Comprehensive Metabolic Profiling of Modified Gegen Qinlian Decoction by Ultra-high-Performance Liquid Chromatography-Diode Array Detection-Q-Exactive-Orbitrap-Electrospray Ionization-Mass Spectrometry/Mass Spectrometry and Application of High-Performance Thin-layer Chromatography for its Fingerprint Analysis

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Abstract

Objective: Gegen Qinlian decoction (GQD) is a classical traditional Chinese medicine formulation which has been used for almost 2000 years. At Guang’anmen Hospital, Beijing, a modified GQD version (mGQD) with seven instead of four herbal ingredients has been applied to treat Type 2 diabetes. Quality control is a crucial prerequisite for the therapeutic application of herbal medicines. For the identification of products derived from classical GQD, the Chinese Pharmacopeia requires the analysis of only three marker compounds. Because mGQD is a more complex mixture containing seven herbs and hundreds of constituents, the pharmacopoeia method for GQD is inadequate.

Materials and Methods: A more comprehensive characterization of the formula’s constituents has been developed using ultra-high-performance liquid chromatography-diode array detection (UHPLC-DAD)-Q-Exactive-mass spectrometry (MS) in electrospray ionization positive and negative mode. Moreover, a new method for the fingerprint analysis of mGQD via high-performance thin-layer chromatography (HPTLC) has been established.

Results: Altogether, 91 compounds have been assigned to their originating plants and 84 substances were identified either by comparison with authentic references or with data from the literature. The HPTLC method is based on the application of two different mobile phases and is able to detect both lipophilic and hydrophilic constituents of mGQD.

Conclusions: The modified GQD was extensively characterized by UHPLC combined with DAD and Q-Exactive Orbitrap high-resolution MS detection, leading to the assignment and identification of compounds present in the decoction. In addition, a new method for the fingerprint analysis of the mGQD using HPTLC was established, which allows fast and simple identification of the herbal ingredients in the mixture.

Keywords: Fructus jujubae, HPTLC, Modified gegen qinlian decoction, Radix et rhizaoma glycyrrhizae praeparata cum melle, Radix puerariae lobatae, Radix scutellariae, Rhizoma coptidis, Rhizoma anemarrhenae, Rhizoma zingiberis, UHPLC-DAD-Q-Exactive-MS
**Introduction**

Gegen Qinlian decoction (GQD) is a classical traditional Chinese medicine (TCM) formulation first documented by Zhang Zhongjing in “Shang Han Lun (Treatise on Febrile Diseases)” at the end of the East Han dynasty (ca. AD 220).[1] The traditional formula contains four herbs, namely Ge-gen (Radix Puerariae lobatae, RP, *Pueraria montana* var. *lobata* (Willd.) Sanjappa and Pradeep), Huangqin (Radix Scutellariae, RS, *Scutellaria baicalensis* Georgi), Huanglian (Rhizoma Coptidis, RC, *Coptis chinensis* Franch., C. deltoidea C. Y. Cheng et P. K. Hsiao or *Coptis teeta* Wall.,) and Zhigancao (Radix et Rhizoma Glycyrrhizae Praeparata cum Melle, RRGP, *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat., or *Glycyrrhiza glabra* L.) in the ratio of 8:3:3:2.[2] GQD is known to have a positive effect on the maintenance and recovery of gut microbial homeostasis and is nowadays clinically used to treat diarrhea, acute enteritis, bacterial dysentery, or *Helicobacter pylori* infection-derived gastritis.[3,4] During the last decade, several studies have demonstrated the positive impact of GQD and its contained herbs in treating type 2 diabetes mellitus (T2DM) by increasing insulin sensitivity, improving glucose metabolism, and protecting pancreatic β-cells.[5,6] There is also evidence that the anti-diabetic mechanism of GQD is related to the modulation of the gut microbiota.[1] At Guang’annen Hospital in Beijing (China Academy of Chinese Medical Sciences, CAMC), GQD and a modified version of GQD (mGQD) have been used for many years for preventing and treating T2DM. In mGQD, the following three herbs have been added to the original composition in order to achieve better therapeutic results: *Zhimu* (Rhizoma Anemarrhenae, RA, *Anemarrhena asphodeloides* Bge.), *Wuweizi* (Fructus Schisandrae chinensis, FS, *Schisandra chinensis* (Turcz.) Baill.), and *Ganjiang* (Rhizoma Zingiberis, RZ, *Zingiber officinale* Rosc.). In the theory of TCM, *Zhimu* has the effect of clearing heat and nourishing yin, *Wuweizi* can benefit *qi* and regenerate body fluid, and *Ganjiang* is used for warming spleen and stomach. The addition of these three herbs to the original formula is supposed not only to strengthen the hypoglycemic effect, but also to reduce side effects such as the impairment of spleen and stomach function.

The chemical composition of the classical GQD formula and of the individual herbs contained in mGQD has been described previously.[7-9] However, no work has been done on the analysis of the chemical composition of mGQD. Herbal formulations, in particular TCM formulas, are highly complex mixtures of natural products that may be significantly affected or changed by the quality of the used starting material, but also by processing (*paozhi*). In order to guarantee its quality, safety, and efficacy in clinical use, it is important to get deeper knowledge about the composition and identity of the chemical constituents contained in mGQD. This can be achieved by a detailed analysis and identification of the compounds contained in the therapeutically used form. Moreover, it is necessary to establish a simple and reliable method for the quality control of mGQD batches. For the identification of classical Gegen Qinlian formulations (tablets and pills), only berberine, puerarin, and baicalin are used as reference standards according to the Chinese Pharmacopoeia. Compounds derived from licorice are completely neglected. The quantitative analysis of the tablets refers to puerarin in Radix Puerariae und berberine in Rhizoma Coptidis and does not consider Radix Scutellariae or Radix et Rhizoma Glycyrrhizae Praeparata cum Melle.[10] However, since the bioactive constituents, pharmacological targets, and mechanisms of action of the formula are not yet fully elucidated, it is highly questionable whether and to which degree two or three markers are sufficient for characterization. Moreover, the therapeutic effects of herbal formulations are most likely not caused by the activity of two or three single compounds, but are supposedly based on multitarget effects in which a wide spectrum of constituents and also synergistic effects may be involved. For these reasons, quality control based on only one or a few single markers may be not sufficient in the case of complex herbal formulations.[11,12]

In this study, we have established two methods for fingerprint-based analysis of mGQD. The first one aims at the comprehensive untargeted characterization of secondary metabolites contained in mGQD by ultra-high-performance liquid chromatography-diode array detection (UHPLC-DAD)-Q-Exactive- mass spectrometry (MS). The second method applies high-performance thin-layer chromatography (HPTLC) for a fast and effective quality control of mGQD.

**Materials and Methods**

**Samples**

Ready-to-use instant granules of mGQD were produced exclusively for clinical research purposes at the Guang’annen Hospital Beijing by Jiangyin Tianjiang Pharmaceutical Co., Ltd (Jiangsu, China) (Batch number 1506339/052018). In brief, the single herbs were mixed, boiled with water, filtered, concentrated under reduced pressure, and granulated using maltodextrin as carrier. The single herbs Radix Coptidis, Radix et Rhizoma Glycyrrhizae praeparata cum Melle, Radix Puerariae lobatae, Radix Scutellariae, Fructus Schisandrae, Rhizoma Anemarrhenae, and Rhizoma Zingiberis were purchased from Plantasia GmbH – Großhandel für asiatische Heilkräuter (Oberndorf bei Salzburg, Austria). Voucher specimens are kept at the Institute of Pharmaceutical Sciences, Section of Pharmacognosy, University of Graz (Specimen no. XG/RC012017, XG/G012017, XG/RS012017, XG/FS052017, XG/RA052017, and XG/ RZ052017).

**Chemicals and reagents**

The phytoproof® reference substances (all pharmaceutical primary reference substance grade) coptisine chloride, genistin, 6-gingerol, 6-shogaol, isoliquiritigenin, isoliquiritin, liquiritigenin, liquiritin, mangiferin, puerarin, sarsasapogenin, schisandrin A, schisandrin B, soyasaponin Bb, and tinosaponin AIII were purchased from PhytoLab GmbH and Co.
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were purchased ≥99.9%, p.a., ≥99.5% p.a., ACS, ISO), Ammonium glycyr rhizate (80.6%) was obtained from the European Directorate for the Quality of Medicines (Strasbourg Cedex, France).

n-Hexane (≥95%, for synthesis), dichloromethane (ROTISOLV® HPLC grade), methanol (ROTIPURAN® ≥99.9%, p.a., ACS, ISO), ethyl acetate (≥99.5%, Ph. Eur., extra pure), n-butanol (ROTIPURAN® ≥99.5% p.a., ACS, ISO), ethanol (96%), toluene (ROTISOLV, HPLC grade), formic acid (≥98%, p.a., ACS), acetic acid (100%, p.a.), sulfuric acid (95-98%, Ph. Eur.), and vanillin (≥99%) were all bought from Carl Roth GmbH (Karlsruhe, Germany). Water was purified using an ELGA Purelab Prima system (Veolia Water Technologies Deutschland GmbH, Celle, Germany). Iron (III) chloride (p.a.) was obtained from Merck KGaA (Darmstadt, Germany). HPLC-grade acetonitrile (HiPerSolv CHROMANORM®) and water (HiPerSolv, CHROMANORM®) were purchased from VWR International SAS (Fontenay Sous Bois, France). Formic acid (eluent additive for LC-MS) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sample preparation
Extracts of mGQD and its contained single herbs were prepared in two different ways.

Classical decoction
Decoctions of the formulation and each contained single herb were prepared as follows: for the single herbs, 50 g dried plant material was immersed in 500 ml cold water for 1 h and decocted by boiling for 50 min. The solution was filtered, and the filtrate was concentrated under reduced pressure to approximately 100 ml extract (Heidolph Instruments, Schwabach, Germany). To obtain mGQD mixture in liquid form, the granules were prepared following the manufacturer’s instructions: four sachets were dissolved in 300-ml boiling water to obtain the dose for 1 day. From each decoction, 10 ml was withdrawn, lyophilized (VirTis BenchTop Pro freeze dryer, SP Scientific, Warminster, PA USA), and analyzed as total decoction. The remaining part was transferred to a separation funnel and subsequently extracted with 3 ml × 30 ml n-hexane, dichloromethane, ethyl acetate, and n-butanol. These subextracts were again concentrated under reduced pressure and dried under nitrogen flow, while the water residues were frozen and lyophilized. All dried extracts were stored at −20°C until use.

