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Short communication

Optimization and validation of HPLC method for determination of related and degradation products in Paracetamol tablets 500 mg

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Introduction

Acetaminophen (N-acetyl-p-amino-phenol, AAP), also known as paracetamol, is a widespread antipyretic and analgesic accepted as an effective treatment for the relief of pain and fever in adults and children. As paracetamol could be hepatotoxic, the concentrations of the related constituents, 4-aminophenol (EP imp K, raw material residue), 4-nitrophenol (EP imp F, raw material residue) and 4-chloracetanilide (EP imp J, byproduct in API synthesis) should be strictly controlled, particularly the 4-aminophenol which is paracetamol main degradation product (Calinescu et al., 2012).

Although paracetamol has been available on the market for decades, new methods for determination of its impurities in dosage forms, following the current legislation, still need to be developed to ensure the safety of drug product. This paper reports the optimization and validation of a new, simple and reliable HPLC method for the simultaneous determination of impurities 4-aminophenol and 4-chloracetanilide in Paracetamol 500 mg tablets. The main challenge was to obtain good peak symmetry for the main component and achieve suitable level of quantification of 4-chloroacetanilde, taking in consideration its extremely low specification limit of NMT 0.005%.

Materials and methods

Chemicals and reference standards

Potassium hydroxide, o-Phosphoric acid 85% and Acetonitrile were supplied from Merck KGaA, Darmstadt, Germany. Reference standard for Paracetamol was supplied from Alkaloid AD, Skopje; 4-Aminophenol and 4-chloroacetanilide were purchased from Merck KGaA, Darmstadt, Germany; 4-Nitrophenol was obtained from LGC Limited, England. The HPLC method has been validated to show specificity, linearity and range, accuracy, precision, limit of quantification (LOQ) and limit of detection (LOD), robustness, stability and filtration of solutions, as per ICH guideline (Q2 (R1) Validation of Analytical Procedures: Text and Methodology, 2005).

Instrumentation and analytical conditions

The analyses were performed on four different HPLC systems: Thermo Ultimate DAD 3000, Agilent 1260 Infinity Quaternary LC with UV-VIS detector, Nexera UHPLC DAD and Hitachi Chromaster 600 bar all controlled with Chromeleon CDS software version 7.2 SR5.

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Results and discussion

The newly developed HPLC method for simultaneous determination of 4-aminophenol and 4-chloroacetanilde was optimized by using column Zorbax Eclipse Plus C18; 250 x 4.6mm, 5 µm; with column temperature of 25 °C, autosampler temperature of 4 °C, at a 1 mL/min flow rate and 245 nm detection. The injection volume was 10 µL. Solution A (phosphate buffer pH=6.3) : Acetonitrile (ACN) gradient method was set: 0–12 min 10% ACN, 12–38 min from 10% to 30% ACN, 38–58 min 30% ACN, 58–60 min from 30% to 10% ACN, and 60–65 min 10% ACN.

The system suitability was evaluated on the basis of resolution between 4-aminophenol and paracetamol peak (Resolution, Rs>5.0). There was no interference from diluent and placebo with paracetamol, 4-aminophenol and 4-chloroacetanilide, indicating the specificity of the method. Linear correlations were obtained between the responses of 4-aminophenol peak related to the concentrations of standards over the range of 0.0005% (0.025 µg/ml) – 0.3% (15 µg/ml), (r=1.00), paracetamol peak related to the concentration of standards over the range of 0.0005% (0.025 µg/mL) – 0.3% (15 µg/mL), (r=1.00), and 4-chloroacetanilide peak related to the concentration of standards over the range of 0.0005% (0.025 µg/mL) – 0.3% (15 µg/mL), (r=1.00). The accuracy of the method was evaluated using an equivalent amount of placebo present in paracetamol tablets 500 mg at working concentration spiked with known quantities of 4-aminophenol, paracetamol and 4-chloroacetanilide at five different levels, in triplicate. The samples were analyzed by the proposed method and the amount of 4-aminophenol, paracetamol and 4-chloroacetanilide recovered was calculated. All the obtained results were into the range of the acceptance criteria for recovery (90.0-110.0%) and RSD (≤10%). The precision of the method was verified by repeatability and intermediate precision. The repeatability was shown by six replicate injections of the standard solution containing 4-aminophenol in the working concentration of 5.0 µg/mL, paracetamol in the working concentration of 10.0 µg/mL, and 4-chloroacetanilide in the working concentration of 0.5 µg/mL.

The intermediate precision of the method was evaluated using different analyst, column and different instrument and the analysis was performed on different days. The LOD and LOQ for impurities were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of diluted solutions with known concentrations (for paracetamol and both impurities LOD is 0.01 µg/mL and LOQ is 0.03 µg/mL). The method was robust for all the varied conditions. Standard solutions of 4-aminophenol at concentration level of 5.0 µg/mL, paracetamol at a concentration level of 10.0 µg/mL, 4-chloroacetanilide at a concentration level of 0.5 µg/mL and sample solutions prepared as per test method were analyzed initially and at different time intervals (24 h, 48 h, 72 h, 96 h, 120 h) by keeping the solutions at temperature of 4 °C and protected from light. From the obtained data, it was concluded that the standard solution is stable for 96 hours and sample solution is stable for 72 hours at temperature of 4 °C and protected from light. The results from the filter study indicated that no differences are observed when the standard solution is filtered through RC filter (0.45µm pore size) and PVDF filter (0.45 µm pore size).

Conclusion

It can be concluded that the defined RP-HPLC method is rapid and efficient for purity testing of commercially available Paracetamol 500 mg tablets.

References

Calinescu, O., Pincu, E., Badea, I.A., 2012. HPLC separation of acetaminophen and its impurities using a mixed-mode Reversed-Phase/Cation Exchange Stationary Phase. J. Chrom. Sci. 50, 335-342.

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