Application Note

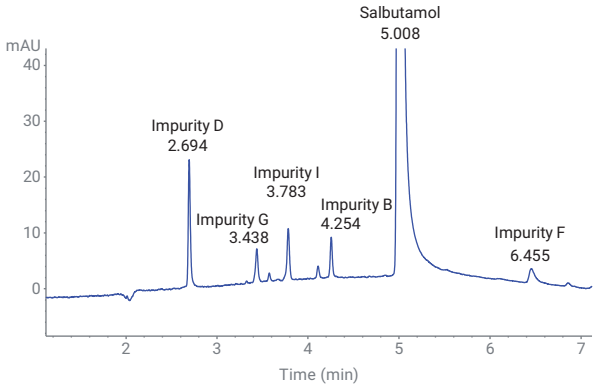
Biopharma/Pharma

Agilent

Trusted Answers

Suitable for Agilent
1260 Infinity III LC

# Detection of Low-Level Impurities in Salbutamol Using the Agilent 1260 Infinity II SFC System with a Variable Wavelength Detector





## Authors

Amandine Dispas
University of Liege, Belgium

Davy Guillarme
Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, Switzerland

Edgar Naegele and Daniel Kutscher
Agilent Technologies, Waldbronn, Germany



## Abstract

The objective of this application note is to demonstrate a competitive trace analysis of impurities in the active pharmaceutical ingredient (API) salbutamol sulfate using the combination of the Agilent 1260 Infinity II SFC System with an Agilent 1290 Infinity II Variable Wavelength Detector (VWD). The detection and quantification of low-level pharmaceutical impurities in an API, especially at low SFC flow rates with elution gradients at elevated temperature and smaller id columns, will be demonstrated by means of the VWD.

## Introduction

Any API could possibly be polluted with potentially harmful organic impurities, mainly due to manufacturing and synthesis.[1]

The present study is focused on a well characterized API, salbutamol, and its impurities with a monograph in the European Pharmacopeia. The tolerated amount of known impurities is defined in the European Directorate for the Quality of Medicines (EDQM) monograph.[2]

This application note demonstrates the detection of impurities in the API salbutamol at a 0.2 to 0.4% level by means of the Agilent 1260 Infinity II SFC equipped with an Agilent 1290 Infinity II VWD. The described results are part of a round robin test and the complete study is published in the scientific literature.[3]

## Experimental

Agilent 1260 Infinity II SFC System:

- 1260 Infinity II SFC Control Module (G4310A)
- 1260 Infinity II SFC Binary Pump (G4782A)
- 1260 Infinity II SFC Multisampler (G4767A)
- 1290 Infinity II Multicolumn Thermostat
- 1260 Infinity II Variable Wavelength Detector (G7114A) with 10 mm path length high pressure flow cell (G1314-60182)

### Column

Hybrid silica based, diethylamine (DEA), 100 × 3.0 mm, particle size 1.7 µm

### Software

Agilent OpenLab CDS ChemStation edition for LC & LC/MS systems, Rev. C.01.08

### Test solutions

**Stock solution:** Dissolve with water/ACN 20/80 v/v, 5 mg of impurity B CRS + 5 mg of impurity D CRS + 5 mg of impurity F CRS and 5 mg of impurity G CRS in a volumetric flask of 50.0 mL.

**Intermediate solution 1:** Dissolve 20 mg of salbutamol sulfate CRS and dilute 600 µL of stock solution in a volumetric flask of 10.0 mL.

**SST solution:** Dissolve the content of one vial of impurity I with 1.0 mL of intermediate solution 1.

**Stock solution of impurity D:** Dissolve with water/ACN 20/80 v/v, 5 mg of impurity D CRS in a volumetric flask of 5.0 mL.

### Calibration

**Level 1 (SC1):** 4 µg/mL

**Level 2 (SC2):** 6 µg/mL

**Level 3 (SC3):** 8 µL/mL

**Quality control solution (QC):** 6 µg/mL

### Sample preparation

Dissolve an accurately weighed amount of 20 mg of salbutamol sample in 2 mL of water in a volumetric flask of 10.0 mL. Fill the flask with acetonitrile. Prepare three independent solutions of each sample.

