Commonalities and characteristics of aqueous extracts from three Uighur medicines were analyzed by using three-stage infrared spectroscopy combined with ultra-performance liquid chromatography-time of flight-mass spectra

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OBJECTIVE: To establish a method to analyze the commonalities and characteristics of the aqueous extracts from three Uighur medicines.

METHODS: In this study, a combination method of three-stage infrared spectroscopy and ultra-performance liquid chromatography-time of flight-mass spectra (UPLC-TOF-MS) method was used to analyze the commonalities and characteristics of the aqueous extracts from Hezi (Fructus Chebulae), Maohezi (Terminalia Belliricae Fructus) and Xiqingguo (Chebulae Fructus Immaturus).

RESULTS: In Fourier transform-infrared spectroscopy spectra, all three samples showed the characteristic absorption peaks of tannins similarly. According to UPLC-TOF-MS data analysis, the difference of the position and intensity of the peaks at 1713-1707 cm\(^{-1}\) in the three samples were related to the difference in the relative content of tannins and pentacyclic triterpenoids. In second derivative infrared spectroscopy spectra, further analysis of the differences in the infrared spectra of the three samples was performed by increasing the apparent resolution. In combination with UPLC-TOF-MS data, some features infrared absorption peaks were assigned. The absorption peaks at 1032-1030 cm\(^{-1}\) assigned to the stretching vibration of C-O-C (ether bond) groups of tannins; 1164-1163, 1063-1062, 1009-1005, 904 cm\(^{-1}\) attributed to the stretching vibration of C-O (ether bond) and C-O-C group of glycosidic compounds. 1385-1383 cm\(^{-1}\) attributed to the bending vibration of C-H (alkyl hydrogen) of methoxyl group of pentacyclic triterpenoids. Peak 835 cm\(^{-1}\) was assigned to the characteristic absorption
peak of bending vibration of = C-H groups of penta-
cyclic triterpenoids. The two dimensional correla-
tion infrared spectroscopy could further quickly dis-
tinguish three samples through the dynamic struc-
tural information of their chemical components
and discrepancy of auto-peaks and cross-peaks intu-
itively in the range of 1720-419 cm⁻¹.

CONCLUSION: By comparing the intensity of the
infrared characteristic absorption peaks, the main
chemical components were assigned, which signifi-
cantly enriched and perfected the data analysis of
the infrared spectra of three Uighur Medicines. It
provided a rationale for the identification of med-
cinal materials with complex and similar chemical
components using the three-stage infrared spect-
roscopy and UPLC-TOF-MS.

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Keywords: Tannins; Terminalia; Spectrophotome-
try, infrared; Chromatography, liquid; Uighur
Medicine

INTRODUCTION

Hezi (Fructus Chebulae) is a dried ripe fruit of Termina-
alia chebula Retz. Maohezi (Terminalia Belliricae Fruc-
ti) is a dried ripe fruit of Terminalia bellirica (Gaertn.)
Roxb. Xiqingguo (Chebulae Fructus Immaturus) is a
dried immature fruitlet of Terminalia chebula Retz.¹³
All these three species of Uighur Medicine belong to
tannins drugs of Combretaceae with convergence,
bleeding, antibacterial and other effects⁶ which are of-
ten single or mutual compatible used in the prepara-
tion of Uighur Medicine.⁷ Due to the similarity of the
plant sources and chemical composition, rapid identifi-
cation is very important for the safety and guidance of
formula prescriptions.

Fourier transform-infrared spectroscopy (FT-IR) spec-
troscopy can provide detailed information of complicat-
ed Chinese medicinal herbs without purification. Re-
cently, a three-stage infrared spectroscopy method, FT-
IR integrated with second derivative infrared spec-
troscopy (SD-IR) and two dimensional correlation infra-
red spectroscopy (2D-IR) was proposed by Sun et al.¹⁴ to
provide a rapid and effective analysis of complicated
and similar mixture systems, such as medicinal herbs
and the extracts. With the continuous development of
chromatography technology, FT-IR combines with
some chromatographic methods, such as TLC,¹⁰ GC,¹¹ and
HPLC,¹²,¹³ begin to apply to analyze medicinal herbs.
However, this chromatography requires stan-
dards for qualitative analysis. And it is hardly to gain a
variety of standards in a complex system of medicinal
herbs.

