Determination of Related Substances of Quercetin Tetrahydrochloride by GC-FID and Structural Confirmation of Unknown Impurities by GC-MS

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Abstract:

A gas chromatography-flame ionization detection (GC-FID) method was established and validated for the determination of related substances of quercetin tetrahydrochloride, and the structural confirmation of unknown impurities generated during synthesis was carried out using gas chromatography-mass spectrometry (GC-MS). In this study, an Agilent CP-Sil 8 CB for Amines (30m × 0.25mm × 0.5μm) column was used, and the temperature programming conditions were optimized to achieve effective separation of ethylenediamine, diethylenetriamine, tris(2-aminoethyl)amine, N-ethylpiperazine, and N-(Piperazinyl)ethyl-1,2-EDA from the main component, quercetin tetrahydrochloride. Systematic validation was conducted to evaluate the specificity, linearity, sensitivity, precision, accuracy, and system suitability of the established method. The results indicated that the impurities showed good linearity within the specified limits, with R^2 values greater than 0.999. The limit of quantification (LOQ) ranged from 0.099 μg/mL to 1.552 μg/mL, and the relative standard deviation (RSD) of precision ranged from 1.2% to 3.0%. The RSD for ruggedness ranged from 2.1% to 3.5%, and the recovery rate was between 91.6% and 105.9%. During the analysis of the synthesis samples, two unknown impurities were detected. After purification, one impurity was effectively removed, and the other impurity was identified by GC-MS database matching and reference substance comparison. The unknown impurity was confirmed as N-(Piperazinyl)ethyl-1,2-EDA, which was suspected to be a by-product introduced during the synthesis process. The GC-FID method established in this study, combined with GC-MS structural confirmation, can be used for the quantitative analysis of related

substances in quercetin tetrahydrochloride and the study of impurity sources, providing reliable technical support for the quality control and process optimization of this drug.

Keywords:
Trientine tetrahydrochloride, Related Substances, Gas Chromatography (GC), Method Development ; Structural Confirmation.