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Complementarity of UV-PLS and HPLC for the simultaneous evaluation of antiemetic drugs

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ABSTRACT

This work was dedicated to the development of a simple and direct multivariate UV spectrophotometric method for the simultaneous determination of three antiemetic drugs (ondansetron, dexamethasone and aprepitant) in a new organogel formulation developed for their simultaneous transdermal administration. This method that does not require separation of the drugs and sophisticated instrument will permit to control quality of this new transdermal form both during the optimization step and for a further routine control of this preparation at the pharmacy department of the hospital. Hence, a partial least squares regression model using the spectral data record from 260 to 288 nm and 5 components, has firstly been validated thanks to the evaluation of the REP% (under 7.9%) and secondly using an accuracy profile approach (acceptance limit of $\pm$ 10%). Thereby, the method allows the quantitation of the drugs in the ranges (5–15 mg L^{-1}), (4–8 mg L^{-1}) and (20–50 mg L^{-1}) for ondansetron, dexamethasone and aprepitant, respectively. An HPLC/UV reference method has also been developed. Optimal separation ($2.52 < Rs < 9.49$) of the three drugs and their internal standards has been obtained in less than 15 min with a C18 stationary phase using a gradient separation protocol. This method has been validated similarly for the quantitation of ondansetron, dexamethasone and aprepitant in the ranges (0.3–3.5 mg L^{-1}), (0.2–10 mg L^{-1}) and (3.5–35 mg L^{-1}), respectively. Both methods used for quality control of an organogel pharmaceutical formulation, have shown recoveries between 95% and 105%, hence validating the UV/PLS method and the formulation preparation process. Lower limits of quantitation obtained with the HPLC/UV method will be in favor of its use for permeation studies.

1. Introduction

Despite the high number of formulations available for a drug on the pharmaceutical market, it can arise that a patient needs a treatment for which none pharmaceutical form or dosage is available. In this case, pharmacy department of the hospital may have to prepare this form, in accordance with health agency, respecting PIC/S guide to "good practices for the preparation of medicinal products in health care establishments" [1]. Oral route is the most often chosen in regard to its ease of use [2] and to the high quantity that can be administrated. Nevertheless, this route exhibits some drawbacks as local gastrointestinal toxicity and first pass metabolism. Moreover, it is sometimes not suitable, especially for all populations like the elderlies [3] or new-borns [4], and for all pathophysiological states such as dysphagia. Although intra-venous route does not display the oral drawbacks, its invasive side and the need of aseptic administration limits its systematic use [5].

In this area, a specific request of an onco-pediatric department has been registered to develop a galenic form allowing the simultaneous administration of three antiemetic drugs currently used to prevent acute or delayed chemotherapy-induced nausea and vomiting (CINV). Hence, ondansetron (Ond), dexamethasone (Dex) and aprepitant (Apt) are included in an antiemetic protocol starting with the chemotherapy and administrated for three days, (Fig. 1) [6]. These drugs are recommended by the American Society of Clinical Oncology (ASCO) [7], the European Society of Medical Oncology (ESMO), the Multinational Association of Supportive Care in Cancer (MASCC) [8] and the National Comprehensive Cancer Network (NCCN) guidelines [9], as they targeted different therapeutic receptors. Despite oral route is recommended for the administration of this antiemetic treatment, it is clearly not convenient. The other alternative, *i.e.* the intra-venous route, results in an important discomfort for the patient, especially for children. To improve the patient quality of life, the development of transdermal formulations containing these three antiemetic drugs has been proposed.

A thorough study of the feasibility of such approach is currently performed in our laboratory. As onco-pediatric department is

Abbreviations: Ond, ondansetron; Dex, dexamethasone; Apt, aprepitant; Met, metoclopramide; Hyd, hydrocortisone; Lop, loperamide

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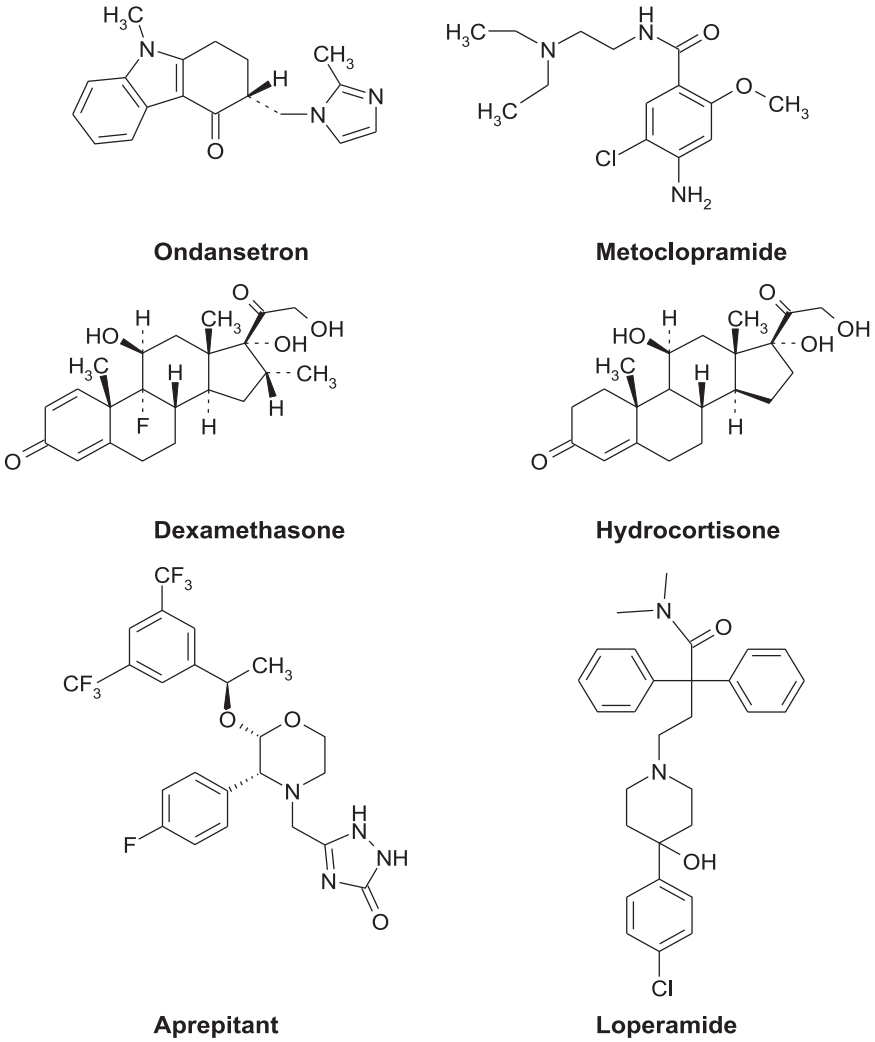

