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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE DETERMINATION OF HYDROCORTISONE SODIUM SUCCINATE

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ABSTRACT

A simple, rapid, sensitive and linear HPLC method was developed for estimation of Hydrocortisone sodium succinate. In this method separation was carried out on HiQSil C8 column (250mm*4.6 mm, 5µm) using potassium dihydrogen orthophosphate Buffer pH 4 (adjusted with orthophosphoric acid) and ACN (30.:70 v/v) as mobile phase at flow rate of 1 ml/min. The quantification was carried out at 242 nm. The retention time (t_R) of drug was 3.4 ± 0.10 min. The method was validated with respect to linearity, precision, assay, accuracy and robustness. The data of linear regression analysis indicated a good linear relationship over the range of 5-30 µg/ml concentrations with a correlation coefficient (r^2) of 0.9914. Method can be used for routine analysis of compounds.

Keywords: Hydrocortisone sodium succinate, High performance liquid chromatography, validation

INTRODUCTION

The molecular composition of Hydrocortisone sodium succinate is represented by the formula C25H33NaO8, and its IUPAC name is sodium; 4-[2-[(8~{S},9~{S},10~{R},11~{S},13~{S},14~{S},17~{R})-11,17-dihydroxy-10,13-dimethyl-3-oxo-2,6,7,8,9,11,12,14,15,16-decahydro-1~{H}-cyclopenta[a]phenanthren-17-yl]-2-oxoethoxy]-4-oxobutanoate. It is the sodium salt of hydrocortisone succinate, possessing glucocorticoid properties. Chemically resembling the endogenous hormone, it promotes anti-inflammatory and immunosuppressive effects, with minor mineralocorticoid influences [1]. The structure of Hydrocortisone sodium succinate is illustrated in Fig. 1.

Literature review indicates that reported methods include HPLC on ontologic preparations containing Hydrocortisone sodium succinate as an ingredient [2] and investigations into the stability of hydrocortisone succinate under various pH and temperature conditions [3]. High-performance thin-layer chromatography methods have also been documented for estimating Hydrocortisone sodium succinate and other forms of Hydrocortisone in pharmaceutical dosage forms [4].

As of our knowledge, there is no reported simple RP-HPLC method for estimating Hydrocortisone Sodium Succinate. This study introduces a straightforward RP-HPLC method for both bulk and pharmaceutical dosage form (Hydrocort-100) following the guidelines of the International Conference on Harmonization (ICH) [5-6].

MATERIALS AND METHODS:

Reagents and Solutions: Methanol (HPLC Grade) and Potassium Dihydrogen Orthophosphate (AR Grade) were procured from LobaChemie (India). HPLC grade water was obtained on-site using an ELGA water purification system (Purelab UHQ-II model). Orthophosphoric Acid (AR Grade) was sourced from SD Fine Chem Ltd. All chemicals were of analytical grade and used as received. Hydrocortisone Sodium Succinate, available in the market under the brand name Hydrocort-100, was used (Label claim: each vial contains Hydrocortisone sodium succinate USP equivalent to Hydrocortisone 100 mg).

Instrumentation and Chromatographic Conditions:

A JASCO HPLC system was utilized, featuring a model PU 2080 Plus pump, Rheodyne sample injection port (20 µl), JASCO PDA MD-2010 Plus detector, and Borwin chromatography software (version 1.5). The chromatographic column used was HiQSil C8 (250 mm*4.6 mm, 5µm). The optimized mobile phase consisted of Phosphate Buffer (pH 4): ACN (30:70 v/v). The pH of the Phosphate Buffer was adjusted using Orthophosphoric Acid. The overall run time was 10 minutes, with a flow rate of 1.0 ml/min. Quantification was performed at 242 nm.

Preparation of Standard Stock Solution:

A Hydrocortisone Sodium Succinate stock solution was prepared by dissolving 10 mg of Hydrocortisone Sodium Succinate in 5 ml of methanol, making the volume up to 10 ml to achieve a concentration of 1000 µg/ml. Further dilutions were made in methanol, and a final concentration of 10 µg/ml was prepared using the mobile phase. A representative chromatograph is presented in Fig. 2.

