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Development and validation of a reverse-phase liquid chromatographic method for the assay of lidocaine hydrochloride in alginate-Gantrez® microspheres

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Abstract

A simple, fast and reliable reverse-phase high-performance liquid chromatographic (HPLC) method was developed for the assay of lidocaine hydrochloride (LH) in Gantrez®-alginate microspheres. Separation was achieved in a LiChrospher C18 column, using a mobile phase consisting of acetonitrile:ammonium acetate (0.0257 M) adjusted to pH 4.85 with acetic acid, in the ratio 70:30 (v/v) and a flow rate of 0.6 mL/min. The detection was made with a diode array detector measuring at the maximum for the compound. The validation study demonstrated that the method was precise, accurate and linear over the concentration range of analysis with a limit of detection of 0.001 mg/mL. The limit of quantification was 0.002 mg/mL. Linear regression analysis in the range of 0.8–2.4 mg/mL gave correlation coefficients higher than 0.995. The method developed was applied to the analysis of lidocaine in microsphere samples in order to evaluate in next papers, the encapsulation efficiency of different formulations.

Keywords: Lidocaine; Validation; HPLC; Gantrez®; Alginate; Microspheres

1. Introduction

Lidocaine hydrochloride (LH) or 2-diethylamine-*N*-(2,6-dimethylphenyl)-ethanamide hydrochloride is a local anaesthetic that reversibly inhibits the nerve impulse transmission. It binds to the receptors in sodium channels and decreases their activity functioning as a cell membrane stabilizer. It has a good superficial activity, penetrating in depth through the mucous membranes and reduces the sensation pain [1]. LH is characterized by a fast onset and an intermediate persistence of activity. Like other local anaesthetics, at relatively high plasma concentrations, lidocaine possesses relevant systemic adverse effects, mainly on the central nervous and cardiovascular systems [2]. When used for topical application, its absorption from the intact skin is poor. However, when applied to damaged skin, the systemic absorption can be more effective [3]. Several approaches have been developed to enhance the local anaesthetic permeation through the use of liposomes [4,5].

Taking into account the possibility to enhance the local action of LH, alginate-Gantrez® microspheres containing LH have been developed, with the aim to apply the drug to the buccal mucosa [6,7]. Alginate is a linear polysaccharide that is composed of homopolymeric blocks and blocks with an alternating sequence [8]. The gelation of alginate is mainly achieved by the exchange of sodium ions with divalent cations such as Ca^{2+}. There is widespread agreement that the gel network, induced by a cooperative binding of Ca^{2+} by polyG chain segments, forms stable junctions consisting mainly of dimers (egg-box model). This phenomenon has been applied for preparing alginate beads employed as drug delivery systems. However, the major disadvantages of calcium alginate beads are its large gel porosity which causes leakage of drugs, low drug entrapment efficiency and rapid release of the encapsulated drug in simulated intestinal fluid. Attempts have been made to reduce and control the permeability and to increase the strength of the gel network structure [9]. With this purpose, Gantrez® MS-955 is added to the formulation. This hydrophilic polymer, a maleic anhydride copolymer,

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is also used because of its mucoadhesive properties within the oral cavity [10,11].

Most of the analytical methods found in the literature, carried out by high-performance liquid chromatographic (HPLC) to determine LH are aimed at quantifying lidocaine in plasma [12–15] and in biological fluids [16], to determine the raw material and its related substances. Few of the methods described by HPLC that are found, are dedicated to the study of lidocaine in final products [17–19].

The development of a new dosage form is subjected to many studies that assure the adequate drug loading into the dosage form. Sometimes, it is necessary to use a specific technique to quantify the drug, due to the multiple composition of the dosage form, which components can exert some interference in the drug quantification. In this paper, microspheres of alginate-Gantrez® containing lidocaine have been elaborated. It is important to develop a specific technique for the drug quantification, because alginate is a polymer that absorbs the ultraviolet light in the same wavelength range than lidocaine. Then, the development and validation of an HPLC analytical technique for this dosage form is crucial to have adequate results.

For this reason, this work describes the validation parameters stated either by USP 29 [20] and by the ICH guidelines [21] to achieve an analytical method with acceptable characteristics of suitability, reliability and feasibility, ensuring that the findings achieved, when this method is applied, are correct.