Fig. 1. Chemical structures of antiemetic investigated (Dex, Ond, and Apt) and their respective internal standard (Hyd, Met, and Lop).

responsible for the treatment of patients from 0 to 18 years old, for whom weight and body surface area can be very different, it appears clearly that transdermal forms prepared and controlled at the hospital will contain a wide range of the antiemetic drugs. Moreover, none information is available concerning the pharmacokinetics of these drugs using a transdermal administration. Firstly, taking into account the dose used, for an adult, to treat acute chemotherapy-induced nausea and vomiting (32 mg of Ond, 12 mg of Dex and 80 mg of Apt, administrated once daily) [6], we chose to prepare a transdermic organogel form containing 3.2% (w/w) Ond, 1.2% Dex and 8% Apt, that will be administrated once daily. Thereafter, according to the patient and to pharmacokinetic data that will be recorded, organogels containing either lower or higher quantities of the three drugs will be formulated.

The purpose of this work was first to develop an analytical tool to control the quality of these new transdermal forms, not only during the optimization step but for a further routine control of these preparations at the pharmacy department of the hospital. The second objective was to establish an analytical method for studying the ability of these organogels to release drugs in the systemic circulation, *i.e.* a suitable method for the determination of drugs during *ex vivo* permeation studies.

Despite the combination of these three antiemetics in the CINV treatment for several years, the MEKC method previously optimized and validated in our team was the only one dealing with the simultaneous determination of ondansetron, dexamethasone and aprepitant. Unfortunately, this method is time consuming, and requires a sophisticated instrument not available at the hospital [10]. Firstly, we also focus on the development of a simple and rapid method not requiring the separation of the drugs, using UV spectrophotometry associated with a chemometric treatment, *i.e.* partial least squares (PLS) regression, as its interest has been widely demonstrated [11–16]. Hence, when more than one light absorbing component are present in a mixture, which is the case with the three antiemetic drugs studied, a direct comparison of the absorption at a certain wavelength with a reference material is not suitable, as co-absorption may occur. PLS is a multivariate analysis method based on factor analysis and involving spectral decomposition, that permits to establish a regression model based on latent variable decomposition relating a block of independent variables, X (spectra), to a block of dependent ones, Y (concentrations). It is therefore a statistical tool to predict concentration of different chemicals in a mixture from its complex spectra [16–18]. This method has been applied for the quantitation of three antiemetic drugs in the organogel.

Moreover, in order to evaluate the results obtained by PLS and to fulfill our second objective, it became essential to have another analytical method allowing simultaneous quantitation of the three antiemetic drugs. Despite MEKC is an alternative methodology, it exhibits quite high quantitation limits not suitable for this purpose [10]. We also focused on another orthogonal methodology, *i.e.* HPLC using UV detection. To our knowledge, no publication describes

simultaneous HPLC quantitation of these drugs. The only few published HPLC methods available in the literature deals with the quantitation of ondansetron [19–21], aprepitant [22–25] or dexamethasone [26–31]. It is noteworthy that the HPLC method has been developed for an eventual coupling to a mass spectrometer (MS) for further pharmacokinetic studies.

The works, presented hereafter, describes the optimization and the validation of the UV-PLS and HPLC methods. Both methods were finally used for the quality control of a new organogel formulation.

2. Materials and methods

2.1. Chemicals

Ondansetron was purchased from Biotrend (Zurich, Switzerland), dexamethasone from Inresa (Bartenheim, Germany), aprepitant from Focus Synthesis LLC (San Diego, United States), hydrocortisone base from the Cooper (Melun, France), metoclopramide from Santa Cruz biotechnology (Santa Cruz, Canada) and loperamide from Sigma Aldrich (Saint Quentin Fallavier, France). Acetonitrile was supplied by VWR (Val de Fontenay, France), ethanol by LiChrosolv (Darmstadt, Germany) and formic acid by Prolabo (Val de Fontenay, France). Ultra-pure 18 MΩ water was produced by a Milli-Q system Millipore (Saint-Quentin en Yvelines, France). All the chemicals and reagents were of chemical analytical grade. The organogel was kindly gifted by Fagron (Paris, France). It is commercialized as a kit composed of two phases to mix extemporary: (1) a pluronic F127 gel and (2) a lecithin solution.

2.2. Apparatus and conditions

2.2.1. Spectrophotometry UV/PLS

A Cary 100 UV–visible spectrophotometer supplied by Varian (Les Ulis, France) was used at 25 °C to record absorption spectra from 200 to 400 nm using a scanning speed of 300 nm min^{-1} and a spectrum data interval of 0.5 nm. Spectral measurements were carried out with 1 cm path length quartz cells and collected with the Cary WinUV software.

