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Développement d'un véhicule de suspension pour formulations extemporanées pédiatriques

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Axe Formulation et Analyse du Médicament

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Résumé

L'utilisation de médicaments hors autorisation de mise en marché, comme la modification de la forme pharmaceutique, est une pratique courante chez les pharmaciens. Ceci est principalement dû au nombre limité de formulations pédiatriques disponibles, ce qui a conduit les pharmaciens à reformuler les produits commercialisés en préparations extemporanées mieux adaptées aux patients jeunes. Cependant, les véhicules de suspension actuellement sur le marché ne sont pas spécifiquement conçus pour l'usage pédiatrique. Nous avons donc émis l'hypothèse qu'il est possible de développer un véhicule de suspension plus sécuritaire pour l'utilisation pédiatrique. Ce véhicule devra être stable, permettre une remise en suspension aisée et permettre de masquer le goût de la formulation.

Après avoir passé en revue les méthodes et excipients actuellement utilisés pour les préparations extemporanées, la composition du véhicule a été élaborée. La version finale du véhicule présentait une viscosité de 85 cP à 25 °C et un pH de 4.5. La stabilité dans le temps de ces paramètres a également été évaluée. Une légère diminution de la valeur de la viscosité avec le temps a été rapportée pour les deux véhicules, mais le pH est resté constant.

Le goût joue un rôle important dans le développement d'une formulation pharmaceutique orale, en particulier en ce qui concerne l'adhésion au traitement par les enfants. Une étude de palatabilité a été menée dans deux groupes (adultes et enfants) pour comparer ce nouveau véhicule de suspension à la version USP. Les participants ont dû évaluer l'odeur, le goût, la sensation en bouche et l'appréciation globale des deux véhicules. Il a été constaté que le goût et l'appréciation générale du nouveau véhicule de suspension ont obtenu une évaluation plus élevée.
La caféine (10 mg/mL), l'hydrocortisone (1 mg/mL), la spironolactone (5 mg/mL) et le tacrolimus (0.5 mg/mL) ont été formulés extemporanément à partir de poudre, de comprimés ou de capsules dans un nouveau véhicule de suspension spécialement conçu pour un usage pédiatrique. La stabilité de ces suspensions a été évaluée dans des bouteilles en plastique ambré ainsi que dans des seringues en plastique ambré à 5 °C et à 25 °C. La caféine était stable pendant 30 jours dans toutes les conditions testées dans les

seringues et jusqu'à 180 jours dans les bouteilles. Pour l'hydrocortisone, la concentration est restée supérieure à 90% dans les bouteilles à 25 °C pendant 180 jours et pendant 90 jours pour les bouteilles à 5 °C. La concentration d'hydrocortisone mesurée dans les seringues était supérieure à 90% après 30 jours pour les deux températures. La spironolactone est restée stable à toutes les conditions pendant toute la durée de l'étude tant dans les bouteilles que dans les seringues. Les suspensions de tacrolimus ont été jugées stables pendant 180 jours entreposées à 5°C et 90 jours à 25°C dans les bouteilles. Lorsqu'entreposés dans des seringues, les suspensions ont été jugées inchangées pendant 14 jours à 5°C et 30 jours à 25 °C. Aucun changement des propriétés organoleptiques ou du pH n'a été observé pour les formulations, à l'exception d'un léger changement de couleur pour la formulation de spironolactone.

Mots-clés: oral, liquide, suspension, solution, pédiatrique, stabilité, caféine, gout, palatabilité, hydrocortisone, spironolactone et tacrolimus.

Abstract

The off-label use of drugs, such as modifying the formulation, is a common practice amongst pharmacists. This is mainly due to the small number of available pediatric formulations, resulting in pharmacists reformulating commercialized products into extemporaneous preparations better suited for young patients. However, the suspension vehicles currently found on the market are not specifically designed for the pediatric use. We therefore hypothesized that it is possible to develop a safer suspension vehicle for the pediatric use. This vehicle will need to be stable, allow a good resuspendability and have some taste masking properties.

After reviewing the currently used methods and excipients for extemporaneous preparations, the vehicle composition was elaborated. The final version of the novel vehicle presented a viscosity of 85 cP at 25°C and a pH of 4.5. The stability of these properties was also evaluated. A slight reduction of the viscosity was reported for both vehicles when stored at room temperature, but the pH remained constant. The developed vehicle was stable for 6 months in refrigerated conditions and at room temperature.

Taste has an important role in the development of an oral pharmaceutical formulation, especially when it comes to the children compliance to the treatment. A palatability study was conducted in two groups (adult and children) to compare this new suspension vehicle to the USP version. The participants evaluated the odour, taste, mouthfeel and overall appreciation of both vehicles. It was found that the taste and overall appreciation were rated higher for the newly developed suspension vehicle.

Caffeine (10 mg/mL), hydrocortisone (1 mg/mL), spironolactone (5 mg/mL) and tacrolimus (0.5 mg/mL) were formulated extemporaneously from commercially available tablets or powder in a novel suspension vehicle specially designed for pediatric use. The stability of these suspensions was evaluated in amber plastic bottles as well as amber plastic syringes at 5 °C and 25 °C. Caffeine was stable for 30 days at all tested conditions in syringes and up to 180 days in bottles. For hydrocortisone, the concentration remained over 90% in bottles at 25°C for 180 days and 90 days for bottles at 5°C. In syringes, the measured concentration was over 90% after 30 days. Spironolactone remained stable at

all conditions for the whole duration of the study in both bottles and syringes. The tacrolimus suspensions were found stable 180 days in plastic bottles stored at 5°C and at least 90 days at 25°C. In syringes, they were stable 30 days when stored 25°C and 14 days at 5°C. No changes in organoleptic properties or pH were observed for the formulations, with the exception of a slight color change for the spironolactone formulation.

Keywords: oral, liquid, suspension, solution, paediatric, stability, caffeine, hydrocortisone, spironolactone and tacrolimus.

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Liste des abréviations

ADME : Absorption, Distribution, Métabolisme, Excrétion

AMM : Autorisation de mise en marché

API : *Active Pharmaceutical Ingredient* – Ingrédient pharmaceutique actif

BHE : Barrière hématoencéphalique

DLU: Date limite d'utilisation

FDA : *Food and Drug Administration* - Administration des aliments et médicaments

EMA : *European Medecines Agency* - Agence Européenne des Médicaments

HPLC : *High Performance Liquid Chromatography* - Chromatographie liquide haute performance

HPMC : Hydroxypropylméthylcellulose

LoQ : *Limit of Quantification* – Limite de quantification

MON : Mode Opératoire Normalisé

NIOSH : *National Institute for Occupational Safety and Health* - Institut national pour la sécurité et la santé au travail

PUMA : *Paediatric-use marketing autorisation* – Autorisation d'usage commercial pédiatrique

RPMQ : Registre des préparations magistrales du Québec

SIM : *Stability indicating method* – Méthode indicatrice de stabilité

USP : *United States Pharmacopeia* – Pharmacopée des États-Unis

UV : Ultraviolet

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Chapitre 1: Introduction

1.1 Mise en contexte

Le développement et la mise en marché d'un médicament est un processus complexe et très réglementé. Une fois développées, la molécule ainsi que sa formulation doivent être évaluées lors d'études cliniques représentant les conditions d'utilisation futures. Une autorisation de mise en marché (AMM) est ensuite émise pour les indications étudiées lors des phases cliniques. Puisque les études cliniques sont généralement effectuées sur une population adulte, peu de formulations pour enfants sont présentement disponibles sur le marché. Les compagnies pharmaceutiques n'ont pas à produire d'informations sur l'innocuité ou l'efficacité d'une molécule pour la population pédiatrique si la population adulte est celle visée. Bien souvent, les médicaments approuvés chez l'adulte sont utilisés en pédiatrie faute d'autres options et ce, malgré un manque flagrant d'informations sur leur utilisation chez l'enfant[1],[2]. Cette pratique expose les cliniciens et les patients à des délais, coûts et risques supplémentaires lors de la préparation des formulations extemporanées. Tout cela pourrait être évité en incluant des sujets d'âge pédiatrique lors de certaines étapes du développement d'une nouvelle molécule à usage thérapeutique. Les différentes autorités gouvernementales adoptent présentement de nouvelles mesures afin de pallier à ce problème, comme par exemple le projet PUMA (paediatric-use marketing authorization) en Europe.

1.1.2 Différences physiologiques entre la population pédiatrique et adulte

Auparavant, lorsqu'un médicament pour adulte était donné à un enfant, la dose était simplement ajustée par rapport au poids de l'enfant[3]. Il est devenu clair que les enfants ne sont pas de petits adultes. Plusieurs différences physiologiques expliquent pourquoi les enfants répondent différemment des adultes à certains traitements pharmacologiques pris oralement ou autrement. Ces différences peuvent être classées en 4 catégories; l'absorption, la distribution, le métabolisme et l'élimination (ADME). Pour ce qui est de l'absorption, la première différence se situe au niveau de l'estomac. À la naissance, le pH

est pratiquement neutre (6-8). Il chute à environ 1-3 dans les 24 premières heures suivant la naissance pour revenir progressivement à la neutralité au jour 10. Il diminue ensuite lentement pour atteindre les valeurs des adultes[4]. Vient ensuite le transit intestinal où d'autres différences peuvent être remarquées au niveau des transporteurs membranaires responsables de l'absorption. Certains de ces récepteurs sont sous-exprimés dû à l'immaturité du système digestif des enfants en bas âge, ce qui limite la biodisponibilité orale de certains médicaments[5].

Une fois absorbé, le médicament est distribué à travers le corps en fonction de ses propriétés physicochimiques. À la naissance, la barrière hématoencéphalique (BHE) n'est toujours pas totalement mature et les médicaments peuvent accéder au système nerveux central causant une possible toxicité. Comme le cerveau est disproportionné chez les jeunes enfants, ce facteur, combiné à l'immaturité de la BHE, cause une distribution différente du médicament à travers le corps[6]. Un autre facteur influençant la distribution est le taux de liaison aux protéines plasmatiques. Fréquemment, la fraction non liée est plus élevée chez les nouveau-nés et les nourrissons pour plusieurs raisons. Premièrement, la concentration des protéines de liaison est moindre que chez l'adulte. De plus, ces protéines sont qualitativement différentes et ont généralement des capacités de liaison plus faibles, en particulier chez les nouveau-nés[7].

Avant même d'être distribué, le médicament est métabolisé par le foie lors du premier passage hépatique. Le flux sanguin et les enzymes du foie métabolisant les médicaments sont réduits chez les enfants, ce qui réduit la clairance hépatique[8]. La demi-vie de différentes molécules en fonction de l'âge est représentée dans le tableau suivant à titre comparatif :

Tableau 1. Comparaison des temps de demi-vies des substrats des principales voies de métabolisme selon l'âge. [Traduit de (20)]

Isoenzyme	Substrat	Nouveau-né	Nourrisson	Enfant	Adulte
CYP1A2	Caféine	95	7	3	4
	Théophylline	24-36			3-9
CYP 2C9	Phénytoïne	30-60	2-7	2-20	20-30
CYP2C19	Phénobarbital	70-500	20-70	20-80	60-160
	Diazépam	22-46	10-12	15-21	24-48
CYP3A	Carbamazépine	8-28	–	14-19	16-36
	Lidocaïne	2.9-3.3	–	1-05	1-2.2

Les médicaments métabolisés ou inchangés sont ensuite excrétés par les reins sous forme d'urine. Un taux d'élimination plasmatique similaire ou supérieur à celui des adultes a été observé chez les enfants en bas âge pour de nombreux médicaments[9]. Par conséquent, de plus grandes doses pour ces médicaments sont nécessaires chez les enfants afin d'atteindre les mêmes concentrations plasmatiques que chez les adultes. Les causes des faibles rapports concentration-dose plasmatiques chez les nourrissons et les enfants sont variables et peuvent être dues à un certain nombre de phénomènes liés à la fonction rénale (comme une augmentation de la capacité de sécrétion tubulaire) ou la liaison plus faible aux protéines plasmatiques.

Ce ne sont là que quelques-unes des différences répertoriées dans la littérature. De nombreuses autres études sont nécessaires afin de répertorier toutes les disparités encore non listées.

Puisque la croissance et le développement constant d'un enfant ajoute une variabilité supplémentaire à toutes ces différences, il est davantage nécessaire d'offrir un traitement personnalisé au patient.

1.1.3 Études cliniques chez la population pédiatrique

Jusqu'à récemment (Paediatric Research Equity Act, 2003, USA), il était considéré comme non-éthique d'inclure des sujets mineurs lors des études cliniques requises pour l'AMM [10]. L'Europe est présentement la référence en ce qui a trait aux lignes directrices à utiliser pour la population pédiatrique. En 2007, l'European Medecines Agency (EMA) a créé le Règlement pédiatrique Européen dont l'objectif était d'améliorer la santé des enfants en Europe en facilitant le développement et la disponibilité des médicaments pour cette population. Depuis, de nombreuses autres recommandations ont été émises. Au Canada, un comité d'experts sur les produits thérapeutiques pour les nourrissons et les enfants a été créé en 2009 afin d'aviser à Santé Canada sur le développement, la délivrance de permis et le suivi post-approbation des médicaments[11]. Bien que les progrès aient été lents, les essais cliniques pédiatriques ont connu une croissance avec la reconnaissance internationale de l'importance des essais chez les enfants [12].

Ces études présentent plusieurs difficultés méthodologiques, comme le recrutement nécessitant l'accord parental, une population hétérogène divisée en plusieurs sous-groupes d'âge ainsi que des sujets peu coopératifs et en constant développement. Les autorités gouvernementales et différentes agences de réglementation reconnaissent maintenant la nécessité d'obtenir davantage d'informations sur l'innocuité et l'efficacité des médicaments chez les enfants et encouragent donc la conduite d'essais cliniques pédiatriques, mais avec une plus grande surveillance réglementaire et éthique que celle prescrite pour les essais cliniques chez les adultes [13]. Malgré tout, le nombre de médicaments qui obtiennent une indication pour usage pédiatrique au Canada reste faible[5].

1.1.4 Pratiques et recommandations en pédiatrie

Face à ce manque, les pharmaciens n'ont d'autres choix que de fournir aux enfants un médicament pour adulte. L'utilisation des médicaments hors indication, c'est-à-dire en dehors des usages listés sur la monographie du produit et pour lesquelles il y a eu des essais cliniques, est donc une pratique très répandue en pédiatrie [14]. Souvent, le

médicament disponible est reformulé en préparation magistrale liquide orale afin de répondre aux besoins spécifiques du jeune patient [15].

Figure 1. Schéma de fabrication d'une préparation magistrale

Les comprimés adultes sont triturés puis mélangés à un véhicule de suspension. Cette forme liquide est davantage appréciée par les enfants en bas âge[16],[17]. La formulation orale liquide présente plusieurs autres avantages; ajustement de la dose, administration plus facile, simple à réaliser et personnalisation possible (ajout de saveur).

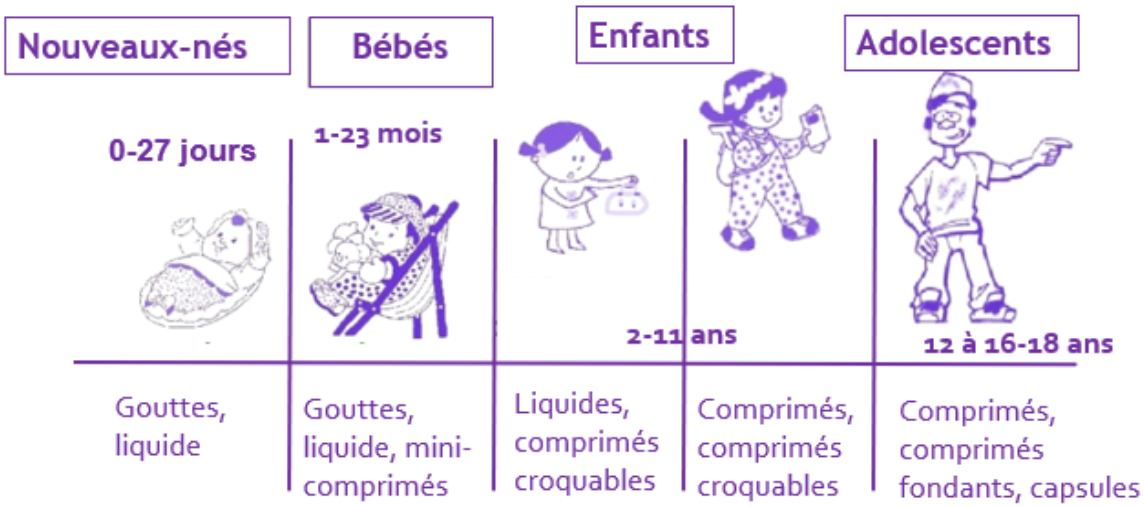

Figure 2. Formulations préférées en fonction de l'âge [Traduite et modifiée de (17)]

Cependant, les véhicules de suspension utilisés pour préparer ces formulations extemporanées n'ont pas été précisément conçus pour les enfants. En effet, les listes d'excipients "generally recognized as safe" (GRAS) ou "Inactive Ingredients Guide" (IIG) de la FDA sont basées principalement sur des études réalisées chez l'adulte[18],[19]. Peu de données sont présentement disponibles sur la sécurité de ces excipients chez l'enfant. Il faut donc choisir les excipients d'une formulation pédiatrique avec une précaution accrue.

Vu le manque flagrant d'informations sur la sécurité de nombreux excipients chez l'enfant, il est important de limiter toute exposition inutile[11, 12, 20]. Les enfants sont en constant développement et leurs caractéristiques physiologiques différentes de celles des adultes ont un impact sur l'absorption, la distribution, le métabolisme et l'élimination des médicaments [15]. De plus, les processus d'ADME varient entre les différents groupes d'âge; nouveau-nés prématurés; nouveau-nés (0–27 jours); bébés (1–23 mois); enfants (2–11 ans); et adolescents (12–16 ans aux U.S ou 12–18 ans dans l'Union européenne) [21]. La perméabilité intestinale, la fonction rénale, l'expression des différents transporteurs membranaires ainsi que le métabolisme hépatique ne sont que quelques-unes des différences majeures qui existent. Puisqu'ils sont en continuel développement, la pharmacocinétique et pharmacodynamique sera également en constant changement; d'où

le besoin d'avoir une formulation appropriée et ajustée pour chaque enfant selon son âge, son poids et sa condition [15].

Il est connu que le goût des enfants diffère de celui des adultes [22]. Les enfants préfèrent davantage les goûts surs et sucrés et ont généralement une aversion marquée pour l'amertume. Pour cette raison, une attention accrue devrait être accordée à la palatabilité des formulations pédiatriques.

Lorsqu'une formulation pédiatrique est disponible, elle sera souvent sous forme liquide comme une solution ou une suspension [19]. Une des différences entre ces deux formulations est que le principe actif est dissout dans la solution alors qu'il ne l'est pas dans la suspension. Il faut donc s'assurer de bien redistribuer ce principe actif en agitant les suspensions avant de prendre une dose. Il y a récemment eu une augmentation du nombre de formulations pédiatriques suite aux nombreuses incitations des autorités réglementaires. Les formulations tels que les films orodispersibles, les granules, les minicomprimés croquables et gouttes concentrées sont maintenant disponible pour les enfants. Les innovations ne se limitent pas qu'à la voie orale, des formulations transdermales, pulmonaires, oculaires et injectables sont également développées [19]. De nouvelles réglementations et recommandations sont implantées afin de répondre aux nombreuses questions demeurant sans réponses considérant les pratiques en pédiatrie [23].

1.2 Véhicules de suspension

1.2.1 Définitions et normes

Les suspensions sont des systèmes hétérogènes constitués de deux phases[24], dans ce cas une phase particulaire solide insoluble dispersée dans une phase aqueuse liquide. La phase aqueuse est le véhicule de suspension. Une solution peut également résulter du mélange dans le cas où tous les excipients et principes actifs contenus dans le comprimé ou la capsule sont solubles à la dose voulue. Il arrive qu'un ou des agents solubilisant soit ajoutés à la composition du véhicule de suspension afin d'augmenter la solubilité des actifs suspendus. Ce n'est toutefois pas toujours nécessaire.

Il est attendu d'un véhicule de suspension qu'il donne un mélange stable physiquement, chimiquement et microbiologiquement[25]. Pour ce faire, la formulation contiendra normalement un agent de suspension qui lui conférera une certaine viscosité et qui évitera la sédimentation trop rapide des particules en suspension. Les suspensions doivent cependant être facilement redispersées suite à la sédimentation. Un véhicule de suspension devrait également contenir un système de sels tampons qui régulera le pH de la formulation. Selon le chapitre 51 de l'USP (United States Pharmacopeia), un agent de conservation est requis pour éviter la prolifération de micro-organismes lorsqu'une formulation orale liquide est contenue dans un contenant multidose. Le véhicule de suspension devrait idéalement masquer le goût amer du principe actif qu'elle contient. Une méthode simple et efficace est l'ajout d'un édulcorant dans la composition[26].

Il existe sur le marché plusieurs véhicules de suspension tels qu'Ora-Blend, Oral Mix, le sirop simple, etc. Ces options n'ont cependant pas spécifiquement été conçues pour l'usage pédiatrique du point de vue des excipients. Il existe également des véhicules de suspension en poudre pouvant être préparés de manière extemporanée au moment désiré tel que Dry Alka (Medisca).

À notre connaissance, très peu de documents relatent le développement et l'évaluation de véhicules de suspension. La plupart du temps, une formulation contenant un principe actif est mise au point et une étude de stabilité s'en suit. Cependant il existe une équipe ayant poussé l'étude de leur formulation un peu plus loin. *Helin-Tanninen et al.* ont développé une formulation avec différentes concentration de nifédipine. Ils ont ensuite évalué la stabilité chimique et microbiologique ainsi que l'uniformité de dose de ces formulations[27]. Une étude de stabilité a ensuite été lancé une fois la suspension jugée satisfaisante[28]. Les objectifs de cette étude ressemblent beaucoup à ceux du présent document. Cependant, nous n'incluront aucun principe actif lors des évaluations préliminaires du véhicule de suspension. Nous obtiendrons ainsi un véhicule de suspension auquel plusieurs principes actifs pourront être ajoutés afin d'obtenir une formulation stable, sécuritaire et efficace.

1.2.2 Formulation et innocuité des excipients

Pour ce qui est du véhicule de suspension développé dans ce projet, seuls les excipients essentiels seront présents dans la composition. Il est important de minimiser le nombre et la quantité d'excipients pour faciliter la fabrication, minimiser les interactions, réduire les coûts et naturellement réduire la toxicité et limiter toute exposition inutile. Idéalement, des excipients qui ne sont pas ou peu absorbés devraient être employés. Un agent épaississant est nécessaire afin d'améliorer la stabilité physique d'une suspension et d'assurer ainsi l'homogénéité du produit en augmentant sa viscosité[29]. L'hydroxypropylméthylcellulose (HPMC) a été choisie pour remplir ce rôle. L'HPMC est un dérivé de la cellulose chimiquement inerte et n'est pas absorbé par la voie orale[30] ,[31].

Même si ce type d'additif n'est pas recommandé dans les préparations pédiatriques[32], un agent de conservation devra être utilisé pour prévenir la prolifération de micro-organismes. Le bicarbonate de sodium, l'acide propionique et sa forme saline, le propionate de calcium, ont été choisis car ce sont des substances endogènes aux propriétés antimicrobiennes[33],[34].L'oxyde de zinc a également été testé pour les mêmes raisons[35].

En raison de l'amertume aversive de la plupart des principes actifs, un édulcorant sera ajouté à la composition pour améliorer le goût des suspensions. Le goût et la palatabilité d'une formulation devraient être agréables. Ces paramètres sont critiques dans l'acceptabilité du produit final par le patient et son adhésion au traitement[36]. Le sucralose a été choisi pour remplir ce rôle. Cet édulcorant est 600 fois plus sucré que le saccharose, mais n'affecte pas la glycémie et est non calorique. Il a fait l'objet d'études approfondies chez les adultes et les enfants et ne présente aucun risque pour la santé[37], d'autant plus que la dose journalière admissible ne devrait pas être dépassée en usage normal[38].