Accelerated solvent extraction
The herbs were ground into powders (0.25 mm sieve; ZM100 Retsch mill, Retsch GmbH, Haan, Germany), mixed 4:1 (w: w) with diatomaceous earth (Thermo Fisher Scientific, Waltham, MA, USA), and extracted successively with n-hexane, dichloromethane, and methanol (Dionex™ ASE™ 200, Thermo Fisher Scientific, Waltham, MA, USA). For the mGQD formula, 15 g granules were directly mixed with diatomaceous earth and extracted without former grinding. The parameters for accelerated solvent extraction (ASE) were set as follows: preheat: 0; heat: 5 min; static: 5 min; flush 40%; purge: 60 s; cycles: 3; pressure: 69 bar; temperature: n-hexane: 72°C, dichloromethane: 44°C, and methanol: 68°C. The extracts were dried under nitrogen flow and stored at −20°C until use.

For LC-MS and HPTLC analysis, the dried extracts were dissolved in methanol p.a. at a concentration of 10 mg/ml, sonicated for 15 min, and filtered through a 0.2 µm filter (Syringe filter RC, Carl Roth, Karlsruhe, Germany). The reference compounds were dissolved in methanol p.a. at a concentration of 1 mg/ml.

Ultra-high-performance liquid chromatography-diode array detection-high-resolution mass spectrometry analyses and metabolite identification
UHPLC-DAD-high-resolution MS analyses of the decoctions prepared from mGQD and the contained single-herbal ingredients were performed on an Ultimate 3000 UHPLC hyphenated with a Q Exactive™ hybrid quadrupole Orbitrap mass spectrometer (Thermo Scientific) in both ESI positive and negative mode. The separation was carried out using an ACQUITY BEH C18 column (1.7 µm, 2.1 mm × 150 mm, Waters Corp., Milford, MA, USA) protected by a VanGuard™ Precolumn (BEH C18 1.7 µm, 2.1 mm × 5 mm, Waters Corp.) as stationary phase. The mobile phase consisted of water +0.1% formic acid (A) and acetonitrile +0.1% formic acid (B). The gradient was set as follows: 0–25 min, 5%–30% B in A; 25–40 min, 30%–100% B in A; 40–45 min, 100% B in A; 45–46 min, 100%–5% B in A; 46–55 min, 5% B in A. Flow rate was 0.45 ml/min and column temperature was 45°C. The mass spectrometer was run in the HESI positive and negative modes using the following parameters: probe heater temperature 400°C; capillary temperature 350°C; spray voltage 4.0 kV for positive and 3.5 kV for negative ion mode; sheath gas flow 50 arbitrary units; auxiliary gas flow 15 arbitrary units; resolution: 70,000 (full MS) and 17,500 (data-dependent MS). The injection volume was 1 µl for reference solutions and 0.5 µl for sample and blank solutions.

Data processing was performed by Compound Discoverer 2.1 software (Thermo Fisher Scientific, Waltham, MA, USA). The workflow was set as follows: retention time window for data processing: 0–50 min; total intensity threshold: 500,000; alignment was performed by using the adaptive curve model; maximum RT shift: 2 min; and maximum mass tolerance: 5 ppm. For detecting and grouping unknown compounds, an S/N threshold of 3, a minimum intensity threshold of 50,000, and a RT tolerance of 0.3 min were applied. For gap filling, the S/N threshold was set to 20. The result file was exported to Microsoft Excel 2016.

The resulting frame reports consisted of all features (i.e., m/z–retention time pairs) detected in the different samples and their respective peak areas. Peaks with areas >2,000,000 were included for identification. Peaks with retention time >35 min
were excluded from metabolite identification because they were also present in chromatograms from blank solutions and therefore considered as analytical artifacts. Metabolites were identified by comparing retention time, precursor monoisotopic mass, and MS/MS fragment ion masses with authentic reference, or by comparing molecular formulas calculated from the exact mass as well as MS/MS fragmentation patterns with existing data from databases and literature data.

**High-performance thin-layer chromatography analyses**

HPTLC analysis was carried out using HPTLC glass plates coated with silica gel 60 F254 (coating layer 200 μm, particle size 10–12 μm, and pore size 60 Å) (Merck KGaA, Darmstadt, Germany) as stationary phase. Samples were applied to the HPTLC plates as lines with 8 mm length in a distance of 5 mm to the lower edge by a CAMAG Automatic TLC Sampler ATS 4 (CAMAG Chemie-Erzeugnisse und Adsorptionstechnik AG, Muttenz, Switzerland). The applied amount of each sample solution was 5 μl. Two different mobile phase systems were developed for the detection of the main constituents of each single herb and of the formulation. For samples containing polar compounds (ASE methanol fraction, total decoction, decoction ethyl acetate fraction, n-butanol fraction, and water residue), a mobile phase system consisting of ethyl acetate: formic acid: water (15:1:1:2) was used. In order to separate the nonpolar samples (ASE n-hexane and dichloromethane fractions, decoction n-hexane, dichloromethane, and ethyl acetate fractions), we employed the upper phase of a mixture of toluene: ethyl acetate: formic acid: water (20:10:1:1). The ethyl acetate samples obtained by liquid–liquid extraction (LLE) of the decoctions were developed with both mobile phases in order to analyze both polar and nonpolar constituents extracted by ethyl acetate.

Development was carried out in a CAMAG Automatic Developing Chamber ADC2 (CAMAG Chemie-Erzeugnisse und Adsorptionstechnik AG, Muttenz, Switzerland). The relative humidity was controlled and kept constant by using oversaturated KSCN solution (rH = 47% ± 3%). After 15 min equilibration with saturation pad and 5 min plate preconditioning, the plates were developed to a final migration distance of 85 mm and dried by air blow.

The plates were photographed with a CAMAG Reprostar 3 documentation system (CAMAG Chemie-Erzeugnisse und Adsorptionstechnik AG, Muttenz, Switzerland) directly after development as well as after derivatization (ultraviolet [UV] 254 nm, 366 nm, and visible [Vis light]). In order to enable the detection of compounds with various chemical properties in the different single herbs, different derivatization methods were applied. RC was directly visualized without derivatization under UV 366 nm. RRGP was derivatized with sulfuric acid 10% and heating for 5 min at 105°C before it was visualized under UV 366 nm. For RB, a solution of 1% iron (III) chloride in ethanol (m/v) was employed and the plate was heated at 100°C for 10 min before the plate was examined in Vis. RP, RA, RZ, and FS were also visualized in Vis after spraying with vanillin-sulfuric acid reagent and heating at 110°C for 5 min.

**RESULTS AND DISCUSSION**

Since mGQD is a very complex mixture containing a broad range of phytochemicals from diverse compound classes, LLE of the decoctions of mGQD and its individual herbs with solvents of increasing polarity was performed in order to allow selective enrichment of constituents. In addition, ASE extracts of the mGQD formula and every contained herb were prepared in order to compare the different extraction methods by HPTLC. The absolute and relative extract yields obtained after extraction and fractionation are shown in Tables 1 and 2.

The highest yields were obtained for all samples by extraction with polar solvents. Both in ASE with solvents of increasing polarity, and in LLE of the classical decoctions, the hydrophilic solvents methanol and n-butanol were able to extract the major part of the compounds, while the amounts extracted with n-hexane were quite low, demonstrating that the majority of compounds contained in the herbs is hydrophilic. Polar constituents were particularly dominant in the decoction-derived LLE extracts (% yield decoctions: mGQD 18.27%, RP 9.40%, RC 7.40%, RS 18.80%, RRGP 33.40%, RA 14.00%, RZ 9.80%, and FS 31.00%) because classical decoctions were prepared by boiling with water.

### Table 1: Extract yields obtained by accelerated solvent extraction of modified Gegen Qinlian decoction and its single herbal components

| Component | Initial weight (g) | Yield n-hexane extract, g (%) | Yield DCM extract, g (%) | Yield MeOH extract, g (%) |
|-----------|-------------------|-------------------------------|--------------------------|--------------------------|
| mGQD      | 12.06             | 0.02 (0.18)                   | 0.03 (0.25)              | 4.06 (33.67)             |
| RP        | 10.01             | 0.03 (0.30)                   | 0.02 (0.20)              | 2.12 (21.18)             |
| RC        | 10.01             | 0.07 (0.70)                   | 0.07 (0.70)              | 1.24 (12.39)             |
| RS        | 8.00              | 0.05 (0.63)                   | 0.04 (0.50)              | 1.20 (15.00)             |
| RRGP      | 10.00             | 0.06 (0.60)                   | 0.08 (0.80)              | 2.77 (27.70)             |
| RA        | 7.99              | 0.08 (1.00)                   | 0.03 (0.38)              | 2.31 (28.91)             |
| RZ        | 8.03              | 0.32 (3.99)                   | 0.06 (0.75)              | 0.63 (7.85)              |
| FS        | 6.01              | 0.80 (13.31)                  | 0.03 (0.50)              | 2.46 (40.93)             |

RP: Radix Puerariae, RC: Rhizoma coptidis, RS: Radix Scutellariae, RRGP: Radix et Rhizoma Glyceryrhizae Praeparata cum Melle, RA: Rhizoma Anemarrhenae, RZ: Rhizoma Zingiberis, FS: Fructus Schisandrae, mGOD: Modified Gegen Qinlian decoction.
Metabolite assignment and identification by ultra-high-performance liquid chromatography-diode array detection-high-resolution mass spectrometry

For the characterization of the chemical constituents of the modified GQD formula, a UHPLC-DAD-HRMS method has been established. Figure 1 shows the base peak chromatograms of mGQD in both ESI positive and negative modes. LC-MS data were processed by Compound Discoverer 2.1 software (Thermo Fisher Scientific, Waltham, MA, USA). A total of 8235 features were detected in ESI positive mode and 9853 were detected in negative mode. Features with retention times from min 0–35 and areas >2,000,000 were included for peak identification, resulting in 220 features in positive and 107 in negative mode. From the features detected in the formula, 84 peaks were identified and assigned to their originating herbs. Seventeen of these constituents could be unambiguously identified by comparing their retention times and m/z values with authentic reference substances, and 67 compounds were tentatively identified by comparing m/z values and fragmentation patterns with data from databases (Metlin, Human Metabolome Database HMDB, and Massbank of North America MoNA) and existing literature [Table 3].

