# A sensitive method for the quantitation of formoterol in human plasma

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## ABSTRACT

Formoterol is a highly effective, long-acting beta-2-selective adrenoceptor agonist used to treat pulmonary disorders. The lowest recommended daily dose of formoterol for children 12 years of age and older is 12 µg for oral administration and 20 µg for aerosol delivery. Therefore, drugs such as formoterol circulate in human blood at relatively low plasma quantities. As drug discovery and development initiatives concentrate on more effective, lower dosage drugs and throughput requirements push the simplicity of sample preparation and analysis, the demand for greater sensitivity in bioanalytical assays continues to increase. The developed assay focuses on measuring formoterol levels in human plasma with a small aliquot volume and a simple extraction procedure.

## INTRODUCTION

As drug discovery and development initiatives concentrate on more effective lower dosage drugs, there is a continual increase in demand for more simple and sensitive bioanalytical methods. The simplest approach to meet these requirements is to utilize a more sensitive mass spectrometer. Bioanalytical scientists have the most flexibility when working with a system that delivers technological advancements that offer sensitivity enhancements across the mass range and in both polarities.[1,2]

Formoterol is a highly effective, long-acting beta-2-selective adrenoceptor agonist used as a therapeutic to treat pulmonary disorders. The lowest recommended daily dose of formoterol for children 12 years of age and older is 12 µg for oral- and 20 µg for aerosol-based administration. During the drug development pipeline, pharmacokinetic studies could require a dosage as low as 9 µg. Since the concentrations available for analysis are particularly low, the quantitation of formoterol in human plasma requires a highly sensitive method.[3,4]

The bioanalytical method described in this here uses 300 µL of plasma and a simple extraction method to detect ultra-low levels of formoterol. A lower limit of quantitation (LLOQ) of 0.05 pg/mL in human plasma was achieved using the SCIEX 7500 system.

## MATERIALS AND METHODS

**Sample preparation:**
Formoterol fumarate was spiked into 300 µL of human plasma at concentrations ranging from 0.05 to 100 pg/mL and 50 pg of internal standard was added. Samples were extracted using liquid-liquid extraction with 2.5 mL of tert-butyl methyl ether and 200 µL of 0.1% (v/v) ammonium hydroxide solution in water.

The samples were vortexed for 3 mins and centrifuged at 3901 RCF for 5 mins. The supernatant was collected and dried under nitrogen at 40° C. Dried samples were reconstituted using 150 µL of 80:20 (v/v), methanol/10mM ammonium acetate in water.

**Chromatography:**
An ExionLC system with a Phenomenex Luna Omega Polar C18 column (2.1 x 100 mm, 3 µm, 100 Å) was used for chromatographic separation at a flow rate of 0.6 mL/min. The column was operated at 40° C. Mobile phase A was 0.1% (v/v) acetic acid in water and mobile phase B was methanol. Table 1 summarizes the LC gradient conditions used.

**Table 1. LC gradient.**

| Time (min) | Mobile phase A (%) | Mobile phase B (%) |
|---|---|---|
| 0.0 | 70 | 30 |
| 2.5 | 70 | 30 |
| 2.6 | 70 | 30 |
| 3.0 | 5 | 95 |
| 6.0 | 5 | 95 |
| 6.2 | 70 | 30 |
| 8.0 | 70 | 30 |

**Mass spectrometry:**
Samples were analyzed using the SCIEX 7500 system equipped with the OptiFlow Pro ion source and the system was controlled by SCIEX OS software. The optimized MS parameters are listed in Table 2.

**Table 2. Optimized MS parameters.**

| Name | Q1/Q3 (*m/z*) | Q0D (V) | CE (V) | CXP (V) |
|---|---|---|---|---|
| *Formoterol* | *345.4/149.0* | *5* | *27* | *15* |
| *Formoterol d6* | *351.2/155.2* | *5* | *27* | *11* |
| **Source parameters** | **Value** | **Source parameters** | | **Value** |
| *Curtain gas* | *40 psi* | *CAD gas* | | *8* |
| *Ion source gas 1* | *40 psi* | *Ion spray voltage* | | *1750 V* |
| *Ion source gas 2* | *90 psi* | *Temperature* | | *500°C* |

**Data processing:**
Data processing was performed using SCIEX OS software, version 3.0. Peaks were automatically integrated using the MQ4 algorithm with a weighting of $1/x^2$.