2. Experimental

2.1. Chemicals

LH was obtained from Sigma–Aldrich (Germany); HPLC-grade acetonitrile, ammonium acetate and acetic acid were used to prepare the mobile phase and were purchased from Panreac (Barcelona). Gantrez® MS-955 was gently supplied by PQS. Sodium alginate (low viscosity; viscosity of 2% solution 25 °C, ≈250 cps), sodium tripolyphosphate pentabasic (TPP) used to dissolve the alginate-Gantrez® microspheres was obtained from Sigma–Aldrich (Germany).

Deionized and purified water using a Milli-Q system (Millipore) was used for the mobile phase and the standard solutions preparation. All other reagents were of analytical grade.

2.2. Chromatographic system

The chromatographic apparatus consisted of a Hitachi system manager D-7000, equipped with a quaternary pump L-7100, a diode array detector L-7455, an automatic injector L-7200 and interfase D-7000.

For data collection and calculation, HSM System Manager Software was used.

The chromatographic conditions were optimized using a column C18 (Merck, LiChrospher 100 RP-18; 125 mm × 4 mm, 5 μm). The mobile phase consisted of acetonitrile:ammonium acetate (0.0257 M) adjusted to pH 4.85 with acetic acid (70:30, v/v). The mobile phase was filtered through a 0.22-μm nitrocellulose-membrane filter (Millipore, Barcelona) and degassed under vacuum prior to use. The flow rate was 0.6 mL/min. The monitoring wavelength was 254 nm and the injection volume was 20 μL. Peak areas were measured and HPLC analysis was conducted at room temperature.

2.3. Preparation of lidocaine-loaded microspheres

Drug-loaded microspheres were prepared by an A/O emulsion with internal ionotropic gelation. These microspheres were prepared as follows. First, an aqueous solution containing the drug (0.3%, w/v) with 2% (w/v) of sodium alginate, was prepared.

Microspheres were prepared by adding of a 5% suspension (w/v) of micronized calcium carbonate into the alginate solution. This aqueous solution was then emulsified in a mixture of 70 mL of isopropyl miristate, 30 mL of dichloromethane and 3 mL of Span® 80 using a mechanical stirring at 1000 rpm. After emulsification, 20 mL of isopropyl miristate containing 500 μL glacial acetic acid was added and the dispersion was stirred for 40 min to enable $CaCO_3$ solubilization and start the internal gelation. Microspheres were separated with 50 mL of ethyl acetate and centrifugation at 3000 rpm for 5 min. They were washed with 200 mL of ethyl acetate. To harden them, different washings with acetone were made. Afterward, Ca-alginate microspheres were recovered by filtration under vacuum and dried at room temperature.

Gantrez® coating (4%, w/v) was incorporated in an intermediate phase, before beginning the ionic gelification process.

2.4. Stock and sample solutions

Standard stock solutions of LH at a concentration of about 1.6 mg/mL were prepared by dissolving the appropriate amount of LH (40 mg) in 5 mL of a solution of TPP 1% (w/v) adjusted to pH 6.85. The volume was completed up to 25 mL with purified water. This standard solution will be used to quantify the active on the final product. These solutions were stored in the dark under refrigeration at 4 °C and were found to be stable for several weeks. The stability of the standard solutions was checked over this period by preparing and injecting daily a solution of the analyte.

To carry out the sample solution (assay of pharmaceutical preparation), an appropriate amount of alginate-Gantrez® microspheres, and equivalent to 40 mg of LH was placed in a 25-mL volumetric flask with 5 mL of TPP 1% (w/v) solution adjusted to pH 6.85. This pH value is required to assure the total disintegration of microspheres. The solution was sonicated for 30 min and diluted to volume with purified water. This solution was then filtered through a 0.45-μm nylon-membrane filter (Millipore, Barcelona). The resulting filtered solution was placed in a HPLC vial.

2.5. Validation study

2.5.1. Selectivity

The selectivity is defined as the capacity of an analytical method to exactly measure the concentration of analyte without

interferences of impurities, products of degradation, excipients or related compounds. In this assay, it was tested by running solutions containing the formulation components in the same quantities and conditions that in samples (placebo sample) to show that there is not peaks in the retention times corresponding to the analyte.

The selectivity of the method was evaluated onto three placebo samples and standard solutions of LH.