2.2.2. HPLC/UV

Chromatographic analyses were performed on a Waters system (Milfors, MA, USA) equipped with a gradient quaternary 600E metering pump, an online degasser apparatus, a 7125 Rheodyne injector (20 μL sample loop) and a 996 photodiode array detector. Data were collected and processed on a computer running with Empower solftware (version 2) from Waters. Separations were carried out on a reversed-phase Kinetex C18 (100 × 4.60 mm *i.d.*, 2.6 μm) column (Phenomenex, Le Pecq, France). After optimization, a gradient separation mode has been used to separate Ond, Dex and Apt as their respective internal standards, *i.e.* Met, Hyd and Lop, respectively, using H_2O (A) and acetonitrile (B), both containing 0.1% formic acid. The gradient elution performed at 1 mL min^{-1} is the following: (1) 20/80 (v/v) mixture of solvents A and B for the first 0.5 min; (2) increase to 80% of B in a linear gradient from 0.5 to 16 min. The column was thermostated at 25 °C. Compounds were dissolved in 20/80 EtOH/H_2O (v/v) and filtered through a 0.45 μm regenerated cellulose membrane prior to loading the column. Detection was performed at 310 nm for Ond and Met; other compounds were detected at 265 nm.

2.3. Preparation of calibration and validation standards

2.3.1. Spectrophotometry UV with PLS

Stock solutions of Ond (100 mg L^{-1}), Dex (100 mg L^{-1}) and Apt (1000 mg L^{-1}), were prepared in EtOH. Matrix stock solution, *i.e.* 2% (w/w) organogel solution was obtained by: (1) dissolution of the organogel in EtOH (50-fold dilution factor), (2) sonication for 15 min and (3) filtration on a 0.45 μm PTFE syringe filter (Alltech, Templemars, France). All these solutions were used to prepare 2.5 ml of ethanolic calibration and validation standards, containing a constant concentration of organogel of 0.033% (w/w) (3000-fold diluted organogel). The dilution factor was chosen so that the concentrations of each drug in the resulting solution were in its own linear dynamic range for the analysis of real samples, according to the expected organogel drugs content. The calibration set was constructed according to a 3^3+1 full factorial design, *i.e.* three factors, (Ond, Dex and Apt concentrations), at three levels (low, median and high concentrations) plus one central point repeated 6 times. Selected concentrations are specified in Table 1. These ranges were selected around the values expected for the final concentration of the analyzed samples, after the dilution of the formulated organogel. Moreover, they take into account the variability of forms which can be prepared according to pediatric doses and pharmacokinetic parameters that will be evaluated. Validation standards containing the three antiemetics were prepared at three concentration levels: low (5, 4 and 20 mg L^{-1}); medium (10, 6 and 35 mg L^{-1}) and high concentration (15, 8 and 50 mg L^{-1}) of Ond, Dex and Apt, respectively. All standards were randomly analyzed according to the design proposed by MODDE™ statistical design program (version 2009, Umetrics, Malmö, Sweden). Thus, 33 calibration standards and 9 validation standards were performed each day and during three consecutive days (*i.e.* 27 validations standards) to validate the spectroscopic UV method modelized by the PLS regression.

Table 1
Values of the variables at factorial (−1; 1) and center (0) points of the experimental design.

Level	[Ond] (mg L^{-1})	[Dex] (mg L^{-1})	[Apt] (mg L^{-1})
−1	2	2	10
0	10	6	35
+1	18	10	60

2.3.2. HPLC/UV

Stock solutions of Ond, Dex, Apt, Met, Hyd and Lop (1000 mg L^{-1}) were prepared in EtOH. Matrix stock solution, *i.e.* 2% (w/w) organogel ethanolic solution, was obtained according the same protocol used for UV/PLS experiments. All these solutions were used to prepare calibration and validation standards, containing constant concentrations of organogel (0.033% (w/w)) and internal standards (3, 10 and 30 mg L^{-1} of Hyd, Met and Lop respectively), in EtOH/H_2O (20/80 – v/v). The organogel content of all the standards was set according to the minimal dilution to perform to analyze organogel formulations (3000-fold dilution). For each drug, the lowest concentration of the calibration standards was set at the LOQ estimated from a signal-to-noise ratio of ten. For Apt, the highest concentration was chosen according to its solubility in EtOH/H_2O – 20/80 (v/v). For Ond and Dex, the highest concentrations were finally set with respect to the highest concentration of Apt, taking into account the expected proportion of these three drugs in the organogel formulations. Therefore, a series of eight calibration standards containing mixtures of the three drugs were prepared; the concentration ranges were 0.05–12 mg L^{-1}, 0.2–4 mg L^{-1} and 1–40 mg L^{-1} for Ond, Dex and Apt, respectively. Four validation standards containing the three drugs were prepared in triplicate; the concentration of Ond, Dex and Apt were respectively (0.2, 0.3 and 1.5 mg L^{-1}) for the low level, (1, 0.9 and 6 mg L^{-1}) and (5, 1.75 and 20 mg L^{-1}) for the two medium levels, and finally (10, 3.5 and 35 mg L^{-1}) for the high level.

2.4. Validation of the methods

The validation of each method was performed according to the validation guidelines proposed by the French Society of Pharmaceutical Sciences and Techniques – SFSTP [31–33]. Its requirements are based on the study of the "accuracy", also called the "total error" which results from the sum of the systematic error (trueness) and the random error (precision) obtained with validation standards. The total error describes the difference between the true value and the value calculated with the analytical method for each concentration level of validation standards allowing drafting an accuracy profile. The accuracy profile is a decision-making tool allowing to ensure the quality of the analytical procedure with a tolerance error (acceptance limits) and a risk (β-expectation tolerance interval) previously defined by the analyst. Hence, β represents the probability that measurements are included inside the acceptance limits.

The validation of the method was carried out on three consecutive days to estimate the prediction errors. Each day, a series of eight calibration standards, four validation standards (each prepared in triplicate) and a blank sample were prepared and analyzed. Finally, selectivity, response function, linearity, trueness, precision (repeatability and intermediate precision) were studied according to the validation requirements of the SFSTP [31–33] and accuracy profile was assessed taking into account the $\pm$10% acceptance limits admitted for quantitation of transdermic preparation [34] at a risk of 5%.

