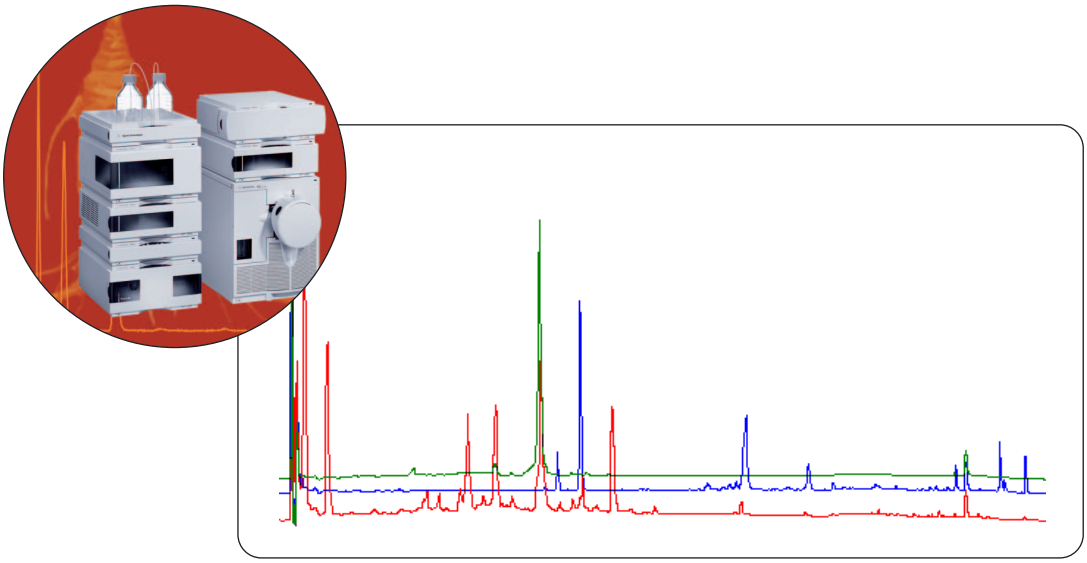
Development of reliable quality control methods for TCM preparations using rapid resolution LC with UV and MS detection

Application Note

Zhixiu Xu



Abstract

This Application Note describes the development of reliable quality control methods for analysis of individual traditional Chinese medicines (TCMs) and TCM formulations using rapid resolution liquid chromatography with UV-visible and quadrupole mass spectrometry detection. The chromatograms obtained with UV and MS detection for different individual TCMs are compared and the combination of UV and MS spectra is used to qualify target compounds.

Agilent Equipment:

1200 Series Rapid Resolution LC system
6140 Quadrupole MS

Application Area:

Traditional Chinese Medicine

Agilent Technologies

Introduction

China has a long history of using TCMs and TCM preparations and has vast experience of the therapeutic effects. However, quality control is based on appearance only. With the development of new technologies, it was recognized that TCMs contain hundreds of compounds and that the concentration levels are wide ranging. Further, differences in composition result from herbal TCMs being grown in different regional areas and harvested at different seasonal times as well as from preparation and manufacturing processes. As a result, controlling the quality of the compounds in TCMs has become a tremendous challenge. Today, tracking only one or two components in TCM preparations is not enough to control the quality because TCM preparations with specific formulas are often made from more than one TCM. For example, Qishenyiqi dripping pills, a traditional patent Chinese medicine used to treat coronary diseases, include four kinds of TCMs:

- Astragali (huangqi)
- Salviae miltiorrhizae (danshen)
- Notoginseng (sanqi)
- Lignum dalbergine ordoriferae (jiangxiang)

Qishenyiqi dripping pills were used as the sample in this study, in which a new method for the quality control of TCMs was developed using using rapid resolution liquid chromatography (RRLC) with UV-visible and MS detection. The main objective was to develop a reliable method to determine the potential target components that do not undergo change during heating or mixing.

Experimental

Equipment

- Agilent 1200 Series Rapid Resolution LC system comprising binary pump SL with vacuum degasser, high performance autosampler SL, thermostatted column compartment SL, and diode array detector SL with micro flow cell (2 µL volume, 3 mm path length)
- Agilent 6140 quadrupole MS with ESI source
- Agilent ChemStation B.02.01 SR1 for data acquisition and evaluation
- Agilent ZORBAX SB C18 RRHT column, 3.0 x 50 mm, 1.8 µm particle size

Standards

Salvianolic acid B, tanshinone I, tanshinone IIA, cryptotansshinone, danshensu, notoginsenoside R1 and astragaloside were purchased from National Institute of Chemical Pharmaceutical and Biological Products (NICPBP), China. Ginsenoside Rg1, ginsenoside Rb1, 3,4-dihydroxybenaldehyde, 3,4-dihydroxybenzoic acid, ginsenoside Re and ginsenoside Rc were purchased from Sigma-Aldrich, USA.

