HPLC Method for Simultaneous Quantitative Detection of Quercetin and Curcuminoids in Traditional Chinese Medicines

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Key Words
curcuminoid, high-performance liquid chromatography (HPLC), international conference harmonisation (ICH), quercetin

Abstract

Objectives: Quercetin and curcuminoids are important bioactive compounds found in many herbs. Previously reported high performance liquid chromatography ultraviolet (HPLC-UV) methods for the detection of quercetin and curcuminoids have several disadvantages, including unsatisfactory separation times and lack of validation according the standard guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

Methods: A rapid, specific, reversed phase, HPLC-UV method with an isocratic elution of acetonitrile and 2% v/v acetic acid (pH 2.6) at a flow rate of 1.3 mL/minutes, a column temperature of 35°C, and ultraviolet (UV) detection at 370 nm was developed. The method was validated and applied to the quantification of different types of market available Chinese medicine extracts, pills and tablets.

Results: The method allowed simultaneous determination of quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin in the concentration ranges of 0.00488 — 200 μg/mL, 0.625 — 320 μg/mL, 0.07813 — 320 μg/mL and 0.03906 — 320 μg/mL, respectively. The limits of detection and quantification, respectively, were 0.00488 and 0.03906 μg/mL for quercetin, 0.62500 and 2.50000 μg/mL for bisdemethoxycurcumin, 0.07813 and 0.31250 μg/mL for demethoxycurcumin, and 0.03906 and 0.07813 μg/mL for curcumin. The percent relative intra day standard deviation (% RSD) values were 0.432 — 0.806 μg/mL, 0.576 — 0.723 μg/mL, 0.635 — 0.752 μg/mL, and 0.655 — 0.732 μg/mL for quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin, respectively, and those for intra day precision were 0.323 — 0.806 μg/mL, 0.805 — 0.854 μg/mL, 0.078 — 0.844 μg/mL and 0.275 — 0.829 μg/mL, respectively. The intra day accuracies were 99.589% — 100.821%, 98.588% — 101.084%, 9.289% — 100.88%, and 98.292% — 101.022% for quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin, respectively, and the inter day accuracy were 99.665% — 103.06%, 97.669% — 103.513%, 99.569% — 103.617%, and 97.929% — 103.606%, respectively.

Conclusion: The method was found to be simple, accurate and precise and is recommended for routine quality control analysis of commercial Chinese medicine products containing the flour flavonoids as their principle components in the extracts.

1. Introduction

Quercetin is a category in the class of flavonoids, and...
Ginkgo biloba, botanicals, such as flowers, barks and leaves. It is also found in medicinal licis found as pigments in fruits, vegetables, seeds, nuts, flowers, barks and leaves. It is also found in medicinal products like Chinese medicinal materials (because such products normally contain more than one herb). For simultaneous determination of quercetin and curcuminoids, HPLC method is the recommended technique because it uses separation, identification and quantification of the analytes from plant extracts, foods, pharmaceutical products, and body fluids.

In the present study, a simple isocratic reversed phase HPLC method was developed according to international conference harmonisation (ICH) guidelines [52] for the simultaneous quantitative detection of quercetin and curcuminoids. The method was also validated by using market available traditional Chinese medicine materials such as granules, pills and tablets.

2. Materials and Methods

Curcumin (mixture of curcumin, demethoxycurcumin, and bisdemethoxycurcumin) was obtained from Acros Organics, USA. Quercetin anhydrous was obtained from Sigma, USA. The HPLC grade acetonitrile and methanol were purchased from JT Baker, USA. Analytical grade acetic acid was obtained from QRëC, Malaysia. Nylon membrane filters 0.45 µm were purchased from Whatman, England.

HPLC analysis was performed using a Shimadzu-LC system (Shimadzu, Japan) equipped with an CBM-20A controller, LC-20AT pump, DGU-20A5 prominence degasser, SIL-20A auto sampler, SPD-20AV detector and CTO-10ASvp column oven.

Chromatographic separations were achieved using a Thermo Hypersil Gold column (250 mm x 4.6 mm I.D.: 5 µm). A security guard column (Zorbax Eclipse Plus) packed with a replaceable C-18 cartridge (12.5 mm x 4.6 mm ID.: 5 mm) was used to protect the analytical column. A reverse phase HPLC assay was carried out using an isocratic elution with a flow rate of 1.3 mL/minutes, a column temperature of 35°C, a mobile phase of acetonitrile and 2% v/v acetic acid (pH 2.60) (40% : 60% v/v) and a detection wavelength of 370 nm. The injection volume was 20 µL of each solutions. The total run time was 18.5 minutes for each injection. Data were acquired and processed with LC-Solution Software. Solvents and distilled water were prior filtered through a 0.45-µm nylon membrane by using a set of glass bottles with the aid of a vacuum pump (Fisherbrand FB 70155, Fisher Scientific, UK).

