Application Note

Small Molecules

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Analysis of Doxorubicin Hydrochloride with the Agilent 1260 Infinity II Prime LC as per USP Monograph Method

Authors

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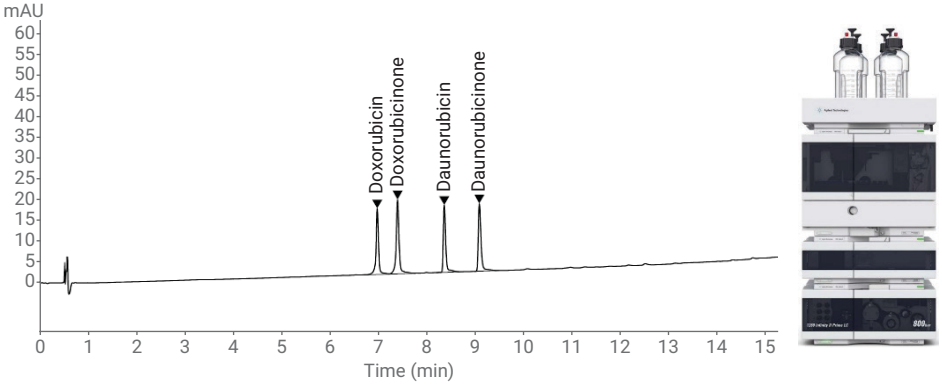
Abstract

This Application Note demonstrates the use of the Agilent 1260 Infinity II Prime LC in the latest compendial method for the analysis of doxorubicin hydrochloride developed on sub-2 µm columns. The United States Pharmacopeia (USP) is pursuing modernization of monographs in its compendium to provide labs with increased efficiency. The doxorubicin hydrochloride monograph is one such example, recently published in USP 39 and developed on an UHPLC platform. With a pressure range of up to 800 bar, a 350 µL dwell volume, and the Agilent intelligent system emulation technology (ISET), the 1260 Infinity II Prime LC can be used for sub-2 µm methods. Seamless transfer of analytical methods from conventional LC systems such as the Agilent 1200 Infinity Series LC to the 1260 Infinity II Prime LC is facilitated.



Introduction

Doxorubicin hydrochloride is one of the most common broad-spectrum anticancer chemotherapy drugs. The classical method for doxorubicin analysis in previous monographs was developed on L13 packing. This required the use of ion-pairing reagents at pH values lower than the column tolerance. This was an example of a monograph requiring modernization due to incompatible method conditions, long run times, and absence of an organic impurities procedure in the previous monograph. Another aim was to introduce an UHPLC method that would be MS-compatible. Conversely, the most recent method has been developed on a sub-2 µm column with L1 packing to be performed on an UHPLC system. The 1260 Infinity II Prime LC is a powerful, robust, and reliable system, having both HPLC and UHPLC capabilities. It is perfectly suited to run a wide range of routine to challenging applications that demand robust performance concerning retention time (RT) and area RSD. The 800 bar backpressure capacity of the Agilent 1260 Infinity II Flexible Pump facilitates analysis using sub-2 µm columns, and increases lab productivity.

This Application Note demonstrates the robust performance of the 1260 Infinity II Prime LC in the analysis of doxorubicin hydrochloride with a new compendial assay and RS method.

Experimental

Instrumentation

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1260 Infinity II Multisampler (G7167A)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity II Diode Array Detector HS (G7117C)

Solvent and samples

All solvents were LC grade sourced from J. T. Baker. Trifluoroacetic acid (TFA) was LC/MS grade, sourced from Sigma-Aldrich. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak).

Software

Agilent OpenLab CDS workstation version 2.3.0 (M8413AA)

Sample preparation

Assay

- **Diluent:** solutions A and B (50:50) (**note:** protect solutions containing doxorubicin from light)
- **System suitability solution:** 0.1 mg/mL each of USP doxorubicin hydrochloride RS and USP epirubicin hydrochloride RS in diluent
- **Standard solution:** 0.1 mg/mL of USP doxorubicin hydrochloride RS in diluent
- **Sample solution:** 0.1 mg/mL of doxorubicin hydrochloride in diluent

Related substances

Standard solution: 0.002 mg/mL each of USP doxorubicin hydrochloride RS, USP doxorubicinone RS, USP daunorubicin hydrochloride RS, and USP daunorubicinone RS in diluent

Sample solution: 0.4 mg/mL of doxorubicin hydrochloride in diluent

Table 1. Chromatographic conditions.