## RESULTS

- A calibration curve was analyzed for concentrations ranging from 0.05 to 100 pg/mL. To evaluate reproducibility, each concentration of formoterol was analyzed in triplicate.
- An LLOQ of 0.05 pg/mL was achieved for formoterol in human plasma. No interferences were observed in the matrix blank (Figure 1). Linearity was observed across concentrations ranging from 0.05 to 100 pg/mL with a regression coefficient ($r^2$) >0.99 and a linear dynamic range (LDR) of 3.2 orders of magnitude (Figure 2). No carryover was observed for the blank injection following analysis of the highest concentration tested.

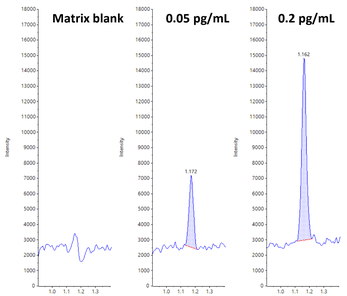


**Figure 1. Representative extracted ion chromatograms (XICs) for formoterol in human plasma.** The left panel shows results for the matrix blank. The middle and right panels show results for formoterol spiked into human plasma at 0.05 pg/mL (middle) and 0.2 pg/mL (right).

- Analytical performance was evaluated based on the requirement that the accuracy of the calculated mean should be between 80% and 120% at the LLOQ and between 85% and 115% at higher concentrations. The %CV of the calculated mean of the concentration should be below 20% at the LLOQ and below 15% at all higher concentrations.[5]
- For this assay, accuracy was within ±8% of the nominal concentration and %CV was <11% for formoterol in human plasma (Table 3). Calculated percent accuracy and %CV values were within the acceptance criteria at each concentration level (Table 3).

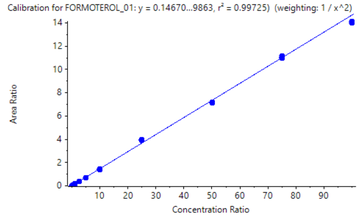


**Figure 2. Calibration curve for the quantitation of formoterol in human plasma.**

**Table 3. Summary of the quantitative performance.** Reproducibility and accuracy results were determined from the calibration curve across 3 replicates.

| Concentration (pg/mL) | CV (%) | Accuracy (%) |
|---|---|---|
| *0.05* | *10.5* | *99.4* |
| *0.2* | *6.90* | *102* |
| *0.5* | *5.50* | *101* |
| *1* | *3.00* | *100* |
| *2.5* | *0.60* | *102* |
| *5* | *0.70* | *97.1* |
| *10* | *1.70* | *96.1* |
| *25* | *0.56* | *108* |
| *50* | *0.40* | *97.9* |
| *75* | *0.90* | *100* |
| *100* | *0.50* | *95.9* |

- SCIEX OS software is a closed system and requires records and signatures to be stored electronically, meeting the regulations outlined by 21 CFR Part 11. SCIEX OS software can open raw data files from any visible storage location within a closed network by using designated processing workstations. Figure 5 illustrates the features of SCIEX OS software that are used for monitoring the audit trail, acquiring and processing data and configuring user access.
- The audit trail feature enables users to audit critical user actions and locks in data integrity. The Central Administrator Console (CAC) feature allows users to centralize acquisition and processing using a single platform to maximize efficiency for multi-instrument laboratories, independent of compliance standards.
- The configuration module allows users to assign roles and access as the administrator, method developer, analyst and reviewer


**Figure 5. Features of the SCIEX OS software for monitoring user access and evaluating the audit trail.**

## CONCLUSIONS

- Ultra-low quantitation levels were achieved for formoterol in human plasma using a simple liquid-liquid extraction sample preparation
- The method demonstrated accurate and highly reproducible quantitative performance at all concentration levels
- The assay exhibited the ability to routinely quantify ultra-low levels of formoterol in an 8-min runtime, enabling bioanalytical labs to deliver high-quality data with excellent throughput
- A single platform for streamlined data acquisition, processing and management with SCIEX OS software was presented

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