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Simultaneous quantification of different cyclodextrins and Gantrez by HPLC with evaporative light scattering detection

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Abstract

A rapid and simple HPLC method with evaporative scattering detection (ELSD) has been developed for the separation and quantitation of β-cyclodextrin (β-CD), 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) and poly(methyl vinyl ether-co-maleic anhydride) (Gantrez). Separation was carried out on a Zorbax Eclipse XDB-Phenyl narrow bore column, with water–acetonitrile in gradient elution as mobile phase at a flow-rate of 0.25 ml/min. Polyethylenglycol 6000 was used as internal standard. The limit of quantification was of about 0.2 mg/ml for cyclodextrins and 0.05 mg/ml for Gantrez. The precision did not exceed 7%. This method was successfully applied to the rapid analysis of CD–Gantrez nanoparticle conjugates without interference from other components of the formulation.

Keywords: β-Cyclodextrin; 2-Hydroxypropyl-β-cyclodextrin; HPLC; ELSD; Nanoparticles; Poly(methyl vinyl ether-co-maleic anhydride)

1. Introduction

Cyclodextrins (CDs) are a group of cyclic oligosaccharides, obtained from the enzymatic degradation of starch, composed of α-1,4-linked glucopyranose units which form a truncated cone with a hydrophobic internal cavity [1,2]. The most abundant natural cyclodextrins are α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD), with six, seven and eight glucopyranose units, respectively. For pharmaceutical purposes, β-CD and its derivatives are widely employed. In particular, hydroxypropyl-β-cyclodextrin (HP-β-CD) deserves special attention because of a higher aqueous solubility, a lower toxicity and a more hydrophobic cavity compared to the parent compound. All of these facts make this derivative as an ideal candidate for the solubilization of hydrophobic drugs in pharmaceutical formulations [1,3,4].

CDs are also widely employed in a number of technological applications, including their use as stationary phases in capillary electrophoresis [5] and HPLC [6–8]. Another interesting application is their use with polymeric drug delivery systems in order to both increase their loading capacity and modify the release of the loaded drug [9,10]. In this context, we have designed and prepared conjugates between PVM/MA nanoparticles and different CDs in order to evaluate their ability as oral drug carriers. Gantrez® AN is a copolymer between methyl vinyl ether and maleic anhydride (PVM/MA), which can be used as a thickening and suspending agent in aqueous solutions and adhesive bases in denture preparations, transdermal patches and buccal tablets [11]. As a first step, we have attempted to develop a suitable analytical method to quantify the copolymer and CDs in the same sample. For Gantrez, a review of the literature showed that currently there are no methods available for the quantification of this copolymer. The analysis of CDs is extremely difficult because, since these carbohydrates contain no chromophores, the UV detection is not applicable. Therefore, the HPLC methods described are mainly based on the use of refractive index detection [12], UV following derivatisation [13], indirect spectrophotometry [14,15] and pulsed amperiometry [16–18].

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All of these problems generate the need for an analytical method able to determine the different CDs and Gantrez in the same sample. In this context, an evaporative light scattering detector (ELSD) was chosen as detector. This technique has been successfully applied to the characterization of some polysaccharides (i.e. sulfobutyl ether-β-cyclodextrin [8]) and is compatible with gradient elution [19].

This work describes the development and validation of a microanalytical high-performance liquid chromatographic technique for the determination of β-CD, 2-HP-β-CD and PVM/MA in nanoparticle formulations, without interference by other substances used in the preparation of these news delivery systems.

2. Materials and methods

2.1. Chemicals, reagents and solutions

β-Cyclodextrin was provided by Sigma–Aldrich (Steinheim, Germany) and 2-hydroxypropyl-β-cyclodextrin by RBI (Massachusetts, USA). Poly(methyl vinyl ether-co-maleic anhydride) or PVM/MA (Gantrez® AN 119; MW 200,000) was kindly gifted by ISP (Barcelona, Spain). 1,3-Diaminopropane by Sigma–Aldrich. Acetone and ethanol were obtained from VWR Prolabo (Fantenay sous Bois, France). Polyethylenglycol (PEG) 6000 (used as internal standard) and acetonitrile (HPLC grade) were supplied by Merck (Darmstadt, Germany). Deionized reagent water (18.2 MΩ cm resistivity) was prepared by a water purification system (Wasserlab, Pamplona, Spain). Nitrogen gas (ultra-pure, >99%) was produced using an Alltech nitrogen generator (Ingeniería Analítica, Barcelona, Spain).