2.5. Pharmaceutical preparation analysis

In order to evaluate the recovery of both validated methods, an organogel pharmaceutical formulation containing 32 mg g^{-1} of ondansetron, 12 mg g^{-1} of dexamethasone and 80 mg g^{-1} of aprepitant has been prepared in our laboratory: 64 mg of Ond, 24 mg of Dex and 160 mg of Apt were introduced in 400 μL of ethanol. After addition of 220 mg of lecithin solution, a suitable mass of pluronic F127 was added to prepare 2 g of organogel. To control the composition of this preparation, 50 mg of this one were 50-fold diluted in EtOH and filtrated on a 0.45 μm PTFE syringe filter. Then, the solution was diluted one more time to obtain 3000-fold diluted samples either in EtOH for UV/PLS experiments or in H_2O/EtOH – 20/80 v/v for HPLC/UV analysis. In this last case, internal standards were obviously added during the last dilution step to obtain final concentration equal to 3, 10 and 30 mg L^{-1} for Hyd, Met and Lop, respectively.

3. Results and discussion

3.1. Spectrophotometry UV/PLS

3.1.1. PLS regression model optimization

In the first step, ethanolic solutions of the three drugs alone and mixed together with the 3000-fold diluted matrix were prepared. The concentrations were chosen in regard to the concentration that will be obtained for the analysis of real samples. The corresponding spectra were recorded from 200 to 400 nm. As can be observed in Fig. 2, presenting the spectra in the spectral range of interest (from 200 to 340 nm), there is a strong overlap of the spectra of the three drugs. Moreover, absorbance of the matrix cannot be overlooked. A multivariate calibration approach is also required for the quantitation of the drugs without a previous separation step. Multivariate calibration methods require a suitable experimental design of the calibration standards to permit a good prediction. Concentrations of drugs were obviously chosen in their linear dynamic range, beyond the limit of quantitation equal

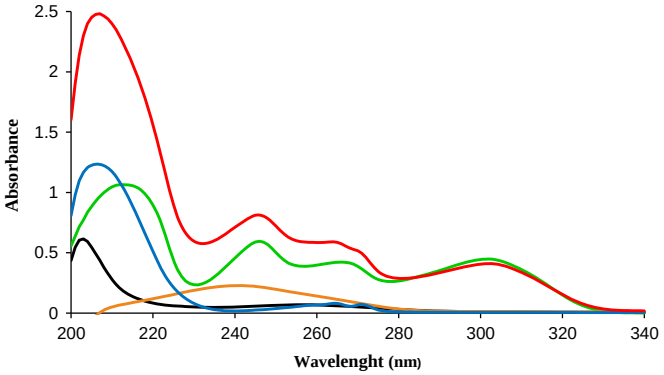

Fig. 2. Spectra of the three antiemetic drugs and of the organogel alone or in mixture in ethanol: orange line – Dex at 6 mg L^{-1}; green line – Ond at 10 mg L^{-1}; blue line – Apt at 35 mg L^{-1}; black line – 3000-fold diluted organogel; and red line – 3000-fold diluted organogel containing a mixture of the three drugs at the same concentrations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to 0.07 mg L^{-1}, 0.1 mg L^{-1} and 2 mg L^{-1} for Ond, Dex and Apt, respectively. These values were determined during the evaluation of the linear dynamic range of each drug, from the standard deviation of the noise (σ_N) and the slope of the calibration curve (a) using the following equation:

$$\mathrm{LOQ} = 10 \times \frac{\sigma_N}{a} \quad (1)$$

The calibration set was built using 33 calibration standards (Table 1). In addition, nine solutions were used as validation standards (three concentration levels prepared in triplicate). A PLS-2 regression model was constructed using PLS module in Excel Stat 2012 software. This construction was performed for different spectral regions used during the calibration to further choose the spectral region leading to the best predictive model. In fact, it may have a great influence on the quality of the model as undesired regions containing needless and interfering information can result in an increase in the prediction errors. First of all, the number of components has to be determined. It is a very important parameter to obtain an optimum prediction with the main information and to avoid over and underfittings [11]. For this purpose a cross-validation method was employed [15]. The parameter examined for the determination of the components number was the Q^2_{cum} index that is expressed by the following equation:

$$Q^2{}_{cum}(h) = 1 - \prod_{a=1}^{h} \frac{\mathrm{PRESS}_a}{\mathrm{RESS}_{a-1}} \quad (2)$$

with a, the component number and h the total component number [37]. The PRESS corresponds to the predicted residual sum of squares and RESS to the residual sum of squares [35]. It corresponds to a measure of the global contribution of the h first components to the predictive quality of the model. The optimal components number is the minimal number of component which yields to the maximum $Q^2{}_{cum}$ (no more improvement for a subsequent component). The number of components selected for each spectral region investigated is presented in Fig. 3. It ranges from five to seven depending on the spectral region. Generally, a high number of components degrades the prediction ability of the model, but the complexity of the mixture containing three compounds without UV specific characteristics (Fig. 2) explains the necessity to have many components [14]. Fig. 3 summarizes the $Q^2{}_{cum}$ values obtained after suppression of the outliers. Best results were obtained for the spectral region ranging from 260 to 288 nm with $Q^2{}_{cum}$ values greater than 0.996 for the three

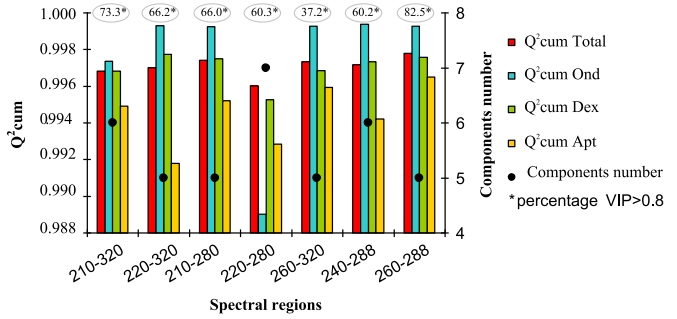

Fig. 3. PLS regression model optimization – components number, Q^2_{cum} index for each drug separately (Q^2_{cum} Dex, Q^2_{cum} Ond and Q^2_{cum} Apt) and for all the drugs (Q^2_{cum} total) and VIP, for the different spectral regions studied.

antiemetic drugs and a $Q^2{}_{cum}$ total equal to 0.997 using 5 components. It is noteworthy that VIP (variable importance for the projection) was evaluated in each case. For the selected spectral region, 82.5% of the explicative variables show a VIP greater than 0.8 in accordance with limit set by Wold [36].