Twenty mg of a mixture of curcumin (containing mainly curcumin, demethoxycurcumin and bisdemethoxycurcumin) and 20 mg of quercetin were accurately weighed using a microbalance (Sartorius, MC5, Germany) and dissolved in 20 mL of HPLC grade methanol in a 20 mL volumetric flask. The mixtures were diluted to 320 µg/mL with HPLC grade methanol; and were then serially doubling diluted to 1.22 ng/mL. These solutions were used as calibration standards for the quantitative determinations of the...
Figure 1 Chemical structures of quercetin, and the curcuminoids: curcumin, demethoxycurcumin and bisdemethoxycurcumin.

limit of detection (LOD), the limit of quantification (LOQ) and the limit of linearity (LOL), and for the linear range analysis. Three quality control (QC) samples at concentrations of 3.75 µg/mL, 100 µg/mL and 160 µg/mL, respectively, were prepared from the stock solution. All solutions were stored in tightened screw cap bottles to avoid evaporation and were protected from light, and were kept in a refrigerator (4°C) for not more than two weeks.

Standard solutions with concentrations in the range from 1.22 ng/mL to 320 µg/mL were injected in duplicate into the HPLC unit. The LOD and LOQ of quercetin (QUE), bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC) and curcumin (CUR) were determined in a at the lower concentration range based on the signal to noise ratio. According to The United States Pharmacopeia (USP), the LOD and the LOQ are in terms of 2 or 3 times, and 10 times the noise level respectively. The LOL was determined by plotting a calibration curve (mean value of the peak areas against the concentrations) beginnings with the LOQ concentration and proceeding to the data point that deviated from the regression line. The coefficient of determination ($R^2 \geq 0.999$) was used as a guideline to evaluate the model fit of a regression equation.

Linear ranges for quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin included concentrations of 1.25, 5, 20, 40, 80, 140 and 200 µg/mL. Separate calibration curves were constructed for quercetin, bisdemethoxycurcumin demethoxycurcumin and curcumin by plotting the peak areas against the concentrations, and the methods were evaluated by determining the coefficient of determination ($R^2$). Unknown assay samples were quantified by referencing them to these calibration curves. QC samples (3.75, 100 and 160 µg/mL) were used to validate intra day and inter day accuracies and precisions. Intra day precisions and accuracies were determined by using a replicate analysis ($n = 6$) of the QC samples on the same day under the same analytical conditions. Inter day

3. Results

The LOD and the LOQ were determined based on the signal to noise (S/N) ratio, with the S/N > 3 and the S/N
> 10 for the LOD and the LOQ, respectively. The LODs of quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin were 0.00488, 0.62500, 0.07813 and 0.03906 µg/mL, respectively. The LOQs of quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin were 0.03906, 2.5000, 0.31250 and 0.07813 µg/mL, respectively (Table 1). The linearity for detecting quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin was tested against a mixture of calibration standards with concentration ranging from 1.22 ng/mL to 320 µg/mL. The LOL of each compound was determined from a separate calibration curve. Quercetin was linear up to 200 µg/mL, while bisdemethoxycurcumin, demethoxycurcumin and curcumin were linear up to 320 µg/mL.

Linear calibration curves in the range from 1.25 to 200 µg/mL were constructed for each compound by plotting the peak area against the concentration. The retention times and the peak areas are tabulated in Table 2. The values of R², the y-intercept and the slope for each compound’s calibration plot are shown in Table 1. A regression analysis of the data showed a linear relationship for quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin, with excellent R² values of 0.99993, 0.99984, 0.99985 and 0.99993 µg/mL, respectively.

The peaks of quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin were well separated at different retention times with resolutions of 32.195, 2.887 and 2.830 for quercetin-bisdemethoxycurcumin, bisdemethoxycurcumin-demethoxycurcumin and demethoxycurcumin-curcumin, respectively. No interferences or excipient peaks co eluted with the analytes were observed, indicating the method is selective and specific in relation to the medium and excipients used in this study (Fig. 2, Table 2).

Precision and accuracy data for the intraday and the inter-day variations for the three QC samples are summarized in Table 3. The RSD values for the intraday and the inter day precisions were < 1%. For the accuracy test, the intraday and the inter day accuracies ranges from 98.292% to 103.617%, confirming the accuracy of the method.

Robustness is a measure of the method’s capability to remain unaffected by small, but deliberate, variations in the method parameters [52]. The robustness parameters tested were the mobile phase’s composition, the concentration of acetic acid (pH effect), the flow rate and the column temperature. The results are tabulated in Table 4(a-d). The retention times for all four compounds due to variations in the parameters were significantly different compared to those for the normal parameters. The peak area for curcumin was not significantly different after changing the acetic acid concentration from 2% to 3%, but was significantly different after changing the concentration from 2% to 1%. Quercetin, bisdemethoxycurcumin and demethoxycurcumin were shown to have significant differences in their peak area when the concentration of acetic acid was changed. Changes in the acetonitrile’s composition and temperature were shown not to cause significant differences in quercetin’s peak areas, however significant differences were seen in curcumin, bisdemethoxycurcumin and demethoxycurcumin peak areas. Increasing or decreasing the flow rate by 0.1 mL/min from normal conditions significantly raised or reduced the values of the peak areas of quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin. Although changes in experimental conditions changed the retention time, the peak area and the values of the system’s suitability parameters, the four analyzed peaks were still well resolved from each other and from additional small peaks and showed good resolution in the tested parameters (Fig. 3).