Within the study of selectivity, a series of degradation studies were carried out, where the standard solutions and the work samples were subjected to different degrees of stress, by following the ICH guidelines [21]:

- *Acid degradation*: in a 25-mL volumetric flask, 40 mg of LH were accurately weighed. They were dissolved in 5 mL of 1% (w/v) TPP pH 6.85. Afterwards, 5 mL of HNO_3 0.1N were added, keeping the solution during 24 h. Then, 1 mL of NaOH 0.1N was added and finally, it was completed to volume with purified water and the mixture was shaken.
- *Basic degradation*: in a 25-mL volumetric flask, 40 mg of LH was accurately weighed. They were dissolved in 5 mL of 1% (w/v) TPP pH 6.85. Afterwards, 5 mL of NaOH 0.1N were added, keeping the solution during 24 h. Then, 1 mL of HNO_3 0.1N was added and finally, it was completed to volume with purified water and the mixture was shaken.
- *Sun light degradation*: in a 25-mL volumetric flask, 40 mg of LH was accurately weighed. They were dissolved in 5 mL of 1% (w/v) TPP pH 6.85. The volumetric flask was exposed to the sun light during 24 h and finally, it was completed to volume with purified water, shaking the mixture.
- *Degradation with temperature (60 °C)*: in a 25-mL volumetric flask, 40 mg of LH were accurately weighed. They were dissolved in 5 mL of 1% (w/v) TPP pH 6.85. The volumetric flask was put under heating to 60 °C during 24 h. Finally it was completed to volume with purified water and the mixture was shaken.

After the stress assay, the samples were analyzed by HPLC as shown in the chromatographic conditions.

2.5.2. Precision

The precision is the parameter that expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple analysis of the same homogenous sample under the prescribed conditions. In our study the repeatability was evaluated as follows:

- *Instrumental precision*: For six consecutive times, a same standard solution prepared according the described method in Section 2.3, was injected. The standard deviation and the relative standard deviation (R.S.D.) were calculated for the six injections. For acceptance, the R.S.D. value must be smaller or equal that 1.5%.
- *Method repeatability*: This parameter was determined by using the obtained results for the accuracy test (low level 50%; middle level 100%; high level 150%) (see Section 2.5.3). The standard deviation and the R.S.D. were calculated. The method repeatability can be accepted when R.S.D. is smaller or equal that 2.0%.
- *Intermediated precision*: The aim of this study consists at establishing the effects of the random events on the analytic method. The intermediated precision was evaluated by analyzing a same sample by different analysts in two different days.

2.5.3. Accuracy (recovery method)

Accuracy of a method is defined as the closeness of the measured value to the true value for the sample. The recovery method was studied at concentration levels of 50%, 75%, 100%, 125% and 150%, where a known amount of the active (0.25, 0.375, 0.50, 0.625 and 0.75 mg) was added to a determined amount of placebo solution to obtain drug concentrations of 0.8, 1.2, 1.6, 2 and 2.4 mg/mL, respectively. The amount of LH recovered in relation to the added amount (recovery percent), was calculated. This study was carried out on the basis of the method describe above.

2.5.4. Linearity

The linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to the concentration. This study was performed by evaluating the system and method linearity. For the system linearity, standard solutions of LH at five concentration levels, from 50% to 150% of the target analyte concentration, were prepared. The concentrations were 0.8, 1.2, 1.6, 2.0 and 2.4 mg/mL. Each level of concentration was prepared in triplicate. The experimental results were graphically plotted, obtaining a calibration curve and carrying out the corresponding statistical study. For the method linearity, the procedure was the same than system linearity, but the sample was a solution containing the alginate-Gantrez® microspheres of LH, dissolved in the medium.

2.5.5. Limit of detection (LOD)

The LOD of a method is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically three times the noise level. To determine this parameter, a battery of different solutions with different LH concentrations, was prepared. This parameter needs to be determined only for impurity methods, in which chromatographic peaks near the detection limit will be observed.

2.5.6. Limit of quantification (LOQ)

The LOQ is the lower level of analyte that can be accurately and precisely measured.

Similarly to LOD assay, a battery of different concentrations diluted was prepared. The range of prepared concentrations was from 0.002 to 0.16 mg/mL. The response factor was calculated (relationship between the area and concentration) for each point studied. Afterwards, the concentrations in relation to the R.S.D. obtained for the response factors from each of the concentrations were plotted. The first point which does not fulfil this R.S.D. corresponds to the LOD, and the first point which fits into this specified value corresponds to the LOQ.