2.2. Calibration standards

Stock solutions of CDs and PVM/MA (with a concentration of 5 mg/ml) were separately prepared by dissolving 25 mg CDs in 5 ml of reagent water and 25 mg PVM/MA in 5 ml of acetonitrile. Finally, eight standard solutions of CDs (0.5, 0.9, 1, 1.5, 1.75, 2, 3.5 and 4 mg/ml) were prepared by dilution of the stock solution with appropriate volumes of water. Similarly, the PVM/MA standard solutions (0.05, 0.1, 0.3, 0.5, 0.6, 0.75, 0.9, 1, 1.5, 1.75, 2, 2.5, 3, 3.5 and 4 mg/ml) were prepared by dilution of stock solution with appropriate volumes of acetonitrile. The standard solution of polyethylenglycol 6000 (10 mg/ml) was the stock solution.

2.3. Instrumentation and chromatographic conditions

The apparatus used for the HPLC analysis was on Agilent model 1100 series LC (Waldbronn, Germany) coupled with an evaporative light scattering detector, ELSD 2000 (Alltech, Illinois, USA). An ELSD nitrogen generator (Alltech) was used as the source of the nitrogen gas. Data acquisition and analysis were performed with a Hewlett-Packard computer using the ChemStation G2171 AA program. Separation was carried out at 50 °C on a reversed-phase Zorbax Eclipse XDB-Phenyl column (2.1 mm × 150 mm; particle size 5 μm) obtained from Agilent Technologies (Waldbronn, Germany). This column was protected by a 0.45 μm filter (Teknokroma, Spain). ELSD conditions were optimized in order to achieve maximum sensitivity: the drift tube temperature was set at 115° C, the nitrogen flow was maintained at 3.2 l/min and the gain was set to 1. The mobile phase composition was a mixture of acetonitrile (A) and water (B) in a gradient elution at a flow-rate of 0.25 ml/min. Complete gradient conditions are summarized in Table 1.

Table 1
Phase mobile conditions for gradient elution

Time (min)	A (%)	B (%)
0	0	100
2	0	100
9	60	40
11	71	29
12	0	100
15	0	100

A, acetonitrile and B, water.

2.4. Preparation of CD–Gantrez nanoparticles

Poly(methyl vinyl ether-co-maleic anhydride) nanoparticles were prepared by a desolvation method and chemical cross-linkage [11]. Two different procedures were assayed. Method A consisted of the preparation of unloaded PVM/MA nanoparticles, which were subsequently incubated with different amounts of CDs for 30 min at room temperature. These coacervates were hardened with 1,3-diaminopropane (DP) for 5 min at room temperature.

Nanoparticles for Method B were prepared by the incubation of either β-CD or 2-HP-β-CD with a Gantrez organic solution. Then, the nanoparticles were formed by the addition of the ethanol–water mixture (1:1). Organic solvents were eliminated by evaporation under reduced pressure and the resulting nanoparticles cross-linked with DP for 5 min.

In all cases, the different CDs–Gantrez nanoparticle conjugates were purified twice by centrifugation at 17,000 rpm for 20 min. The recovered supernatants were diluted to 10 ml in water and stored at −20 °C until analysis.

2.5. Sample preparation

A 1-ml aliquot of the supernatants spiked with the internal standard (50 μl of 10 mg/ml of PEG 6000) were transferred to autosampler vials, capped and placed on the HPLC autosampler. A 5-μl aliquot of the supernatant was injected onto the HPLC column.

2.6. Quantitation

For β-CD and 2-HP-β-CD calibration curves were determined by potential and polynomial regression analysis,

respectively. For PVM/MA, calibration curve was divided into two ranges. The first range (0.05–1 mg PVM/MA/ml) was determined by polynomial regression and the second one (1–4 mg PVM/MA/ml) by potential regression. For this purpose, the peak area ratio between the corresponding CDs or PVM/MA and PEG 6000 versus the corresponding analyte concentration was plotted. A Student's *t*-test was used to compare the back-calculated concentrations with each calibration curve, which consisted of eight calibration points. For cyclodextrins, the following concentrations were selected: 0.5, 0.9, 1, 1.5, 1.75, 2, 3.5 and 4 mg CDs/ml. For Gantrez, two different calibration curves were performed, the first one ranged from 0.05 to 1 mg PVM/MA/ml, whilst the second ranged from 1 to 4 mg PVM/MA/ml.

