# Separation and Detection of the Antioxidant Flavonoids, Rutin and Quercetin, Using HPLC Coupled on-line With Colorimetric Detection of Antioxidant Activity

Nitra Nuengchamnong, Ank Hermans-Lokkerbol and Kornkanok Ingkaninan*

Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand
*Corresponding author. E-mail address: kornkanoki@nu.ac.th (K. Ingkaninan)
Received 20 January 2004; accepted 28 July 2004

**Abstract**

The HPLC system coupled on-line with colorimetric detection of antioxidant activity was developed in order to separate antioxidants especially the flavonoids, rutin and quercetin, and to determine their activities in a single step. In the system developed, the eluate from the HPLC column was split into two flows at the ratio of 8:2. The major part flowed to the UV-detector set at 220 nm, while the minor part was mixed with a free radical, 2,2-diphenyl-1-picrylhydrazyl, to perform a color reaction with the eluted antioxidants. This reaction occurred in a knitted-shape reactor (70 cm 0.8 mm I.D. PTFE). The peaks indicating antioxidant activity were monitored from the decrease of absorbance at 515 nm. The results showed that the on-line HPLC coupled with antioxidant activity detection developed could separated the flavonoids, rutin and quercetin in plant extracts such as *Sophora japonica* and *Morus alba* and simultaneously determine their antioxidant activity. The detection limits for the antioxidant activity determination of these two compounds were 500 and 200 ng, respectively. This method could be applied for rapid determination of rutin, quercetin in complex mixtures.

**Keywords**: antioxidant, flavonoids, rutin, quercetin, on-line separation, activity determination method

## Introduction

Natural antioxidants are known to prevent many physiological and pathological processes caused by free radical reactions. They can prevent some diseases such as cancer, cardiovascular diseases, as well as aging. Therefore, they can serve as leads for the development of new drugs with the prospect of improving the treatment of several diseases. Moreover, they can be used as an alternative to the synthetic food additives such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA).

Several assays have been developed to measure the activity of antioxidants. These methods focus on different mechanisms of the antioxidant defense system. In some assays the reduction of molybdenum (Prieto et al., 1999) or ferric-tripyridyltriazine ($Fe^{3+}$-TPTZ) (Benzie and Strain, 1999) is used. Some used the scavenging of free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aquino et al., 2001; Joyeux et al.,1995; Nakagawa et al., 2000; Schmeda-Hirschmann et al., 1999; Sing et al., 2002; Tocharus et al., 2000; Yen and Duh, 1994), 2,2 azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)(Miller et al., 1993), or *N*,*N*-dimethyl-*p*-phenyldiamine (DMPD) (Fogliano et al.,1999).

Another method is the determination of the capacity of antioxidants to bleach β-carotene (Sing et al., 2002; Velioglu et al., 1998). In the thiobarbituric acid (TBA) assay, thiobarbituric acid reacts with malondialdehyde, a secondary product of lipid oxidation, to form a pink colored di-adduct (Karastogiannidou, 1999).

Some high-performance liquid chromatography (HPLC) separation systems coupled on-line with antioxidant activity determination have been reported. These methods were aimed for rapid detection and separation of antioxidants in complex mixtures. Dapkevicius et al. (1999) used HPLC with on-line antioxidant activity detection through a post column reaction with luminol. The resulting chemiluminescence was measured. Koleva et al. (2000, 2001) developed the on-line detection of radical scavengers in HPLC eluates using the stable free radicals, DPPH and ABTS. The applications of the on-line HPLC-DPPH method for the screening of antioxidants in plants extracts were reported by Bandoniene and Murkovic (2002a, b), Pukalskas et al. (2002) and Kosar et al. (2003). However, there are no reports on the application of this methods for the detection of the commonly known antioxidant flavonoids, rutin and quercetin.

In this study, we developed an HPLC coupled on-line with colorimetric detection of antioxidant activity using DPPH as a free radical. This method can be used for separation and detection of antioxidant flavonoids, such as rutin and quercetin. It can be applied for dereplication of these compounds in complex mixtures during lead finding procedures.