3. Results and discussion

3.1. Method development

The introduction of new HPLC methods for a routine quality control of pharmaceutical preparations begins with a series of preliminary investigations, which enables establishing the optimal experimental conditions and provide maximum relevant information by analyzing the experimental data [22–25]. In this study, a RP-HPLC method for the determination of LH in alginate-Gantrez® microspheres, was developed and validated. A simple sample preparation, short separation time and a low LOQ were considered when the study started.

The aim for sample preparation method was to remove the interferences from the other microsphere constituents to be reproducible with a high recovery involving a minimum number of working steps.

3.2. Validation study

3.2.1. Selectivity

From the LH chromatogram, it was observed that the drug eluted at a retention time of 3.47 min (Fig. 1A). The study of the purity of the peak showed that the three spectrums obtained at different times are within the established threshold for this peak.

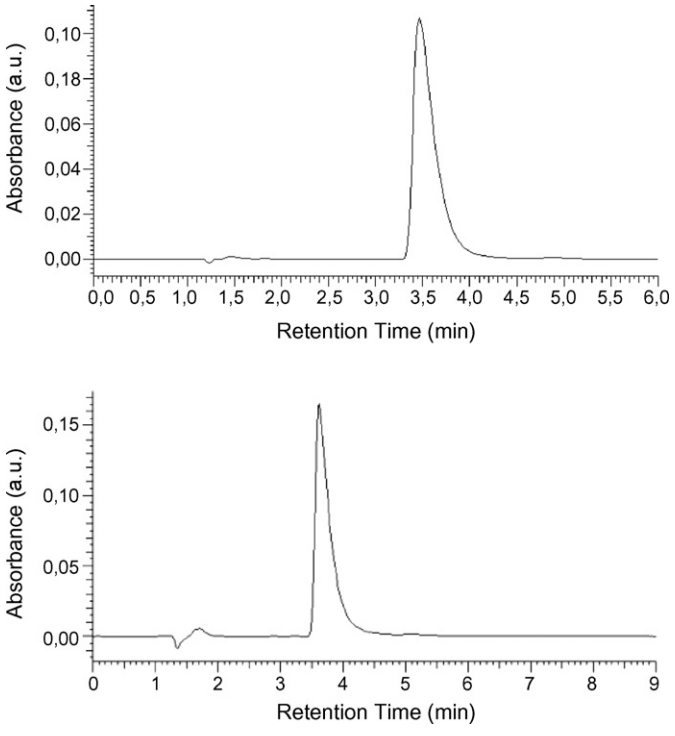

Fig. 1. Representative chromatogram of (A) the standard solution of LH and (B) microsphere sample containing LH. The retention time was about 3.6 min. Chromatographic conditions: reverse-phase HPLC on a column C18 (Merck, LiChrospher 100 RP-18; 125 mm × 4 mm, 5 μm); mobile phase: acetonitrile:ammonium acetate (0.0257 M) adjusted to pH 4.85 with acetic acid (70:30, v/v); flow rate, 0.6 mL/min; detection wavelength, 254 nm; injection volume, 20 μL.

Table 1
Data of the peak areas corresponding to the placebo samples submitted to the specified conditions

Sample	Peak area	S.D.	Purity (%)
Standard	1,646,836	1122	99.0
Light sun	1,667,838	5869	99.0
60 °C	1,649,698	9536	99.0
HNO_3	1,654,498	2522	99.0
NaOH	1,648,148	1025	99.0

It was observed the absence of interferences of the excipients for pharmaceutical preparation and the absence of impurity interferences provided by the supplier of the raw material, because none of the peaks appears at the same retention time than LH peak (Fig. 1B). Then, it was concluded that the developed method is selective in relation to the excipients of the final preparation and the impurities provided by the supplier of raw materials.

Another study carried out to check the selectivity of the method was the degradation test submitted to the samples under different stress conditions, as described in Section 2.5.1. Table 1 summarizes the results obtained for LH standard and the samples analyzed. The mean purity value obtained was 99.0%, indicating that the LH peak is pure.

According to the areas obtained, it can be concluded that LH is stable in these conditions. The purity factor for the drug assures that there is no co elution of other peaks. Therefore, the method is selective and suitable for routine work.

3.2.2. Precision

3.2.2.1. Repeatability. As defined in the International Conference on Harmonization (ICH) guidelines [21], repeatability expresses the precision under the same operating conditions over a short interval of time. ICH guidelines suggest a minimum of six readings of a single sample at 100% of target concentration.