UPLC-TOF-MS is a modern separation and analysis
 technique developed at the beginning of the 21st centu-
ry.¹⁰ It is combined with the fast and efficient separa-
tion ability of UPLC and the wide testing range, high
resolution and specificity of TOF-MS, which has been
widely used in research on medicinal herbs chemical in-
gredients, providing more accurate qualitative and
quantitative analysis for complicated systems.

Because of the high similarity of chemical ingredients
in the three medicines, it has brought certain difficul-
ties in the analysis and identification of medicinal ma-
terials. In this study, we aimed at establishing a method
combined with the three-stage infrared spectroscopy
and UPLC-TOF-MS method to analyze the common-
alities and characteristics of the aqueous extracts from
three Uighur medicines.

MATERIALS AND METHODS

Apparatus

Spectrum GX FT-IR spectrometer (PerkinElmer, Man-
chester, UK) equipped with DTGS detector in determina-
tion range of 4000-400 cm⁻¹. All IR spectra were re-
corded from an accumulation of 32 scans with a resolu-
tion of 4 cm⁻¹ and 0.2 cm/s of OPD speed. The inter-
fences of H₂O and CO₂ were subtracted while scan-
ing a portable programmable temperature controller
(Model 50-886, Love Control Corporation, Milford,
MA, USA) is the variable temperature attachment.

The chromatography was performed on an Aqueous
ACQUITY UPLC system combined with Aqueous
LCT Premier XE TOF-MS. Column: ACQUITY
UPLC BEH C₂₈ (2.1 mm × 50 mm, 1.7 µm; Waters,
Milford, MA, USA). Column temperature: 30 °C.

Reagents

KBr (specpure) was purchased from (Sigma, St. Louis,
MO, USA); chromatographic grade of Methanol and
Formic acid (Sigma, St. Louis, MO, USA); others re-
agents were all analytical purity (Tianjin Chemical Re-
tant Co., Tianjin, China).

Samples

Hezi (Fructus Chebulae) (HZ), Maohezi (Terminalia
Belliricae Fructus) (MHZ) and Xiqingguo (Chebulae
Fructus Immaturus) (XQG) were purchased in
Urumqi, identified by Professor Yun Zhu (College of
Pharmacy, Shihezi University, Xinjiang, China).
Each material 14 g was extracted with distilled water (3 ×
300 mL). The aqueous extracts (HZ-W; MHZ-W and
XQG-W) were evaporated to dryness until the weight
of concentrate close to the original material amount of
weight and through vacuum drying processing into sol-
id samples.

Procedure

IR: Each solid sample (1-2 mg) was blended with KBr,
thoroughly grinded the mixture evenly and pressed in-
to tablets, and the spectra were collected at room temperature. To obtain the 2D-IR spectra, the sample tablets were put into the sample pool with a temperature controller. The temperature was from 50 to 120 °C with an increasing rate at 2 °C/min, the dynamic spectra were collected at each interval of 10 °C. The full temperature scan lasted for a total time of 50 min.

