

Research Article

High-Performance Thin-Layer Chromatographic Quantification of Rosmarinic Acid and Rutin in Abnormal Savda Munziq

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Received 16 January 2012; Revised 4 June 2012; Accepted 19 June 2012

Academic Editor: Sevgi Kolayli

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A high-performance thin-layer chromatographic (HPTLC) method has been established for simultaneous analysis of rosmarinic acid and rutin in Abnormal Savda Munziq (ASMq). A methanol extract of ASMq was used for quantification. The compounds were separated on silica gel H thin layer plate with ethyl acetate-formic acid-acetic acid-water 15 : 1 : 1 : 1.5 (v/v) as a developer, trichloroethanol as the color reagent. The plates were scanned at 365 nm. The linear calibration data of rosmarinic acid and rutin were in the range of 0.0508 to 0.2540 μg ($r = 0.9964$), 0.2707 to 1.35354 μg ($r = 0.9981$), respectively. The recovery rate of rosmarinic acid was 99.17% (RSD = 2.92%) and rutin was 95.24% (RSD = 2.38%). The method enables rapid screening, precise, selective, and sensitive quantification for pharmaceutical analysis.

1. Introduction

Abnormal Savda Munziq (ASMq), one of the Uighur medicinal herbal preparations, is made up of ten medicinal species, which are *Cordia dichotoma* Forst. f., *Anchusa italica* Retz., *Glycyrrhiza uralensis* Fisch., *Adiantum capillus-veneris* L., *Euphorbia humifusa* Willd., *Ziziphus jujuba* Mill., *Lavandula angustifolia* Mill., *Foeniculum vulgare* Mill., *Melissa officinalis* L., and *Alhagi pseudalhagi* Desv. These herbs are widely distributed in the Xinjiang region of China. ASMq has long been used in Traditional Uighur Medicine for the treatment of several diseases such as digestive cancer, diabetes, cardiovascular diseases or chronic asthma [1], the effects on nonspecific immunity, humeral immunity, and cell immunity functions of cyclophosphamide-induced immune-suppressed mice [2]. It is regularly used by Uighur physicians for the treatment of cancer, and in southern Xinjiang many ordinary people regularly self-medicate with it to prevent cancers. To date, these putative anticancer effects of ASMq have been little studied and its potential mechanism of action is still unclear. A possible anticancer effect of ASMq clearly warrants further investigation. In previous studies, ASMq was proved to possess strong free radical scavenging effects, decrease biological markers of oxidative stress in man [3], protect mitochondria

and DNA against OH-induced oxidative damage in a cell-free system [3, 4], and inhibit cancer cells proliferation and viability in vitro [5].

Rutin [6] ($\text{C}_{27}\text{H}_{30}\text{O}_{16}$; Figure 1) is a kind of flavonoids, which is found in the Labiatae, Scrophulariaceae, Compositae, and other plants. Rutin has been reported to have antidiabetic, antithrombotic, anti-inflammatory, and anticarcinogenic activities [7]. The literature determined rutin in flavanoid fraction of stem extract of *Hibiscus micranthus* (FFHM) by HPTLC [8]. Rosmarinic acid [9] ($\text{C}_{18}\text{H}_{16}\text{O}_8$; Figure 2) has antioxidant, anti-inflammatory, and antimicrobial activities. The antioxidant activity of rosmarinic acid was found to be stronger than vitamin E. Rosmarinic acid helps to prevent cell damage caused by free radicals, thereby reducing the risk for cancer and atherosclerosis. Rosmarinic acid is also used for food preservation. In Japan the perilla extracts, rich in rosmarinic acid, are used in garnish and improve the shelf life of fresh seafood. Besides, rosmarinic acid is used to treat peptic ulcers, arthritis, cataract, cancer, rheumatoid arthritis, and bronchial asthma as discussed elsewhere [10].

Different analytical methods for the determination of rutin and rosmarinic acid have been reported, which include high-performance liquid chromatography (HPLC) with UV

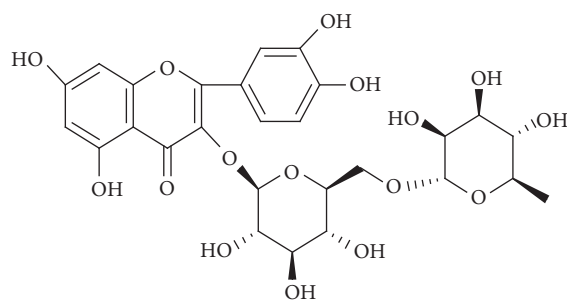


FIGURE 1: Chemical structure of rutin.