Identification of compounds from Radix Puerariae

Radix Puerariae lobatae (Kudzuvine Root) is the dried root of P. mantana var. lobata (Willd.) Maesen and S. M. Almeida ex Sanjappa and Predeep (Fabaceae). Isoflavones and isoflavone glucosides are the main compounds in RP. More than twenty isoflavones have been isolated from the plants of genus Pueraria, including puerarin as the most abundant one, which has also been reported to be responsible for RP’s antidiabetic effects.[34-36]

We were able to identify twenty compounds derived from RP in mGQD, rendering RP one of the two most dominant

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**Table 2: Extract yields obtained by classical decoction combined with liquid-liquid extraction of modified Gegen Qinlian decoction and its single herbal components**

| Initial weight crude drug (g) | Yield decoction | Yield n-hexane extract | Yield DCM extract | Yield EtOAc extract | Yield BuOH extract | Yield water residue |
|-------------------------------|----------------|------------------------|--------------------|---------------------|--------------------|-------------------|
| mGQD 60                      | 1.10 (18.27)   | 0.03 (0.06)            | 0.12 (0.22)        | 0.23 (0.43)         | 2.14 (3.96)        | 8.53 (15.80)      |
| RP 50                        | 0.47 (9.40)    | 0.01 (0.02)            | 0.01 (0.02)        | 0.05 (0.11)         | 0.96 (2.13)        | 3.33 (7.40)       |
| RC 50                        | 0.37 (7.40)    | 0.01 (0.02)            | 0.04 (0.09)        | 0.02 (0.04)         | 0.96 (2.13)        | 3.03 (6.73)       |
| RS 50                        | 0.94 (18.80)   | 0.01 (0.02)            | 0.19 (0.42)        | 0.20 (0.44)         | 0.70 (1.56)        | 4.72 (10.49)      |
| RRGP 50                      | 1.67 (33.40)   | 0.01 (0.01)            | 0.03 (0.07)        | 0.11 (0.24)         | 0.68 (1.51)        | 7.35 (16.33)      |
| RA 50                        | 0.70 (14.00)   | 0.01 (0.02)            | 0.02 (0.04)        | 0.03 (0.07)         | 1.53 (3.40)        | 12.72 (28.27)     |
| RZ 50                        | 0.49 (9.80)    | 0.02 (0.04)            | 0.03 (0.07)        | 0.02 (0.04)         | 0.12 (0.27)        | 1.94 (4.31)       |
| FS 50                        | 1.55 (31.00)   | 0.03 (0.07)            | 0.04 (0.09)        | 0.12 (0.27)         | 2.13 (4.73)        | 8.53 (18.96)      |

*The extract after decoction is produced by using an aliquot of the whole decoction (1/10), hence, the yield is calculated using 1/10 of the initial weight of the crude drug and the yields of the other extracts (LLE with the remaining decoction) are calculated based on 9/10 of the weight of the initial crude drug. RP: Radix Puerariae, RC: Rhizoma Coptidis, RS: Radix Scutellariae, RRGP: Radix et Rhizoma Glycyrrhizae Praeparata cum Melle, RA: Rhizoma Anemarrhenae, RZ: Rhizoma Zingiberis, FS: Fructus Schisandrae, mGOD: Modified Gegen Qinlian decoction.
Table 3: Compounds identified in modified Gegen Qinlian decoction by UHPLC-DAD-Q-Exactive-HRMS/MS

| Peak number | Elemental composition | Monoisotopic mass (Da) | UV (nm) | RT (min) | ESI positive mode | ESI negative mode | Occurrence | Identification | Literature | Classification |
|-------------|-----------------------|------------------------|---------|----------|------------------|------------------|------------|---------------|------------|----------------|
| 1 | C_{12}H_{22}O_{11} | 342.116 | | 1.05 | 381.0797 | [M+K] | 260, 216, 186, 148 | 341, 215, 197, 179, 161 | Sucrose | [13] | Disaccharide |
| 2 | C_{12}H_{22}O_{11} | 342.116 | | 1.08 | 360.1048 | [M+NH_4]^+ | 325, 277, 180, 163, 145, 127 | | | | |
| 3 | C_{18}H_{30}O_{10} | 504.169 | | 1.08 | 522.2029 | | 325, 272, 180, 163, 145, 127 | | | | |
| 4 | C_{22}H_{34}O_{14} | 578.164 | 245, 305 | 3.16 | 579.1714 | | 561, 459, 417, 399, 381, 363, 351, 321, 297, 267 | | | |
| 5 | C_{22}H_{34}O_{16} | 584.138 | 255, 315, 355 | 3.54 | 585.1453 | | 567, 549, 519, 489, 465, 435, 405, 387, 369, 327, 303, 273, 261 | | | |
| 6 | C_{26}H_{48}O_{16} | 578.164 | 250, 300 | 3.94 | 579.1712 | | 417, 255 | | | |
| 7 | C_{26}H_{52}O_{10} | 432.106 | 245, 290 | 4.14 | 433.1129 | | 415, 397, 379, 367, 351, 337, 323, 313, 283, 271, 198, 115 | | | |
| 8 | C_{20}H_{28}O_{16} | 564.148 | 4.57 | 565.1555 | | | 433, 415, 397, 379, 367, 351, 337, 323, 313, 283, 271, 198, 115 | | | |
| 9 | C_{17}H_{22}O_{9} | 368.111 | 325 | 4.70 | 369.1177 | | 194, 177, 145 | | | |
| 10 | C_{20}H_{32}O_{16} | 564.148 | 290, 370 | 4.89 | 565.1555 | | 433, 415, 397, 379, 367, 351, 337, 323, 313, 283, 271, 198, 115 | | | |
| 11 | C_{24}H_{31}NO_{10} | 505.195 | 5.26 | 506.2027 | | | 344, 326, 190 | | | |
| 12 | C_{19}H_{30}O_{11} | 422.085 | 255, 320, 365 | 5.36 | 423.0921 | | 405, 387, 369, 357, 351, 339, 327, 303, 273 | | | |
| 13 | C_{24}H_{32}O_{9} | 416.111 | 250, 305 | 5.54 | 417.1178 | | 399, 381, 363, 351, 335, 327, 321, 297, 267 | | | |
| 14 | C_{19}H_{30}O_{11} | 422.085 | 255, 315, 365 | 5.73 | 423.0922 | | 405, 387, 369, 357, 351, 339, 327, 303, 273 | | | |

Contd...
| Peak number | Elemental composition | Monoisotopic mass (Da) | UV (nm) | ESI positive mode | ESI negative mode | Occurrence | Identification | Literature | Classification |
|-------------|----------------------|------------------------|---------|-----------------|-----------------|------------|---------------|------------|----------------|
|             | C_{20}H_{28}O_{15}   | 548.153                | 250, 305| 5.84            | 549.1605        | 531, 417, 399, 381, 363, 351, 335, 321, 297, 279, 267 | 5.93      | 547.1464 | 295, 267 | RP Puerarin 6''-O-xyloside | [7,14,16] | Isoflavone |
| 16          | C_{20}H_{28}O_{15}   | 548.153                | 250, 305| 6.21            | 549.1605        | 417, 399, 381, 363, 351, 335, 321, 297, 279, 267 | 6.30      | 547.1461 | 295, 267 | RP Minifecin | [7,14,16] | Isoflavone |
| 17          | C_{22}H_{32}O_{10}   | 446.121                | 270, 300| 6.22            | 447.1285        | 250 | 6.36      | 445.1142 | 325, 297 | RP 3'-Methoxypuerarin | [7,14,16] | Isoflavone |
| 18          | C_{20}H_{26}NO_{4}+  | 342.171                | 270, 300| 6.32            | 342.1692        | [M]^+ 297, 282, 265, 237 | RC        | Magnoflorine | [7,17,20,21] | Aporphin alkaloid |
| 19          | C_2H_12O_9           | 368.111                | 325 | 6.91            | 369.1180        | 194, 177, 145 | 6.93      | 367.1035 | 191, 173 | RC O-Feruloylquinic acid | [18] | Quinic acid |
| 20          | C_{20}H_{30}O_{9}    | 416.111                | 250, 310| 7.14            | 417.1180        | 255 | 19.34     |        | 253 | RP Daidzin | [7,14,16] | Isoflavone |
|             | C_{25}H_{30}NO_{10}  | 504.187                | 7.42    | 504.1868        | 342, 324        | 7.47      | 502.1726 | 340, 322 | RC 11-Hydroxy-stepholidine glucoside | [8,19] | Quaternary protoberberine alkaloid |
|             | C_{22}H_{32}O_{10}   | 446.121                | 8.06    | 447.1286        | 285 | 8.10      | 491.1204 | 445, 430, 283, 268 | RP 3'-Methoxydaidzin | [7,14] | Isoflavone |
|             | C_{20}H_{26}O_{14}   | 564.148                | 260 | 8.15            | 565.1554        | 433, 415, 397, 379, 367, 351, 337, 323, 313, 395, 283, 271, 198, 115 | 8.22      | 563.1420 | 341, 311, 283 | RP Genistein 8-C-xylosyl/apiosyl-(1-6)-glucoside | [7,14,17] | Isoflavone |
| 24          | C_{21}H_{30}O_{9}    | 416.111                | 8.42    | 417.1180        | 399, 381, 363, 351, 335, 321, 307, 297, 267, 257 | 8.54      | 415.1040 | 295, 267, 253 | RP Puerarin isomer | [7] | Isoflavone |
|             | C_{21}H_{30}O_{9}    | 416.111                | 265, 310| 8.83            | 607.2023        | 299 | 8.81      | 605.1884 | 297, 253 | RP Pueroside A | [7,14,17] | Aromatic glycoside |
| 26          | C_{21}H_{30}O_{9}    | 418.126                | 275, 310| 8.97            | 257.0805        | 239, 211, 163, 147, 137, 123 | 9.06      | 417.1193 | 255 | RRGP Liquiritin* | Flavanone |
| 27          | C_{20}H_{30}O_{15}   | 550.169                | 275, 310| 9.13            | 257.0807        | 239, 211, 163, 147, 137 | 9.24      | 549.1620 | 417, 295, 255, 135 | RRGP Liquiritin apioside | [14,16] | Flavanone |
### Table 3: Contd...