2.7. Validation

The method was fully validated by analysis of calibrators prepared at five different concentrations. In supernatants, for PVM/MA, two different concentration ranges were analysed: 0.05–1 mg/ml and 1–4 mg PVM/MA/ml. For both CDs, the range between 0.5 and 4 mg of each CDs/ml were analysed. The quality control samples were prepared as a single batch on the same day at each concentration. Precision and accuracy were also determined. The precision was expressed as the coefficient of variation (CV). Within-day variability was determined by measuring five replicate measurements at four concentration levels: 0.05, 0.1, 0.5 and 0.9 mg/ml for low Gantrez concentrations and 1, 1.5, 2 and 3 mg/ml for high Gantrez concentrations, and 0.5, 0.9, 1.75 and 3.5 mg/ml for cyclodextrins. Similarly, between-day variability, on three different days, was determined by repeated analysis of four quality control samples at low, medium and high concentration levels: 0.05, 0.5 and 0.9 mg/ml for low Gantrez concentrations, 1, 2 and 3 mg/ml for high Gantrez concentrations and 0.9, 1.75 and 3.5 mg/ml for cyclodextrins. Accuracy was determined according to the following equation:

$$\text{difference from theoretical value (\%)} = \frac{X - C_T}{C_T} \times 100 \quad (1)$$

where X is the estimated concentration of the analysed molecule and C_T the theoretical concentration. To be acceptable, all the differences should be lower than 15%.

The selectivity of the assay was determined by the individual analysis of blank samples. In all cases, no interference from substances used in the preparative process was observed (i.e., 1-3-diaminopropane).

The limit of detection (LOD) was defined as the lowest concentration of analyte able to be clearly detected. The limit of quantitation (LOQ) was defined as the lowest drug concentration, determined with a precision of 20% and accuracy ranged from 80 and 120%. In this work, LOD and LOQ were determined by serial dilution of sample preparations containing the lowest level of CDs and Gantrez.

2.8. Application of the method

This analytical HPLC method using ELSD was applied to determine the CDs and polymer content in particulate dosage forms. The amount of CDs associated to nanoparticles was calculated as the difference between the initial CDs and the amount of CDs recovered in the supernatants. Similarly, the amount of PVM/MA in the nanoparticles was estimated by difference in the same way. The yield of the process of preparing the nanoparticles was calculated by gravimetry. For this purpose, PVM/MA nanoparticles, freshly prepared, were freeze-dried. Then, the yield was calculated as the difference between the initial amount of the polymer used to prepare nanoparticles and the weight of the freeze-dried carriers.

The encapsulation efficiency (%) was expressed as the percentage of CDs associated to nanoparticles with respect to the initial amount of CDs added in the formulation, whereas the CD loading was calculated in accordance with Eq. (2) and expressed in percentage.

$$\text{CD loading (\%)} = \frac{\text{amount of CD in nanoparticles (}\mu\text{g)}}{\text{PVM/MA nanoparticle yield (mg)}} \times 100 \quad (2)$$

3. Results and discussion

The general aim of our research was to combine the inclusion properties of cyclodextrins with the ability of PVM/MA nanoparticles to modify drug release and bioadhesion in specific sites of the gastrointestinal tract. Gantrez AN is a polyanhydride characterized by its ability to easily react in aqueous environments with molecules containing $-NH_2$ or $-OH$ residues [20]. This fact permits the development of new nanoparticulate devices with different physico–chemical or biological properties. In this context, the association between CDs and Gantrez nanoparticles provides a good strategy to both increase the loading capacity of lipophilic drugs by "traditional" Gantrez nanoparticles and modulate their release from these pharmaceutical forms.

The first step in this development would be quantification of both materials (CDs and PVM/MA) in nanoparticles. However, no analytical method specifically designed for this purpose has been described in the literature. The main difficulty in the analysis of CDs and other saccharides is the lack of suitable both spectral and electrochemical properties, which dramatically restricts the possibilities for detection and analysis. Furthermore, quantification based on the use of refractive index (RI) is also limited because of its inability to be used with gradient elution systems. During the last decade, a quasi-universal detector based on the evaporative light scattering detection (ELSD) has been increasingly used for the quantification of non-absorbing analytes by liquid chromatography [21]. Moreover, ELSD is at least 100 times more sensitive than the RI detector and is compatible with HPLC elution gradient [19].

