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Research Paper

Development and Validation of the RP-HPLC Method for Dexamethasone Sodium Phosphate Determination in Nasal Chitosan Microsphere Preparations

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Abstract
The purpose of this work was to provide a robust, sensitive, accurate, and straightforward analytical method for measuring dexamethasone sodium phosphate (DSP) in chitosan microspheres prepared using the spray drying method. DSP was quantitatively analyzed using RP-HPLC with an ultraviolet detector at 254 nm, a mobile phase that contained a mixture of acetonitrile and 0.1% sodium dihydrogen phosphate monohydrate (50:50) operating isocratically at a flow rate of 1.0 mL/min, and a stationary phase that was a C18 PrincetonSPHER-100 C18-QB 100A HPLC Column (250 × 4.6 mm, 5 μm). The ICH recommendations were followed in the validation of the analytical method. DSP had a retention duration of 2.899 minutes and a tailing factor of 0.827. The RP-HPLC method was linear (R = 0.9992) in the 15-60 μg/mL concentration range. The limits of quantitation (LOQ) and detection (LOD) were 4.425 μg/mL and 1.327 μg/mL, respectively. The relative standard deviations for the intra-day and inter-day precisions were 0.057-0.876% and 0.780-0.949%, respectively. The recovery percentages at 50, 100, and 200% concentration levels were within the 99.269-100.980% range. The validated method has been successfully applied to determine DSP entrapment efficiency in chitosan microspheres. A linear, sensitive, accurate, precise, and robust technique of determining DSP in chitosan microsphere preparations is offered by the established RP-HPLC method.

Keywords
Method Development and Validation, Dexamethasone Sodium Phosphate, Microspheres, Entrapment Efficiency, Chitosan

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1. INTRODUCTION

Synthetic adrenal corticosteroid dexamethasone has strong anti-inflammatory qualities. Intranasal use of dexamethasone has been proven to suppress 75% of fever symptoms in ragweed hay fever sufferers, which is more effective than dexamethasone given orally (Norman et al., 1966). Through the intranasal route, therapeutic levels of dexamethasone in the central nervous system even at low doses showed better results compared to intravenous administration in the therapy of COVID-19 patients (Cárdenas et al., 2024). Dexamethasone administered nasally was also more effective in controlling lipopolysaccharide-induced neuroinflammation in rats, compared with intravenous administration of the drug at the same dose (Meneses et al., 2017). However, intranasal use of dexamethasone has been linked with iatrogenic Chusing's syndrome and adrenal insufficiency. When dexamethasone nasal drops are used repeatedly and uncontrollably over an extended time to treat sinusitis and rhinitis, the medication enters the bloodstream and causes these side effects. To reduce the side effects of intranasal dexamethasone administration, a delivery system that can maintain dexamethasone's action locally in the nose and minimal systemic absorption is needed (Fuchs et al., 1999; Joshi and Maresh, 2018).

In the nasal cavity, the mucociliary transport mechanism occurs, in which the mucosa cleans the surface of the nasal cavity by moving foreign particles trapped in the mucus towards the nasopharynx. This mechanism serves as a local defense to maintain the health of the nose. The transit time of particles in the nasal mucociliary tract in humans has been reported to be 12-15 minutes (Chhajed et al., 2011; Merkus et al., 1998). Drugs administered intranasally may have a shorter residence time due to nasal mucociliary transport, which may impact the drug's presence at the location where it acts and its capacity to function locally in the nose.