| Peak number | Elemental composition | Monoisotopic mass (Da) | UV (nm) | ESI positive mode | ESI negative mode | Occurrence | Identification | Literature | Classification |
|-------------|-----------------------|------------------------|---------|------------------|------------------|------------|----------------|------------|----------------|
| 28          | C₂₁H₂₀O₃             | 416.111                |         | 9.24             | 417.1180         | 9.36       | 415.1038       | 295, 255   | RP  Puerarin isomer [7] Isoflavone |
| 29          | C₂₆H₂₉O₁₅            | 548.153                | 275, 315| 9.50             | 549.1606         | 9.60       | 547.1464       | 487, 457, 427, 367, 337 | RS  Chrysin 6-C-arabinoside 8-C-glucoside [22,23] Flavone |
| 30          | C₂₁H₂₀O₁₀            | 432.106                | 275, 315| 9.64             | 433.1130         | 9.71       | 477.1042       | 431, 269   | RP  Genistin* Isoflavone |
| 31          | C₁₉H₁₀NO₄⁺           | 322.108                | 270, 355| 10.04            | 322.1073 [M]⁺    |           |               |            | RC  Tetrahydrocouchliner or tetrahydrocouchlinolium [17,20,21] Quaternary protoberberine alkaloid |
| 32          | C₁₉H₁₈NO₄⁺           | 324.124                | 270, 340| 10.33            | 324.1229 [M]⁺    |           |               |            | RC  Demethylenberberine [17,20,21] Quaternary protoberberine alkaloid |
| 33          | C₂₆H₂₉O₁₅            | 548.153                | 275, 315| 10.51            | 549.1606         | 10.64      | 547.1464       | 529, 457, 427, 367, 337 | RS  Chrysin 6-C-glucoside 8-C-arabinoside [22,23] Flavone |
| 34          | C₂₀H₁₈NO₄⁺           | 352.188                | 265, 345| 10.63            | 352.1179 [M]⁺    |           |               |            | RC  Neo-oxyberberine or berberastine [17] Quaternary protoberberine alkaloid |
| 35          | C₂₁H₂₀O₃             | 562.169                |         | 11.06            | 563.1762         | 11.21      | 561.1624       | 339, 309, 281 | RP  Formononetin 8-C-xylosyl-[1-6]-glucoside [7] Isoflavone |
| 36          | C₁₉H₁₄NO₄⁺           | 320.092                | 265, 355| 12.19            | 320.0916 [M]⁺    |           |               |            | RC  Coptisine* Quaternary protoberberine alkaloid |
| 37          | C₂₀H₂₀NO₂⁺           | 338.139                | 270, 345| 12.4             | 338.1338 [M]⁺    |           |               |            | RC  Columbamine [7,16,17,20,21] Quaternary protoberberine alkaloid |
| 38          | C₁₉H₁₈NO₄⁺           | 336.124                | 270, 345| 12.48            | 336.1229 [M]⁺    |           |               |            | RC  Epiberberine [7,16,17,20,21] Quaternary protoberberine alkaloid |
| 39          | C₂₀H₂₀NO₂⁺           | 338.139                | 275, 345| 12.78            | 338.1384 [M]⁺    |           |               |            | RC  Jatrorrhizine* Quaternary protoberberine alkaloid |
| 40          | C₁₉H₁₈O₄             | 346.069                |         | 13.04            | 347.0761         | 13.19      | 345.0618       | 330, 315, 298, 165 | RS  Viscidulin III [7,16,23] Flavone |
| Peak number | Elemental composition | Monoisotopic mass (Da) | UV (nm) | ESI positive mode | ESI negative mode | Occurrence | Identification | Literature | Classification |
|-------------|----------------------|------------------------|---------|-------------------|-------------------|------------|----------------|------------|----------------|
| 41          | C₁₉H₁₅NO₄⁺           | 322.108                | 307     | 307, 279          |                   | RC         | Thalifendine or berberubine | [20,21]   | Quaternary protoberberine alkaloid |
| 42          | C₁₉H₁₅NO₅⁺           | 418.126                | 13.58   | 13.58             |                   | RRGP       | Isoquercitrin*            |           | Chalcone        |
| 43          | C₁₅H₁₇NO₂⁺           | 676.239                | 13.66   | 676.238           | 496, 334, 319     | RC         | Copticine-quinic acid conjugate - CO + 2H | [8]       |                  |
| 44          | C₁₉H₁₇NO₄⁺           | 254.058                | 13.76   | 255.060           | 227, 199, 172, 137, 111 | RRGP       | Daidzein*             |           | Isoflavone       |
| 45          | C₁₅H₁₇O₂₀⁺           | 936.493                | 13.77   | 919.4897 [M + H-H₂O]⁺ | 757, 595, 433, 397, 289, 271, 253 | RA         | Timosaponin N or isomer | [15,24]   | Furostanol saponin |
| 46          | C₁₉H₁₇O₂₀⁺           | 430.126                | 14.02   | 431.1336          | 269               | RRGP, RP   | Ononin               |           | Isoflavone       |
| 47          | C₁₅H₁₇O₁₁⁺           | 446.085                | 14.14   | 447.0919          | 271               | RS         | Baicalin               |           | Flavone         |
| 48          | C₁₅H₁₇NO₂⁺           | 334.107                | 14.24   | 334.1067          |                   | RC         | Worenine              |           | Quaternary protoberberine alkaloid |
| 49          | C₁₅H₁₇NO₂⁺           | 336.124                | 14.81   | 336.1228 [M⁺]     | 321, 292          | RC         | Berberine*             |           | Quaternary protoberberine alkaloid |
| 50          | C₁₅H₁₇NO₂⁺           | 352.155                | 15.07   | 352.1541 [M⁺]     | 337, 308, 245, 162 | RC         | Palmarine*             |           | Quaternary protoberberine alkaloid |
| 51          | C₁₅H₁₇O₂₀⁺           | 936.493                | 15.19   | 981.4967 [M+HCOO]⁺ | 773, 611, 449     | RA         | Timosaponin E1 or Macrostemonoside J | [15]      | Furostanol saponin |
| 52          | C₁₅H₁₇O₁₁⁺           | 446.085                | 15.54   | 447.0921          | 271               | RS         | Norwogonin 7-O-glucuronide | [7,22]    | Flavone         |
| 53          | C₁₅H₁₇O₁₆⁺           | 920.498                | 16.44   | 903.4943 [M + H-H₂O]⁺ | 741, 579, 417, 399, 325, 285, 273, 255 | RA         | Timosaponin BII       | [15]      | Furostanol saponin |
| 54          | C₁₅H₁₇O₁₀⁺           | 430.090                | 16.56   | 431.0972          | 269, 255, 147     | RS         | Chrysin 7-O-glucuronide |           | Flavone         |
| 55          | C₁₅H₁₇O₁₀⁺           | 920.498                | 16.56   | 903.4946 [M + H-H₂O]⁺ | 742, 579, 417, 399, 320, 285, 273, 255 | RA         | 25R-Timosaponin BII    | [15]      | Furostanol saponin |
| 56          | C₁₅H₁₇O₁₁⁺           | 460.101                | 16.68   | 461.1077          | 285, 270          | RS         | Oroxyn A 7-O-glucuronide | [7,22]    | Flavone         |
| 57          | C₁₅H₁₇O₁₀⁺           | 920.498                | 16.83   | 903.4946 [M+H-H₂O]⁺ | 741, 579, 417, 399, 381, 355, 273, 255 | RA         | 25S-Officinalisin I    | [15]      | Furostanol saponin |
| Peak number | Elemental composition | Monoisotopic mass (Da) | UV (nm) | ESI positive mode | ESI negative mode | Occurrence | Identification | Literature | Classification |
|-------------|-----------------------|------------------------|---------|------------------|------------------|------------|----------------|------------|----------------|
| 58          | C_{21}H_{39}NO_{10}   | 350.139                | 16.83   | 350.1385 [M]     | 335, 320, 306, 286, 282, 271, 265, 261, 211 | RC         | 13-Methylberberine | [17,21,25] | Quaternary protuberberine alkaloid |
| 59          | C_{8}H_{14}O_{10}     | 920.498                | 17.04   | 906.4945 [M + H-H_2O]^+ | 741, 579, 417, 399, 381, 325, 285, 273, 255 | RA         | Officinalisin I | [15]      | Furostanol saponin |
| 60          | C_{30}H_{57}NO_{18}   | 527.158                | 17.25   | 528.1657         | 334, 319         | RC         | Demethylcoptichine | [8,17]    | Benzylisoquinoline alkaloid |
| 61          | C_{25}H_{41}O_{10}    | 460.101                | 17.70   | 461.1076         | 285, 270         | RS         | Wogonoside    | [7,22]    | Flavone |
| 62          | C_{3}H_{6}O_{2}       | 270.053                | 20.34   | 271.0599         | 253              | RS         | Baicalein* |          | Flavone |
| 63          | C_{35}H_{78}O_{15}    | 984.457                | 20.79   | 985.4641         | 906, 615, 471, 453, 435, 407 | RRGP      | Licorice saponin A3 | [14,16,17,26] | Triterpen saponin |
| 64          | C_{3}H_{6}O_{1}       | 880.409                | 21.33   | 881.4163         | 529, 511, 493, 451, 405, 285, 233, 177 | RRGP      | 22β-Acetoxyglycyrrhizin | [14,16,17,27] | Triterpen saponin |
| 65          | C_{3}H_{6}O_{1}       | 265.074                | 21.57   | 266.0806         | 254, 213         | RP         | Formononetin*  |          | Isoflavone |
| 66          | C_{3}H_{6}O_{1}       | 902.488                | 21.98   | 903.4944         | 741, 579, 417, 273, 255 | RA         | Timosaponin C | [15]      | Furostanol saponin |
| 67          | C_{3}H_{6}O_{1}       | 902.488                | 22.28   | 903.4948         | 741, 579, 417, 273, 255 | RA         | Timosaponin BIII | [15]      | Furostanol saponin |
| 68          | C_{3}H_{6}O_{1}       | 820.388                | 23.96   | 821.3963         | 451              | RRGP      | Yunganoside E2 | [27]      | Triterpen saponin |
| 69          | C_{3}H_{6}O_{1}       | 838.399                | 24.29   | 839.4062         | 663, 469         | RRGP      | Licorice saponin G2 | [7,14,16, 26-28] | Triterpen saponin |
| 70          | C_{3}H_{6}O_{1}       | 284.068                | 25.02   | 285.0757         | 270              | RS         | Wogonin *     |          | Isoflavone |
| 71          | C_{3}H_{6}O_{1}       | 822.404                | 26.38   | 823.4108, 647.37  | 453, 435, 217, 149 | RRGP      | Glycyrrhizinic acid * | [14,16,17,27] | Triterpen saponin |
| 72          | C_{3}H_{6}O_{1}       | 374.100                | 26.60   | 375.1073         | 360, 345, 327, 283, 227, 195 | RS         | Skullcapflavone II or isomer | [7,22]    | Flavone |