## Materials and Methods

### Reagents

2, 2-Di(tert-octylphenyl)-1-picrylhydrazyl (DOPH) was purchased from Aldrich (Milwaukee WI, USA). DPPH, rutin hydrate, and quercetin dihydrate were from Sigma-Aldrich Chemie GmbH (Germany). Ascorbyl palmitate and trolox were from Fluka (Switzerland). Potassium dihydrogen phosphate, AR grade, was from Merck (Germany). Phosphoric acid 85%, AR grade, came from Carlo Erba (Italy). Acetonitrile and methanol, both HPLC grade, were purchased from Labscan Asia Co. Ltd. (Thailand). All water used was deionized.

### Apparatus

The HPLC coupled on-line with colorimetric detection for antioxidant activity (Figure 1) is as follows.

*Line A* represents the HPLC system. It consisted of a pump, type 3200 (TSP, USA), an injector, type 7725i with 10 µl loop (Rheodyne, USA), a UV-VIS detector model 785A (Perkin Elmer, USA) and a flatbed, double pen recorder, model BD 112, (Kipp & Zonen, the Netherlands). The I.D. of the post-column tubing was 0.254 mm. A 20-cm length of 0.064 mm I.D. PEEK tubing coupled the line A to the line B via a T-connector; the column effluent was split into two streams with the flow ratio of 8:2, with the minor stream going to line B.

*Line B* represents the flow injection analysis (FIA) system for antioxidant activity detection. It consisted of a pump, type 3500 (TSP, USA), a home-made knitted-shape reactor, UV-VIS detector model 9050 (Varian, USA), which was connected to the double pen recorder of line A.

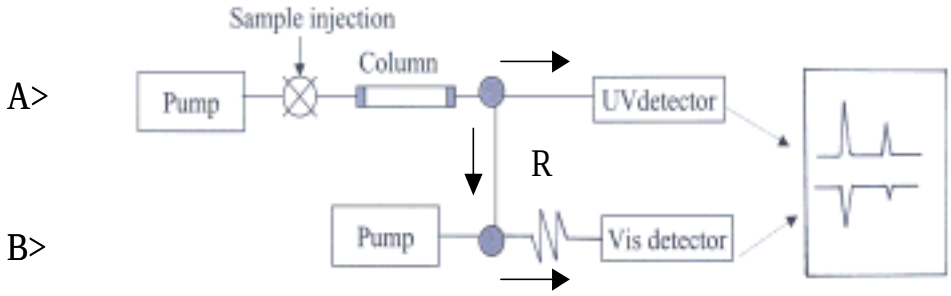


**Figure 1** Scheme of the HPLC coupled with FIA detection system; A is the HPLC-line; B is the FIA-line. R is the reactor. The arrows indicate flow directions.

Conditions of HPLC and FIA systems

HPLC: a LiChrospher 60 RP-8 select B column (125 x 4 mm, 5 μm, Merck, Germany) was used, combined with a precolumn, (45 x 3.9 mm), home-packed with LiChrosorb RP-18, 10 μm (Merck, Germany). The mobile phase was MeCN:MeOH:25mM $KH_2PO_4$, pH 3.0, 20:15:65 (v/v/v) or 0:50:50 (v/v/v). The pump in the HPLC-line was set at 1ml $min^{-1}$. The detector was set at 220 nm.

FIA: After optimization, the conditions for the FIA were as follow: the reactor consisted of a 70 cm long, 0.8 mm I.D., knitted PTFE tubing. The carrier stream solution was 0.1 mM DOPH or DPPH in MeOH. The pump was set at 0.5 ml $min^{-1}$. The detector in line B was set at 515 nm for the experiments using DPPH and at 538 nm for the experiments using DOPH as the FIA reagent.

Sample Preparation

*Sophora japonica* L. dried flowers and *Morus alba* L. dried leaves were from Phitsanulok, Thailand. The plant materials were ground and macerated with methanol at ambient temperature (about 28°C) for 3 days. The ratio of methanol:plant material was 50 ml $g^{-1}$. The plant extracts were filtered and evaporated to dryness under reduced pressure at temperature below 50°C. The dried extracts were dissolved in methanol to a concentration of 10 mg $ml^{-1}$ and filtered over 45 μm nylon membrane before injected into HPLC system.

**Results and Discussion**

The determination of antioxidant activity in plant extracts was developed using the free radicals DPPH and DOPH as color reagents in the FIA system. The FIA system was then coupled to an HPLC for the on-line separation and activity determination of antioxidant.