UPLC-TOF-MS: The mobile phase was gradient elution which was mixed with solvent A (methanol) and B (0.1% formic acid aqueous). The gradient program was as follows: 0.7-4.1 min, 10%-30% A; 4.1-10.7 min, 30%-45% A; 10.7-15.7 min, 45%-75% A; 15.7-16.1 min, 75%-95% A. The flow rate was 0.2 mL/min. The auto-sampler pool was conditioned at room temperature. To obtain the spectra, the auto-sampler pool was conditioned at room temperature (Figure 1). The correlation coefficients of three samples were 1.0000 (HZ-W); 0.9467 (MHZ-W); 0.9610 (XQG-W) respectively. The preliminary assignments of characteristic absorption peaks for FT-IR spectra of HZ-W, MHZ-W and XQG-W were summarized in Table 1. According to the literature, FT-IR spectra showed obvious characteristic absorption peaks of tannins. The strong and broad peaks at 3399-3380 cm⁻¹ are almost generated by the stretching vibration of O-H groups. The strong peaks at 1713-1707 cm⁻¹ are characteristic peaks of the stretching vibrations of carbonyl groups. The three representative peaks in the range of 1615-1614, 1534-1518 and 1449-1447 cm⁻¹ are mainly caused by phenol ring frame vibration. The obvious difference could be found in the range of 1534-1518 cm⁻¹. A weak absorption peak was observed at 1534 cm⁻¹ in HZ-W and 1518 cm⁻¹ in XQG-W respectively. However, MHZ-W has no absorption in this position. The two peaks in the range of 1347-1343 and 1211-1208 cm⁻¹ were attributed to the bending vibration of O-H groups and the stretching vibration of C-O groups of phenolic acids respectively. And the peaks in the range of 1037-1034 cm⁻¹ were mostly attributed to the stretching vibration of C-O-C groups. A great deal of weak absorption appears in the range of 900-400 cm⁻¹ while a weak absorption peak only appeared at 924 cm⁻¹ in MHZ-W. Collectively, FT-IR spectra of the aqueous extracts of three samples (HZ-W, MHZ-W and XQG-W) were extremely similar so that it’s difficult to distinguish the three samples only using FT-IR.

RESULTS

FT-IR spectra

FT-IR spectra of the aqueous extracts of three samples (HZ-W, MHZ-W and XQG-W) were collected in the range of 4000-400 cm⁻¹ at room temperature (Figure 1). The correlation coefficients of three samples are 1.0000 (HZ-W); 0.9467 (MHZ-W); 0.9610 (XQG-W) respectively. The preliminary assignments of characteristic absorption peaks for FT-IR spectra of HZ-W, MHZ-W and XQG-W were summarized in Table 1. According to the literature, FT-IR spectra showed obviously the characteristic absorption peaks of tannins. The strong and broad peaks at 3399-3380 cm⁻¹ are almost generated by the stretching vibration of O-H groups. The strong peaks at 1713-1707 cm⁻¹ are characteristic peaks of the stretching vibrations of carbonyl groups. The three representative peaks in the range of 1615-1614, 1534-1518 and 1449-1447 cm⁻¹ are mainly caused by phenol ring frame vibration. The obvious difference could be found in the range of 1534-1518 cm⁻¹. A weak absorption peak was observed at 1534 cm⁻¹ in HZ-W and 1518 cm⁻¹ in XQG-W respectively. However, MHZ-W has no absorption in this position. The two peaks in the range of 1347-1343 and 1211-1208 cm⁻¹ were attributed to the bending vibration of O-H groups and the stretching vibration of C-O groups of phenolic acids respectively. And the peaks in the range of 1037-1034 cm⁻¹ were mostly attributed to the stretching vibration of C-O-C groups. A great deal of weak absorption appears in the range of 900-400 cm⁻¹ while a weak absorption peak only appeared at 924 cm⁻¹ in MHZ-W. Collectively, FT-IR spectra of the aqueous extracts of three samples (HZ-W, MHZ-W and XQG-W) were extremely similar so that it’s difficult to distinguish the three samples only using FT-IR.

UPLC-TOF-MS outcomes

UPLC-TOF-MS data showed that the main components of HZ-W, MHZ-W and XQG-W were tannins.
such as 1, 3, 6-tri-O-galloyl-β-D-glucose, pentacyclic triterpenoids and arjunglucoside I. In addition, the sample also contained other types of minor chemical ingredients such as organic acids, flavonoids (not listed).

