Research Article

Spray-Dried Cascara sagrada Extract for Direct Compression: Tablet Formulation and a Simple HPLC Method for Tablet Performance Evaluation

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ABSTRACT

Cascara sagrada bark is extensively used in the treatment of occasional constipation. Despite its wide diffusion, Cascara sagrada dried extracts with good flow and compactability properties for tablet formulation by direct compression and simple methods for the determination of Cascara sagrada extract release from tablets are not available. To overcome this lack of information, this article is focused on the design of tablets based on a Cascara sagrada extract obtained by spray-drying and on the quantification of its active constituents using a novel and isocratic HPLC-UV technique, which was validated. Flow properties of the Cascara sagrada spray-dried extract and of the physical mixtures of it with conventional excipients were evaluated. The different formulated powders were compacted and their properties (i.e., hardness, friability, disintegration and dissolution) were studied.

The proposed tablet formulations, based on the Cascara sagrada spray-dried extract, reached the pharmacopoeial requirements for immediate release tablets. In particular, the formulas containing a superdisintegrant showed the best pharmaceutical performance. Nevertheless, the use of excipients to improve the powder flow and/or the tablets properties was not strictly necessary.

Besides, the HPLC-UV method used to monitor the extract release from tablets was validated, proving to be linear within the concentration range adequate for therapeutic purposes and appropriate for routine analysis due to its simplicity, good sensitivity, accuracy and precision.

Keywords: Spray drying, Cascara sagrada bark, tablets, direct compression, HPLC method.

INTRODUCTION

In average, more than 80% of the pharmaceutical industries production is based on powders in tablet form[1]. The principally reasons for their continued popularity are the ease of manufacture, low-cost production, accurate dosage and large storage stability in comparison with oral liquid and semi-solid pharmaceutical forms[2] .Direct compression (DC) is the most efficient process used in tablet manufacturing. The advantages of DC, among others, are the use of fewer processing steps, lower labor costs, processing time and energy consumption compared with wet or dry granulation processes[3]. Nevertheless, this technique is limited to powders with good flow properties and low segregation tendency[4].

In the production of phytomedicinal tablets, dried medicinal plant extracts are usually used as the therapeutically active ingredient. Tablet formulations usually contain a high dose of dried medicinal plant extracts that are generally poorly compressible and hygroscopic powders[5]. Consequently, dried medicinal plant extracts cannot be compressed directly, making tablet formulation a complex step. Appropriate excipients have to be added to plant extracts and preferably co-processed

by means of a proper technology[6]. Thus, the development of new technologies to obtain dried medicinal plant extracts with accurate quality characteristics is an important issue for the herbal processing industry. Several drying techniques can be utilized. In particular, the spray drying method is the preferred route due its capacity to produce powders with precise specifications (i.e., moisture content, solubility and bulk density) in continuous operations[7]. This technique also offers the advantages of relatively low temperatures and short particle residence times, both conditions that preserve the powder stability[8]. Nevertheless, it is important to fine-tune the drying process conditions in order to obtain products with the desired flow properties and appropriate process yields. In a previous paper, Gallo et al.[9] reported a study of the necessary operating conditions and liquid feed formulation to obtain a spray-dried medicinal extract of *Rhamnus purshiana* (Cascara sagrada bark) with good physical properties for DC (i.e., good flow properties, low moisture content and hygroscopicity).

Cascara sagrada is widely used in the treatment of occasional constipation[10]. The active constituents of the herb are hydroxyanthracene glycosides (6-9%), within this group about 70-90% are *C*-10 glycosides with 8-*O*-glycosides and the remaining 10-30% is constituted by aloins A and B, and chrysaloins A and B (Fig. 1). The laxative action is primarily due to cascarosides (Fig. 1), which comprise around 60-70% of the total *O*- and *C*-glycosides[11]. These components excite peristalsis in the colon, inhibiting the absorption of electrolytes and water from large intestine with a consequent increase in the volume contents of the intestine and thereby stimulating peristalsis[12].

The bark of the Cascara sagrada is generally used by patients as a finely cut crude drug or as dried extracts, fluid extracts and other liquid and solid preparations[10]. Even though, to the best of our knowledge there is a lack of production of Cascara sagrada dried extracts with good flowability and compactability to be manufacture in the form of tablets by the advantageous DC technology. In this context, the significance of the Cascara sagrada spray-dried extract powder developed by Gallo et al.[9] is to provide a raw material with adequate physical properties to be directly compressed. Nevertheless, the feasibility of its use as an active ingredient for tablets manufactured by DC technology has not been previously studied.

In addition, the evaluation of the formulation or the quality control of tablets requires a specific analytical method applicable to end-products. The quantitative analysis of Cascara sagrada active components in raw material has been determined spectrophotometrically following a standard pharmacopoeial method[13]. Even though this method is very appropriate for quality control of raw material, it is highly time consuming for routine tablets evaluation. In fact, this method involves several extractions and hydrolysis steps to evaluate the content of Cascara sagrada active constituents. In addition, some high performance liquid chromatography (HPLC) methods for the quantitative analysis of cascarosides have also been reported. These chromatographic methods require either the compounds transformation by oxidative hydrolysis to their aglycones prior to the quantification[14] or the use of a gradient program[15]. These techniques are complex and also time consuming. Hence, a simple method for routine analysis of Cascara sagrada tablets is needed.

