Determination of Methylprednisolone Antiderivatives By HPLC Analysis Method And Its Practical Application

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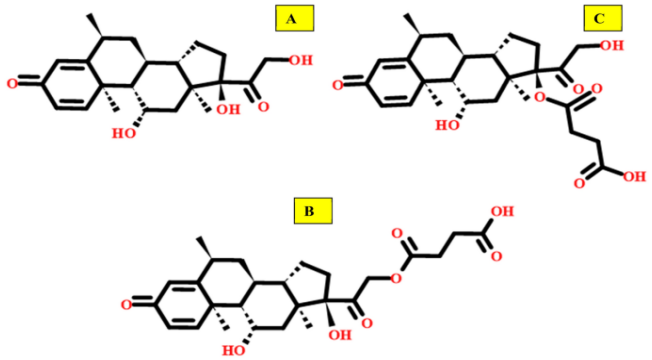
Abstract- *A parenterally administered water-soluble corticosteroid ester is methylprednisolone sodium succinate, or MPSS. The assay determines total MP by sharing the three peaks that are produced: methylprednisolone (MP), 17-methylprednisolone hemisuccinate (17-MPHS), and methylprednisolone hemisuccinate (MPHS). It is frequently found in prescription anti-inflammatory medications, where it is used extensively. The goal of the current study was to develop a quick RP-HPLC method for the analysis of MP and its derivatives that doesn't require any special chemical reagents and has high linearity, repeatability, sensitivity, and selectivity. In a short amount of time, the MP, 17-MPHS, and MPHS could be found and separated with a satisfactory outcome when using the current method. The BDS column is used in RP-HPLC, which is the chromatographic system. It is 250 mm×4.6 mm×5 µm. To prepare the mobile phase, a volume ratio of 63:2:35 was used to mix WFI, glacial acetic acid, and acetonitrile at a flow rate of 2.0 mL/min, room temperature detection wavelength of 254 nm, and injection volume of 20 µL. The approach demonstrated a satisfied linearity regression R2 (0.9998–0.99999) for MP and MPHS, respectively, with LOD 143.97 ng/mL and 4.49 µg/mL and LOQ 436.27 ng/mL and 13.61 µg/mL. By achieving system suitability in the robustness and ruggedness conduction in accordance with the validation guidelines, the method demonstrated its effectiveness. Excellent sensitivity based on its LOD and LOQ. Therefore, the pharmaceutical industry may take into consideration the current approach. The recommended approach has been effectively put into practice. adopted for the quantitative evaluation of the final product's assay in the local Egyptian market.*

I. INTRODUCTION

The sodium salt of methylprednisolone hemisuccinate (MPHS) is called methylprednisolone sodium succinate (MPSS). MPHS is known by the IUPAC name 4-[2-[(6S,8S,9S,10R,11S,13S,14S, and 17R)Phenanthren-17-yl]-11,17-dihydroxy-6,10,13-trimethyl-3oxo7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a][2-oxoethoxy]Fig. 1: 4-oxobutanoic acid. The water-soluble corticosteroid ester of methylprednisolone, known as MPSS, is used to treat a variety of conditions, including heart problems, severe allergic reactions, hypoxic emergencies, respiratory, ophthalmic, and dermatological disorders; it is also used to treat antineoplastic, hormonal, anti-inflammatory, and neoplastic diseases; additional uses include conditions of the nervous system, endocrine, and immunological disorders. When given parenterally and in equal doses, MPSS and methylprednisolone (MP) have similar anti-inflammatory and metabolism effects; their biologic actions are the same1.



Structure of (**A**) methylprednisolone [MP],
(**B**) methylprednisolonehemisuccinate [MPHS],
(**C**) methylprednisolone 17-hemisuccinate [17-MPHS].

Since MPSS drugs have a wide range of applications in the pharmaceutical industry, it is imperative to develop efficient, straightforward, quick, and easy methods for assay determination. Additionally, when this method is used to estimate MPSS after washing cleaning machines and production lines, a sensitive method should be carried out at low concentrations of this drug preparation. To guarantee that the cleaning process is effective in eliminating any drug residues that might inadvertently contaminate the next product being produced, a cross-contamination process that is wholly unacceptable, the sensitive method should be used. According to the quality standards outlined in the guidelines for good manufacturing practice, this kind of contamination2, 3, and 4

For the assay determination of MP, a variety of techniques are used in multiple analysis tools. These include flow injection analysis using LC-Q-TOF MS5, HPLC-MS1, RP-HPLC6,7,8,9, voltammetric techniques10, SWNTs/EPPGE11, and spectrophotometrically12.