3.1.2. Validation of the UV/PLS method

The external validation of the PLS method was performed using the standards of the validation set. The prediction ability of a PLS method is most often described in terms of the relative error of prediction (REP) and is also expressed as a percentage [34]. Despite some authors suggest the calculation of a mean value for all the standards included in the validation set, a relative error of prediction was calculated for each drug at each concentration level (k) separately, according to the following equation:

$$\%\ \mathrm{REP}_k = \frac{100}{C_{true}}\sqrt{\frac{1}{n}\sum_{i=1}^{n}(C_{true}-C_{pred})^2} \qquad (3)$$

where C_{true} is the true concentration of the standard i, C_{pred} the corresponding predicted concentration determined using the model obtained from the calibration set and n the number of validation standards with the same drug concentration. The relative error of prediction was also calculated for each drug and at three concentration levels from the 9 validation standards respectively performed. They are varying from 2.8% to 7.9%, from 4.0% to 5.6% and from 2.7% to 6.0% for Ond, Dex and Apt, respectively. According to the acceptance limits chosen for these works ($\pm$10%), the prediction quality of the method can be regarded as being good. Anyway, this approach does not permit to evaluate the total error (trueness and precision) at various concentration levels unlike the accuracy profile. For this reason and to further compare HPLC and UV/PLS methods using the same parameters, the accuracy profile of the UV/PLS method has been established for each drug (9 validation standards each day and during three consecutive days). In this goal, the concentration of validation standards was firstly back-calculated using the PLS model, before to be used to evaluate both the trueness and the precision (repeatability and the intermediate precision) (Table 2). Relative biases that range from $-$1.91% to 4.03%, are in accordance with the pharmaceutical criteria used for the control of extemporaneous preparation for hospital requirement [34] and attest the trueness of the method. Moreover, precision values evaluated by means of relative standard deviation are lower than 4.51%. From these data, the upper and the lower boundaries of the tolerance interval were determined for each validation standard and accuracy profiles were established. As illustrated in Fig. 4, whatever the concentration level, the tolerance interval (β=5%) is included in the acceptance limits set at $\pm$10% for the three drugs. In other words, for a β-expectation tolerance interval and an acceptance limit set at 5 and 10%, respectively, no more than 5% of our samples will have a total error higher than 10%.

According to the approach chosen, the low and high quantitation limits are clearly the extremes that can be quantified with a defined accuracy, and are consequently the extreme concentrations investigated during the validation experiments, *i.e.* (5 and 15 mg L^{-1}), (4 and 8 mg L^{-1}) and (20 and 50 mg L^{-1}) for Ond, Dex and Apt, respectively.

3.2. HPLC/UV

3.2.1. Optimization of the HPLC method

The three active ingredients of interest have different physicochemical properties [38–40] such as their ionization capacity (pKa$_{Ond}$=7.7, pKa$_{Dex}$=13.5, and pKa$_{Apt}$=9.7), or log P (log P_{Ond}=2.00, log P_{Dex}=1.83, and log P_{Apt}=4.80) and as a result different solubilities. These differences have led us to use a specific internal standard for each drug. Despite it complicates the separation, it will permit the use of this methodology for the simultaneous quantitation of these drugs in biological matrix after a purification step if necessary. After few assays, hydrocortisone, metoclopramide and loperamide were finally selected as internal standard of ondansetron, dexamethasone and aprepitant, respectively (Fig. 1). In regard to literature, a C18 stationary phase and acetonitrile-based mobile phase have been mostly used to achieve the analysis of the drugs [19–31]. In order to allow a further detection using MS, 0.1% of formic acid has been added to water and acetonitrile to obtain A and B solvents. Optimization was focused on the acetonitrile proportion, on the sample solubilization solvent and on the temperature. The goal of this optimization is to separate the three active ingredients, the matrix constituents and their respective internal standard in an appropriate run time. The ACN content was firstly varied from 35% to 60%. Whatever the mobile phase composition, the elution order is the following: (1) Met, (2) Ond, (3) Hyd, (4) Dex, (5) Lop and (6) Apt. This result is in accordance with their hydrophobic character and their ionization state. Retention factor (k') and resolution (Rs) are decision-making parameters to assess quality of separation and are so presented for each analyte for different proportion of ACN in Fig. 5. As illustrated, whatever the percentage of ACN, k' values observed for the three drugs are very different. For example, using 35% of ACN, k' values for Ond, Dex and Apt are 0.34, 2.51 and 44.39, respectively. Thus, their separation using an isocratic mode is impossible in an appropriate run time. Finally, compounds of interest were separated at 25 °C using a gradient phase ((1) 20/80 (v/v) mixture of solvents A and B for the first 0.5 min; (2) increase to 80% of B in a linear gradient from 0.5 to 16 min). The chromatograms obtained at 310 and 265 nm are shown in Fig. 6a. Antiemetic compounds and their respective internal standard are eluted in less than 15 min with resolution between 2.52 and 9.49. As none significant difference between the separation performances has been noticed by varying temperature from 20 to 40 °C, the column temperature was maintained equal to 25 °C. Moreover, it is interesting to underline that attention must be paid on the composition of sample solubilization solvent. Indeed, ethanol content higher than 20% resulted in splitting of metoclopramide peak. Thus, the sample solubilization solvent composed of EtOH/H_2O (20/80, v/v) both enable to solubilize all drugs and to obtain a suitable chromatogram.

3.2.2. Validation of the HPLC method

Specificity was assessed by comparing chromatograms obtained by injecting a solution containing organogel matrix (3000-fold dilution; Fig. 6b) and the same solution spiked with antiemetics and their respective internal standards (Fig. 6a). The absence of coelution between the matrix constituents and the analytes revealed the specificity of the method.