Microspheres can be used in various drug delivery systems,

when used intranasally, microspheres can be used to increase the residence time of the drug at the site of action (Desnita et al., 2023). Microspheres in dry powder have been proven to overcome mucociliary clearance and extend the contact time of the drug and the nasal mucosa (Pandey and Tripathi, 2016; Pereswetoff-Morath, 1998; Suresh and Bhaskaran, 2005). Intranasal administration of mucoadhesive microspheres using chitosan polymer is considered quite effective. One of the influencing factors is the concentration of chitosan used (Sapra et al., 2024). The intranasal microsphere device may help reduce the quantity of dexamethasone that enters the bloodstream and improve the effectiveness of dexamethasone therapy in treating localized nasal cavity inflammation. Chitosan in particular has good potential as a carrier material for intranasal dexamethasone sodium phosphate microspheres (Mouez et al., 2014; Wang et al., 2020). An extended residence period in the nasal cavity can be made possible by the positive electrical charge on chitosan polymer causing significant electrostatic interactions with mucus or negatively charged sialic acid residues on the surface of the nasal mucosa (Hagesaether, 2011).

The ability to determine the concentration of dexamethasone in chitosan microspheres is critical for evaluating the microsphere formulations. Unfortunately, analytical methods for this specific application have not been reported before. Therefore, this research was carried out to develop a high-performance liquid chromatography (HPLC) method for quantifying dexamethasone sodium phosphate (DSP) in chitosan microsphere preparations. The chitosan microspheres were prepared using the spray dry method.

2. EXPERIMENTAL SECTION

2.1 Materials and Instruments

Dexamethasone sodium phosphate (Indonesian compendia standard) was purchased from the Republic of Indonesia Food and Drug Supervisory Agency (BPOM), and Chitosan was purchased by CV. Bio Chitosan Indonesia. Several chemicals and equipment were purchased through PT Almaas Borneo Jaya such as Lactic acid (Merck), Acetonitrile HPLC (Merck), Sodium dihydrogen phosphate monohydrate (Merck), Orthophosphoric acid (Merck), Whatman® brand filter membrane no. 4 (pore size 0.45 μm, diameter 47 mm), Whatman® brand membrane filter no. 41 (0.20 μm pore size, 90 mm diameter), Millipore filter, HPLC (Shimadzu Corporation). HPLC Column C18 PrincetonSPHER-100 C18-QB 100A 250 × 4.6 mm, 5 μm purchased through PT. Alphasains Dynamics in Jakarta. The Sonicator uses the Bronson brand and the spray dryer is a Mini Buchi B 290 type.

2.2 Preparation of Microspheres

Microspheres were made by first dissolving 400 mg DSP into 100 mL aquabidest. Then 800 mg chitosan was dissolved in 300 mL of 2% lactic acid solution. The two solutions were combined and stirred at a speed of 400 rpm for 5 minutes to continue with the spray dry process using a Mini Spray Dryer B-290, with a nozzle diameter of 0.7 mm. The inlet and outlet temperatures were set at 140°C and 85°C, respectively. The aspirator was set at 95% with a pump flow speed of 6 mL/min. Blank chitosan microspheres were also prepared using the same method in the absence of DSP.

2.3 Chromatographic System

HPLC uses Shimadzu Corporation UFLC with Prominence UV Detector (SPD-20A), and the stationary phase is the Princet onSPHER-100 C18-QB 100A HPLC Column. 250 × 4.6 mm, 5 μm at a wavelength of 254 nm. Elution was performed isocratically using a mixture of acetonitrile and 0.1% sodium dihydrogen phosphate monohydrate solution (adjusted to pH 3 by adding ortho-phosphoric acid) with a ratio of 50:50 v/v at a flow rate of 1 mL/min. Before use, the mobile phase of the solution had been filtrated using a 0.45 μm filter. The injection volume was 20 μL and the column was equilibrated for approximately 60 min before injection.

2.4 Preparation of Standard Solutions

Standard solutions were prepared by first making a stock solution containing 3.4 mg DSP that was dissolved in a 10 mL mobile phase. The mixture was stirred thoroughly and sonicated for 5 minutes. Then 0.5 mL of the solution was taken and diluted to 5 mL in a volumetric flask using the mobile phase. The standard solution was filtered through a 0.2 μm membrane filter (Whatman) before being injected into the HPLC system.

