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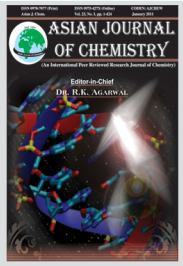

NOTE

RP-HPLC Determination of Hydrocortisone in Parenterals

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A precise reverse phase HPLC method has been developed for the determination of hydrocortisone in parenteral dosage forms. The chromatography was carried out on an ODS column using a mixture of phosphoric acid, acetonitrile and water (0.5:380:620 v/v) as the mobile phase at a flow rate of 1.0 mL/min. The detection of the drug in the eluates was done at 245 nm. The retention time obtained for the drug was 4.38 min. The method produced linear responses in the concentration range of 2-20 µg/mL of hydrocortisone. The method was also found to be applicable for determination of the drug in parenteral preparations with an average per cent recovery of 99.83.

Key Words: Hydrocortisone, Estimation, Injections, RP-HPLC.

Hydrocortisone[1-3] (10R,11S,13S,17R)-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclo penta(a)phenanthrene-3-one, is a corticosteroid drug used to treat inflammatory and rheumatoid diseases, Addison's disease and allergies. A literature survey revealed that only a few HPLC methods are available for estimation of hydrocortisone[4-7]. The authors now propose a new validated, sensitive and reproducible HPLC method for the determination of hydrocortisone. The applicability of this method in determining the drug in commercial dosage forms was also studied.

A working standard sample of hydrocortisone obtained from East India Pharmaceutical works limited and two commercial samples of injections containing the drug namely Hycort (Ind Swift) and Primacort (Macleods) were used in the study. HPLC grade acetonitrile (Qualigens), AR grade phosphoric acid and Milli Q water (E Merck) were used for preparing the mobile phase.

Chromatographic conditions: A Shimadzu LC-2010 CHT high-performance liquid chromatographic instrument provided with a Shimadzu LC 2010 C series HPLC pump and a SIL LC 2010 C series auto sampler equipped with a 20 µL sample loop was employed in the study. A Kromasil ODS reverse phase column (250 mm × 4.6 mm; 5 µ) was used for the separation. Detection was done using an SPD LC 2010 C dual absorbance detector and the output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SPI software.

A freshly prepared 0.5:380:620 (v/v/v) mixture of phosphoric acid, acetonitrile and Milli Q water (E Merck) was used as the mobile phase. The solvents and water were filtered through a 0.45 µm membrane filter and sonicated before use. Enertech ultra sonicator was used for this purpose. The flow rate of the mobile phase was maintained at 1.0 mL/min. The detection of the eluates was monitored at 245 nm.

Estimation of hydrocortisone: About 100 mg of hydrocortisone was weighed accurately and transferred into a 100 mL volumetric flask and dissolved in 25 mL of the mobile phase. The solution was sonicated for 15 min and then the volume made up with a further quantity of the mobile phase to get a 1 mg/mL solution. From this solution, further dilutions ranging from 2-20 µg/mL were prepared in 50 mL volumetric flasks with the mobile phase. 20 µL of the solution was injected each time into the column. Each of the dilutions was injected five times into the column and the corresponding chromatograms were obtained. From these chromatograms, the retention times and the areas under the peaks of the drug were noted (Table-1) and the relevant calibration curve was constructed. The regression equation of the curve was computed. This equation was later used to estimate the amount of hydrocortisone in parenterals.

To check the intra-day and inter-day variation of the method, solutions containing 4, 8 and 12 µg/mL of hydrocortisone were subjected to the proposed HPLC method of analysis and the recoveries obtained were noted.