Le véhicule de suspension sera tamponné à l'aide d'un tampon citrate pour assurer que le pH des suspensions reste constant car cela pourrait affecter la stabilité des suspensions composées[39]. Le tapon citrate est un sel régulièrement utilisé dans les préparations

pharmaceutiques. Vu son occurrence naturelle dans le corps humain, il ne présente aucun risque pour la santé lorsqu'il est utilisé dans les dosages recommandés[40].

1.2.3 Caractérisation physicochimique

Différentes compositions de véhicule de suspension seront développées et leurs paramètres seront évalués afin de s'assurer de leur efficacité. Les aspects rhéologiques du véhicule et sa capacité à suspendre adéquatement un ingrédient actif sont des paramètres importants qui seront étudiés. La viscosité, le comportement rhéologique ainsi que le volume de sédimentation et la capacité de remise en suspension seront mesurés.

L'équation de Stokes exprime le taux de sédimentation des particules en suspension dans un liquide dans le temps :

Équation de Stokes : $\frac{dx}{dt} = \frac{d^2(\rho_i - \rho_e)\mathrm{g}}{18\eta}$

Cette équation estime le taux de sédimentation en fonction des caractéristiques physiques du véhicule de suspension. Ces caractéristiques incluent le diamètre des particules en suspension (d), la densité des particules (ρ_i) et celle de la phase externe (ρ_e), l'accélération due à la gravité (g) ainsi que la viscosité du véhicule (η) [41]. Certaines de ces variables sont elles-mêmes également dépendantes d'autres facteurs, comme la température, qui peuvent influer sur la vitesse de sédimentation. En supposant que la taille des particules et la température soient constantes, l'équation ne laisse qu'un facteur pouvant être affecté; la viscosité. Cependant, si le véhicule est de l'eau, cette hypothèse n'est plus valide. L'eau a une viscosité constante et demeure la même, même si elle est agitée ou mélangée, comme tous les fluides newtoniens. Néanmoins, la viscosité d'une solution aqueuse est affectée par la quantité et la nature des particules solides ajoutées à sa composition[42]. C'est pourquoi différents agents épaississants seront testés, afin d'obtenir un véhicule de suspension aqueux avec des propriétés rhéologiques adéquates. Un véhicule de suspension devrait donner des formulations homogènes lorsque mélangé à un principe actif. On ne devrait idéalement pas observer de sédimentation rapide du principe actif, mais si le cas est, celui-ci devrait être facilement remis en suspension. La viscosité du véhicule de suspension ne devrait également pas empêcher de manipuler

aisément la formulation lors des mesures de dose ou du transfert d'un contenant à un autre.

L'osmolalité est un autre paramètre important d'une suspension orale puisque celle-ci peut influencer la tolérabilité gastro-intestinale[43]. La concentration en particules dissoutes dans une solution est exprimée en osmole de soluté par kilogramme de solvant et est appelée "osmolalité". Dans le plasma humain, l'osmolarité est de 290 mOsm / L (285 - 310 mOsm / L)[44]. Celle-ci sera déterminée et ajustée autour du point iso-osmotique afin d'éviter les problèmes dus à l'osmolalité et de convenir à un possible usage chronique.

Le pH est un facteur majeur influençant la stabilité d'un principe actif. La dégradation des médicaments se produit souvent par hydrolyse, qui peut se produire plus facilement à certains pH. Des profils de dégradation en fonction du pH sont généralement produits afin de déterminer le pH auquel un médicament est plus sensible à la dégradation. Le pharmacien peut utiliser ces profils pH /stabilité pour déterminer le pH qui assurera la stabilité maximale de la préparation[45]. Un système tampon est utilisé pour maintenir le pH pendant la durée de vie prévue de la préparation. Le pH des véhicules développés sera ajusté à 4.5 et 7.5 afin de convenir à un maximum de principes actifs, qu'ils soient stables en milieu acide ou alcalin[37].

Tous les paramètres précédemment mentionnés seront mesurés et comparés à ceux des véhicules de suspension trouvés commercialement. Des valeurs similaires seront établies afin de s'assurer de l'efficacité du produit final.

1.2.4 Évaluation de la palatabilité

Un véhicule de suspension doit avoir un goût, une odeur et une texture agréables afin de masquer le goût amer qu'ont la plupart des ingrédients actifs[46].

La palatabilité est l'un des principaux facteurs qui influent sur l'acceptabilité d'un médicament par voie orale. Il est crucial qu'elle soit plaisante pour l'adhérence au traitement, en particulier chez les enfants. L'évaluation de la palatabilité devrait être une étape critique dans le développement d'une formulation orale[47],[48].

La palatabilité est définie comme l'appréciation globale d'un médicament pris oralement et de ses propriétés telles que l'apparence, l'odeur, le goût, l'arrière-goût et la sensation en bouche[49].

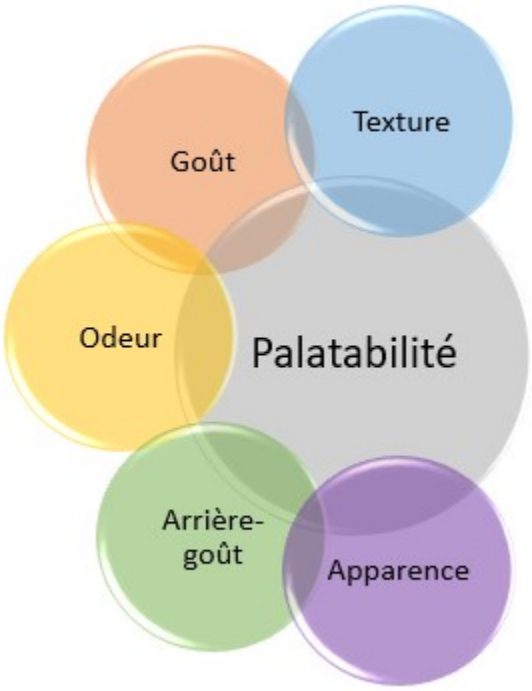

Figure 3. Définition de la palatabilité

Ces caractéristiques seront évaluées par des participants adultes et enfants. L'inclusion des enfants dans cette évaluation a été jugée essentielle car il est bien connu que les préférences gustatives des enfants diffèrent de celles des adultes[50]. Différentes méthodes peuvent être employées pour l'évaluation de la palatabilité comme des tests animaux, des méthodes analytique et/ou in vitro (e-tongue). La langue électronique n'est toujours pas entièrement optimisée et certaines propriétés comme la sensation en bouche et l'arrière-goût ne peuvent pas être évaluées par cet instrument. Les évaluations animales restent subjectives et il est difficile de comparer différentes formulations similaires. L'évaluation humaine est la méthode privilégiée pour déterminer la palatabilité d'une formulation, malgré quelques difficultés [41]. En effet, le goût peut être différemment perçu selon le sujet.

1.3 Stabilité des formulations avec principes actifs suspendus

1.3.1 Définition

Un des problèmes que l'on retrouve avec les formulations extemporanées est l'absence de données sur la stabilité de celles-ci. Une date limite d'utilisation (DLU) est fournie pour chaque produit commercial. Cependant, le produit commercial est altéré lorsque reformulé en préparation extemporanée. Une nouvelle date limite d'utilisation doit être déterminée en fonction du principe actif utilisé, des excipients ajoutés, des conditions d'entreposage, du mode de préparation, etc.

Pour ce faire, les modes opératoires normalisés (MON) de la plateforme de biopharmacie sont utilisés. Ce sont les critères généralement utilisés pour évaluer la stabilité des préparations magistrales et ils sont basés sur les lignes directrices de l'ICH. Durant toute la durée de l'étude de stabilité, l'apparence des formulations ne doit pas changer. Si une sédimentation des particules en suspension est observée, elle doit être facilement redispersée manuellement. Le pH initial des formulations ne doit pas changer de plus qu'une unité de pH. La concentration du principe actif doit demeurer au-dessus de 90% de la valeur initiale. Afin d'évaluer ce dernier critère de stabilité, une méthode chromatographique liquide à haute performance (HPLC) appropriée pour chaque principe actif a été développée et validée.

1.3.2 Principes actifs étudiés

Différents principes actifs seront donc étudiés afin de produire des données de stabilité sur les formulations développées en utilisant le nouveau véhicule de suspension. Ces données faciliteront l'implantation du nouveau véhicule de suspension en milieu hospitalier et communautaire.

Les principes actifs évalués sont: caféine, hydrocortisone, spironolactone et tacrolimus suite à la demande du centre hospitalier pour enfants Ste-Justine. Les concentrations des formulations sont basées sur les pratiques standards de la communauté des pharmaciens hospitaliers américains [51],[52].

La caféine est un méthylxanthine largement utilisé dans le traitement de l'apnée chez les prématurés en première ligne d'intention[53],[54]. La dose de maintien actuelle recommandée par la Food and Drug Administration (FDA) est de 5 mg / kg de caféine base, une fois par jour, sous forme liquide orale ou intraveineuse[55]. Des formulations commerciales sont maintenant disponibles, mais elles ne sont pas approuvées dans tous les pays. Les formulations extemporanées faites à partir d'excipient en poudre sont encore couramment trouvées dans la pratique[56]. Des solutions de caféine ayant une stabilité comprise entre 3 et 6 mois ont été rapportées dans des conditions ambiantes et réfrigérées dans différents véhicules de suspension[57],[58].

Pour les patients d'âge pédiatriques ou néonatals, l'hydrocortisone est utilisée par voie orale comme première ligne de traitement pour l'hyperplasie surrénale congénitale ainsi que beaucoup d'autres conditions. La posologie recommandée dépend de la condition et est ajustée par rapport au poids de l'enfant[59,60]. Commercialement, ce médicament peut être trouvé sous forme de comprimés, une forme posologique inappropriée pour les nouveau-nés, nourrissons et jeunes enfants. Une forme galénique liquide est donc nécessaire. Les pharmaciens doivent préparer une formulation extemporanée à partir du produit disponible dans le commerce en utilisant un véhicule de suspension[61]. Des suspensions avec une DLU entre 4 semaines et 3 mois à température ambiante et réfrigérée ont été formulées avec les véhicules de suspension Ora-Plus et Ora-Sweet SF [62],[63]. Une formulation préparée à partir d'excipients en poudre a été décrite avec une stabilité de 30 jours à 5 °C et 25 °C [64].

La spironolactone est un diurétique fréquemment utilisé pour traiter l'hypertension chez les nouveau-nés et les enfants et est également utilisée pour l'insuffisance cardiaque [65]. Comme il n'y a pas de produit homologué sous forme liquide orale, les préparations extemporanées sont souvent préparées à partir de comprimés triturés afin de mieux répondre aux besoins des patients pédiatriques. Plusieurs formulations extemporanées peuvent être trouvées dans la littérature et leur stabilité a également été évaluée. Une suspension de spironolactone fait avec du sirop de cerise s'est avérée avoir une DLU d'au moins 4 semaines à 30 °C[66]. Une étude a également rapporté une stabilité d'au moins 90

jours pour des suspensions de spironolactone lorsqu'elles étaient entreposées à température réfrigérée et à température ambiante[67]. Une autre étude similaire a évalué des suspensions de spironolactone dans Oral Mix entreposé à 5 °C et 25 °C pendant une période de 90 jours et a constaté qu'elles étaient chimiquement stables[68].

Le tacrolimus est un agent immunosuppresseur utilisé pour prévenir et traiter le rejet d'organes transplantés[69]. Le tacrolimus est disponible dans le commerce sous la forme de capsules de 0,5, 1 et 5 mg pour administration orale, dans des ampoules contenant 5 mg / ml de tacrolimus pour injections intraveineuses et sous forme topique[70]. Aucune formulation posologique liquide orale n'est disponible dans le commerce ou décrite dans la littérature. Par conséquent, le pharmacien ou le soignant doit mélanger le contenu des capsules avec un liquide avant l'administration. Cette préparation requiert de suivre les précautions émises par le National Institute for Occupational Safety and Health (NIOSH). La stabilité de certaines de ces suspensions a été étudiée. Une stabilité d'au moins 56 jours a été rapportée pour une suspension extemporanée faite avec des quantités égales d'Ora-Plus et de sirop simple NF lorsqu'elle est conservée à 24-26 °C dans des flacons de verre ou de plastique ambré[71]. Un autre groupe a démontré que le tacrolimus formulé sous forme de suspension à 1 mg / ml avec quantités égales d'Ora-Plus et d'Ora-Sweet était stable pendant au moins 4 mois à la température ambiante[72].

Ces formulations ont été préparées en utilisant des véhicules à suspension qui n'étaient pas spécialement conçus pour l'utilisation pédiatrique. De plus, la concentration, les conditions de stockage et les contenants varient d'une étude à l'autre. Compte tenu de la variation des préparations, nous avons mené une étude de stabilité de 6 mois à deux températures contrôlées (5 °C et 25 °C) dans des seringues en plastique ambré et des bouteilles en polyéthylène téréphtalate (PET). Toutes les formulations ont été préparées en utilisant le nouveau véhicule de suspension, spécialement conçu pour la sécurité des enfants.

1.4 Méthode HPLC et analyse

1.4.1 Définition

Les conditions environnementales (par exemple la température, l'humidité, etc.) qui varient pendant la fabrication, le transport, la distribution et le stockage de médicaments ont un grand effet sur la qualité du produit pharmaceutique. De plus, les impuretés et/ou les produits de dégradation de la formulation peuvent altérer l'effet pharmacologique de l'ingrédient actif[73].

Plusieurs paramètres sont observés pour déterminer la stabilité d'une formulation. La teneur ou concentration en principe actif est sans aucun doute l'un des plus importants. Les appareils HPLC sont fréquemment utilisés pour déterminer la concentration d'une molécule dans un échantillon donné.

Un appareil HPLC est normalement constitué d'une pompe, d'un autoéchantillonneur tempéré, d'un four à colonnes, d'une valve solénoïde, d'une colonne HPLC, d'un dégazeur ainsi que d'un détecteur UV (Ultra-violet) ou à fluorescence. La pompe sert à fait migrer les phases mobiles utilisées dans l'élution du composé étudié et crée une pression suffisante pour faire éluer l'injection au travers de la colonne HPLC. Cette colonne repose dans un four où il est possible de faire varier la température. Ce paramètre, comme la pression causée par le débit, peut faire varier le temps de rétention du composé à l'étude. Une fois le composé élué, l'intensité du pic est mesuré par le détecteur. Toutes ces composantes sont reliées entre elles par un réseau de tubulures comportant des valves empêchant le retour des phases mobiles. Les phases mobiles se mélangent à la solution analysée une fois que celle-ci est prélevée et injectée dans le système. Les phases mobiles peuvent être mélangées et dégazées manuellement ou automatiquement à l'aide de la valve solénoïde et du dégazeur. Les composantes de l'échantillon analysé sont séparées en fonction de leurs propriétés physicochimiques et de leur affinité avec la phase mobile par une colonne HPLC. L'intensité de chaque pic est ensuite mesurée par un détecteur UV. Un faisceau de lumière d'une longueur d'onde préalablement déterminée est concentré au travers du tubule et est ensuite redirigé vers le détecteur. En fonction du nombre de molécules passant dans la tubule, l'intensité du faisceau de lumière varie[74]. La concentration de l'échantillon peut être connue à l'aide

d’une courbe de calibration dont la concentration des standards est connue. Pour chaque molécule ou famille de molécule, une méthode avec des conditions bien précises doit être optimisée.

Les agences réglementaires exigent actuellement que les méthodes d'analyse utilisées pour les produits pharmaceutiques soient des méthodes indicatrices de stabilité, c’est à dire spécifiques pour détecter non seulement le principe actif principal, mais aussi les impuretés et les produits de dégradation qui pourraient apparaitre pendant une étude de stabilité accélérée ou à long terme[75]. L’ICH et la FDA sont en accord pour ce qui est de la méthode analytique et offrent tous deux une certaine flexibilité dans le design de l’étude de stabilité. L’ICH accorde davantage d’importance aux conditions d’entreposage alors que la FDA dicte les essaies à effectuer lors des échantillonnages[76,75].

1.4.2 Validation

Après la mise en place initiale des conditions chromatographiques, la linéarité, la LoQ (Limite de quantification), la précision (répétabilité), la robustesse et la spécificité devront être évaluées afin de valider la méthode. La linéarité d’une méthode est atteinte si l’intensité du signal détecté augmente de manière linéaire avec la concentration du composé détecté. Il s’agit d’habitude d’un intervalle de concentration. La limite de détection est, comme son nom l’indique, la concentration minimale permettant la détection du composé par l’appareil. La robustesse est la capacité de la méthode de donner un résultat constant malgré de petits changements tels que l’usage de la colonne, les variations de la composition de la phase mobile ou les conditions atmosphériques ambiantes. La précision est quant à elle la répétabilité de la méthode. Ce paramètre est mesuré par le coefficient de variation de mêmes injections répétées. La spécificité des méthodes indicatrices de stabilité (SIM) est validée lors de tests de dégradation forcée, qui ont comme principal objectif d'évaluer la pureté des pics du principe actifs et celles des produits de dégradation. La méthode analytique obtenue de cette manière sera en mesure de détecter de telles substances si elles apparaissent pendant les études de stabilité[77].

La dégradation forcée est effectuée dans des conditions plus rudes que celles utilisées dans une étude de stabilité accélérée. Les procédures de laboratoire devraient provoquer la dégradation du médicament dans des conditions spécifiques (acide, alcaline, lumière/photostabilité, oxydation et température).

1.5 Hypothèse

À la suite d'une revue de la littérature sur les méthodes de préparation de formulations extemporanées ainsi que sur la sécurité des excipients employés, nous pensons qu'il est possible de développer un nouveau véhicule de suspension mieux adapté à la population pédiatrique. Ce véhicule devra posséder une bonne palatabilité, une bonne redispersabilité, en plus d'être stable chimiquement, physiquement et microbiologiquement.

1.6 Objectifs

1. La première étape sera la définition et la mise au point du véhicule de suspension. Une revue de littérature servira à établir les principaux excipients utilisés et quelles sont leurs alternatives. Les différentes méthodes de formulation extemporanée et pratiques utilisés en pédiatrie seront également étudiées.

2. Une fois les excipients choisis, leur efficacité devra être prouvée. Les propriétés physicochimiques et microbiologiques seront évaluées pour s'assurer de la pertinence de chaque excipient.

2.1 Évaluation des propriétés physicochimiques : La viscosité, le volume de sédimentation, la redispersabilité et la stabilité de ces paramètres seront mesurés sur des solutions aqueuses contenant divers agents de suspension afin de déterminer la nature et la concentration de celui qui sera utilisé.

2.2 Évaluation de la stabilité microbiologique : Le véhicule devra passer le test USP <51> d'efficacité des agents de conservation. Un agent de conservation ou une combinaison d'agents de conservation devront être soumis au test d'efficacité antimicrobienne si la solution échoue sans leur présence.

2.3 Palatabilité : Le véhicule final devra avoir un goût et une texture agréable. Pour ce faire, différents édulcorants seront utilisés afin de trouver une combinaison qui rendra la palatabilité acceptable.

3. Stabilité chimique : Une fois la composition finale du véhicule déterminée, divers principes actifs seront suspendus dans celui-ci et leur stabilité chimique sera monitorée dans le temps. Des méthodes HPLC-UV seront développées et validées pour chaque principe actif.

Chapitre 2: Development of a safe and versatile suspension vehicle for paediatric use.

Part 1: Formulation development.

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2.1 Résumé en français

Le but de ce projet était de développer un nouveau véhicule de suspension spécialement conçu pour un usage pédiatrique. Une attention particulière a été accordée à la sélection des excipients en ce qui concerne leur sécurité ainsi que leur utilisation enregistrée en pédiatrie. Deux véhicules de suspension ont été conçus au pH neutre et acide.

Quelques compositions ont été déterminées et leurs propriétés physicochimiques ont été évaluées et comparées aux véhicules commerciaux actuels.

Tel que requis pour une formulation orale multidose, un test d'efficacité antimicrobienne a été réalisé selon le protocole USP. Différentes souches microbiennes ont été inoculées individuellement dans les différentes formulations testées. La concentration en micro-organismes a ensuite été mesurée au jour 7, 14 et 28.

L'acide propionique s'est révélé être un agent de conservation efficace contre toutes les souches testées à pH 4.5. Tous les agents de conservation testés à pH 7.5 ont échoué au test, une composition alcaline du véhicule n'a donc pas pu être établie.

La version finale du nouveau véhicule présentait une viscosité de 85 cP à 25 °C. Un comportement clair d'amincissement par cisaillement a pu être observé pour le véhicule développé. Ces propriétés ont été évaluées pour garantir une stabilité physique et une remise en suspension adéquates.

La stabilité de ces propriétés a également été évaluée. Une légère réduction de la viscosité a été rapportée pour les deux véhicules lorsqu'entreposés à température ambiante, mais le pH est demeuré constant. Le véhicule développé était stable 6 mois dans des conditions réfrigérées et à température ambiante. Le résultat est un véhicule de suspension prêt à l'emploi contenant un minimum d'excipients. Cet article est le premier d'une série de trois et détaille le développement de la formulation. Le second article détaille l'évaluation de la palatabilité du véhicule développé et la troisième porte sur les études de stabilité.

Mots-clés: formulation pédiatrique, formulation orale, suspension orale, véhicule de suspension, formulation extemporanée, préparation, liquide oral.

2.2 Résumé en anglais

The purpose of this project was to develop a novel suspension vehicle specially designed for pediatric use. Special care was accorded to the selection of the excipients regarding their safety as well as their recorded use in pediatrics. Two suspension vehicles were designed at a neutral and acidic pH.

A few compositions were determined, and their physicochemical properties were assessed and compared to the current commercial vehicles.

As required for a multidose oral formulation, an antimicrobial effectiveness test was conducted following the USP protocol. Different microbial strains were inoculated individually in the different tested formulations. The concentration of micro-organisms was then monitored on day 7, 14 and 28.

Propionic acid proved to be an effective preservative against all tested strains at pH 4.5. Every tested preservative failed the test at pH 7.5.

The final version of the novel vehicle presented a viscosity of 85 cP at 25°C. A clear shear-thinning behaviour could be observed for the developed vehicle. These properties were assessed to make sure that an adequate physical stability and resuspendability would be obtained.

The stability of these properties was also evaluated. A slight reduction of the viscosity was reported for both vehicles when stored at room temperature, but the pH remained constant. The developed vehicle was stable for 6 months in refrigerated conditions and at room temperature. The result is a ready to use compounding vehicle containing minimal excipients. This part is the first one of a three parts article and details the formulation development. The second part will detail the palatability evaluation of the developed vehicle and the third will address the stability studies of compounded pharmaceutical ingredients.

Keywords: pediatric formulation, oral formulation, oral suspension, suspension vehicle, extemporaneous formulation, compounding, oral liquid.

2.3 Introduction

[In this first article of a three-part series, we discuss the formulation development of suspension vehicle intended for compounding paediatric oral formulations. A palatability study and the stability studies of compounded drugs will be addressed in the second and third parts respectively.]

The off-label use of drugs for paediatric formulation is a very common practice amongst pharmacists (1). Off-label use includes prescribing a drug for unapproved indications or age group. The administration of extemporaneous formulations with untested bioavailability or stability is another off-label practice. Due to the small number of available pediatric formulations, pharmacists have no better options but to reformulate commercialized products into liquid extemporaneous preparations better suited for young patients (2). These preparations are often formulated from levigated commercial tablets or capsules combined with a suspension vehicle.

Suspensions are heterogeneous systems consisting of two phases (3), in this case, a solid insoluble particulate phase dispersed in a liquid aqueous phase. The liquid formulation offers a greater dose flexibility, an appreciated advantage when administered to a pediatric population. However, the suspension vehicles currently found on the market are not specifically designed for pediatric use. Children metabolism differs greatly from the adult one since they do not possess fully developed organs (4). Hence, the chronic administration of an active or non-active ingredient could result in a higher exposition than expected. The excipients used in a pediatric formulation must, therefore, be chosen with an increased cautiousness.