Parameter	Value
Column	2.1 mm × 10 cm, 1.7 mm packing L1
Mobile phase	A) 0.1 % TFA prepared by transferring 1.0 mL of TFA to 1 L of water B) Acetonitrile (80 %), methanol (20 %), and TFA (0.1 %)
Gradient	Time (min) % B 0.0 10 15.0 75 16.0 75 16.1 10 18.0 10
Flow rate	0.5 mL/min
Injection volume	2 µL
Column temperature	35 °C
Detection	254 nm

Results and discussion

Assay

Doxorubicin RS and assay method analysis was performed under chromatographic conditions as per the USP method given in Table 1. For precision of area and retention time, six consecutive standard replicates of doxorubicin standard were injected. System suitability solution was injected to check the resolution between doxorubicin and epirubicin (Figure 1).

As per the latest USP chapter ⟨621⟩ guidelines, the %RSD limit for five replicate standard injections is <0.73 % for the assay method. The precision values were calculated based on six consecutive runs. The RT RSDs and area RSDs for doxorubicin hydrochloride were excellent, and much lower than the specified limits of less than 0.73 %. To display the precision of the 1260 Infinity II Multisampler (Figure 2), 25 consecutive injections of doxorubicin standard were performed. An area %RSD of 0.07 % and RT %RSD of 0.13 % were achieved for 25 replicate injections of the standard.

Table 2. Specifications of the USP monograph method compared with experimental results.

SST criteria	Expected	Observed	Pass/fail
RRT epirubicin	1.05 minutes	1.05 minutes	Pass
Resolution	NLT 1.5	2.9	Pass
%RSD area	NMT 0.73	0.11	Pass
%RSD RT	NMT 0.73	0.14	Pass

RRT: relative retention time; NLT: not less than; NMT: not more than

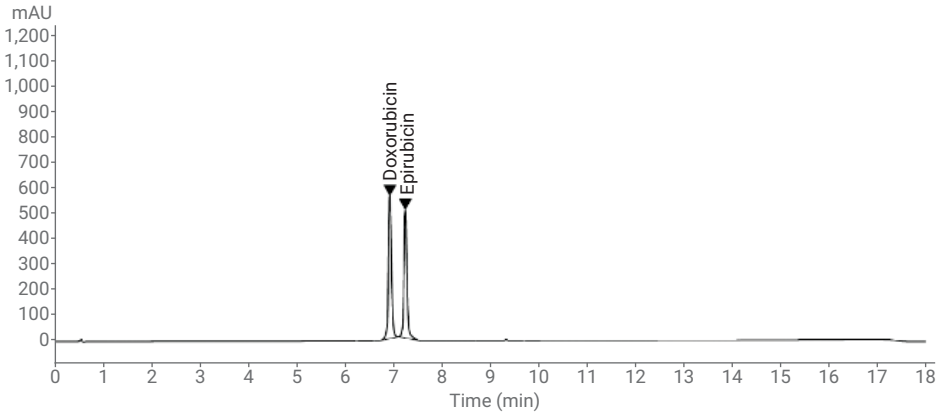


Figure 1. System suitability solution injected to observe resolution between doxorubicin and epirubicin.

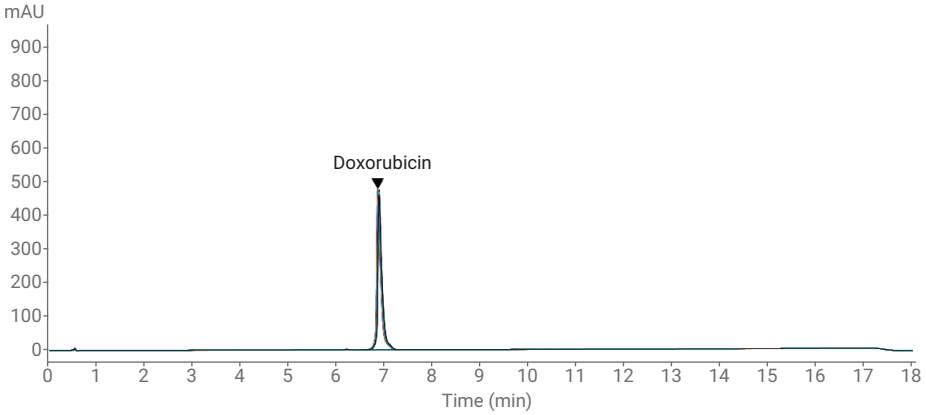


Figure 2. Overlay of 25 injections for repeatability.