We selected nine chemical components with more obvious differences for analysis shown in Figures 2, 3 and Table 1. 1, 3, 6-tri-O-galloyl-β-D-glucose, corilagin, chebulin, punicalin and chebulinic acid are the tannins which are the main components in three medicines. However, the relative contents of these ingredients in each medicine are significantly different. For example, the relative content of 1, 3, 6-tri-O-galloyl-β-
D-glucose in HZ-W and MHZ-W is similar, which is about higher 4-5 times in XQG-W. In addition, the levels of Chebulanic, punicalin, and chebulin acid in the XQG-W are much higher than that of HZ-W and MHZ-W. Arjungenin, arjunglucoside I and chebuloside I belong to pentacyclic triterpenoids and pentacyclic triterpene glycosides. These components were detected in a large amount in XQG-W and HZ-W, and the relative content in XQG-W was higher than that in HZ-W. However, these ingredients were extremely low in MHZ-W and even couldn’t be detected. 3,3’-di-O-methyl-4-O-(β-D-xlyopyranosyl) ellagic acid was detected in a large amount in MHZ-W, but was very low in HZ-W and XQG.

**Differences of FT-IR spectra in combination with UPLC-TOF-MS data**

As shown in Figure 1, the position and intensity of the carbonyl stretching vibration absorption of HZ-W and XQG-W at 1713 cm⁻¹ were almost coincident. The carbonyl absorption of MHZ-W appears at 1707 cm⁻¹, and its peak intensity was significantly smaller than that of the HZ-W and XQG-W. We combined the UPLC-TOF-MS data to analyze the possible explanations for these differences as follows: (a) The material base of HZ-W and XQG-W absorption position peak and intensity were similar: although the total amount of the tannins contained in HZ-W was larger than that of XQG-W, the content of the constituents containing more carbonyl groups in the molecular structure was higher in XQG-W than that of the HZ-W such as chebulanic acid and chebulin (Table 2). In addition, HZ-W and XQG-W contained similar triterpenoid components, and their carbonyls have similar chemical environments. (b) The absorption peak position and intensity of MHZ-W at 1707 cm⁻¹ are different from the HZ-W and XQG-W. Combined with UPLC-TOF-MS data analysis, the main difference between MHZ-W and the former is the triterpenoids.

**Table 2 Ultra-performance liquid chromatography-time of flight-mass spectra data of the main chemical constituents in three samples**

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Sample</th>
<th>tᵢ (s)</th>
<th>Ion peak area (a.u.)</th>
</tr>
</thead>
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<tr>
<td>1,3,6-tri-O-galloyl-β-D-glucose</td>
<td>HZ-W</td>
<td>4.44</td>
<td>1403.94</td>
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<td></td>
<td>MHZ-W</td>
<td>4.43</td>
<td>1343.90</td>
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<td></td>
<td>XQG-W</td>
<td>4.48</td>
<td>330.74</td>
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<td>Corilagin</td>
<td>HZ-W</td>
<td>4.53</td>
<td>1057.84</td>
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<td></td>
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<td>4.53</td>
<td>1552.40</td>
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<td></td>
<td>XQG-W</td>
<td>4.56</td>
<td>997.77</td>
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<td>HZ-W</td>
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<td></td>
<td>MHZ-W</td>
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<td></td>
<td>XQG-W</td>
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<td>0.68</td>
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<td>MHZ-W</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>XQG-W</td>
<td>0.70</td>
<td>66.35</td>
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<td>Chebulinic acid</td>
<td>HZ-W</td>
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<td>16.30</td>
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<td></td>
<td>MHZ-W</td>
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<td>70.83</td>
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<td></td>
<td>XQG-W</td>
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<td>185.13</td>
</tr>
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<td>Arjungenin</td>
<td>HZ-W</td>
<td>14.81</td>
<td>158.33</td>
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<tr>
<td></td>
<td>MHZ-W</td>
<td>14.81</td>
<td>5.24</td>
</tr>
<tr>
<td>Arjunglucoside</td>
<td>HZ-W</td>
<td>11.46</td>
<td>216.81</td>
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<td></td>
<td>MHZ-W</td>
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<td>0.47</td>
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<tr>
<td></td>
<td>XQG-W</td>
<td>11.50</td>
<td>297.08</td>
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<td>HZ-W</td>
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<td></td>
<td>MHZ-W</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>XQG-W</td>
<td>15.09</td>
<td>54.38</td>
</tr>
<tr>
<td>3,3’-di-O-methyl-4-O-(β-D-xlyopyranosyl) ellagic acid</td>
<td>HZ-W</td>
<td>9.18</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>MHZ-W</td>
<td>9.14</td>
<td>394.87</td>
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<td></td>
<td>XQG-W</td>
<td>9.19</td>
<td>8.88</td>
</tr>
</tbody>
</table>

Notes: using the peak area as a relative content. HZ-W: aqueous extracts of Hezi (Fructus Chebulae); MHZ-W: the aqueous extracts of Maohezi (Terminalia Belliricae Fructus); XQG-W: the aqueous extracts of Xiqingguo (Chebulae Fructus Immaturus).