Design and optimization of the FIA system for antioxidant activity detection

In the FIA system, the analyte reacted with the free radical present in the reactors. The change caused by the reaction was then measured. The sensitivity of the measurement depends on the choice of free radicals, the reaction time, the length and the shape of the reactor in combination with the flow rate.

Choice of test substances and reagents for the FIA

Rutin and quercetin, together with the well known antioxidants trolox and ascorbyl palmitate, were used as test substances for FIA system optimization. The antioxidant activity can be measured from the decrease of an absorption of a free radical (R●) after reacting with an antioxidant (AH):

$$R\bullet + AH \longrightarrow R\text{-}H + A\bullet$$

In our study the free radicals DPPH and DOPH were used as free radical reagents in the carrier stream. Both compounds were purple in the radical form and colorless in reduced form. The absorption was measured at 515 or 538 nm, corresponding to the maximum absorbance of DPPH or DOPH, respectively. The decrease of the absorption correlated to the antioxidant activity of the sample.

The structures of the free radicals and antioxidant compounds are shown in Figure 2.

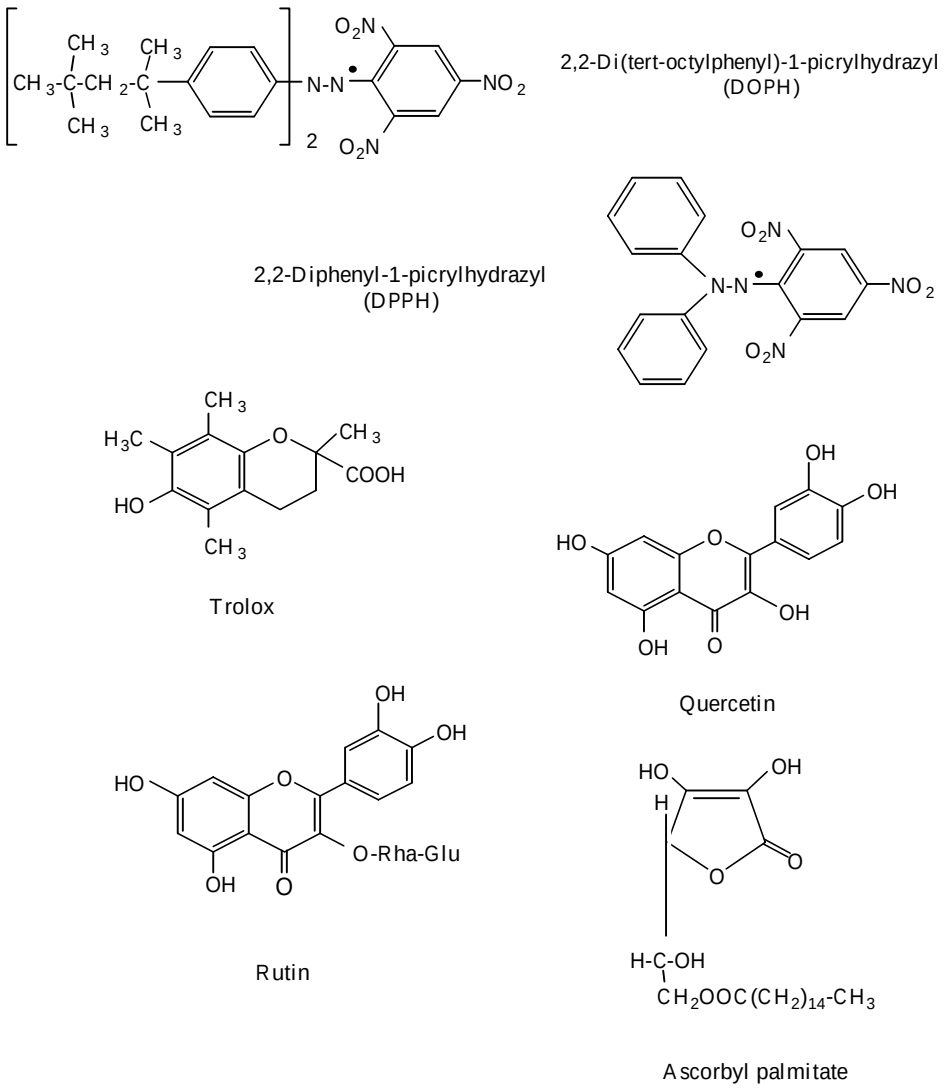


**Figure 2** The structures of the free radicals DPPH and DOPH and four antioxidants: rutin, quercetin, trolox and ascorbyl palmitate.