Solvents

Acetonitrile was purchased from Fisher, USA. Water was prepared with a Milli-Q pure water system.

Samples and sample preparation

Qishenyiqi dripping pills, intermediate extractions of huangqi, danshen, sanqi and essential oil of jiangxiang were kindly provided by the TASLY Pharmaceutical Company, China. The raw TCMs were purchased from a local TCM store.

The dripping pills, TCM extractions and raw TCMs were prepared by dissolving in a 70 % methanol/water solution, treating ultrasonically for 30 minutes, and filtering through a 0.22 µm membrane.

RRLC method

- Mobile phase:
  A: Water with 0.1 % formic acid
  B: ACN with 0.1 % formic acid
- Gradient: 0 min, 10 %B;
  8 min, 38 %B; 12 min, 100 %B;
  hold for 3 min, then 10 %B
- Flow rate: 1.0 mL/min
  (passive splitter reduces flow to MS to about 0.4 mL/min)
- Column temperature: 45 °C
- Detection wavelength: 203 nm
- Peak width: 0.5 s
- Slit width: 4 nm
- Spectra: 190-400 nm, step 2 nm

MS method

- Scan: 80-1400 (pos/neg)
- Fragmentor: 70 (pos/neg)
- Drying gas: 12 L/min
- Nebulizer pressure: 50 psi
- Drying gas temperature: 50 °C
- Cap. Voltage: 3200 V (pos/neg)

Results and discussion

The Agilent method translation tool (available on CD pub. no. 5989-5130EN) was used to convert the traditional LC method to a rapid resolution method.

Comparison of danshen sanqi, huangqi and jiangxaing extracts

Figure 1 shows that the chromatograms of danshen and sanqi were very different from the combined extracts when detected at 203 nm. After mixing and heating, some peaks disappeared and new peaks emerged.

This means that target compound screening during quality control is an important step to make sure the compounds do not undergo changes during manufacturing. Most researchers use detection at 203 nm because the main active compounds are saponins, which have weak absorbance at 203 nm. But large peaks appearing at 203 nm might not be relevant for quality control and small peaks might be important for the research of active components. As a consequence, a complementary detector with higher sensitivity is needed to provide more information about the components.

Comparison between different detectors and conditions

Figure 2 shows the differences in the results from UV and MS detection. More peaks appeared in the MS total ion chromatogram (TIC) because some components had weak or no UV absorbance. Using MS detection added an extra dimension and gave more information about the structures of the different compounds. The negative polarity mode gave more complete information about the peaks and enabled the target compounds to be identified for quality control.

Comparison of qishenyiqi dripping pills and different extracts

Figure 3 shows chromatograms of danshen sanqi, huangqi and jiangxiang extracts, as well as the qishenyiqi dripping pills, which are made from these three extracts and other additives. The numbered peaks are the target compounds that were screened for quality control of this TCM preparation. Based on the UV and MS data, different peaks were studied to determine whether the compounds had undergone changes. Table 1 shows the results of the qualifying process.

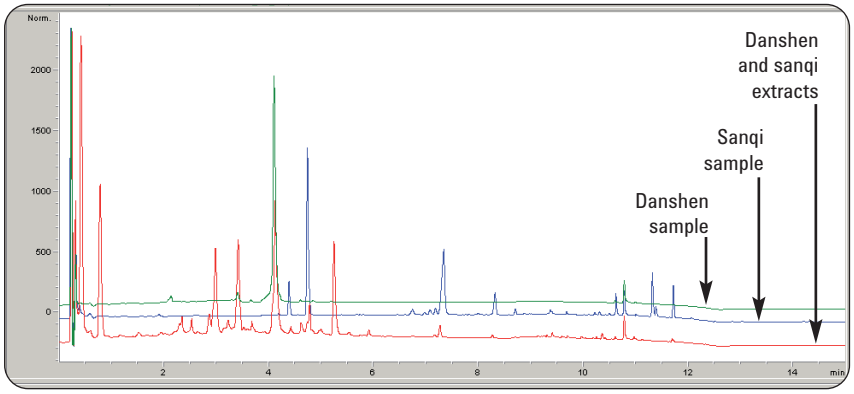


Figure 1
Chromatograms of individual TCMs danshen and sanqi, and of the combined extracts.

