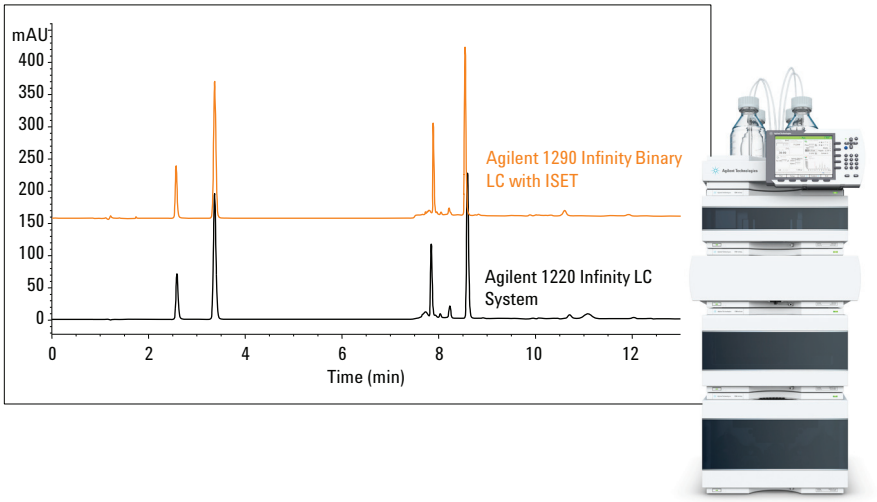
# Seamless instrument-to-instrument method transfer of an USP/EP method from an Agilent 1220 Infinity LC to an Agilent 1290 Infinity Binary LC using Intelligent System Emulation Technology (ISET)

## Application Note

Pharmaceuticals





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## Abstract

This Application Note presents an Application Solution for the analysis of antituberculosis drugs. The seamless method transfer of the USP method from the Agilent 1220 Infinity LC System to the Agilent 1290 Infinity Binary LC using the Agilent Intelligent System Emulation Technology (ISET) is shown.

The US EP method 29-NF 24 for the analysis of the antituberculosis drug preparation of rifampicin, isoniazid, and pyrazineamide is applied onto the different LC instruments. Differences between retention times after transfer of the US Environmental Protection Agency (EPA) method to the 1290 Infinity Binary LC with and without ISET are determined. US EPA acceptance criteria are proven for compliance.


Agilent Technologies

## Introduction

Isoniazid, pyrazinamide, and rifampicin are used in combination for the treatment of *Mycobacterium tuberculosis*. The US EP method 29-NF 24[1] was developed to analyze these compounds. Typically, EPA methods are isocratic methods, but in this case, there is an isocratic part at the beginning, followed by a short-steep gradient step in the middle and an additional isocratic part at the end of the method, see Figure 1. This decreases the elution time of rifampicin. An additional difficulty is that rifampicin shows strong degradation in solution if isoniazid is present[2]. Therefore, the analysis should be done as fast as possible after preparation of the standard solutions. Isoniazid and pyrazinamide are far more stable in solution.

The US EPA method was first applied to the 1220 Infinity LC System and fulfillment of the acceptance criteria were proven. The same chromatographic conditions were then transferred on to the 1290 Infinity Binary LC with and without enabling ISET. The limits allowed for the retention time shift on the 1290 Infinity Binary LC with ISET are < ±5%. In addition, it was proven whether the US EP acceptance criteria were fulfilled on the 1290 Infinity Binary LC with and without ISET.