Shape and length of the reactor

To avoid a high degree of dispersion in the FIA system, a good mixing of analyte and reagent was required. Since the shape of the reactor affects the mixing, we compared a coiled-shape reactor with a knitted-shape reactor, both at 50 and 100 cm tubing lengths.

The results are shown in Figure 3. In all cases, the knitted-shape reactors gave greater peak heights indicating less band broadening comparing to that of the coiled-shape reactors.

To find the optimal tubing length, the four tested substances were injected into the system, using knitted-shape reactors with various lengths. The result is shown in Figure 4. The knitted-shape reactor with the length of 70 cm was chosen as it gave satisfactory peak heights of the four tested substances.

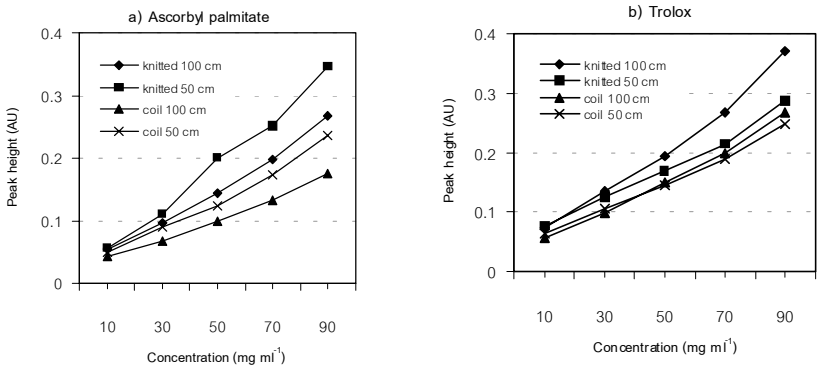


**Figure 3** Effect of the shape of the reactor on the peak heights of a) ascorbyl palmitate b) Trolox. Carrier stream 0.1 mM DOPH; flow rate 0.5 ml $min^{-1}$; injection volume 10 µl; I.D. of reactor 0.8 mm; detection at 538 nm.

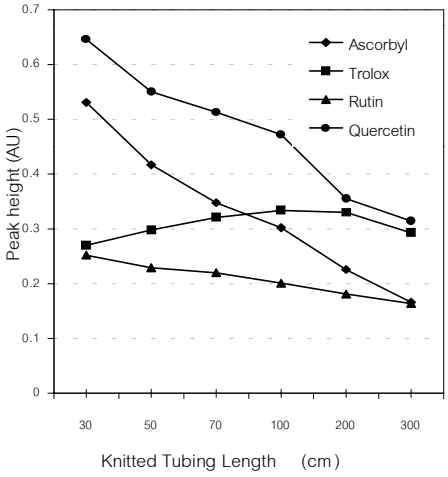


**Figure 4** The peak heights of ascorbyl palmitate, trolox, rutin and quercetin when injected into FIA system with various lengths of knitted-shape reactors. Sample concentration 100 µg $ml^{-1}$. Other conditions as in Figure 3.

Concentration of the color reagent

Various DOPH concentrations were studied. The results are shown in Figure 5. The highest peak height was obtained when 0.1 mM DOPH was used as a color reagent in the carrier stream.