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Conc. of hydrocortisone (µg/mL)	Peak area	Conc. of hydrocortisone (µg/mL)	Peak area
2	2797663	12	12507615
4	4095335	14	19583640
6	6190670	16	22381310
8	8381332	18	25178966
10	10398092	20	27976628

Estimation of the drug in injections: Two commercial samples of injections containing the drug (Hycort of Ind Swift and Primacort of Macleods) were chosen for testing the suitability of the proposed method to estimate hydrocortisone in the injections. For this, 20 injections were taken and the contents were pooled up. An accurately measured portion of this liquid equivalent to 100 mg of hydrocortisone was accurately measured and mixed with 50 mL of the mobile phase in a 100 mL volumetric flask. The solution was shaken well and allowed to stand for 0.5 h with intermittent sonication to ensure complete solubility of the drug. The mixture was then thoroughly mixed and made upto the mark with the mobile phase and filtered through a 0.45 µ membrane filter. From this filtrate, different dilutions ranging from 2-20 µg/mL were prepared in 10 mL volumetric flasks with the mobile phase. Twenty microlitres of each of these prepared samples were then injected 5 times and chromatographed. The average peak areas were calculated. The concentration of the drug was then calculated from the corresponding regression equation.

The present study was aimed to develop a more sensitive, precise and accurate HPLC method for the analysis of hydrocortisone in parenteral dosage forms. For this, a mixture of phosphoric acid, acetonitrile and water in the ratio 0.5:380:620 (v/v/v) was found to be the most suitable mobile phase as the chromatographic peaks obtained with this system were better defined, resolved and were almost free from tailing. Under the above mentioned conditions, the retention time obtained for hydrocortisone was 4.38 min. A typical chromatogram showing the separation of the drug is given in Fig. 1.

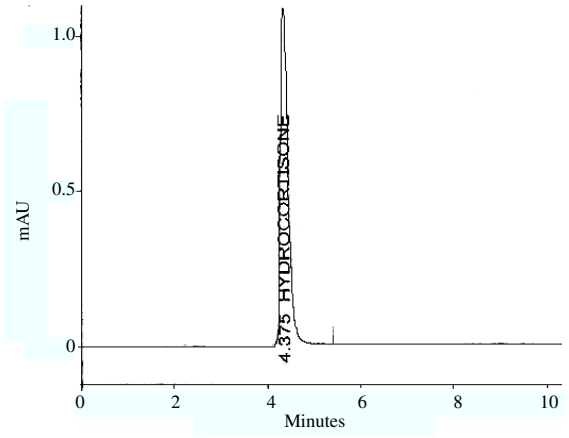

Fig. 1. A typical chromatogram for estimation of hydrocortisone

A good linear relationship (r = 0.999) was observed between the concentrations of hydrocortisone and the corresponding peak areas. The regression of hydrocortisone concentration over its peak area was found to be Y = 984442x + 520267 (where y is the peak area and x is the concentration of hydrocortisone). The intra-day and inter-day drug variation studies by the proposed method showed low coefficient of variation as shown in Table-2. The drug content in the injections was quantified using the proposed method of analysis. The mean amount of hydrocortisone obtained in the injections is shown in Table-3. This reveals that the method is quite precise and accurate. The absence of additional peaks in the chromatogram indicated no interference of the common excipients used in the injections.

TABLE-2
INTRA- AND INTER-DAY PRECISION OF THE PROPOSED METHOD

Conc. of hydrocortisone (µg/mL)	Observed concentration of hydrocortisone (µg/mL)			
	Intra-day		Inter-day	
	Mean (n = 5)	RSD (%)	Mean (n = 5)	RSD (%)
4	3.978 ± 0.062	1.559	3.944 ± 0.072	1.826
8	7.972 ± 0.051	0.640	7.916 ± 0.055	0.695
12	11.908 ± 0.109	0.915	11.978 ± 0.062	0.518

TABLE-3
DETERMINATION OF HYDROCORTISONE IN INJECTIONS

Brand name of the injection	Labelled amount of the drug (mg)	Mean (± SD) amount found by the proposed method (n = 5)	Mean (± SD) % labeled amount
Hycort	100	99.970 ± 0.055	99.970 ± 0.055
Primacort	200	199.932 ± 0.066	99.966 ± 0.035

It can be concluded that the proposed HPLC method is sensitive and reproducible for analysis of hydrocortisone in parenteral dosage forms in a short analysis time. The method was duly validated by evaluation of the required parameters.

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