### Injection sample table

- Blank solution (water/ACN 20/80 v/v)
- SC1 (two injections)
- SC2 (two injections)
- SC3 (two injections)
- Blank solution (water/ACN 20/80 v/v)
- QC
- Sample A replicate 1
- Sample A replicate 2
- Sample A replicate 3
- Sample B replicate 1
- Sample B replicate 2
- Sample B replicate 3
- Sample C replicate 1
- Sample C replicate 2
- Sample C replicate 3
- QC
- Blank solution (water/ACN 20/80 v/v)
- SC1
- SC2
- SC3
- Blank solution (water/ACN 20/80 v/v)

### SFC method

| Parameter | Value |
|---|---|
| Flow Rate | 1.5 mL/min |
| Modifier | Ammonium hydroxide 0.1% in methanol (MeOH) |
| Gradient Mode | 0 min: 3% B<br>6.5 min: 35% B<br>7 min: 3% B<br>10 min: 3% B |
| BPR | 135 bar, BPR temperature 60 °C |
| Column Temperature | 55 °C, postcolumn temperature 38 °C |
| Autosampler Temperature | 6 °C |
| Injection | Operation mode in SFC |
| Feed Injection | 2 µL injection, feed speed 100 µL/min, overfeed volume 4 µL, feed solvent MeOH/$H_2O$ 90/10 |
| Needle Wash | Standard wash, 20 s, solvent MeOH/$H_2O$ 50/50 |
| Sampling | Draw speed 75 µL/min, eject speed 400 µL/min, wait time after draw 5 s |
| UV Detection | 220 nm, data rate 20 Hz, dual wavelength collection not used |

### Chemicals

- Methanol gradient grade, acetonitrile gradient grade, and 2-propanol analytical grade were purchased from Merck KGaA, Darmstadt, Germany.
- Fresh ultrapure water was obtained from a Milli-Q integral system equipped with LC-Pak polisher and a 0.22-µm membrane pointofuse cartridge (Millipak).
- Ammonium hydroxide 28% was purchased from Merck KGaA, Darmstadt, Germany.
- Carbon dioxide 99.998% was purchased from prxair, Düsseldorf, Germany.
- Salbutamol test samples were provided by the study initiator.
- EDQM Impurity D CRS (10 mg), EDQM Impurity B CRS (10 mg), EDQM Impurity F CRS (10 mg), EDQM Impurity G CRS (10 mg), EDQM Impurity I CRS (one vial of 0.006 mg), and salbutamol sulfate CRS (100 mg) were purchased from and provided by the study initiator.

## Results and discussion

### Preliminary test of the SFC system

The achiral SFC method involved in this interlaboratory study consists in the simultaneous separation of salbutamol sulfate and its specified impurities B, D, F, I, and G. This method was developed by a screening of different stationary phases and by means of an analytical quality-by-design strategy including a full validation.[4] The separation of salbutamol and its impurities takes only 7 minutes and is seven times faster than the normative HPLC method from European Pharmacopeia (Figure 1).

For the determination of the system performance prior to the sample measurement, one injection of the diluent followed by six injections of SST solution was performed. This was done by using the SFC method described above (see Experimental) and the separation between all peaks has been verified. For the verification, all measured retention time and peak area values were inserted in a given calculation Excel sheet (Table 1). For the measurement of the SST solution, it was required that the RSD values should be <1% for retention times (for all compounds) and <2% for peak areas (only for impurities). All retention time areas showed RSDs typically below 0.03%. The determined area RSDs were below the required 2%, typically below 1%, with the exception of Impurity F. This late-eluting impurity showed an RSD value of 2.09%.

