ABSTRACT

Ultra Performance Liquid Chromatography (UPLC), is a new class of separation technique which gives fast, improved resolution, speed and sensitivity. There is a great demand for UPLC in pharmaceutical analysis because of the importance in quality control of drug products. Stability indicating methods accurately measures the changes in active ingredients concentration without interference from other degradation products, impurities and excipients. The development of suitable stability indicating method provides a background for the preformulation studies, stability studies and the development of proper storage requirements.

Analytical Method Development and Validation for the Simultaneous Estimation of Domperidone and Rabeprazole in Bulk and its Dosage forms by UPLC technique:

The UPLC method is used for simultaneous determination of Domperidone and Rabeprazole in bulk and its pharmaceutical dosage forms. The chromatographic separation was carried out on a Hypersil gold C$_{18}$ (3.0 x 100 mm, 1.9 μ) column with a mixture of Potassium dihydrogen ortho phosphate buffer (pH: 4.5 – 4.7 adjusted with o-phosphoric acid): Methanol (50:50, v/v) as mobilephase at a flow rate of 1 ml/min. Isocratic mode was used for the separation of Domperidone and Rabeprazole. UV-Spectroscopic detection at a wavelength of 215 nm was performed and the column oven temperature was maintained at 40°C. The mobile phase was used as a diluent.

The retention times of Domperidone 60 μg/ml and Rabeprazole 40 μg/ml in the standard solution were found to be around 2.391 minute and 4.603 minute, respectively. This method has been applied successfully for the fast analytical procedure of the simultaneous quantitation of Domperidone and Rabeprazole in bulk and its combined dosage form. Domperidone and Rabeprazole show the percentage purity values are 99.13 % w/v and 98.55 % w/v respectively.

System suitability parameters were calculated. The Tailing factor for Domperidone and Rabeprazole was found to be 1.54 and 1.34 respectively. The Theoretical plates per unit for Domperidone and Rabeprazole was found to be 4025 and 6424.8
respectively. The resolution for Domperidone and Rabeprazole was found to be 4.12. The method showed satisfactory results for system suitability parameters.

The developed method was specific as no interference of excipients was found. Calibration plots were linear ($r^2 > 0.999$) over the concentration range of 48-72 μg/ml for Domperidone and 32-48 μg/ml for Rabeprazole. The analytical procedure was validated and it showed satisfactory results for all the validation parameters.

The sample solutions were subjected to stress conditions like acidic, basic, peroxide, water and light. The developed method has the ability to separate the drugs from degradation products and excipients found in the pharmaceutical dosage form.

**Analytical Method Development and Validation for the Simultaneous Estimation of Metolazone and Spironolactone in Bulk and its Dosage forms by UPLC Technique:**

The UPLC method is used for simultaneous determination of Metolazone and Spironolactone in bulk and its pharmaceutical formulations. The method was developed on a reversed-phase Hypersil Gold C$_{18}$ (2.1×100 mm, 2.7 μm) column and the mobile phase was optimised with Methanol: Acetonitrile: Potassium dihydrogen ortho phosphate buffer (pH 3.5) (50:32:18 %v/v) at a flow rate of 1 ml/min. Isocratic mode was used for the separation of Metolazone and Spironolactone. UV-Spectroscopic detection at a wavelength of 235 nm was performed and the column oven temperature was maintained at 40 °C. The mobile phase was used as a diluent.

The retention times of Metolazone 20 μg/ml and Spironolactone 200 μg/ml in the standard solution were found to be around 2.888 minute and 3.835 minute, respectively. Metolazone and Spironolactone shows the percentage purity values are 99.0 % w/v and 100.04 % w/v respectively. This method has been applied successfully for the fast analytical procedure of the simultaneous quantitation in bulk and its combined dosage form.

System suitability parameters were calculated. The Tailing factor for Metolazone and Spironolactone was found to be 1.017 and 0.90 respectively. The Theoretical plates per unit for Metolazone and Spironolactone was found to be 5907 and 6717.3
respectively. The resolution for Metolazone and Spironolactone was found to be 3.08. The method showed satisfactory results for system suitability parameters.

The developed method was specific as no interference of excipients was found. Calibration plots were linear ($r^2 > 0.999$) over the concentration range of 12 - 28 μg/ml for Metolazolone and 120 - 280 μg/ml for Spironolactone. The analytical procedure was validated and it showed satisfactory results for all the validation parameters.

The sample solutions were subjected to stress conditions like acidic, basic, peroxide, water and light. The obtained results showed developed method has the ability to separate the drugs from degradation products and excipients found in the pharmaceutical dosage form.

**Analytical Method Development and Validation for the Simultaneous Estimation of Duloxetine and Mecobalamin in Bulk and its Dosage forms by UPLC Technique.**

The UPLC Method is used for simultaneous determination of Duloxetine and Mecobalamin in bulk and its pharmaceutical formulations. The chromatographic separation was carried out on an Acquity UPLC BEH C18 (1.0 × 100 mm, 1.7μm) column with a mixture of Methanol: Water (55:45 % v/v) as mobilephase at a flow rate of 1 ml/min. Isocratic mode was used for the separation of Duloxetine and Mecobalamin. UV-Spectroscopic detection at a wavelength of 320 nm was performed and the column oven temperature was maintained at 40 °C. The mobile phase was used as a diluent.

The retention times of Duloxetine 3 μg/ml & Mecobalamin 75 μg/ml in the standard solution were found to be around 4.348 minute and 5.342 minute respectively. Duloxetine & Mecobalamin showed the percentage purity values as 99.71 % w/v and 99.02 % w/v respectively. This method has been applied successfully for the fast analytical procedure of the simultaneous quantitation of Duloxetine and Mecobalamin in bulk and its combined dosage form.
System suitability parameters were calculated. The Tailing factor for Duloxetine and Mecobalamin was found to be 1.0402 and 0.918 respectively. The Theoretical plates per unit for Duloxetine and Mecobalamin was found to be 3594.333 and 3334.33 respectively. The resolution value of Duloxetine and Mecobalamin was found to be 3.746. The method showed satisfactory results for system suitability parameters.

The developed method was specific as no interference of excipients was found. Calibration plots were linear ($r^2 > 0.999$) over the concentration range of 1.5 – 5.25 μg/ml for Duloxetine and 37.5 – 131.25 μg/ml for Mecobalamin respectively. The analytical procedure was validated and it showed satisfactory results for all the validation parameters.

The sample solutions were subjected to stress conditions like acidic, basic, peroxide, water and light. The obtained results showed developed method has the ability to separate the drugs from degradation products and excipients found in the pharmaceutical dosage form.