In general, as the degree of α, β-unsaturation increases, the absorption peak position moves to a low wave number. The main types of carbonyl moieties of tannins are α,
β-unsaturated esters or α, β-unsaturated aromatic esters, while the main carbonyl type of triterpenoids is carboxylic acid and ester groups. The chemical environment of the carbonyl groups was different. Combined with the above analysis, we boldly speculated that HZ-W and XQG-W at 1713 cm⁻¹ [ν, (C = O)] assigned to α, β-unsaturated ester and to carboxylic acid or ester. Due to the extremely low content of triterpenoids contained in MHZ-W, the absorption peak at 1707 cm⁻¹ is only affected by α, β-unsaturated ester, which may cause the position of absorption peak to shift slightly to low wave number.

The chemical structure type of tannins in three herbs belong to gallotannins and ellagitannins, which contain large amounts of phenolic hydroxyl groups, ester bonds and glycosidic bonds. Figure 1 showed three strong characteristic absorption bands: 1347-1343 cm⁻¹ attributed to the bending vibrations of O-H groups, 1211-1208 cm⁻¹ belong to the stretching vibrations of C-O groups and 1037-1034 cm⁻¹ attributed to the stretching vibrations of C-O-C groups.

In combination with UPLC-TOF-MS data analysis

**SD-IR spectra difference**

Each band represents an overall overlap of some characteristic absorption peaks of functional groups in the sample. Figures 4 and 5 reveal the second derivative spectra in the range of 1620-1300 and 1200-800 cm⁻¹ of three samples.

Tannins: many chemical compositions in plants contain C-O-C groups which produce a large number of peaks in the range of 1100-900 cm⁻¹, such as lignin, glycosides, tannins, etc.

Table 2 shows that the relative content sequences of tannins are as followed:

![Figure 4 Second derivative IR spectra at 1620-1300 cm⁻¹](image)

*a*: HZ-W; *b*: MHZ-W; *c*: XQG-W. HZ-W: aqueous extracts of Hezi (Fructus Chebulae); MHZ-W: the aqueous extracts of Maohezi (Terminalia Belliricae Fructus); XQG-W: the aqueous extracts of Xiqingguo (Chebulae Fructus Immaturus).

![Figure 5 Second derivative IR spectra at 1200-800 cm⁻¹](image)

*a*: HZ-W; *b*: MHZ-W; *c*: XQG-W. HZ-W: aqueous extracts of Hezi (Fructus Chebulae); MHZ-W: the aqueous extracts of Maohezi (Terminalia Belliricae Fructus); XQG-W: the aqueous extracts of Xiqingguo (Chebulae Fructus Immaturus).
HZ-W = MHZ-W > XQG-W. The content changes are consistent with the relative intensity of peaks at 1032-1030 cm⁻¹ in Figure 5. These characteristic absorption peaks are caused by the stretching vibration of C-O-C groups of tannins. Figure 4 shows a complex and numerous absorption peaks in the range of 1600-1540 cm⁻¹ which are difficult to be assigned, many functional groups share absorbance in this range, such as ν₁(ν(C-O)), δ(C-H). Figure 4b shows the peaks of MHZ-W in the range of 1453-1489 cm⁻¹ split into two absorption peaks 1453 and 1447 cm⁻¹. With reference to UPLC-TOF-MS data, MHZ-W contains numerous 3, 3'-di-O-methyl-4-O-(β-D-xylopyranosyl) ellagic acid with many methoxyl groups. 1453 cm⁻¹ can be assigned to the bending vibration of C-H of methoxy lgroup. HZ-W and XQG-W almost don’t contain this component, so there are no splitting peaks in this region.