2.5 Preparation of Test Sample Solution

A total of 100 mg of sample was dissolved in 20 mL of 2% lactic acid solution, stirred at 400 rpm for 10 minutes, then diluted with distilled water to 100 mL. 0.5 mL of this solution was taken and diluted in 10 mL of aquabidest then sonicated for 15 minutes then filtered using a 0.2 μm membrane filter (Whatman) before being injected into the HPLC.

2.6 System Suitability Testing

System suitability is determined to ensure that the chromatography method used produces good and optimum separation for analyte measurement. System suitability parameters include retention time (minutes), tailing/asymmetry factor, and theoretical plates to determine system suitability. Quantification is carried out with a UV detector at 254 nm to then determine the retention time according to the Chromatographic system (Amjad and Hussain, 2020).

2.7 Method Validation

2.7.1 Linearity

Standard solutions of DSP with a concentration range of 15-60 μg/mL were prepared. Each solution was injected into the HPLC instrument three times. Plotting the peak area against the concentration allowed for the establishment of the calibration curve (González and Herrador, 2007). The full separation of the analyte peak from other peaks from the sample matrix ensures specificity in HPLC. The chromatographic apparatus is

injected with distinct doses of a standard, the sample, a placebo, and an unfilled solution to guarantee this. Selectivity was examined and found unaffected by solvent signals, mobile phase solution, or baseline aberrations by identifying chromatograms representing the analyte. Reproducibility linearity is the ability of an analytical method to yield data proportionate to the analyte concentration within a given range dependent on the mitigation that is carried out (Gupta, 2020; Kowalska et al., 2022; Le et al., 2019; McMillan, 2016; Patil and Chalikwar, 2024).

2.7.2 Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were estimated using the standard curve according to the International Council for Harmonisation (ICH) guidelines (González and Herrador, 2007).

2.7.3 Accuracy and Precision

To evaluate accuracy and precision, spiked matrices at three concentration levels (50, 100, and 200%) of DSP were prepared. Each concentration level was prepared in triplicate. To prepare spiked matrices, the DSP standard was added at the indicated concentration into 15 mg blank chitosan microspheres. Spiked microspheres were dissolved in 20 mL of 2% lactic acid solution, stirred at 400 rpm for 10 minutes, then diluted with distilled water to 100 mL. 0.5 mL of this solution was taken and diluted in 10 mL of aquabidest, followed by filtration using 0.2 μm membrane and degassing using an ultrasonicator for 10 min. The solutions were then injected into the HPLC system. Intra and interday precision were evaluated based on the relative standard deviation (RSD) percentages from replicates run on the same day and three different days, respectively. Accuracy was evaluated based on the percent recovery (Sonar et al., 2022).

2.7.4 Robustness

The method's robustness was evaluated by making several small intentional changes in the column temperature, pump flow speed, and mobile phase composition. Data were evaluated based on the %RSD for each parameter that changes.

2.8 Determination of Entrapment Efficiency

The validated HPLC method was used to determine drug entrapment efficiency by quantifying the DSP amount in the DSP loaded microspheres. 100 mg of DSP-loaded microspheres were dissolved in 20 mL of 2% lactic acid solution, stirred at 400 rpm for 10 minutes, then diluted with distilled water to 100 mL. 0.5 mL of this solution was taken and diluted in 10 mL of aquabidest then sonicated for 15 minutes. The solution was then filtered, degassed, and injected into the HPLC instrument using the procedures described above. The peak area from the chromatogram was then used to calculate the concentration of the DSP and the entrapment efficiency. The entrapment efficiency was calculated by comparing the amount of DSP successfully loaded into the microspheres to the amount of DSP added to the microsphere preparation formula (Brugnera et al., 2022).