However, at both the academic and commercial levels, HPLC-UV detection is a simple, accurate, and affordable method. The analysis method for determining MP and MPHS was published by the United States Pharmacopoeia (USP44-NF 39 2021)13. With a stationary phase column measuring 3.9 mm × 30 cm, the mobile phase is made up of butyl chloride, water-saturated butyl chloride, Fluorometholone should be used as the internal standard when dissolving the standard and test in a diluent of glacial acetic acid and chloroform (97:3). About 25 minutes are allotted for MPHS retention. tetrahydrofuran, methanol, and glacial acetic acid in the following ratios: 95:95:14:7:6. L3 is packed at a flow rate of approximately 1.0 mL per minute.

A high percentage of organic modifiers from methanol, acetonitrile, special reagents like chloroform, tetrahydrofuran, butyl chloride, tetrabutylammonium hydroxide, adjusted pH buffer solutions, gradient program7,8,14,15,16,17, and a special type of separation HPLC column6 using guard column cartridge14 were used in the HPLC analysis method for the majority of MP and its derivatives for MPHS and 17-MPHS. Furthermore, the process of separation takes time. Furthermore, certain techniques employed a high flow rate of 4.0 mL/min and a Zorbax Eclipse XDB-C18 (250 mm × 9.4 mm; 5 μm) special column1. These variables are used to obtain the ideal tailing and peak shape18,19.

Recent advances in science have tended to focus on antibiotic purification of hospitals, pharmaceutical plants, and industrial wastewater. Finding simple, quick, accurate, and affordable ways is therefore becoming critically important.2, 3, 4.

In this manuscript, we describe a proposed protocol for the detection and assessment of the medication methylprednisolone and its derivatives, utilising an easy, quick, and reliable methodological approach. Furthermore, the analysis method is easily accessible to any general laboratory and works under simple chemical conditions. Additionally, an analytical comparison of the various methods used to determine methylprednisolone was conducted.

Methods and materials:-

Assuit, Egypt-based UP Pharma supplied the following: Methylprednisolone sodium succinate working standard (MPSS), Methyl Prednisolone hemisuccinate reference standard (MPHS), and Methyl Prednisolone reference standard (MP). Hydrochloric acid 37%, sodium hydroxide, glacial acetic acid 99%, hydrogen peroxide 30%, acetonitrile HPLC-grade, and disodium hydrogen phosphate and sodium dihydrogen phosphate (Scharlau, Spain). Prior to use, the water for injection (WFI) in the analysis was filtered using a 0.45 μm nylon membrane filter. 1000 millilitres of WFI were used to weigh 1.6 grammes of disodium hydrogen phosphate to create phosphate solution (1). To make phosphate solution (2), weigh approximately 0.3 g of sodium dihydrogen phosphate in 1000 mL of water for irrigation.

Chromatographic system configuration:-

In contrast to the earlier HPLC techniques and the present analysis approach, we achieved the optimal separation for the achievement of the ideal system suitability without the need for a special chemical reagent, a dedicated pH solution adjustment, or a high percentage of the organic modifier of acetonitrile.

The MP, 17-MPHS, and MPHS assay determination was carried out utilising the variable wavelength HPLC model HP 1100 series. Thermo Scientific's RP-BDS column, measuring 250 mm×4.6 mm×5 μm, was utilised for the current methodology. WFI: glacial acetic acid: acetonitrile in a volume ratio (63:2:35) was used to prepare the mobile phase. It had an injection volume of 20 μL, a detection wavelength of 254 nm at room temperature, and a flow rate of 2.0 mL/min.

Parameters methods of validation:-

Regarding parameters like system suitability, range of linearity, the limit of detection (LOD), the limit of quantification (LOQ), repeatability (precision), recovery and accuracy, robustness, ruggedness, the stability of the solution, specificity, and selectivity, the HPLC validation method was carried out in accordance with the International Conference on Harmonisation (ICH) guidelines20,21, 22.