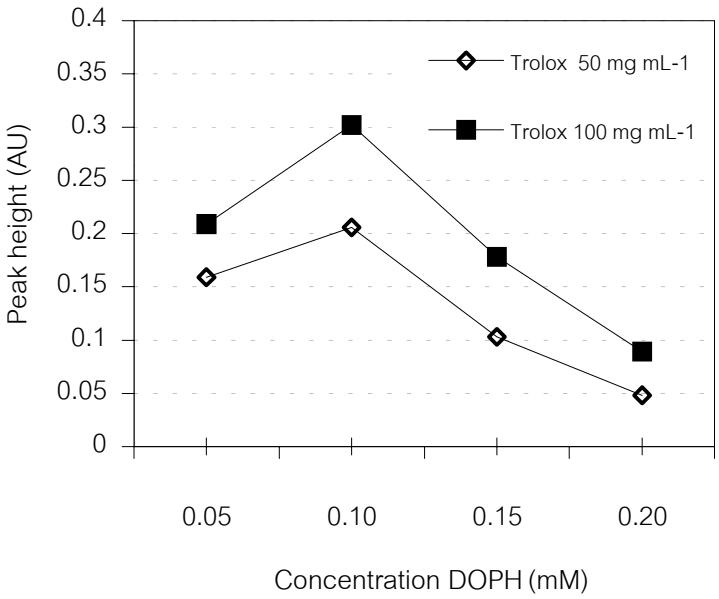


**Figure 5** The peak heights of 50 and 100 μg $ml^{-1}$ trolox obtained from FIA using various concentrations of DOPH as a carrier stream. Other conditions as in Figure 3.

Working range for quantitative analysis

After the FIA system had been developed, the system, as described in Materials and Methods, was tested for its application on quantitative analysis. The relationship between peak height and concentration of the four tested compounds were investigated. The results are summarized in Table 1. For all compounds, the linear range was around 10-100 mg $l^{-1}$. The correlation coefficient (r) was more than 0.999 in all experiments.

**Table 1** The linear ranges, limits of detection (LOD), correlation coefficient (r), and regression equation, of the four antioxidants.

| Antioxidant | Linear ranges (mg $l^{-1}$) | LOD [a)] (mg $l^{-1}$) | r | Regression equation |
|---|---|---|---|---|
| Rutin | 10-100 | 5.9 | 0.9996 | y= 0.23x+0.74 |
| Quercetin | 10-110 | 6.5 | 0.9993 | y= 0.57x-2.11 |
| Trolox | 10-90 | 7.5 | 0.9991 | y= 0.29x+1.30 |
| Ascorbyl palmitate | 10-100 | 6.5 | 0.9995 | y = 0.604x-2.21 |

Note: [a)] LOD is defined as signal-to-noise ratio = 3

HPLC coupled on-line with the colorimetric determination of antioxidant activity

In order to find the suitable conditions for the separation of two flavonoids, rutin and quercetin, a $C_8$ analytical column, combined with a $C_{18}$ guard column was used, and various mobile phase compositions were tested. Complete separation of the two compounds within 10 minutes was obtained using MeCN:MeOH:25 mM $KH_2PO_4$ pH3.0 (20:15:65 v/v/v) as a mobile phase.

For the on-line determination of the antioxidant activity after separation in HPLC, the FIA system was coupled to the HPLC by a 20 cm length of 0.064 mm I.D. of PEEK tubing. The T-connector split the column effluent into two streams with the ratio of 8:2 (v/v). The major stream went to the UV detector set at 220 nm while the minor stream went to the FIA line (Figure 1).

However, when the FIA reagent stream was mixed with the HPLC mobile phase mentioned above, precipitation occurred. A solubility study of 0.1 mM solution of DOPH in MeOH with various amounts of buffer using visual detection, showed that precipitation occurred when the amount of the buffer exceeds 35% v/v. So, the mobile phase was then changed to 25 mM $KH_2PO_4$ pH3.0:MeOH 50:50 v/v. This eluent has a higher organic solvent content, but about the same eluting force. Using this mobile phase, complete separation within 8 minutes was obtained.

Optimization after coupling HPLC to FIA

Some parameters for the on-line coupling of HPLC and FIA for the separation and determination of antioxidants were varied and optimized as follows. The flow rate of HPLC was set at 1 ml $min^{-1}$. The sample was a solution of 500 μg $ml^{-1}$ of rutin and quercetin in MeOH. Injection volume was 10 μl. After the column, the flow was split into two streams; 0.8 ml $min^{-1}$ to the UV detector, set at 220 nm, and 0.2 ml $min^{-1}$ to the reactor. The latter one merged to DOPH, which was pumped from the FIA line.

Flow rate in FIA line

The various flow rates of DOPH, i.e., 1.0, 0.7, 0.5 and 0.3 ml $min^{-1}$ were tested. The FIA flow rate of 0.3 ml $min^{-1}$ showed the highest peak height. The total flow rate in the reactor was then 0.5 ml $min^{-1}$.