3. RESULT AND DISCUSSION

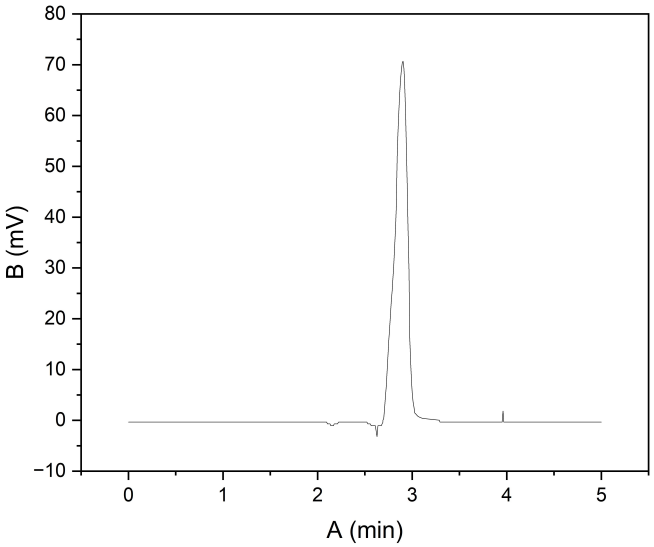

Figure 1. Chromatogram of Dexamethasone Sodium Phosphate Standard Solution

This research was conducted to develop an analytical method method for determining DSP in chitosan microspheres. This method will be applicable for further studying the microsphere characteristics such as drug entrapment efficiency and drug release profile. In the specificity test, the result was obtained that the peak of the standard solution appeared at 2.899 minutes (Figure 1). For the selectivity test, the sample solution, the peak appeared as shown in Figure 2 where the peak DSP as an active substance appeared at 2.924 minutes. The placebo and carrier solution used in this process did not show a peak at the retention time of DSP and at the scanning time range. From the system suitability testing which was performed by six replicate injections of the standard solution, the following results were obtained: retention time of dexamethasone sodium phosphate was 2.899 minutes (can be seen in Figure 2), with a tailing factor (TF) of 0.827 and the number of theoretical plates (N) of 2107.691. This result is not much different from a previously published method for the determination of dexamethasone sodium phosphate in a veterinary injection suspension. The previous method reported 2.25 minutes retention time using a reverse phase HPLC utilizing a C18 column (250 × 4.6 mm, 5 μm) as the stationary phase, mobile phase containing a mixture of acetonitrile: 0.1% phosphate buffer (50:50) pH 3.0, operated in isocratic mode at a flow rate of 1.0 mL/min, and UV detector at 254 nm (Amjad and Hussain, 2020). The selection of the mobile phase greatly affects the wavelength and retention time that will be used in the analysis of DSP levels in preparation. This can be seen in previous studies to determine

Table 1. Summary of Validation Method Parameters

Parameters	Information	Results
Suitability Testing	Number of Replications	Six Replicate
	Tailing factor (TF)	0.827
	Number of theoretical plates (N)	2107.691
Specificity	DSP standard solution	retention time 2.899 min
Selectivity	Sample	peak appeared at 2.924 min
	Placebo	no peak in DSP retention time
	Mobile phase solution	no peak in DSP retention time
Linearity	Range (μg/mL)	15-60
	Regression equation	$y = 19357x + 8565.4$
	Coefficient Correlation	0.9992
Limit of Detection	μg/mL	1.327
Limit of Quantification	μg/mL	4.425
Precisions	Intraday	RSD 0.057-0.876 %
	Interday	RSD 0.780-0.949 %
Accuracy	Intraday	Recovery 99.269-99.877 %
	Interday	Recovery 99.381-100.980 %
Robustness	Variation column temperature	RSD 1.212 %, Recovery 100.768 %
	Variation flow rate	RSD 9.624 %, Recovery 103.460%
	Variation of mobile phase composition	RSD 7.066 %, Recovery 100.112 %

Table 2. Intraday Precision and Accuracy

Concentration (%)	Time to	Microspheres and Dexamethasone Sodium Phosphate	
		Average Peak Area	% Recovery
50	1	297763.567	99.602
	2	294912.667	98.620
	3	299959.767	100.358
	Mean	297545.333	99.526
	SD	2530.617	0.872
	RSD	0.009	0.009
	%RSD	0.850	0.876
100	1	589214.933	99.990
	2	580829.433	98.546
	3	585042.300	99.271
	Mean	585028.889	99.269
	SD	4192.766	0.722
	RSD	0.007	0.007
	%RSD	0.717	0.727
200	1	1167907.667	99.821
	2	1169208.467	99.933
	3	1168371.867	99.861
	Mean	1168496.000	99.872
	SD	659.225	0.057
	RSD	0.001	0.001
	%RSD	0.056	0.057

