**Quantification of galantamine in *Narcissus tazzeta* and *Galanthus nivalis* (Amaryllidaceae) populations growing wild in Iran**

M. Rahimi Khonakdaria, M. H. Mirjalilia,\*, A. Gholipourb, H. Rezadoostc, M. Moridi Farimanic

a *Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C., Evin, 1983963113, Tehran, Iran*

*b Department of Biology, Payam Noor University (PNU), Sari, Mazandaran, Iran*

*c Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C., Evin, 1983963113, Tehran, Iran*

\*Corresponding author. E-mail: m-mirjalili@sbu.ac.ir

**Experimental**

 **Chemicals**

Standard of galantamine (GAL) was purchased from Sigma (Sigma-Aldrich Corporation, MO, USA). Methanol, HPLC grade methanol, acetonitrile and ammonium acetate of analytical grade were obtained from Merck (Darmstadt, Germany). HPLC grade water was used throughout the analysis.

**HPLC UV/MS analysis**

A KNAUER liquid chromatography apparatus consisting of a 1000 Smartline Pump, a 5000 Smartline Manager Solvent Organizer and a 2800 Smartline Photo- diode Array Detector was used for the HPLC analysis. Injection was carried out through a 3900 Smartline Autosampler injector equipped with a 100 µl loop. The temperature of the column was controlled with a Jet Stream 2 Plus oven (Knauer, advanced scientific instrument, Berlin, Germany). Separation was achieved on a 25 cm×4.6 mm with a pre-column, Eurospher 100-5 C18 analytical column provided by Knauer (Berlin, Germany). Data acquisition and integration was performed with EZchrom Elite software. The chromatographic assay was performed on a 25 cm×4.6 mm with pre-column, Eurospher 100-5 C18 analytical column provided by KNAUER (Berline, Germany) reversed phase matrix (5 μm) (Waters) and elution was carried out in a gradient system with acetonitrile as the organic phase (solvent A) and 1% (w/v) ammonium acetate buffer adjusted to pH 6.6 with acetic acid (solvent B) with the flow-rate of 1 mL min-1. Peaks were monitored at 280 nm wavelength. Injection volume was 20 µL and the temperature was maintained at 25°C. All injections were repeated three times (*n*=3). Calibration graphs were plotted subsequently for linear regression analysis of the peak area with concentration 5, 10, 25, 50, 80, 120, 150 and 200 mg L–1.

**LC–MS instrumentation and conditions**

HPLC separations were carried out on an Agilent series 1200 system equipped with degasser, binary high pressure mixing pump, column thermostat and variable-wavelength UV-Vis absorbance detector (Agilent Technologies; Waldbronn, Germany). Determination was performed using a Knauer C18 analytical column (Eurospher 100-5, 4.6 × 250 mm) and an gradient elution with a mixture of acetonitrile and water (containing 1 % ammonium acetate) at the ratio of 80/20. The flow rate of 1 mL min-1 was applied. The maximum absorption wavelength was 288 nm, and 20 µL of the sample was injected. All eluted peaks were transferred to a Finnigan TM LCQ TM DECA instrument, comprising an ion trap. An ionization device was used for sample analyses (sheath gas: 80 mL/min; auxiliary gas: 20 mL min; spray voltage: 5 kv; capillary temperature: 300°C; capillary voltage: 46 kv; tube lens: -60 kv). The Xcalibur 2.0 SR2 software (copyright Thermo Electron Corporation 1998–2006) was used.