3.1. Optimization of the chromatographic system

In this work, different packing materials were tested for the separation of CDs. Other authors have previously employed an anion-exchange column with this objective [8]. However, this column is not suitable as the compounds included in the mobile phase (e.g. buffers or ion-pair agents) can induce the aggregation and the precipitation of polymers or macromolecules employed in the preparation of nanoparticulate dosage forms. Such precipitation inside the columns, results in a reduction in the column separative capacity, loss of peak quality and a dramatic increase in the back pressure [22]. As a first approach, a highly non-polar Alltima C18 column (150 mm × 2.1 mm) was chosen. However, the use of acetonitrile–water mixtures resulted in rapid elution of the CDs and polymer with very poor resolution between chromatographic peaks. Similarly, a C8 column, which was previously proposed for the resolution of CDs [23], did not show acceptable chromatographic behavior. Finally, a Zorbax Eclipse XDB-Phenyl was selected which gave peak shapes at acceptable elution times for both CDs. The use of this column enabled us to determine CDs and Gantrez in small sample volumes without the need for buffered mobile phases because of the high purity of the silica employed in this packing and the end-capping process applied to inactivate the free silanol groups. In addition, the narrow bore of these columns led to a higher detector response than standard columns due to the decreased diffusion of the sample as it passes through the column.

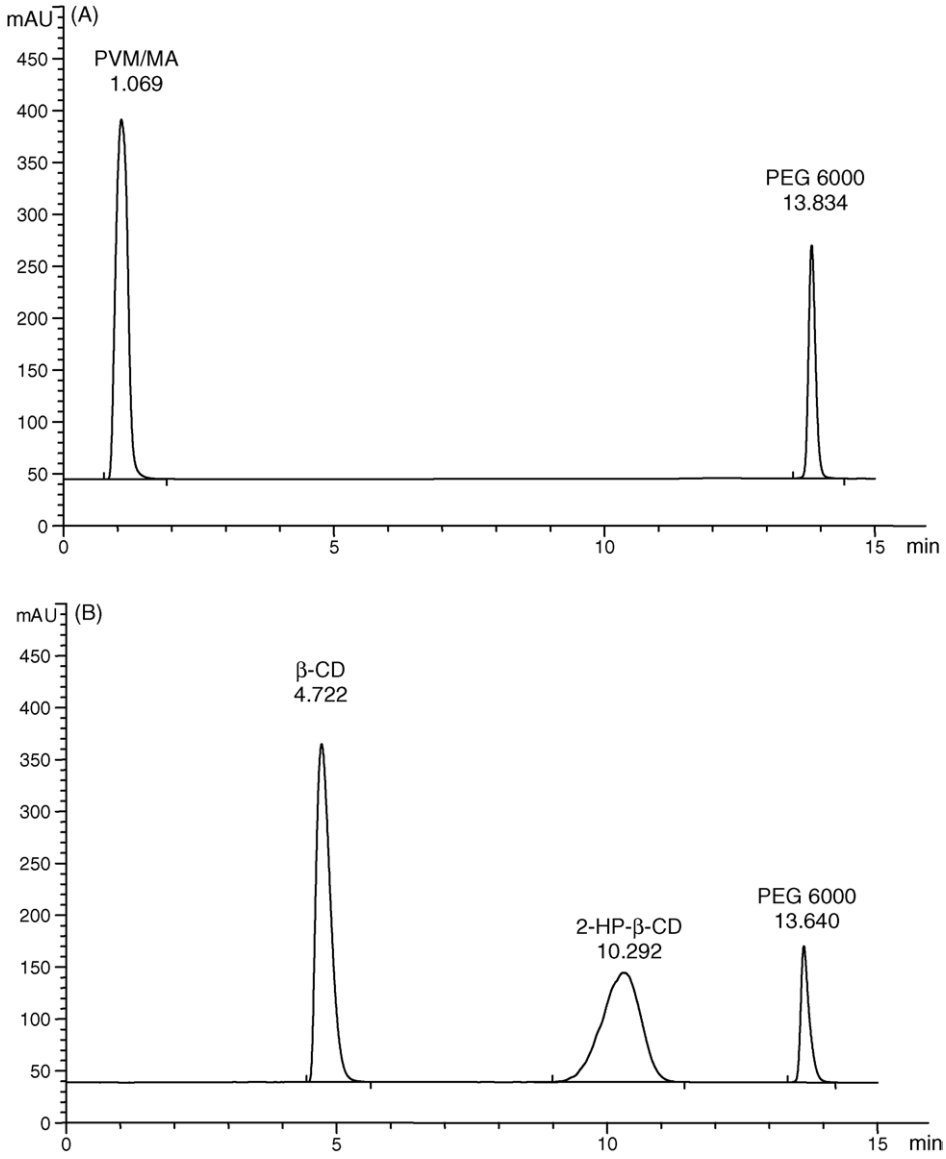

Fig. 1. Chromatogram obtained from the analysis of a PVM/MA solution in acetonitrile at 2 mg/ml (A) and a synthetic mixture of β-CD and 2-HP-β-CD solution in water at 2 mg/ml and PEG 6000 as internal standard (B). PVM/MA: poly(methyl vinyl ether-co-maleic anhydride); β-CD: beta-cyclodextrin; 2-HP-β-CD: 2-hydroxypropy-β-cyclodextrin; PEG 6000: polyetylenglycol 6000.