For the above-mentioned reasons, the objectives of this investigation were: (a) the formulation of phytomedicine tablets by DC technology using the Cascara sagrada spray-dried extract powder previously obtained as the active ingredient and (b) the development and validation of an HPLC analytical procedure, which can be used to easily determine the extract release from the tablets.

MATERIALS AND METHODS

Cascara sagrada (*Rhamnus purshiana* D.C.) (Droguería Argentina) and colloidal silicon dioxide (SiO_2, Aerosil 200®, Degussa AG, Germany) were used as received from the suppliers. Distilled water was employed for the preparation of the fluid plant extract and the aqueous dispersion to be spray dried.

Methanol (HPLC grade), acetic acid (AR grade), iron (III) chloride (AR grade), hydrochloric acid (AR grade), methylene chloride (AR grade), ethyl acetate (AR grade) and magnesium acetate (AR grade) were purchased from Sintorgan, Argentina. Ultrapure water was obtained by means of a Milli-Q apparatus (Millipore Co., Bedford, USA) and was used for HPLC experiments.

Lactose monohydrate (Lactose DC), dicalcium phosphate dihydrate (Emcompress), microcrystalline cellulose (Avicel PH 101), crosslinked polymer of carboxymethylcellulose sodium (Ac-Di-Sol) and magnesium stearate (Parafarm-USP grade) were purchased from Droguería Saporiti, Argentina and were used for tablet formulation.

Preparation of Cascara sagrada spray-dried extract

Cascara sagrada fluid plant extract was prepared from Cascara sagrada bark and boiling water; 0.9 kg of Cascara sagrada bark were weighted accurately and mixed with 4000 ml of boiling water. This mixture was macerated for 3 h and then transferred to a percolator. Extra boiling water, used as menstruum, was continuously poured into the percolator until 5000 ml of the fluid plant extract were collected[13].

The solid residue (SR) content of the fluid plant extract was determined by evaporation of the solvent under reduced pressure, followed by drying in an oven at 80 ºC to constant weight. The fluid plant extract SR value, measured in triplicate, was 3.80 ± 0.12% (w/v)[9].

A laboratory-scale Mini Spray Dryer Büchi B-290 (Büchi Labortechnik AG) was employed for the Cascara sagrada spray-dried extract production. A two-fluid nozzle with a cap orifice diameter of 0.5 mm was used and the air atomizing pressure was kept constant at 6 bars. Spray drying was carried out at the operating conditions established by Gallo et al. (9) to obtain a Cascara sagrada powder with appropriate flow properties and stability attributes. The best set of operating conditions were: drying air inlet temperature 130 ºC, atomization air volumetric flowrate 400 l/h, feed volumetric flowrate 15% (expressed as % of the maximum pump rate, equal to 3 ml/min), drying air volumetric flowrate 100% (given as % of the maximum aspiration rate, about 35-38 m^3/h). The Cascara sagrada spray-dried extract was obtained by spray-drying a dispersion of the fluid plant extract and SiO_2. The proportion of SR:SiO_2 was 1:1. This dispersion was mixed for 30 min before the atomization and during the spray drying process, using a magnetic stirrer bar rotating at 1000 rpm to keep it homogenized.

In addition, the fluid plant extract without adjuvant (i.e., without SiO_2) was also spray dried at the same operating conditions for comparative purposes. The active compounds (i.e., hydroxyanthracene derivatives and cascarosides) in this product were determined according to the method described in the USP 30/NF 25[13]. Total concentration of active components in the spray-dried extract was 41.2 % (w/w)[9].

Carr's compressibility index (CI)

In order to determine the density of the extract powders, these were gently poured into a 10 cm^3 graduate cylinder. Bulk density (D_B) was calculated as the ratio between the weight (g) of the sample contained in the cylinder and the occupied volume. Tap density (D_T) was estimated by tapping the cylinder until no measurable change in volume was noticed. Powder flowability was evaluated using Carr's compressibility index (CI)[16], which is calculated as follows:

$$CI = \frac{(D_T - D_B)}{D_T} \times 100 \qquad (1)$$

According to USP 30/NF 25[13], Carr's compressibility indexes below 10% denote excellent flow, values between 11 and 15% are representative of good flowability, from 16 to 20% the powder flow is fair, between 21 and 25% the product has acceptable flow properties and powder flow will be poor for values of the 26-31%.

Angle of repose (α)

The angle of repose was determined by pouring around 7 g of powder through a funnel located at a fixed height on a graph paper flat horizontal surface and measuring the height (h) and radius (r) of the conical pile formed. The tangent of the angle of repose is given by the h/r ratio[17].

