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Validated capillary electrophoresis method the quantification of sodium metabisulfite in cephalosporin using indirect UV detection

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ABSTRACT

The capillary electrophoresis (CE) method for the determination of sodium metasbisulfite content in cephalosporin drugs was developed using potassium hydrogen phthalate (5mM) and cetyl trimethyl ammonium bromide (0.25mM) as a flow modifier at pH 7.2 along with the application of an electric field of 20kV in 0.4 minute, using a 40 cm a fused silica capillary. Indirect UV detection was performed at a wavelength of 210 nm. Linearity and accuracy were performed in the concentration range of 4-24μg mL^{-1} and 1-2.4μg mL^{-1} respectively. The method was validated to show specificity, linearity, accuracy, precision, ruggedness, robustness and stability in analytical solution. The limit of quantification and detection were 1.673μg mL^{-1} and 1.402μg mL^{-1} respectively.

KEYWORDS

Capillary electrophoresis;
Indirect UV detection;
Sodium metabisulfite.

INTRODUCTION

In the synthesis of cephalosporine drug, sodium metabisulfite was used for the conversion of 7-Amino cephalosporonic acid to cefpodoxime proxetil and therefore the quantification of residual sodium metabisulfite is essential in the drug as it is harmful to the human body. Quantification of Sodium metabisulfite has been reported by Amperometric method in literature[1]. In pharmaceutical formulations its estimation is reported by capillary electrophoresis method[2]. A rapid and more sensitive method by capillary electrophoresis has been developed for the quantification of sodium metabisulfite at the trace level in cephalosporin active pharmaceutical ingredient.

EXPERIMENTAL

Chemicals

Cephalosporin and its related impurities were synthesized in Maulana azad college, Aurangabad, India. Fluka grade sodium metabisulfite (Steinheim Germany) was used. AR grade Potassium Hydrogen Phthalate was obtained from Sigma (Steinheim Germany) and Cetyl trimethyl ammonium bromide from Acros (New Jersey, USA). HPLC grade methanol was obtained from Merck Limited, USA. The water used for the study was obtained from a Milli-Q System (Millipore, Bedford, MA, USA).

Instrumentation

Capillary electrophoresis analysis was performed

on a HP 3D CE Instrument (Hewlett Packard, USA, Model No. G1600), coupled with PDA and an automated vial replenishment system. The data acquisition and processing were done using HP Chemstation enabling electropherogram with enhanced integrator and peak purity plots. Analysis was performed using an uncoated fused silica capillary with a total length of 48.5 cm, effective length of 40cm and internal diameter of 50μm (SRi capillary). The capillary was thermostated in a capillary cassette at 30°C using peltier cooling system. Indirect UV detection was performed by using the detection wavelength as 340nm and reference wavelength as 210nm. Sample, Standard and blank were injected using the hydrodynamic mode of injection by applying a pressure of 50 mbars for 10 seconds, and the Voltage was ramped to 20kV in 0.4 min. The required background electrolyte was prepared by dissolving 1.02gm of potassium hydrogen phthalate and 91.12mg of Cetyl trimethyl ammonium bromide in 100mL water, adjusted to pH to 7.2 with dilute KOH. One part of this solution was diluted with one part of methanol and eight parts of water. Prior to use, the capillary was flushed with 1M NaOH and followed by water for 10 min each using a pressure of 940 mbar.

RESULTS AND DISCUSSION

Sodium metabisulfite content

As sodium metabisulfite is a UV inactive compound, indirect UV detection mode was selected. Initially, Pyromeltic acid was used as a visualizing agent with cetyl trimethyl ammonium bromide as flow modifier along with a reverse polarity setup (Cathode at the inlet side). Various buffers were tried with variable concentration of flow modifier along with different detection wavelength and reference wavelength but no peak were observed[3,4]. The detection relies on the use of UV active buffer component with the same charge as electrolyte (Phthalate buffer). Also the EOF modifier (Cetyl trimethyl ammonium bromide) with reversed polarity (Cathode at inlet side) was applied to ensure the movement of metabisulfite ions towards the detector. Capillary electrophoresis technique therefore was chosen to determine sodium metabisulfite content in Cephalosporin A.