Optimized gradient conditions are described in Section 2 and Table 1. The gradient started with 100% water for 2 min. Seven minutes later (time: 9 min) the composition of the mobile phase was water–acetonitrile 40:60 (v/v) and at 11 min it changed to a water–acetonitrile mixture of 29:31 (v/v). The re-equilibration of the column was performed during the following 3 min (mobile phase: 100% water). In ELSD detection, the intensity of the scattered light is proportional to the size of the solutes particles. This particle size is influenced by the solute concentration along the peak profile. In fact, the use of a high gradient slope has been proved to be an advantage resulting in sharp peaks, and thus contributing to increased sensitivity [19].

In ELSD, the nebulizer-gas pressure and drift tube temperature are the mayor instrumental parameters affecting the signal response. Using the optimized mobile phase, the influence of evaporation temperature and nebulizer-gas pressure on the shape and area of peaks was studied. Concerning the drift tube temperature, the range 95–120 °C was analysed and 115 °C was found to be the best temperature to obtain a good chromatographic response. With respect to the nebulizer-gas pressure, in general, large droplets are formed at low gas pressure, which results in spikes and noisy signals [24]. On the other hand, increasing the gas pressure results in a marked decrease of the signal response. The optimum nebulizer-gas pressure in this case was set at 3.2 bar.

Under these chromatographic conditions the CDs, Gantrez and the internal standard (PEG 6000) were well resolved within 15 min. PEG 6000 was chosen as internal standard for its retention time, which was suitable for our analysis and system of detection. Furthermore, PEG 6000, as β-cyclodextrins, can be used as coating agent of nanoparticles in order to modify their behavior and bioadhesive properties [25]. The retention time for Gantrez was 1.08 ± 0.05 min, for β-CD 4.58 ± 0.07 min, 10.27 ± 0.06 min for 2-HP-β-CD and for PEG 13.60 ± 0.04 min (Fig. 1).

3.2. LC–ELSD method validation

The selectivity of the assay was studied by the analysis of supernatants of both loaded and unloaded PVM/MA nanoparticles. Under these chromatographic condition, no