With the limited information on the safety of these excipients in children, it is important to limit any unnecessary exposition (5,6,7). Indeed, most of the excipient safety databases are based on studies including healthy adult volunteers. A special attention was given to this criterion for the selection of the excipients used in the composition of the novel vehicle as it could be used in a chronic treatment of a pediatric patient. Few guidelines are currently found on pediatric formulations, but all are in consensus to limit the number and quantity of excipients (8). Therefore, to comply with this guideline, a suspension vehicle intended for paediatric use should be water-based, flavor-free, dye-free and sugar-free and ideally

will not contain any surfactant or anti-foaming agent. Only the essential excipients should be included in the composition, such as a thickening agent to improve the physical stability of the suspension by increasing its viscosity (9). The microbiological stability of the aqueous vehicle is another essential parameter to investigate. Preservatives are frequently used to prevent the growth of microorganisms. This type of additive is not recommended in pediatric formulations (10). Nevertheless, any suspension vehicle needs to comply with the USP <51> requirements and pass a preservative effectiveness test if it is intended to be used in a multi-dose container. Recent concerns have been raised concerning the safety of parabens (11), a widely used preservative in pharmaceutical products. Methylparaben can be found in the composition of some of the most commonly used commercially available suspension vehicles. Safer options were explored as an alternative, such as sodium bicarbonate, propionic acid and its salt form, calcium propionate which are all endogenous substances with antimicrobial properties (12, 13). Zinc oxide was reported to be an efficient and non-toxic preservative and will also be tested (14).

Due to the aversive bitterness of most active pharmaceutical ingredients (API), a sweetening agent is often required to be added in the composition to increase the taste-masking properties of the suspension vehicle. The taste and palatability of a formulation should be pleasant. These parameters are critical in the patient acceptability of the final product and his compliance with the treatment, especially for children (15).

Finally, a suspension vehicle should be buffered to ensure that the suspension pH remains constant, as this parameter could affect the stability of the compounded suspensions (9).

With these constraints in mind, we set out to design a suspension vehicle that met the specific requirements of the pediatric population. Moreover, this vehicle had to be physically, chemically and microbiologically stable, allow a good resuspendability and display some taste masking properties. Our objectives were to develop such a suspension vehicle and assess its physicochemical properties and stability. In this study, the different requisites for an oral suspension were explored and addressed with a specific emphasis on the pediatric final usage.

2.4 Materials and methods

2.4.1 Materials

Sodium citrate dihydrate (≥99% FG), dibasic sodium phosphate (anhydrous), sucralose (≥98.0% HPLC), zinc oxide (ACS reagent, ≥99.0%), calcium propionate (99.0-100.5%), sodium chloride (anhydrous) and glucose (D-(+)-) were purchased from Sigma-Aldrich (St-Louis, MO, USA). Anhydrous citric acid (ACS certified), tryptone, polysorbate 80, dextrose (anhydrous, certified ACS) and dibasic potassium phosphate (certified ACS) were obtained from Fisher Chemical (Fair Lawn, NJ, USA).

Microcrystalline cellulose (Vivapur 101), carboxymethylcellulose sodium (high viscosity, NF) and sodium benzoate were purchased from Galenova (St-Hyacinthe, QC, Canada).

Carrageenan, xanthan gum, propionic acid and potassium sorbate were purchased from TCI America (Portland, OR, USA).

Methylparaben (NF) was obtained from Pharmascience Inc. (Montreal, QC, Canada).

Hydroxypropyl methylcellulose (HPMC, Methocel K100M) (Methocel K4M) were donated by Dow Chemical Company (Midland, MI, USA).

Soytone, granulated agar and polypeptone were purchased from Becton, Dickinson and co (Sparks, MD, USA).

Acetaminophen (tablets, 325 mg) and prednisone (tablets, 5 mg) were obtained from Apotex (Toronto, ON, Canada).

The reference, ready-to-use vehicles used in this study were Oral Mix SF (Medisca Pharmaceutique Inc., Montreal, QC, Canada), Ora Blend (Perrigo, Minneapolis, MN, USA) and simple syrup (Laboratoire Atlas Inc, Montreal, QC, Canada).

Staphylococcus aureus (ATCC No. 6538), Pseudomonas aeruginosa (ATCC No. 9027), Escherichia coli (DH5α), Candida albicans (ATCC No. 10231) and Aspergillus brasiliensis (ATCC No. 16404) were used. All strains were acquired from ATCC (Manassas, VA, USA) except for Escherichia coli which was kindly donated by Prof. Marc Servant (Faculty of Pharmacy, University of Montréal, Montréal, QC, Canada).

Milli-Q water (Synthesis A10 system, Millipore, Etobicoke, ON, Canada) was used in this study.

2.4.2 Excipient selection

A search on PubMed was performed using the cross-referenced keywords: oral AND liquid AND/OR suspension AND/OR solution AND/OR stability. Out of 439 papers, 38 were retained. After reviewing the currently used methods and excipients for extemporaneous preparations, the vehicle composition was elaborated. The principal excipients were selected and their safety assessed using the scientific literature as well as the STEP (Safety and Toxicity of Excipients for Paediatrics) database (16) and the GRAS (Generally Recognised As Safe) (17) databases. All of the excipients used in the composition of the vehicle have been evaluated and listed in the except for propionic acid which is currently under revision.

2.4.3 Compounding of the suspension vehicles

For the general practices in pediatric formulations, the guidelines of major regulatory agencies (Health Canada, EMA and FDA) were used as references. Compounding journals were also included.

For all solutions and developed formulations, the excipients were accurately weighed and tumbled in a closed container (50 tumbles over five minutes). They were then slowly sprinkled into one third of the final volume of heated water (80°C) under magnetic agitation to ensure an adequate dispersion of the HPMC. Once the excipients were solubilized, the remaining two thirds of cold water were added. The solution was stirred overnight before any further analysis.

The USP vehicle was prepared as described in the NF Monographs: Vehicle for Oral Suspension (18).

2.4.4 Physicochemical characterization

Viscosity and rheological behaviour

The apparent viscosity of the suspension vehicles was determined. Certified viscosity standards No. B200 and B2000 (Brookfield, Middleboro, MA, USA) were first measured at 25°C using different spindles (CP40, CP41, CP42 et CP51) to ensure the proper calibration of the rheometer (Brookfield LVDV-III ultra CP). All data were processed with the Rheocalc software (Brookfield). The spindle CP51 was selected for the measurements of the test samples.

For each measurement, the sample (500 µL) was added into the receptacle. After one minute of stabilization, the measurement was taken at 90% of torque. Three samples per batch were measured for three independent batches at both 5°C and 25°C.

Commercially available and USP suspension vehicles were also measured to establish the range of adequate viscosity. Their rheological behaviour was also evaluated by increasing the shear rate and observing the viscosity in function of this increment.

Different solutions of HPMC K100M and K4M at different concentrations (0.25%, 0.4% and 0.5% w/v) were then produced and evaluated to determine the adequate concentration of the suspension agent.

The remaining excipients, like the sweetening agent, preservatives and salt buffers, were thereafter added to these solutions to observe any impact on the viscosity.

pH

The pH was measured at room temperature using a pH meter (Accumet, model AP61, Fisher Scientific). Three samples for three batches were measured (n=9).

Osmolality

This parameter was evaluated in triplicate with a Micro-Osmette Automatic (model 5004, Precision System Inc, Natick, MA, USA). This apparatus measures the osmolality using

the freezing-point depression method. The osmometer was first calibrated using standard reference solutions of 100 and 500 mOsm/kg (Precision System Inc, Natick, MA, USA). 50 µL of test samples were placed in a 1.5-mL centrifuge tube and measurements were taken.

Density

The density was measured by precisely weighing 10.0 mL of the desired solution in a 10.0-mL graduated cylinder.

2.4.5 Microbiological stability

Grow media

The growth media used with each strain was prepared as described in the USP <51> (19).

Turbidimetry

An aliquot (1 mL) of each strain was quickly thawed and 100 µL were transferred in 15 mL of appropriate growth media. The tubes were then placed in a rotative incubator at 250 RPM and 37°C overnight. On the following day, a series of dilution was performed using growth media. The optical density (OD600) of each of these dilutions was measured at 600 nm using a UV spectrophotometer (Ultrospec 2100 pro, Biochorm LTD, Cambridge, England). Of each of these dilutions, 100 µL were transferred on a Petri dish containing 25 mL of the adequate growth media for each strain and were platted using sterile, 5-mm platting beads (Zymo research, CA, USA). This procedure was performed in triplicate. The Petri dishes were then incubated at 30°C for 24-48h until all colonies had appeared and were of adequate size for counting.

Preservative effectiveness test

The USP <51> Preservative effectiveness test protocol was used. Each strain was grown in the proper growth medium. The culture tubes were placed in a rotative incubator at 250 RPM and 37°C overnight. On the following day, the OD600 was measured every hour until the concentration of microorganisms reached 1x108 CFU/mL (Colony Forming Unit). The concentration was confirmed by the plate count technique as mentioned above.

A quantity of 500 µL of inoculum was added to 50 mL of the tested formulations to obtain a concentration between 105 and 106 CFU/mL.

For Aspergillus brasiliensis, the samples were diluted using a saline solution containing 0.1% v/v of polysorbate 80. Of each of these dilutions, 100 µL were transferred on a Petri dish and quantified as described in the turbidimetry section. This procedure was performed in triplicate for each independent batch.

The positive control was the adequate growth media without preservatives.

The negative controls were the solutions containing 0.1% of sodium benzoate or 0.1% methylparaben and 0.1% potassium sorbate.

All formulations were tested in triplicate. The formulations were stored at room temperature in 60 mL amber plastic bottles (PolyEthylene Terephthalate with black phenolic cap, Medisca Pharmaceutique).

2.4.6 Sedimentation volume and redispersibility

Suspensions of acetaminophen (32 mg/mL) and prednisone (5 mg/mL) were compounded from commercial tablets of acetaminophen (325 mg, Apotex inc, Toronto, ON, Canada) or prednisone (5 mg, Apotex inc, Toronto, ON, Canada) using the final formulation of the developed vehicle, the 180-day-old final formulation or the USP vehicle. Three batches of 125 mL each (acetaminophen) and 100 mL each (prednisone) for each vehicle were evaluated.

These suspensions were produced by crushing tablets in a mortar with a pestle and by adding the suspension vehicle by geometric dilution. The suspensions were stirred and then transferred into a graduated cylinder. After 10 minutes, the initial volume of sedimentation (Vi) was measured using the graduation of the cylinder. The sedimentation volume was measured after 16h, 24h, 48h and 7 days. The final sedimentation volumes (Vf) were compared to the total volume of the suspension (Vsusp) and the percentage of sedimentation was then determined and compared between the two suspension vehicles using equation 1 (20):

$$\text{Equation 1: \% sedimentation} = (Vf / Vsusp) \times 100$$

Once the final measurements were taken (day 7), the redispersability was evaluated. The cylinders were slowly inverted by an inversion of 180°. The number of rotations required to resuspend the sedimented volume was noted. One rotation was considered as an inversion of 180° and then back to the initial position.

2.4.7 Physicochemical stability

To assess the stability of the suspension vehicles, their viscosity, pH and appearance were evaluated at different time points. Three batches of the final formulation were monitored at 5°C and 25°C/60% RH for 180 days. Measurements at t0 (initial time) were considered the references for all evaluated parameters.

2.5 Results and discussion

2.5.1 Excipients selection

Extemporaneous suspensions consist in most cases of crushed commercial tablets mixed with a suspension vehicle. A small quantity of the vehicle and the finely triturated active ingredient are normally combined in a mortar. Additional vehicle is added by geometric dilution until the final volume is obtained. Some suspensions are also prepared from powdered mixed excipients (21). The most widely used suspension vehicles are simple syrup, Ora-blend (Perrigo, Minneapolis, MN, USA) or home-made cellulose-based suspension vehicles (22). Commercial vehicles like Ora-Blend are widely employed as they are ready-to-use options. The formulation composition will depend of the compounder and his location since most guidelines on the subject are not harmonized, and most of suspension vehicles are not available worldwide (23,24). Normally, the development of medicinal products with a neutral taste should be considered, especially for formulations used in the treatment of chronic conditions, as strong flavors can become unpalatable after repeated administrations (8).

Among all listed ingredients to act as a thickening agent, hydroxypropyl methylcellulose (HPMC) was chosen to fulfill this role. HPMC is a chemically inert cellulose derivative and is not absorbed by the body (25,26). Therefore, the risk of unintended side effects was considered minimal and safe for children's use.

As for preservatives, safer options than parabens were explored. Sodium bicarbonate, propionic acid and its salt form, calcium propionate, were retained since they are endogenous substances with antimicrobial properties (12, 27). Zinc oxide was also selected for its known innocuity by the oral route (14).

Finally, among all sugar options, sucralose was chosen as a relevant sweetening agent. Indeed, this molecule is 600 times sweeter than sucrose, but does not affect glycemia and is non-caloric. It has been extensively studied in both adult and children and does not present any concern for health (28), especially as the Acceptable Daily Intake (ADI) is not likely to be exceeded in normal use (29). Sucralose concentration was set at 0.0225% w/v, within the recommended dietary range (28).

Since this suspension vehicle could be used in chronic treatment, the exposure of certain excipients could be higher than expected due to the duration of the treatment. We paid a special attention to selecting excipients known for their innocuity and long-term safety.

2.5.2 Evaluation of commercial options and initial formulations

Following the excipients safety, their efficacy should be the main concern. The thickening agent nature and its concentration are the main parameters governing the physical stability of a suspension. This excipient was therefore studied first as it would serve as the basis of the suspension vehicle. The viscosities of the commercial options were measured to serve as reference values. Different HPMC grades were then used to reproduce a similar viscosity and rheological behaviour. The spindles CP42 and CP51 were the most accurate. However, a broad range of viscosities was required to measure this parameter at a lower temperature. Therefore, the spindle CP51 was chosen for the rest of the measurements. The viscosity, density and osmolarity data are summarized in Table 2.1.

Table 2.1. Physicochemical characteristics of different suspension vehicles and formulations. n=3

		Viscosity ± SD (cP)		Density ± SD (g/cm^3)	Osmolarity ± SD (mOsm/kg)
	Solution	**5°C**	**25°C**	**25°C**	**25°C**
Reference vehicles	Oral Mix SF	<10	<10	1.035 ± 0.004	987 ± 3
	Ora blend	133 ± 12	17 ± 0.4	1.101 ± 0.002	167 ± 18
	Simple syrup	1172 ± 75	164 ± 3	1.326 ± 0.005	-
	Syrspend SF	956 ± 27	398 ± 22	1.005 ± 0.005	41 ± 4
	USP vehicle	16 ± 1	11 ± 1	1.026 ± 0.004	100 ± 22
HPMC K4M	HPMC 2% pH 6.5	293 ± 4	74 ± 0.2	1.006 ± 0.005	47 ± 5
HPMC K100M	HMPC 0.5% pH 6.5	356 ± 20	119 ± 6	1.008 ± 0.004	58 ± 7
	HMPC 0.5% pH 3	335 ± 17	112 ± 5	1.032 ± 0.003	87 ± 2
	HMPC 0.5% pH 7.5	350 ± 5	114 ± 4	1.009 ± 0.004	306 ± 4
	HPMC 0.5% + sucralose 0.0225% pH 6.5	349 ± 8	117 ± 5	1.009 ± 0.002	62 ± 3
	HPMC 0.5% + sucralose 0.0225% pH 3	343 ± 19	116 ± 6	1.013 ± 0.003	105 ± 5
	HPMC 0.5% + sucralose 0.0225% pH 7.5	351 ± 13	118 ± 4	1.005 ± 0.004	307 ± 21
	HMPC 0.4% pH 6.5	112 ± 18	43 ± 4	1.007 ± 0.005	56 ± 4
	HMPC 0.25% pH 6.5	61 ± 12	21 ± 1	1.005 ± 0.002	36 ± 9

The viscosity of commercial vehicles ranged from lower than 10 cP up to 398 cP when the measurements were taken at 25°C. These values logically increased at a lower temperature. First, an HPMC K4M was tested, at a concentration of 2% w/v. The resulting viscosity at 25°C was found somehow low compared to the relatively high concentration of thickening agent. Therefore, an HPMC with a higher molecular weight, namely HPMC K100M, was subsequently tested. Increasing concentration of HPMC K100M from 0.25% to 0.5% allowed us to increase the apparent viscosity at 25°C from about 20 to 120 cP, which was considered suitable for further investigation (*i.e.,* in the same range as simple syrup). Next, the effects of varying the pH and adding a sweetening agent were evaluated on the apparent

viscosity values. Acidic pH was adjusted using a citrate buffer, whereas basic pH was obtained with a phosphate buffer. Obviously, neither these factors had a significant impact on the viscosity, whether at 25 or 5°C (Table 2.1).

In parallel, the density of most suspension vehicles and solutions ranged from 1.005 to 1.101 g/cm^3, lower than the value of simple syrup, due to its high concentration of sugar.

Finally, the range of osmolality found in commercial vehicles was also quite wide, ranging from 41 to 987 mOsm/kg, with 300 mOsm/kg being the iso-osmotic point. The osmolality of the tested solutions also fall within this range, with the combination HPMC/sweetener at pH 7.5 being the closest to the iso-osmotic point. The osmolality was evaluated to ensure that the developed vehicle osmolality would be within the range of the commercial ones. This is indeed an important factor to consider for an oral suspension since it can influence the gastrointestinal tolerance (30).

2.5.3 Evaluation of the proposed new vehicles

Physicochemical characterization

Based on the results from the evaluation of the tested solutions and references as presented in Table 1, two suspension vehicles were elaborated. An acidic (F4.5) and an alkaline (F7.5) working version were developed to suit a larger variety of active ingredients.

Table 2.2. Initial composition and physicochemical properties of the proposed new vehicles. n=3

Excipient	Purpose	F4.5	F7.5
HPMC K100M	Thickening agent	5 g/L	4 g/L
Sucralose	Sweetening agent	0.225 g/L	0.2 g/L
Citric acid	Salt buffer	6 g/L	1.33 g/L
Sodium citrate	Salt buffer	6.9 g/L	0 g/L
Sodium phosphate	Salt buffer	0 g/L	18 g/L
Water	Solvent	q.s. 1 L	q.s.1 L
Properties	**Units**	**F4.5**	**F7.5**
Viscosity ± SD 5°C	cP	236 ± 14	108 ± 5
Viscosity ± SD 25°C	cP	82 ± 6	39 ± 2
Density ± SD 25°	g/cm^3	1.034 ± 0.003	1.010 ± 0.004
Osmolarity ± SD	mOsm/kg	117 ± 10	350 ± 20
pH		4.5 ± 0.2	7.5 ± 0.3

The adequate concentration of citric acid and sodium citrate were determined to obtain a pH of 4.5 for the F4.5 formulation, whereas a citric acid/sodium phosphate buffer was used for the F7.5 formulation to yield a pH of 7.5. Both vehicles displayed osmolality values within the range of acceptability to reduce the risk of gastrointestinal issues or irritations (31,32).

The viscosity of both vehicles was found to be in the adequate range of viscosity, being 39 cP for the alkaline version and 82 cP for the acidic version. The viscosity of these formulations was also measured at different shear rates. By plotting the viscosity as a function of shear rate, it is possible to establish the rheological behaviour of the suspension vehicles. Results are shown in Figure 1 for Ora Blend, simple syrup and both developed formulations at 25°C.

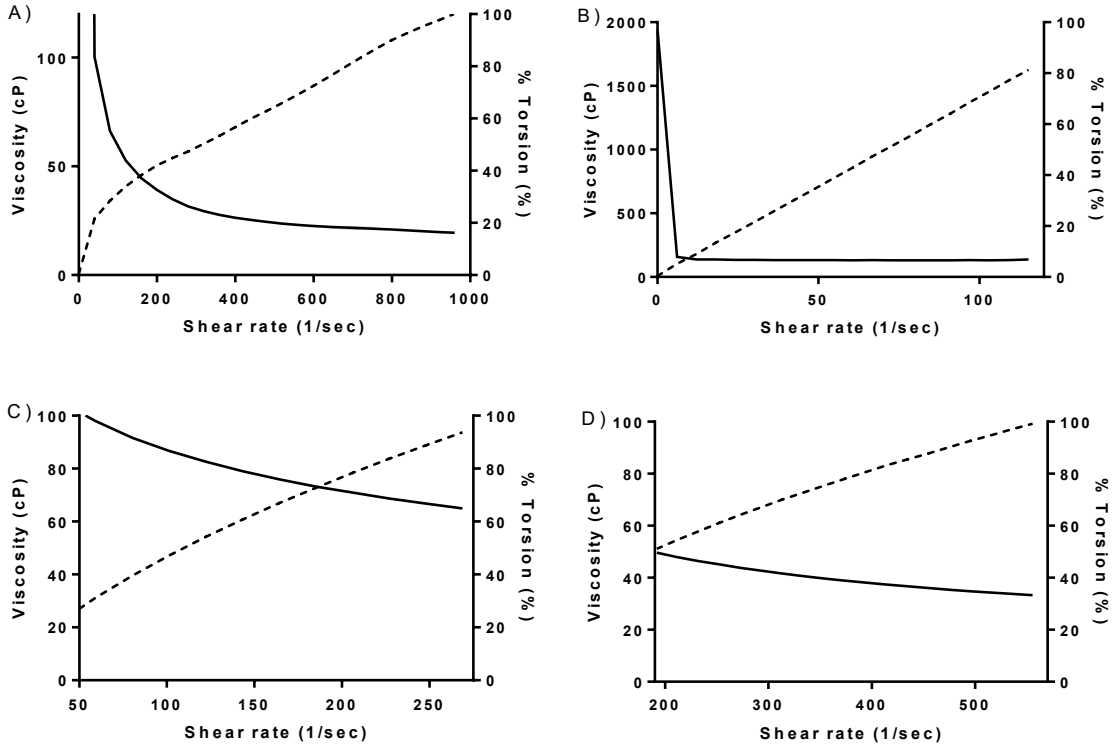

Figure 2.1. Rheological behaviour of **A)** Ora Blend, **B)** simple syrup, **C)** F4.5 and **D** F7.5 at 25°C. The full curves represent the viscosity (scale on the left) while the dotted curve represents the torsion (scale on the right).

As seen in Figure 1A, Ora-Blend appeared more viscous at a lower shear rate. Its viscosity decreased exponentially with the increasing shear rate. Ora-Blend is a shear-thinning suspension vehicle since it was specially designed to be viscous at rest and more fluid during the agitation. A shear-thinning behaviour is an appreciated characteristic in a suspension vehicle as it eases the redispersion of sediments during agitation (32). Meanwhile, as expected from previous works (33), simple syrup was found a typical Newtonian liquid (Figure 1B). Its apparent viscosity remained similar regardless of the shear rate exerted. As soon as the shear threshold was reached (more than 20% of torsion), a plateau appeared and the viscosity remained constant.

A shear-thinning behaviour was also observed for F4.5 and F7.5 since the viscosity decreased as the shear rate increased. This result was expected since HPMC, the employed thickening agent, is a cellulose-derived compound like methylcellulose, which

is found in Oral Mix and Ora Blend. This behaviour has already been largely reported for cellulose-based solutions (34,35).

2.5.4 Microbiological stability

Turbidimetry

For all strains, the OD600 was measured after a series of dilutions of microorganism cultures. These dilutions were then plated on agar Petri dishes to assess the CFU per mL.

The bacterial strains had a concentration of about 1.0 x 10^8 CFU/mL when their OD_{600} was around 1.5; meanwhile, for the same OD_{600}, the fungi strains displayed a concentration of around 1.0 x 10^7 CFU/mL. Additional details are presented in the supplementary information (Table S2).

After a dilution of 1:100, the microorganism concentrations were found in the range of 1.0 x 10^5 to 9.9 x 10^6 CFU/mL, which is the required concentration of microorganisms at t_0 according to the USP <51> protocol. The concentration was always counter-verified by plate counting after inoculation.