Table 2
Validation results of the UV/PLS method: response function, trueness, precision and accuracy for the analysis of ondansetron (Ond), dexamethasone (Dex) and aprepitant (Apt).

Validation parameters		Ond		Dex		Apt	
Response function	**Y=BX+E**[a]	PLS model with 5 components; Q^2_{cum} total=0.996 ± 0.002					
	Q^2_{cum}	0.999 ± 0.001		0.999 ± 0.001		0.992 ± 0.002	
Trueness[b]		$C_{calculated}$ (mg L^{-1})	Relative bias (%)	$C_{calculated}$ (mg L^{-1})	Relative bias (%)	$C_{calculated}$ (mg L^{-1})	Relative bias (%)
	QC1	4.94	−1.16	4.03	0.80	20.74	3.70
	QC2	9.99	−0.11	6.08	1.29	35.34	0.98
	QC3	14.71	−1.91	8.32	4.03	49.84	−0.32
Precision[b]		Repeatability (% RSD)	Intermediate precision (% RSD)	Repeatability (% RSD)	Intermediate precision (% RSD)	Repeatability (%RSD)	Intermediate precision (% RSD)
	QC1	1.36	3.42	1.56	2.16	1.08	3.26
	QC2	1.30	1.49	0.98	1.32	3.72	4.51
	QC3	2.84	4.25	1.63	2.77	0.93	3.11
Accuracy[b]	**Tolerance interval 95%**	**Lower limit**	**Upper limit**	**Lower limit**	**Upper limit**	**Lower limit**	**Upper limit**
	QC1	−4.86	2.53	−1.78	3.39	−0.43	7.84
	QC2	−2.28	2.06	−1.04	3.62	−1.75	3.70
	QC3	−4.92	1.11	0.95	7.11	−4.42	3.78

[a] **Y**: concentration matrix; **X**: absorbance matrix; and **E**: residual matrix.
[b] Ond, Dex and Apt concentrations of the validation standards were respectively: QC1 (5, 4 and 20 mg L^{-1}); QC2 (10, 6 and 35 mg L^{-1}); and QC3 (15, 8 and 50 mg L^{-1}).

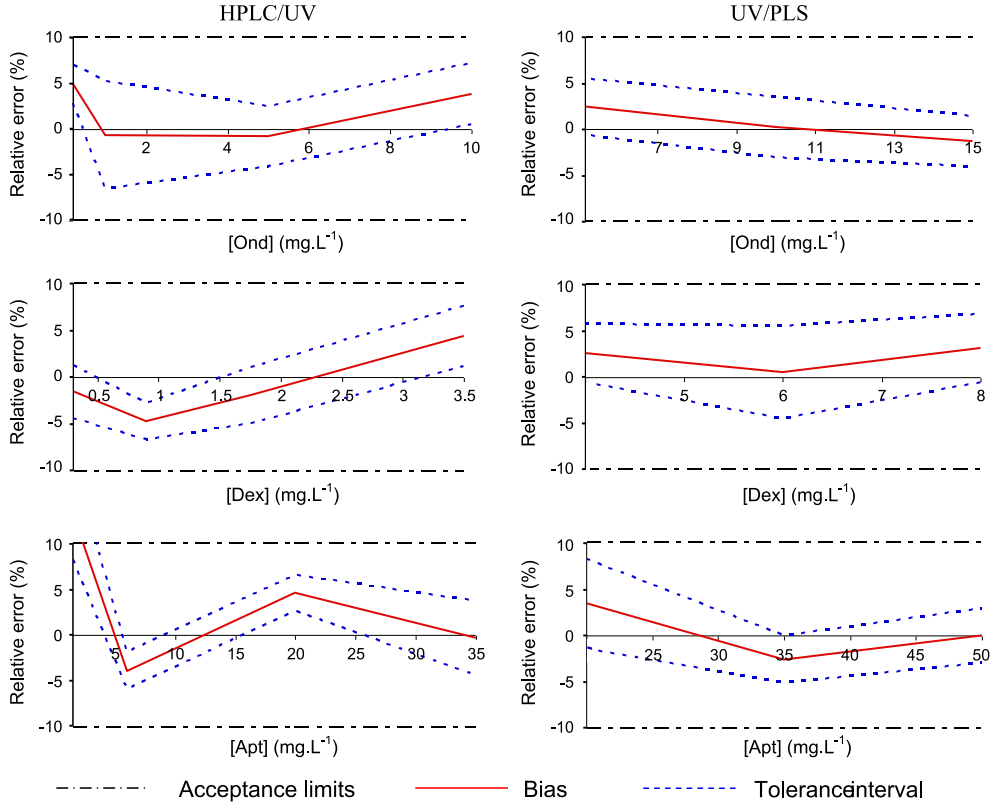

Fig. 4. Accuracy profiles of the HPLC/UV and UV/PLS methods for the quantitation of ondansetron, dexamethasone and aprepitant in organogel formulations.The dashed lines represent the acceptance limits of 10%, the red plain line corresponds to the bias and the tolerance interval of the bias for a risk of 5% was materialized by dotted blue lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In first intention, LOQ was evaluated considering a ratio of 10. The obtained value was selected as the lowest calibration standard, *i.e.* 0.05, 0.2 and 1 mg L^{-1} for Ond, Dex and Apt, respectively. Calibration curves, expressing the peak areas ratio (antiemetic drug/IS) *versus* antiemetic drug concentration, were then obtained from eight calibration standards. These calibration curves were used to assess the response function using linear least square regression model. Obtained results are summarized in Table 3. The validity of the model is attested by the determination coefficient obtained ($R^2 > 0.995$). Secondly, linearity of the method was evaluated. Whatever the drug investigated, the introduced concentrations and the back-calculated one can be expressed by linear models ($R^2 > 0.995$) with slopes

close to the unit and *y*-intercept that can be considered to be equal to zero.