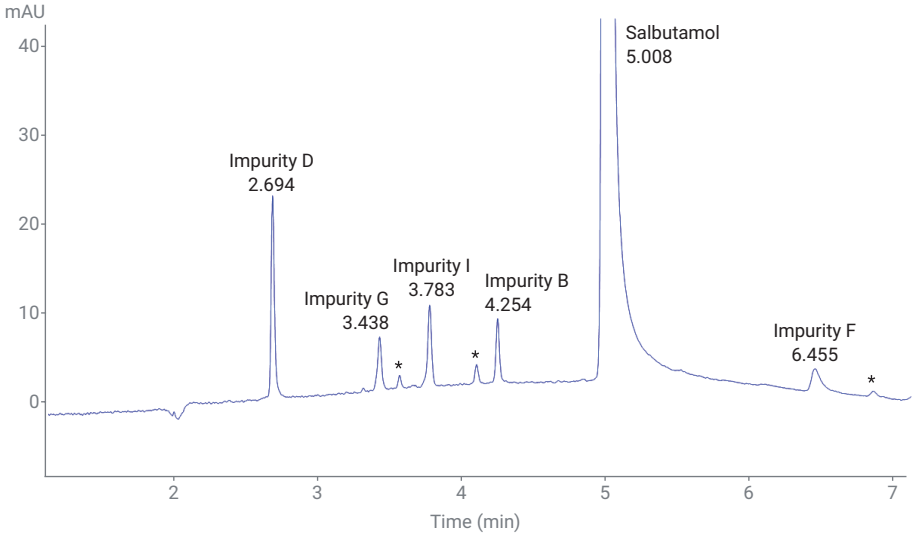



**Figure 1.** Separation of salbutamol and salbutamol impurities by SFC and VWD detection (* unknown impurity).

## Quantification of impurities in the API sample

Since SFC has gained a lot of interest in recent years, it is not only important to demonstrate reliability and robustness of an SFC method in one laboratory: it is also of interest to demonstrate the reproducibility of a developed method within different laboratories across different industries. Such a demonstration can open doors for the application of SFC in quality control laboratories.[5] For the expansion of the described study, which was carried out with only one SFC vendor, a new version of the study was featured comparing different vendors of instruments.[4]

**Table 1.** Calculation of the retention time and peak area RSDs of the salbutamol impurities and confirmation of requirements.

| | Theoretical RT (min) | Observed RT (min) | Peak Area | Mean RT (min) | RSD RT (min) | RSD Peak Area (%) |
|---|---|---|---|---|---|---|
| Impurity D | 2.6 | 2.696 | 36.845 | 2.70 | 0.02 | 0.88 |
| | | 2.695 | 36.178 | | | |
| | | 2.695 | 36.127 | | | |
| | | 2.695 | 36.699 | | | |
| | | 2.695 | 36.821 | | | |
| | | 2.695 | 36.663 | | | |
| Impurity G | 3.3 | 3.437 | 12.406 | 3.44 | 0.02 | 0.94 |
| | | 3.437 | 12.211 | | | |
| | | 3.438 | 12.321 | | | |
| | | 3.437 | 12.336 | | | |
| | | 3.438 | 12.095 | | | |
| | | 3.438 | 12.368 | | | |
| Impurity I | 3.8 | 3.785 | 18.727 | 3.78 | 0.02 | 0.78 |
| | | 3.784 | 19.056 | | | |
| | | 3.784 | 18.937 | | | |
| | | 3.784 | 18.662 | | | |
| | | 3.784 | 18.941 | | | |
| | | 3.783 | 18.879 | | | |
| Impurity B | 4.1 | 4.256 | 12.482 | 4.26 | 0.02 | 1.15 |
| | | 4.255 | 12.396 | | | |
| | | 4.256 | 12.436 | | | |
| | | 4.255 | 12.716 | | | |
| | | 4.255 | 12.314 | | | |
| | | 4.254 | 12.353 | | | |
| Salbutamol Sulfate | 4.8 | 5.012 | nd | 5.01 | 0.03 | nd |
| | | 5.01 | | | | |
| | | 5.01 | | | | |
| | | 5.009 | | | | |
| | | 5.009 | | | | |
| | | 5.008 | | | | |
| Impurity F | 6.2 | 6.457 | 13.061 | 6.46 | 0.02 | 2.09 |
| | | 6.455 | 13.641 | | | |
| | | 6.456 | 13.593 | | | |
| | | 6.456 | 13.912 | | | |
| | | 6.456 | 13.558 | | | |
| | | 6.454 | 13.723 | | | |

In this study, three samples of salbutamol were provided for the quantification of Impurity D. According to the given method (see Experimental), three concentration levels at 4, 6, and 8 µg/mL were created and measured together with the samples and a quality control sample at 6 µg/mL (see injection sequence in Experimental). All calibration curves showed excellent linearity and RSD values typically better than 1% (Figure 2, Table 2). The quality control sample showed a recovery of 98.93%.