Preservative effectiveness test

In order to comply with the USP and EMA guidelines, a multi-dose oral formulation has to be proved microbiologically stable (19,36).

The formulations containing different preservatives were first tested on *E. Coli*. If any growth inhibition was observed, the formulation was further tested on the remaining strains. Figure 2 represents a summary of the different conditions tried with the developed vehicles. Detailed data are provided in Figure S1.

In our experiments, unmodified F4.5 and F7.5 solutions were considered as positive controls, whereas F4.5 + 0.1% sodium benzoate and F7.5 + methylparaben 0.1% + potassium sorbate 0.1% were used as negative controls. The positive and negative controls showed the expected results at both pHs.

A small deviation to the USP protocol was made for *A. Brasiliensis*; 0.9% saline containing 0.1% of polysorbate 80 instead of 0.05% was used for the dilutions. This is due to the fact that, when using a 0.05% concentration of polysorbate 80 as suggested by the USP

protocol, the spores would not spread evenly on the agar Petri, resulting in non-reproductive data.

The three selected preservatives intended for formulation F4.5 (*i.e.* compatible with an acidic pH) were found efficient in preventing the microorganism growth. However, it should be noted that the minimal concentration of propionic acid required to pass the test was lower for *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* (0.1%). The fungi strains seemed to be more resistant to propionic acid as the concentration of propionic acid had to be raised to 0.2% to comply with the USP requirements (19). Microorganisms of the bacterial type seemed to be more vulnerable to this compound (12, 37). Therefore, either 0.2% of propionic acid or 0.3% of calcium propionate proved to be efficient preservatives against all strains.

The overall results were expected. Propionic acid and its calcium propionate salt are extensively used in the food industry and their antimicrobial properties have been well documented (12). It is also known that organic acids, like propionic and benzoic acid, are only effective in acidic conditions (pH < 5) (37). Propionic acid is an intermediary product of the normal human metabolism and the selected concentration was found acceptable (Concentration < 0.3%) (38). Calcium propionate was hence selected as the preservative for the F4.5 formulation. Propionic acid (PA) is a naturally occurring carboxylic acid, which in its pure state exists as a colorless corrosive liquid with an unpleasant odor. The salt form was preferred as it is found in a solid state less difficult to handle than the corrosive acid form and it also displays a fainter odor and lesser taste than the acid form (39).

As far as the basic F7.5 formulation was concerned, the absence of preservatives, as well as the presence of 4% of sodium bicarbonate or 15% of zinc oxide, failed the test. In fact, the combination of methylparaben (0.1%) and potassium sorbate (0.1%) was the only formulation made with F7.5 that showed antimicrobial efficacy. It was not possible to find another preservative that could both maintain a basic pH and respect the safety concerns prevalent in this study. Consequently, the F7.5 formulation was not retained as a viable formulation and its development was stopped.

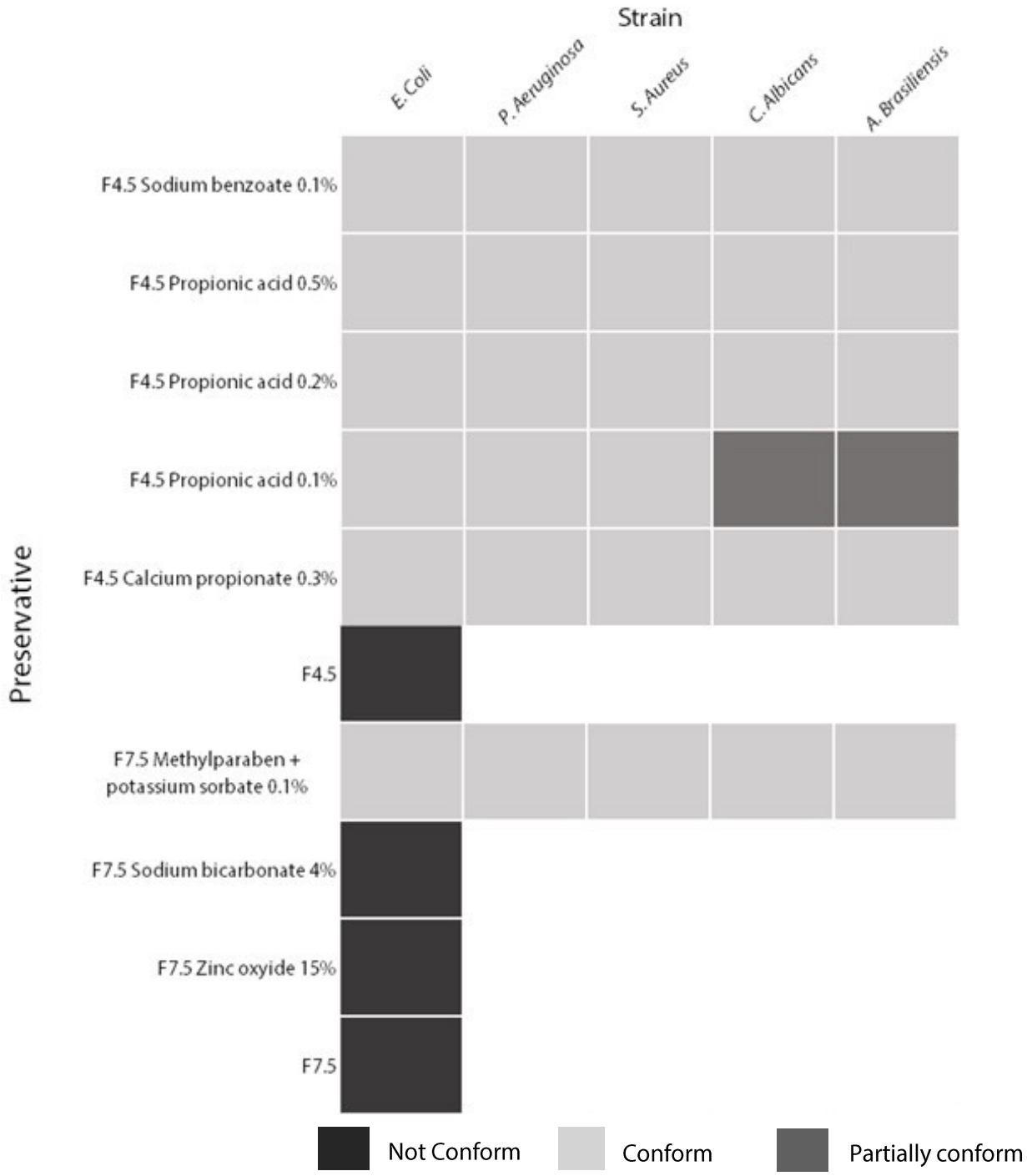

Figure 2.2. Preservative effectiveness test results

The black color indicates a failed test according to the USP guidelines, whereas the pale grey indicates a successful assay. The dark grey color is used to indicate partially successful assays (see Figure S1 for more details).

2.5.5 Evaluation of the final formulation

Once the nature and concentration of the preservative were determined, the preservative was added to the composition of F4.5. The complete and final formulation, namely F4.5+ (Table 3), was then evaluated.

The addition of calcium propionate did not significantly change the physicochemical properties of F4.5+ compared to F4.5 (Table 2).

Table 2.3. Final composition of the suspension vehicle (F4.5+)

Excipient	Purpose	pH 4.5
HPMC K100M	Thickening agent	5 g/L
Sucralose	Sweetening agent	0.225 g/L
Citric acid	Salt buffer	6 g/L
Sodium citrate	Salt buffer	6.9 g/L
Calcium propionate	Preservative	2.83 g/L
Water	Solvent	q.s. 1 L

Table 2.4. Physicochemical properties and characteristics of F4.5+

Properties/test	Value		SD
Viscosity 5°C	236	±	14
Viscosity 25°C	84	±	5
Density 25°C	1.034	±	0.003
Osmolality	124	±	9
pH	4.5	±	0.2
Stability 5°C	6 months		
Stability 25°C	6 months		

The addition of calcium propionate did not significantly change the physicochemical properties of F4.5.

Physicochemical properties stability

To evaluate the stability of F4.5+, the viscosity, pH and appearance of three batches were monitored for 180 days. The results are summarized in Figure 2.3.

No change in odor or appearance could be noted during the entirety of the observation period for all tested solutions. A statistically significant reduction in viscosity could be observed at 25°C (from 84 ± 5 cP at t0 to 70 ± 1 cP at t = 180 days) but not at 5°C. The pH values remained stable at both temperatures for 180 days. A possible explanation for the slight drop in viscosity could be linked to the introduction of calcium ions in the solution (under the form of calcium propionate). These bivalent ions are known to interact with carboxylate groups, presenting more affinity with them than monovalent ions such as sodium (40). Compared to sodium cellulose salts, divalent salts were recently shown to display lower viscosities at low concentrations (in the non-entangled regime), suggesting less expanded chains (40). In our case, this transformation could occur slowly within the storage period at room temperature but not at lower temperatures (less favorable thermodynamic conditions).

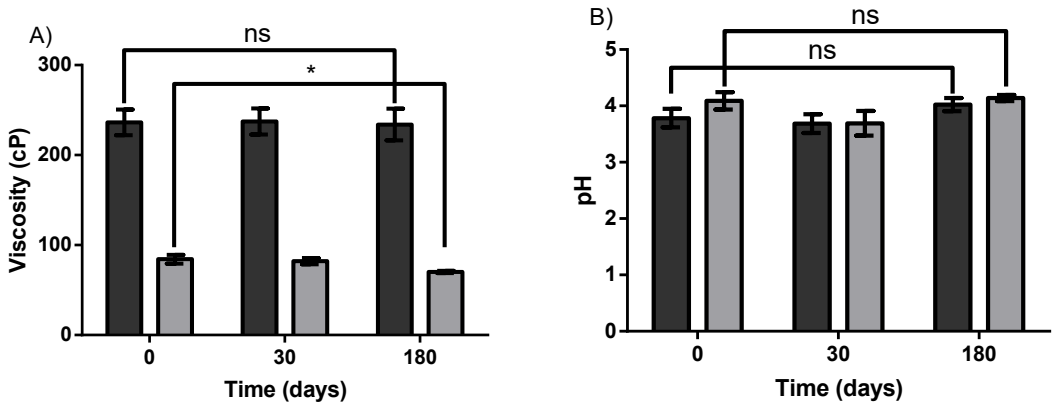

Figure 2.3. A) Viscosity and **B)** pH of the final formulation in time at 5°C (dark grey bars) and 25°C (pale grey bars) (n = 3, three measurements per batch)

Sedimentation volume and redispersibility

There is no established procedure, nor official guideline to evaluate the redispersibility of a suspension (41). We therefore designed a protocol for this purpose, mimicking the way a patient's caregiver would resuspend a medicinal suspension (by bottle inversion).

Acetaminophen and prednisone were selected for the sedimentation volume assay as they both have low solubility in water (42,43) and their compounding would subsequently result in a suspension. Crushed tablets were used instead of bulk powder because it is a more common and easy way to compound a medicinal suspension for community pharmacists. Therefore, the prepared suspensions also contained insoluble excipients contributing differently to the global suspension environment (44).

For all formulations, no sedimentation could be seen at the first time point (10 minutes). For the acetaminophen formulations, the final sedimentation volume was reached after 48 h for F4.5+ (12.5 ± 1.2 % v/v) and after 7 days for the reference USP vehicle (2.7 ± 0.9% v/v). This latter discrepancy could be explained by the difference of solubility of the various tablet excipients in the two vehicles.

For the prednisone formulations, the final sedimentation volume was reached after 7 days for F4.5+ (11.6 ± 0.5 % v/v) as well as for the reference USP vehicle (13.7 ± 1.5% v/v).

The redispersibility is a critical parameter and was evaluated at the end of the sedimentation volume test, by a normalized bottle inversion shaking. All formulations were easily redispersed in a comparable manner (Figure 2.4C and D).

A 180-day-old F4.5+ suspension vehicle was also used in the study, in order to verify its redispersability properties, as the viscosity was found slightly (but statistically significantly) reduced after a 180-day storage period (Figure 2.3A). As evidenced in Figure 2.4, there was no difference in behaviour between the freshly prepared and the 180-day-old F4.5+ formulations, in terms of sedimentation volumes or redispersability.

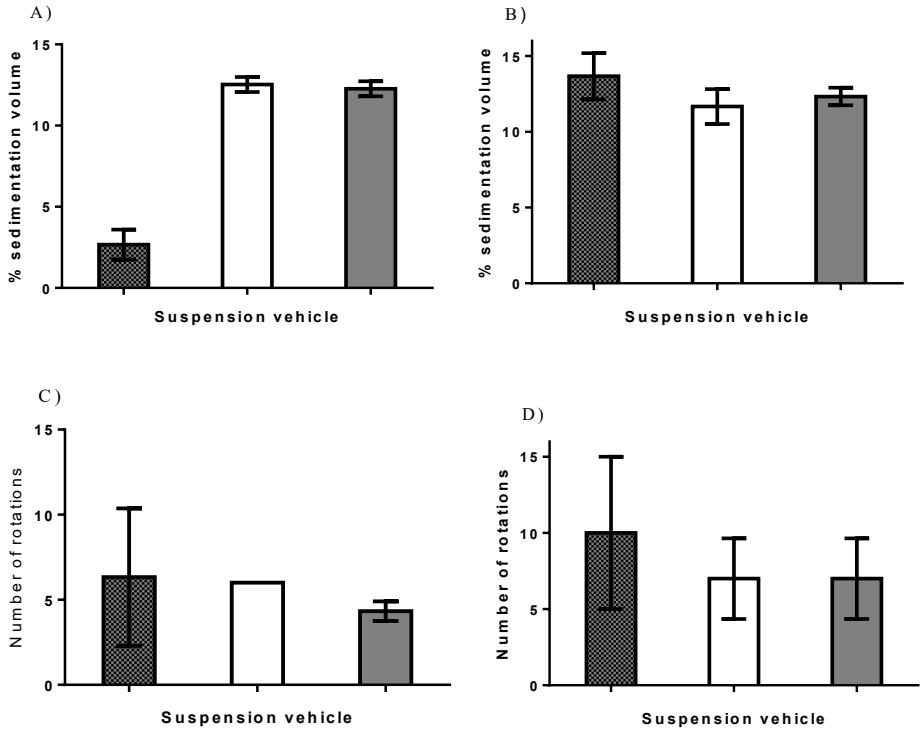

Figure 2.4. Sedimentation volume of **A)** acetaminophen and **B)** prednisone suspension and redispersibility of **C)** acetaminophen and D**)** prednisone suspension using the USP vehicle (checkered pattern bars), the fresh novel suspension vehicle (white bars) or the 180-day-old novel vehicle (grey bars) and at 25°C. n = 3

2.6 Conclusion

A safe and versatile suspension vehicle specially designed for children was developed. The excipients used in the composition were carefully chosen for their known safety in children, as well as their commercial availability. The physicochemical and microbiological stabilities of this new vehicle were assessed and found suitable for the intended use. The rheological behaviour, as well as the resuspendability, were also evaluated and corresponded to their application purpose.

The F4.5+ formulation containing HPMC, sucralose, citric acid, sodium citrate and calcium propionate was found stable 180 days in refrigerated conditions, as well as at room temperature (25ºC). Actually, despite a slight decrease in the apparent viscosity

values after 180 days, the new vehicle was judged suitable and stable enough to warrantee an unmodified performance.

The taste masking properties of the vehicle will be assessed in the second article of this three-part series via a clinical palatability study, while chemical stability studies of suspended active ingredients will be reported in the third and final part of this series, providing the pharmacist with a safe, ready-for-use formulae. Perspectives also include a scale-up process for the developed formulations containing an active ingredient. The pediatric population represents a smaller and less lucrative market share than adults and therefore suffers from inadequate or missing age-appropriate formulations. Thus, the role of hospital or community pharmacists should be to drive changes and embrace safer, scientifically evidenced compounding practices.

2.7 Acknowledgments

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2.8 Disclosure of interest

The authors report no conflicts of interest.

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Chapitre 3: Development of a safe and versatile suspension vehicle for paediatric use.

Part 2: Palatability study in paediatric and adult subjects

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3.1 Résumé en français

Le goût joue un rôle important dans le développement d'une formulation pharmaceutique orale, en particulier en ce qui concerne l'adhésion au traitement par les enfants. Comme les enfants ne sont pas toujours capables d'avaler des formes pharmaceutiques solides, un véhicule de suspension masquant le goût est souvent utilisé pour reformuler les comprimés sous une forme liquide. Les études de palatabilité sont l'un des paramètres importants de contrôle de qualité pour évaluer une formulation pédiatrique. À ce jour, la méthode la plus utilisée pour évaluer ce paramètre est l'évaluation de la palatabilité à l'aide de volontaires humains. Un nouveau véhicule de suspension a été développé spécialement pour un usage pédiatrique. Une attention particulière a été accordée aux excipients entrant dans la composition. Une étude de palatabilité a été menée dans deux groupes (adultes et enfants) pour comparer ce nouveau véhicule de suspension à la version USP. Le sulfate de quinine a été utilisé comme standard d'amertume dans les deux véhicules à la même concentration. Les participants ont dû évaluer l'odeur, le goût, la sensation en bouche et l'appréciation globale des deux véhicules. Des statistiques ont été effectuées sur les résultats pour distinguer toutes différences significatives entre les deux véhicules de suspension pour chaque critère. Il a été constaté que le goût et l'appréciation générale étaient mieux notés pour le nouveau véhicule de suspension.

Mots-clés: formulation pédiatrique, formulation orale, suspension orale, véhicule de suspension, formulation extemporanée, préparation, liquide oral, gout, palatabilité.

3.2 Résumé en anglais

Taste plays an important role in the development of oral pharmaceutical formulations, especially when it comes to the children's compliance to treatments. Since children are not always able to swallow solid dosage forms, a taste masking suspension vehicle is often used to reformulate tablets or capsules into a liquid form. Palatability studies are one of the key development steps for evaluating a paediatric formulation. In a previous article, we reported the development of a new suspension vehicle, specifically intended for paediatric use. An ensuing palatability study was conducted in two groups (adult and children) to compare this new suspension vehicle to the USP version. Quinine sulphate was employed as a bitterness standard in both vehicles at the same concentration to mimic a drug compound. The participants evaluated the smell, taste, mouthfeel and overall appreciation of both vehicles. Results showed that the taste and overall appreciation was statistically in favour of the newly developed suspension vehicle.

Keywords: paediatric formulation, oral formulation, oral suspension, suspension vehicle, extemporaneous formulation, compounding, oral liquid, taste, palatability.

3.3 Introduction

[In this second article of a three-part series, we discuss the palatability assessment of the newly developed suspension vehicle intended for compounding paediatric oral formulations. The formulation development of the suspension vehicle was discussed in the first article of this series and the stability studies of compounded drugs will be addressed in the third part.]

Although the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have urged, for a decade now, pharmaceutical companies to specifically design drug products adapted to children, paediatric formulations still remain a clear unmet medical need. It is therefore unavoidable, if not encouraged, that drug dispensed through compounded, validated formulations may be considered as a viable alternative. Among all paediatric formulations, oral liquids are traditionally considered the formulation of choice for children, who are generally unable to swallow tablets or capsules (1). In 2016, Pinto and Selen defined the FDA expectations for liquid formulations intended for paediatric patients. Among the listed characteristics of those formulations, the palatability was the first mentioned, along with stability and age-appropriate excipients for safety considerations (2). However, until recently, no clear consensus was reached as far as the notion of palatability was concerned (3).

In 2013, the Committee for Medicinal Products for Human Use of the European Medicines Agency (EMA) produced a guideline concerning the pharmaceutical development of medicines for paediatric use. In this document, palatability is also pointed out as a crucial aspect of paediatric patients' acceptability of a drug product. Palatability is defined as '*the overall appreciation of an (often oral) medicinal product in relation to its smell, taste, aftertaste and texture (i.e. feeling in the mouth)*' (4). The palatability criterion is essential to the patient's adherence and observance of the treatment, especially among children. In a reference article published in 2007, Matsui reported that compliance rates in children ranged from 11-93%, with major factors attributed to formulation and palatability (5). This aspect was also reported by Venables *et al.*, who conducted an interview-based study on the refusal of medicines in children/caregivers (0-18 years) and youngsters (12-18 years)

(6). One third of the patients included in this study refused to take a medicine. (Bad) taste was significantly the most common cause of this refusal (64% of refusals).

To prevent this phenomenon, palatability must be evaluated early in the development of a drug product (7, 8). Recent *in vitro* tests were designed to gather information about the palatability of a formulation, such as the electronic tongue (e-tongue). Based on the use of several sensors that are able to specifically interact with the chemical compounds contained in the formulation, the applications of this analytical device seem limitless (9). However, their performances sometimes remain basic and taste-centered (10), whereas palatability is a more global notion. Other current approaches being developed include *in silico* bitterness prediction, which relies on the chemical structure of drug compounds to calculate a predictive bitterness factor (11). This very encouraging approach can thus be implemented early in the drug development process. Nevertheless, it still fails to accurately predict the overall palatability of the final drug product. Human evaluation is therefore the preferred method for assessing the palatability of a complete formulation, despite some difficulties linked to the subjectivity of this global perception (7).

We have previously reported the development of a suspension vehicle made from simple ingredients, specially selected for their established innocuity in children (submitted for publication). Since the physicochemical stability and microbial challenge test were conclusive, we sought to evaluate its palatability. This characteristic was evaluated by a human taste panel of both adult and children participants. Including children in this assessment was judged essential as it is well known that the taste preferences of children differ from those of adults (12, 13). A sweetening agent was included in the composition of the developed suspension vehicle, which is generally considered a simple and effective way of masking the bitter taste of the suspended active principles. In our study, the newly developed suspension vehicle was compared to a reference and broadly used suspension vehicle, the USP suspension vehicle. quinine sulfate was used as a known bitterness standard (14).

3.4 Material and methods

3.4.1 Materials

Citric acid (anhydrous, USP/FCC), sodium citrate (anhydrous, USP), sodium chloride (crystals, USP), microcrystalline cellulose (50um, NF), xanthan gum (NF), sodium phosphate (dibasic, anhydrous, USP), potassium sorbate (FCC) and sodium carboxymethylcellulose (high viscosity, NF) were purchased from Galenova (Sainte Hyacinthe, QC, Canada).

Sucralose (NF), calcium propionate (powder, FCC), quinine sulfate (dihydrate, USP) and carrageenan (NF) were obtained from Spectrum Chemicals (New Brunswick, NJ, USA).

Methylparaben (NF) was purchased from Pharmascience (Montréal, QC, Canada).

Hydroxypropyl methylcellulose (HPMC, Methocel K100M) was donated by Dow Chemical Company (Midland, MI, USA).

Sterile water (USP) was a kind gift of the Centre Hospitalier Universitaire de Sainte-Justine (Montreal, QC, Canada).

All specific details about manufacturers and lot numbers are given in Table S1.

3.4.2 Methods

Formulation of suspension vehicles and standard taste solutions

The evaluated suspension vehicle (F4.5+) and the USP (FUSP) vehicles were prepared according to specifications of Table 1. Quinine sulfate (100 µg/mL) was used as a bitterness standard, in order to mimic a drug molecule bitterness. In order to calibrate the participant's tasting accuracy, four aqueous solutions displaying four basic tastes (sour, salty, sweet and bitter) were also prepared (Table 1). Briefly, excipients were accurately weighed and tumbled in a closed container (50 tumbles over five minutes). They were then slowly sprinkled into one third of the final volume of heated water (80°C) under magnetic agitation. Once the excipients were solubilized, the remaining two thirds of cold water were added. The solution was stirred overnight before measurements were made.

All solutions were prepared in a clean and safe environment specially designed for the compounding of extemporaneous formulations. The materials and excipients used were dedicated to this study.