Free radical reagents

Due to the poor solubility of DOPH in water, we studied the other free radical, DPPH (2,2-Diphenyl-1-picrylhydrazyl), which has a higher solubility. The standard sample solution was injected. It was found that DPPH gave higher peak heights than DOPH. Therefore, DOPH was replaced by DPPH in the further studies.

0.10 and 0.15 mM DPPH solution in MeOH were pumped through the FIA line at the previously described condition. Rutin and quercetin, at

concentrations of 50 μg $ml^{-1}$ or 500 μg $ml^{-1}$, were injected to the HPLC system. The results showed that 0.15 mM DPPH gave higher peak heights than 0.10 mM. However, the noise was also higher. We, therefore, selected DPPH at the concentration of 0.10 mM for the further works.

Split ratio

We injected 10 μl of the mixed standard antioxidants at the concentration of 500 μg $ml^{-1}$ into the HPLC system. The ratios of the flows mobile phase split to UV detector and FIA system varied from 8:2 to 6:4 by changing the length of the connection tubing between the HPLC and FIA system. The non-splitting system in which all of the eluate flowed through UV detection and then went to FIA system was also tested. The flow rate in the FIA line was set at 0.3 ml $min^{-1}$. The results showed that the non-splitting gave the lowest peak height. A split ratio of 6:4 gave the same peak height of quercetin and higher peak height of rutin when compared to that of at the ratio of 8:2. However, precipitation might occur when the DPPH mixes with HPLC mobile phase. The split ratio of 8:2 was, therefore, selected for the on-line system.

Reactor length

We injected the mixed standard antioxidants at the concentrations of 100 μg $ml^{-1}$ and 50 μg $ml^{-1}$ into the on-line system with various lengths of the reactor i.e. 50, 70, and 100 cm. A tubing length of 70 cm was selected, as it gave the highest peak heights for both flavonoids.

Detection limits

The detection limits of rutin and quercetin, defined as the amount that gives a signal to noise ratio of 3, were 500 ng and 200 ng respectively, corresponding to the concentrations of 82 and 60 μM of rutin and quercetin in the injected sample. Conditions were as described in Materials and Methods, using the mobile phase, 25 mM $KH_2PO_4$ pH 3.0:MeOH 50:50 (v/v) and DPPH as the carrier reagent. Injection volume was 20 μl. The detection limit of rutin and quercetin were higher than those of reported by, Koleva et al. (2000) (44 and 6.6 ng, respectively). This may be due to the lower sensitivity in data processing of the recorder compare to the integrator. Another reason for the lower sensitivity observed might be due to the less solubility of DPPH in aqueous system.

Delay time

We injected rutin, quercetin and trolox into the developed on-line HPLC coupled with FIA system. Two chromatograms were obtained (Figure 6). The lower shows the peaks of the compounds detected at 220 nm, and the upper shows their antioxidant activities with a delay time of around 50 seconds. The delay times are shown in Table 2.

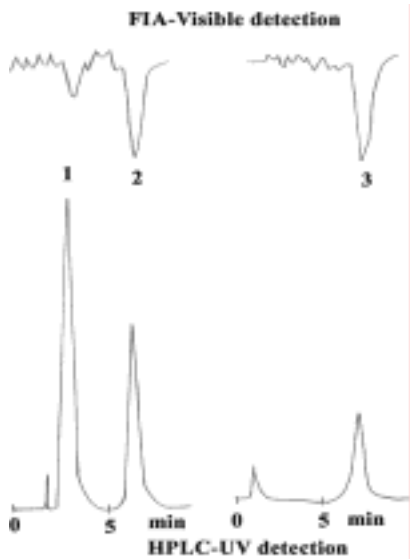


**Figure 6** Chromatograms of a) 200 μg $ml^{-1}$ of rutin (1) and 100 μg $ml^{-1}$ of quercetin (2), b) 100 μg $ml^{-1}$ of trolox (3). DPPH was used as the carrier reagent. The chromatograms on the lower part were from HPLC-UV detector set at 220 nm. The chromatograms on the upper part were from FIA detector set at 515 nm; the chart speed was 2 mm $min^{-1}$. Other conditions as in Figure 3.