Repose angles between 25-30º indicate excellent flow, between 31-35º the flow is good, and within the range 36-40º the flow is fair and does not need aids. For values above 41º the powder has bad flow properties.

Tablet preparation

The recommended dose of hydroxyanthracene glycosides per day for adults and children of 12 years and above is between 20-30 mg (10, 18-20). Based on the total concentration of active components in the spray-dried extract without SiO_2 estimated in the above section (i.e., 41.2 % w/w) and the recommended daily dose of Cascara sagrada active ingredients, 146 mg of the Cascara sagrada spray-dried extract containing SiO_2 (i.e., SR:SiO_2 1:1, 73 mg dried extract and 73 mg of SiO_2) were established per tablet. Although the spray-dried extract presented good flow and compactability properties for DC[9], compression processing requires at least the addition of a lubricant (as Mg stearate) to prevent solid blend sticking to the punches and dies during compression. For this reason, as shown in Table 1, the first formulation (F1) contains the spray-dried extract and Mg stearate. Besides, different excipients (which are described below) were physically mixed with the Cascara sagrada spray-dried extract leading to the other four tablet formulations (Table 1).

Lactose DC, Avicel PH 101, Emcompress and Ac-Di-Sol were selected to study their influence on the spray-dried extract flowability and tablets properties (i.e., friability, hardness, disintegration time and dissolution rates). Lactose DC is the excipient most widely used as filler in tablet formulation due to its good flow properties. Avicel PH 101 is used mostly as filler for DC and also acts as a disintegrant by swelling mechanism. Its capillarity allows the penetration of water into the tablet, destroying the cohesive bonds between particles[21]. Avicel PH 101 poor flow properties can be offset by mixing it with other filler with good flowability[22]. Emcompress is an insoluble and non-elastic tablet diluent[23], being also a flow enhancer[24]. Ac-Di-Sol is widely used as superdisintegrant[25-28]. This excipient has a fiber like nature; each fiber can act as a hydrophilic channel to facilitate water uptake into the tablet, swelling to 4-8 times its original volume[26]. All these excipients

were utilized to identify if their addition made tablets with better characteristics than those corresponding to the basic F1. In F2, Lactose DC and Avicel PH 101 were incorporated to establish if there were improvements in the powder flowability and tablets disintegration time, respectively. In F3, the amount of diluent was increased with respect to F2 by adding Lactose DC and Emcompress. F4 and F5 were designed with the excipients utilized in F2 and F3, and a superdisintegrant (Ac-Di-sol) in order to determine if this type of carrier led to significant enhancements in the tablets disintegration and influenced the extract dissolution.

For F1, the corresponding dose of spray-dried extract was mixed with Mg stearate during 4 min and the powder blend was compacted in an excentric tablet press (SP1, Talleres Sanchez) equipped with 8 mm circular edged punches. For F2 to F5, the spray-dried extract was blended with the different excipients (except Mg stearate) for 12 min. Then, Mg stearate was added to the blend and mixed for 4 additional minutes. The obtained solid blends were compacted in an excentric tablet press equipped with 12 mm circular edged punches. The hardness of each tablet was measured with a hardness tester (Scout).

Friability test

Tablet friability was measured as the percentage of weight loss of a total of 6.5 g of tablets tumbled in a friabilator (Scout)[29]. After 5 minutes of rotation at 25 rpm, the loose dust from the tablets was removed and the tablets were reweighted. The weight loss (% Friability) was calculated by the following equation:

$$\%\ Friability = \frac{W_i - W_r}{W_i} \times 100 \qquad (2)$$

where W_i and W_r are the initial and final weight of the tablets, respectively[30].

Disintegration time (DT)

The tablets disintegration time was determined according to FNA[29] (Disintegration tester Scout). The tests were carried out in 800 ml of distilled water at 37 ± 0.5 ºC. For each formulation, 6 randomly selected tablets were tested. The disintegration time was defined as the time necessary for complete disintegration of the total number of tablets.

Dissolution profiles

The release of the Cascara sagrada spray-dried extract from the tablets was determined in an Erweka dissolution apparatus according to the USP 30/NF 25[13] specifications (Apparatus II, rotational speed of 100 rpm). The dissolution medium was 500 ml of distilled water. Samples of 5 ml were removed and replaced with an equal volume of fresh medium at specified time intervals. The samples were filtered and the concentration of Cascara sagrada spray-dried extract was analyzed using the HPLC method described in next section. The major peak of cascarosides was used as reference peak to study the Cascara sagrada extract release from tablets. All the experiments were conducted in triplicate. The release was stated as the percentage of dissolved Cascara sagrada extract after 5 minutes (PD_5)[27].

Analytical HPLC method

Cascarosides isolation and identification

Cascarosides are not commercially available as reference compounds. Thus, Thin Layer Chromatography (TLC) was used to isolate cascarosides from the fluid plant extract using the method described by Wagner and Bladt[31]. A mixture of ethyl acetate:methanol:water (100:13.5:10) was used as mobile phase and 1 ml of the fluid plant extract was spotted on a silica gel TLC plate (20 × 20 cm) (Silica gel 60 F_{254}, Merck). The assay was performed by triplicate. After elution, the plate was sprayed with 10 ml of 10 % ethanolic potassium hydroxide reagent and visualized under 365 nm UV. At this wavelength, cascarosides fluoresce bright yellow.