Table 3.1. Composition of the liquid preparations used in the present palatability study

Excipient	Purpose	Concentration (mg/mL)
FUSP (USP suspension vehicle)		
Quinine sulfate	Bitterness standard	0.100
Microcrystalline cellulose	Thickening agent	8.0
Xanthan gum	Thickening agent	2.0
Carrageenan	Thickening agent	1.5
Carboxymethylcellulose	Thickening agent	0.25
Citric acid	Salt buffer	2.5
Sodium phosphate (dibasic)	Salt buffer	1.2
Potassium sorbate	Preservative	1.0
Methylparaben	Preservative	1.0
F4.5+ (test suspension vehicle)		
Quinine sulfate	Bitterness standard	0.100
Hydroxypropylmethylcellulose	Thickening agent	5.0
Sucralose	Sweetening agent	0.225
Citric acid	Salt buffer	6.0
Calcium propionate	Preservative	2.83
Citrate de sodium	Salt buffer	6.9
Bitter taste standard solution		
Quinine sulfate	Bitterness standard	0.025
Sour taste standard solution		
Citric acid	Sourness standard	1.0
Salty taste standard solution		
Sodium chloride	Salinity standard	4.5

Sweet taste standard solution		
Sucralose	Sweetening agent	0.075

In vivo palatability study

Study populations

The study was carried out on two distinct groups, adults and children, after approval by the the "Comité d'éthique de la recherche en santé" (CERES, Ethics committee in health research) of the University of Montreal (protocol # 17-150-CERES-P). Moreover, the assessment complied to the principles of the Declaration of Helsinki and regulations concerning clinical trials, as well as the 'Requirements for Informed Consent Documents' from Health Canada (2014).

- Adult group.

Adults were eligible if aged between 18 and 35 years old. Exclusion criteria included: known food or drug allergies, smoking, consuming tobacco products or using an electronic cigarette in the last 30 days, pregnancy or breastfeeding, a condition of cold or any other condition that could affect the perception of taste and smell on the day of the test, inability to recognize different tastes during the preliminary calibration phase. Adults were recruited among the Pharm.D., graduate students and research associates of the faculty of pharmacy, University of Montreal.

- Children group.

The children's group consisted of individuals aged between 6 and 12 years.

Exclusion criteria included: known food or drug allergies, smoking, consuming tobacco products or using an electronic cigarette in the last 30 days, a condition of cold or any other condition that could affect the perception of taste and smell on the day of the test, inability to recognize different tastes during the preliminary calibration phase.

Both groups had a similar number of male and female subjects. The participants were asked not to eat or drink anything except water, an hour before the start of the evaluation. Recruited children were the offspring or relatives of staff members of the faculty of pharmacy, University of Montreal.

Table 3.2. Demographic characteristics of subjects enrolled in the palatability study.

Study group	Characteristics:	
Adults	Male:	11
	Female:	10
	Age range:	18 – 35 yr
	Age mean ± sd:	24.6 ± 2.9
	Exclusion :	1 out of 21
Children	Male:	3
	Female:	9
	Age range:	6 – 12 yr
	Age mean ± sd:	8.3 ± 2.0
	Exclusion :	2 out of 12

Study design of the palatability testing

The palatability assessment was a randomized, crossover study.

Prior to the start of the study, informed consent was signed by either the adult participants or, for the children group, by the legal guardian(s) of each child. Immediately prior to the testing, children were asked for their oral confirmation for participating in the test and were explained they were entitled to stop the evaluation whenever they felt like it.

The protocol was carried out with the adult group first, to ensure the assay's safety and feasibility.

Participants were individually placed in a quiet, private room. The test was conducted by two independent evaluators, of which only one knew the tested formulae (partially double-blind test). After filling the consent form and other health-related questionnaires, the taste perception was first evaluated. The sour, bitter, sweet and salty solutions (Table 1) were tasted (~ 5 mL in a plastic medical cup) in a random manner. To neutralize taste before and between samplings, subjects were offered to eat an unsalted cracker and rinsed their mouth with spring water. They spat the liquids back after each sampling. This calibration was performed to ensure their ability to perceive and name correctly the different tastes.

Candidates who could not correctly identify different tastes were not selected for the next step (one in the adult group, two in the children group).

After this selection, the F4.5+ and FUSP (~ 5 mL in a plastic medical cup) were randomly assessed in a swirl-and-spit manner. Each participant was asked to look at the preparation, smell it, taste it and spit it after 5 seconds. Participants had to rinse their mouths with water and could eat a cracker during the five-minute wait period between the evaluation of each preparation. Participants had to evaluate the smell, taste and mouthfeel immediately and 2 minutes after taking the sample, using the hedonic faces scale represented in Figure 1. To confirm the participant's evaluation, both evaluators separately noted the participant's facial expression at the moment of tasting (satisfied, neutral, grimacing).

Finally, the participants were asked if they would accept to take this medicine again to cure a condition. The children were also asked to select the preferred formulation.

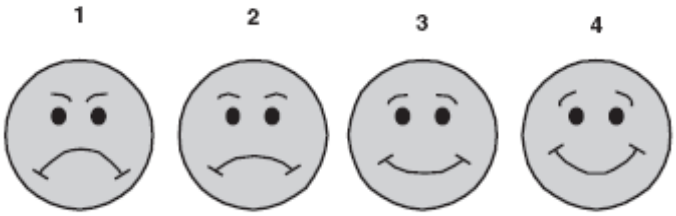

Figure 3.1. Hedonic faces scale used during the evaluation of palatability

Data processing and analysis

The difference in palatability of the test formulation (F4.5+) and the reference formulation (FUSP) as indicated by the adults and the children on the hedonic faces scale was considered the primary outcome. A two-way ANOVA with intra-subject repeated measures was used to calculate any significant difference between the two formulations for every criterion (smell, taste and mouthfeel, immediately and 2 minutes after spitting). Preference between the two formulations and willingness to use the vehicle to cure a disease were used as secondary outcomes. Statistical analysis was performed using SPSS 25 software (2019 license).

3.5 Results and discussion

3.5.1 Study design

In the course of developing a new suspension vehicle, the design of a palatability study immediately followed the development of the optimized suspension vehicle (Figure 2). Once the microbiological and physicochemical stabilities were assessed, our concern turned toward the acceptability of this new vehicle. Unfortunately, not many palatability studies in children have been published to date and the employed methods and study designs are still not harmonized (15).

Based on an extensive search of the literature, several criteria were selected to build the assessment protocol. First, as the assay was designed in healthy children in a swirl-and-spit manner, a safe drug mimetic was chosen. Indeed, the EMA considers ethical to include healthy children for palatability studies under these conditions only (16). In our case, the bitterness was provided by quinine sulfate, a molecule which is both a drug used to treat paludism and a flavoring agent in soda beverages, depending on the dose. The used dose of quinine for medical indications in children is 24 mg/kg/day (17). In a 355 mL can of tonic soda, approximately 30 mg of quinine hydrochloride can be found (18). During the whole test, the maximal exposure was 150 µg, therefore 200 times less than in a tonic soda drink.

The evaluation method was then selected. An hedonic faces scale was used, as recommended for children (19). Based on the study by Davies and Tuleu, (20) a 4-point hedonic scale was proposed (Figure 1) as it seemed more appropriate for children aged 6-12 years. We decided to include a discriminatory taste assay as a way to cross-validate the relevance of children choice and judgment. Indeed, in many cases, parents/caregivers are often included in palatability evaluations in children (21). The principle behind this participation is that they are a more reliable source of information about overall acceptability (*i.e.* including other notions than palatability such as appearance, swallowability, frequency and ease of administration, device used for administration and packaging), especially for small children. In this study however, the palatability evaluation was not based on repeated administrations of a medicine but in a single comparison with an existing and validated suspension vehicle, namely the USP suspension vehicle. Children aged 6-12 were supposed to be articulate enough to express correctly their likings by

themselves through hedonic scales and verbal judgment (19). Assessment of palatability was deconvoluted into three specific points: smell, taste and texture (mouthfeel) in order to encompass the global notion of palatability. The aftertaste was also evaluated by asking the participants to renew their judgments on the same three points two minutes after spitting. In this way, our study conformed to international guidelines regarding the appropriateness of the palatability assessment (22).

Finally, in order to ensure the greatest possible safety for healthy child volunteers, we decided to evaluate the F4.5+ vehicle first in a panel of adults. Indeed, the evaluation of the benefits/risks balance, which was mandatory at this stage of the development of our vehicle,(23) indicated a very minimal risk of adverse reactions following exposure to this vehicle. The vehicle ingredients had been specially selected for their known safety in humans, particularly in children. In addition, people most likely to present adverse reactions, *i.e.* those with a known history of food allergies or intolerance, were excluded from the study. Thus, by ensuring that no adults developed any reactions following the evaluation (Figure 2, go / no go stage), we ensured that children's exposure was the safest possible prior the assay in children.

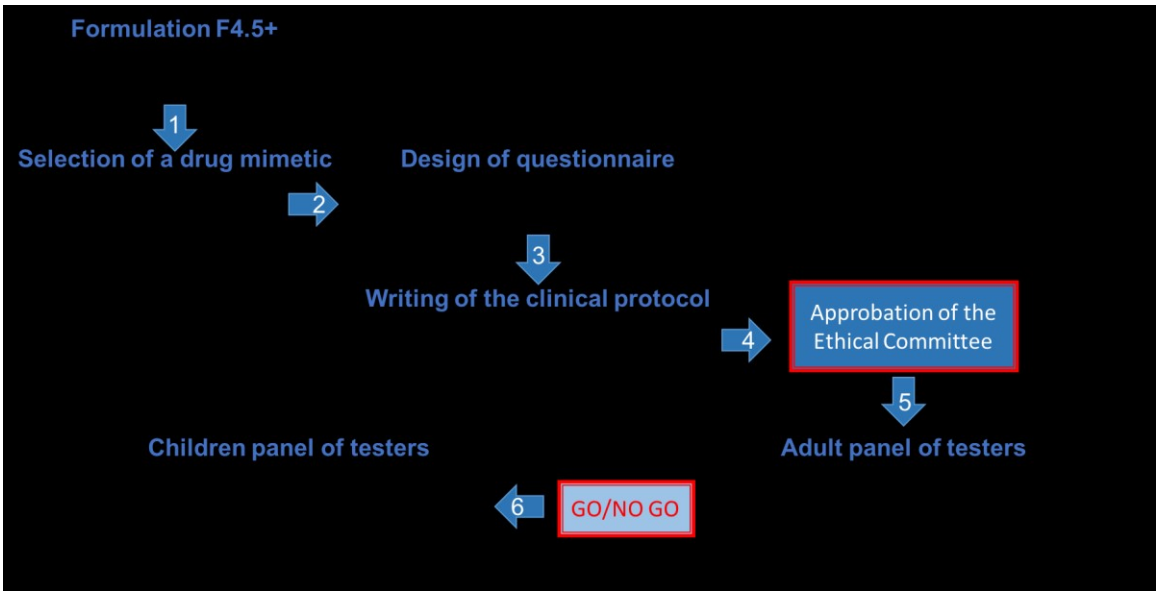

Figure 3.2. Schematic representation of the process of the palatability study design.

3.5.2 Adult group

In this study, 21 adults were recruited and 20 were selected to complete the evaluation. The scores for smell, taste and mouthfeel are displayed in Figure 3. No adverse reaction was noted or reported by any volunteer.

After completing the questionnaires, 17 volunteers out of 20 (85%) found the F4.5+ formulation acceptable. Nineteen adults (95%) answered that they would be ready to take this formulation again if needed to cure a condition.

For the reference formulation (FUSP), 12 volunteers (60%) found the preparation acceptable. If needed, 18 adults (90%) answered that they would be ready to take this formulation again to cure a condition.

When comparing the formulations between themselves, a significant difference could be observed for the taste immediately and 2 minutes after spitting in favor of F4.5+ when compared to FUSP. The smell and mouthfeel of the two formulations at both times were judged similar by the adult volunteers. The overall appreciation of the adult group was slightly greater for the first formulation.

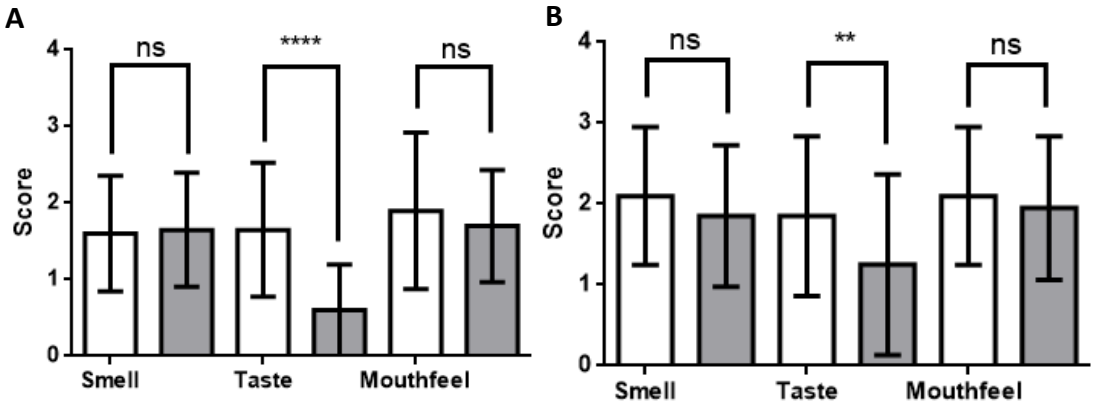

Figure 3.3. Evaluation of smell, taste and mouthfeel of the adult group A: immediately after spitting and B: 2 minutes after spitting. White bars: F4.5+; Grey bars: FUSP.

3.5.2 Children group

For the children group, 12 children were recruited and 10 were selected to complete the evaluation. The scores for smell, taste and mouthfeel are displayed in Figure 4. No adverse reaction was noted or reported by any volunteer.

Overall, the smell, taste and mouthfeel scores of the children group were lower than those of the adult group immediately after spitting (Figure 4A versus 3A) but this tendency was reversed in the 2-min later evaluation (Figure 4B versus 3B). Children were more readily prone to use the extreme parts of their hedonic scales to rate the preparations. Their facial expressions, as recorded by both evaluators, as well as their verbal judgments always concurred with the rating of the hedonic scales.

After completing the questionnaires, 8 children out of 11 (73%) preferred the F4.5+ over the FUSP. Seven children (64%) answered that they would accept to take this formulation again if needed to cure a condition, whereas only 5 of them (45%) declared to be ready to take again the FUSP if required.

Two noteworthy difficulties were encountered during this assessment phase with the children group. The terms ‘sour’ and ‘bitter’ were not always distinguishable by the younger ones (6 to 8 years old). Evaluators therefore used food analogies to describe all the tastes: crackers and sea water for salty, biscuits and juices for sweet, lemon juice and vinegar for sour, and dark chocolate and Brussels sprouts for bitter. This proved to be helpful for children to correctly discriminate the different taste standard solutions. Another difficulty was experienced by the younger ones to differentiate the ‘taste’ and ‘mouthfeel’ notions. Once again, verbal precisions by the evaluators were necessary to explain those notions through food examples. Globally, children were very satisfied of their experience and felt elated to have participated in a study aiming to design better medicines for sick children. This notion was, however, better understood by children over 8. This triggers the question of the inclusion of children under 8 in palatability studies, as previously mentioned by others (21).

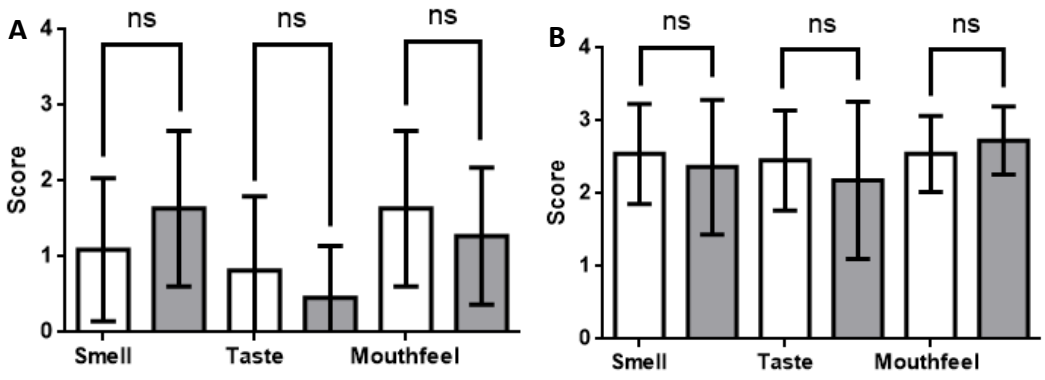

Figure 3.4. Evaluation of smell, taste and mouthfeel of the children group A: immediately after spitting and B: 2 minutes after spitting. White bars: F4.5+; Grey bars: FUSP.

In a recent palatability study by Bastiaans *et al*., the authors examined the taste score of a valacyclovir liquid formulation against a reference formulation (in Ora-Sweet SF) in both children and their parents (24). They reported that there was no correlation between the liking of the formulations by the children (4-12 years old) and their parents (34-54 years old). In our case, the primary outcomes of the study, namely the differences in smell, taste and mouthfeel between the test and reference formulations, were also not correlated between the adult and the children groups. However, the overall preference for the test formulation, as established by the concluding question, was noted in both study populations, even if the specific scores were not statistically different. Nonetheless, based on our results and observations, we agree with Bastiaans *et al*. (24) and current international guidelines (22) that the palatability of new paediatric formulations should be assessed in children and not deduced or extrapolated from adult testing, whenever possible. Palatability studies are time-consuming. The methodology is not yet standardized nor amenable to screening very large numbers of samples in a rapid and economical way (21). Human panels present several limitations including health concerns, poor memory, tiredness and desensitisation of tasters, personal preferences and maintaining the motivation for tasting unpleasant compounds. The data analysis and interpretation present certain difficulties due to genetic variations in taste, individual perception of bitter taste and age dependency on the perception of bitter taste (12, 25-27). These are difficult-to-control factors, even in a clinical study. In our case, the number of children enrolled in the

study was also debatable. Indeed, previous studies showed that a group of 15-50 children was preferable in order to benefit from a clear statistical discrimination (20). This was our primary target, which we could not reach from our recruiting population. Two main reasons prevented us from achieving this goal: some parent's reluctance to expose their healthy children to a product not yet marketed and the relatively high prevalence of children with allergies which were judged incompatible with the inclusion criteria in our study for safety concerns. This latter fact corroborates with increasing reports of food intolerances and allergies, especially in western countries (28, 29). Both factors can prove challenging to overcome in a context of self-funded academic study.

3.6 Conclusion

Based on the observed results, we can conclude that the newly developed vehicle for children, containing less and safer excipients, had a similar, if not better, palatability than the USP vehicle. The F4.5+ liquid formulation, despite the absence of a flavoring agent, enabled to mask the bitterness of quinine sulfate, an established bitterness marker. The results of this palatability study further support the development of this new paediatric suspension vehicle as an alternative to the currently available suspension vehicles, all primarily intended for adult uses. Based on the primary results of physicochemical stability and acceptable palatability, the stability of compounded formulations using different test drugs is needed to definitively establish the utility of this paediatric suspension vehicle.

3.7 Acknowledgments

The authors would like to thank Alexandre Melkoumov for the tasting of preliminary formulations, Maria Vershinin for participating in collecting data and Steven Sanche for advice in statistical data processing.

3.8 Disclosure of interest

The authors report no conflicts of interest.

3.9 References

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Chapitre 4: Development of a safe and versatile suspension vehicle for paediatric use.

Part 3: Stability studies of compounded formulations. Caffeine, hydrocortisone, spironolactone and tacrolimus.

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4.1 Résumé en français

La caféine (10 mg/mL), l'hydrocortisone (1 mg/mL), la spironolactone (5 mg/mL) et le tacrolimus (0.5 mg/mL) ont été formulés extemporanément à partir de comprimés ou de poudre disponibles dans le commerce dans un nouveau véhicule de suspension spécialement conçu pour un usage pédiatrique. La stabilité de ces suspensions a été évaluée dans des bouteilles en plastique ambré ainsi que dans des seringues en plastique ambré à 5 °C et à 25 °C. Les propriétés organoleptiques, le pH et la concentration des principes actifs ont été évalués à des moments prédéterminés allant jusqu'à 180 jours pour les bouteilles et à 30 jours pour les seringues. Une méthode HPLC indicatrice de stabilité a été développée. La caféine était stable pendant 30 jours dans toutes les conditions testées dans les seringues et jusqu'à 180 jours dans les bouteilles. Pour l'hydrocortisone, la concentration est restée supérieure à 90% dans les bouteilles à 25 °C pendant 180 jours et pendant 90 jours pour les bouteilles à 5 °C., La concentration d'hydrocortisone mesurée dans les seringues était supérieure à 90% après 30 jours pour les deux températures. La spironolactone est restée stable à toutes les conditions pendant toute la durée de l'étude tant dans les bouteilles que dans les seringues. Les suspensions de tacrolimus ont été jugées stables pendant 180 jours entreposées à 5°C et 90 jours à 25°C dans les bouteilles. Lorsqu'entreposés dans des seringues, les suspensions ont été jugées inchangées pendant 14 jours à 5°C et 30 jours à 25 °C. Aucun changement des propriétés organoleptiques ou du pH n'a été observé pour les formulations, à l'exception d'un léger changement de couleur pour la formulation de spironolactone.

Mots-clés: formulation pédiatrique, formulation orale, suspension orale, véhicule de suspension, formulation extemporanée, préparation, liquide oral, caféine, hydrocortisone, spironolactone, tacrolimus.

4.2 Résumé en anglais

Caffeine (10 mg/mL), hydrocortisone (1 mg/mL), spironolactone (5 mg/mL) and tacrolimus (0.5 mg/mL) were formulated extemporaneously from commercially available tablets, capsules or powder in a novel suspension vehicle specially designed for paediatric use (the F4.5+ vehicle). The stability of these suspensions was evaluated in amber plastic bottles as well as amber plastic syringes at 5 °C and 25 °C. Organoleptic properties, pH and drug concentrations were assessed at predetermined time points up to 180 days for bottles and 30 days for syringes. Validated, stability-indicating HPLC methods were developed for each drug. Caffeine and spironolactone were stable for 30 days at all tested conditions in syringes and up to 180 days in bottles. Hydrocortisone concentrations remained over 90% in all tested conditions, except for bottles at 5°C (90 days). The tacrolimus suspensions were found stable 180 days in plastic bottles stored at 5°C and at least 90 days at 25oC. In syringes, they were stable 30 days when stored 25°C and 14 days at 5°C. A preliminary scale up was conclusively conducted from 100-mL to 3.0-L batches, without altering the physicochemical properties of the model suspensions.

Keywords: paediatric formulation, oral formulation, suspension vehicle, extemporaneous formulation, compounding, caffeine, hydrocortisone, tacrolimus, spironolactone.

4.3 Introduction

[In this third and final article of a three-part series, we focus on the stability assessment of four model drugs compounded in the newly developed suspension vehicle (F4.5+) intended for paediatric oral formulations. The formulation development of this vehicle is related in the first part of this series and the second part concerns the palatability study of the vehicle.]

Recent improvements in the field of paediatric formulations arise from an increased number of guidelines issued from international regulatory authorities aiming to harmonize practices around the world (1). Consequently, more paediatric formulations are being developed, even if their number is still significantly lower than the number intended for adults (2). Therefore, compounding, even if disputable, remains a valid alternative to accurately dose newborns and children. These compounded formulations are often prepared from adult commercial tablets (3). Many issues result from these practices, one of them being the lack of information about their stabilities.

For this purpose, we tested the stability of four compounded oral formulations made from a new suspension vehicle that we specifically developed for children (submitted). For that purpose, we selected four active ingredients, namely caffeine, hydrocortisone, spironolactone and tacrolimus. The concentrations of the formulations were based on the standard practices of the compounding community (4,5, CHU Sainte-Justine).

Caffeine is a methylxanthine used as a first-line treatment for the apnea in premature infants (6,7). The current maintenance dose recommended by the FDA is 5 mg/kg of caffeine base, once daily, in oral liquid form (8). Commercial forms are now available; however, they are not approved in every country. Extemporaneous formulations made from bulk powder are still commonly found in practice (9). Stability between 3 to 6 months has been reported at ambient and refrigerated conditions in different suspension vehicles (10,11).

