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Application News

Ultra High Performance Liquid Chromatograph

# Simultaneous Analysis of API and Related Impurities Using SPD-M40 X4 Photodiode Array Detector

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## User Benefits

- Using SPD-M40 X4, which provides linearity up to 3 AU, enables reliable quantification over a wide concentration range from low to high levels.
- In impurity analysis, the API and its related impurities can be simultaneously analyzed and quantified in a single injection.

## ■ Introduction

In pharmaceutical impurity analysis, it is desirable to quantify both the API and its related impurities. When the analysis is performed using a highly concentrated sample solution to ensure the detectability of impurities, the detector signal for the API may become saturated, preventing accurate determination of its peak area. In such cases, the sample must be diluted so that the API peak height falls within the linear dynamic range of the detector. However, this requires multiple analyses at different sample concentrations for the API and impurities, and the associated dilution steps can be labor-intensive. Nexera™ X4 (Fig. 1) is a next-generation UHPLC system that inherits the technologies established in Shimadzu's Nexera series and delivers top-class analytical performance. The photodiode array detector SPD-M40 X4 achieves linearity up to 3 AU through improvements in the optical system, enabling reliable quantification over a wide concentration range from low to high levels. In this article, an example of simultaneous analysis and quantification of an API and impurities using Nexera X4 is presented.

## ■ Analytical Conditions and Target Compounds

The analytical conditions and the compound used in this study are summarized in Table 1. Salicylic acid, a small-molecule pharmaceutical compound, was employed as a model sample. The linearity of the calibration curve in the high-concentration range and the repeatability of retention time and peak area for the impurity were evaluated for this sample.

Table 1 Analytical Conditions and Target Compounds

| | |
|---|---|
| System : Nexera X4 | |
| Sample : Salicylic acid | |
| Mobile phase | |
| Pump A : 0.1% formic acid in water | |
| Pump B : Acetonitrile | |
| Column : Shim-pack Scepter™ C18-120 *1 (50 mm × 2.1 mm I.D., 1.9 µm) | |
| Analytical conditions | |
| B Conc. | : 5%(0 min)→95%(1.4 min)→5%(1.4-2.6 min) |
| Column Temp. | : 40 °C |
| Flow rate | : 0.5 mL/min |
| Mixer | : Micro mixer |
| Sample loop Vol. | : 15 µL |
| Injection Vol. | : 1 µL |
| Detection | : 299 nm (SPD-M40 X4, STD cell) |

*1 P/N : 227-31012-03

Fig. 1 Nexera™ X4

## ■ Linearity of the Calibration Curve in the High-Concentration Range

To confirm the linearity in the high-concentration range, a calibration curve (Fig. 2) was constructed for salicylic acid with peak heights in the range of 1–3 AU. The concentrations corresponding to each peak height and the percent errors are summarized in Table 2. The calibration curve exhibited excellent linearity ($r^2$ > 0.9999), and small percent errors were obtained even in the high-concentration region (~3 AU), indicating high quantification accuracy. Chromatograms for each calibration level are shown in Fig. 3.

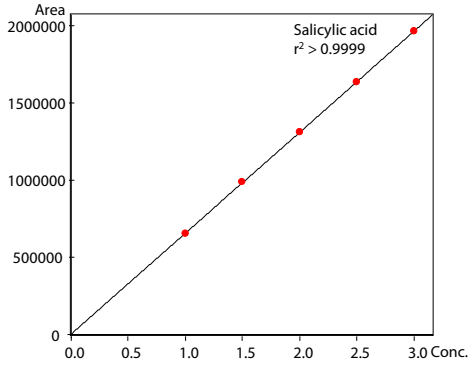


Fig. 2 Calibration Curve of Salicylic Acid in the High-Concentration Range

Table 2 Peak Heights, Corresponding Concentrations, and Relative Errors

| | Peak height (AU) | Calibration point concentration (mg/L) | Relative error*1 (%) |
|---|---|---|---|
| 1 | 1 | 148 | 0.31 |
| 2 | 1.5 | 296 | 0.37 |
| 3 | 2 | 444 | 0.27 |
| 4 | 2.5 | 592 | 0.26 |
| 5 | 3 | 740 | 0.33 |

*1 : the percent error between the back-calculated concentration and the nominal concentration

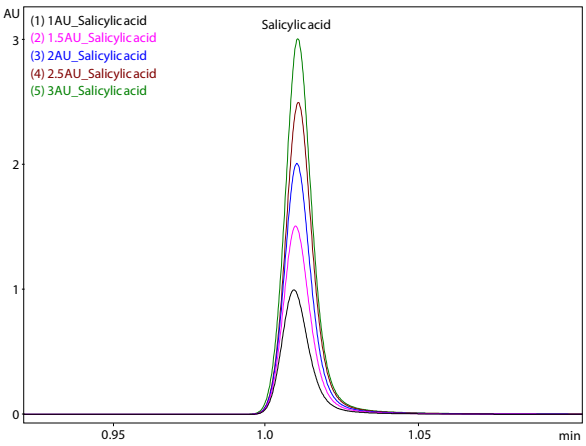


Fig. 3 Chromatograms at Each Calibration Level

## ■ Reproducibility of Impurity Analysis

Fig. 4 shows the overlaid chromatograms obtained from six replicate analyses of a high-concentration salicylic acid sample containing an impurity. The reproducibility (%RSD) of the retention time and peak area for both the impurity and salicylic acid in the replicate analyses is summarized in Table 3. Although the peak height of salicylic acid was approximately 2.75 AU, accurate quantification was achieved without dilution because it remained within the linear range of the SPD-M40 X4 (~3 AU). The impurity, present at approximately 0.05% in terms of area percentage (Impurity highlighted by the red circle in Fig. 4), was clearly detected, and both the retention time and peak area exhibited relative standard deviations below 1%, indicating excellent reproducibility. These results demonstrate that the SPD-M40 X4 provides a wide linear dynamic range up to 3 AU, enabling high-accuracy simultaneous analysis and quantification of the impurity and the API.

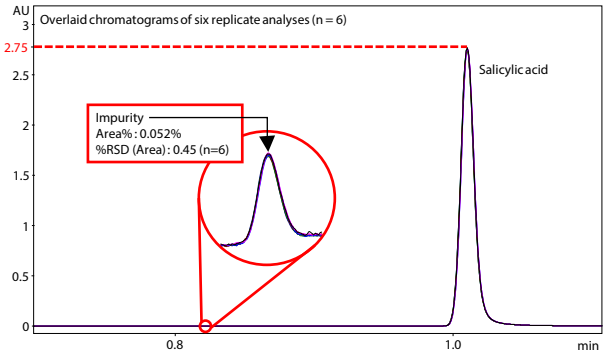


Fig. 4 Overlaid Chromatograms From Six Replicate Analyses

Table 3 Impurity Reproducibility in Six Replicate Analyses (%RSD)

| | Retention time | Peak area |
|---|---|---|
| Impurity | 0.032 | 0.45 |
| Salicylic acid | 0.016 | 0.38 |

## ■ Conclusion

An example of simultaneous analysis and quantification of an active pharmaceutical ingredient (API) and impurities using SPD-M40 X4 photodiode array detector on Nexera X4 is presented. The SPD-M40 X4 provides linearity up to 3 AU, enabling quantification over a wide concentration range from low to high levels. Consequently, high-concentration samples containing trace impurities can be analyzed in a single injection without dilution, allowing both the API and impurities to be quantified simultaneously. This capability contributes to improved efficiency in analytical workflows.



01-01126-EN First Edition: Feb. 2026

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