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High-performance liquid chromatography method for determination of 1-(2-phenylethyl)-5-(quinaldin-4-yl) biuret (a potential cytotoxic agent) in rat plasma

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Background and Aims: Recently, biuret derivatives have been reported as showing moderate to good cytotoxic effect against certain cancer cell lines. In this study, a high-performance liquid chromatography method was developed for determination of 1-(2-phenylethyl)-5-(quinaldin-4-yl) biuret in rat plasma to use in future studies on this compound and related derivatives .

Methods: Different stationary phases such as C18, C8 and CN were tested, and finally , separations were performed on a Nucleosil CN HPLC column (150×3.9 mm) (5 µm), using a mixture of acetonitrile: methanol: potassium dihydrogen phosphate buffer(0.05 M, pH 3.5) (10:10:80) as mobile phase delivered at a flow rate of 1mL/minute. Detection of the biuret derivative and internal standard (1-({[3-(1,3-benzothiazol-2-ylsulfanyl) propyl]carbamoyl}amino)-N-(2-phenylethyl)formamide was done at 235 nm and ambient temperature. Plasma samples (200 µL) were prepared by addition of 40 µL internal standard (100 µg/mL) and 400 µL acetonitrile. After vortex mixing and centrifugation at 10000 g, 50 µL of the clear supernatant was directly injected onto the chromatography column. Calibration curves were constructed by fitting the peak area ratio of the biuret to internal standard against concentration of biuret to a power model using generalized least squares non-linear regression method.

Results: Under the above condition, biuret compound and the internal standard were detected at 4.5 and 13.5 minutes, respectively. Limit of quantitation of the 1-(2-phenylethyl)-5-(quinaldin-4-yl) biuret was 0.1 µg/mL. Accuracy of the method over the concentration range of 0.1-100 µg/mL was between 88-109%. Inter- and intra-day precisions were 4 and 19% and 6-8%, respectively. A good relationship in the form of a power model was found for two separate concentration ranges of 0.1-1 and 2.5-100 µg/mL(R^2>0.99).

Conclusions: The presented simple HPLC method is sufficiently accurate, precise and sensitive for the quantitation of 1-(2-phenylethyl)-5-(quinaldin-4-yl) biuret in rat plasma .

Keywords: 1-(2-Phenylethyl)-5-(quinaldin-4-yl) biuret; HPLC; Rat plasma