The accuracy of the model was assessed with the total error criteria (trueness and precision) and the construction of accuracy profiles. The concentration of validation standards was back-calculated from the response function to evaluate both the trueness and the precision. As shown in Table 3, trueness evaluated for Ond and Dex by measuring relative bias is varied from −4.65 to 4.83% and is also in accordance with chosen criteria. Moreover, precision values (repeatability and intermediate precision) evaluated by measuring the RSD are lower than 9.71%. From these data, the upper and the lower confidence limits for validation standard were determined leading to the establishment of accuracy profiles (Fig. 4). Whatever the concentration level, the tolerance interval (β=5%) is included in the acceptance limits set at ±10% for Ond and Dex. Results obtained for Apt are quite different. Whereas relative biases are in accordance with established criteria for the highest calibration standard concentrations, it was around 14% for the smallest. Similarly, precision is not acceptable for the lowest calibration standard concentration, as intermediate precision reaches 15%. The accuracy profile established according to these data exhibits a tolerance interval excluded from the acceptance limits for the lowest aprepitant concentration. A quantitation limit can then be calculated by taking the intersection point between the acceptability limit and the tolerance interval; it is equal to 3.5 mg L^{-1}.

Nevertheless, according to the approach chosen, the quantitation limits are clearly the extremes that can be quantified with a defined accuracy. As a conclusion, for Ond and Dex the low and high quantitation limits are the extremes concentration investigated during the validation experiments, *i.e.* (0.2 and 10 mg L^{-1}) and (0.3 and 3.5 mg L^{-1}), respectively. For Apt, they are equal to 3.5 mg L^{-1} and 35 mg L^{-1} as determined previously.

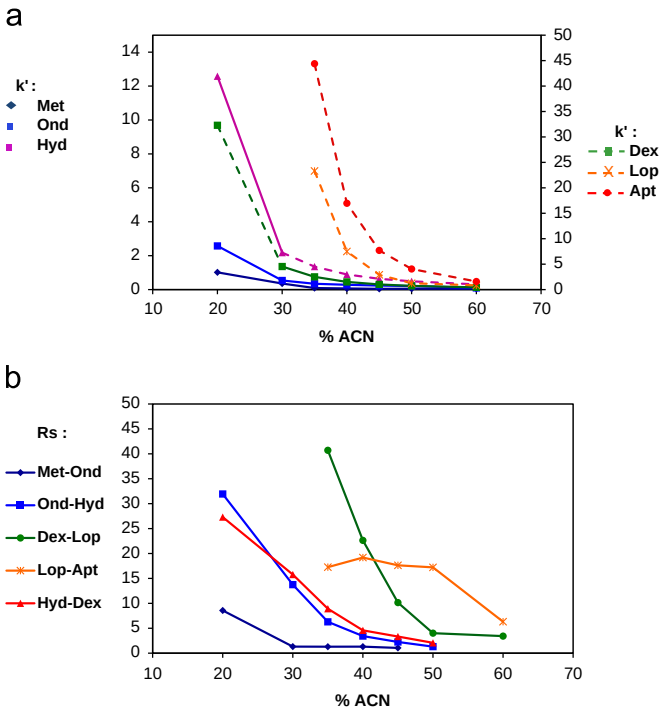

Fig. 5. HPLC method optimization – (a) retention factors (k') of antiemetic drugs and their respective internal standards and (b) resolution (Rs) *versus* ACN proportion in the mobile phase. D=1 ml min^{-1}, 25 °C, Kinetex C18, Mobile phase: ACN/H_2O both containing 0.1% formic acid, V_{inj}=20 μL.

3.3. Application of the UV/PLS and HPLC/UV methods to the quality control of a real organogel

In order to evaluate the recovery of the two methods and to demonstrate their applicability for real samples, quantitation of Apt, Dex and Ond was achieved in an organogel pharmaceutical formulation prepared in our laboratory and containing 32 mg $g^{-1}$of Ond, 12 mg $g^{-1}$of Dex and 80 mg $g^{-1}$of Apt. A slight amount of the organogel (around 50 mg) was 50-fold diluted in