The system suitability criteria were in accordance with the Centre for Drug Evaluation and Research (CDER) guidelines [53] and are summarized in Table 5. The mean values of the six replicate injections of 160 µg/mL QC standards

![Figure 2 Chromatograms of quercetin and curcuminoids. QUE, quercetin; BDMC, bisdemethoxycurcumin; DMC, demethoxycurcumin; CUR, curcumin.](http://www.journal.ac/039)

| Compounds | LOD (µg/mL) | LOQ (µg/mL) | LOL (µg/mL) | Regression analysis (1.25 — 200 µg/mL) |
|-----------|-------------|-------------|-------------|---------------------------------------|
|           | slope       | y-intercept | Coefficient of determination (R²) |
| QUE       | 0.00488     | 0.03906     | 200         | 70055.85913 | 1521.41433 | 0.99993 |
| BDMC      | 0.62500     | 2.50000     | 320         | 1807.72930 |  — 440.28180 | 0.99984 |
| CUR       | 0.07813     | 0.31250     | 320         | 10011.55795 | 40.13501    | 0.99985 |
| DMC       | 0.03906     | 0.07813     | 320         | 34176.44088 | 3645.08890  | 0.99993 |

LOD, limit of detection; LOQ, limit of quantification; LOL, limit of linearity; QUE, quercetin; BDMC, bisdemethoxycurcumin; DMC, demethoxycurcumin; CUR, curcumin.
Table 2 Retention times and responses data of calibration standards of QUE, BDMC, DMC, and CUR

| Concentration (µg/mL) | Retention time (n = 5)               | Peak area (n = 5)               |
|-----------------------|-------------------------------------|---------------------------------|
|                       | Mean (min) RSD (%)                  | Mean (min) RSD (%)              |
| QUE                   |                                     |                                 |
| 1.25                  | 3.970 0.117                         | 94937 0.676                     |
| 5                     | 3.972 0.066                         | 367965 0.739                    |
| 20                    | 3.972 0.041                         | 1438240 0.624                   |
| 40                    | 3.973 0.055                         | 2781685 0.508                   |
| 80                    | 3.972 0.029                         | 5582929 0.437                   |
| 140                   | 3.972 0.048                         | 9735618 0.866                   |
| 200                   | 3.972 0.053                         | 14073938 0.368                  |
| BDMC                  |                                     |                                 |
| 1.25                  | 13.823 0.308                        | 1859 1.611                      |
| 5                     | 13.840 0.095                        | 8843 1.181                      |
| 20                    | 13.842 0.093                        | 37086 1.089                     |
| 40                    | 13.843 0.087                        | 71560 1.044                     |
| 80                    | 13.846 0.117                        | 143659 1.073                    |
| 140                   | 13.846 0.134                        | 249462 1.835                    |
| 200                   | 13.849 0.060                        | 363457 0.850                    |
| DMC                   |                                     |                                 |
| 1.25                  | 15.214 0.227                        | 14705 0.273                     |
| 5                     | 15.229 0.096                        | 52692 0.540                     |
| 20                    | 15.230 0.074                        | 204602 0.665                    |
| 40                    | 15.232 0.073                        | 398446 0.436                    |
| 80                    | 15.237 0.099                        | 798153 0.867                    |
| 140                   | 15.236 0.120                        | 1384220 1.416                   |
| 200                   | 15.242 0.039                        | 2015583 0.158                   |
| CUR                   |                                     |                                 |
| 1.25                  | 16.708 0.199                        | 46645 0.856                     |
| 5                     | 16.718 0.077                        | 182515 0.901                    |
| 20                    | 16.719 0.061                        | 701982 0.700                    |
| 40                    | 16.720 0.064                        | 1358591 0.299                   |
| 80                    | 16.725 0.096                        | 2737751 0.423                   |
| 140                   | 16.725 0.108                        | 4749355 0.897                   |
| 200                   | 16.734 0.067                        | 6866971 0.313                   |

RSD, relative standard deviation; QUE, quercetin; BDMC, bisdemethoxycurcumin; DMC, demethoxycurcumin; CUR, curcumin.

were used to evaluate the retention time, the peak area, the resolutions for the analyte peaks, the tailing factor, the number of theoretical plates and the capacity factor. The results for the system suitability parameters are shown in Table 6. The RSD values for the tested parameters were < 1%, indicating the precision of the method. The tested parameters passed the criteria under the CDER guidelines except for the capacity factor value for quercetin (< 2) [53]. This is because the retention time of quercetin was quite fast and just 1 minute behind the solvent peak. However, the quercetin peak was well resolved from the solvent peak and from the front additional small peak.