Contd...
| Peak number | Elemental composition | Monoisotopic mass (Da) | UV (nm) | ESI positive mode | ESI negative mode | Occurrence Identification | Literature | Classification |
|-------------|----------------------|------------------------|--------|------------------|------------------|-------------------------|------------|----------------|
| 73          | C_{20}H_{33}O_{14}   | 758.445                | 26.82  | 741.4417 [M + H-H_{2}O] | 417, 399, 381, 289, 271, 253 | 26.82 | 803.4469 [M+HCOO], 757.4401 | RA | Anemarrhasaponin II or isomer [15] | Furostanol saponin |
| 74          | C_{14}H_{33}O_{4}    | 294.183                | 26.87  | 294.1825          | 277, 177          | RZ | 6-Gingerol* |
| 75          | C_{20}H_{35}O_{10}+  | 352.188                | 26.91  | 352.1179          | 337, 336, 322, 308, 290 | RC | Neo-oxyberberine or berberastine [17] | Phenolic compound Quaternary protoberberine alkaloid |
| 76          | C_{20}H_{20}O_{7}    | 432.215                | 27.7   | 478.2801 [M + 2Na-H] | 415, 384, 346 | FS | Schisandrol A [29-32] | Lignan |
| 77          | C_{4}H_{6}O_{4}      | 822.404                | 27.81  | 823.4109, 647.3793 [M-H-GlC-A], 433.2221 | 471, 453, 435 | 27.75 | 821.3998, 734, 394, 351, 289, 193, 175 | RRGP | Glycyrrhizinic acid isomer | Triterpen sapoin |
| 78          | C_{2}H_{4}O_{10}     | 824.419                | 28.28  | 825.4268          | 613, 455, 437, 409 | 28.23 | 823.4152, 453, 351, 289, 193 | RRGP | Licorice saponin J2 [26,33] | Triterpen sapoin |
| 79          | C_{2}H_{2}O_{7}      | 416.184                | 29.06  | 462.2486 [M + NH_{4}] | 399, 384, 368, 357, 330 | FS | Schisandrol B [30,31] | Lignan |
| 80          | C_{20}H_{30}O_{5}    | 500.241                | 30.28  | 546.3062 [M + NH_{4}], 523.2304, 511.2480 | 483, 401, 370 | FS | Tigloylgomisin H or Angeloylgomisin H [30,31] | Lignan |
| 81          | C_{2}H_{3}O_{2}      | 530.252                | 30.91  | 576.3166 [M + NH_{4}] | 431, 400, 387, 372, 356 | FS | Angeloylgomisin Q [30,31] | Lignan |
| 82          | C_{20}H_{30}O_{8}    | 740.435                | 31.20  | 758.4688 [M + NH_{4}], 579.3895, 417.3364 | 417, 399, 381, 273, 255, 163, 145 | 31.11 | 785.4363, 739, 577, 351, 209, 179, 161, 143 | RA | Timosaponin AIII* | Spirostanol sapoin |
| 83          | C_{2}H_{2}O_{2}      | 416.220                | 33.45  | 417.2272          | 402, 386, 347, 316, 301 | FS | Schisandrin A* | Lignan |
| 84          | C_{20}H_{30}O_{4}    | 312.230                | 34.03  | 311.2021          | 149 | RA | Dihydroxy-octadecenoic acid | Fatty acid |

*Compound identified by comparison of retention time and MS data with authentic standard. RT: Retention time, RP: Radix Puerariae, RC: Rhizoma Coptidis, RS: Radix Scutellariae, RRGP: Radix et Rhizoma Glycyrrhizae Praeparata cum Melle, RA: Rhizoma Anemarrhenae, RZ: Rhizoma Zingiberis, FS: Fructus Schisandraceae, UV: Ultraviolet, ESI: Electrospray ionization, Glc: glucose, GlcA: glucuronic acid.
herbs in the formula. All detected compounds except for puerarin A (25) were isoflavones or isoflavan glycosides. The identities of puerarin (13), genistin (30), daidzein (44), and formononetin (65) were unambiguously confirmed by comparing their retention times and m/z values with reference standards. Compounds 4, 6–8, 10, 15–17, 20, 22–25, 28, and 35 were identified by interpreting and comparing their UV spectra, retention times, and fragmentation patterns with existing data from literature.

Analyzing the MS/MS fragmentation of a given flavonoid glycoside enables distinction between O-and C-glycosides. C-glycosidic bonds are stronger than O-glycosidic bonds, hence no aglycones of C-glycosides are produced, but the sugar moieties are subject to ring cleavage in different positions of the ring. Therefore, the presence of a C-glycoside is characterized by the loss of fragments with 150, 120, and 90 Da for hexoses and 60 and 90 Da for pentoses, while O-glycosides usually produce an abundant aglycone fragment resulting from the cleavage of the complete glycosyl moiety.[37,38]

Discrimination between the different flavonoid types was achieved by interpretation of their UV spectra. Flavonoids exhibit two major UV absorption bands: band I corresponds to the absorption caused by the B ring, and band II represents A ring absorption. The absorption maxima for the two bands are characteristic for the flavonoid structure and can be used for the tentative assignment of the position of functional groups.[39]

Compound 4 showed a [M+H]+ parent ion at m/z 579.1714 and a [M-H]- parent ion at 577.1572, as well as a similar MS/MS fragmentation pattern as puerarin. A neutral loss of 162 Da indicated the additional presence of an O-glucose moiety. The UV spectrum showed absorption maxima at 245 nm and 305 nm. Comparison of the UV spectrum, the MS/MS fragmentation pattern, and order of elution with the existing data led to the tentative identification of compound 4 as puerarin 4′-O-glucoside.[7,13,14]

Compound 6 showed DAD λ_{max} at 250 nm and 300 nm. It produced a [M+H]+ parent ion at m/z 579.1712 and fragments at m/z 417 and m/z 255, corresponding to the loss of two O-glucose moieties. Other fragments smaller than 255 Da showed the same fragmentation pattern as daidzein. Hence, compound 6 was tentatively identified as daidzein 4′,7-di-O-glucoside.[7,13,14]

Compound 7 was tentatively identified as 3′-hydroxypuerarin. In ESI positive mode, its parent ion m/z 433.1129 produced a fragment at m/z 313, corresponding to [M+H-C_{4}H_{11}O_{4}]^{+}, and the fragments m/z 415, m/z 397, and m/z 379 were caused by the step-wise loss of three H_{2}O molecules. In the DAD spectrum, absorption maxima at 245 nm and 290 nm were detected.[7,14,16]

Compounds 8 and 10 both showed parent ions with m/z 563.1414 in the ESI negative mode and fragments at m/z 311, corresponding to the loss of an O-pentose, together with the C_{4}H_{9}O_{4} fragment of a C-glucose. In ESI positive mode, the respective parent ion with m/z 565.1555 fragmented to m/z 433 [M-O-pentose] and m/z 313 [M-O-pentose-C_{4}H_{11}O_{4}]^{−}. Neither [M+H-120] nor [M+H-90] fragments were detected, leading to the suggestion that the C-glucose is not free, but obviously connected to the pentose moiety. Moreover, after a neutral loss of 132 Da (O-pentose), the remaining fragments exactly corresponded to the fragments of puerarin but with a difference of 16 Da, indicating an additional hydroxyl moiety in the aglycone. Both compounds showed DAD absorption maxima at 209 nm and 370 nm. By comparing these data with that of compounds known from RP, compounds 8 and 10 were tentatively identified as the two epimeric isoflavone glycosides 3′-hydroxypuerarin 6"-O-xiloside and 3′-hydroxypuerarin 6"-O-apioside, respectively.[7,14,16]

In analogy to compounds 8 and 10, compounds 15 and 16 were identified as puerarin 6"-O-xiloside and mirificin (puerarin 6"-O-apioside), respectively. For both compounds, absorption maxima at 250 nm and 305 nm were observed. Both showed parent ions at m/z 549.1605 in ESI positive mode and fragmented to m/z 417 corresponding to [M+H-pentose]^{−}, m/z 297 corresponding to [M+H-pentose-C_{4}H_{11}O_{4}]^{−}, and m/z 399 and m/z 381 due to the loss of two H_{2}O molecules. Because there is no way to differentiate between the apiosyl-glycoside and the xylosyl-glycoside by MS, the compounds were tentatively identified by comparing their elution orders with the existing data from literature.[7,14,16]

Compound 20 showed DAD absorption maxima at 250 nm and 310 nm. It produced a parent ion at m/z 417.1180 in ESI positive mode and fragmented to m/z 255. In ESI-mode, compound 20 showed parent ions at m/z 461.1094 [M+HCOO]^{−} and m/z 415.1039 [M-H]^{−}, and fragmented to m/z 253. It was identified as daidzin.[7,14,16]

Compound 23 showed an absorption maximum at 260 nm. It generated a [M+H]+ ion at m/z 565.1554 and fragmented to m/z 433 [M+H-pentose]^{−}, m/z 313 [M+H-pentose-C_{4}H_{11}O_{4}]^{−}, and m/z 283 [M+H-pentose-C_{4}H_{9}O_{4}]^{−}. Fragments m/z 415, m/z 397, and m/z 379 were produced by loss of three H_{2}O molecules. According to Rong et al., the minus 120 Da fragment is more abundant in 8-C-glucosides, while 6-C-glucosides produce a more prominent [M+H-150]^{−} ion.[38] Fragment m/z 313 showed a relative abundance of nearly 100%, while the abundance of fragment m/z 283 was 20%. Thus, compound 23 was identified as genistein 8-C-xilosyl/ apiosyl-(1-6)-glucoside.[7,14,17]

Identification of compounds from Radix Scutellariae

Radix Scutellariae (Baical Skullcap Root) is the dried root of S. baicalensis Georgi from the Lamiaceae family. The main constituents of Scutellaria species are flavonoids, mainly of the flavone type, and derivatives thereof. Until now, more than 120 flavonoids have been isolated only from S. baicalensis, with baicalin and baicalin representing the major constituents.[40-42] Other components, including trans-martynoside, trans-verbascoside, and viscidullin III, could be detected in small amounts in S. baicalensis root.[43]
In mGQD, 11 compounds originating from Radix Scutellariae were detected and characterized. All of them were flavones. Baicalin (47), baicalein (62), and wogonin (70) were identified by comparing their retention times and UV and MS spectra with authentic references.

The isomers 29 and 33 both showed absorption maxima at 275 nm and 315 nm, respectively, and obviously only differed in the positions of their glycosyl moieties. They generated parent ions at m/z 547.1464 in ESI negative mode and fragmented to m/z 487 corresponding to [M-H-C$_{2}$H$_{2}$O$_{2}$]$^{-}$ and m/z 457 correlating with [M-H-C$_{3}$H$_{4}$O$_{3}$]$^{-}$, indicating the presence of at least one C-pentoside. The ion m/z 427 could be derived either by neutral loss of 120 Da from a C-hexose moiety or by the simultaneous loss of C$_{2}$H$_{4}$O$_{2}$ fragments from two C-linked pentoses. However, the parent mass of 547 substantiated the suggestion of the presence of one pentose and one hexose moiety. Hence, m/z 367 was due to the loss of two −90 Da fragments, and m/z 337 indicated the synchronous cleavage of a −90 Da and a −120 Da fragment. By searching the existing data for the literature for already-isolated substances, the compounds were tentatively identified as chrysín-6-C-arabinoside-8-C-glucoside and chrysín-6-C-glucoside-8-C-arabinoside.$^{[22,23]}

Compound 52 exhibited $\lambda_{\text{max}}$ at 280 nm and 315 nm and showed a [M-H]$^{-}$ ion at m/z 445.0781 and fragmented to m/z 269, corresponding to the loss of an O-glucuronide moiety and a remaining trihydroxyflavone structure. Compound 52 was therefore tentatively identified as norwogonin 7-O-glucuronide.$^{[7,22]}

Compound 54 showed $\lambda_{\text{max}}$ at 270 nm and 305 nm in the UV spectrum. In the MS spectra, it produced a parent ion at m/z 429.0828 in ESI negative mode and at m/z 431.0972 in ESI positive mode, corresponding to C$_{24}$H$_{24}$O$_{10}$. The neutral loss of 176 Da (ESI negative mode) indicated the presence of a glucuronic acid moiety. The remaining fragment m/z 253 exactly corresponded to the mass of a dihydroxyflavone-H$^{-}$. By comparison with the existing UV spectra of chrysin and literature on compounds isolated from $S$. baicaleinis, compound 54 was tentatively identified as chrysin 7-O-glucuronide.

