

# **Abstract**

The Agilent 1120 Compact LC is the system of choice for conventional, analytical-scale liquid chromatography. It is an integrated LC designed for ease of use, performance, and reliability. It is well-suited for the analysis of drugs due to highly precise retention times and peak areas and excellent detector linearity. This Application Note shows:

- Excellent retention time precision, with relative standard deviation (RSD) < 0.07 % for all six linearity levels.
- $\bullet$  Excellent area precision, with RSD < 0.25 % for all six levels.
- Excellent linearity, with coefficient of correlation > 0.9999. This study covers a wide range of ~ 150 ng to 5,000 ng.



# Agilent Equipment

- Agilent 1120 Compact LC
- Agilent TC C18(2) column

#### **Application Area**

• Pharmaceutical industry: QA/QC

# **Introduction**

In the development of an HPLC method for analysis, the detector plays a very important role as it defines the limits of concentrations over which the method can perform satisfactorily.

The purpose of this Application Note was to evaluate the detector linearity and precision of peak areas and retention times for the Agilent 1120 Compact LC system using the antipsychotic drug Aripiprazole (figure 2) as a test compound.

# **Experimental**

## Equipment

The Agilent 1120 Compact LC system included:

- A gradient pump with low-pressure mixing
- An autosampler with vial tray
- A column compartment for a column up to 250 mm in length
- A variable wavelength detector (VWD)

An Agilent TC (Typical Carbon load) C18(2) column,  $150 \ge 4.6$  mm,  $5 \ \mu m$  particle size, was used for all separations.

The instrument was controlled by Agilent EZChrom Elite Compact Compliance software.

## **Chromatographic parameters**

The chromatographic conditions were as follows:

- Sample: Aripiprazole
- Column: Agilent TC-C18(2), 150 x 4.6 mm, 5 μm
- Mobile phase:
  - A = water + 0.2 % TFA,
  - B = acetonitrile + 0.16 % TFA



Agilent 1120 Compact LC.



## Structure of Aripiprazole.

Serial #	Weight or volume	Diluted to	Expected concentration	Solution name
1	20.00 mg of Aripiprazole	10 mL	2.000 mg/mL or 2,000 ng/µL	A ( Stock)
2	500 µL of A	1 mL	1.000 mg/mL or 1,000 ng/µL	B, Level-6
3	500 µL of B	1 mL	0.500 mg/mL or 500 ng/µL	C, Level-5
4	250 µL of B	1 mL	0.250 mg/mL or 250 ng/µL	D, Level-4
5	125 µL of B	1 mL	0.125 mg/mL or 125 ng/µL	E, Level-3
6	62.5 µL of B	1 mL	0.0625 mg/mL or 62.5 ng/µL	F, Level-2
7	31.25 µL of B	1 mL	0.03125 mg/mL or 31.25 ng/µL	G, Level-1

Note: To prepare the stock solution, Aripiprazole was initially dissolved in methanol (20 % of the total make-up volume) and the solution was then made up to the mark with diluent.

#### Table 1 Sample preparation.

- Flow rate: 1.0 mL/min
- Gradient: at 0 min 30 %B, at 7 min 70 %B, then hold the ratio for another two minutes
- Injection volume: 5 µL
- Autosampler programmed with a wash vial (using acetonitrile) for

rinsing exterior of the needle

- Run time: 9 min
- Post time: 5 min
- Column oven: 40 °C
- VWD: 254 nm, peak width (PW) > 0.05 min
- Diluent / blank: 60:40 acetonitrile:water

Line Location S		Sample name	# Injections	Injection volume (µL)		
1	Vial 1	Blank	2	5		
2	Vial 2	Linearity Level-1	6	5		
3	Vial 3	Blank	2	5		
4	Vial 4	Linearity Level-2	6	5		
5	Vial 5	Blank	2	5		
6	Vial 6	Linearity Level-3	6	5		
7	Vial 7	Blank	2	5		
8	Vial 8	Linearity Level-4	6	5		
9	Vial 9	Blank	2	5		
10	Vial 10	Linearity Level-5	6	5		
11	Vial 11	Blank	2	5		
12	Vial 12	Linearity Level-6	6	5		

#### **Sample preparation**

The samples for the linearity test were prepared as per table 1.

## Sequence table

Table 2 shows the sequence table that was set up in the Agilent EZChrom Elite Compact Compliance software.

# **Results and discussion**

Figure 3 shows the chromatogram of Aripiprazole. The mobile phase contained trifluoroacetic acid as modifier, which improved retention and peak shape.

Figure 4 shows the chromatographic overlay of all six linearity levels. The results of the linearity results are summarized in table 3, and the linearity plot is displayed in figure 5. The observed linearity correlation was r-squared > 0.9999.













# **Conclusion**

The variable wavelength detector within the Agilent 1120 Compact LC gives excellent linearity over a very wide range of concentrations. The instrument is able to analyze Aripiprazole with high precision for retention times and peak areas. In this study, the precision for retention times was < 0.07 % RSD and for areas of baseline-separated peaks was < 0.25 % RSD.

The Agilent 1120 Compact LC system is well-suited for this application, as it delivers the needed data quality and is based on a proven robust design. This Application Note also demonstrates that the Agilent 1120 Compact LC system meets the detector linearity requirements of a pharmaceutical QA/QC lab.

Results	Level-1	Level-2	Level-3	Level-4	Level-5	Level-6
RSD of area	0.163 %	0.139 %	0.141 %	0.145 %	0.230 %	0.244 %
RSD of retention time	0.041 %	0.067 %	0.020 %	0.015 %	0.011 %	0.007 %

Table 3 Linearity results.





Linearity curve for Aripiprazole from ~ 150 to 5,000 ng injected.

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