RESULTS AND DISCUSSION

Specificity: The method's specificity was confirmed through peak purity profile studies, with peak purity values indicating no interference from degradation products, impurities, or matrix (995).

Linearity: From the standard stock solution (1000 µg/ml), solutions ranging from 5 to 30 µg/ml were prepared. The linearity was determined, yielding the calibration curve equation: $y = 28982x + 36707$. The calibration curve is shown in Fig. 3.

Precision: Intraday and interday variation studies were conducted, with percentage RSD calculated. Results are detailed in Table 1.

Assay: For Hydrocortisone sodium succinate in Hydrocort-100, the assay was performed, and results are presented in Table 2.

Accuracy: Recovery studies at three different levels (50%, 100%, and 150%) were carried out. Percentage recovery was determined using the linearity equation, and results are shown in Table 3.

Sensitivity/Limit of Quantification (LOQ) and Limit of Detection (LOD): LOD and LOQ were calculated from the linearity data, with LOD at 0.37 µg/ml and LOQ at 1.13 µg/ml.

Robustness: The method's robustness was assessed by deliberately altering conditions, and the impact on peak areas was noted.

Hydrocortisone sodium succinate exhibited absorbance maxima at 242 nm. After optimization, Phosphate Buffer pH 4 and ACN (30:70 v/v) were chosen as the mobile phase. The developed method showed good linearity, recovery, precision, and accuracy within the specified limits. The validation parameters are summarized in Table 4.

Conclusion

The conclusion drawn is that the method is a simple, sensitive, selective, accurate, and repeatable approach for analysing hydrocortisone sodium succinate in bulk and pharmaceutical dosage forms without interference from excipients.

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Table 1: Precision of Hydrocortisone sodium succinate

Concentration (µg/ml)	Intra-day Precision*		Inter-day Precision*	
	Average	% RSD	Average	% RSD
10	101.32	0.320	100.12	1.351
20	101.31	0.730	100.07	1.125
25	99.13	0.488	98.41	1.107

*- Average of 3 denominators

Table 2: Assay of Hydrocortisone sodium succinate

Drug	Peak Area (Avg.)	Amount Recovered (µg/ml)	% Recovery	Mean % Recovery	SD	% RSD
Hydrocortisone sodium succinate	330714.005 325968.25 324034.932 329681.36 325896.25 331512.47	10.144 9.981 9.914 10.109 9.978 10.172	101.44 99.81 99.14 101.09 99.78 101.72	100.49	1.055	1.05

Table 3: Accuracy of Hydrocortisone sodium succinate

Level % of Accuracy	Sample (µg/ml)	Standard (µg/ml)	% Recovery*	% RSD*
50	10	5	100.04	1.59
100	10	10	100.19	1.27
150	10	15	99.13	0.48

*- Average of 3 denominators.

Table 4: Summery of Validation Parameters

Sr.No.	Parameter	Results
1	Linearity	y = 28982x + 36707 R^2 = 0.9914
2	Range	5-30 µg/ml
3	Precision	%RSD
	Intraday	0.32 - 0.73
	Interday	1.10 – 1.35
4	Assay	100.49 %
5	Accuracy	% Recovery
	50%	100.04
	100%	100.19
	150%	99.13
6	LOD	0.37 µg/ml
7	LOQ	1.13 µg/ml.
8	Specificity	Specific
9	Robustness	Robust