Oróxylin A 7-O-glucuronide (56) and wogonoside (61) are structural isomers and only differ by the positions of their methoxy groups. Both produced a [M-H]$^{-}$ ion at m/z 459.0936 and fragments at m/z 283 [M-H-glcA]$^{-}$ and m/z 268 [M-H-glcA-CH$_{2}$]$^{-}$. Compound 56 showed $\lambda_{\text{max}}$ at 270 nm and 310 nm. In the UV spectrum of compound 61, a bathochromic shift in the second UV maximum was observed, due to the change of position of the methoxy group, leading to $\lambda_{\text{max}}$ at 275 nm and 340 nm. The compounds were finally identified by comparing their UV spectral data and MS fragmentation data with the existing data from literature.$^{[7,22]}

Identification of compounds from Rhizoma Coptidis

Rhizoma coptidis (Golden Thread) is the dried rhizome of $C$. chinensis Franch., $C$. deltoidea C. Y. Cheng et P. K. Hsiao or $C$. teeta Wall. ($R$.anunculaceae). Protoberberine-type alkaloids represent the major bioactive constituents in RC, including berberine, cryptopine, palmatine, jatrorrhizine, and columbamine.$^{[44]}$. Among them, berberine is considered the main contributor to the hypolipidemic and hypoglycemic activities of RC.$^{[45,46]}$ Aside, lignans, flavonoids, phenolic compounds, saccharides, and steroids have been isolated from RC.$^{[47]}$

With twenty assigned compounds, Rhizoma coptidis represented one of the two most dominant herbs in mGQD, next to RP. Within the twenty identified compounds, four were unambiguously identified and sixteen were tentatively characterized.

The identities of coptisine (36), jatrorrhizine (39), berberine (49), and palmatine (50) were confirmed by comparison of their retention times and parent ion exact masses with authentic references.

Compound 32 presented a parent ion at m/z 324.1229 in ESI positive mode and showed fragment ions at m/z 309, corresponding to [M-CH$_{3}$]$^{+}$, m/z 308 [M-CH$_{2}$-H]$^{+}$, m/z 294 [M-CH$_{3}$]-CH$_{3}$]$^{+}$, m/z 292 [M-CH$_{3}$]-CH$_{2}$H]$^{+}$, m/z 280 [M-CH$_{3}$]-H$\equiv$CO]$^{+}$, and m/z 266 [M-CH$_{2}$-CH$_{2}$]-CO]$^{+}$. By comparing this fragmentation pattern with data from literature, compound 32 was tentatively identified as demethylberberine.$^{[17,20,21]}

The isomeric compounds 34 and 75 were tentatively identified as neo-oxyberberine or berberastine, respectively. Both presented parent ions at m/z 352.1179 in ESI positive mode and produced fragments at m/z 337 [M-CH$_{3}$]$^{+}$, m/z 336 [M-CH$_{3}$-H]$^{+}$, m/z 322 [M-CH$_{3}$]-CH$_{3}$]$^{+}$, m/z 308 [M-CH$_{3}$]-H$\equiv$CO]$^{+}$, and m/z 290 [M-CH$_{2}$-CH$_{2}$-CO]-H$^{+}$.$^{[17]}

Compounds 31 and 41 both generated M$^{+}$ ions at m/z 322.1173. According to Liu et al., a [M-CO]$^{+}$ fragment is only produced when the methylenedioxy group is positioned at C-9 and C-10 of the protoberberine skeleton, while a methylenedioxy group at C-2 and C-3 is too stable to lose a CO fragment.$^{[21]}$ A −28 Da fragment was only detected in compound 31, hence it was tentatively identified as tetrahydrodocoulerine or tetrahydrocheilanthifolinium. Compound 41 showed no-28 Da fragment and was therefore tentatively annotated as thalifendine or berberrubine.$^{[17,20,21]}

Compound 37 showed DAD $\lambda_{\text{max}}$ at 270 nm and 345 nm and generated an M$^{+}$ ion at m/z 388.1383. It showed the same fragmentation pattern as jatrorrhizine, however it did not show the same retention time as the jatrorrhizine reference. Thus, compound 40 was tentatively identified as the structural isomer of jatrorrhizine, columbamine.$^{[17,21]}

Compound 38 showed an M$^{+}$ ion at m/z 336.1229 and fragmented to m/z 321 [M-CH$_{3}$]$^{+}$, m/z 320 [M-CH$_{2}$-H]$^{+}$, m/z 308 [M-CO]$^{+}$, and m/z 292 [M-CH$_{3}$-H$\equiv$CO]$^{+}$. Comparison with data from literature led to its identification as epiberberine.$^{[17,16,17,20,21]}$
The fragmentation pattern of compound 58 was very similar to that of berberine. The mass difference of the corresponding fragments generated from compound 58 and from berberine was all exactly 14 Da, indicating the presence an additional methyl group in 58. By comparing the spectra with the existing data of compounds described from RC, the compound was tentatively identified as 13-methylberberine.\textsuperscript{[17,21,25]}

Magnoflorine (18) is the only nonprotoberberine-type alkaloid identified in mGQD. The aporphine alkaloid presented a M⁺ ion at m/z 342.1692 and generated fragment ions at m/z 297 [M-C₆H₇N], m/z 282 [M-C₆H₇N-CH₂], m/z 265 [M-C₆H₇N-MeOH], and m/z 237 [M-C₆H₇N-MeOH-CO].\textsuperscript{[7,17,20,21]}

**Identification of compounds from Radix et Rhizoma Glycyrrhizae Praeparata cum Melle**

According to Chinese Pharmacopoeia, honey stir-baked licorice root is the dried root and rhizome of *G. uralensis* Fisch. ex DC, *G. inflata* Bat., or *G. glabra* L. (Fabaceae). The major active components in licorice are triterpenoids and flavonoids. The triterpenoids are from oleanane type and are mainly derived from glycyrrhetinic acid (3-hydroxy-11-o xoolean-12-en-30-oic acid) as aglycon. The major glycoside glycyrrhizin, which is a mixture of potassium and calcium salts of glycyrrhizinic acid (syn. glycyrrhizin), is believed to be the main bioactive substance exhibiting sweet taste, anti-inflammatory, anti-ulcer, and immunomodulatory activities.\textsuperscript{[48-50]} In addition, several minor triterpenoids were isolated. Their aglycon structures represent glycyrrhetinic acid, deoxy-glycyrrhetinic acid, glycyrrhetinic acid 22-lactone, 24-hydroxy glycyrrhetinic acid, deoxy-24-hydroxy glycyrrhetinic acid, and 22-acetoxy glycyrrhetinic acid. In addition, flavonoids and chalcones as well as their derivatives, such as liquiritin, isoliquiritin, liquiritigenin, and licursides, show remarkable antioxidant effects. Other constituents of licorice include coumarins, stilbenoids, and phenolic compounds.\textsuperscript{[51]} However, by processing the crude slices according to TCM guidelines, the qualitative and quantitative composition of the material can be modified. Therefore, processing can also influence the pharmacological effects of the drug. According to TCM, RRGP is prepared by soaking crude Radix Glycyrrhizae slices in refined honey (diluted in boiling water) and gently frying the pieces until the drug become “yellow and not sticky to the fingers.”\textsuperscript{[10]} After honey-roasting, saponins and flavonoid glycosides are cleaved into aglycones and sugars.\textsuperscript{[52]} Sung et al. showed that honey-roasted licorice samples contain higher amounts of the aglycone 18-β-glycyrrhetinic acid, while the content of the intact glycyrrhizin in the drug decreases.\textsuperscript{[53]} Another study demonstrated that during honey-roasting, the isomeric flavaneone glycosides liquiritin and isoliquiritin, as well as their aglycones liquiritigenin and isoliquiritigenin, are converted reversibly into each other. In a second step, more of the aglycones liquiritigenin and isoliquiritigenin are formed from their glycosides due to the presence of heat and honey.\textsuperscript{[54]}

In the studied mGQD decoction, 11 compounds derived from RRGP were detected and characterized. Liquiritin (26), isoliquiritin (42), and glycyrrhizinic acid (71) were identified by comparing their retention times and m/z data with that of reference standards.

Compound 27 presented a [M-H]⁻ ion at m/z 549.1620 and showed the same fragmentation pattern as liquiritin. The difference between their precursor ions was exactly 132 Da, indicating the presence of an additional O-pentosyl moiety. In addition, there was no change in the UV spectrum compared to liquiritin. λ\textsubscript{max} were at 275 nm and 310 nm, which is characteristic for flavanones. Thus, the compound was tentatively identified as liquiritin apioside.\textsuperscript{[14,16]}

Both in the literature and in our experiments, ononin (46) has been found in both RP and RRGP. It produced a parent ion at m/z 475.1249 in ESI negative mode and m/z 431.1336 in ESI positive mode, indicating that the molecular mass of the compound is 430 Da and the precursor ion in ESI negative mode is the formic acid adduct. The compound fragmented to m/z 267 in ESI negative and m/z 269 in ESI positive mode, corresponding to the loss of a glucose moiety.\textsuperscript{[7,14]}

Compounds 63 generated a parent ion in ESI negative mode at m/z 983.4547 and fragmented to m/z 821 (glycyrrhizinic acid) by losing 162 Da, which corresponds to a glucose moiety. The other fragments are exactly the same as in glycyrrhizinic acid. Thus, compound 63 was tentatively identified as licorice saponin A3.\textsuperscript{[14,16,17,26]}

Compounds 64 and 69 were tentatively identified as 22-β-acetoxyglycyrrhizin and licorice saponin G2, respectively. Both showed the same fragment ions as glycyrrhizinic acid. Compound 64 generated a [M-H]⁻ ion at m/z 879.4058 and a [M+H]⁺ ion at m/z 881.4163. The mass difference compared to glycyrrhizinic acid was exactly 58 Da, indicating the presence of an additional acetoxy group. Compound 69 presented a [M-H]⁻ ion at m/z 837.3953 and a [M+H]⁺ ion at m/z 839.4062, corresponding to the mass of glycyrrhizinic acid with one additional hydroxy group.\textsuperscript{[14,16,27]}

Compound 78 also showed the same fragmentation pattern as glycyrrhizinic acid. It generated a [M-H]⁻ ion at m/z 823.4152 and a [M+H]⁺ ion at m/z 825.4268. The mass difference between compound 78 and 69 (licorice saponin G2) was exactly 14 Da, which led to the suggestion that compound 78 is the deoxy-analog to licorice saponin G2. Hence, compound 78 was tentatively identified as licorice saponin J2.\textsuperscript{[26,33]}

**Identification of compounds from Rhizoma Anemarrhenae**

Common Anemarrhena rhizome is the dried rhizome of *A. asphodeloides* Bge. (Asparagaceae). It has been reported that the main components found in Rhizoma Anemarrhenae are steroidal saponins, flavonoids, phenylpropanoids, and alkaloids. The major biologically active compounds are steroidal saponins, including timosaponin AIII, BII, BIII, and E1, and the xanthone derivatives mangiferin and neomangiferin.\textsuperscript{[55]} Recently published results have shown a broad spectrum of pharmacological activities for these two compound classes, including improvement of memory skills, anti-inflammatory, anti-tumor, and anti-diabetic effects.\textsuperscript{[56-62]}

Nöst, et al. Analysis of modified Gegen Qinlian decoction
In the mGQD formulation, 14 constituents derived from RA were assigned: three xanthone derivatives, ten steroidal saponins, and one fatty acid.