DSP levels in eye lens contact preparations, where the use of a mixture of 0.01 M phosphate buffer pH 6.0 and acetonitrile in a ratio of 80:20 v/v caused the DSP detection wavelength to be at 242 nm with a retention time of 2.9 min (Toffoletto et al., 2024).

The linearity test in this study was carried out by ICH

Table 3. Inter-Day Precision and Accuracy

Concentration (%)	Day to	Microspheres and Dexamethasone Sodium Phosphate	
		Average Peak Area	% Recovery
50	1	299477.000	100.192
	2	304863.067	102.047
	3	300959.767	100.702
	Mean	301766.611	100.980
	SD	2782.207	0.958
	RSD	0.009	0.009
	%RSD	0.922	0.949
100	1	581147.833	98.600
	2	589881.600	100.104
	3	587416.833	99.680
	Mean	586148.756	99.462
	SD	4502.853	0.775
	RSD	0.008	0.008
	%RSD	0.768	0.780
200	1	1152800.9	98.520
	2	1171241	100.108
	3	1164340.167	99.514
	Mean	1162794.022	99.381
	SD	9316.772187	0.802
	RSD	0.008	0.008
	%RSD	0.801	0.807

Table 4. Robustness Data of the Proposed HPLC Method

Parameter	Condition	% RSD Retention Time	% RSD Peak Area	% Recovery
Column Temperature	20°C 25°C 30°C	1.212	0.66	100.768
Flow Rate	0.9 (mL/min) 1 (mL/min) 1.1 (mL/min)	9.624	7.107	103.460
Mobile Phase Composition	-5 % Acetonitrile Normal +5 % Acetonitrile	7.066	0.667	100.112

recommendations using seven concentrations which were processed statistically to show linearity. This research obtained an R^2 value of 0.9992 with $y = 19357x + 8565.4$, presented in Table 1. This is by the provisions which state that a good correlation value is close to 1 (Prasada Rao, 2022). The correlation coefficient shows a linear relationship between the area under the peak and the analyte concentration. The lowest limit of analyte concentration that can be found and measured with adequate precision and accuracy is indicated by these LOD and LOQ values. The signal-to-noise approach is used to measure this number based on data from the calibration curve (Boros et al., 2014; Naseef et al., 2018). In this research, the LOD value was 1.327 μg/mL and the LOQ value was 4.425 μg/mL. These values are relatively high when compared with the results from a previously published method by Al Aani and Alnukkary which reported LOD and LOQ of 0.04 μg/mL and 0.13 μg/mL, respectively (AlAani and Alnukkary, 2016). These lower detection and quantification limits were achieved using a Thermo Hypersil C18 column (250 mm, id 4.6 mm, 5 μm) as the stationary phase and an isocratic mixture of acetonitrile - potassium dihydrogen phosphate buffer (pH 4.0; 0.05 M) (30:70, v/v) at detection wavelength 230 nm (AlAani and

Alnukkary, 2016). Good phase mobility can enhance signal quality by lowering noise and raising resolution. This implies that a lower LOD is possible due to improved analyte detection. The appropriate wavelength can lower the LOD by improving analyte detection and the detector's sensitivity. By increasing the detector's specificity, the appropriate wavelength can lower the LOQ, enabling more precise and accurate measurement of the analyte.