3.2.2.2. Instrumental precision. The repeatability of the instrumental system was evaluated with this parameter. In this study, a R.S.D. of 0.709% was obtained for the area corresponding to the first day (1759667.833 ± 12487.91, $n = 6$), by injecting a standard solution of 1.6 mg/mL. Moreover, the retention time repeatability was determined, showing a mean value of 3.47 (R.S.D. = 0.109%).

On the basis of the obtained results, we can conclude that the repeatability of the system is satisfactory (R.S.D. < 1.5%).

3.2.2.3. Method repeatability. This parameter was evaluated using the same data obtained for the accuracy study. Table 2 summarizes these results. R.S.D.s for the six recovery values for levels I, III and V of the accuracy test are less than 2.0%. For this reason, the method repeatability was considered validated.

3.2.2.4. Intermediate precision. ICH recommendations for this precision parameter are to study the effect of random events during the analysis. In this precision study, two random events were considered: the analysis of microspheres on two different

Table 2
Recovery results of LH in alginate-Gantrez® microspheres

Level	Theoretical	Peak area	Experimental	Recovery (%)
I	20.8	856,956	20.9	100.4
	20.5	857,342	20.9	101.9
	20.4	852,770	20.8	101.8
	20.4	843,448	20.5	100.7
	20.6	844,080	20.6	99.8
	20.1	812,722	19.8	98.4
	Mean	844,553	20.6	100.5
	S.D.	16730.6	0.4	1.3
	R.S.D.	2.0	2.0	1.3
II	30.1	1,218,312	29.9	99.4
	30.0	1,217,158	29.9	99.6
	30.5	1,240,594	30.5	99.9
	30.7	1,254,792	30.8	100.4
	31.2	1,287,057	31.6	101.4
	30.4	1,252,556	30.8	101.2
	Mean	1245078.2	30.6	100.3
	S.D.	26186.1	0.7	0.8
	R.S.D.	2.1	2.1	0.8
III	40.9	1,684,011	41.5	101.6
	40.8	1,666,352	41.1	100.8
	40.7	1,680,698	41.5	101.9
	41.3	1,692,166	41.7	101.1
	40.4	1,628,874	40.2	99.4
	40.2	1,626,843	40.1	99.8
	Mean	1663157.3	41.0	100.8
	S.D.	28594.9	0.7	1.0
	R.S.D.	1.7	1.7	1.0
IV	53.0	2,181,084	53.9	101.9
	50.2	1,997,602	49.4	98.4
	51.8	2,122,964	52.5	101.4
	53.4	2,186,108	54.1	101.3
	51.0	2,097,806	51.9	101.8
	50.0	1,997,757	49.4	98.8
	Mean	2097220.2	51.9	100.6
	S.D.	84159.3	2.1	1.6
	R.S.D.	4.0	4.1	1.6
V	64.8	2,658,037	65.9	101.7
	60.6	2,441,329	60.5	99.8
	62.1	2,553,419	63.3	101.9
	62.6	2,548,918	63.2	100.9
	60.0	2,441,329	60.5	100.8
	60.4	2,478,630	61.4	101.7
	Mean	2,520,277	62.5	101.1
	S.D.	83781.3	2.1	0.8
	R.S.D.	3.3	3.4	0.8

Concentration range: 0.8–2.4 mg/mL (level I: 0.8 mg/mL; level II: 1.2 mg/mL; level III: 1.6 mg/mL; level IV: 2.0 mg/mL; level V: 2.4 mg/mL). Theoretical: theoretical amount of LH (mg). Experimental: amount of LH obtained (mg). S.D.: standard deviation; R.S.D.: relative standard deviation.

days and two analysts performing the analysis on the same day [26].

The results obtained were expressed as the drug recovery percent and R.S.D.%. They are summarized in Table 3. All results are below the established limit according to the variation

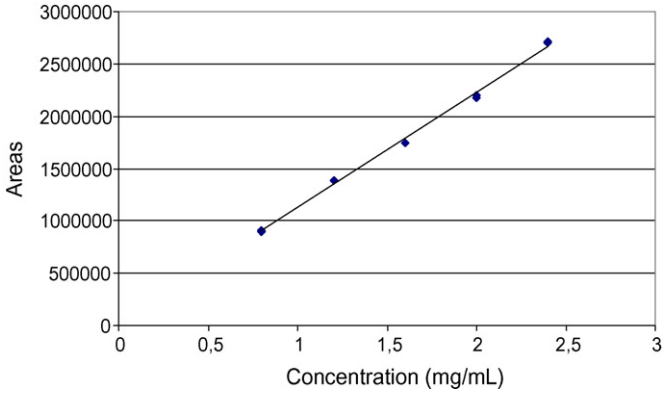

Fig. 2. System linearity corresponding to the concentration range of 0.8–2.4 mg/mL of the LH standard solution.