**Table 2** Delay times between the HPLC-UV detector set at 220 nm and visible detector for antioxidant activity detection set at 515 nm of the HPLC coupled on-line with colorimetric detection for antioxidant activity. The results shown are the means$^{+}$ S.D. of five experiments.

| Analyte | Retention time (sec) of the analyte detected from | | Delay time (sec) |
|---|---|---|---|
| | UV detector | Vis detector | |
| rutin | 166 $\pm$ 0.005 | 214 $\pm$ 0.033 | 47 $\pm$ 0.01 |
| quercetin | 370 $\pm$ 0.012 | 419 $\pm$ 0.253 | 49 $\pm$ 0.25 |
| trolox | 435 $\pm$ 0.017 | 485 $\pm$ 0.028 | 50 $\pm$ 0.03 |

## Applications

The method was tested on extracts from flowers of *S. japonica* and leaves of *M. alba*. These two plant extracts are known to contain rutin and quercetin (Ng et al., 2000; Lee et al., 2002). The chromatograms of two plant extracts are shown in Figure 7. The lower chromatograms were obtained from the HPLC-UV detector, while the upper chromatograms were obtained from the FIA detector. The UV chromatograms show the complexity of the two extracts while

there are fewer peaks of antioxidant activity in the FIA chromatograms. In the analysis of the *S. japonica* crude extract, the two peaks of antioxidant activity at 213 and 419 sec correspond to the UV peak at 166 and 370 sec. They could be identified as the peaks of rutin and quercetin, respectively. In the same manner, the analysis of *M. alba* crude extract indicated the presence of only rutin.

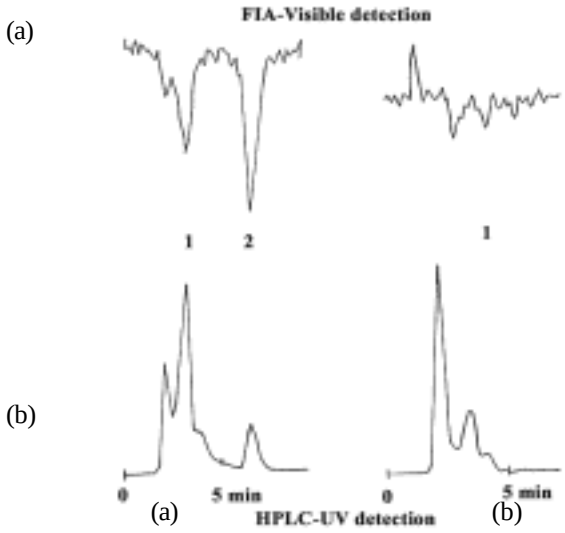


**Figure 7** Chromatograms of a) 10 mg $ml^{-1}$ of the flower extract of *S. japonica* b) 10 mg $ml^{-1}$ of the leave extract of *M. alba*. The chromatograms on the lower part were from HPLC - UV detector set at 220 nm. The chromatograms on the upper part were from FIA-Visible detector set at 515 nm; the chart speed was 2 mm $min^{-1}$. Numbers 1 and 2 indicated the peaks of rutin and quercetin, respectively.

This experiment demonstrates the rapid identification of the antioxidant flavonoids, rutin and quercetin from complex mixtures. This can be used as method of dereplication for these two flavonoids in drug discovery processes. By this method, rutin and quercetin can rapidly be identified in antioxidant mixtures, and excluded from further study for new antioxidants. Only sample mixtures showing unidentified antioxidant peaks should be further investigated. This method can also be used for qualitative and quantitative analysis of the two flavonoids in complex mixtures. The system can be further developed for on-line coupling to an identification unit such as NMR or MS. In that way, both activity determination and spectral data of the compounds isolated from HPLC will be obtained almost at the same time.

## Conclusion

A simple and rapid method for on-line separation and antioxidant activity determination was developed. The method can be applied for the separation and selective detection of flavonoids with antioxidant activity. The detection limits for

rutin and quercetin are 500 and 200 ng, respectively. The method can be used during lead finding procedures for the dereplication of these compounds in complex mixtures.

**References**

Aquino, R., Morelli, S., Lauro, M. R., Abdo, S., Saija, A., & Tomaino, A. (2001). Phenolic constituents and antioxidant activity of an extract of *Anthurium versicolor* leaves. *Journal of Natural Products*, 64, 1019-1023.

Bandoniene, D., & Murkovic, M. (2002a). On-line HPLC screening method for evaluation of radical scavenging phenols extracted from apples (*Malus domestica* L.) *Journal of Agricultural and Food Chemistry*, 50, 2482-2487.