In pediatric or neonatal populations, hydrocortisone is used orally as a first-line of treatment for congenital adrenal hyperplasia. The recommended dose will depend of the condition and weight of the child (12, 13, 14). Commercially, the drug can be found in

tablets, an unsuitable dosage form for neonates, infants and young children. A liquid dosage form is, therefore, necessary. Pharmacists must prepare extemporaneous formulations from the commercially available products (13). Suspensions with a stability of 4 weeks to 3 months at room and refrigerated temperatures have been formulated in Ora-Plus and Ora-Sweet SF (15,16). A formulation reconstituted from powdered excipients was reported with a stability of 30 days at 5°C and 25°C (17).

Spironolactone is a diuretic frequently used to treat hypertension in neonates and children and is also used for cardiac failure (18). As there is no licensed product in oral liquid form, extemporaneous preparations are often prepared from crushed tablets to better suit the paediatric patients' needs. Several extemporaneous formulations can be found in the literature, as well as their stability. Suspensions of spironolactone have been shown to maintain a shelf-life of at least 4 weeks at 30°C (19). A study also reported a stability of at least 90 days when the suspension was stored under refrigeration and at room temperature (20). Another similar study evaluated spironolactone suspensions stored at 5°C and 25°C during a 90-day period and found that they were chemically stable for this duration (21).

Tacrolimus is an immunosuppressive agent used to prevent and treat the rejection of transplanted organs (22). Tacrolimus is commercially available as 0.5, 1 and 5 mg gelatin capsules for oral administration, in ampules containing tacrolimus 5 mg/mL for intravenous injections and in topical form (23). No oral liquid dosage formulation is commercially available or described in the literature. Therefore, the pharmacist or caregiver must mix the content of the capsules with a liquid before administration. The stability of some of these suspensions has been studied. A stability of at least 56 days was reported for an extemporaneous suspension compounded with equal amounts of Ora-Plus and Simple Syrup NF when stored at RT in both glass and plastic amber bottles (24). Another group demonstrated that tacrolimus formulated as a 1 mg/mL suspension in equal amounts of Ora-Plus and Ora-Sweet had a shelf-life of 4 months at room temperature (25).

These formulations were made using suspension vehicles that were not expressly designed for paediatric uses. Concentration, storage and conditioning are parameters that

vary a lot depending on local practices. We therefore conducted a 6-month stability study at two controlled temperatures (5°C and 25°C) in both amber plastic syringes and polyethylene terephthalate (PET) bottles, according to a standard protocol from our laboratory (26,27,28,29). All formulations were compounded using the novel F4.5+ suspension vehicle, designed for the safety of children. An appropriate stability-indicating HPLC method was developed and validated for each of the model active ingredients. The linearity, precision, variability and specificity of these methods were evaluated. The drug concentrations were monitored over 180 days in bottles and 30 days for syringes. Additionally, organoleptic changes and pH were recorded for the whole duration of the study. Finally, a preliminary scale up production was performed, from 100 mL to 3.0 L.

4.4 Materials and Method

4.4.1 Materials

Sodium citrate dihydrate (≥ 99% FG), dibasic sodium phosphate (anhydrous), sucralose (≥ 98.0% HPLC), calcium propionate (99.0-100.5%) and sodium chloride (anhydrous) were purchased from Sigma-Aldrich (St-Louis, MO, USA). Potassium phosphate monobasic (crystal) was purchased from JT Baker Inc. (Phillipsburg, NJ, USA). Citric acid (anhydrous, ACS certified), glacial acetic acid (ACS certified), methanol (HPLC), acetonitrile (HPLC), hydrochloric acid aqueous solution (1 M), sodium hydroxide aqueous solution (1 M) and hydrogen peroxide aqueous solution (3%) were obtained from Fisher Scientific (Ottawa, ON, Canada). Hydroxypropyl methylcellulose (HPMC, Methocel K100M) was donated by Dow Chemical Company (Midland, MI, USA). Caffeine (anhydrous), hydrocortisone (micronized, USP), tacrolimus (monohydrate), 60-mL amber plastic bottles (PET bottle with black phenolic cap) and 3-mL amber plastic syringes (Precise Dose Dispenser with tip cap) were purchased from Medisca (Montreal, QC, Canada). Spironolactone was purchased from TCI America (Portland, OR, USA). Spironolactone tablets (100 mg) were obtained from Teva (Toronto, ON, Canada). Hydrocortisone tablets (20 mg, Cortef) were obtained from Pfizer (Kirkland, QC, Canada). Tacrolimus capsules (5 mg, Prograf) were obtained from Astellas Pharma

(Markham, ON Canada). Milli-Q water (Synthesis A10 system, Millipore, Etobicoke, ON, Canada) was used in this study. Details about manufacturers and lot numbers may be found in Table S1.

4.4.2 Instruments and equipment

The HPLC system (Prominence UFLC, Shimadzu, Laval, QC, Canada) consisted of a SIL-20AC HT refrigerated autosampler, a DGU-20A5 solvent degasser, a LC-20AD binary pump, a CTO-20AC column oven and a SPD-M20A photodiode array detector; HPLC column, Kinetex XB-C18 (4.6 × 100 mm, 5 µm, P/N 00D-4605-E0, S/N 640207-11, Phenomenex, Torrance, CA, USA); HPLC column, Sorbax, SB-C18 (4.6 × 100 mm, 5 µm, P/N 883975-902, S/N USCM010053, Agilent, Mississauga, ON, Canada); HPLC column, Kinetex XB-C18 (3.0 × 100 mm, 5 µm, S/N H 5203685, P/N 5705-0037, Phenomenex, Torrance, CA, USA). pH was measured using a Accumet pH meter (model AP61, Fisher Scientific);

4.4.3 Extemporaneous preparations

Preparation of the new suspension vehicle F4.5+

The excipients were accurately weighed and tumbled in a closed container (50 tumbles over five minutes). They were then slowly sprinkled into one third of the final volume of heated water (80°C) under magnetic agitation to ensure an adequate dispersion of the HPMC. Once the excipients were solubilized, the remaining two thirds of cold water were added.

Caffeine

Suspensions were compounded to a target concentration of 10 mg/mL from powder (base, 1000 mg) which was first triturated using a mortar and a pestle prior to geometric incorporation of the suspension vehicle (100 mL).

Hydrocortisone

Suspensions were compounded to a target concentration of 1 mg/mL from tablets (5 x 20 mg tablets) which were first pulverized using a mortar and a pestle prior to geometric incorporation of the suspension vehicle (100 mL).

Spironolactone

Suspensions were compounded to a target concentration of 5 mg/mL from tablets (5 x 100 mg tablets) which were first pulverized using a mortar and a pestle prior to geometric incorporation of the suspension vehicle (100 mL).

Tacrolimus

Suspensions were compounded to a target concentration of 0.5 mg/mL from capsules (10 x 5 mg capsules) which were first emptied in a mortar prior to geometric incorporation of the suspension vehicle (100 mL).

Suspension concentrations and drug initial forms are summarized in Table 1.

Table 4.1. Extemporaneous preparations compounding

Formulation	Concentration (mg/mL)	Quantity used (mg)	Final volume (mL)
Caffeine	10	1000	100
Hydrocortisone	1	5 x 20 mg tablets	100
Spironolactone	5	5 x 100 mg tablets	100
Tacrolimus	0.5	10 x 5 mg capsules	100

4.4.4 Design of the stability study

Based on the ICH guidelines (30), the standard of procedure followed by our laboratory biopharmacy was used to assess the suspension stability (26-29). The preparations were packaged in 60-mL amber plastic bottles (30-mL fill volume, 6 bottles per preparation) and 3-mL amber plastic syringes (1-mL fill volume, 48 syringes per preparation). The formulations were stored at 5 ± 2 °C and 25 ± 2 °C/60 ± 5 %RH for up to 180 days. For each formulation, three bottles and three syringes for each time point were stored at both

conditions. At predetermined time points (0, 7, 14, 30, 45, 60, 75, 90 and 180 days), an aliquot (1 mL) from each bottle and three syringes were retrieved from each temperature condition. Bottles and syringes were vigorously shaken and vortexed for 20 seconds prior to sampling. The organoleptic properties of each test sample were inspected, pH was measured and the active principle concentration was determined using the HPLC-UV method described below.

4.4.5 Preparation of samples for HPLC injection

Caffeine

Test samples (100 µL) were diluted using methanol (900 µL) in a 1.5-mL centrifuge tube. The mixture was vortexed (20 s) and then centrifuged (10000 g, 10 min). Supernatant (50 µL) was further diluted using water (950 µL), vortexed (20 s) and transferred to a sealed 96-well plate. These solutions for injection had a nominal concentration of 0.05 mg/mL and were analyzed immediately after preparation (duplicated injections).

Hydrocortisone

Test samples (200 µL) were diluted using a mixture of methanol: water 30:70 (3800 µL) in a 5-mL centrifuge tube. The mixture was vortexed (20 s) and then centrifuged (10000 g, 10 min). Supernatant (250 µL) was transferred to a sealed 96-well plate. These solutions for injection had a nominal concentration of 0.05 mg/mL and were analyzed immediately after preparation (duplicated injections).

Spironolactone

Test samples (200 µL) were diluted using acetonitrile (800 µL) in a 1.5-mL centrifuge tube. The mixture was vortexed (20 s) and then centrifuged (10000 g, 10 min). Supernatant (50 µL) was further diluted using methanol (950 µL), vortexed (20 s) and transferred to a sealed 96-well plate. These solutions for injection had a nominal concentration of 0.05 mg/mL and were analyzed immediately after preparation (duplicated injections).

Tacrolimus

Test samples (200 µL) were diluted using acetonitrile (800 µL) in a 1.5-mL centrifuge tube. The mixture was vortexed (20 s) and then centrifuged (10000 g, 10 min). Supernatant (150 µL) was further diluted using water (150 µL), vortexed (20 s) and transferred to a sealed 96-well plate. These solutions for injection had a nominal concentration of 0.05 mg/mL and were analyzed immediately after preparation (duplicated injections).

4.4.6 HPLC-UV method

Parameters used for the HPLC methods developed for each active ingredient are summarized in Table 4.2.

Table 4.2. HPLC methods parameters

Parameters	Active ingredient			
	Caffeine	**Hydrocortisone**	**Spironolactone**	**Tacrolimus**
Column	Kinetex XB C18	Agilent C18 Sorbax SB 4,6 mm	Agilent C18 Sorbax SB 4,6 mm	Kinetex XB C18
Flow rate	1 mL/min	0,9 mL/min	0,75 mL/min	0,9 mL/min
Wavelenght	273 nm	245 nm	239 nm	210 nm
Injection volume	10 µL	10 µL	10 µL	10 µL
Mobile phase	91% H_20, 8% ACN, 1% acetic acid	50% MeOH 50% H_20	70% MeOH 30% H_2O	70% H_20 30% ACN
Oven temperature	40°C	40°C	25°C	50°C

4.4.7 HPLC-UV method – Linearity

Caffeine

Stock caffeine solutions (15 mg/mL) were prepared in the suspension vehicle F4.5+ from bulk powder. 750 mg were first pulverized using a mortar and a pestle prior to geometric incorporation of the vehicle (*q.s. ad* 50 mL). The stock solution was diluted with the vehicle to obtain solutions having concentrations of 8, 9, 10, 11, 12 mg/mL. These

solutions (100 µL) were diluted using methanol (900 µL) in a 1.5-mL centrifuge tube. The mixture was vortexed (20 s) and then centrifuged (10000 g, 10 min). Supernatant (50 µL) was further diluted using water (950 µL) and vortexed (20 s) to obtain standard solutions having concentrations of 40, 45, 50, 55, 60 µg/mL. These solutions were analyzed using the HPLC method (triplicated injections).

Hydrocortisone

Stock hydrocortisone suspensions (1.5 mg/mL) were prepared in the suspension vehicle F4.5+ from tablets. 3 x 20 mg tablets were first pulverized using a mortar and a pestle prior to geometric incorporation of the vehicle (q.s. ad 40 mL). The stock suspension was diluted with the vehicle to obtain suspensions having concentrations of 0.8, 0.9, 1.0, 1.1, 1.2 mg/mL. These suspensions (200 µL) were diluted using a mixture of methanol: water 30:70 (3800 µL) in a 5-mL centrifuge tube, vortexed (20 s) and then centrifuged (10000 g, 10 min). Supernatants were used as standard solutions having concentrations of 40, 45, 50, 55, 60 µg/mL. These solutions were analyzed using the HPLC method (triplicated injections).

Spironolactone

Stock spironolactone suspensions (7.5 mg/mL) were prepared in the suspension vehicle F4.5+ from tablets. 3 x 100 mg tablets were first pulverized using a mortar and a pestle prior to geometric incorporation of the vehicle (q.s. ad 40 mL). The stock suspension was diluted with the vehicle to obtain suspensions having concentrations of 4, 4.5, 5, 5.5, 6 mg/mL. These suspensions (200 µL) were diluted using acetonitrile (800 µL) in a 1.5-mL centrifuge tube. The mixture was vortexed (20 s) and then centrifuged (10000 g, 10 min). Supernatant (50 µL) was further diluted using methanol (950 µL) and vortexed (20 s) to obtain standard solutions having concentrations of 40, 45, 50, 55, 60 µg/mL. These solutions were analyzed using the HPLC method (triplicated injections).

Tacrolimus

Stock tacrolimus suspensions (0.75 mg/mL) were prepared in the suspension vehicle F4.5+ from capsules. 6 x 5 mg capsules were first emptied and pulverized using a mortar and a pestle prior to geometric incorporation of the vehicle (q.s. ad 40 mL). The stock

suspension was diluted with the vehicle to obtain suspensions having concentrations of 0.4, 0.45, 0.5, 0.55, 0.6 mg/mL. These suspensions (200 µL) were diluted using acetonitrile (800 µL) in a 1.5-mL centrifuge tube. The mixture was vortexed (20 s) and then centrifuged (10000 g, 10 min). Supernatant (150 µL) was further diluted using water (150 µL) and vortexed (20 s) to obtain standard solutions having concentrations of 40, 45, 50, 55, 60 µg/mL. These solutions were analyzed using the HPLC method (triplicated injections).

4.4.8 HPLC-UV method – Sample preparation recovery

Standard solutions of caffeine, hydrocortisone, spironolactone and tacrolimus were prepared at concentrations of 40, 45, 50, 55, 60 µg/mL in water, MeOH, ACN and ACN respectively and analyzed using the HPLC-UV method. The resulting peak areas were compared with the areas obtained after sample preparation of the standard samples described in the HPLC-UV method – Linearity section.

4.4.9 HPLC-UV method – Variability

Intraday variability was calculated from the triplicated injections described in the HPLC-UV method – Linearity section.

Interday variability was calculated from the injection of standard samples described in the HPLC-UV method – Linearity on three different days.

4.4.10 HPLC-UV method – Specificity

Caffeine

Stock caffeine solutions (10 mg/mL) were prepared in the suspension vehicle F4.5+ from bulk powder as described above. Aliquots of this suspension (0.5 mL) were mixed with water (0.5 mL), aqueous hydrogen peroxide 3% (0.5 mL), aqueous hydrochloric acid 1 M (0.5 mL) and aqueous sodium hydroxide 1 M (0.5 mL). The NaOH solution was stored

for 1 h at 60 °C and the three other solutions were stored for 4 h at 60 °C. The acidic solution (100 µL) was neutralized using aqueous sodium hydroxide 1 M (50 µL) and diluted using methanol (850 µL). Similarly, the alkaline solution was neutralized using aqueous hydrochloric acid 1 M (50 µL) and diluted using methanol (850 µL). The water and peroxide solutions were directly diluted using methanol (900 µL). All these solutions were vortexed (20 s) and centrifuged (10000 g, 10 min). Supernatants (100 µL) were recovered and diluted in water (900 µL) to achieve a nominal concentration of 50 µg/mL (prior to degradation) and analyzed by HPLC. The chromatograms obtained from these analyses were compared with the chromatograms obtained from non-degraded caffeine solution (10 mg/mL) submitted to sample preparation for HPLC injection.

Hydrocortisone

Stock hydrocortisone suspensions (1 mg/mL) were prepared in the suspension vehicle F4.5+ from tablets as described above. Aliquots of this suspension (0.5 mL) were mixed with water (0.5 mL), aqueous hydrogen peroxide 3% (0.5 mL), aqueous hydrochloric acid 1 M (0.5 mL) and aqueous sodium hydroxide 1 M (0.5 mL). The NaOH solution was stored for 1 h at 60 °C and the three other solutions were stored for 4 h at 60 °C. The acidic solution (100 µL) was neutralized using aqueous sodium hydroxide 1 M (50 µL) and diluted using a mixture of methanol: water 30:70 (850 µL). Similarly, the alkaline solution was neutralized using aqueous hydrochloric acid 1 M (50 µL) and diluted using a mixture of methanol: water 30:70 (850 µL). The water and peroxide solutions were directly diluted using a mixture of methanol: water 30:70 (900 µL). All these solutions were vortexed (20 s) and centrifuged (10000 g, 10 min). Supernatants (250 µL) having a nominal concentration of 50 µg/mL (prior to degradation) were analyzed by HPLC. The chromatograms obtained from these injections were compared with the chromatograms obtained from non-degraded hydrocortisone suspension (1 mg/mL) submitted to sample preparation for HPLC injection.

Spironolactone

Stock spironolactone suspensions (5 mg/mL) were prepared in the suspension vehicle F4.5+ from tablets as described above. Aliquots of this suspension (0.5 mL) were mixed with water (0.5 mL), aqueous hydrogen peroxide 3% (0.5 mL), aqueous hydrochloric

acid 1 M (0.5 mL) and aqueous sodium hydroxide 1 M (0.5 mL). These solutions were stored for 3 h at 60 °C. The acidic solution (100 µL) was neutralized using aqueous sodium hydroxide 1 M (50 µL) and diluted using ACN (850 µL). Similarly, the alkaline solution was neutralized using aqueous hydrochloric acid 1 M (50 µL) and diluted using ACN (850 µL). The water and peroxide solutions were directly diluted using ACN (900 µL). All these solutions were vortexed (20 s) and centrifuged (10000 g, 10 min). Supernatants (200 µL) were recovered and diluted in methanol (800 µL) to achieve a nominal concentration of 50 µg/mL (prior to degradation) and analyzed by HPLC. The chromatograms obtained from these injections were compared with the chromatograms obtained from non-degraded spironolactone suspension (5 mg/mL) submitted to sample preparation for HPLC injection.

Tacrolimus

Stock tacrolimus suspensions (0.5 mg/mL) were prepared in the suspension vehicle F4.5+ from capsules as described above. Aliquots of this suspension (0.5 mL) were mixed with water (0.5 mL), aqueous hydrogen peroxide 3% (0.5 mL), aqueous hydrochloric acid 1 M (0.5 mL) and aqueous sodium hydroxide 1 M (0.5 mL). These solutions were stored for 3 h at 60 °C. The acidic solution (100 µL) was neutralized using aqueous sodium hydroxide 1 M (50 µL) and diluted using ACN (850 µL). Similarly, the alkaline solution was neutralized using aqueous hydrochloric acid 1 M (50 µL) and diluted using ACN (850 µL). The water and peroxide solutions were directly diluted using ACN (900 µL). All these solutions were vortexed (20 s) and centrifuged (10000 g, 10 min). Supernatants (200 µL) were recovered and diluted in methanol (800 µL) to achieve a nominal concentration of 50 µg/mL (prior to degradation) and analyzed by HPLC. The chromatograms obtained from these injections were compared with the chromatograms obtained from non-degraded tacrolimus suspensions (0.5 mg/mL) submitted to sample preparation for HPLC injection.

4.4.11 Definition of stability

Based on previously published studies (26-29), stability was defined as the absence of noticeable changes in the organoleptic properties, a pH variation of no more than 1.0 unit of pH relative to the initial pH and a concentration of active ingredient not less than 90.0% of the initial concentration.

4.4.12 Scale up process

For each batch, the excipients required to prepare 3.0 L of either formulation (caffeine, hydrocortisone and spironolactone) were accurately weighted and mixed by hand in a closed container (50 tumbles over 5 minutes). They were then slowly sprinkled in one liter of heated water (80°C) under mechanical agitation. After the dissolution of the ingredients, the remaining water was added to obtain a final volume of 3.0 L. The suspensions remained under agitation overnight. On the following day, samples were taken and their pH, concentration and viscosity were measured and compared to the values of the smaller volumes made for the stability studies (100 mL).

4.5 Results and discussion

4.5.1 HPLC-UV method – Linearity

A linearity of not less than 0.9995 was achieved for all methods for both solvent and extracted calibration curves.

4.5.2 HPLC-UV method- Sample preparation recovery

Recoveries between 98.8% and 109.6% were observed for all methods.

4.5.3 HPLC-UV method – Variability

Intraday variability of less than 3.78% was observed for all methods. The highest interday variability was of 3.59% for all methods.

4.5.4 HPLC-UV method – Specificity

Validation of the HPLC-UV methods

In general, testing an analytical procedure encompasses validating the following analytical characteristics: specificity, precision, linearity, accuracy, suitability range, limit of detection, limit of quantitation and stability of solutions (31).

For all active ingredients, a linearity of not less than 0.9995 was achieved, whether the calibration curves were prepared from solvent or extracted from calibration curves made in the F4.5+ vehicle. Recoveries between 98.8% and 109.6% (Tables S2 to S5) were observed for all methods and were found satisfactory, according to ICH guidelines (32). Intraday variability of less than 3.78% was observed for all methods (Tables S6 to S9). The highest interday variability was of 3.59% for all methods (Tables S10 to S13). These values are within the 5% range, as specified by ICH guidelines (32).

For caffeine, recoveries of 96%, 101%, 98% and 82% were observed after degradation in water, hydrogen peroxide, acidic and alkaline conditions, respectively. Thus, caffeine, a methylxanthine alkaloid, was mainly degraded by alkaline conditions, as already reported by others (33). No peak overlap of caffeine with excipients, impurities or degradation products were observed. Caffeine peak purity index, calculated between 243 and 303 nm, was not less than 0.9999 in all cases.

For hydrocortisone, recoveries of 99%, 86%, 97% and 78% were observed after degradation in water, hydrogen peroxide, acidic and alkaline conditions, respectively. Both hydrogen peroxide and alkaline conditions were able to degrade hydrocortisone. Indeed, hydrocortisone is known to be sensitive to oxidizing conditions (34). No peak overlap of hydrocortisone with excipients, impurities or degradation products were

observed. Hydrocortisone peak purity index, calculated between 215 and 275 nm, was not less than 0.9999 in all cases.

For spironolactone, recoveries of 110%, 93%, 94% and 12% were observed after degradation in water, hydrogen peroxide, acidic and alkaline conditions, respectively. Alkalinisation had a strong impact on spironolactone stability, as previously reported by Ram et al (35). No peak overlap of spironolactone with excipients, impurities or degradation products were observed. Spironolactone peak purity index, calculated between 209 and 269 nm, was not less than 0.9999 in all cases.

For tacrolimus, recoveries of 61%, 67%, 93% and 44% were observed after degradation in water, hydrogen peroxide, acidic and alkaline conditions, respectively. Except for acidic conditions, all other media strongly impacted on tacrolimus chemical stability, as previously demonstrated (36). No peak overlap of tacrolimus with excipients, impurities or degradation products were observed. Hydrocortisone peak purity index calculated between 200 and 240 nm was not less than 0.999 in all cases.

From these results, all methods were therefore found suitable for the appropriate quantification of the four active ingredients. Typical calibration curves in the 80-120% of the targeted drug concentrations in the F4.5+ vehicle are shown in Figure 4.1.