The TLC analysis revealed two yellow bands with retention factors (R_f) of approximately 0.05 and 0.14, which are in good agreement with R_f values reported by Wagner and Bladt[31] for cascarosides A and B, respectively, under the same experimental conditions. In order to isolate the cascarosides, the cascarosides bands were scraped off. The silica was transferred to a vial, 7 ml of water:methanol (80:20) were then added and the sample was thoroughly vortexed. The vial was then centrifuged and the supernatant was collected, filtered (Durapore filter), transferred to autosampler vials and injected (25 µl) into the HPLC system in order to determine the retention times of cascarosides. The cascaroside presenting the major area peak was used as the reference peak to study the release of the Cascara sagrada extract from the tablets.

Apparatus and chromatographic conditions

The HPLC system (Brezee) consisted of a Waters 1525 pump, a Waters 717 plus autosampler, a Waters 1500 series column heater, a Waters 2475 multi λ fluorescence detector (λex 290 nm, λem 410 nm) and a Waters 2996 photodiode array detector (PDA) (Waters Corp., Milford, USA). Separation was accomplished on a column Symmetry™ C_{18}, 150 × 3.9 mm i.d., 5 µm particle size; which was maintained in the column oven at 25 ºC and protected by a SecurityGuard™ precolumn. Data acquisition was performed by the Empower Software data registration TM.

The mobile phase consisting of water:methanol:acetic acid (75:25:1, v/v/v) was filtered through a 0.45 µm Millipore™ Durapore filter and degassed by vacuum prior its use. Elution was performed isocratically at 25 ºC at a flow-rate of 1 ml/min. The injection volume was 25 µl. The wavelength of PDA was set at 297 nm, the maximum wavelength absorption of cascarosides. All injections were performed in triplicate unless otherwise stated.

Method validation

The HPLC method was validated with respect to the following parameters: linearity, accuracy, precision, and stability of sample solution[32-33].

Preparation of standard solutions

Stock standard solutions of 5 mg Cascara sagrada spray-dried extract/ml were prepared in Milli-Q water. The suspensions were shaken and stored at room temperature for 1 h (to allow SiO_2 precipitation). The suspensions were filtered (Durapore filter) to eliminate the residual SiO_2 and calibration standards were prepared. Calibration standards were made by appropriate dilutions of the stock solutions with Milli-Q water. The concentrations of the Cascara sagrada extract in the solutions were of 0.0015, 0.0029, 0.0044, 0.0073, 0.0146, 0.0292, 0.0438, 0.0584, 0.0730, 0.0876, 0.1022, 0.1165 and 0.146 mg/ml. Calibration standards were filtered (Durapore filter), transferred to autosampler vials and injected into the HPLC in order to obtain calibrations curves. Duplicate solutions were prepared for each concentration level.

Linearity

In order to evaluate the linearity of the analytical procedure, four calibration curves (cc_1, cc_2, cc_3, and cc_4) were analyzed. Each calibration curve was constructed by plotting the concentration of Cascara extract spray-dried extract (mg/ml) as a function of the highest peak area (i.e., the area corresponding to the cascaroside selected as reference). For each calibration curve different experimental points were used, and a linear least-squares regression analysis was conducted to determine the slope, intercept and coefficient of determination (r^2) (Table 2). The high r^2 coefficients demonstrate the linearity of the calibration curves. The calibration curve cc_5 was obtained considering all the experimental points, and this linear function was used to estimate the concentration of the unknown samples.

Accuracy and precision

The accuracy and precision of the method were determined by replicate analysis of samples at high, medium and low concentration levels in intra-day and inter-day (Table 3). The method accuracy was calculated by Eq. (3):

$$Accuracy\ (\%) = \frac{C_c}{C_n} \times 100 \qquad (3)$$

where C_c is the mean of the concentration calculated from the chromatograms and calibration curves and C_n is the mean nominal concentration.

The precision was expressed as the % coefficient of variation (CV) and was calculated by Eq. (4):

$$CV = \frac{\sigma}{C_c} \times 100 \qquad (4)$$

where σ is the standard deviation and C_c is the mean of the concentration calculated from the replicates.

Cascara sagrada extract sample stability

The stability of the extract samples in the conditions used during the analytical process was evaluated by analysis of samples kept at 25 ºC for 6 h (as in the HPLC autosampler) and at -20 ºC for 5 weeks.