Related substances

The related substances test was performed as per the chromatographic conditions mentioned in the USP monograph in Table 1. Six replicate injections of the doxorubicin RS standard mix (containing doxorubicin, doxorubicinone, daunorubicin, and daunorubicinone)(Figure 3) were performed for repeatability. Excellent %RSD was achieved for all four compounds (Table 3). Doxorubicin sample solution was also injected, and all three known impurities, doxorubicinone, daunorubicin, and daunorubicinone, were detected (Figure 4).

Conclusion

This Application Note shows the use of the 1260 Infinity II Prime LC system for assay and related substance analysis of doxorubicin. Use of a sub-2 µm column requires an UHPLC system. The 1260 Infinity II Prime LC system meets this need, accommodating the method backpressure of <800 bar. The 1260 Infinity II Prime LC is the best fit for all analytical labs, as it can be used as a conventional LC with ISET. It can also be used in UHPLC mode with a pressure range of up to 800 bar.

References

1. 3602 Official Monographs/Doxepin – USP 39.
2. Physical Tests/⟨621⟩ Chromatography 431 – USP 38.

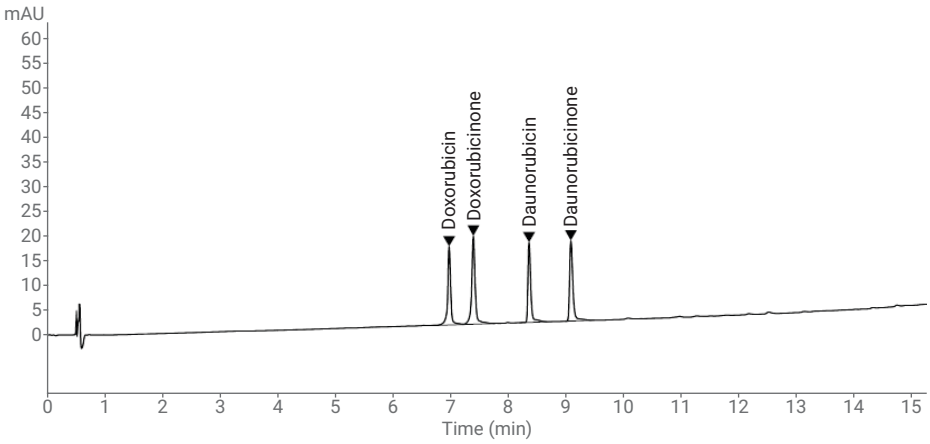


Figure 3. Doxorubicin RS standard mix.

Table 3. %RSD of RT and area.

	RT RSD (%)	Area RSD (%)	Resolution
Doxorubicin	0.09	0.54	–
Doxorubicinone	0.07	0.78	4.74
Daunorubicin	0.04	0.2	8.05
Daunorubicinone	0.03	0.31	10.85

Acceptance criteria: RSD not more than 5 %.

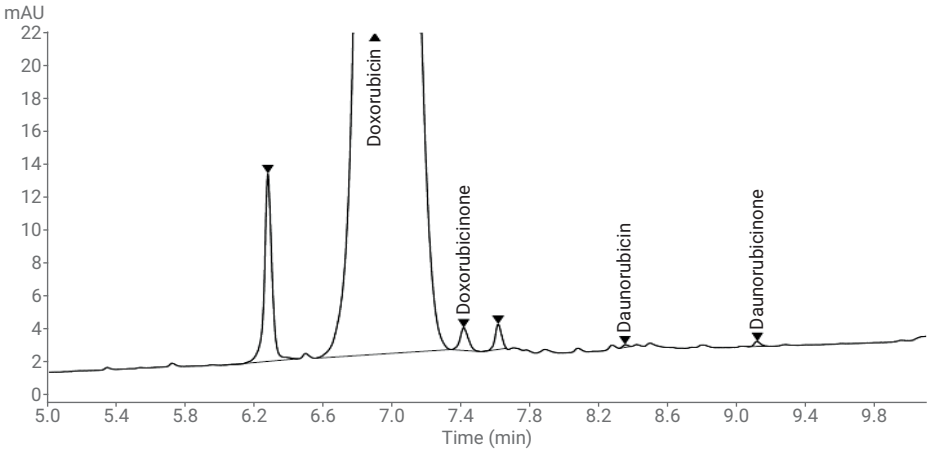


Figure 4. Doxorubicin sample injection.

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