Pentacyclic triterpenoids: Table 2 shows the chemical structure type of triterpenoid compounds contained in the three samples are pentacyclic triterpenoids, such as arjunogenin, arjunoglucoside I and chebuloside I. Their contents are lower than the tannins compounds while the relative content sequence of pentacyclic triterpenoids are as followed: XQG > HZ > MHZ. The pentacyclic triterpenoid compounds usually contain multiple methyl groups, and the UPLC-TOF-MS data shows the relative content of it also corresponding to the relative intensity of the peaks in the range of 1385-1383 cm⁻¹ which attributed to the bending vibration of C-H of methyl groups shown in Figure 4. Arjunoglucoside I and chebuloside I both belong to pentacyclic triterpenoids glycoside compounds. In 2D-IR spectra, the relative intensity of the peaks at 1164-1163, 1063-1062, 1009-1005 and 904 cm⁻¹ were consistent with the relative content of pentacyclic triterpenoids. These absorption peaks assigned to the vibration of C-O and C-O-C groups of pentacyclic triterpenoids glycosidic compounds. The relative intensity of absorption peak at 835 cm⁻¹ which assigned to the characteristic absorption peak of bending vibration of = C-H groups in Δ 12 of pentacyclic triterpenoids matched with the relative content sequence (XQG > HZ > MHZ).

In addition, in the range of 1515-1514, 1400-1396, 987-984, 924-922, 816-812 cm⁻¹ all showed similar peak shape but intensity are different among three samples. HZ-W (1418 cm⁻¹) and MHZ-W (1417 cm⁻¹) have similar shape and position of the peaks, they are different from XQG-W (1412 cm⁻¹). A low intensity peak shown at 1338 cm⁻¹ appears in the MHZ-W, while HZ-W and XQG-W have no peak in this range. XQG-W had a weak absorption peak at 1332 cm⁻¹ while HZ-W and MHZ-W no absorption in this position.

**2D-IR spectra**

For a simple comparison, the information of positions and correlations of the auto-peaks were summarized in Table 3.

Table 3 showed the 2D-IR correlation synchronous spectra of the three samples in the range of 1720-1200 cm⁻¹. HZ-W has ten auto-peaks at 1288, 1345, 1456, 1471, 1507, 1559, 1623, 1674, 1683, 1701 cm⁻¹, which formed the 10×10 peak cluster. The strongest auto-peak at 1674 cm⁻¹ and the cross-peaks of (1507 cm⁻¹, others) are negative, while others are positive. XQG-W has ten auto-peaks at 1245, 1335, 1342, 1454, 1508, 1555, 1602, 1620, 1680, 1688 cm⁻¹, which formed the 10×10 peak cluster. The strongest auto-peak at 1680 cm⁻¹ and the cross-peaks of (1508 cm⁻¹, others) are negative, while others are positive. In this region, the functional groups of HZ-W and XQG-W have similar sensitivity for external thermal perturbation. In contrast, MHZ-W could be easily distinguished. MHZ-W has eight auto-peaks at 1250, 1346, 1420, 1455, 1552, 1612, 1624, 1676 cm⁻¹. The strongest auto-peak at 1455 cm⁻¹ (8(CHO)) and their cross-peaks are all positive.

Table 3 showed the 2D-IR correlation synchronous spectra of the three samples in the range of 1200-800 cm⁻¹. This area display some carbohydrate ring skeleton vibration related absorption peak. HZ-W has seven auto-peaks at 877, 911, 999, 1018, 1075, 1091, 1157 cm⁻¹, the strongest auto-peak at 1157 cm⁻¹ and their cross-peaks are all positive. XQG-W has seven auto-peaks at 880, 901, 995, 1026, 1066, 1074, 1152 cm⁻¹, the strongest auto-peak at 1152 cm⁻¹ and their cross-peaks are all positive. The carbohydrate ring skeleton vibration related absorption peak of HZ-W and XQG-W have similar sensitivity for external thermal perturbation. It illustrates that their carbohydrate ring type is resemblance, which is consistent with the result drawn from UPLC-TOF-MS data of Pentacyclic triterpenoids glycoside compounds. In Figure 5a, Figure 5b, and Figure 5c, different functional groups of HZ-W and XQG-W could be easily distinguished.
penoid glycosides. It is easy to distinguish MHZ-W from others. MHZ-W has nine auto-peaks at 817, 866, 924, 989, 1050, 1059, 1075, 1105, 1170 cm$^{-1}$, which forms the 9 × 9 peak cluster. The strongest auto-peak at 817 cm$^{-1}$ and the cross-peaks of (1170 cm$^{-1}$, others) are negative, while others are positive. It reveals that the related absorption peak of MHZ-W is different from HZ-W and QXG-W in sensitivity for temperature.