To study the extract stability in the autosampler, three samples in the low and high concentration levels were analyzed before (zero time) and after storage at 25 ºC for 6 h, which were the temperature and run time required for the analysis of all the samples (Table 4). In order to investigate the stability of extract solutions kept at -20 °C before quantifying, three samples (at the same concentrations tested at 25 ºC) were stored in a freezer (-20 ºC) for 5 weeks (Table 4) and subsequently analyzed. The stability percentage was calculated by Eq. (5):

$$Stability = \frac{C_{tt}}{C_{t0}} \times 100 \qquad (5)$$

where C_{tt} is the mean measured concentration at the selected analysis time (for both storage temperatures) and

C_{t0} is the mean concentration measured at zero time.

RESULTS AND DISCUSSION

Validation of HPLC analytical method

Within the wide studied concentration range of (0.0015-0.146 mg Cascara sagrada extract/ml), the relationship between the peak area of the cascaroside selected as reference (y) and the concentration in mg/ml (x) presented good linearity. In fact, the calibration curves showed correlation coefficients higher than 0.993 (Table 2). The chromatographic system (i.e. mobile phase, type of column, flow rate, etc.) was adequate for the analysis. The total run time for each sample was 25 min (Fig. 2), allowing the analysis of a large number of samples per day. In addition, for all the

tested tablet formulations, the tablets HPLC chromatograms showed cascarosides patterns similar to that of the Cascara sagrada extract (without excipients), indicating that the HPLC method was not affected by the presence of the selected excipients (data not shown).

The accuracy of the assay method, defined as the closeness of agreement between the mean of the calculated concentrations and the nominal concentrations, is considered to be acceptable if it is within ± 15% of the actual value at all concentrations (i.e. between 85-115%). The intra-day and inter-day accuracy at low, medium and high concentrations were in the range of 95-100 % (Table 3), demonstrating a good accuracy of the method.

The precision should not exceed 15% of the CV. In the present study, precision of the method was tested by both intra- and inter-day repeatabilities. The intra-day precision was between 2.00 and 3.87% while the inter-day precision was among 1.52 and 2.60% (Table 3). All these values were well below 15%, suggesting an adequate repeatability of the assay method.

The extract solutions were found to be stable for at least 6 h at 25 ºC since the concentration did not decrease below 99.5 % (Table 4). This result allows concluding that is possible to process a large batch of samples in one assay run. Moreover, extract solutions were found to be stable when kept for 5 weeks at -20 ºC as the concentration did not decrease below 97% (Table 4). This suggests that, if necessary, the extract solutions obtained from the in vitro dissolution test could be freeze-stored before HPLC quantification. In addition, the cascarosides retention times and peak areas remained almost unchanged for the two studied storage conditions.

Evaluation of the formulations flowability and tablets properties

Regarding the rheological properties of the five selected spray-dried extract tablet formulations (including different excipients), the results indicated that the flow properties of all the formulations were adequate for DC (Table 5). For comparative purposes, the flow properties were evaluated for the spray-dried extract without SiO_2, and the spray-dried extract (without SiO_2) mixed with the excipients selected for F3 (Lactosa DC and Emcompress, excipients with good flowability). The spray-dried extract powder without SiO_2 showed poor flow properties (Table 5). In addition, the blend of Cascara sagrada spray-dried extract and excipients of F3 showed CI and angle of repose values indicative of bad flowability (Table 5). On the other hand, the Cascara sagrada spray-dried extract (SR:SiO_2, 1:1) demonstrated suitable rheological and compactability properties, which allow its use for direct compression (Table 5). Therefore, the production of an extract powder with satisfactory flow and compactation properties became essential for tablet production. In fact, the addition of excipients with good rheological properties did not improve the poor flow of the dried extract powder obtained by spray drying without any adjuvant.

According to the values presented in Table 5, the addition of Lactose DC with or without Emcompress (F2 and F3) decreases the angle of repose and causes no appreciable change in the CI values compared to F1. Therefore, Lactose DC and Emcompress could not be suggested as adequate excipients to improve the formulation flow properties. However, the biopharmaceutical properties (tablets disintegration time and extract dissolution rate) should be evaluated before concluding the convenience of including diluents. On the other hand, the addition of disintegrants and superdisintegrants combined with diluents demonstrates an improvement in the CI and α compared to F1.

Friability test indicates if tablets possess a suitable mechanical resistance to avoid crumbling or breaking on handling or subsequent processing in the manufacture of tablets[34]. As shown in Table 5, all the formulations presented friability percentages well below 1%, the maximum allowable value[13,30,35]. According to these results, both the Cascara sagrada spray-dried extract only with lubricant (F1) or with other excipients gave tablets with appropriate friability.

Besides, tablets should be sufficiently hard to resist breaking during normal handling and yet soft enough to disintegrate properly after swallowing [36]. In the present work, the height of the upper punch of the excentric tablet press was adjusted to obtain tablets with a mean hardness value around 5 kg_f (Table 5), which is considered satisfactory for tablets[36].