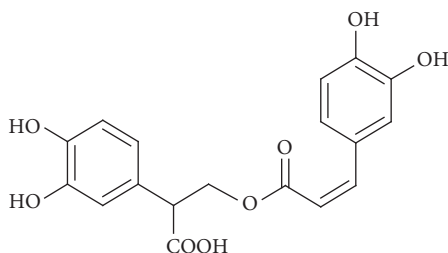


FIGURE 2: Chemical structure of rosmarinic acid.

detection (HPLC-UV) [11, 12]. At present, the researches on ASMq mainly have focused on its pharmacological action/immunity [1–5], immunity functions [2, 13], and extraction technology [14]. However, there is little study on simultaneous quantitative analysis of rutin and rosmarinic acid with validated high-performance thin-layer chromatographic (HPTLC) in the pharmaceutical dosage form. The objective of the present work is to develop a simple, economical, precise, and accurate HPTLC method for simultaneous quantitation of rutin and rosmarinic acid in ASMq.

2. Experimental

2.1. Chemicals and Materials. Rutin standard was purchased from China Pharmaceutical and Biological Products, 100080, $\geq 92.5\%$; rosmarinic acid standard was purchased from Sigma-Aldrich, CFAE-536954, $\geq 97.00\%$.

Methanol used was of HPLC grade (Fisher Chem Alert Guide, USA); ethyl acetate (Fuyu Fine Chemical Company, China), formic acid (Beijing Fine Chemical Company, China) and acetic acid (Nanda Chemical Company, China) used were of analytical grade. Ultrapure water was obtained by a direct-Q system (Millipore, USA). Abnormal Savda Munziq (ASMq) was supplied by Uygur Medicine Department of Xinjiang Medical University. Silica gel H plates (10 cm \times 20 cm), purchased from Huoshan Chemical material factory, China.

CAMAG Linomat 5, CAMAG TLC Scanner 3 was purchased from KAMA GmbH, Switzerland; Milli-Q3 Ultrapure Water Purification Systems purchased from Millipore, USA; Electronic balance with XS105 and AL204 (purchased from Mettler Toledo, Switzerland; Ultrasonic cleaning machine

KQ-200KDE purchased from Kunshan Ultrasonic Instruments Co., Ltd., China, vacuum oven DZF-6021 were purchased from precision equipment Co., Ltd., China.

2.2. Preparation of Standard Solution. Stock solutions of rutin ($0.88 \text{ mg}\cdot\text{mL}^{-1}$) and rosmarinic acid ($6.6 \text{ mg}\cdot\text{mL}^{-1}$) were prepared in methanol. Standard solutions of rutin $100 \mu\text{L}$ and rosmarinic acid $25 \mu\text{L}$ were mixed with $200 \mu\text{L}$ methanol and filtered through $0.45 \mu\text{m}$ filter paper, which were then used for subsequent chromatographic analysis.

ASMq was weighed accurately (2.0000 g) then transferred to a flask and appropriate amount of methanol was added and sonicated (150 w , 60 Hz) for 30 min at room temperature then filtered. Methanol was added to residue and sonicated (150 w , 60 Hz) for 40 min . Filtrates were combined and filtered through $0.45 \mu\text{m}$ filter paper. The solvent was evaporated to dryness. Then the residue was dissolved in methanol and diluted to 25 mL with methanol to obtain a stock solution.

2.3. Chromatography. HPTLC was performed on silica gel H plates, which were activated at 105°C for 10 min in advance. Ethyl acetate-formic acid-acetic acid-water $15:1:1:1.5$ (v/v) was utilized as a developer.

3 batches of samples ($5 \mu\text{L}$) and standards were applied to the plates as 8 mm bands, 6 mm apart, using a CAMAG Linomat 5 sample applicator equipped with a $100 \mu\text{L}$ syringe. The plates were developed in a $200 \text{ mm} \times 100 \text{ mm}$ twin-trough chamber; the development distance was 18 cm . The plates were removed from the chamber and dried. The densitometric determination was performed at 365 nm with a CAMAG TLC scanner 3 in absorbance mode. The slit dimensions were $10.00 \text{ mm} \times 0.20 \text{ mm}$ and the scanning speed was $50 \text{ mm}\cdot\text{s}^{-1}$. The source of radiation was a deuterium lamp emitting a continuous UV spectrum from 200 nm to 400 nm .