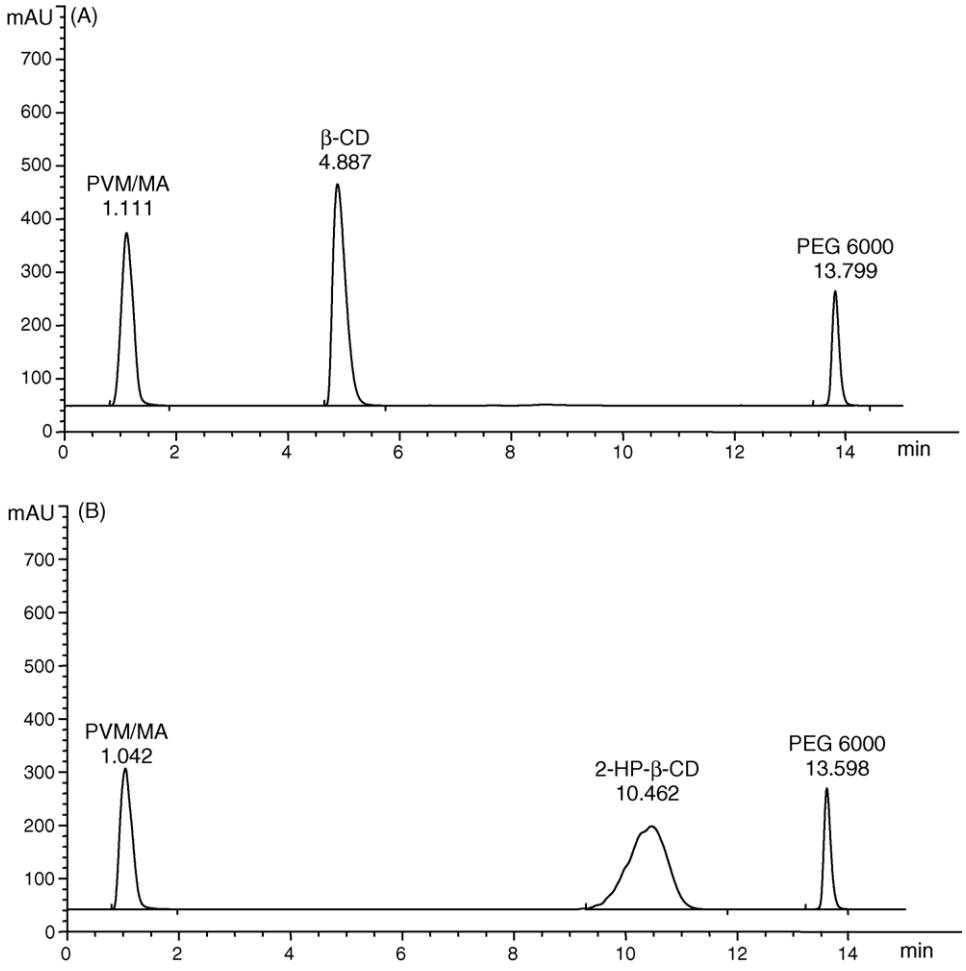

Fig. 2. Chromatograms resulting from the analysis of supernatants obtained during the purification step of the preparation process of β-CD—Gantrez nanoparticles (A) and 2-HP-β-CD–Gantrez nanoparticles (B). PVM/MA: poly(methyl vinyl ether-co-maleic anhydride); β-CD: beta-cyclodextrin; 2-HP-β-CD: 2-hydroxypropyl-beta-cyclodextrin; PEG 6000: polyethylenglycol 6000.

Table 2
Standard curves for cyclodextrins in water

	N	Regression equation	r
β-CD			
Day 1	8	$y = -0.449x^2 + 1.9817x - 0.3808$	0.9999
Day 2	8	$y = 0.0083x^2 + 2.0229x - 0.4391$	0.9998
Day 3	8	$y = 0.0224x^2 + 2.0726x - 0.4097$	0.9997
2-HP-β-CD			
Day 1	8	$y = 1.0811x^{1.499}$	0.9989
Day 2	8	$y = 1.0611x^{1.519}$	0.9999
Day 3	8	$y = 1.0415x^{1.497}$	0.9988

interferences were observed and the resolution between the peaks was satisfactory (Fig. 2).

3.2.1. Cyclodextrins

3.2.1.1. Sensitivity of the assay. Detection and quantification limits (LOD and LOQ, respectively) of the HPLC assay were determined by the analysis of the peak baseline noise in six blank samples. Thus, LOD defined as the lowest drug concentration which can be calculated as three times the variation in the measured response, was calculated to be 0.03 mg/ml for β-CD and 0.05 mg/ml for 2-HP-β-CD. Similarly, the LOQ, estimated as 10 times the variation in the measured response, was calculated to be 0.2 mg/ml for both CDs. The mean assay result was 0.213 ± 0.004 mg/ml ($n = 5$) with a relative standard deviation of 6.42% for 2-HP-β-CD and 0.209 ± 0.012 mg/ml ($n = 5$) with a relative standard deviation of 4.90%.

3.2.1.2. Linearity of the assay. Linearity was determined by plotting a standard curve from the ratios between peaks areas of either β-CD or 2-HP-β-CD and that of PEG 6000 (I.S.) versus the corresponding CDs concentration. A strong relationship between chromatographic response and CD concentration was observed on three different days over the range 0.5–4 mg/ml (see Table 2). In all cases, polynomial regression for 2-HP-β-CD and potential regression for β-CD, displayed correlation coefficients greater than 0.998 and 0.999, respectively. In addition, relative error in each concentration was calculated in the mean curve and did not exceed 8% in all cases. The absence of a linear response when ELSD is used appears to be a characteristic of this detector [24].

Furthermore, for both cyclodextrins (β-CD and 2-HP-β-CD), the calculated and the nominal values were found to be statistically similar when a Student's *t*-test was applied ($p < 0.05$).

Table 3
Cyclodextrin analysis: accuracy of the method, expressed as relative error in percent

Concentration added (mg/ml)	Concentration found (mean ± S.D.; mg/ml)		Relative error (%)	
	β-CD	2-HP-β-CD	β-CD	2-HP-β-CD
0.9	0.87 ± 0.04	0.88 ± 0.01	−2.74	1.71
1.75	1.68 ± 0.09	1.82 ± 0.06	−3.74	−3.89
3.5	3.32 ± 0.07	3.59 ± 0.04	−5.22	−2.54

Accuracy ($n = 5$).