Even though the hardness was adjusted to the desired value, it is necessary to simultaneously confirm the tablets disintegration at appropriate times. As it can be seen in Table 5, all the studied formulations fulfilled the USP 30/NF 25[13] requirement for Cascara sagrada tablets, being the disintegration times lower than 60 min. In particular, the formulations without disintegrant agents, F1 and F3, exhibited the higher disintegration time values (about 12 min). In F3, the presence of Emcompress allowed water penetration into the tablet but did not produce a disintegration force large enough to modify the disintegration time[22]. The disintegration behavior could thus be optimized with the incorporation of others excipients. In fact, F2 showed an intermediate value between those of F1 and F3 due to the addition of Avicel PH 101. Moreover, as expected, F4 and F5 containing a superdisintegrant (Ac-Di-Sol), exhibited faster disintegration times

than F2 and F3. Although, the incorporation of disintegrants enhanced the disintegration time, F1 also disintegrated rapidly. Therefore, the addition of disintegrants was not a necessary requisite for the production of tablets containing only the Cascara sagrada spray-dried extract.

For all the formulations, the dissolution assay showed a fast release of the extract (Fig. 3). F1 released more than 37% of the extract within 5 min (Table 5), exhibiting a satisfactory release profile for an immediate release formulation (Fig. 3). Except for F3, all the others formulations showed faster release rates than F1 (Table 5). Indeed, the formulations containing disintegrants and superdisintegrants presented lower disintegration times and faster extract release rates than F1. On the other hand, even though F1 and F3 had similar disintegration times, F3 exhibited a slower extract release rate. As described by other authors[23,37], the presence of Emcompress in the tablet affected the extract dissolution, probably due to the high proportion of this insoluble excipient.

In general, F1 showed appropriate flow properties for DC and satisfied the requirements to become an immediate-release tablet (adequate friability, hardness and disintegration time, and rapid extract release). Furthermore, its pharmaceutical performance could be improved by using the proportions of excipients utilized in F4 and F5.

CONCLUSIONS

The Cascara sagrada spray-dried extract obtained by spray drying according to Gallo et al.[9] proved to be a good starting material for the production of phytomedicine tablets by DC technology. In fact, it was shown that it is not possible to obtain powders of good flow properties by the physical mixture of the spray-dried fluid plant extract (without adjuvant) and conventional excipients for tablets production.

The proposed formulations, based on the Cascara sagrada spray-dried extract, reached the pharmacopoeial requirements for immediate release tablets. In particular, the formulas containing superdisintegrants (Ac-Di-Sol) showed the best performance. Nonetheless, it was evident that the use of excipients to improve the powder flowability and/or disintegration of the tablets containing the Cascara sagrada spray-dried (F1) is not necessary.

The selected HPLC method was successfully applied to quantify the extract release from Cascara sagrada tablets in the dissolution test. Additionally, the validation study showed that the method was linear in the proposed concentration range and appropriate for routine quality control analysis of the Cascara sagrada extract in pharmaceutical preparations. In fact, the analytical method is simple, has good accuracy and precision, and reduces analysis time and costs. Besides, no stability problems were detected during sample processing procedures and freeze storage.

Summarizing, the Cascara sagrada spray-dried extract was successfully formulated as immediate-release tablets overwhelming one of the main challenges that herbal medicines face nowadays: the dosage measurement or quality control by means of affordable analytical methods.

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Table 1: Composition of tablet formulations

	F 1	F 2	F 3	F 4	F 5
Cascara sagrada spray-dried extract (SR:SiO_2, 1:1)	146 mg	146 mg	146 mg	146 mg	146 mg
Lactose DC	-	70 mg	70 mg	70 mg	70 mg
Avicel PH 101	-	70 mg	-	70 mg	-
Emcompress	-	-	70 mg	-	70 mg
Ac-Di-Sol	-	-	-	70 mg	70 mg
Mg stearate	1 mg	4 mg	4 mg	4 mg	4 mg
Total tablet weight	147 mg	290 mg	290 mg	360 mg	360 mg

F: formulation

Table 2: Results of regression analysis of linearity

$y = bx + a$	cc_1	cc_2	cc_3	cc_4	cc_5
a (intercept)	-5143.3	-2039.9	-1529.8	-3083.2	-1994.6
b (slope)	1.96×10^6	2.12×10^6	2.22×10^6	2.21×10^6	2.14×10^6
r^2	0.998	0.998	1.000	0.999	0.993
Number of points of the calibration curve	6	7	13	13	39

cc: calibration curve

Table 3: Accuracy and precision of the HPLC assay

NNominal concentration (mg/ml)	Calculated concentration (mean ± σ, mg/ml)	Accuracy (%)	Precision (CV, %)
Intra-day variability (number of replicates for each concentration=5)			
0.0146 (low concentration)	0.0138 ± 0.0003	95	2.00
0.0730 (medium concentration)	0.0710 ± 0.0018	97	2.59
0.1165 (high concentration)	0.1166 ± 0.0045	100	3.87
Inter-day variability (number of replicates for each concentration=5)			
0.0146 (low concentration)	0.0138 ± 0.0003	95	1.98
0.0730 (medium concentration)	0.0711 ± 0.0018	97	2.60
0.1165 (high concentration)	0.1167 ± 0.0018	100	1.52