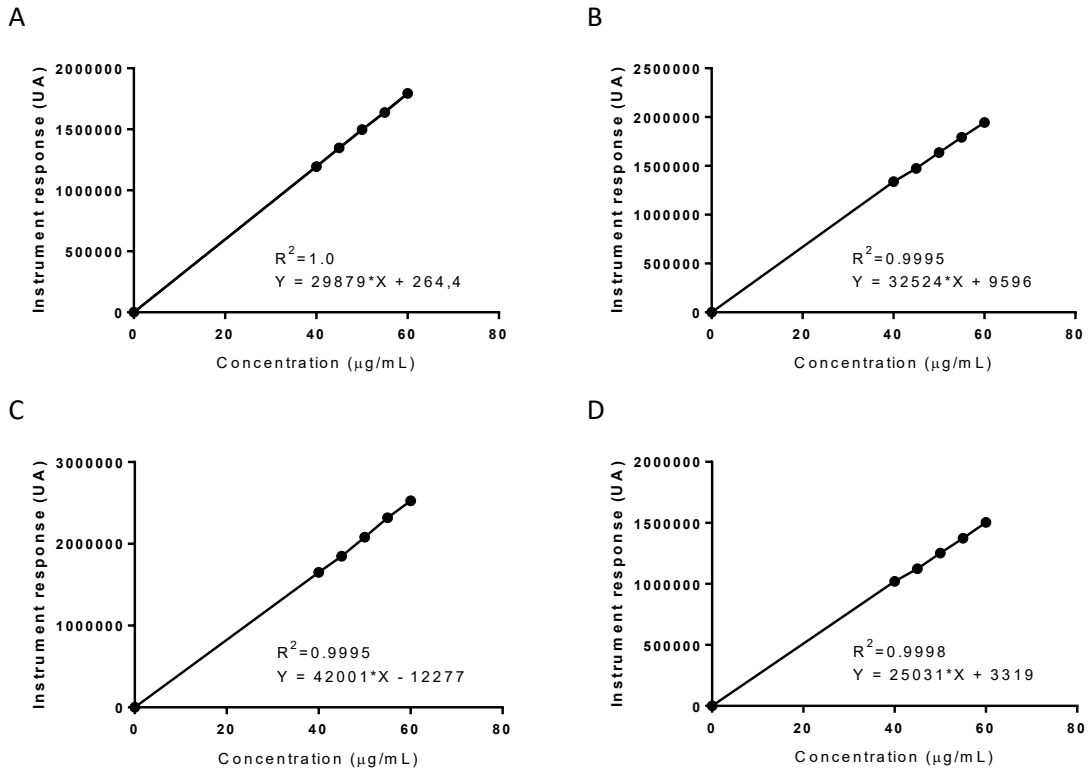

Figure 4.1. Typical calibration curves of active ingredients compounded in the F4.5+ vehicle. A- caffeine ; B- hydrocortisone ; C- spironolactone and D- tacrolimus.

4.5.5 Stability study

Studying the chemical stability of a compounded drug is critical. Indeed, various drugs intended for pediatric patients are commonly extemporaneously as drug products are mostly designed for the adult population. Therefore, it is still often required to compound these adult forms into liquid oral formulations (solutions or suspensions) in order to ensure that children receive the right dose. However, growing concerns arise as to whether compounded medications are similar in efficacy to FDA-approved products (37). In particular, the stability parameter may be essential for drug formulations that are prepared to be administered over weeks or months. The objective of this study was to further assess the variability in potency during the storage period of the four compounded formulations in the new F4.5+ suspension vehicle. In order to better mimic the most common storage conditions, we evaluated the stability of the compounded suspensions in amber plastic 60-mL bottles and 3-mL syringes, at room temperature and 5°C.

For caffeine solutions, no significant change in pH (Table S14) or appearance was observed for the duration of the study. Concentrations remained in the 95-105% range of the initial value (table 3), up to 180 days in bottles and 30 days in syringes both at 5°C and 25°C. Although there is a small- but constant- need for caffeine oral liquids in children (38), the majority of compounded caffeine is given as caffeine citrate solutions (9-11). Apart from using caffeine citrate solutions which taste poorly, only one reported compounded formulation of a 10 mg/mL caffeine suspension was found in the literature, using SyrSpendSF PH4 (Fagron) as a suspension vehicle (39). The authors found a 90-day stability at 5 and 25°C.

Organoleptic properties and pH (Table S15) of hydrocortisone suspensions also remained unchanged for the duration of the study. Hydrocortisone concentrations remained over 90% at 25°C in bottles for 180 days and 90 days at 5°C (table 4). A sedimentation phenomenon was observed in the refrigerated bottles after 180 days of storage; most of the excipients could be resuspended but not entirely. However, the formulations stored in syringes remained stable for 30 days at both tested temperatures. Hydrocortisone stability has already been reported when compounded in various suspension vehicles, either home-made (40) or in commercially available ones, such as Oral Mix (41), Ora-Sweet and Humco simple syrup (42) or InOrpha® (INRESA) (43). Our stability results are consistent, and even slightly improved, with the reported stabilities in the 70-90 days range.

Spironolactone suspensions (Tables 5 and S16) remained stable in all conditions for the whole duration of the study in both bottles and syringes at both temperatures. Nonetheless, some variability could be observed at a few time points for the bottle-conditioned suspensions (25°C, days 14 and 60; 5°C, day 75). The retrieved concentrations for these time points, over 110%, could be explained by the insolubility of the active ingredient in aqueous media (22 mg/L, PubChem CID 5833) which made the sampling problematic. Only a slight change of color was noticed for the spironolactone suspension turning from very pale to light yellow. Others have also published compounded formulations of spironolactone, for human or veterinary uses. Mathur et Vickman reported a Cherry Syrup NF formulation from spironolactone tablets, with

concentrations ranging from 2.5 to 10 mg/mL, whose stabilities in amber glass bottles were successfully evaluated for 4 weeks (44). More recently, a 5 mg/mL formulation from bulk powder compounded in Oral Mix was shown to display a 90-day stability in both plastic and glass bottles (45).

Finally, tacrolimus suspensions displayed no change of appearance nor pH (table S17) for the whole duration of the study. Tacrolimus concentrations ranged from the 90-110% of the nominal initial value for 90 days at 25°C and 180 days at 5°C for suspensions conditioned in bottles (table 6). The syringe-conditioned suspensions were stable 14 days at 5°C and 30 days at 25°C (table 6). The same intermittent variability in concentrations were also noticed for tacrolimus suspensions with some quantified remaining percentages over 110% (25°C, day 30; 5°C, days 14 and 30). Once again, the quasi-insolubility of tacrolimus in water (<1 mg/L, PubChem CID 445643) could be pointed out as a cause for problematic sampling. Tacrolimus compounded suspensions had already been established at the same target concentration, using either a 50:50 mixture of OraPlus/simple syrup (stable for 56 days at RT NF) (46) or Oral Mix (stable for 90 days at 5 and 25°C) (47). Compared to both formulations, the F4.5+/tacrolimus formulation compares favourably in terms of acceptability for children and stability duration.

The pH of all formulations was found stable in all conditions (4.6 for caffeine, 4.2 for hydrocortisone, 4.5 for hydrocortisone and 4.2 for tacrolimus). The maximal pH variation observed was of 0.16 pH unit for the caffeine solution (Tables S14 to S16), which was judged acceptable to ensure the stability of both drugs and excipients (48).

Table 4.3. Chemical stability of caffeine solutions prepared from tablets

	Mean Concentration ± SD (mg/mL) and Mean % Remaining*			
Study day	**Packaged in Amber Plastic Bottles**		**Packaged in Amber Plastic Syringes**	
Storage at 5°C, ambient RH				
Initial	10.01 ± 0.06			
7	9.78 ± 0.25	(97.7)	9.73 ± 0.27	(97.2)
14	10.28 ± 0.24	(102.7)	10.10 ± 0.50	(100.9)
30	9.61 ± 0.28	(96.0)	9.72 ± 0.20	(97.1)
45	9.68 ± 0.18	(96.7)		
60	9.82 ± 0.28	(98.1)		
75	9.75 ± 0.25	(97.4)		
90	10.28 ± 0.12	(102.7)		
180	9.67 ± 0.26	(99.6)		
Storage at 25°C, 60% RH				
Initial	10.01 ± 0.06			
7	9.72 ± 0.23	(97.2)	9.74 ± 0.24	(97.4)
14	10.27 ± 0.35	(102.6)	10.16 ± 0.29	(101.5)
30	9.85 ± 0.36	(98.4)	9.74 ± 0.25	(97.3)
45	9.77 ± 0.25	(97.6)		
60	9.85 ± 0.24	(98.4)		
75	9.89 ± 0.23	(98.8)		
90	9.58 ± 0.26	(95.7)		
180	10.38 ± 0.37	(103.7)		

Table 4.4. Chemical stability of hydrocortisone suspensions prepared from tablets

	Mean Concentration ± SD (mg/mL) and Mean % Remaining*			
Study day	**Packaged in Amber Plastic Bottles**		**Packaged in Amber Plastic Syringes**	
Storage at 5°C, ambient RH				
Initial	1.02 ± 0.02			
7	0.93 ± 0.02	(91.0)	1.00 ± 0.01	(98.3)
14	0.96 ± 0.02	(94.0)	0.95 ± 0.04	(93.6)
30	0.98 ± 0.04	(96.6)	0.95 ± 0.02	(93.7)
45	0.98 ± 0.04	(95.8)		
60	1.00 ± 0.02	(98.3)		
75	1.04 ± 0.05	(101.5)		
90	1.03 ± 0.02	(101.0)		
180	0.88 ± 0.14	(86.4)		
Storage at 25°C, 60% RH				
Initial	1.02 ± 0.02			
7	0.94 ± 0.01	(92.4)	0.99 ± 0.09	(97.1)
14	0.96 ± 0.02	(93.8)	0.96 ± 0.03	(94.3)
30	0.95 ± 0.03	(93.0)	0.95 ± 0.03	(93.2)
45	1.00 ± 0.03	(98.0)		
60	1.03 ± 0.10	(101.0)		
75	1.02 ± 0.02	(99.9)		
90	0.97 ± 0.06	(94.9)		
180	0.93 ± 0.05	(91.3)		

Table 4.5. Chemical stability of spironolactone suspensions prepared from tablets

	Mean Concentration ± SD (mg/mL) and Mean % Remaining*			
Study day	**Packaged in Amber Plastic Bottles**		**Packaged in Amber Plastic Syringes**	
Storage at 5°C, ambient RH				
Initial	4.97 ± 0.08			
7	5.10 ± 0.20	(102.6)	4.90 ± 0.26	(98.6)
14	5.86 ± 0.36	(117.8)	5.10 ± 0.66	(102.6)
30	4.86 ± 0.27	(96.9)	4.66 ± 0.05	(93.7)
45	4.84 ± 0.18	(97.3)		
60	5.71 ± 0.66	(114.6)		
75	5.46 ± 0.14	(109.7)		
90	5.10 ± 0.20	(102.6)		
180	5.17 ± 0.10	(103.9)		
Storage at 25°C, 60% RH				
Initial	4.97 ± 0.08			
7	5.27 ± 0.04	(105.9)	5.27 ± 0.06	(106.0)
14	5.46 ± 0.32	(109.8)	4.83 ± 0.44	(97.1)
30	4.93 ± 0.26	(99.2)	5.08 ± 0.19	(102.1)
45	5.00 ± 0.07	(100.5)		
60	5.43 ± 0.41	(109.3)		
75	5.62 ± 0.21	(113.0)		
90	5.40 ± 0.16	(108.6)		
180	5.46 ± 0.03	(109.9)		

Table 4.6. Chemical stability of tacrolimus suspensions prepared from tablets

	Mean Concentration ± SD (mg/mL) and Mean % Remaining*			
Study day	**Packaged in Amber Plastic Bottles**		**Packaged in Amber Plastic Syringes**	
Storage at 5°C, ambient RH				
Initial	0.49 ± 0.02			
14	0.53 ± 0.02	(107.2)	0.52 ± 0.03	(106.4)
30	0.56 ± 0.07	(113.3)	0.53 ± 0.01	(109.1)
45	0.51 ± 0.03	(105.1)		
60	0.52 ± 0.01	(105.4)		
75	0.51 ± 0.02	(104.0)		
90	0.52 ± 0.01	(106.1)		
180	0.38 ± 0.14	(78.1)		
Storage at 25°C, 60% RH				
Initial	0.49 ± 0.02			
14	0.55 ± 0.02	(113.0)	0.47 ± 0.07	(95.6)
30	0.57 ± 0.04	(115.9)	0.37 ± 0.01	(76.4)
45	0.54 ± 0.01	(110.1)		
60	0.49 ± 0.02	(101.0)		
75	0.52 ± 0.06	(105.8)		
90	0.53 ± 0.01	(108.0)		
180	0.50 ± 0.05	(101.2)		

4.5.6 Scale up

One important factor in the practicality of compounding a liquid vehicle relates to ensuring that a sufficient quantity of the compounded formulation can be prepared at the same time. This aspect is of primordial importance to hospital pharmacists who sometimes have to prepare large quantities of formulations, with an expected stability of over a few months. Therefore, we chose to evaluate the scale up feasibility of the compounding process with three of the active ingredients, caffeine, hydrocortisone and spironolactone. Due to its high cost and relative toxicity, tacrolimus was not retained for this part of the study.

Therefore, three 100-mL batches and three 3-L batches were prepared and characterized for appearance, viscosity, pH and concentrations (Table 7). The concentration and viscosity did not seem to be impacted by the method of fabrication nor the final volume. Viscosity values were found very similar between 100-mL and 3-L batches, with no more than a 2.1% difference (for hydrocortisone). The pH and appearance were also comparable for all formulations, except for the spironolactone one, for which the pH of the 3-L batches was found significantly higher. These differences could be explained by the fact that the 100-mL batches were compounded from tablets whereas the 3-L batches were prepared from bulk powders. This could also explain the color difference, as the tablets contain a yellow dye.

100-mL batches are typically prepared in a community pharmacy, or even a hospital for rare formulations. On the contrary, the 3-L batches represent bigger hospital batches or the pilot scale of an industrial process. Our results proved the possible scale-up of these formulations. Maintaining and documenting consistent quality and performance in the presence of scale change remains a challenge, due for instance to the different process equipment (49). In particular, suspensions, as any thermodynamically unstable system, require careful adjustment of the mixing phase (50). Our preliminary results demonstrate that the F4.5+ formulation is compatible with the scale-up process and displays similar physicochemical characteristics as the 100-mL, bench formulation. This holds great promises for the globalization of use of this safer, paediatric-oriented oral vehicle.

Tables 4.7. Scale up process

Batch volume	Formulation	Concentration (mg/mL)	pH	Viscosity (cP)	Appearance
100 mL	Caffeine	10.01±0.06	4.57±0.02	85.75±1.34	Transparent solution
	Hydrocortisone	1.02±0.02	4.22±0.01	80.16±0.85	White suspension
	Spironolactone	4.97±0.08	4.46±0.02	84.45±2.35	Yellow suspension
3 L	Caffeine	9.72±0.11	4.49±0.07	85.57±1.65	Transparent solution
	Hydrocortisone	1.02±0.02	4.60±0.02	81.86±0.99	White suspension
	Spironolactone	5.04±0.08	4.44±0.05	83.36±1.66	White suspension

4.6 Conclusion

Using stability-indicating, validated HPLC methods, this study demonstrated the stability of 10 mg/mL caffeine solutions, 1 mg/mL hydrocortisone, 5 mg/mL spironolactone and 0.5 mg/mL tacrolimus suspensions prepared from the new F4.5+ suspension vehicle, specially designed for the paediatric use. Caffeine and spironolactone remained stable at 5 °C and 25 °C when stored in amber plastic bottles for 180 days and up to 30 days in amber plastic syringes. Hydrocortisone was found stable in bottles for 180 days at 25°C and 90 days at 5°C. When stored in syringes, its stability was established for 30 days at both temperatures. Finally, tacrolimus, when stored at 5ºC, was stable for 180 days in bottles and 14 days in syringes. When stored at 25ºC, its stability was ascertained up to 90 days in plastic bottles and 30 days in syringes.

Taken together with the formulation development study (submitted), as well as the palatability evaluation in children and adults (submitted), these series of articles demonstrate unambiguously that the F4.5+ vehicle is a safe and suitable alternative to commercially-available suspension vehicles for newborns and children. Its low production cost and process simplicity should ensure an easy translation from benchtop to clinical practice.

4.7 Acknowledgments

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4.8 Disclosure of interest

The authors report no conflicts of interest.

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Chapitre 5: Discussion et Conclusion

Les objectifs énumérés au chapitre 1 ont été réalisés, excepté pour ce qui est de la réalisation de la version alcaline du véhicule de suspension. Premièrement, un véhicule de suspension a été développé et caractérisé. Pour ce faire, différentes formulations furent testées afin d'obtenir les caractéristiques voulues. Une fois la composition déterminée, le pH, la viscosité, le comportement rhéologique ainsi que l'osmolalité de ces solutions ont été déterminés et ajustés afin de convenir à l'utilisation future en milieu pédiatrique. La stabilité des propriétés physicochimiques ainsi que microbiologiques du véhicule final a ensuite été évaluée. Aucun changement n'a été observé durant les 6 mois de stabilité et ce à température pièce et réfrigérée.

Lorsqu'une formulation est développée pour une sous-population, il est important de considérer les vulnérabilités de ce groupe dans toutes les étapes du développement. Puisque les enfants sont en constante croissance, une formulation offrant une flexibilité de dose est nécessaire. Ils ont également de la difficulté à avaler. Une formulation liquide a donc été envisagée. Vu la faible quantité d'information disponible chez les enfants quant à l'innocuité des divers excipients utilisés, une approche conservatrice a été utilisée pour établir la composition de cette formulation. Viennent ensuite les recommandations et exigences des autorités gouvernementales. Nous avons combiné les recommandations en pédiatrie de différents gouvernements pour les formulations pédiatriques aux exigences de l'USP en ce qui a trait aux suspensions.

Il aurait été intéressant de développer un véhicule de suspension au pH alcalin afin de convenir aux principes actifs plus stables à pH élevé. Aucun des agents de conservation testés au pH 7.5 ne fut efficace. Ce critère a été jugé essentiel pour poursuivre le développement du véhicule et c'est pourquoi la version alcaline du véhicule de suspension a été laissée de côté. Pour le moment, les principaux agents de conservation utilisés en milieu alcalin sont une combinaison de parabènes. De récentes évidences ont semé le doute quant à l'innocuité de cette substance. De récentes études ont démontré une activité agoniste sur les récepteurs de l'œstrogène ainsi que des signes de génotoxicité et de carcinogénicité [78,79]. Bien qu'utilisés en tant que contrôle négatif pour le test

d'efficacité antimicrobien, il a été jugé inadéquat d'inclure des parabènes dans la composition du véhicule de suspension par raison de sécurité. Il est évident que la découverte de nouveaux agents de conservation efficaces en milieu alcalin bénéficierait à la communauté scientifique. La plupart des agents de conservation utilisée pour inhiber la croissance microbienne dans le domaine pharmaceutique sont efficaces en milieu acide [80].Considérant ce manque, il est difficile de développer une formulation liquide au pH alcalin qui répondra aux exigences des autorités réglementaires tout en étant sécuritaire. Malgré tout, l'acide propionique s'est révélé être un agent de conservation sécuritaire et efficace pour la version acide du véhicule de suspensions. Une formulation prévenant la croissance microbienne a donc pu être établie.

Une évaluation de la palatabilité a ensuite été réalisée chez des volontaires enfants et adultes. Le véhicule développé contenant de la quinine comme standard d'amertume fut comparé à un autre véhicule de suspension contenant la même quantité de quinine. Dans le groupe adulte, une différence significative au niveau du goût a été remarquée en faveur du véhicule développé. L'appréciation générale a été plus grande pour le véhicule développé dans les 2 groupes. Vu le faible nombre de participants, peu de différences significatives ont pu être observées. Un nombre plus important de sujets aurait pu être envisagé. L'échelle de visages hédoniques pour évaluer les différents critères aurait pu comporter plus de points, ou être de nature différente. La présence des parents lors de l'évaluation aurait également pu influencer l'enfant en le mettant en confiance. Dû à la difficulté de recruter des enfants et d'obtenir le consentement des parents, les sujets d'âge pédiatrique sont souvent laissés de côté lors des études cliniques, nous avons dû également faire face à ce problème. Il a été jugé essentiel d'inclure un groupe pédiatrique dans l'évaluation de la palatabilité. En effet, bien que les tendances en termes d'appréciation soient similaires, les résultats se sont révélés différents en fonction des groupes d'âge enfant et adulte. Les enfants ont évalué les préparations plus sévèrement en général. Une formulation jugée acceptable par des adultes pourrait donc déplaire complètement aux enfants.

Finalement, différents principes actifs ont été ajoutés au véhicule de suspension développé et la stabilité de ces formulations a été déterminée. Les résultats présentés dans cet article concordent avec ceux trouvés dans la littérature lorsque des conditions de stockage similaires sont utilisées (bouteilles et seringues ambrées entreposées à 5 °C et 25 °C). Des stabilités de 3 à 6 mois ont été rapportées pour différentes formulations de caféine, ce qui est identique à la stabilité de 180 jours trouvée dans notre étude. Pour l'hydrocortisone, un résultat similaire a été trouvé par rapport aux études de stabilité rapportées dans la littérature (4 semaines à 3 mois avec Ora-Blend). Les études de stabilité des formulations de spironolactone rapportés dans la littérature sont limitées à une période de 90 jours. Nous avons mené une étude de stabilité de 180 jours et avons constaté que la spironolactone était stable pendant toute la durée de cette étude. De même, la plus longue stabilité constatée pour le tacrolimus était de 4 mois lorsqu'Ora-Blend est employé comme véhicule de suspension. Nos résultats montrent que ce principe actif est stable jusqu'à 6 mois lorsqu'il est entreposé dans des bouteilles en plastique ambré et entreposé à 25°C ou 3 mois lorsqu'entreposé dans des seringues et bouteilles à 5°C et 25°C. Il serait avantageux d'étudier davantage de principes actifs, ce qui augmenterait la pertinence du nouveau véhicule de suspensions et bénéficierait aux pharmaciens des différents milieux en leur fournissant des données fiables. Pour répondre à ce besoin, d'autres études de stabilité pourraient être réalisées dans le futur en fonction des besoins de la population pédiatrique.

Des procédés de fabrication à petite et plus grande échelle ont été comparés pour ces formulations. Le pH, l'apparence et la concentration en principes actifs des suspensions ont été utilisés comme critères de comparaison. La taille des lots et le procédé de fabrication utilisé n'ont pas influencé les caractéristiques des formulations, ce qui montre une possibilité de fabrication à l'échelle industrielle.

En conclusion, un véhicule de suspension stable, efficace et sécuritaire a été développé spécialement pour l'usage pédiatrique. Différentes formulations ont été réalisées et leur

stabilité a été déterminée afin de fournir une date limite d'utilisation fiable aux pharmaciens ainsi qu'aux patients.