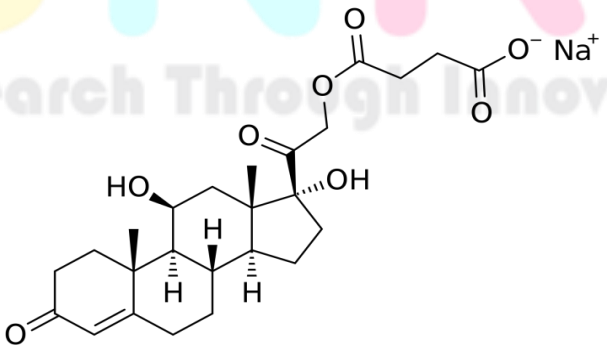

Fig.1: Chemical structure of Hydrocortisone Sodium Succinate

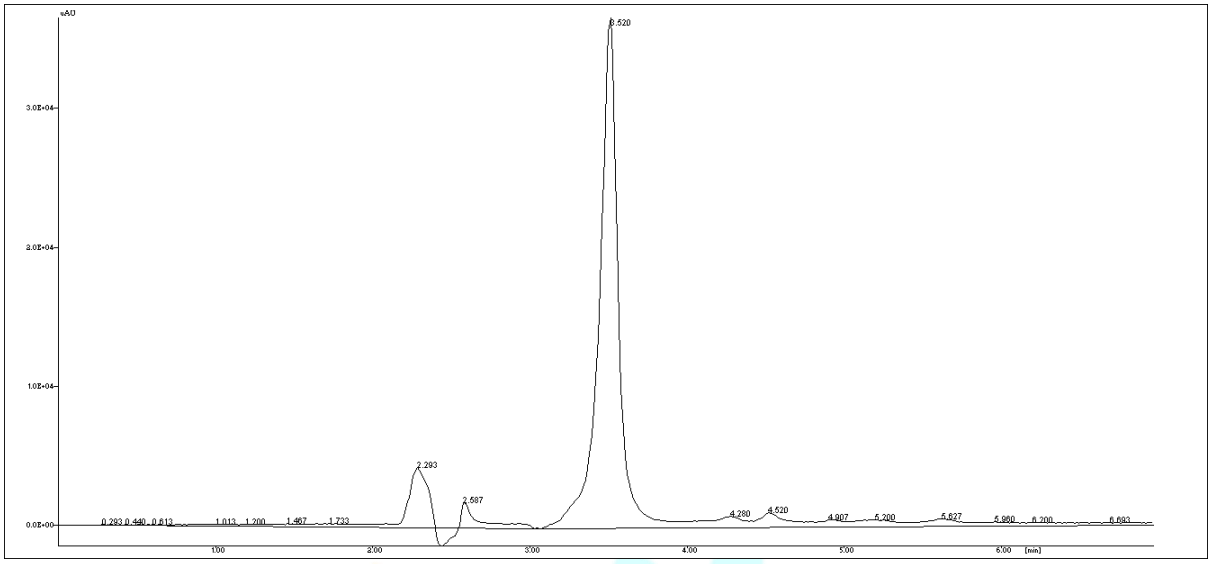

Fig. 2: Chromatogram of Hydrocortisone sodium succinate (10 µg/ml)

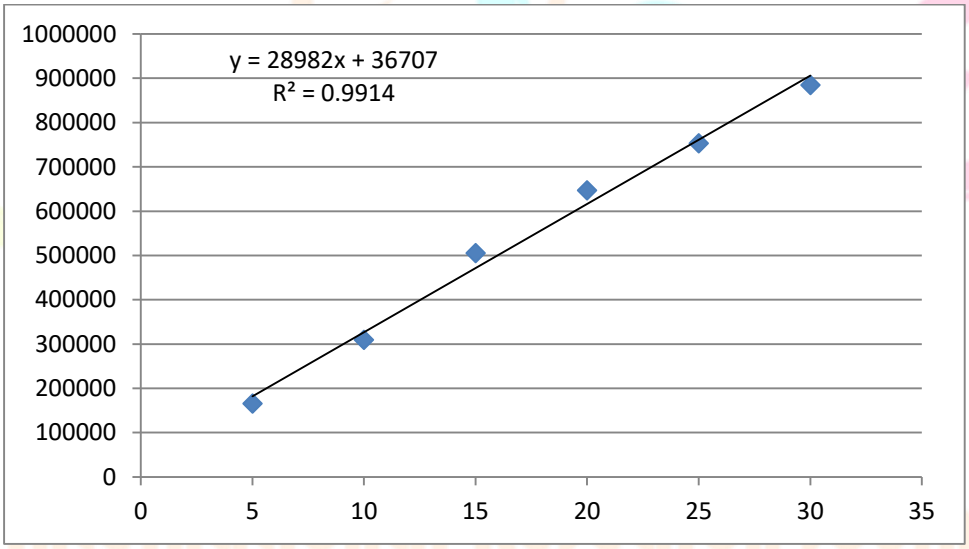

Fig. 3: Calibration Curve of Hydrocortisone sodium succinate