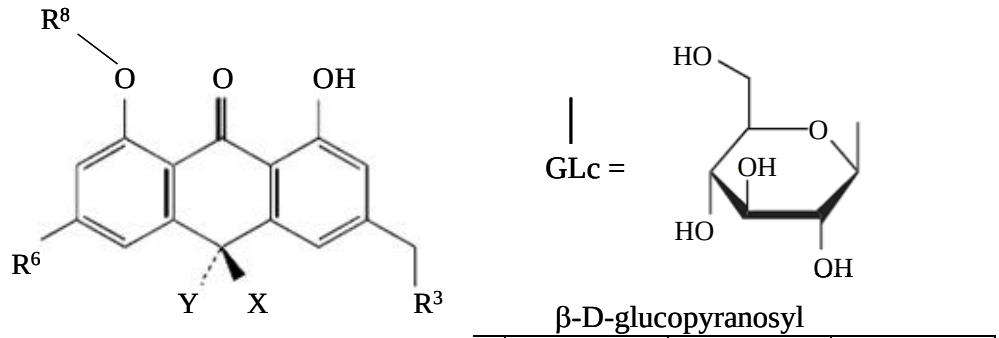
Table 4: Stability of Cascara sagrada extract samples under different conditions

Nominal concentration (mg/ml)	Calculated concentration (mean ± σ, mg/ml)	Calculated concentration (mean ± σ, mg/ml)	Calculated concentration (mean ± σ, mg/ml)	Stability (%)	
	zero time	6 h (25 ºC)	5 weeks (-20 ºC)	6 h (25 ºC)	5 weeks (-20 ºC)
0.0146 (low concentration, number of replicates=3)	0.0130 ± 0.0007	0.0130 ± 0.002	0.0126 ± 0.0008	100	97.4
0.1165 (high concentration, number of replicates=3)	0.1216 ± 0.0003	0.1209 ± 0.001	0.1190 ± 0.0007	99.5	97.8

Table 5: Dried extracts flowability and tablets properties

	CI (%)[a]	α (º)[b]	Friability (%)	Hardness (kg_f)[c]	DT (min)	PD_5 (%)
Cascara sagrada spray-dried extract (SR:SiO_2, 1:1)	20.03 ± 3.08	27 ± 2	-	-	-	-
Cascara sagrada spray-dried extract (SR:SiO_2, 1:0)	33.80 ± 4.39	63 ± 1	-	-	-	-
Cascara sagrada spray-dried extract (SR:SiO_2, 1:0) and F3 excipients	28.13 ± 3.20	59 ± 2	-	-	-	-
F 1	20.30 ± 3.11	27 ± 1	0.50	5.1 ± 0.2	12.30	37.51 ± 1.30
F 2	20.41 ± 3.63	24 ± 1	0.48	5.3 ± 0.4	5.42	52.86 ± 1.39
F 3	19.12 ± 3.39	23 ± 2	0.56	5.0 ± 0.4	12.43	28.14 ± 0.12
F 4	15.51 ± 3.27	23 ± 1	0.53	5.1 ± 0.4	2.19	75.90 ± 3.90
F 5	13.54 ± 2.07	23 ± 2	0.61	5.0 ± 0.6	2.12	79.02 ± 0.07

DT= disintegration time, PD_5= percentage of dissolved extract after 5 minutes
[a] The values represent the mean of sixteen determinations ± standard deviation.
[b] The values represent the mean of twenty determinations ± standard deviation.
[c] The values represent the mean of ten determinations ± standard deviation.



			R^3	R^6	R^8
Aloin A	GLc	H	OH	H	H
Aloin B	H	GLc	OH	H	H
Cascaroside A	GLc	H	OH	H	GLc
Cascaroside B	H	GLc	OH	H	GLc
Cascaroside C	GLc	H	H	H	GLc
Cascaroside D	H	GLc	H	H	GLc
Cascaroside E	GLc	H	H	OH	GLc
Cascaroside F	H	GLc	H	OH	GLc
Chrysaloin A	GLc	H	H	H	H
Chrysaloin B	H	GLc	H	H	H