The proposed method was applied to quantitatively detect the quercetin and curcuminoids in Chinese medicines.
Table 3  Precisions and accuracies for intraday and interday repetitions for the quantitative detection of QUE, BDMC, DMC and CUR

| Concentration (µg/mL) | Peak Response | Intra day* Precision (RSD, %) | Accuracy (%) | Peak Response | Inter day† Precision (RSD, %) | Accuracy (%) |
|-----------------------|---------------|--------------------------------|--------------|---------------|-------------------------------|--------------|
| QUE                   |               |                                |              |               |                               |              |
| 3.75 263151           | 0.432         | 99.589                         |              | 263350        | 0.323                         | 99.665       |
| 100 7064599           | 0.717         | 100.821                        |              | 7221470       | 0.646                         | 103.060      |
| 160 11221611          | 0.806         | 100.010                        |              | 11218287      | 0.968                         | 100.070      |
| BDMC                  |               |                                |              |               |                               |              |
| 3.75 6243             | 0.576         | 98.588                         |              | 6181          | 0.854                         | 97.669       |
| 100 182293            | 0.723         | 101.084                        |              | 186683        | 0.878                         | 103.513      |
| 160 286851            | 0.654         | 99.32746                       |              | 288040        | 0.805                         | 99.738       |
| DMC                   |               |                                |              |               |                               |              |
| 3.75 37700            | 0.635         | 100.310                        |              | 37687         | 0.466                         | 100.276      |
| 100 1010004           | 0.752         | 100.880                        |              | 1037410       | 0.078                         | 103.617      |
| 160 1590498           | 0.651         | 99.5584                        |              | 1594989       | 0.844                         | 99.569       |
| CUR                   |               |                                |              |               |                               |              |
| 3.75 129618           | 0.655         | 98.292                         |              | 129152        | 0.297                         | 97.929       |
| 100 3456218           | 0.732         | 101.022                        |              | 3545353       | 0.275                         | 103.606      |
| 160 5448675           | 0.711         | 99.5764                        |              | 5454012       | 0.829                         | 99.673       |

*Intra day repetitions for each concentration were analyzed on the same day. †Inter day repetitions for each concentration, were analyzed on six consecutive days. RSD, relative standard deviation; QUE, quercetin; BDMC, bisdemethoxycurcumin; DMC, demethoxycurcumin; CUR, curcumin.

Table 4(a) Robustness – change in organic composition

| System suitability | Compound | Change in the normal organic composition of acetonitrile: 2% acetic acid |
|--------------------|----------|------------------------------------------------------------------------|
|                    |          | (A) Normal condition Mean (n = 6) RSD (%)                              |
| Retention time, tR (minutes) |          | (B) 38% : 62% v/v Mean (n = 6) RSD (%)                               |
| QUE                | 3.993    | 0.690                                                                  |
| BDMC               | 13.951   | 0.342                                                                  |
| DMC                | 15.340   | 0.291                                                                  |
| CUR                | 16.829   | 0.245                                                                  |
| Peak area          |          | (C) 42% : 58% v/v Mean (n = 6) RSD (%)                               |
| QUE                | 68.53044 | 0.433                                                                  |
| BDMC               | 167.417  | 0.647                                                                  |
| DMC                | 94.0836  | 0.404                                                                  |
| CUR                | 330.2593 | 0.236                                                                  |
| Resolution, R      |          | (Continued)                                                            |
| QUE                | 32.498   | 0.379                                                                  |
| BDMC               | 2.908    | 0.208                                                                  |
| DMC                | 2.850    | 0.237                                                                  |
| CUR                | 1.371    | 0.254                                                                  |
| Tailing factor, Tf |          | (Continued)                                                            |
| QUE                | 1.533    | 0.364                                                                  |
| BDMC               | 1.160    | 0.484                                                                  |
| DMC                | 1.094    | 0.094                                                                  |
| CUR                | 1.371    | 0.115                                                                  |
| System suitability | Compound | Change in the normal organic composition of acetonitrile: 2% acetic acid |
|--------------------|----------|****************************************************************************|
|                    |          | (A) Normal condition | (B) 38% : 62% v/v | (C) 42% : 58% v/v |
|                    |          | Mean (n = 6) | RSD (%) | Mean (n = 6) | RSD (%) | Mean (n = 6) | RSD (%) |
| Theoretical plate, N | QUE      | 8752.133   | 1.463   | 8857.791   | 0.312   | 8520.171   | 0.238   |
|                      | BDMC     | 15931.889  | 1.147   | 16311.011  | 0.058   | 16303.130  | 0.103   |
|                      | DMC      | 14298.287  | 1.761   | 16569.474  | 1.029   | 14210.321  | 0.233   |
|                      | CUR      | 16000.049  | 1.120   | 16543.754  | 0.535   | 15157.508  | 0.340   |
| Peak area            | QUE      | 0.680      | 0.344   | 0.777      | 0.906   | 0.601      | 0.327   |
|                      | BDMC     | 4.878      | 0.020   | 3.800      | 0.209   | 3.800      | 0.209   |
|                      | DMC      | 5.463      | 0.232   | 7.214      | 1.592   | 4.247      | 0.206   |
|                      | CUR      | 6.097      | 0.253   | 8.038      | 0.481   | 4.729      | 0.209   |