Precision measures the closeness of results obtained from replicate measurements, the result expressed as the Relative Standard Deviation (RSD). They performed according to ICH recommendations, repeating the procedure three times on the same day and three days apart (Ahuja, 2007). In this study, a precision test was carried out by adding dexamethasone sodium phosphate, the concentration of which was known, to the preparation of chitosan microspheres without the active ingredient with the RSD percent results as shown in Tables 2 and 3. The test results showed that the chitosan microspheres with dexamethasone sodium phosphate showed a value of % The RSD is quite good, that is, nothing exceeds 2 (Sellappan and Devakumar, 2021). It can be said that the analysis and preparation methods used were quite precise to determine the levels of dexamethasone sodium phosphate in the microsphere preparation. The high repeatability value shows During the analysis process, the capacity of the equipment to produce accurate data will also be displayed (Apridamayanti et al., 2023). Percent recovery in the accuracy analysis procedure is carried out in the same manner and method as the precision analysis process. The chitosan microsphere samples were confirmed by adding three levels of standard dexamethasone sodium phosphate concentrations. From Tables 2 and 3 it can be seen that the average recovery percentage was found to be within acceptable limits, namely 99.269 to 100.980% with a relatively small standard deviation value compared to the average value of the data (González and Herrador, 2007; Puti, 2023).

Determining the entrapment efficiency capability of dexamethasone sodium phosphate microspheres was carried out by first preparing microsphere samples, this was necessary to ensure the extraction process of the active substance from the encapsulation of chitosan as the polymer that makes up the microspheres. Chitosan is a polymer with excellent solubility in acidic pH fluids. To release dexamethasone sodium phosphate from chitosan microspheres, a 2% lactic acid solution is needed so that the walls of the microspheres open (Bratskaya et al., 2019; Czechowska-Biskup et al., 2007; Wu et al., 2018). Using a 2% lactic acid solution makes the sample have a very low pH below the resistance pH of the chromatography column used as the stationary phase. For this reason, multilevel dilution is carried out by dissolving the sample with a little acid solution and then diluting it with aquabidest until it reaches a pH of 3 to 4 and is safe for the column used. In this study, the results of HPLC analysis can be seen in Figure 2, where there is a perfect separation between the peak area of dexamethasone sodium phosphate at a retention time of 2.924 minutes and chitosan at 5.983 minutes. From the peak area, it was found that the dexamethasone sodium phosphate chitosan microsphere formula had an entrapment percentage of 87.7% ± 0.71.

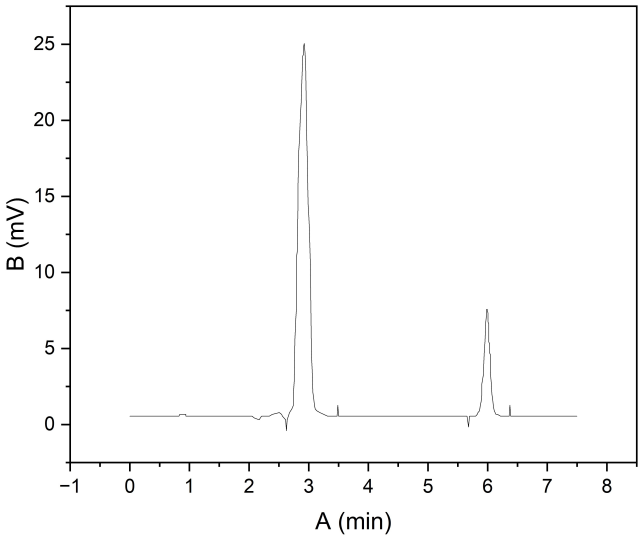

Figure 2. Chromatogram of Dexamethasone Sodium Phosphate Chitosan Microspheres

4. CONCLUSIONS

An RP-HPLC method has been developed for the determination of dexamethasone sodium phosphate in chitosan microspheres and validated according to ICH guidelines. All parameters including linearity, sensitivity, precision, accuracy, and robustness have met the requirements. The validated method has been successfully applied to determine entrapment efficiency in dexamethasone sodium phosphate-loaded microspheres, with the efficiency found to be 87.7% ± 0.71. The method is simple, fast, and potentially can be applied to other drug delivery systems containing dexamethasone sodium phosphate with no or minimal adjustments in the sample preparation procedure and chromatography conditions.

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