For the quantification of Impurity D, the three samples were individually weighed three times for the measurement of the replicates (Table 2). Besides the measured concentration of Impurity D as well as the calculation of the relative concentration, the RSD values were calculated for each series to be in the order of 1%.

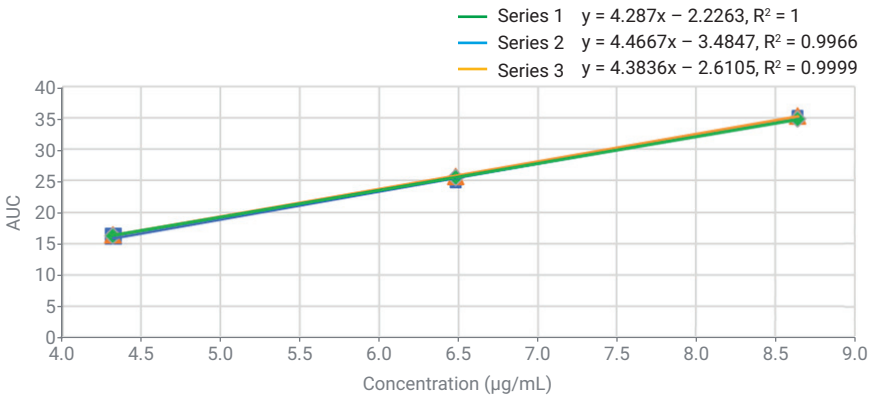



**Figure 2.** Calibration curves for Impurity D in salbutamol at 4, 6, and 8 µg/mL.

**Table 2.** Summary of the quantitative measurements of Impurity D in the three salbutamol samples including calibration and quality control (0.2 % of Impurity D in salbutamol sulfate (sample A), 0.3 % of Impurity D (sample C), and 0.4 % of Impurity D (sample B)).

| Calibration | | | |
|---|---|---|---|
| | SC1 | SC2 | SC3 |
| Theoretical Concentration (µg/mL) | 4 | 6 | 8 |
| Real Concentration (µg/mL) | 4.32 | 6.48 | 8.64 |
| AUC Injection 1 | 16.139 | 24.804 | 35.435 |
| AUC Injection 2 | 16.368 | 25.712 | 35.305 |
| AUC Injection 3 | 16.294 | 25.553 | 34.814 |
| AUC Mean | 16.267 | 25.356 | 35.185 |
| RSD AUC (%) | 0.72 | 1.91 | 0.93 |
| **QC Solution** | | | |
| Theoretical Concentration (µg/mL) | 6 | | |
| Real Concentration (µg/mL) | 6.48 | | |
| AUC Injection 1 | 25.075 | | |
| AUC Injection 2 | 25.525 | | |
| Measured Concentration (µg/mL) | 6.41 | | |
| Recovery (%) | 98.93 | | |
| **Sample A** | | | |
| | Replicate 1 | Replicate 2 | Replicate 3 |
| Weight (mg) | 19.9 | 20.4 | 19.9 |
| AUC | 19.788 | 20.009 | 19.879 |
| Impurity D Concentration | 5.15 | 5.20 | 5.17 |
| % Impurity D in Salbutamol API | 0.26 | 0.26 | 0.26 |
| Mean % Impurity D | 0.26 | | |
| RSD (%) | 1.00 | | |