Table 4(b) Robustness – change in acetic acid concentration

| System suitability | Compound | Change in the acetic acid concentration (% v/v) |
|--------------------|----------|****************************************************************************|
|                    |          | (A) Normal condition | (B) 1.0% (pH 2.73) | (C) 3.0% (pH 2.48) |
|                    |          | Mean (n = 6) | RSD (%) | Mean (n = 6) | RSD (%) | Mean (n = 6) | RSD (%) |
| Retention time, t0 (minutes) | QUE      | 3.972      | 0.175   | 4.054      | 0.064   | 3.893      | 0.071   |
|                      | BDMC     | 13.868     | 0.310   | 14.549     | 0.086   | 13.177     | 0.167   |
|                      | DMC      | 15.255     | 0.265   | 16.017     | 0.085   | 14.542     | 0.153   |
|                      | CUR      | 16.743     | 0.213   | 17.590     | 0.084   | 16.028     | 0.141   |
| Peak area            | QUE      | 7039483    | 0.562   | 6966950    | 0.525   | 6952833    | 0.630   |
|                      | BDMC     | 180475     | 0.541   | 176885     | 0.575   | 152439     | 0.895   |
|                      | DMC      | 1000716    | 0.736   | 987128     | 0.551   | 956266     | 0.670   |
|                      | CUR      | 3433379    | 0.754   | 3428762    | 0.533   | 3428762    | 0.558   |
| Resolution, R        | QUE      | 3.166      | 0.077   | 3.136      | 0.215   | 1.370      | 0.110   |
|                      | BDMC     | 32.327     | 0.172   | 33.254     | 0.244   | 31.950     | 0.268   |
|                      | DMC      | 2.900      | 0.370   | 2.974      | 0.303   | 3.033      | 0.527   |
|                      | CUR      | 2.840      | 0.429   | 2.904      | 0.339   | 2.966      | 0.608   |
| Tailing factor, T0   | QUE      | 1.366      | 0.077   | 1.364      | 0.215   | 1.370      | 0.110   |
|                      | BDMC     | 1.493      | 1.377   | 1.463      | 0.331   | 1.060      | 0.139   |
|                      | DMC      | 1.160      | 1.075   | 1.137      | 0.103   | 1.325      | 0.823   |
|                      | CUR      | 1.085      | 0.148   | 1.092      | 0.050   | 1.083      | 0.108   |
| Theoretical plate, N | QUE      | 8711.993   | 0.267   | 8877.546   | 0.460   | 8548.948   | 0.269   |
|                      | BDMC     | 15740.557  | 0.397   | 16067.808  | 0.689   | 16308.146  | 0.664   |
|                      | DMC      | 14041.181  | 0.701   | 14691.580  | 0.675   | 14241.082  | 1.031   |
|                      | CUR      | 15793.019  | 0.472   | 16098.239  | 0.701   | 15531.342  | 0.811   |
| Capacity factor, k'  | QUE      | 6.056      | 1.783   | 0.680      | 1.484   | 0.610      | 0.803   |
|                      | BDMC     | 4.798      | 1.202   | 5.036      | 0.658   | 4.449      | 0.511   |
|                      | DMC      | 5.333      | 0.988   | 5.637      | 0.698   | 5.014      | 0.478   |
|                      | CUR      | 6.016      | 1.416   | 6.295      | 0.628   | 5.629      | 0.443   |  