The structure of mangiferin (12) was confirmed by comparing its retention time, DAD spectrum, exact mass, and fragmentation pattern with an authentic standard.

Compound 5 was tentatively identified as neomangiferin. It produced a [M-H]⁻ ion at m/z 583.1315 and fragmented to m/z 493, corresponding to the loss of a 90 Da element from a C-glucose, m/z 463, indicating the loss of 120 Da from a C-glucose, and m/z 421, representing the loss of an O-glucose. The fragmentation pattern of fragments smaller than 421 again matches the pattern of mangiferin. The presence of the additional glucose moiety is also visible in the hypsochromic λmax shifts in the UV/Vis spectrum.

Compound 14 eluted 40 s after mangiferin and also gave a [M-H]⁻ ion at m/z 421.0781 and a [M+H]⁺ ion at m/z 423.0922, and displayed exactly the same fragmentation pattern as mangiferin. In the UV/Vis spectrum, a hypsochromic shift of λmax due to the change of position of the glucose moiety could be observed. Thus, it was tentatively identified as isomangiferin.

Timosaponin AIII (82), a spirostanol-type steroid saponin, was identified by comparing its retention time and fragmentation data with a reference standard.

All other characterized steroidal saponins in RA were from furostanol type. Compound 45 was tentatively identified as timosaponin N or its isomer. It presented a [M+H-HO]⁻ ion at m/z 919.4917 and fragmented to m/z 757, indicating the loss of an O-hexose moiety, m/z 595, representing the loss of a second O-hexose, m/z 433 for losing an O-hexose, m/z 289 for losing C₁₁H₂₀O₄, m/z 271, and m/z 253 by step-wise loss of two H₂O moieties.

Compound 51 showed exactly the same parent ion and fragmentation pattern as timosaponin N and was therefore tentatively identified as its isomer timosaponin E1 or macrostemonoside J.

Compounds 53, 55, 57, and 59, all presented a [M+H]⁺ ion at m/z 903.49 and showed the same fragmentation patterns. All generated a main fragment at m/z 741, corresponding to [M+H-hex⁻]. A second highly abundant fragment at m/z 579 indicates the loss of a second O-hexose. The fragment at m/z 417 represents [M+H-3 hex⁻], m/z 273 is derived from the loss of a C₁₁H₂₀O₄, fragment and m/z 255 is generated by further loss of H₂O. The compounds were tentatively identified by comparing their order of elution with data from the literature as timosaponin BII and its 25R isomer, 25S officinalisin I and officinalisin I, respectively.

Compounds 66 and 67 showed [M+H]⁺ ions at m/z 903.494. Both fragmented to m/z 741 [M+H-hex⁻], m/z 579 [M+H-2 hex⁻], 417 [M+H-3 hex⁻], 273 [M+H-3 hex-C₁₁H₂₀O₄], and 255 [M+H-3 hex-C₁₁H₂₀O₂H₂O]⁻. By comparing their retention times with data from the literature, the compounds were tentatively identified as timosaponin C and timosaponin BIII, respectively.

Compound 73 generated a parent ion at m/z 741.4417 [M+H-HO]⁻ in mode and fragmented to m/z 417, m/z 399 corresponding to [M+H-HO-2 hex⁻], m/z 381, m/z 289 [M+H₂O-H-2 hex-C₁₁H₂₀O], and m/z 271 and m/z 253 due to the further loss of two H₂O moieties, respectively. By comparison with existing data from literature, compound 73 was tentatively identified as anemarrhenasaponin II or its isomer.

The only fatty acid component detected in mGQD is compound 84. It showed a parent ion at m/z 311.2021, and was tentatively identified as dihydroxy-octadecadienoic acid by comparing the retention times and MS data with data from the literature.

Identification of compounds from Fructus Schisandrae
Fructus Schisandrae chinensis (Bei Wuweizi) is the dried ripe fruit of S. chinensis (Turcz.) Baill. (Schisandraceae). Previous studies have demonstrated its antioxidant, hepatoprotective, anti-inflammatory, anti-diabetic, and anti-cancer activities. It has also been reported to exhibit sedative and anxiolytic effects on the central nervous system. Until 2000, the Chinese Pharmacopoeia accepted both, the fruits of S. chinensis and of S. sphenanthera as Fructus Schisandrae. However, the chemical composition of both plants differs, and the fruits of S. chinensis are considered to be of higher quality. Therefore, since 2000, fruits from the two species are listed as two different crude drugs in the Chinese Pharmacopoeia. The main constituents of Fructus Schisandrae chinensis are dibenzocyclooctene lignans such as deoxyschisandrin, schisantherin A, and schisandrol A and B. Altogether, more than 100 lignans have been isolated from this herbal drug. In addition, Fructus Schisandrae chinensis contains organic acids, fatty acids, volatile oil, and sugars.

In mGQD, Wuweizi is a minor component with the lowest share. Nonetheless, five compounds derived from Fructus Schisandrae chinensis could be assigned. They were characterized as dibenzocyclooctene lignans by comparing their retention times and MS/MS data with data from literature. Schisandrin A (83) was identified by comparing its retention time and MS/MS data with an authentic standard.

Compound 76 generated a parent ion at 433.2221 in ESI positive mode. Fragment ions at m/z 415 [M+H-HO]⁻, m/z 384 [M+H-H₂O-OCH₃]⁻, and m/z 346 [M+H-H₂O-OCH₃]⁻ were detected, and the compound was tentatively identified as schisandrol A.

Compound 79 showed a [M+NH₄]⁺ ion at m/z 462.2486 and fragmented to m/z 399 [M+H-HO]⁻, m/z 368 [M+H-H₂O-OCH₃]⁻, and m/z 330 [M+H-H₂O-C₁₁H₂₀]. This fragmentation pattern was very similar to the one displayed by schisandrol A. The mass differences of the respective fragments between the two compounds were 16 Da, indicating the presence of a dioxy structure instead of two methoxy groups. Thus, compound 79 was tentatively assigned to be schisandrol B.
Compounds 80 and 81 both generated a typical neutral loss of 100 Da (C₇H₇COOH), indicating that both compounds possess either a tigloyl or an angeloyl moiety. Peak 80 showed a [M+NH₄]⁺ ion at m/z 546.3062 and fragmented to m/z 483 [M-H₂O]⁺, 401 [M-C₆H₅COOH]⁺, and 370 [M-C₆H₅COOH-CH₃O]⁺, and was tentatively assigned to be tigloylgomisin H or angeloylgomisin H. Compound 81 produced a ESI+ parent ion at m/z 576.3166 and was tentatively identified as angeloylgomisin Q.²⁹,³⁰ It is worth mentioning that these compounds can be used as chemical markers to differentiate between S. chinensis (Wuweizi) and S. sphenanthera (Nanweizi). Because the presence of tigloylgomisin H, angeloylgomisin H, and angeloylgomisin Q has only been reported for S. chinensis, the compounds are very suitable to be used as marker compounds for quality control.²²

**Identification of compounds from Rhizoma Zingiberis**

Rhizoma Zingiberis is the dried rhizome of Z. officinale Rosc. belonging to the family of Zingiberaceae. In Asian countries, it is commonly used for both dietary and medicinal purposes. Recently, ginger has gained huge scientific interest due to its diverse pharmacological effects including anti-nausea and anti-vomiting, immunomodulatory, anti-inflammatory, antioxidant, and anti-cancer activities.²⁷⁻²⁹ Moreover, ginger has been reported to benefit the treatment of T2DM by improving insulin sensitivity.²⁷⁻⁴¹ The pungent phenolic compounds gingerol and shogaol are considered the major active constituents.

In mGQD, ginger is one of the three newly added components in order to improve the efficacy of the classical formula. Because the amount of ginger in the formula is comparatively small, only one compound derived from Rhizoma Zingiberis, 6-gingerol (74), could be identified by comparing retention time and MS fragmentation pattern to an authentic reference.

**Development of a fast and straightforward high-performance thin-layer chromatography method for fingerprint analysis of modified Gegen Qinlian decoction**

HPTLC is a modern TLC technique which combines the advantages of TLC (simple, rapid) with improved efficiency and automatization possibilities. The application of precoated plates with a small average particle size (5−7 μm), narrow size distribution, and thinner coating layer (50−200 μm) leads to enhanced separation quality in an even shorter separation time. Semi-automated instruments provide better reproducibility by keeping development conditions constant between different analyses. Therefore, high separation efficiency and reproducibility make HPTLC a state-of-the-art analytical method for the quality analysis of complex TCM formulations containing up to hundreds of compounds.²⁵³

An efficient method for thin-layer chromatographic analysis has been developed for the qualitative analysis of mGQD, allowing to assign bands from analytical marker compounds of all herbal components of the formulation. Classic silica gel F₂₅₄ was used as stationary phase, and by applying only two different mobile phases, the major compounds detectable in each individual herb making up the mGQD formulation could be detected in the decoction of the mixture. For the analysis of nonpolar constituents which are primarily contained in ASE n-hexane (AH), ASE dichloromethane (AD), decoction n-hexane (DH), decoction dichloromethane (DD), and decoction ethyl acetate (DE) extracts, a mobile phase system containing the upper phase of a mixture of toluene: ethyl acetate: formic acid: water (20:10:1:1) was applied. The polar constituents in the ASE methanol extract (AM), total decoction (D), decoction ethyl acetate (DE), decoction n-butanol (DB), and decoction water residue (DW) fractions were separated by using a mobile phase system consisting of ethyl acetate: formic acid: glacial acetic acid: water (15:1:1:2).

**Constituents of Radix Puerariae**

With the nonpolar mobile phase, the reference compounds genistin, puerarin, and soyasaponin became visible as violet zone under UV light (254 nm), but could not be clearly assigned in the single herb or the mixture due to the weak signal intensity. After derivatization with vanillin-sulfuric acid, the reference compound soyasaponin became visible as violet zone (Rₓ = 0.13), which was detectable in both the n-butanol fraction of mGQD decoction and n-butanol fraction of RP decoction [Figure 2].