System suitability check:-

To ensure system suitability, six replicate injections of the same sample solution were made. This solution was made by dissolving 5 mg of MP reference standard in 100 mL of mobile phase, combining 10 mL of this solution with 65 mg of MPSS working standard, 1 mL of each phosphate buffer solution, and filling the flask with 100 mL of mobile phase to

obtain a concentration of approximately 500 µg/mL of total MP.

Range and lineraity:-

If the response and the claimed working concentration have a significant portion that starts at the lowest point in the tested range and increases to the highest point with $R2 \geq 0.999$[22,23,24,25,26,27], then the analytical approach is considered to be linear.

Regression linearity equation:-

$$Y = a X \pm b$$

where (X) is the claimed working concentration in percentage, (Y) is the average peak area response, (a) is the calibration curve's slope, and (b) is the calibration curve's intercept.

Five distinct concentrations within the range of the MP working standard (50–150%) were used to submit the linearity parameter. The stock solutions included the MPSS working standard, which is equivalent to 640 mg/100 mL in the mobile phase, and the WFI up to 1000 mL. The stock solutions also contained 48.9 mg of the MP reference standard in 100 mL of the mobile phase. After that, take 5 mL, 7 mL, 10 mL, 12 mL, and 15 mL from each solution of the stock solutions, and complete to 100 mL with mobile phase. Then, inject 2 replicates of each concentration to obtain concentrations (50%, 70%, 100%, 120%, and 150%).

Lime of detection:-

It was described as the lowest known analyte concentration in the matrix that the instrument's detection system could detect. Every time LOD concentration is injected, it shouldn't go through the accuracy, precision, and linearity ranges.22, 23, 24, 25, 26, 27, and 28.

Limit of quantitation :-

It was described as the lowest known analyte concentration in the matrix that the instrument's detection system could detect. Each time LOQ is injected, it must pass the linearity, accuracy, and precision ranges22,23,24,25,26, and 27.

Based on the slope and standard error information derived from the calibration's linearity, LOD and LOQ could be computed as follows:

$$\sigma LOD = 3.3\sigma/S \quad (2)$$

$$\sigma LOQ = 10\sigma/S \quad (3)$$

where (σ) is the standard error of (X & Y) arrays and (S) represents the slope of the linearity calibration curve.

Accuracy and recovery:-

The terms accuracy and recovery are used interchangeably28. The accuracy of a measurement is determined by how close the measured concentration (actual concentration) is to the true concentration (theoretical concentration) (18, 20, 29).

Three distinct stock solutions of the MP reference standard were prepared at 3.74, 5.49, and 6.64 mg in 100 mL of mobile phase, respectively, in order to implement accuracy. The MP concentrations were then combined with 10 mL of each of the 45.7 mg, 64.8 mg, and 77.4 mg/100 mL WFI of the MPSS working standard separately and 1 mL of each phosphate buffer solution. Three replicates of each concentration were injected, resulting in concentrations of total MP of 70%, 100%, and 120%.

Accuracy % could be estimated using the linearity equation:

Accuracy (%)= Actual Conc.(%)/Theoretical Conc.(%)×100

Repeatability and precision:-

Six different methods of determining the 100% test concentration were used to ensure repeatability. First, a quantity of MP reference standard equivalent to 5 mg/100 mL of mobile phase was dissolved. Ten millilitres of this solution were combined with one millilitre of each phosphate buffer solution and a weight of MPSS working standard equivalent to 65 mg in a 100 millilitre volumetric flask. Finally, mobile phase was added to achieve a concentration of approximately 500 µg/mL of total MP22,30.

Robustness:-

Robustness was submitted with planned minor modifications, such as minor adjustments to the mobile phase's composition, temperature, etc. 22.

A distinct organic solvent ratio (acetonitrile) at (± 1%) and a flow rate (± 0.005 mL/min) were used for the planned minor adjustments.

Ruggedness:-

The submission of ruggedness was based on planned and significant observable changes, such as analyst-analyst, column-column, and day-to-day, while preserving all analysis method parameters and conditions in their original state23.