(Continued)
### Table 4(c) Robustness – change in flow rate

| System suitability | Compound | (A) Normal condition | | | (B) 1.2 mL/minutes | | | (C) 1.4 mL/minutes | |
|-------------------|----------|---------------------|-----------------|---------------------|-----------------|-----------------|-----------------|-----------------|
|                   |          | Mean (n = 6)        | RSD (%)         | Mean (n = 6)        | RSD (%)         | Mean (n = 6)    | RSD (%)         |
| Retention time, $t_R$ (minutes) | QUE      | 3.972               | 0.175           | 4.291               | 0.105           | 3.696           | 0.130           |
|                   | BDMC     | 13.868              | 0.310           | 14.953              | 0.321           | 12.909          | 0.333           |
|                   | DMC      | 15.255              | 0.265           | 16.442              | 0.284           | 14.235          | 0.279           |
|                   | CUR      | 16.743              | 0.213           | 18.038              | 0.262           | 15.668          | 0.298           |
| Peak area         | QUE      | 7039483             | 0.562           | 7606272             | 0.662           | 6530571         | 0.497           |
|                   | BDMC     | 180475              | 0.541           | 194216              | 0.753           | 167111          | 1.593           |
|                   | DMC      | 1000716             | 0.736           | 1078076             | 0.714           | 928707          | 1.345           |
|                   | CUR      | 3433379             | 0.754           | 3700134             | 0.690           | 3185325         | 1.198           |
| Resolution, $R$   | QUE      | -                   | -               | -                   | -               | -               | -               |
|                   | BDMC     | 32.327              | 0.172           | 32.779              | 0.199           | 32.047          | 0.928           |
|                   | DMC      | 2.900               | 0.370           | 2.921               | 0.608           | 2.936           | 2.014           |
|                   | CUR      | 2.840               | 0.429           | 2.864               | 0.723           | 2.868           | 1.647           |
| Tailing factor, $T_f$ | QUE      | 1.366               | 0.077           | 1.360               | 0.183           | 1.371           | 0.287           |
|                   | BDMC     | 1.493               | 1.377           | 1.490               | 1.891           | 1.539           | 1.614           |
|                   | DMC      | 1.160               | 1.075           | 1.157               | 1.447           | 1.181           | 2.364           |
|                   | CUR      | 1.085               | 0.148           | 1.081               | 0.101           | 1.087           | 0.207           |
| Theoretical plate, $N$ | QUE      | 8711.993            | 0.267           | 9148.347            | 0.429           | 8249.430        | 0.420           |
|                   | BDMC     | 15740.557           | 0.397           | 16035.103           | 1.342           | 15696.046       | 2.851           |
|                   | DMC      | 14041.181           | 0.701           | 14374.944           | 1.036           | 13420.220       | 0.844           |
|                   | CUR      | 15793.019           | 0.472           | 16216.013           | 1.854           | 15379.165       | 2.364           |
| Capacity factor, $k'$ | QUE      | 0.656               | 1.783           | 0.661               | 0.832           | 0.627           | 0.762           |
|                   | BDMC     | 4.798               | 1.202           | 4.780               | 0.942           | 4.750           | 3.067           |
|                   | DMC      | 5.351               | 0.661           | 5.355               | 0.497           | 5.350           | 2.846           |
|                   | CUR      | 5.966               | 0.632           | 5.985               | 0.500           | 5.862           | 0.427           |

### Table 4(d) Robustness – change in column temperature

| System suitability | Compound | (A) Normal condition | | | (B) 30°C | | | (C) 40°C | |
|-------------------|----------|---------------------|-----------------|-----------------|----------------|----------------|----------------|----------------|
|                   |          | Mean (n = 6)        | RSD (%)         | Mean (n = 6)        | RSD (%)         | Mean (n = 6)    | RSD (%)         |
| Retention time, $t_R$ (minutes) | QUE      | 3.956               | 0.031           | 4.063               | 0.074           | 3.861           | 0.162           |
|                   | BDMC     | 13.673              | 0.070           | 14.647              | 0.174           | 12.810          | 0.268           |
|                   | DMC      | 15.037              | 0.064           | 15.980              | 0.153           | 14.167          | 0.236           |
|                   | CUR      | 16.502              | 0.064           | 17.423              | 0.143           | 15.657          | 0.196           |
| Peak area         | QUE      | 7628483             | 0.252           | 7620525             | 0.254           | 7633341         | 0.259           |
|                   | BDMC     | 196493              | 0.261           | 202870              | 0.253           | 172397          | 0.136           |
|                   | DMC      | 1091099             | 0.300           | 1124567             | 0.281           | 1058404         | 0.205           |
|                   | CUR      | 3738544             | 0.244           | 3836306             | 0.285           | 3643910         | 0.196           |
| Resolution, $R$   | QUE      | -                   | -               | -                   | -               | -               | -               |
|                   | BDMC     | 31.946              | 1.437           | 32.560              | 0.233           | 31.471          | 0.267           |
|                   | DMC      | 2.872               | 1.359           | 2.698               | 0.334           | 3.155           | 0.481           |
|                   | CUR      | 2.829               | 0.575           | 2.718               | 0.305           | 3.106           | 0.559           |
such as plant granule extracts, tablets and pills. The results of 19 samples are summarized in Table 7. In the tested samples, BDMC had the highest concentration compared to the other two curcuminoids tested (DMC and CUR), and was found in the formulations of granule extracts, tablets and pills (such as samples 12, 13, 15, 16, 18 and 19) (Table 7). The preference of BDMC over CUR in the medicine might be due to its strong biological properties, which

### Table 5 System suitability parameters, calculation formula and recommendations

| Parameter                | Formula                                    | Recommendation       |
|--------------------------|--------------------------------------------|----------------------|
| Precision                | \( \text{RSD} = \frac{S}{x} \times 100 \) | \( \text{RSD} \leq 1\% \text{ for } n \geq 5 \) |
| Resolution, \( R \)      | \( R = \frac{(t_{R2} - t_{R1})}{1/2(t_{w1} - t_{w2})} \) | \( R > 2 \) |
| Tailing factor, \( T_f \) | \( T_f = \frac{W_x}{2f} \)               | \( T_f \leq 2 \) |
| Theoretical plates, \( N \) | \( N = 16(t_s/t_w)^2 \)               | \( N \geq 2000 \) |
| Capacity factor, \( k' \) | \( k' = \frac{(t_R - t_0)}{t_0} \)           | \( k' > 2 \) |