Chapitre 6: Informations supplémentaires

Table S1. Matériel Chapitre 2

Item	Supplier	Grade	Catalogue #	Lot #
sodium citrate	Sigma-Aldrich	≥99% FG dihydrate	W302600	28H0209
dibasic sodium phosphate	Sigma-Aldrich	anhydrous	RES20908	115K0034
sucralose	Sigma-Aldrich	≥98.0% HPLC	69293	BCBP3048V
zinc oxide	Sigma-Aldrich	ACS reagent, ≥99.0%,	96479	BCBQ4754V
calcium propionate	Sigma-Aldrich	99.0-100.5%	18104	BCBQ9781V
sodium chloride	Sigma-Aldrich	anhydrous	793566	SLBN2865V
glucose	Sigma-Aldrich	D-(+)-	G8270	SLBM4390V
citric acid	Fisher Chemical	Anhydrous ACS certified	A940	165877
tryptone	Fisher Chemical		BP1421	165824
polysorbate 80	Fisher Chemical		BP338	170526
dextrose	Fisher Chemical	Anhydrous, Certified ACS	D16	101149
dibasic potassium phosphate	Fisher Chemical	Certified ACS	P288	161283
microcrystalline cellulose	Galenova	Vivapur 101	4V901-0100	12490-3232
carboxymethylcellulose sodium	Galenova	high viscosity, NF	C4327-0100	14724-7331
sodium benzoate	Galenova		SO291	14325-5191
carrageenan	TCI America		C1805	28VYJ-QR
xanthan gum	TCI America		X0048	OXSPK-FO

propionic acid	TCI America		P0500	67ATKDB
potassium sorbate	TCI America		S0057	UY45M-BE
methylparaben	Pharmascience	NF	AJ0511	567250
hydroxypropyl methylcellulose	Dow Chemical Company	Methocel K100M		D180G1D001
hydroxypropyl methylcellulose	Dow Chemical Company	(Methocel K4M		PL13012N01
soytone	Becton, Dickinson		243620	5091836
granulated agar	Becton, Dickinson		214530	4239505
polypeptone	Becton, Dickinson		211910	6354833
acetaminophen	Apotex	Tablets, 325 mg		NC5882
prednisone	Apotex	Tablets, 5 mg		MV8205
Oral Mix SF	Medisca		2600-08	611850/A
Ora-Blend	Perrigo			605682/B
simple syrup	Laboratoire Atlas			8GF
Staphylococcus aureus	ATCC		6538	64457140
Pseudomonas aeruginosa	ATCC, Laboratories Jeanne Leblond	product was received on 2017-07-11	9027	
Escherichia coli	Laboratories Marc Servant	DH5α, product was received on 2017-05-29		
Candida albicans	ATCC Laboratories Martine Raymond	product was received on 2017-06-1	10231	
Aspergillus brasiliensis	ATCC		16404	64463882

Table S2. Matériel Chapitre 3

Item	Supplier	Grade	Catalogue #	Lot #
Citric acid	Galenova	anhydrous, USP/FCC	cr480-0100	06992-8037
Sodium citrate	Galenova	anhydrous, USP	so380-004s	00871-7290
Sodium chloride	Galenova	crystals, USP	so370-0100	07028-7300
Microcrystalline cellulose	Galenova	50um, NF	4V901-0100	02127-7311
Xanthan gum	Galenova	NF	xA200-0100	00503-8045
Sodium phosphate dibasic	Galenova	anhydrous, USP	so461-0100	14926-6279
Potassium sorbate	Galenova	FCC	PO281-0100	14457-7292
Sodium carboxymethylcellulose	Galenova	high viscosity, NF	c4327-0100	14724-7331
Sucralose	Spectrum chemicals	NF	S1416-100GM	2GL0277
Calcium propionate	Spectrum chemicals	FCC, powder	CA166-500GM	2GJ0313
Quinine sulfate	Spectrum chemicals	dihydrate, USP	QU110-10GM	2FG0215
Carrageenan	Spectrum chemicals	NF	C1107-25GM	1HC0622
Hydroxypropyl methylcellulose	Dow Chemical Company	Methocel K100M		D180G1D001
Methylparaben	Pharmascience	NF		567250
Sterile water	Baxter	USP		W8D05Q0F

Table S3. Matériel Chapitre 4

Item	Supplier	Grade	Catalog #	Lot #
Sodium citrate	Sigma-Aldrich	≥99% FG dihydrate	W302600	28H0209
Dibasic sodium phosphate	Sigma-Aldrich	Anhydrous	RES20908	115K0034
Sucralose	Sigma-Aldrich	≥98.0% HPLC	69293	BCBP3048V
Calcium propionate	Sigma-Aldrich	99.0-100.5%	18104	BCBQ9781V
Sodium chloride	Sigma-Aldrich	Anhydrous	793566	SLBN2865V
Citric acid	Fisher Chemical	Anhydrous ACS certified	A940	165877
Dibasic potassium phosphate	Fisher Chemical	Certified ACS	P288	161283
Glacial acetic acid	Fisher Chemical	ACS certified	A38	176643
Methanol	Fisher Chemical	HPLC	A412-4	144689
Acetonitrile	Fisher Chemical	HPLC	A4998-4	141693
Hydrochloric acid aqueous solution	Fisher Chemical	1 M	. SA48-1	160432
Sodium hydroxide aqueous solution	Fisher Chemical	1 M	SS266-1	143309
Hydrogen peroxide aqueous solution	Fisher Chemical	3%	H323-500	135515
Carboxymethyl cellulose sodium	Galenova	High viscosity, NF	C4327-0100	14724-7331

sodium benzoate	Galenova		SO291	14325-5191
Hydroxypropyl methylcellulose	Dow Chemical Company	Methocel K100M		D180G1D001
Potassium phosphate monobasic	JT Baker		3246-19	Y22465
Caffeine	Medisca	Anhydrous, USP	0419	51224/A
Hydrocortisone	Medisca	Micronised, USP	0009	613163/A
Tacrolimus	Medisca	Monohydrate	2698	48905/A
60-mL amber plastic bottles	Medisca	PET bottle with black phenolic cap	7294	54755
3-mL amber plastic syringes	Medisca	Precise Dose Dispenser with tip cap	8152-02	601901R/D
Spironolactone	TCI America		S0260	VEWQF-PK
Spironolactone tablets	Teva	100 mg	311324	35211318A
Hydrocortisone tablets	Pfizer	20 mg, Cortef		WNMW
Tacrolimus capsules	Astellas Pharma	5 mg, Prograf		5E3030T

Table S4. Turbidity measurement of micro-organism cultures and associated concentration. n=3.

Strain	OD600		SD	CFU/mL		SD
E. Coli	1.5	±	0.4	2.2	±	1.1×10^8
P. Aeruginosa	1.6	±	0.4	6.3	±	2.5×10^8
S. Aureus	1.6	±	0.2	4.0	±	0.7×10^8
C. Albicans	1.5	±	0.1	6.6	±	2.6×10^7
A. Brasiliensis	1.7	±	0.2	3.0	±	0.8×10^7

Only the formulations showing a minimum of efficacy are displayed on the figure 3 A) to E).

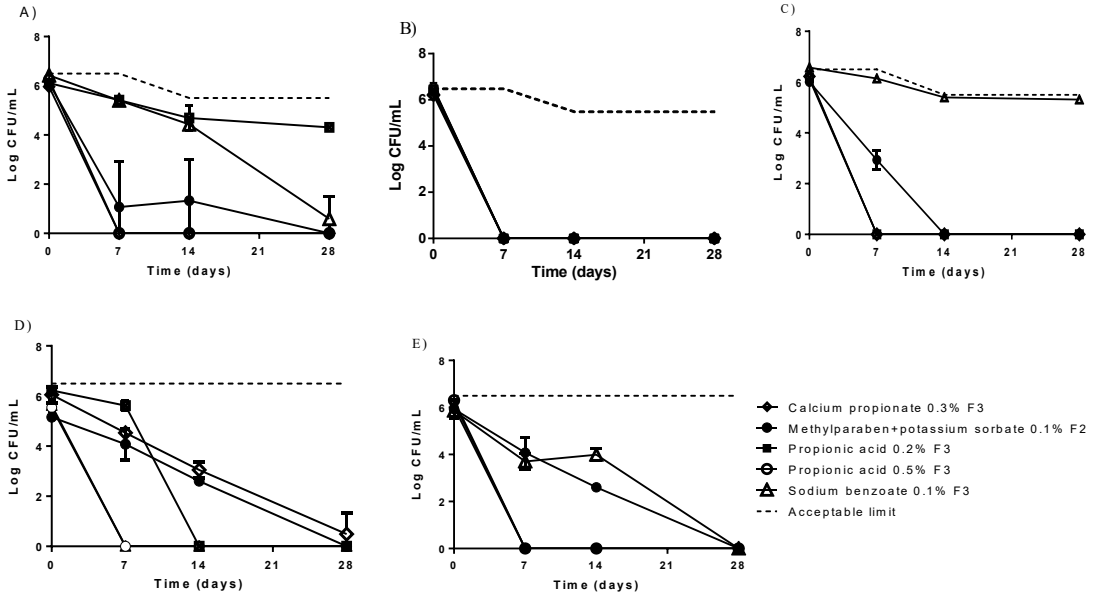

Figure S1. Concentration (Log_{10}) of **A)** *E. Coli,* **B)** *P. Aeruginosa,* **C)** *S. Aureus*, **D)** *C. Albicans* and **E)** *A. Brasiliensis* after inoculation (0, 7, 14 and 28 days) with different preservatives; calcium propionate 0.3% in empty lozenge, methylparaben+potassium sorbate 0.1% in full circle, sodium benzoate 0.1% in empty triangle, propionic acid 0.2% in full square, propionic acid 0.5% in large full circle. The acceptable upper limit of the concentration at each time point is the dotted line.

HPLC-UV method – Linearity

Caffeine

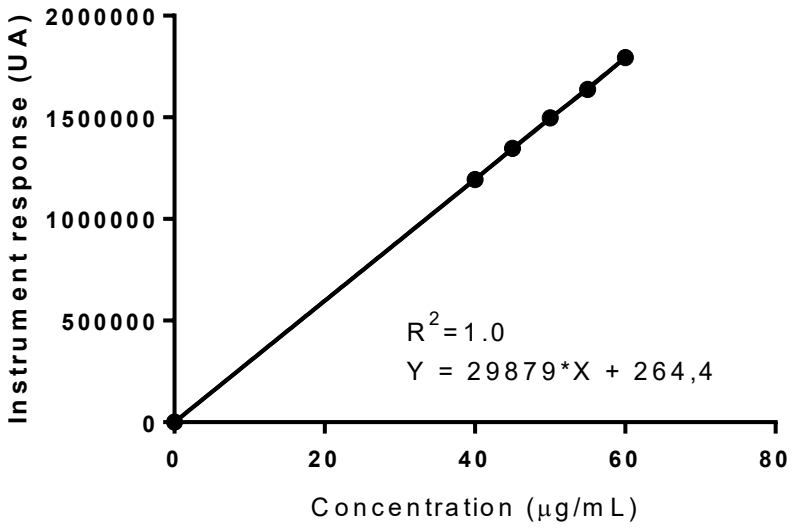

Figure S2. Calibration curve of caffeine in the suspension vehicle

Hydrocortisone

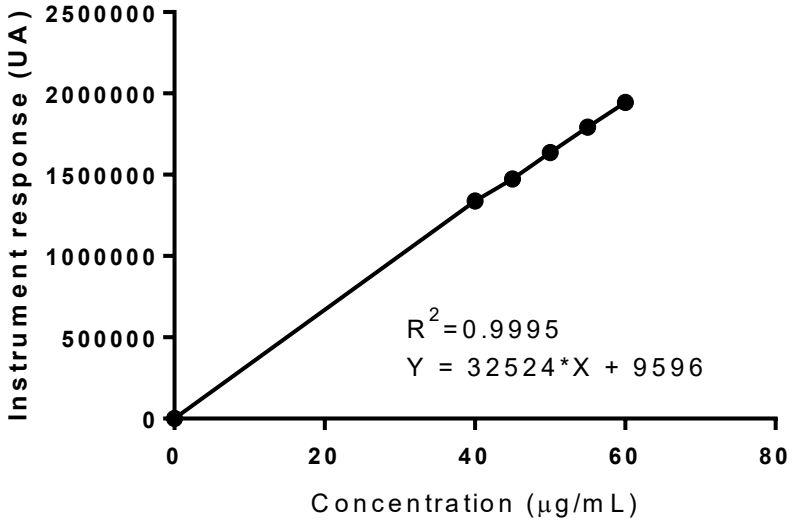

Figure S3. Calibration curve of hydrocortisone in the suspension vehicle

Spironolactone

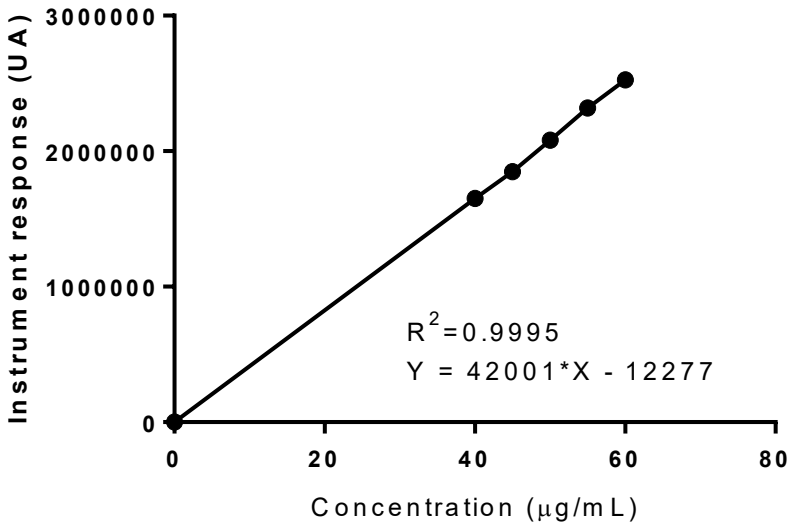

Figure S4. Calibration curve of spironolactone in the suspension vehicle

Tacrolimus

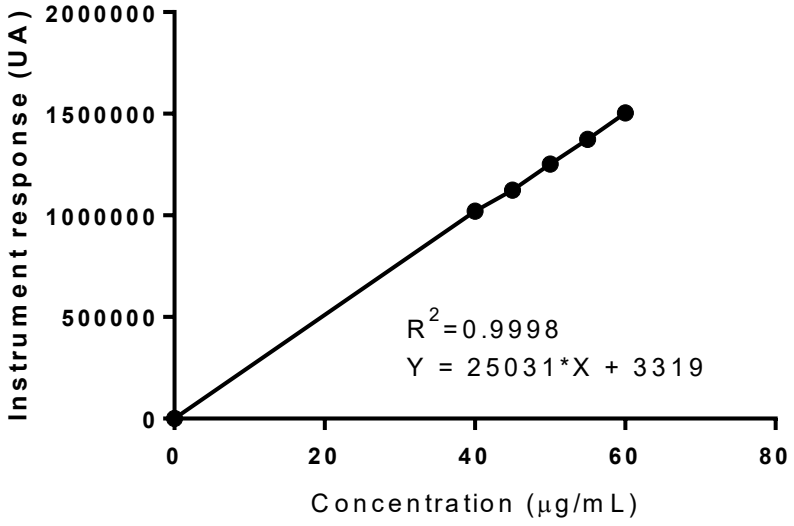

Figure S5. Calibration curve of tacrolimus in the suspension vehicle

HPLC-UV method- Sample preparation recovery

Table S5. Caffeine recovery after sample preparation

Conc. (mg/mL)	Mean area		Recovery
	Mobile Phase	Suspension vehicle	
8	1 194 404	1 275 021	106.7%
9	1 347 497	1 441 497	107.0%
10	1 496 731	1 610 232	107.6%
11	1 637 824	1 747 151	106.7%
12	1 794 895	1 915 410	106.7%

Table S6. Hydrocortisone recovery after sample preparation

Conc. (mg/mL)	Mean area		Recovery
	Mobile Phase	Suspension vehicle	
0.8	1 287 859	1 343 147	104.3%
0.9	1 479 013	1 480 469	100.1%
1	1 651 132	1 647 611	99.8%
1.1	1 816 138	1 794 791	98.8%
1.2	1 960 663	1 947 090	99.3%

Table S7. Spironolactone recovery after sample preparation

Conc. (mg/mL)	Mean area		Recovery
	Mobile Phase	Suspension vehicle	
4	1 578 122	1 650 475	104.6%
4.5	1 773 113	1 848 676	104.3%
5	1 908 151	2 082 492	109.1%
5.5	2 124 311	2 317 604	109.1%
6	2 305 542	2 527 429	109.6%

Table S8. Tacrolimus recovery after sample preparation

Conc. (mg/mL)	Mean area		Recovery
	Mobile Phase	Suspension vehicle	
0.4	1 010 353	1 064 230	105.3%
0.45	1 130 670	1 180 046	104.4%
0.5	1 257 362	1 304 652	103.8%
0.55	1 383 332	1 432 489	103.6%
0.6	1 508 224	1 555 012	103.1%

HPLC-UV method – Variability

Table S9. Intraday variability: Caffeine

Conc. (µg/mL)	Mean area	SD	RSD
0	0	-	-
8	1 275 021	574.61	0.05%
9	1 441 497	921.82	0.06%
10	1 610 232	197.06	0.01%
11	1 747 151	147.78	0.08%
12	1 915 410	666.07	0.03%

Table S10. Intraday variability: Hydrocortisone

Conc. (µg/mL)	Mean area	SD	RSD
0	0	-	-
0.8	1 343 147	1821.33	0.14%
0.9	1 480 469	2160.43	0.15%
1	1 647 611	3012.70	0.18%
1.1	1 794 791	947.50	0.05%
1.2	1 947 090	854.34	0.04%

Table S11. Intraday variability: Spironolactone

Conc. (µg/mL)	Mean area	SD	RSD
0	0	-	-
4	1 650 475	5267.89	0.32%
4.5	1 848 676	8265.12	0.45%
5	2 082 492	6952.36	0.33%
5.5	2 317 604	10720.89	0.46%
6	2 527 429	13466.20	0.53%

Table S12. Intraday variability: Tacrolimus

Conc. (µg/mL)	Mean area	SD	RSD
0	0	-	-
0.4	1 064 230	40198.23	3.78%
0.45	1 180 046	39163.48	3.32%
0.5	1 304 652	34675.25	2.66%
0.55	1 432 489	31088.96	2.17%
0.6	1 555 012	30293.87	1.95%

Table S13 Interday variability: Caffeine

Conc. (µg/mL)	Mean area			Mean Interday	SD Interday	RSD Interday
	Day 1	Day 2	Day 3			
0	0	0	0	0	-	-
8	1 275 021	1 188 522	1 202 223	1 195 050	6872.97	0.58%
9	1 441 497	1 339 455	1 351 102	1 346 018	5962.88	0.44%
10	1 610 232	1 495 540	1 499 311	1 497 194	1927.99	0.13%
11	1 747 151	1 628 989	1 646 363	1 637 725	8687.09	0.53%
12	1 915 410	1 786 295	1 804 826	1 795 339	9273.62	0.52%

Table S14. Interday variability: Hydrocortisone

Conc. (µg/mL)	Mean area			Mean Interday	SD Interday	RSD Interday
	Day 1	Day 2	Day 3			
0	0	0	0	0	-	-
0.8	1 343 147	1 346 792	1 346 792	1 345 577	2104.83	0.16%
0.9	1480469	1 485 377	1 485 377	1 483 741	2833.44	0.19%
1	1647611	1 653 859	1 653 859	1 651 776	3607.67	0.22%
1.1	1794791	1 798 935	1 798 935	1 797 553	2392.35	0.13%
1.2	1 947 090	1 949 031	1 949 031	1 948 384	1120.64	0.06%

Table S15. Interday variability: Spironolactone

Conc. (µg/mL)	Mean area			Mean Interday	SD Interday	RSD Interday
	Day 1	Day 2	Day 3			
0	0	0	0	0	-	-
4	1 650 475	1 691 456	1 653 267	1 665 066	22897.20	1.38%
4.5	1 848 676	1 903 394	1 800 289	1 850 787	51584.72	2.79%
5	2 082 492	2 133 401	2 006 164	2 074 019	64040.43	3.09%
5.5	2 317 604	2 395 585	2 227 070	2 313 420	84335.39	3.65%
6	2 527 429	2 616 734	2 435 175	2 526 446	90783.49	3.59%

Table S16. Interday variability: Tacrolimus

Conc. (µg/mL)	Mean area			Mean Interday	SD Interday	RSD Interday
	Day 1	Day 2	Day 3			
0	0	0	0	0	-	-
4	987 431	977 483	1 021 289	995 401	21737.75	2.18%
4.5	1 109 660	1 104 943	1 123 429	1 112 677	10834.19	0.97%
5	1 253 534	1 250 611	1 253 278	1 252 474	6079.07	0.49%
5.5	1 387 146	1 413 536	1 374 866	1 391 849	17597.96	1.26%
6	1 509 939	1 512 404	1 504 742	1 508 914	4901.89	0.32%

Table S17. Caffeine formulation pH

	Mean caffeine solution pH ± SD	
Study day	**Packaged in Amber Plastic Bottles**	**Packaged in Amber Plastic Syringes**
Storage at 5°C, ambient RH		
Initial	4.57 ± 0.02	
7	4.42 ± 0.01	4.42 ± 0.01
14	4.41 ± 0.02	4.42 ± 0.0
30	4.41 ± 0.01	4.42 ± 0.01
45	4.42 ± 0.01	
60	4.43 ± 0.01	
75	4.42 ± 0.01	
90	4.42 ± 0.01	
180	4.42 ± 0.01	
Storage at 25°C, 60% RH		
Initial	4.57 ± 0.02	
7	4.43 ± 0.01	4.41 ± 0.01
14	4.42 ± 0.0	4.43 ± 0.01
30	4.42 ± 0.01	4.43 ± 0.01
45	4.42 ± 0.01	
60	4.43 ± 0.01	
75	4.42 ± 0.01	
90	4.43 ± 0.01	
180	4.43 ± 0.0	

Table S18. Hydrocortisone formulation pH

	Mean hydrocortisone suspensions pH ± SD	
Study day	**Packaged in Amber Plastic Bottles**	**Packaged in Amber Plastic Syringes**
Storage at 5°C, ambient RH		
Initial	4.22 ± 0.01	
7	4.22 ± 0.01	4.21 ± 0.0
14	4.22 ± 0.01	4.24 ± 0.01
30	4.23 ± 0.0	4.22 ± 0.01
45	4.22 ± 0.01	
60	4.23 ± 0.0	
75	4.24 ± 0.01	
90	4.24 ± 0.01	
180	4.24 ± 0.01	
Storage at 25°C, 60% RH		
Initial	4.22 ± 0.01	
7	4.22 ± 0.01	4.22 ± 0.01
14	4.24 ± 0.01	4.22 ± 0.01
30	4.22 ± 0.01	4.22 ± 0.0
45	4.22 ± 0.0	
60	4.22 ± 0.01	
75	4.23 ± 0.01	
90	4.24 ± 0.01	
180	4.24 ± 0.01	

Table S19. Spironolactone formulation pH

	Mean spironolactone suspensions pH ± SD	
Study day	**Packaged in Amber Plastic Bottles**	**Packaged in Amber Plastic Syringes**
Storage at 5°C, ambient RH		
Initial	4.46 ± 0.02	
7	4.45 ± 0.01	4.45 ± 0.01
14	4.45 ± 0.01	4.45 ± 0.01
30	4.45 ± 0.01	4.45 ± 0.01
45	4.46 ± 0.02	
60	4.45 ± 0.01	
75	4.45 ± 0.01	
90	4.45 ± 0.01	
180	4.46 ± 0.01	
Storage at 25°C, 60% RH		
Initial	4.46 ± 0.02	
7	4.45 ± 0.01	4.45 ± 0.01
14	4.45 ± 0.01	4.45 ± 0.01
30	4.45 ± 0.01	4.45 ± 0.01
45	4.46 ± 0.02	
60	4.46 ± 0.01	
75	4.46 ± 0.01	
90	4.47 ± 0.01	
180	4.47 ± 0.01	

Table S20. Tacrolimus formulation pH

	Mean tacrolimus solution pH ± SD	
Study day	**Packaged in Amber Plastic Bottles**	**Packaged in Amber Plastic Syringes**
Storage at 5°C, ambient RH		
Initial	4.21 ± 0.02	
7	4.22 ± 0.0	4.22 ± 0.0
14	4.21 ± 0.0	4.21 ± 0.0
30	4.23 ± 0.01	4.23 ± 0.01
45	4.22 ± 0.01	
60	4.22 ± 0.0	
75	4.21 ± 0.01	
90	4.23 ± 0.0	
180	4.22 ± 0.0	
Storage at 25°C, 60% RH		
Initial	4.21 ± 0.02	
7	4.22 ± 0.0	4.22 ± 0.0
14	4.21 ± 0.0	4.21 ± 0.0
30	4.23 ± 0.01	4.23 ± 0.01
45	4.22 ± 0.01	
60	4.23 ± 0.01	
75	4.22 ± 0.01	
90	4.23 ± 0.0	
180	4.23 ± 0.01	

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