accepted (R.S.D. < 2.0%), concluding that the variations introduced in the test have no influence on the experimental results. In addition, the MANOVA statistical test revealed that no statistical differences between days and analysts can be appreciated ($p = 0.778$). Therefore, the proposed analytical technique has a good intermediate precision.

3.2.3. Accuracy

The results obtained for the accuracy study in the samples ranging a LH concentration between 0.8 and 2.4 mg/mL and being the 100% corresponding to 1.6 mg/mL ($n = 6$ for 50%, 75%, 100%, 125% and 150%) indicated that the recovery percent was between 98.4% and 101.9% of recovery (Table 2), being the mean relative standard deviation, R.S.D. = 1.09%.

According to the obtained results, it would not be necessary to make an additional statistical test, since the defect acceptance number of the ICH for pharmaceutical formulations in this parameter establishes that the percentage of recovery must be between 98% and 102%, which is equivalent to ±2.0% of the relative error [21].

3.2.4. Linearity

Linearity is the ability of the method to respond proportionally to the changes in concentration or amount of the analyte in a sample. In routine, univariate calibration method linearity is established within a specific range.

The calibration curve obtained by plotting the LH peak area versus the concentration of standard solution was linear in the above mentioned concentration range (Fig. 2). The equation of the regression line obtained (Table 4), with all the values, relating the tested concentrations and the response obtained corresponds to $y = 9.046\text{E}{-7}\ x - 0.0144$ (y: LH concentration (mg/mL); x: peak area, with a standard error of 0.0356 and a correlation coefficient that exceeds 0.995 ($n = 15$).

With respect to the method linearity (Table 5), the regression line ($y = 9.363\text{E}{-7}x + 0.0337$) showed a good linearity in the concentration range of 0.8–2.4 mg/mL (Fig. 3), obtaining a R^2 of 0.9967 ($n = 15$).

Also, the statistical analysis of ANOVA corresponding to the collected data for both system and method linearity, were reported. The F test statistic (F) and its corresponding p-value

Table 3
Method repeatability, developed by two different analysts (1 and 2 before/), in two different days (1 and 2 after/) to two replicated samples (S1 and S2)

Analyst/day	Area	mg/mL	mg obtained	mg theoretical	%	Average	S.D.	R.S.D.
1/1 S1	1,625,736	1.60	40.10	39.22	102.23			
1/1 S2	1,621,975	1.60	40.00	39.16	102.15	40.05	0.07	0.17
2/1 S1	1,639,756	1.62	40.45	40.00	101.11			
2/1 S2	1,614,308	1.59	39.81	40.37	98.61	40.13	0.45	1.12
1/2 S1	1,622,322	1.60	40.01	39.67	100.86			
1/2 S2	1,621,472	1.60	39.99	39.38	101.54	39.99	0.02	0.04
2/2 S1	1,623,917	1.60	40.05	39.78	100.68			
2/2 S2	1,627,571	1.61	40.14	38.81	103.43	40.10	0.07	0.16
Average	1624632.1	1.60	40.07	39.55	101.33	40.07	0.15	0.37
S.D.	7251.3	0.00	0.18	0.50	1.42			
R.S.D.	0.4	0.45	0.45	1.28	1.39			

S.D.: standard deviation; CV: variation coefficient; R.S.D.: relative standard deviation (%).

Table 4
Regression statistics for the system linearity

Regression statistics	
Multiple R	0.9983
R^2	0.9966
Adjusted R^2	0.9963
Standard error	0.0356
Observations	15

ANOVA

	d.f.	SS	MS	F	Significance F
Regression	1	4.7835	4.7835	3769.4337	2.10E−17
Residual	13	0.0165	0.0013		
Total	14	4.8			

	Coefficient	Standard error	t-Stat	p-Value	Lower 95%	Upper 95%
Intercept	−0.0144	0.0279	−0.5154	0.6149	−0.0745	0.0458
Area	9.05E−07	1.47E−08	61.3957	2.10E−17	8.73E−07	9.36E−07

Concentration range: 0.8–2.4 mg/mL; d.f.: degrees of freedom; SS: sum of squares; MS: mean of squares.