Bandoniene, D., & Murkovic, M. (2002b). The detection of radical scavenging compounds in crude extract of borage (*Borago officinalis* L.) by using an on-line HPLC-DPPH method. *Journal of Biochemical and Biophysical Methods*, 53, 45-49.

Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In L. Packer (Ed.), *Methods in Enzymology* (vol.299, pp.15-27). Orlando, FL: Academic Press.

Dapkevicious, A., van Beek, T. A., Niederlander, H. A. G., & Groot A. D. (1999). On-line detection of antioxidative activity in High-Performance Liquid Chromatography eluates by chemiluminescence. *Analytical Chemistry*, 71, 736-740.

Fogliano, V., Verde, V., Randazzo, G., & Ritieni, A. (1999). Method for measuring antioxidant and its application to monitoring the antioxidant capacity of wines. *Journal of Agricultural and Food Chemistry*, 47, 1035-1040.

Joyeux, M., Lobstein, A., Anton, R., & Montier, F. (1995). Comparative antilipoperoxidant, antinecrotic and scavenging properties of terpenes and biflavones from Ginkgo and some flavonoids. *Planta Medica*, 61, 126-129.

Karastogiannidou, C. (1999). Effects of onion quercetin on oxidative stability of cook-chill chicken in vacuum-sealed containers. *Journal of Food Science*, 64, 978-981.

Koleva , I. I., Niederlander, H. A. G., & van Beek, T. A. (2000). An on-line HPLC method for detection of radical scavenging compounds in complex mixtures. *Analytical Chemistry*, 72, 2323-2328.

Koleva , I. I., Niederlander, H. A. G., & van Beek, T. A. (2001). Application of ABTS radical cation for selective on-line detection of radical scavengers in HPLC eluates. *Analytical Chemistry*, 73, 3373-3381

Kosar, M., Dorman, H. J. D., Bachmayer, O., Baser, K. H. C., & Hiltunen, R. (2003). An improved on-line HPLC-DPPH method for the screening of free radical scavenging compounds in water extracts of Lamiaceae plants. *Chemistry of Natural Compounds*, 39, 161-166.

Lee, S. H., Choi, S. Y., Kim, H., Hwang, J. S., Lee, B. G., Gao, J. J., & Kim,S. Y. (2002). Mulberroside F isolated from the leaves of Morus alba inhitbits melanin biosynthesis. *Biological & Pharmaceutical Bulletin*, 25, 1045-1048.

Miller, N. J., Rice-Evans, C., & Davies, M. J. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science*, 84, 407-412.

Nakagawa, K., Hawagoe, M., Yoshimura, M., Arata, H., Minamikawa,T., Nakamura, M., et al. (2000). A differential effects of flavonoid quercetin on oxidative damages induced by hydrophillic and lipophillic radical generators in hepatic lysosomal fractions of mice. *Journal of Health Science*, 46, 509-512.

Ng, T. B., Liu, F., & Wang, Z. T. (2000). Antioxidative Activity of natural products from plants. *Life Science*, 66, 709-723.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, 269, 337-341.

Pukalskas, A., van Beek, T. A., Venskutonis, R. P., Linssen, J. P. H., van Veldhuizen, A., & de Groot, A. (2002). Identification of radical scavengers in Sweet Grass (*Hierochloe Odorata*). *Journal of Agricultural and Food Chemistry*, 50, 2914-2919.

Schmeda-Hirschmann, G., Razmilic, I., Gutierrez, M. I., & Loyola, J. I. (1999). Proximate composition and biological activity of food plants gathered by chilean amerindians. *Economic Botany*, 53, 177-187.

Sing, R. P., Chidambara, K. N., & Jayaprakasha, G. K. (2002). Studied on the antioxidant activity of Pomegranate (*Punica granatum*) peel and seed extracts using in vitro model. *Journal of Agricultural and Food Chemistry*, 50, 81-86.

Tocharus, J., Boonsong, T., & Surangkul, D. (2000). Antioxidative capacity of indigenous vegetable plants in Northern Thailand. *Naresuan University Journal*, 8, 36-40.

Velioglu, Y. S., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry*, 46, 4113-4117.

Yen, G. C., & Duh, P. D. (1994). Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. *Journal of Agricultural and Food Chemistry*, 42, 629-632.