Research Article

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

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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 (r² = 0.9964), and 0.2707 to 1.3535 μg (r² = 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., Anthesis 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] (C₂₇H₃₀O₁₄; 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 flavonoidal fraction of stem extract of Hibiscus micranthus (FFHM) by HPTLC [8]. Rosmarinic acid [9] (C₁₈H₁₄O₇; 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
2. Experimental

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

Methanol 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 × 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 mg·mL⁻¹) and rosmarinic acid (6.6 mg·mL⁻¹) were prepared in methanol. Standard solutions of rutin 100 μL and rosmarinic acid 25 μL were mixed with 200 μL methanol and filtered through 0.45 μm 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 μm 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 μL) 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 μL syringe. The plates were developed in 200 mm × 100 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 mm × 0.20 mm and the scanning speed was 50 mm·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 mg·mL⁻¹ and rosmarinic acid 0.66 mg·mL⁻¹ were used to contrast calibration plots by applying 1.0, 2.0, 3.0, 4.0, 5.0 μL 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).

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

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.

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 per band 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
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 ng μL⁻¹ for rutin and 0.0051 ng μL⁻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 Rf 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
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References

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

<table>
<thead>
<tr>
<th>ASMq no.</th>
<th>Rutin (mg/g)</th>
<th>Rosmarinic acid (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>201005</td>
<td>1.751 ± 0.0027</td>
<td>0.685 ± 0.0023</td>
</tr>
<tr>
<td>201006</td>
<td>1.825 ± 0.0031</td>
<td>0.692 ± 0.0021</td>
</tr>
<tr>
<td>201007</td>
<td>1.898 ± 0.0029</td>
<td>0.702 ± 0.0027</td>
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