| Calibration | | | |
|---|---|---|---|
| | SC1 | SC2 | SC3 |
| **Sample B** | | | |
| | Replicate 1 | Replicate 2 | Replicate 3 |
| Weight (mg) | 19.9 | 20.4 | 20.5 |
| AUC | 35.039 | 35.511 | 36.635 |
| Impurity D Concentration | 8.63 | 8.74 | 9.00 |
| % Impurity D in Salbutamol API | 0.43 | 0.43 | 0.44 |
| Mean % Impurity D | 0.43 | | |
| RSD (%) | 1.20 | | |
| **Sample C** | | | |
| | Replicate 1 | Replicate 2 | Replicate 3 |
| Weight (mg) | 20.4 | 20.7 | 20.1 |
| AUC | 24.294 | 24.828 | 24.236 |
| Impurity D Concentration | 6.18 | 6.30 | 6.17 |
| % Impurity D in Salbutamol API | 0.30 | 0.30 | 0.31 |
| Mean % Impurity D | 0.30 | | |
| RSD (%) | 0.64 | | |

To get quantification data for interday comparability, the complete quantification was repeated on three consecutive days with the same instrument. The quantitative results for Impurity D in one individual salbutamol sample were identical for all performed measurements (Table 3). The calculated RSD values for repeatability and intermediate precision are outlined in Table 4.

## Conclusion

This application note presents the data measured in one laboratory during a round robin test of the Agilent 1260 Infinity II SFC with Agilent 1260 Infinity II VWD. The data show the separation for salbutamol and five related impurities in a 7-minute run time, which is seven times faster than the classical HPLC method. This separation provides excellent retention time repeatability, with RSD values below 0.03% and peak area repeatability with RSDs typically below 2%. The absolute and relative quantification of one impurity in salbutamol samples showed intraday RSDs at the 1% level and excellent quantitative interday repeatability with RSDs typically between 0.44% and 1.76%.

**Table 3.** Quantitative results for the determination of Impurity D in salbutamol.

| Summary of Three Interday Measurements | | | |
|---|---|---|---|
| **Sample A** | | | |
| | Series 1 | Series 2 | Series 3 |
| Impurity D Content (%) | 0.26 | 0.26 | 0.23 |
| RSD Intraseries (%) | 1.00 | 2.50 | 1.16 |
| **Sample B** | | | |
| | Series 1 | Series 2 | Series 3 |
| Impurity D Content (%) | 0.43 | 0.43 | 0.44 |
| RSD Intraseries (%) | 1.20 | 1.44 | 0.42 |
| **Sample C** | | | |
| | Series 1 | Series 2 | Series 3 |
| Impurity D Content (%) | 0.30 | 0.31 | 0.30 |
| RSD Intraseries (%) | 0.64 | 0.41 | 0.43 |

**Table 4.** Repeatability and intermediate precision RSD values (%).

| RSD (%) | Concentration Level | | |
|---|---|---|---|
| | 0.20% | 0.30% | 0.40% |
| Repeatability | 1.76 | 0.44 | 1.1 |
| Intermediate Precision | 6.27 | 2.14 | 1.98 |

## References


1. Roy, J. Pharmaceutical Impurities: A Mini-Review. *AAPS PharmSciTech* **2002**, *3(2)*, 1–8, article 6.
2. ICH Harmonized Tripartite Guideline: Impurities in New Drug Products Q3B(R2).
3. Dispas, A. *et al.* Interlaboratory Study of a Supercritical Fluid Chromatography Method for the Determination of Pharmaceutical Impurities Evaluation of Multi-Systems Reproducibility. *J. Pharm. Biomed. Anal*. **2021**, *203*, 114206.
4. Dispas, A. *et al.* Quantitative Determination of Salbutamol Sulfate Impurities Using Achiral Supercritical Fluid Chromatography. *J. Pharm. Biomed. Anal.* **2017**, *134*, 170–180.
5. Dispas, A.; Marini, R.; Desfontaine, V. First Inter-Laboratory Study of Supercritical Fluid Chromatography Method for the Determination of Pharmaceutical Impurities. *J. Pharm. Biomed. Anal.* **2018**, *161*, 414–424.


**www.agilent.com**


DE44427.2275578704





Printed in the USA, October 15, 2024
5994-3982EN


Agilent
Trusted Answers