Development of suitable HPLC method for the analysis of drugs in urine by using ODS-RPS column and metronidazole as a model

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Abstract;

The suitability of the column of HPLC for the analysis of drugs in urine is so important to get good resolution and to obtain high accuracy in quantitative determination. It has been found that the use of reverse phase mode of chromatography is most preferable for the analysis of drugs, but it mostly suffers of interference of protein constituents, when it is applied on urine sample. Attempts to overcome these problems leads to use of ion-pair reverse phase mode of chromatography which turns the protein constituents more water soluble and can be eluted rapidly. However, this method needs very low pH mobile phase and long time of running to saturate the surface of the stationary phase with the ionic substance.

In this work, an HPLC simple and fast method for the quantitative determination of drug (metronidazole) in urine was developed by using the commercially available column ODS-RPS (4.6 x 150 mm),the mobile phase consists of phosphate buffer 0.1M (pH 4.50) containing acetonitrile 20% ,the detection was by UV at 320nm. The retention time of metronidazole peak was at 3.5 minutes at flow rate 1.0 ml/minute, while all the endogenous substances of urine are eluted within the first 2 minutes. Validation tests for this method are also carried; the precision of analysis for quantitative determination was approved by establishment a straight line relationship between the different dilutions of drug and the peaks areas in the chromatogram with a correlation coefficient of (0.998), the relative standard deviation for five replicate tests of the same concentration was < 1.2% indicating good accuracy.

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