3.2.1.3. Accuracy of the assay. Accuracy values during the same day (intra-day assay) at low, medium and high concentrations of both CDs were always within the acceptable limits (−5.22 and 1.71%) at all concentrations tested (Table 3).

3.2.1.4. Precision of the method. To calculate the precision of the method, "within-day" and "between-day" test were performed. The values are summarized in Table 3. These data clearly indicate that the assay method was reproducible within the same day. From these results (Table 4), it also appears that the analytical method was reproducible between different days.

3.2.2. PVM/MA

Assay performance of PVM/MA was assessed, in the same way as CDs, by all the following criteria: LOD, LOQ, linearity, accuracy, precision and applicability in characterization studies of the drug delivery systems (DDS) formulation development.

3.2.2.1. Sensitivity of the assay. The LOD of PVM/MA, determined as three times the variation in the measured response ($S/N = 3$), was calculated to be 0.02 mg/ml and the estimated LOQ was calculated as 0.05 mg/ml ($S/N = 10$). The mean assay result was 0.052 ± 0.002 mg/ml ($n = 5$) with a relative standard deviation of 4.40%.

3.2.2.2. Linearity of the assay. The assay exhibited linearity between the response (y) and the corresponding concentration of Gantrez (x), over the first range 0.05–1 mg/ml and from 1

Table 4
Cyclodextrin analysis: between- and within-day variability of the HPLC method

Concentration added (mg/ml)	Concentration found (mean ± S.D.; mg/ml)			
	Between-day variability ($n = 5$)		Within-day variability ($n = 5$)	
	β-CD	2-HP-β-CD	β-CD	2-HP-β-CD
0.5	0.49 ± 0.01 (3.55)[a]	0.49 ± 0.02 (4.81)	0.48 ± 0.01 (2.91)	0.47 ± 0.01 (0.83)
0.9	0.87 ± 0.03 (4.36)	0.89 ± 0.04 (5.11)	0.86 ± 0.03 (0.36)	0.87 ± 0.01 (0.42)
1.75	1.68 ± 0.09 (5.93)	1.81 ± 0.06 (3.28)	1.68 ± 0.02 (1.29)	1.76 ± 0.02 (1.22)
3.5	3.32 ± 0.07 (2.08)	3.58 ± 0.05 (1.30)	3.34 ± 0.02 (0.67)	3.65 ± 0.01 (0.40)

[a] CV expressed in percentage.

Table 5
Standard curves for PVM/MA in acetonitrile

	N	Regression equation	r
0.05–1			
Day 1	8	$y = 0.2528x^2 + 1.1922x - 0.0608$	0.998
Day 2	8	$y = 0.228x^2 + 1.1783x - 0.0526$	0.997
Day 3	8	$y = 0.1367x^2 + 1.2984x - 0.058$	0.999
1–4			
Day 1	8	$y = 1.2935x^{1.054}$	0.998
Day 2	8	$y = 1.4525x^{0.915}$	0.997
Day 3	8	$y = 1.3808x^{0.972}$	0.998

to 4 mg/ml in the second range. In both cases, polynomial regression for 0.05–1 mg/ml range and potential regression for the 1–4 mg/ml, displayed correlation coefficients greater than 0.997 (Table 5). Moreover, when a Student's *t*-test was used, the calculated values were not found to be statistically different from nominal ones ($p = 0.571$).

3.2.2.3. Accuracy and precision of the method. Accuracy values, calculated as the percentage difference between the expected and measured concentrations, were within −8.52 and 8.38% (see Table 6). The results for intra-assay variability and between-day precision are summarized in Table 6. These data clearly indicate that the assay method was reproducible within the same day and between different days (see Table 7).

3.3. Application of the method

The reported method was used for the determination of CDs content in PVM/MA nanoparticles. However, this method can be also used to estimate the yield of

Table 6
Analysis of PVM/MA: accuracy of the method, expressed as relative error in percent

Concentration added (mg/ml)	Concentration found (mean ± S.D.; mg/ml)	Relative error (%)
0.05–1		
0.05	0.045 ± 0.001	−8.52
0.5	0.538 ± 0.012	7.64
0.9	0.835 ± 0.035	−7.18
1–4		
1	0.964 ± 0.02	2.16
2	2.06 ± 0.03	1.56
3	2.95 ± 0.11	3.87

Accuracy ($n = 5$).