**Constituents of Radix Scutellariae**

In the nonpolar fractions of Radix Scutellariae and mGQD, the reference compounds baicalein and wogonin could be identified at Rₓ = 0.24 and Rₓ = 0.48, respectively. In addition, an unknown compound below the wogonin zone (Rₓ = 0.41) became visible as grayish zone in both the individual herb and in the mixture (decoction dichloromethane fraction), [Figure S1]. After development of the polar fractions with the polar mobile phase, the rather lipophilic compounds baicalein (Rₓ = 0.81) and wogonin (Rₓ = 0.90) could only be detected in the ethyl acetate fraction in Radix Scutellariae and mGQD. Baicalin, the glucuronide of baicalein, could be detected in all polar fractions derived from the single herb and was also detectable in the ASE methanol fraction, the decoction and decoction n-butanol extract of mGQD, although only weakly visible (Rₓ = 0.25) [Figure 3].

**Constituents of Rhizoma Coptidis**

The four reference compounds could not be eluted by the nonpolar mobile phase. However, one unidentified compound in the decoction dichloromethane fraction of Rhizoma Coptidis could also be detected in the same fraction of the mixture (Rₓ = 0.23) [Figure S2]. By development with the polar mobile phase, the reference compounds berberine, coptisine, jatrorrhizine, and palmatine with Rₓ = 0.32, Rₓ = 0.25, Rₓ = 0.28, and Rₓ = 0.24, respectively, became visible as the
main constituents of Rhizoma Coptidis. They all could be identified as fluorescent zones in the polar fractions of mGQD mixture, both in the ASE and the decoction extracts. Moreover, an additional unassigned yellow fluorescent compound with an $R_f$ value of 0.19 could be detected in all polar fractions of the single herb and the mixture [Figure 4].

**Constituents of Radix et Rhizoma Glycyrrhizae praeparata cum Melle**

The reference compounds glycyrrhizinic acid, liquiritin, isoliquiritin, and isoliquiritigenin are major constituents of Radix et Rhizoma Glycyrrhizae praeparata cum Melle. After development of the nonpolar fractions, isoliquiritin showed a $R_f$ value of 0.32 and could be detected in both the single herb and mGQD. Isoliquiritigenin showed a $R_f$ value of 0.37 but could not be unambiguously detected in the fractions as there were too many other co-eluting zones [Figure S3]. All reference substances could be identified in the polar fractions of the single herb ($R_f = 0.25, R_f = 0.47, R_f = 0.55, \text{and } R_f = 0.94$, respectively). Glycyrrhizinic acid could not be unambiguously assigned in the mGQD decoction fractions because it co-eluted with the strongly fluorescent Coptis alkaloids and may therefore be overlaid. Liquiritin could be identified in all polar fractions of mGQD, and isoliquiritin was visible in the $n$-butanol fraction of mGQD decoction. Due to the better separation by the polar mobile phase, also isoliquiritigenin could be detected in the ethyl acetate and $n$-butanol fractions of mGQD decoction. Moreover, a blue fluorescent compound
with an $R_f$ value of 0.84 could be detected in the ethyl acetate fraction of RRGP and also in the ethyl acetate and n-butanol fractions of mGQD decoction [Figure 5].

**Constituents of Rhizoma Anemarrhenae**

In the nonpolar fractions of Rhizoma Anemarrhenae and mGQD, sarsasapogenin, a steroidal sapogenin, could be detected as dark brown zone in all extracts except n-hexane fractions of RA ($R_f = 0.47$). In the mixture, the intensity is apparently lower, though sarsasapogenin could be identified in the decoction dichloromethane and ethyl acetate fractions. Moreover, two unknown compounds in mGQD could be detected as violet zones in the upper middle part of the chromatogram and assigned to Rhizoma Anemarrhenae ($R_f = 0.59$ and 0.49) [Figure S4]. By applying the polar mobile phase, mangiferin could not be definitely identified in mGQD because the alkaloid berberine from RC showed a similar yellow zone almost at the same position ($R_f = 0.29$, data not shown). Timosaponin AIII was detected in the decoction ethyl acetate and n-butanol fractions of the single herb and the mixture with an $R_f$ value of 0.17. Sarsasapogenin was eluted to the upper part of the chromatogram ($R_f = 0.84$) and was detected in the ethyl acetate fractions of both RA and mGQD. One of the two unidentified substances from the nonpolar chromatogram showed up as violet zone directly above sarsasapogenin ($R_f = 0.87$, marked in green circle) [Figure 6].

**Constituents of Fructus Schisandrae chinensis**

The compounds from FS were detected in UV 254 nm directly after developing the plates. In the nonpolar fractions, schisandrin A could be identified in both the n-hexane fractions of Fructus Schisandrae chinensis and mGQD with an $R_f$ value of 0.54. Schisandrin B, which is contained to a lower extent, could be only detected in the single herb, while it was not visible in the mixture ($R_f = 0.61$). Additionally, a third compound in mGQD derived from Fructus Schisandrae chinensis could be detected in the lower middle section of the chromatogram with an $R_f$ value of 0.28 [Figure 7]. Because Fructus Schisandrae chinensis is mainly composed of nonpolar constituents such as lignans and fatty oil, there were only limited spots detectable after developing with the polar mobile phase system. The reference compounds eluted almost at the solvent front and therefore could not be evaluated. In the upper section of the chromatogram, one compound in the ethyl acetate fraction seems to be derived from Fructus Schisandrae chinensis. However, it could not be definitely assigned because the detection method is quite unspecific and Rhizoma Anemarrhenae showed a zone in similar position (data not shown).

**Constituents of Rhizoma Zingiberis**

In the nonpolar fractions of mGQD and Rhizoma Zingiberis, 6-gingerol could be detected with an $R_f$ value of 0.38 in all sub-extracts except the ethyl acetate fraction of the decoction in both pure ginger and mGQD. 6-shogaol was visible in the n-hexane fractions of the single herb and the mixture ($R_f = 0.54$) [Figure 8]. Because most of the compounds in RZ are volatile oil components, only few compounds were visible after development with the polar mobile phase. A characteristic...
Using the nonpolar mobile phase, schisandrin A from FS could be detected in the n-hexane fraction of mGQD in UV 254 nm. In addition, a second very prominent zone derived from FS was detected in the lower middle section of the chromatogram ($R_t = 0.28$), which can also be used as even more specific marker for FS as its intensity is much higher than that of schisandrin A. After spraying with vanillin-sulfuric acid reagent, 6-gingerol and 6-shogaol from RZ could be clearly identified as greenish-gray zones in the n-hexane extract of mGQD. From RS, baicalein and wogonin were detected as grayish-green and brown zones in the ethyl acetate fraction of mGQD.

**Conclusions**

In the present study, UHPLC combined with DAD and Q-Exactive Orbitrap high-resolution MS detection was employed to intensively characterize a modified version of the classical TCM formula GQD, consisting of seven herbal components. A total of 91 peaks could be traced back to their originating plant, 84 of which could tentatively or unambiguously be identified in the decoction. This means that these components could be detected in both mGQD and in the corresponding individual herb. Seventeen compounds were unambiguously identified by comparison of retention time and MS data with authentic standards, while 67 were tentatively identified based on comparison of their UV/Vis spectra and MS fragmentation patterns obtained in both ESI positive and negative modes with the existing data from the literature. The identified compounds included flavonoids, alkaloids, triterpene saponins and steroidal saponins, lignans, and phenolic compounds. This in-depth analysis as well as the assignment of a high number of mGQD constituents to their originator herbs provides valuable information for the quality assessment and a reproducible production of this formulation.

Moreover, a new HPTLC method for the efficient qualitative analysis of the modified GQD formula was established. As the mixture consists of seven herbal ingredients, all of them containing substances with different chemical properties, it was necessary to apply two different mobile phases in order to analyze both the lipophilic and hydrophilic compounds in mGQD decoction. Altogether, 18 compounds in mGQD could be identified by comparing their $R_t$ values and color with authentic references. For each individual herb, at least one characteristic marker compound could be defined from the identified compounds. In addition, several unknown compounds in mGQD could be assigned to their originating...
herb. This newly established method allows the fast and efficient qualitative analysis of mGQD by detecting and identifying all the seven herbal ingredients at once instead of applying specific methods for each of the individual herbs. Eleven major compounds, including at least one compound derived from each single plant, were selected as marker compounds for the quality assessment of mGQD in order to ensure the presence of each individual herbal component.

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Conflicts of interest

There are no conflicts of interest.

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Figure S1: HPTLC of nonpolar fractions of mGQD (mG) compared with Radix Scutellariae in VIS after derivatization with iron-III-chloride. Red circles: compounds identified by authentic references; Green circles: unidentified compound in mGQD deriving from RS. R1: Baicalein, R2: Baicalin, R3: Wogonin, AH: ASE hexane fraction, AD: ASE dichloromethane fraction, DH: Decoction hexane fraction, DD: Decoction dichloromethane fraction, DE: Decoction ethyl acetate fraction, HPTLC: High-performance liquid chromatography

Figure S2: HPTLC of nonpolar fractions of mGQD (mG) compared with Rhizoma coptidis (RC) detected in ultraviolet 366 nm. Green circles: unidentified compound in mGQD deriving from RC. R1: Berberine, R2: Coptisine, R3: Jatrorrhizine, R4: Palmatine, AH: ASE hexane fraction, AD: ASE dichloromethane fraction, DH: Decoction hexane fraction, DD: Decoction dichloromethane fraction, DE: Decoction ethyl acetate fraction, HPTLC: High-performance liquid chromatography

Figure S3: HPTLC of nonpolar fractions of mGQD (mG) compared with Radix et Rhizoma Glycyrrhizae Praeparata cum Melle (RG) in ultraviolet 366 nm after derivatization with sulfuric acid. Red circles: compounds identified by authentic references. R1: Ammonium glycyrrhizinate, R2: Liquiritin, R3: Isoliquiritin, R4: Isoliquiritigenin, AH: ASE hexane fraction, AD: ASE dichloromethane fraction, DH: Decoction hexane fraction, DD: Decoction dichloromethane fraction, DE: Decoction ethyl acetate fraction, HPTLC: High-performance liquid chromatography

Figure S4: HPTLC of nonpolar fractions of mGQD (mG) compared with Rhizoma Anemarrhenae (RA) in VIS after derivatization with vanillin-sulfuric acid. Red circles: compounds identified by authentic references, Green circles: unidentified compound in mGQD deriving from RA. R1: Mangiferin, R2: Timosaponin AIII, R3: Sarsasapogenin, AH: ASE hexane fraction, AD: ASE dichloromethane fraction, DH: Decoction hexane fraction, DD: Decoction dichloromethane fraction, DE: Decoction ethyl acetate fraction, HPTLC: High-performance liquid chromatography