RP-HPLC Method for the Estimation of 6-Mercaptopurine in Spiked Human Plasma and Pharmaceutical Formulations

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Abstract—A simple, rapid and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method for the determination of 6-mercaptopurine (6-MP) in pharmaceutical formulations and spiked human plasma samples has been developed and validated. The assay of the drug was performed on a CLC C\textsubscript{18} (5 μm, 25 cm × 4.6 mm i.d.) with UV detection at 325 nm. The mobile phase consisted of methanol–water mixture in the ratio of 90 : 10, and a flow rate of 1 mL/min was maintained. The standard curve was linear over the range of 25–150 μg/mL (r\textsuperscript{2} = 0.9964). Analytical parameters have been evaluated. Between and within-day precision and accuracy were acceptable down to the limit of quantification of 9.5 μg/mL in plasma. The proposed method was validated for accuracy and precision. Statistical analysis proves that the method was found to be simple, precise, accurate, rapid and reproducible and can be used for the routine determination of 6-mercaptopurine. The proposed method was successfully applied to the determination of 6-mercaptopurine in spiked human plasma and pharmaceutical formulations. The method will be useful for routine quality control analysis.

Keywords: 6-mercaptopurine, human plasma, RP-HPLC, pharmaceutical samples
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6-Mercaptopurine (6-MP) (3,7-dihydropurine-6-thione) is a purine analogue that has been used in cancer chemotherapy, primarily in childhood leukemia [1] (see Fig. 1). The bioavailability of 6-MP is low and highly variable, with only 16% of an orally administered dose of 6-MP gaining access to the systemic circulation [2], that has been suggested as one of the possible causes of a relapse in children with leukemia receiving maintenance chemotherapy [3, 4]. The importance of optimizing 6-MP therapy and achieving high and predictable systemic drug exposure has encouraged the use of i.v. (intra-venous) 6-MP in patients with acute lymphoblastic leukemia [5]. The half-life of 6-MP in plasma is short, ranging from 1–3 h. It is usually used in combination with other anticancer drugs and interferes with the synthesis of adenine and guanine ribonucleosides, which are important precursors of DNA and RNA. 6-MP is cytotoxic because it acts predominantly on rapidly dividing cells such as the T lymphocytes [1]. A number of analytical studies have been done to determine the drug content in drug products. 6-MP can be estimated by the UV spectroscopic method [6]. The HPLC methods are useful in determination of drug in pharmaceutical dosage form and biological samples. 6-MP and its metabolites have been determined by HPLC [7, 8]. There are various HPLC methods published for the estimation of 6-MP in human erythrocytes, plasma, and serum [9, 10]. These procedures show long retention times, do not give results rapidly, tedious extraction procedure, require expensive solvents, use of organic modifiers viz. triethylamine and glacial acetic acid in the mobile phase composition, and maintenance of pH [11], which finally limits column life. Metabolism of 6-MP in the erythrocytes, liver, and kidney of rates during multiple-dose regiments is also reported [12] in which 6-MP was administrated i.p. (intraperitoneal). The objective of the present study is to develop a sensitive, precise, selective, specific, reproducible, fully validated, easy to perform, and low cost routine RP-HPLC UV detection method for estimation of 6-MP in spiked human plasma and pharmaceutical formulations.

Fig. 1. Chemical structure of 6-mercaptopurine.
Experimental

Instrumentation. All HPLC measurements were made on a Shimadzu Corporation system (Analytical Instruments division, Kyoto, Japan) consisting of a LC10AT solvent pump, SPD10AVP detector and a data station with win-chrome software version 3.1. The separation was performed on a CLC C18 column (5 μm, 25 cm × 4.6 mm i.d.). A CLC ODS (4 cm × 4.6 mm i.d.) was used as a guard column to protect analytical column. Hamilton 702 μR injector with a 25 μL loop was used for the injection of the samples.

Chemicals and reagents. Pure drug sample of 6-MP was purchased from Sigma-Aldrich and used as such. The water and methanol used were of HPLC grade. The 6-MP containing tablets Purinethol (Genuine Pharmaceuticals Ltd., Bangalore, India) were purchased from a local pharmacy.

Chromatographic conditions. Chromatographic separation was achieved at ambient temperature (25°C) on a RP-HPLC by using a mobile phase consisting of methanol and water in the ratio of 90:10 (v/v) by 10 min. The mobile phase was pumped at a rate of 1.0 mL/min. The detector wavelength was set at 325 nm.

Throughout the study, the suitability of the chromatographic system was monitored by calculating the capacity factor (k'), the resolution (R), the selectivity (α) and the peak asymmetry (T).

Working standard of drug solution. A stock solution (100 μg/mL) was prepared by dissolving 10 mg of 6-MP in 10 mL of methanol and then volume was made up to 100 mL with mobile phase. The working solution of 6-MP was prepared by diluting the stock solution with mobile phase. Studies on the stability of analytes in the working solution showed that there were no decomposition products in the chromatogram and also no difference in area-ratio during analytical procedure even after storing for two days at 4°C.

Assay procedure. Mobile phase methanol:water 90:10 was passed through a 0.45 μm membrane filter and delivered at 1.0 mL/min for column stabilization. During this period, the base line was continuously monitored. The wavelength selected for the detection was 325 nm. The prepared dilutions containing concentrations of 6-mercaptopurine in the range of 25 to 150 μg/mL were injected into the column. The peak areas were recorded for all the chromatograms. The chromatogram of 6-MP is shown in Fig. 2. Calibration curve was constructed by plotting peak areas vs. concentration.

Plasma sample preparation. Blood plasma: human blood samples were collected in dry and evacuated tubes (which contained saline and sodium citrate solution) from some healthy volunteers. The samples were handled at room temperature and centrifuged for 10 min at 1500 rpm for the separation of plasma within 1 h of collection. The samples were then transferred to polypropylene tubes and stored at 20°C until analysis. The plasma samples, 0.2 mL, were deproteinized with 2 mL of methanol and water mixture (1:1, v/v), vortexes for 5 min, centrifuged at 6000 rpm for 15 min, and supernatants were collected. The supernatants were spiked with an appropriate volume of suitably diluted stock solutions of 6-MP, giving final concentrations of 25–150 μg/mL. Samples containing 20 μL were injected through a Rheodyne injector, and the effluent was monitored at 325 nm. The above procedure was repeated five times and the plot of peak area of 6-MP and its concentration was plotted in the range of 25–150 μg/mL in plasma.

Estimation of 6-mercaptopurine from commercially available tablets by the proposed method. Twenty tablets of 6-MP each containing 50 mg were accurately weighed (average weight was determined) and crushed into fine powder. An accurately weighed quantity of powder equivalent to 50 mg of 6-MP was transferred into a 50 mL volumetric flask, dissolved in 25 mL of methanol and sonicated for 5 min. The solution was filtered through filter paper no. 41. The residue was washed with 5 mL portions of methanol three times and the total volume of the filtrate was made up to 50 mL with methanol (1 mg/mL). The final concentration was brought to 100 μg/mL with mobile phase. The solution after dilution was analyzed by RP-HPLC method. All determinations were conducted with five runs. The assay was calculated from the equation of regression line for each drug. The results are presented in Table 1. The results of analysis show that the amounts of drug were in good agreement with the label claim of the formulations and also added known amount of drug.