Asian Journal of Chemistry Vol. 21, No. 1 (2009), 101-104

Reversed-Phase HPLC Method for Determination of Quercetin in Human Plasma

K. VIJAYA SRI, J.VIJAYA RATNA*, A. ANNAPURNA and B.V.V. RAVI KUMAR†
Division of Pharmaceutical Technology, College of Pharmaceutical Sciences
Andhra University, Visakhapatnam-530 003, India
Fax: (91)(891)2755324; Tel: (91)(891)2754446
E-mail: vijaya_ratna@yahoo.com

A simple reversed-phase liquid chromatography method was developed for the quantitative determination of quercetin. The method is simple, sensitive and highly selective and involves single extraction of drug from plasma in (4:1) ratio of methanol: DMSO. The mobile phase was pumped at a flow rate of 1.0 mL/min and the effluent was monitored at 370 nm. The retention time of quercetin was 2.72 min. The limit of detection of drug in plasma was found to be 0.2 µg/mL. The method was validated for its linearity, precision and accuracy.

Key Words: Quercetin, RP-HPLC, UV Detection, Human plasma.

INTRODUCTION

Quercetin, chemically is 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one, the major representative of the flavonol subclass of flavonoids, is a common dietary component. Quercetin has been frequently used as a model compound showing the protective properties of flavonoids.

Quercetin has a wide range of biological activities, which include a strong and prolonged antiinflammatory effect, inhibition of histamine release from mast cells and prevention of the oxidation of low-density proteins resulting in the prevention of atherosclerosis plaque formation[1]. Quercetin has antiproliferative activity *in vitro* against ovarian, breast and stomach cancer cell lines[2]. All these activities suggest that quercetin could be a compound with potential clinical application.

Several methods were cited in the literature to determine quercetin and its metabolites in biological fluids.These include HPLC[3-7], LC-MASS[8], spectroscopic and fluorimetric methods[9,10]. Some of the reported HPLC

†Roland College of Pharmaceutical Sciences, Berhampur-760 010, India.

methods required colorimetric and electrochemical detectors and the process is considered tedious. The present method is aimed at developing a single step extraction process. A simple and rapid RP-HPLC method for the determination of quercetin in human plasma using UV detection.

EXPERIMENTAL

Quercetin received as a gift sample from AIE chemicals (CA, USA). Methanol, water HPLC grade (Qualigens), orthophosphoric acid and dimethyl sulphoxide analytical grade (Qualigens) were used.

A gradient high pressure liquid chromatograph (Shimadzu HPLC Classs VP series) with two LC-10AT VP pumps, variable-wave length programmable UV/Vis Detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and RPC-18 column (250 mm × 4.6 mm I.D.; particle size 5 µm; YMC, Inc., Wilimington, Nc2840, USA) was used. The HPLC system was equipped with the software class-VP series version (Shimadzu).

HPLC conditions: The mobile phase was prepared with methanol and phosphoric acid (0.1 % orthophosphoric acid in water) in the ratio of 77:23. The components of the mobile phase were filtered before use through 0.45 µm membrane filter and degassed with a helium spurge for 15 min and the respective solvent reservoirs were pumped to the column at a flow rate of 1.0 mL/min which yielded a column back pressure of 135-150 kg/ cm. The column temperature was maintained at 30 ºC and the volume of the injection loop was 20 µL. Prior to injecting solutions, the column was equilibrated for at least 0.5 h with the mobile phase. The UV detector sensitivity was set at 0.0001 AUFS.

Preparation of standard solutions: The quantification of quercetin in human plasma was carried out by HPLC method using an external standard method. Stock solution of drug was prepared by taking 100 mg of quercetin in a 100 mL volumetric flask containing 70 mL of methanol, sonicated for about 15 min and made up to volume with methanol. The working standard solutions of quercetin were prepared by suitable dilutions of the stock solution with mobile phase.

Procedure: Human plasma was spiked with varying quantities of quercetin stock solution, prepared as above, so as to give a series of drug concentrations ranging from 0.2 to 30 µg/mL of quercetin in human plasma. An aliquot (0.1 mL) of such spiked plasma containing 0.2-30 µg/mL was taken into each of the six stopperd test tubes (capacity-10 mL) followed by the addition of 0.5 mL of 4 methanol:1 DMSO. The mixture was vortexed for 2 min and centrifuged at 3000 rpm for 3 min. The resultant supernatant liquid

was filtered through 0.2 µm membrane filter and the filtrate was injected into the column loop volume 20 µL: (RP-18e:250 × 4.0 mm I.D particle size 5 µm). The eluents were detected by UV detector at 370 nm and the data acquired, stored and analyzed with the software class-VP series version 5.03 (Shimadzu). Each sample was injected six times. The peak area of the drug for each of the concentrations as set up above was recorded.

