
Example #13: Testing Precision of Peak Retention Times and Areas of an HPLC System

The following is an example of an operating procedure for the testing of an HP 1050 Series HPLC system for precision of peak areas and retention times. This is a proposal and starting point only and may need adaptation to different HPLC systems. There is no assurance expressed that the operating procedure will pass a regulatory inspection.

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1. Scope

Testing the precision of peak areas and retention times of an HP 1050 Series HPLC system.

2. Purpose

The precision of peak areas and retention times are important characteristics for qualitative and quantitative measurements in HPLC. This operating procedure provides chromatographic conditions and key sequences to verify these characteristics of a complete HPLC system, comprising an HP 1050 Series Autosampler, a Quaternary Pump and a Variable Wavelength Detector.

3. Frequency

The precision should be verified at least once a year or after the repair of one or more modules.

4. Instrumentation

- a) Quaternary HP 1050 Series Pump
 - b) HP 1050 Series Autosampler
 - c) HP 1050 Variable Wavelength Detector
- HP 3396 Integrator

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5. Columns, chemicals

- Column: 100 mm x 4.6 mm Hypersil ODS, 5 μ (HP P/N 79916OD-554).
- Solvents: Water and Methanol, HPLC grade.
- Sample: Isocratic standard sample (Hewlett-Packard part number 01080-68704) that contains 0.15 wt.% dimethylphthalate, 0.15 wt.% diethylphthalate, 0.03 wt.% biphenyl, 0.03 wt.% o-terphenyl dissolved in methanol.

6. Preparation of the Variable Wavelength Detector

- Switch lamp ON.
- Set the wavelength to 254 nm.
- Set the response time to 1 SEC.

7. Preparation of the Pump

- Prime the pump (use appropriate 1050 SOP): "Priming a Quaternary Pump."
- Fill solvent reservoirs: A with water, B with water, C with methanol.
- Degas solvents (see appropriate 1050 SOP): "Priming a Quaternary Pump."
- Set UPPER LIMIT to 400 (bar).
- Set the FLOW rate to 3.00 ml/min.
- Set the temperature of the column oven to 45°C.
- Set the solvent composition: A = off, B = 15%, C = 70% (channel A will be changed automatically according to %B and %C settings).
- Set the STOP TIME to 5.00 minutes.
- Switch pump ON.

8. Preparation of the Autosampler

- Make sure that the air pressure needed for the solenoid valves is about 5 bar.
- Switch the autosampler on.
- Put sample vial with isocratic sample into the vial tray, position number 10.
- Set up vial numbers: FIRST 10 LAST 10.
- Set the number of injections/vial to 6.
- Set the injection volume to 10 μ l.

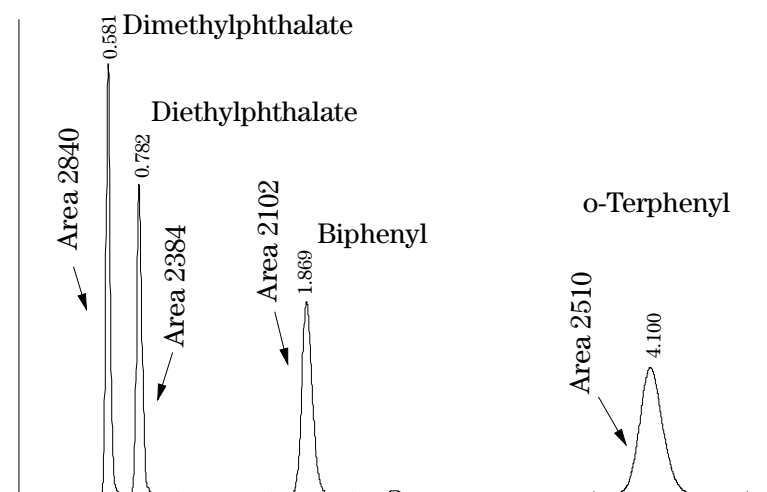
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9. Set parameters for the HP 3396 Integrator

- Attenuation: 10.
- Chart speed: 1 cm/min.
- Zero: 10.
- Threshold: 10.

10. Analysis of Isocratic Standard

- When the baseline is stable, start the analyses.
- As a result, 6 chromatograms similar to the figure below should be obtained (differences in retention times and areas due to variations between different column batches and to variations in the concentration of the sample from batch to batch).



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11. Acceptance

a) Calculate the precision of retention times and peak areas.

$$\text{RSD} = \frac{\sqrt{\frac{1}{n-1} \sum (x-\bar{x})^2}}{\bar{x}} \cdot 100$$

where:

n is the number of injections

x is area or retention time of peak

$$\text{Mean} = \bar{x} = \frac{1}{n} \sum x$$

b) The precision for the peak areas should be < 1.5 % RSD.

c) The precision for retention times should be < 0.5 % RSD.

12. Further Action

If the 1050 HPLC system does not fulfill the given specification, do the following:

- Check the performance of the detector (noise and drift) using appropriate SOP: "Checking Noise and Drift on the HP 1050 Series Variable Wavelength Detector."
- Check whether the pump is leak-tight using appropriate SOP: "Leak Test for the Quaternary HP 1050 Series Pump."
- Check whether the autosampler is leak-tight using appropriate SOP: "Checking the Pressure Tightness on HP 1050 Series Autosampler."

If following these procedures does not result in an improvement, call Hewlett-Packard service.

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13. Appendix - Protocol example for results

Instrument identification

Serial number pump: _____
 Serial number autosampler : _____
 Serial number detector: _____
 Serial number integrator: _____

Date: _____

Results

Precision of peak areas: _____ (spec <1.5 % RSD)
 Precision of retention times: _____ (spec <0.5 % RSD)

Comment:

Further actions (in case the equipment is out of specification)

Approvals

	Name	Signature	Date
Laboratory supervisor	_____	_____	_____
Test engineer	_____	_____	_____