2.4. Calibration Plots (Linearity). A working standard solution of rutin $0.88 \text{ mg}\cdot\text{mL}^{-1}$ and rosmarinic acid $0.66 \text{ mg}\cdot\text{mL}^{-1}$ were used to contrast calibration plots by applying 1.0 , 2.0 , 3.0 , 4.0 , $5.0 \mu\text{L}$ each (corresponding to 270.7 , 541.4 , 812.1 , 1082.8 , 1353.5 ng/spot of rutin; 50.7 , 101.4 , 152.1 , 202.8 , 253.5 ng/spot of rosmarinic acid) to a plate. Chromatographic plates were developed and scanned as described above. The responses of rutin and rosmarinic acid were linear functions of concentration over the range of 270.7 – 1353.5 ng/spot and 50.7 – 253.5 ng/spot , separately; the equations of the calibration plots for rutin and rosmarinic acid were $Y = 1075.08 + 14232.21X$ ($r = 0.9981$), $Y = 393.37 + 50878.94X$ ($r = 0.9964$). The chromatograms are shown in Figures 3, 4, and 5.

3. Results and Discussion

3.1. Optimization of the Developer. The composition of the developer was optimized by testing different solvent systems of different polarity. The best resolution was obtained by use of ethyl acetate-formic acid-acetic acid-water $15:1:1:1.5$ (v/v), and the obtained R_F values were 0.31 ± 0.03 for rutin and 0.82 ± 0.03 for rosmarinic acid.

TABLE 1: Results of recovery test of Rutin and Rosmarinic acid ($n = 6$).

| No. | Amount of drug taken (g) | Amount of rutin added (mg) | Amount of rosmarinic acid added (mg) | Recovery (rutin) (%) | Recovery (Rosmarinic acid) (%) | RSD (Rutin) (%) | RSD (rosmarinic acid) (%) |
|-----|--------------------------|----------------------------|--------------------------------------|----------------------|--------------------------------|-----------------|---------------------------|
| 1 | 1.0149 | 1.76 | 0.66 | 101.67 | 97.73 | | |
| 2 | 1.0152 | 1.76 | 0.66 | 98.34 | 96.79 | | |
| 3 | 1.0064 | 1.76 | 0.66 | 95.70 | 98.47 | 2.38 | 2.92 |
| 4 | 1.0076 | 1.76 | 0.66 | 98.10 | 96.53 | | |
| 5 | 1.0085 | 1.76 | 0.66 | 95.12 | 103.42 | | |
| 6 | 1.0378 | 1.76 | 0.66 | 97.44 | 102.11 | | |

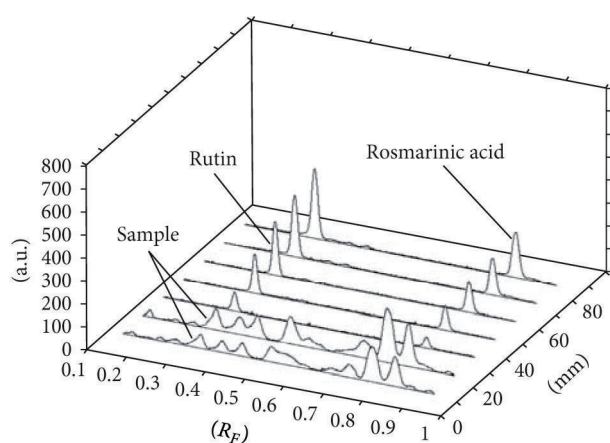


FIGURE 3: HPTLC chromatograms of rutin and rosmarinic acid mixture and ASMq samples.

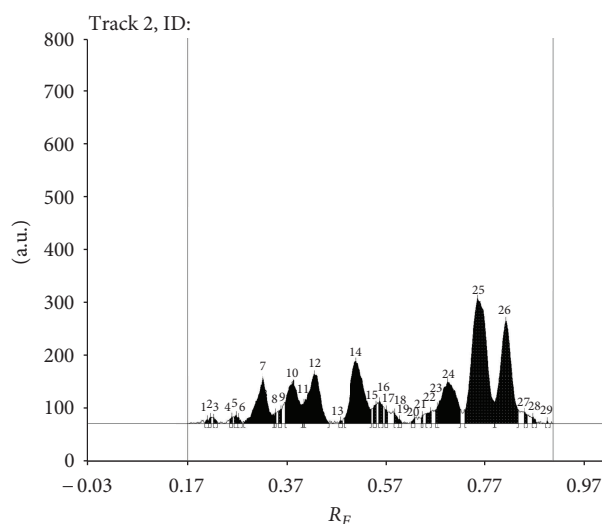


FIGURE 5: HPTLC chromatograms of rutin and rosmarinic acid mixture in ASMq.