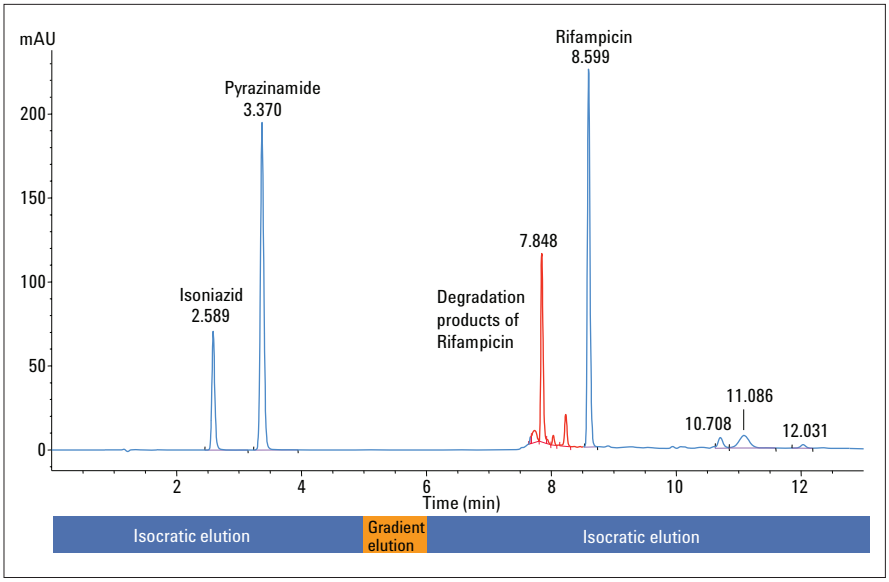



**Figure 1**
**Analysis of antituberculosis drugs applying US EPA method 29-NF 24 on the Agilent 1220 Infinity LC System.**

# Experimental

## Instruments

The instruments used are listed in Table 1.

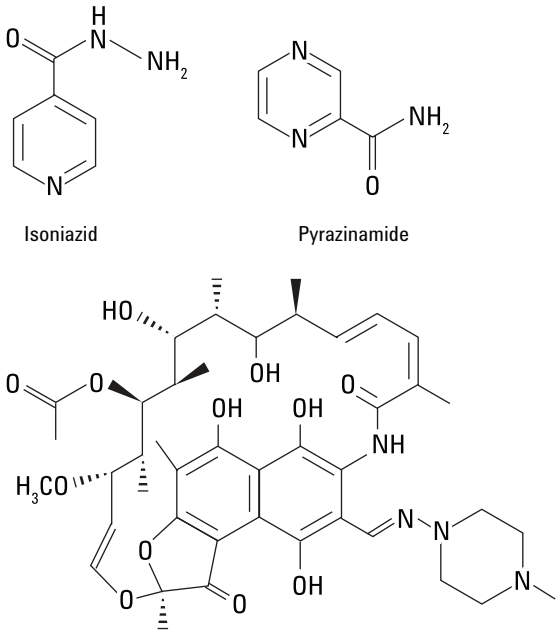
## Acquisition and Evaluation Software

Agilent OpenLAB CDS ChemStation version C.01.04 and ISET

## Chromatographic conditions

| | |
|---|---|
| Sample: | 0.16 mg/mL rifampicin, 0.08 mg/mL isoniazid, 0.43 mg/mL pyrazineamide dissolved in water |
| Column: | Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 3.5 µm (p/n 993967-902) |
| Mobile phases: | Buffer: 1.4 g/L $Na_2HPO_4$, pH = 6.75<br>Organic phase: Acetonitrile<br>Solution A = Buffer/ACN= 96/4<br>Solution B = Buffer/ ACN=45/55 |
| Gradient: | at 0 minutes 0 % B<br>at 5 minutes 0% B<br>at 6 minutes 100% B<br>at 13 minutes 100% B |
| Stop time: | 13 minutes |
| Post time | 3 minutes |
| Flow rate: | 1.2 mL/min |
| Detection VWD: | 238 nm, 10 Hz, 10-mm path length |
| Detection DAD: | 238/6 nm, Reference OFF, 10 Hz, 10-mm path length |
| Column temperature: | 27 °C |
| Injection volume: | 20 µL |

### Analyzed compounds




| | Agilent 1290 Infinity Binary LC | Agilent 1220 Infinity LC |
|---|---|---|
| **Module** | **Product number** | **Product number for integrated LC G4290B** |
| Binary pump | G4220A | Dual gradient pump |
| Autosampler | G4226A | Autosampler |
| ALS cooler | G1330B | n/a |
| Column compartment | G1316C | Column compartment |
| Diode-array detector | G4212A | VWD |

**Table 1**
**Instrumentation used.**

## Results and discussion

The following experiments were performed:

- Applying the US EPA method to the 1220 Infinity LC System
- Transferring the method to the 1290 Infinity Binary LC without ISET using the same chromatographic conditions as on the 1220 Infinity LC System
- Enabling ISET on the 1290 Infinity Binary LC and repeating the analysis
- Reviewing the deviation of retention times and fulfillment of US EPA acceptance criteria

The ISET function was enabled in the ChemStation, see Figure 2. The *1220 LC System GradPumpV1.0* was selected as pump and the *G1329A* as autosampler. The method was saved as a new method and applied within a sequence on the 1290 Infinity Binary LC.