Specificity and selectivity:-

The subsequent solutions were injected one at a time to confirm selectivity:

• Buffer of phosphate.
• The mobile stage.
• MP + MPHS standard; • MP reference standard.
• Phosphate buffer plus the MP reference standard and MPHS reference standard.
• Working standard for MPSS.
• The MPSS working standard and the MP reference standard.
• MPSS working standard, phosphate buffer, and MP reference standard.
•Acid hydrolysis and base H2O2 oxidation hydrolysis were used in forced degradation studies to show the stability, properties, selectivity, and specificity of the process.
c • The acid hydrolysis test for MP was carried out as a recovery test at 100%. The last step involved adding 10 mL of HCl (0.1 M), leaving it for 30 minutes, and then finishing with WFI to 100 mL.
• The MP base hydrolysis was carried out as a recovery test at 100%. The last step involved adding 10 mL of NaOH [0.1 M], leaving it for 30 minutes, and then finishing with WFI to 100 mL.
• The H2O2 hydrolysis test for MP was carried out as a recovery test at 100%. In the last step, 10 mL of H2O2 [3.0%] was added, and it was left for 30 minutes before being finished with WFI to 100 mL.
Test of the approved procedure for UP Pharma's locally marketed product in Egypt

Methylprednisolone 0.1g vilas batch number (221160) afer the constitution stability studies:-

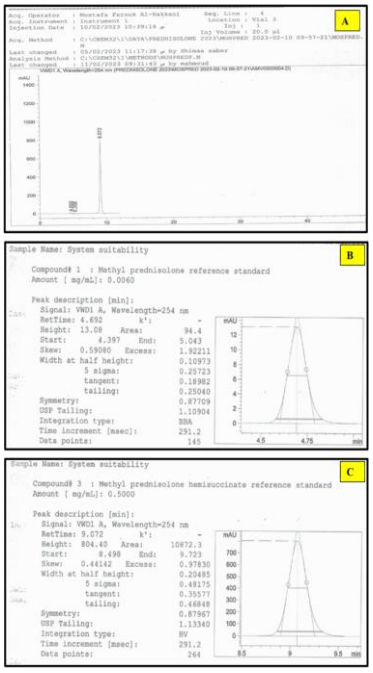
Using the provided solvent WFI, the after-constitution stability study was carried out at zero time and for 24 hours at a temperature of 5 ± 3 °C in the refrigerator.

16 mL of the WFI were used to constitute the vial, and all of its contents were then transferred into a 200 mL volumetric flask. After that, 10 mL of the constituted solution (1 mg/mL) was diluted using WFI in a 100 mL volumetric flask, and the final theoretical concentration (0.5 mg/mL of MP) was added to the HPLC for assay.

Experimental and work methods:-

I certify that all procedures were followed in accordance with all applicable rules and regulations.

Table 1 Molecular data of the Mp, MPHS, and 17-MPHS:-



(**A**) MP, 17-MPHS, and MPHS chromatogram at an optimum HPLC parameter, USP tailing factor, and theoretical plates of (**B**) MP, (**C**) MPHS.

II. CONCLUSION

After being evaluated, the validated method was found to be sensitive to detecting low concentrations of free and hemi-succinate methylprednisolone at LODs of 143.97 ng/mL and 4.49 µg/mL, respectively, and LOQs of 436.27 ng/mL and 13.61 µg/mL. Furthermore, it was discovered that the procedure was exact, repeatable over a two-day period with intra- and inter-precision, and accurate for free and hemi-succinate methylprednisolone (98.8–99.4% and 99.4–99.9%, respectively) at concentration levels of 70 µg/mL to 120 µg/mL with high accuracy. The method's linearity was tested between 250 µg/mL and 750 µg/mL, yielding excellent regression coefficients (R2 = 0.9998–0.99999) for both free and hemi-succinate methyl prednisolone. A variety of carefully considered implementation modifications, including variations in flow rates, mobile phase compositions, days, and analysts, were used to assess the robustness of the method. It demonstrated a high degree of ability to meet the requirements for the suitability of the chromatographic system, including theoretical plates and column efficiency of at least 2000 and USP tailing at 2.0. Ultimately, the minimum resolution

between the Methyl Prednisolone principal peak and the closest related impurity peak at 2.54 verified the current method's selectivity and specificity. The verified procedure demonstrated its effectiveness in separating the methyl distinct from any other appearance-forced degradation peaks, the principal peak of prednisolone

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