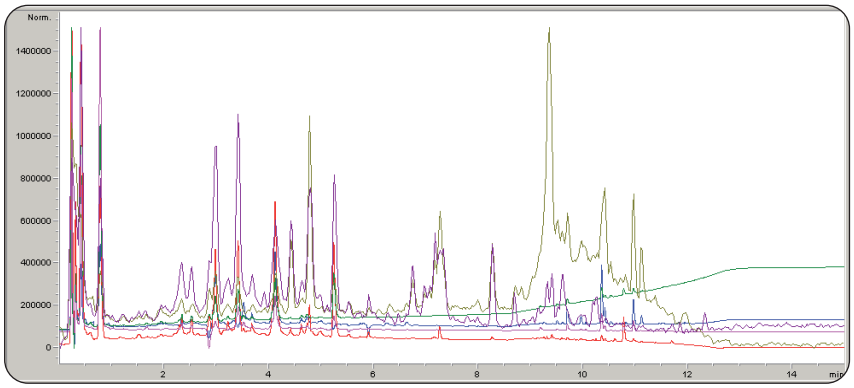


Figure 2
Chromatograms of danshen sanqi combinations, showing the different results obtained by UV and MS detection.

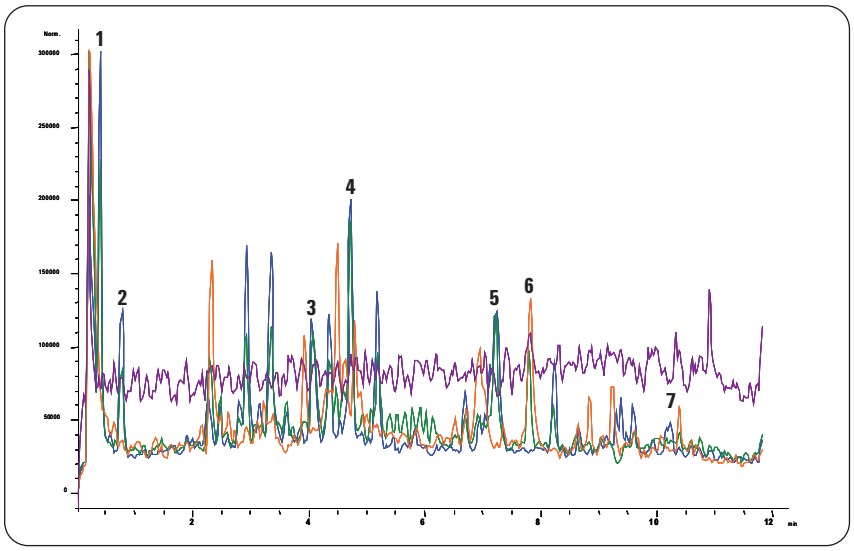


Figure 3
Comparison of the negative TICs for the three TCMs combinations and the dripping pills sample.

Conclusion

The RRLC method with UV and MS detection described in this Application Note is more reliable than the current pharmacopeia quality control method because the quality of several components in the TCM can be tracked based on the information provided by the UV and MS detectors. Tracking these components is important because the components could undergo changes during manufacture and preparation. The Agilent 6140 quadrupole MS used for the mass spectrometry measurements is easy to use and integrates seamlessly with the Agilent 1200 Series RRLC system and Agilent ChemStation sofware.

Peak	Compound	MS	UV λ_{max}(nm)
1	Danshensu	197 $[M–H]^-$, 395 $[2M–H]^-$	280
2	Procatechualdehyde	137 $[M–H]^-$, 275 $[2M–H]^-$	225, 280, 310
3	Salvianolic acid B	717 $[M–H]^-$	260, 280
4	Ginsenoside Rg1	845 $[M+HCOO]^-$	210
5	Ginsenoside Rb1	11071$[M–H]^-$	210
6	Astragaloside IV	829 $[M+HCOO]^-$	210
7	Tanshinone I	295137 $[M+H_2O]^-$	230

Table 1
Target compounds with structural formulas and detection details (numbers refer to the peaks in figure 3).

References

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