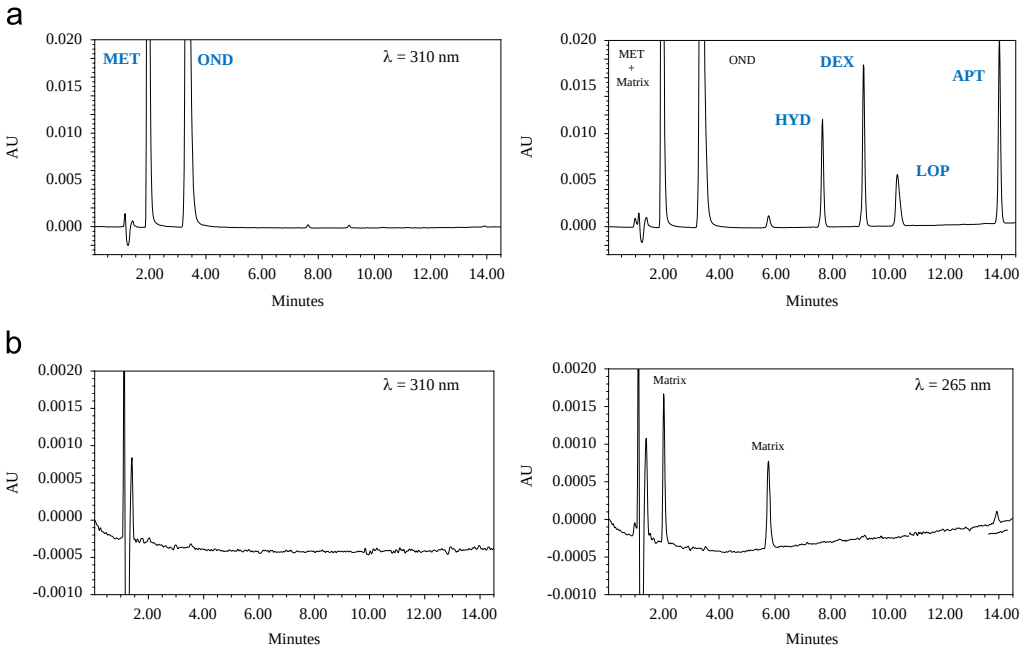

Fig. 6. Validation of the HPLC/UV method – specificity assessment: (a) chromatograms of a calibration standard ([Ond]=12 mg L^{-1}, [Dex]=4 mg L^{-1}, [Apt]=40 mg L^{-1}, [Met]=10 mg L^{-1}, [Hyd]=3 mg L^{-1}, [Lop]=30 mg L^{-1}, 3000-fold diluted organogel in EtOH/H_2O (20/80 – v/v)) at 310 nm (on the left) and 265 nm (on the right) and (b) blank organogel sample (3000-fold diluted organogel in EtOH/H_2O (20/80 – v/v)). Compounds appear in blue at the wavelength used for their quantitation. Separation performed at 25 °C, 1 ml min^{-1} using the optimized gradient elution ((1) 20/80 (v/v) mixture of solvents A and B for the first 0.5 min; (2) increase to 80% of B in a linear gradient from 0.5 to 16 min). Other experimental conditions: see Fig. 5.

Table 3
Validation results of the HPLC/UV method: response function, linearity, trueness, precision and accuracy for the analysis of ondansetron (Ond), dexamethasone (Dex) and aprepitant (Apt).

Validation parameters		Ond		Dex		Apt	
Response function[a]	$f(x)$	$Y=0.1191x-0.0439$		$Y=0.4210x+0.0024$		$Y=0.0504x-0.0012$	
	r^2	0.999 ± 0.001		0.999 ± 0.001		0.995 ± 0.006	
Linearity	Slope	1.002		1.001		0.999	
	Y intercept	0.019		−0.004		0.037	
	r^2	0.999 ± 0.001		0.999 ± 0.001		0.995 ± 0.006	
Trueness[b]		$C_{calculated}$ (mg L^{-1})	Relative bias (%)	$C_{calculated}$ (mg L^{-1})	Relative bias (%)	$C_{calculated}$ (mg L^{-1})	Relative bias (%)
	QC1	0.21	4.83	0.30	−1.53	1.70	13.63
	QC2	0.99	−0.66	0.86	−4.65	5.77	−3.87
	QC3	4.96	−0.80	1.72	−1.91	20.91	4.55
	QC4	10.38	3.80	3.65	4.35	35.30	−0.33
Precision[b]		Repeatability (%RSD)	Intermediate precision (%RSD)	Repeatability (%RSD)	Intermediate precision (%RSD)	Repeatability (%RSD)	Intermediate precision (%RSD)
	QC1	2.01	2.01	4.11	4.71	6.34	14.88
	QC2	3.11	9.71	0.68	0.68	1.65	1.65
	QC3	2.32	3.80	1.17	2.17	0.94	0.94
	QC4	4.66	6.47	2.76	3.75	4.58	7.55
Accuracy[b]	**Tolerance interval 95%**	**Lower limit**	**Upper limit**	**Lower limit**	**Upper limit**	**Lower limit**	**Upper limit**
	QC1	2.77	6.89	−4.36	1.31	8.18	19.7
	QC2	−6.51	5.19	−6.58	−2.73	−5.89	−1.85
	QC3	−4.08	2.49	−4.87	1.05	2.60	6.51
	QC4	0.49	7.11	1.20	7.51	−4.34	3.67

[a] Calibration curves, expressing the peak areas ratio (antiemetic drug/IS) *versus* antiemetic drug concentration were obtained from eight calibration standards by using ordinary least square regression.

[b] Ond, Dex and Apt concentrations of the validation standards were respectively: QC1 (0.2, 0.3 and 1.5 mg L^{-1}); QC2 (1, 0.9 and 6 mg L^{-1}); QC3 (5, 1.75 and 20 mg L^{-1}); and QC4 (10, 3.5 and 35 mg L^{-1}).

EtOH. This solution was then treated according to distinct methodologies, as described in the experimental section, to obtain samples to analyze either by HPLC or by UV/PLS. The concentration of each drug in the organogel was calculated with reference to calibration models constructed in the same day. Each organogel sample was prepared and analyzed individually three times ($n=3$). The results of recovery were $99 \pm 3\%$ for Ond, $102 \pm 2\%$ for Dex and $105 \pm 4\%$ for Apt using HPLC method, *versus* $97 \pm 5\%$ for Ond, $95 \pm 3\%$ for Dex and $95 \pm 4\%$ for Apt using UV/PLS method. These results are consistent with the European pharmacopeia recommendations admitted for quantitation of drugs in transdermal forms and prove that the process of organogel preparation is suitable.

4. Conclusion

A simple and direct multivariate calibration spectrophotometric method has been developed for the determination of ondansetron, dexamethasone and aprepitant in an organogel. This method which does not involve a pre-treatment of the sample, is based on the measurement of the electronic absorption spectrum of the sample diluted in ethanol, in the 260–288 nm region, followed by a data processing using a partial least squares calibration designed with a series of reconstituted samples. This method has been validated both thanks to the evaluation of the relative error of prediction and to the establishment of the accuracy profile. An HPLC/UV reference method has also been optimized and validated using a similar accuracy profile approach. Both methods were used for the determination of the three antiemetic drugs in an organogel pharmaceutical formulation prepared in our laboratory. Concentrations predicted by these methods were comparable and have proven the suitability of the formulation preparation process.

In conclusion, from an experimental point of view, despite the preparation of the samples are quite equivalent in term of complexity and time consuming, HPLC analysis requires globally 30 min *versus* 5 min for UV/PLS. UV/PLS method may be also easier to perform at the hospital, especially for routine control. However, the UV/PLS method can only be used for the evaluation of the organogel selected for our experiments. Quantitation of others gels should require to develop a new model, whereas the HPLC method could be directly used for another matrix, after evaluation of the specificity of the method. Additionally, the HPLC/UV method meanwhile exhibits smaller quantitation limits making this method more efficient for the further *ex vivo* and *in vivo* permeation studies.

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