Linearity: The regression of plasma concentration of the quercetin over its peak area was calculated by the least square method. The fitness of the data to the linear relationship was assessed by calculating correlation coefficient.

Precision: Aliquots of 100 µL drug spiked plasma containing 2, 5 or 10 µg of quercetin were mixed with 0.5 mL of 4 methanol:1 DMSO and treated as described above. The clear filtrate was injected into the HPLC column (n = 6).

Accuracy: Known quantity (2, 5 or 10 µg) of quercetin, as a solution was added to the reanalyzed drug spiked plasma samples containing 10 µg of quercetin and quantified by the present HPLC method to determine the accuracy of the analytical method. Accuracy was expressed in terms of the percent recovery.

RESULTS AND DISCUSSION

The run time was set at 10 min and quercetin appeared on the chromatograph at 2.72 min. There was no interference of any other peak with the drug peak. When the same filterate sample containing quercetin was injected 6 times, the retention time of the drug was same. The mean peak area of quercetin and its respective peak area were subjected to regression analysis by least squares method and high correlation coefficient was observed (r = 0.997) in the range of 0.2-30 µg/mL only. The regression of quercetin concentration over its peak area was found to be Y = 29454X + 24598 with a high correlation coefficient, where Y = peak area and X = plasma concentration of quercetin. This regression equation was used to estimate the amount of quercetin either in plasma or in validation study (precision and accuracy) (Tables 1 and 2).

TABLE-1
RECOVERY OF QUERCETIN FROM PLASMA CONTAINING 10 µg QUERCETIN BY HPLC METHOD

Amount of Quercetin added (µg)	Mean percent of recovery (± SD) (n = 6)
2	82.70 ± 2.25
5	83.95 ± 1.41
10	85.28 ± 1.52

TABLE-2
PRECISION OF THE HPLC METHOD USED FOR THE ESTIMATION OF QUERCETIN (n = 6) IN PLASMA

Concentration of Quercetin plasma (µg/mL)	Intra-day		Inter-day	
	Observed	CV (%)	Mean of peak area ratio	CV (%)
2	1.99 ± 1.1	0.9	2.01 ± 1.2	1.7
5	5.01 ± 1.6	1.6	4.99 ± 1.2	2.2
10	9.96 ± 1.3	0.1	9.92 ± 1.5	0.1

Conclusion

The HPLC assay described here is a simple, selective, precise and accurate means of quantification of quercetin in human plasma. Sensitivity, simplicity and rapidity are the main advantages of this method.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the generous supply of Quercetin, from AIE Chemical USA and Roland College of Pharmaceutical Sciences, Berhampur, for providing necessary facilities and encouragement.

REFERENCES

1. C. Kandaswami and E. Middleton, *Advan. Exper. Med. Biol.*, **366**, 351 (1994).
2. J.V. Formica and W. Regeson, *Food Chem. Toxicol.*, **33**, 1061 (1995).
3. I. Erlund, G. Alfthan, H. Siren, K. Ariniemi and A. Aro, *J. Chromatogr. B Biomed. Sci. Appl.*, **727**, 179 (1999).
4. B. Liu, D. Anderson, D.R. Ferry, L.W. Seymour, P.G. De Takats and D.J. Kerr, *J. Chromatogr. B Biomed. Sci. Appl.*, **666**, 149 (1995).
5. F.M. Wang, T.W. Yao and S. Zeng, *J. Pharm. Biomed. Anal.*, **33**, 317 (2003).
6. K. Ishii, T. Furuta and Y. Kasuya, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, **794**, 9 (2003).
7. K.A. Khaled, M.E. Yousry and A. Sayed, *J. Liq. Chromatogr. Rel. Technol.*, **23**, 455 (2000).
8. L. Wang and M.E. Morris, *J. Chromatogr B.*, **821**, 194 (2005).
9. D.A. Kostic, G.Z. Miletic, S.S. Mitic, I.D. Rasic and V.V. Zivanovic, *Chem. Papers*, **61**, 73 (2007).
10. R. Gugler and H.J. Dengler, *Clin. Chem.*, **19**, 36 (1973).

(*Received*: 19 October 2007; *Accepted*: 11 August 2008) AJC-6745