Table 3 showed HZ-W has a strong auto-peak at 419 cm$^{-1}$ and QXG-W has a strong auto-peak at 489 cm$^{-1}$. Although the position of auto-peaks and the positive or negative of the cross-peaks of HZ-W and XQG-W in the region of 1720-1200 and 1200-800 cm$^{-1}$ are similar, they could be distinguished by two strong peaks at 419 and 489 cm$^{-1}$.

**DISCUSSION**

In this study, a combined method by integrating the three-stage infrared spectroscopy and the UPLC-TOF-MS method was used to analyze the commonalities and characteristics of the extract from the three Uighur medicines.

Firstly, FT-IR spectra showed obviously the characteristic absorption peaks of tannins compounds. The UPLC-TOF-MS technique was used to qualitative and relative content analysis the main chemical components contained in HZ-W, MHZ-W and XQG-W. The difference of carbonyl absorption peaks in the range of 1713-1707 cm$^{-1}$ is due to the differences in the contents of tannins and triterpenoids in the three samples analyzed by combining FT-IR spectra and UPLC-TOF-MS data. Secondly, the SD-IR spectra could effectively enhance the apparent resolution by revealing many overlapping and masked peaks, obtaining more absorption peak information. Combined with the UPLC-TOF-MS data, the main infrared characteristic absorption peaks are further analyzed and assigned. Such as the peaks at 1032-1030 cm$^{-1}$ assigned to the stretching vibration of C-O-C groups of tannins; 1164-1163, 1063-1062, 1009-1005, 904 cm$^{-1}$ attributed to the vibration of C-O and C-O-C groups of Pentacyclic triterpenoids glycosidic compounds. 835 cm$^{-1}$ assigned to the characteristic absorption peak of bending vibration of = C-H groups in Δ 12 of pentacyclic triterpenoids. 1453 cm$^{-1}$ belonged to the bending vibration of = C-H of methoxylgroup of 3, 3′-di-O-methyl-4-O-(B-D-xylpyranosyl) ellagic acid. Thirdly, the infrared two-dimensional correlation spectra further reveal the differences in HZ, MHZ and QXG by providing the correlation of the dynamic structure information among the chemical groups and combined with the UPLC-TOF-MS data can more comprehensively and reasonably analyze the relevant structural information. In the 2D-IR spectra, HZ-W and QXG-W in the range of 1720-1200 and 1200-800 cm$^{-1}$ display similar sensitivity for external thermal perturbation, so that MHZ-W can be easily distinguished. HZ-W and XQG-W have strong absorption peaks at 419 and 489 cm$^{-1}$ respectively and can be used as a marker for distinguishing the two samples.

HZ, MHZ and XQG are usually used in Uighur formula prescription. Due to their similar plant origins and chemical compositions, it is difficult to identify and distinguish them by infrared spectroscopy alone.

In conclusion, the three-stage infrared spectroscopy and UPLC-TOF-MS method were combined to analyze the similarities and differences of water extracts from the three Uighur medicines and attributed the characteristic absorption peaks. It provides a rationale for the analysis of medical herbs using infrared spectroscopy methodology.

**REFERENCES**

11. Shafer KH, Lucas SV, Jakobsen RJ. Application of the combined analytical techniques of HPLC/FT-IR, GC/FT-IR, and GC/MS to the analysis of real samples. J Chro-