Figure 3 shows that the resulting chromatograms are overlaid. For the isocratic part at the beginning of the chromatogram, the retention times were the same for all three instrument configurations, as expected. After the gradient step, the retention times shifted significantly for the 1290 Infinity Binary LC chromatogram without ISET. With ISET, the 1220 Infinity LC and the 1290 Infinity Binary LC chromatogram showed nearly the same retention times for all peaks.

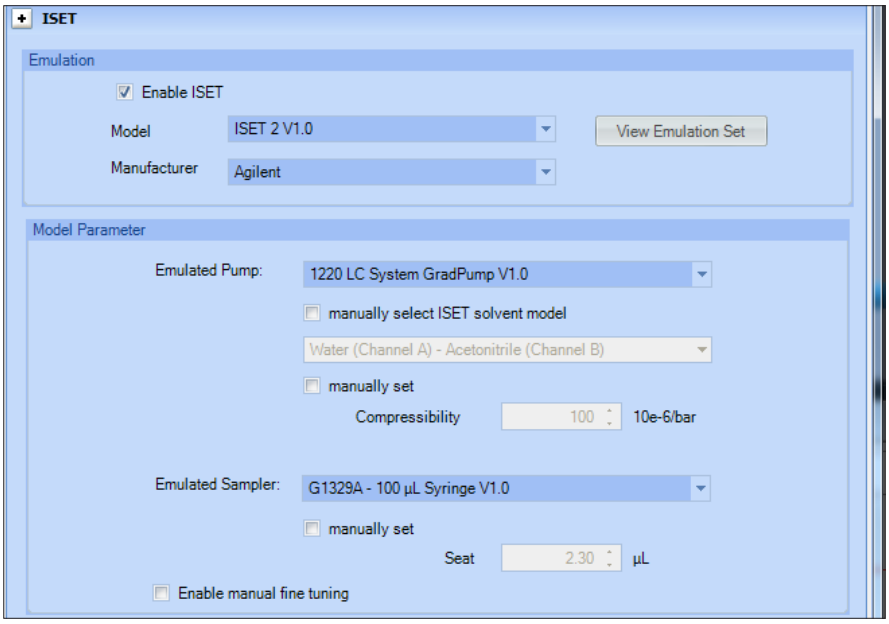



**Figure 2**
**Enabling ISET in the Agilent 1290 Infinity Binary Pump setup screen.**

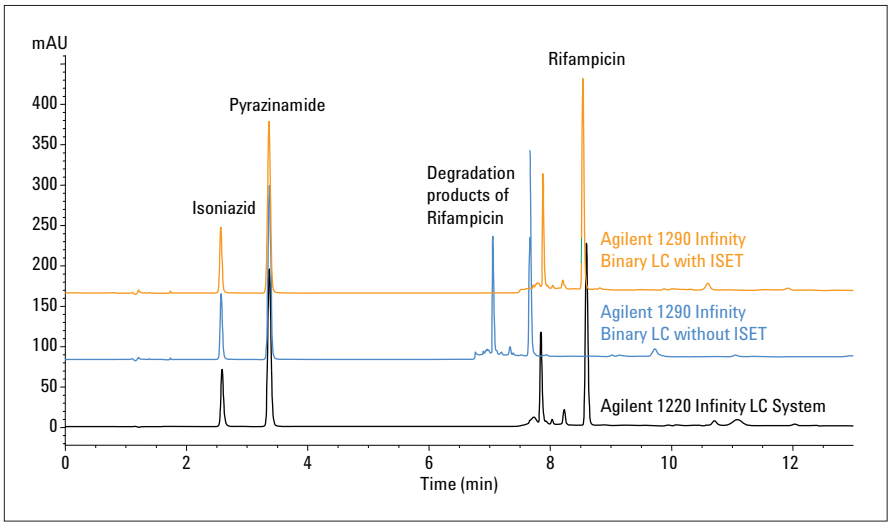



**Figure 3**
**Overlay of original chromatogram with the chromatograms obtained on the Agilent 1220 Infinity LC and the Agilent 1290 Infinity Binary LC with and without ISET.**

Figure 4 shows the differences between the retention times expressed as percentages.

With ISET, the differences between the retention times was less than 1% compared to the original retention times obtained on the 1220 Infinity LC. Without ISET, the deviation of retention times for rifampicin and its main degradation product at 7.848 minutes was approximately 11%.

The US EPA acceptance criteria were fulfilled for all three instrument configurations, see Table 2.