Fig. 1: Cascara sagrada bark active constituents

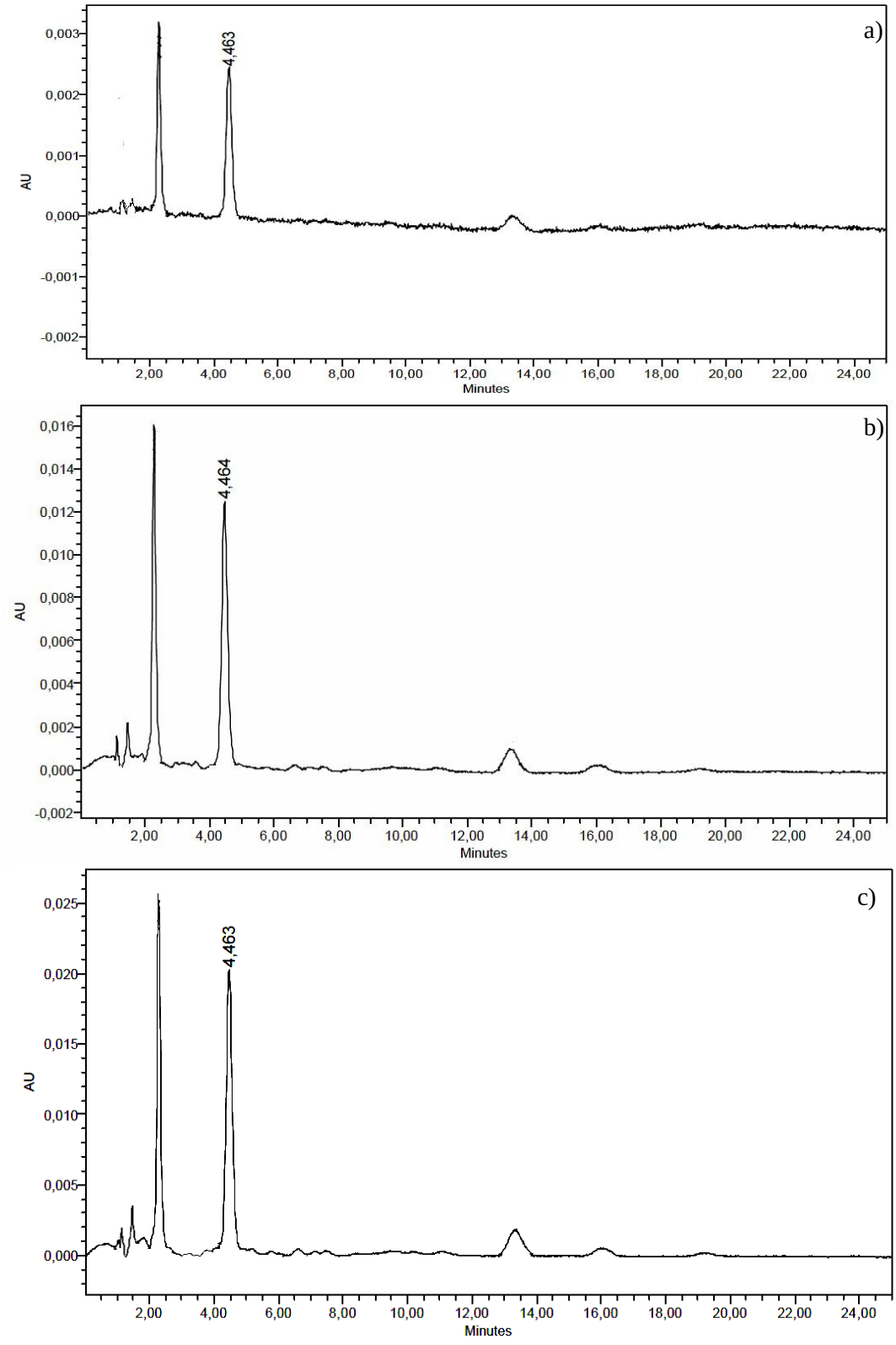


Fig. 2: HPLC chromatograms: a) Spray-dried extract at low concentration 0.0145 mg/ml, b) Spray-dried extract at medium concentration 0.0730 mg/ml and c) Spray-dried extract at high concentration 0.1165 mg/ml

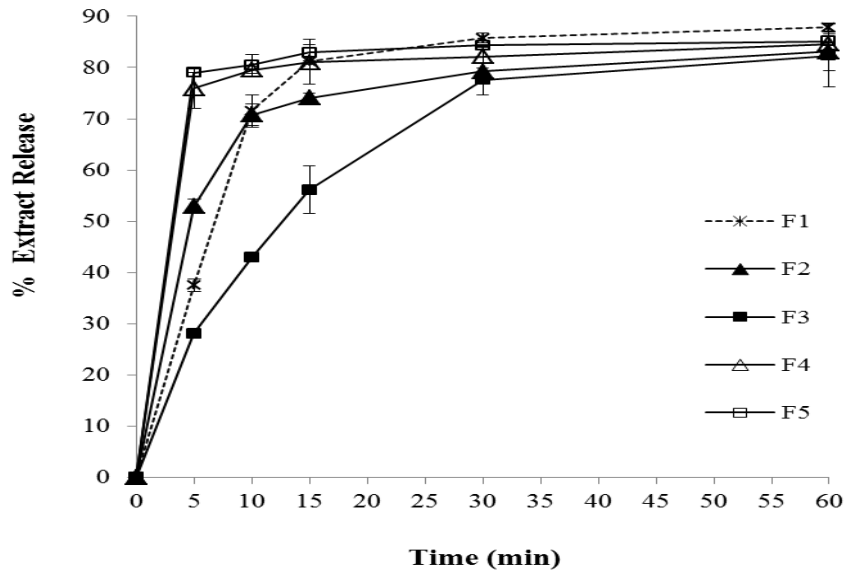


Fig. 3: Dissolution profiles

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