Table 7
Analysis of PVM/MA: between- and within-day variability of the HPLC method

Concentration added (mg/ml)	Concentration found (mean ± S.D.; mg/ml)	
	Between-day variability ($n = 5$)	Within-day variability ($n = 5$)
0.05–1		
0.05	0.050 ± 0.001 (2.17)[a]	0.046 ± 0.001 (2.17)
0.1	0.0908 ± 0.006 (6.91)	0.095 ± 0.005 (5.26)
0.5	0.506 ± 0.011 (2.18)	0.503 ± 0.021 (4.25)
0.9	0.838 ± 0.003 (3.58)	0.899 ± 0.024 (2.79)
1–4		
1	1.013 ± 0.017 (5.30)	1.014 ± 0.017 (1.70)
1.5	1.426 ± 0.09 (6.43)	1.444 ± 0.026 (1.85)
2	1.982 ± 0.053 (2.70)	1.974 ± 0.088 (4.46)
3	2.793 ± 0.077 (2.77)	2.788 ± 0.019 (0.68)

[a] CV expressed in percentage.

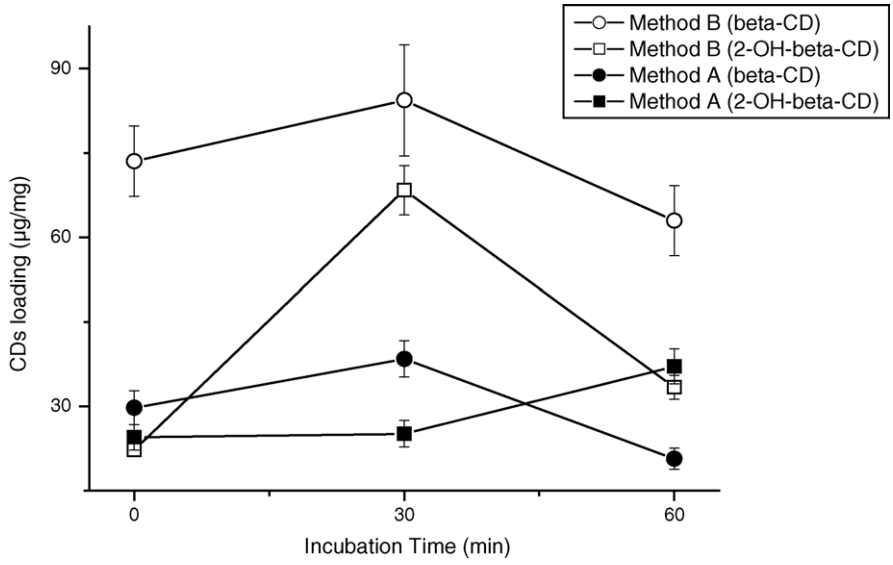

Fig. 3. Application of the method: influence of preparation method on CDs loading (µg CDs/mg nanoparticles). Data express the mean ± S.D. ($n = 5$). The yield of the process was calculated to be 94.7 ± 3% for Method A and 90.9 ± 8% for Method B. β-CD: beta-cyclodextrin; 2-OH-β-CD: 2-hydroxypropyl-beta-cyclodextrin.

the nanoparticle process. Thus, using data obtained from these HPLC analysis, the yield of nanoparticles was calculated to be close to 90.29 ± 8.77%. This value is in the same order than that obtained by gravimetry (84.93 ± 4.29%).

The CDs loading was also calculated by means of Eq. (1) (see Section 2.7) and this parameter was plotted versus the ratio between the initial amount of CDs and the initial amount of PVM/MA. Fig. 3 shows the influence of the preparative method on the CD loading. From these results, it appears to be clear that the incubation between CD and PVM/MA for 30 min before nanoparticle formation by desolvation (Method B) enabled the improvement of the CDs content. Under these conditions, the maximum capacity of β-CD and 2-HP-β-CD loading in PVM/MA nanoparticles was calculated to be around 84.4 and 68.4 μg/mg nanoparticle, respectively.

4. Conclusion

From the above experimental results, it can be concluded that ELSD shows adequate sensitivity for the detection of β-CD, 2-HP-β-CD and PVM/MA in nanoparticle formulations. Moreover, the developed method is able to separate and quantify these three molecules. Finally, the simplicity of the technique, short time analysis, high sensitivity, accuracy and precision, makes this technique suitable for the quality control of CDs–Gantrez nanoparticles conjugates.

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