S, standard deviation; \( \bar{x} \), mean of the data; \( t_s \), retention time of analyte 1; \( t_w \), peak width measured to the baseline of the extrapolated straight sides to baseline; \( W_x \), width of the peak determined at either 5\% (0.05) or 10\% (0.10) from the baseline of the peak height; \( f \), distance between peak maximum and peak front at \( W_x \); \( t_0 \), elution time of the void volume or non retained components.

### Table 6 System suitability testing

| Parameter                | QUE Mean (n = 6) | RSD (%) | QUE Mean (n = 6) | RSD (%) | QUE Mean (n = 6) | RSD (%) | QUE Mean (n = 6) | RSD (%) | BDMC Mean (n = 6) | RSD (%) | DMC Mean (n = 6) | RSD (%) | CUR Mean (n = 6) | RSD (%) |
|--------------------------|------------------|---------|------------------|---------|------------------|---------|------------------|---------|------------------|---------|-----------------|---------|-----------------|---------|
| Retention time, \( t_s \) | 3.970            | 0.021   | 13.840           | 0.027   | 15.230           | 0.025   | 16.723           | 0.021   | 15.230           | 0.025   | 16.723           | 0.021   |
| Peak area                | 11221611         | 0.806   | 286851           | 0.654   | 1590498         | 0.651   | 5448675         | 0.711   | 5448675         | 0.711   | 5448675         | 0.711   |
| Resolution, \( R \)      | -                | -       | 32.195           | 0.321   | 2.887           | 0.364   | 2.830           | 0.370   | 2.830           | 0.370   | 2.830           | 0.370   |
| Tailing factor, \( T_f \) | 1.369            | 0.108   | 1.501            | 0.261   | 1.165           | 0.144   | 1.081           | 0.051   | 1.081           | 0.051   | 1.081           | 0.051   |
| Theoretical plate, \( N \) | 8803.785         | 0.359   | 15552.398        | 0.865   | 13763.145        | 0.646   | 15568.252        | 0.910   | 15568.252        | 0.910   | 15568.252        | 0.910   |
| Capacity factor, \( k' \) | 0.684            | 0.846   | 4.870            | 0.415   | 5.460           | 0.406   | 5.093           | 0.391   | 5.093           | 0.391   | 5.093           | 0.391   |

RSD, relative standard deviation; QUE, quercetin; BDMC, bisdemethoxycurcumin; DMC, demethoxycurcumin; CUR, curcumin. N, number of theoretical plates; \( k' \), capacity factor; Mean of six replicate injections of quality control (QC) standard of 160 \( \mu \)g/mL.
its use as a cure for diseases or as a supplement for certain purposes. Quercetin was found in most of the tested samples, indicating that this compound is common and useful for treatment. Fig. 4 shows the chromatograms for the quercetin and the curcuminoids found in the tested samples.

4. Discussion

The HPLC method was developed by optimization of the mobile phase conditions so that quercetin, bisdemethoxycurcumin, demethoxycurcumin and curcumin peaks could be simultaneously detected by using the same solvent system and an isocratic method. The flow rate, acetic acid concentration and column temperature were varied to determine the chromatographic conditions giving the best separation and the shortest analysis time. UV visible spectrophotometry in the wavelength from 200 to 500 nm was used for the detection of quercetin and curcuminoids; 370 nm was chosen as appropriate wavelength for the analysis of quercetin and curcumin derivatives.

The retention times for quercetin (3.97 minutes), bisdemethoxycurcumin (13.84 minutes), demethoxycurcumin (15.23 minutes) and curcumin (16.72 minutes) were reasonable because the method is simple and general. The chromatograph peaks for mixtures of curcumin were identified based on their percentages in the mixtures. Most of the commercially available curcumin/turmeric products contain mixtures of curcumin, demethoxycurcumin and bisdemethoxycurcumin. Among these, curcumin (46% — 72%) is the major compound, followed by demethoxycurcumin (11% — 28%) and bisdemethoxycurcumin (3% — 14%). All four analyte peaks were well separated from each other and from small additional peaks.

The linear ranges of quercetin (0.039 — 200 μg/mL), bisdemethoxycurcumin (2.500 — 320 μg/mL), demethoxycurcumin (0.313 — 320 μg/mL) and curcumin (0.078 — 320 μg/mL) are suitable for the analysis of most the pharmaceutical products, containing the compounds and for the analysis of crude herbs. The low LOD and LOQ values indicate that the method provides adequate sensitivity. The R² values > 0.999 for the regression model for the calibration curves confirm the good linearity of the method.