Table 5
Regression statistics for the method linearity

Regression statistics	
Multiple R	0.9984
R^2	0.9967
Adjusted R^2	0.9965
Standard error	0.0349
Observations	15

ANOVA

	d.f.	SS	MS	F	Significance F
Regression	1	4.7842	4.7842	3930.6857	1.60E−17
Residual	13	0.0158	0.0012		
Total	14	4.8			

	Coefficient	Standard error	t-Stat	p-Value	Lower 95%	Upper 95%
Intercept	0.0337	0.0266	1.2676	0.2272	−0.0237	0.0910
Area	9.36E−07	1.49E−08	62.6952	1.60E−17	9.04E−07	9.69E−07

Concentration range: 0.8–2.4 mg/mL; d.f.: degrees of freedom; SS: sum of squares; MS: mean of squares.

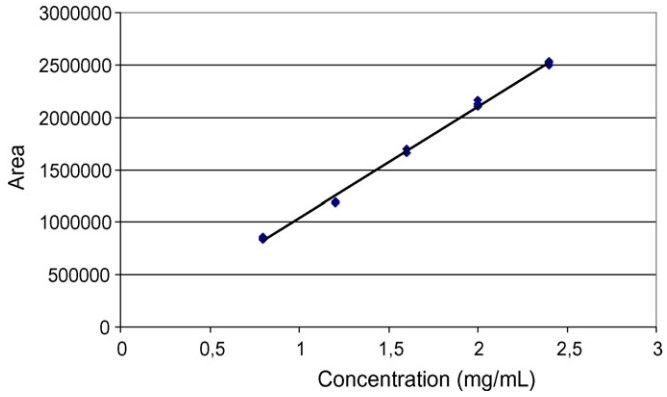

Fig. 3. Method linearity corresponding to the concentration range of 0.8–2.4 mg/mL of LH in alginate-Gantrez® microspheres.

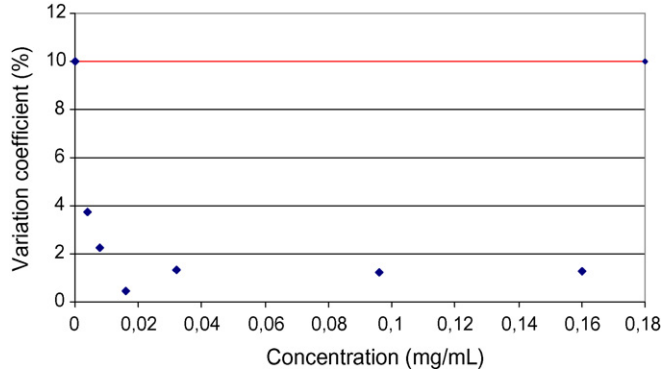

Fig. 4. Limit of quantification of the chromatographic method used to determine the LH in alginate-Gantrez® microspheres.

(significance F) certainly indicate an overall goodness of fit for the model ($p = 2.1\text{E}{-17}$ for system linearity and $p = 1.60\text{E}{-17}$ for method linearity).

3.3. Limit of detection (D_L) and limit of quantification (Q_L)

The D_L is determined by the analysis of samples with known concentrations of LH and by establishing the minimum level at which this analyte can be reliably detected [21].

The visual observations of the sample chromatograms showed that the lower drug concentration which produces a signal different to that noise signal emitted by the HPLC equipment was 0.001 mg/mL.

On the other hand, the LOQ was based on visual evaluation, as recommended the ICH guideline [21]. This parameter is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can by quantified with acceptable accuracy and precision. The results obtained were plotted in Fig. 4 showing that for this analytical technique, a Q_L of 0.002 mg/mL was obtained.

4. Conclusions

The proposed high-performance liquid chromatographic method has been evaluated over the linearity, precision, accuracy and selectivity, and proved to be convenient and effective for the quality control of LH in alginate-Gantrez® microspheres. It has been proved that it was selective, linear between 50% and 150% of the work concentration (1.6 mg/mL) for LH, with a correlation coefficient higher than 0.995, exact and precise. Limits of detection and quantification for the drug were 0.001 and 0.002 mg/mL, respectively, and these values are under the lowest expected concentrations in the samples.

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