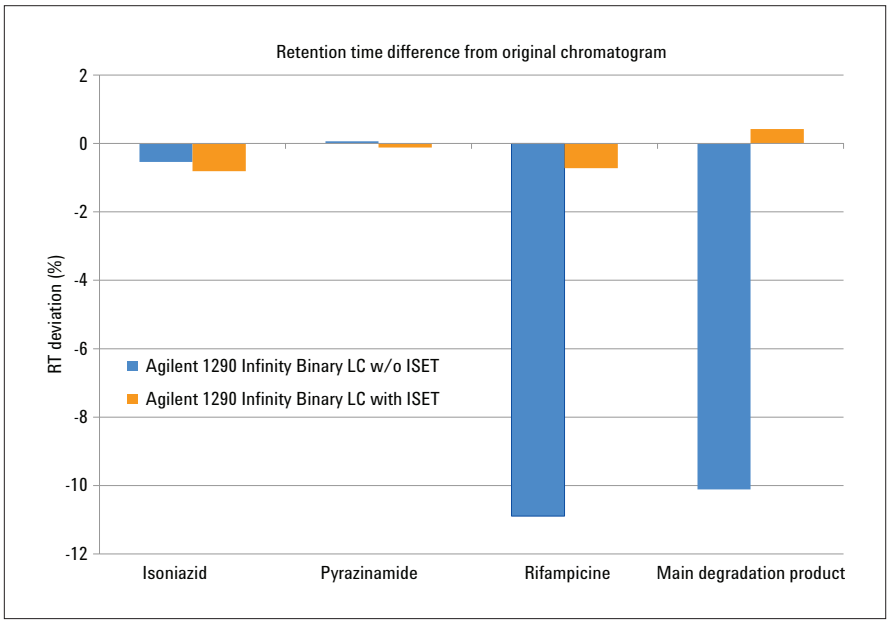



**Figure 4**
**Deviation of retention times from original chromatogram obtained on the Agilent 1220 Infinity LC System.**

| Parameter | US EPA criteria | Agilent 1220 Infinity LC System | Agilent 1290 Infinity System without ISET | Agilent 1290 Infinity System with ISET |
|---|---|---|---|---|
| Rs for pyrazinamide | > 4 | 7.8 | 9.26 | 9.18 |
| RRT isoniazid | ~ 0.7 | 0.76 | 0.76 | 0.76 |
| RRT pyrazinamide | 1 | 1 | 1 | 1 |
| RRT rifampin | ~ 1.8 | 2.5 | 2.27 | 2.53 |
| RSD area isoniazid | < 2 | 0.052 | 0.056 | 0.05 |
| RSD area pyrazinamide | < 2 | 0.029 | 0.057 | 0.14 |
| RSD area rifampin | < 2 | 1.13 | 0.6 | 0.31 |
| N isoniazid | > 6,000 | 12,277 | 17,368 | 17,248 |
| N pyrazinamide | > 10,000 | 16,248 | 20,659 | 19,987 |
| N rifampin | > 50,000 | 180,968 | 208,784 | 262,643 |
| USP tailing isoniazid | < 2 | 1.16 | 1.12 | 1.12 |
| USP tailing pyrazinamide | < 2 | 1.12 | 1.06 | 1.05 |
| USP tailing rifampin | < 2 | 1.19 | 1.23 | 1.22 |

**Table 2**
**Compliance with US EPA acceptance criteria.**

## Conclusions

Method transfer of the US EPA method 29-NF 24 for the analysis of the antituberculosis drugs isoniazid, pyrazinamide, and rifampicin from the Agilent 1220 Infinity LC to the Agilent 1290 Infinity Binary LC with and without applying ISET was performed. Using ISET, the original chromatogram and the chromatogram obtained on the 1290 Infinity Binary LC agreed almost 100%. The deviation of retention times was less than 1%. If ISET was not applied, the deviation of retention times was approximately 11%. The US EPA acceptance criteria were fulfilled for all three instrument configurations.

## References


1.
US EPA method 29-NF 24, http://www.pharmacopeia.cn/v29240/usp29nf24s0_m73666.html

2.
Bhavika Mohan, Nishi Sharda, Saranjit Singh, "Evaluation of the recently reported USP gradient HPLC method for the analysis of antituberculosis drugs fir its ability to resolve degradation products of rifampicin", Journal of pharmaceutical and biomedical analysis, 31, 607-612, **2003**.

**www.agilent.com/chem/ISET**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