The accuracies ranged from 98.292% — 103.617%, and the precisions were less than 1% which indicate that the proposed method is well validated and suitable for quantitatively detecting curcuminoids and quercetin simultaneously in pharmaceutical products, herb materials and various turmeric and quercetin containing products.

System suitability testing is important to ensure the performance of the system before and during the analysis. As defined in the United States Pharmacopeia/National Formulary (USP/NF) [54] system suitability parameters were established as a direct result of the ruggedness and the robustness of the experiments. The system suitability testing proved that the proposed method will allow the separation of all four analytes and will produce satisfactory peak shapes.
Table 7 Concentration of QUE, CUR, DMS and BDMC in Chinese medicines

| No | Chinese medicine       | Type                | Concentration (mean ± S.D.) (µg/100 mg) |
|----|------------------------|---------------------|-----------------------------------------|
|    |                        | QUE                | BDMC         | DMC         | CUR         |
| 1  | Gao liang jiang (高良姜) | Single plant granule extract | 0.7532      | N.D         | 134.8739    | 0.5270      |
| 2  | Jin qian cao (金钱草)   | Single plant granule extract | 4.0618      | N.D         | N.D         | 0.8263      |
| 3  | Yu jin (莪术)           | Single plant granule extract | 0.3195      | 69.1060     | 27.2286     | 27.1020     |
| 4  | E su (莪术)             | Single plant granule extract | 0.5983      | 79.5922     | 42.6982     | 8.6812      |
| 5  | Jiang huang (姜黄)      | Single plant granule extract | 3.6523      | N.D         | 933.8122    | 796.0621    |
| 6  | Yu xing cao (鱼腥草)    | Single plant granule extract | 1.7930      | N.D         | N.D         | 1.3424      |
| 7  | Ting li zi (薏苡子)     | Single plant granule extract | 1.3604      | N.D         | N.D         | N.D         |
| 8  | Tu si zi (菟丝子)       | Single plant granule extract | 3.9300      | N.D         | N.D         | N.D         |
| 9  | Di yu (地榆)            | Single plant granule extract | 0.8962      | N.D         | N.D         | N.D         |
| 10 | Kui hua (槐花)          | Single plant granule extract | 311.0307    | N.D         | N.D         | N.D         |
| 11 | Sang ju yin (桑菊饮)    | Formulation granule extract | 0.7402      | N.D         | 0.3558      | 0.2537      |
| 12 | Chai hu su gan san (柴胡疏肝散) | Formulation granule extract | 0.2029      | 126.8843    | 48.3408     | 1.6417      |
| 13 | Xiao yao san (逍遥散)   | Formulation granule extract | 0.4991      | 97.9203     | 2.5534      | 0.4301      |
| 14 | Long dan xie gan tang (龙胆泄肝汤) | Formulation granule extract | 11.1482     | 5.2111      | 1.2817      | 0.1236      |
| 15 | Sang ju gan mao pian (桑菊感冒片) | Tablet           | 17.3489     | 173.6155    | 2.8579      | N.D         |
| 16 | Dan zhi xiao yao pian (丹栀逍遥片) | Tablet           | 7.8101      | 135.1892    | 1.0883      | 0.2624      |
| 17 | Long dan xie gan pian (龙胆泄肝片) | Tablet           | N.D         | 5.5352      | 6.7428      | 0.2378      |
| 18 | Bu zhong yi qi (补中益气) | Tablet           | 0.9052      | 623.1338    | 5.9485      | 0.5964      |
| 19 | Xiao yao wan (逍遥丸)   | Pill               | 12.015      | 79.7951     | 11.7471     | 1.1516      |

‘n = 3; N.D, not detected; QUE, quercetin; BDMC, bisdemethoxycurcumin; DMC, demethoxycurcumin; CUR, curcumin.

Figure 4 Chromatograms for Chinese medicinal plant extracts (a) containing quercetin and (b) containing curcuminoids. QUE, quercetin; BDMC, bisdemethoxycurcumin; DMC, demethoxycurcumin; CUR, curcumin.

5. Conclusions

A simple isocratic RP-HPLC method with UV detection has been developed for simultaneous detection of quercetin, curcumin, demethoxycurcumin and bisdemethoxycurcumin. The analytes were well separated and detected
within 19 minutes. This method was validated for specificity, linearity, precision, accuracy and robustness as per ICH guidelines. The data showed good selectivity and sensitivity, a wide linear range, precision and accuracy. The method was sensitive to HPLC conditions; that is, changes in the mobile phase's composition, the pH, the column temperature and the flow rate affected the retention time and response, but did not affect the separation of the compounds. In addition, each parameter showed good repeatability of the retention time and response. In conclusion, the proposed method is simple, easy and cost effective, no specific solvent is involved and it utilizes common HPLC instruments with UV detectors. Hence, this UV-HPLC method is suitable for routine analysis of quercetin and curcuminoid formulations or products.

Conflict of interest

The authors declare that there are no conflict of interest.

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