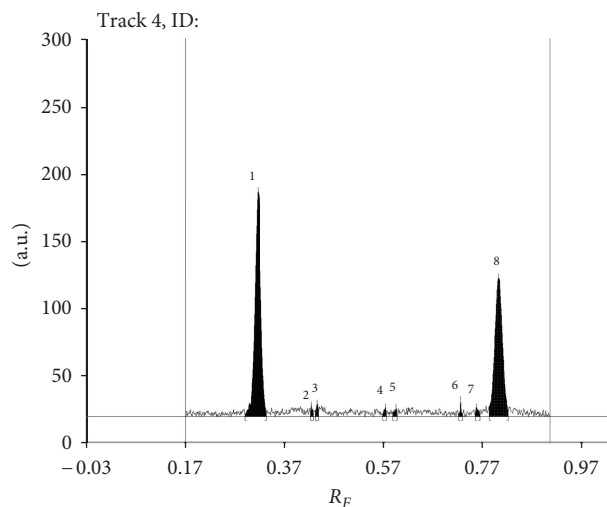


FIGURE 4: HPTLC chromatograms of standard samples.

3.2. Precision. The precision of the method was determined by analyzing standard solutions in triplicate at three concentrations on the same day and on different days for interday and intraday precision, respectively. Precision was expressed as RSD of the series of measurements.

The samples were checked repeatedly by measuring the five spots for the same concentration having same concentration of rutin and rosmarinic acid (1.354 mg per band for rutin and 0.254 mg per band for rosmarinic acid mixed standards) applied on the same plate without changing the position of the plate. The relative standard deviations (RSDs) of intra-day were 2.0% and 3.0% for rutin and rosmarinic acid samples, respectively. The relative standard deviations (RSDs) of inter-day for the rutin and rosmarinic acid samples were 1.98% and 1.26%, respectively.

3.3. Stability. Stability of the rutin and rosmarinic acid was assessed by use of quality-control samples (1.354 mg per band for rutin and 0.254 mg for rosmarinic acid mixed standards); the measurement of the peak area and the peak height were checked at 1, 2, 4, 8, 24, 48 h. The RSDs for the rutin and rosmarinic acid samples are 1.83% and 1.56%, respectively.

3.4. Recovery. Recovery of rutin and rosmarinic acid was determined by the standard addition method. Known amount of standards of rutin and rosmarinic acid (1.67 mg per band for rutin, 0.66 mg per band for rosmarinic acid) were added to six prequantified samples. The amounts of

TABLE 2: Quantification of rutin and rosmarinic acid in three batches of ASMq.

| ASMq no. | Rutin (mg/g) | Rosmarinic acid (mg/g) |
|----------|----------------|------------------------|
| 201005 | 1.751 ± 0.0027 | 0.685 ± 0.0023 |
| 201006 | 1.825 ± 0.0031 | 0.692 ± 0.0021 |
| 201007 | 1.898 ± 0.0029 | 0.702 ± 0.0027 |

rutin and rosmarinic acid were determined by measuring the peak areas and by fitting these values into the regression equation of the calibration plot. The results were shown in Table 1.

3.5. Quantification Limits. The limits of quantification (LOQ) were calculated by preparing solutions at six volumes (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 μL , 0.0027 $\text{ng}\cdot\mu\text{L}^{-1}$ for rutin and 0.0051 $\text{ng}\cdot\mu\text{L}^{-1}$ for rosmarinic acid) in the lower range of linear regression curve. The LOQ were 4.1 ng/spot and 5.1 ng/spot for rutin and rosmarinic acid, respectively.

3.6. Quantification of Rutin and Rosmarinic Acid in Samples. Amounts of rutin and rosmarinic acid in three batches of ASMq were shown in Table 2.

4. Conclusion

A rapid, precise, and simple HPTLC method has been established, which has good specificity, for simultaneous analysis of rutin and rosmarinic acid in ASMq for the first time. Use of silica gel H plants with ethyl acetate-formic acid-acetic acid-water 15 : 1 : 1 : 1.5 (v/v) as developer enables good resolution with R_F values of 0.31 ± 0.03 , 0.82 ± 0.03 for rutin and rosmarinic acid, respectively.

Two color-developing agents were compared in this study, because of different principles. The air bleached the bands' color out after a while when alcoholic solution of sulfuric acid was used. It brought difficulties for stability of experiment. However, when aluminum chloride-ethanol solution (1.5%) was used the bands' colors were clear and results were stable. The study offered basic research data for the establishment of quality standard of ASMq.

Acknowledgment

This work was supported by the Program for Changjiang Scholar and Innovative Team IRT0977.

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