Method validation

Specificity

Sample spiked with sodium metabisulfite was analysed as per method. Further, known related substances of cephalosporin along with sodium metabisulfite were spiked in the sample to check their interference if any with sodium metabisulfite peak. The area of the analyte peak in the sample spiked with sodium metabisulfite and other known related impurities remains same compared to the area in the sample spiked with sodium metabisulfite indicating that there is no interference of cephalosporin related substances with sodium metabisulfite peak proving that the method is specific for the determination of sodium metabisulfite in cephalosporine.

Precision

System precision was established by making replicate injections of standard solution (20μg mL^{-1}) of sodium metabisulfite. The % RSD value of 1.60 for the corrected peak area counts and 0.57 for migration time indicates an acceptable level of precision for the analytical system. Method precision was determined by analyzing six samples of single batch of cephalosporin A as per the proposed method. The RSD value obtained for the six samples was 3.80% for sodium metabisulfite content. Intermediate precision was determined by analyzing six samples of a single batch of cephalosporin A by a second analyst on a different day using a different capillary and the RSD value obtained was 3.02% for sodium metabisulfite content. Inter and intraday precision of the method was checked by comparing the content of sodium metabisulfite in a cephalosporin sample. The sample was analyzed on a capillary electrophoresis using one set of capillary by an analyst on one day. A similar analysis was performed by another analyst using a different capillary and on a different day. Data shows insignificant difference between the two sets of data (Method precision and Intermediate precision) as determined by the overall RSD value of 3.28% for sodium metabisulfite.

Linearity

The linearity of response for sodium metabisulfite was determined by injecting sodium metabisulfite standard solution in duplicate in the range of about 4-24μg/

mL. The detector response was found to be linear over the specified range as determined from the coefficient of correlation value of 0.99768.

The equation representing the calibration curve is y = 0.00553x-0.01366.

Accuracy

A known amount of sodium metabisulfite was added to cephalosporin at three levels i.e. about 0.20% w/w, 0.40%w/w, 0.48%w/w (with respect to cephalosporin A) in triplicate. The samples were analysed as per the proposed method. Mean recovery of 88.19% with RSD of 1.47% (Individual observation in the range of 80-120%) indicates an acceptable level of accuracy for the proposed method.

Sensitivity

The Limit of detection and quantification for sodium metabisulfite were determined by injecting solutions of various concentrations of sodium metabisulfite and showing the precision at these concentrations. Experimentally determined- LOQ and LOD for sodium metabisulfite are 1.673μg mL^{-1} and 1.402μg mL^{-1} respectively.

Stability in analytical solution

A sample of cephalosporin spiked with sodium metabisulfite was prepared and kept at ambient room temperature. The sample solution was analysed initially and at different time intervals. The cumulative RSD upto 408 min was found to be 9.88%. It was therefore concluded that the sample is stable at room temperature for at least 7 Hours.

Robustness

Robustness of the method for quantification of sodium metabisulfite was checked by analyzing a sample of cephalosporin, by varying the capillary cassette temperature (± 5°C), pH of BGE (± 0.2), voltage (±2kV), and wavelength (±5nm). Robustness of the method is indicated by the insignificant difference between the two sets of data (Control and variable conditions) as determined by overall RSD values. These have been observed to be below 10%.

CONCLUSION

Thus it is concluded that the method developed for the quantification of sodium metabisulfite is specific, precise, linear and accurate in the concentration range of 4-24μg mL^{-1} and can be used for the routine analysis for the quantification of sodium metabisulfite in any cephalosporin drug up to 1.67μg mL^{-1} level.

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