placed 48 h at 37 °C before CFU counting.

Discussion

The preparation of liquid oral forms is essential in pediatrics to enable better doses adaptation than with commercially available drugs. They also facilitate the administration of drugs to infants and children. Indeed, several issues are commonly encountered with the drugs that are currently available:

- *Non-suitable forms.* It is not advisable to prescribe tablets for children under six-year-old, because of a significant risk of going down the wrong pipe [3]. However, few active ingredients are available in oral, which disperses in the mouth, or in powder form. In the long term, an injectable route is difficult to consider. Without alternative, doctors are forced to prescribe tablets. Therefore, hospital pharmacists have a key role in terms of optimization of the galenic form of drugs. Furthermore, an American study has shown bioequivalence between solid and liquid oral forms such as Ursofalk® [17].
- *Non-adapted doses.* Since children's maximum doses are lower than adults' ones, it is generally necessary to cut the tablets. In practice it is complicated to cut a tablet into more than two equal parts. To overcome this issue, caregivers often crush the tablets and make manual dilutions, but some forms of tablets cannot be

crushed. In addition, dose uniformity is not guaranteed and mistakes can be made during the calculation or during the preparation of the dilution.

- *Contraindicated excipients.* Ethanol, propylene glycol and benzoic acid are often present in drugs formulation, which prevent their use for children. Since therapeutic alternatives are not always available, pharmacists may have to find raw materials to prepare an appropriate drug, free of non-wanted ingredients.

The constant request of pediatricians and neonatologist from our pediatric hospital for UDCA treatment adapted for babies before 56 days of life has led us to develop this new formulation, all the more so as very few oral suspensions have been developed with this active ingredient. Despite the use of an universal suspension vehicle, the development of the formula was challenging. Indeed, the low solubility of the active ingredient forced us to adapt the formulation. In practice, we tested different concentrations and add an excipient to facilitate UDCA resuspension.

The second and most challenging step of our project was to develop an assay to analyze this absorbance molecule with HPLC-DAD, the only available material in our lab. The bibliography concerning the dosage of UDCA using an HPLC coupled with DAD is poor, and many attempts were necessary to optimized and validate our analytical method.

This work has led to significant changes in our practices. The production campaigns are more easily planned, with less risk of stock shortage because there is only one dosage left. After a need assessment, it was defined that one batch would represent 600 mL of suspension, distributed in 10 bottles of 60 mL. The switch from capsules to drinkable suspensions brings an appropriate response to the requests of the medical professionals as well as for the caregivers who noticed a time saving.

Three months after providing the neonatal department with our new formulation, the feedbacks are highly positive. One batch has been made monthly since the beginning of the production and demand increases. Requests originate mainly from our neonatal care service and from home hospitalization, which takes care of patients leaving this unit. Besides, another hospital, for which our pharmacy is used to subcontracting, has also made their own request, without any information given on the availability of this product.

Conclusion

This work led to a new galenic formulation with low dosed oral form of UDCA at 50 mg/ml appropriate for the treatment of infants. Since this drug is used in hospital, it already has very positive implications for the treatment of neonatal jaundice. It required substantial work upstream in order to be able to assess its feasibility and prove its stability. Although the addition of universal suspension adjuvants improves palatability, evaluation of sedimentation and agglomeration must be systematically performed at all pre-formulating stages. This study shows that an appropriate development results in a formulation suitable for the caregivers who requested it.

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