# High Sensitivity and Extended Linear Range Quantification of Inhalation Drugs in Dried Blood Spots

***High Sensitivity QTRAP® 5500 System and Advanced SignalFinder™ Algorithm***

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## The Challenge of DBS Bioanalysis

Dried blood spot analysis (DBS) offers advantages for the reduction of sampling volume and simplification of sample handling and storage. At the same time, it presents new bioanalytical challenges. For example, the small amount of sample available places increased demands on assay sensitivity especially for drugs with very low systemic circulation such as inhalation drugs. Another challenge is assay dynamic range. Due to the nature of DBS, performing dilutions and ensuring their integrity is challenging. Conventional plasma samples can be diluted with blank plasma prior to extraction. This is not possible with a dried blood sample, therefore developing assays with a wide linear dynamic range gains additional importance.

Advanced technology in both hardware and software offers new solutions to these challenges. The high sensitivity and dynamic range of the QTRAP® 5500 system, combined with UHPLC chromatography makes DBS analysis of inhalation drugs feasible. In addition, advanced peak modeling capabilities of the SignalFinder™ Algorithm allow for more consistent peak integration with higher precision, and enables the innovative approach of applying software saturation correction to extend assay linear dynamic range[1].

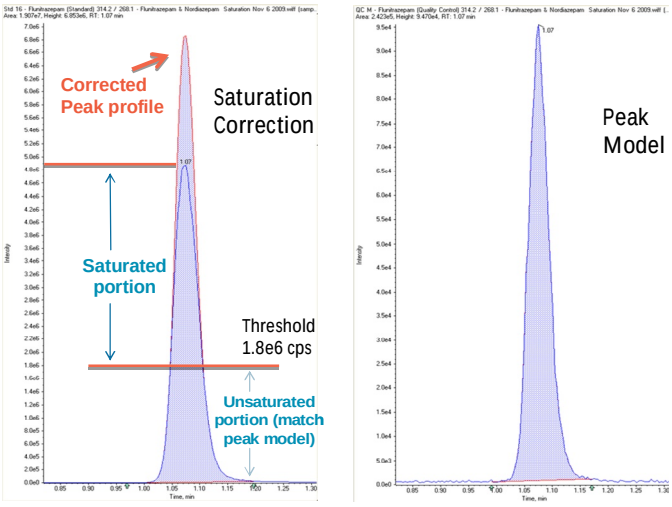



**Figure 1. Saturation Correction using the SignalFinder™ Algorithm.** (Left) A saturated peak consists of a saturated portion (above the saturation threshold of the detector), and an unsaturated portion below the saturation threshold. Using an unsaturated peak as a model (right), the algorithm can extrapolate the correct peak profile from the unsaturated portion.


In this technical note, we demonstrate the quantification of Budesonide in dried blood spots with a very sensitive LOQ of 5 pg/mL using the QTRAP 5500 system, and successful extension of assay linear range by five-fold using the SignalFinder Algorithm in MultiQuant™ Software 2.0.

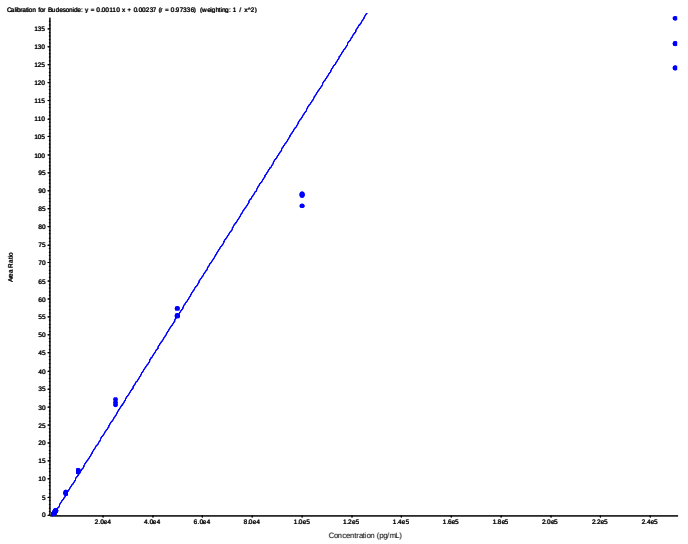
## Key Features of the QTRAP® 5500 System and MultiQuant™ Software for DBS Analysis

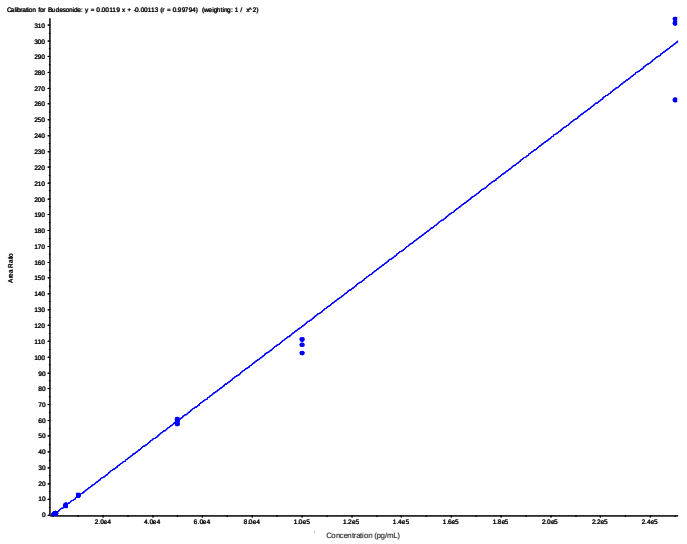
### QTRAP® 5500 System

- State of the art ion path and QJet® Ion Guide provides ultra high sensitivity to achieve the lowest limits of quantification.
- Proven Turbo V™ Source technology for rugged bioanalysis and efficient handling of high flow rates and compatibility with UHPLC.
- Fast scanning speed with LINAC® Collision Cell allows monitoring of multiple analytes and internal standards in a DBS sample under UHPLC conditions without sacrificing sensitivity.
- MRM$^3$ capability for eliminating challenging interferences.

### MultiQuant™ Software with SignalFinder™ Algorithm

- SignalFinder algorithm in MultiQuant software 2.0 provides more reproducible peak integration with reduced need for operator intervention.
- Software saturation correction for extending assay linear range.





| Actual Concentration (pg/mL) | Number of Values | Mean | Standard Deviation | %CV | %Accuracy |
|---|---|---|---|---|---|
| 5 | 3 of 3 | 4.67E+00 | 1.83E+00 | 39.2 | 93.4 |
| 10 | 3 of 3 | 1.11E+01 | 2.29E+00 | 20.6 | 111.4 |
| 50 | 3 of 3 | 5.09E+01 | 1.59E+00 | 3.1 | 101.9 |
| 100 | 3 of 3 | 1.11E+02 | 9.82E+00 | 8.8 | 111.2 |
| 500 | 3 of 3 | 5.58E+02 | 1.73E+01 | 3.1 | 111.6 |
| 1000 | 3 of 3 | 1.08E+03 | 1.46E+01 | 1.4 | 107.7 |
| 5000 | 3 of 3 | 5.55E+03 | 2.26E+02 | 4.1 | 110.9 |
| 10000 | 3 of 3 | 1.10E+04 | 2.02E+02 | 1.8 | 110.3 |
| 50000 | 3 of 3 | 5.07E+04 | 1.07E+03 | 2.1 | 101.4 |
| 100000 | 3 of 3 | 7.95E+04 | 1.65E+03 | 2.1 | 79.5 |
| 250000 | 3 of 3 | 1.19E+05 | 6.22E+03 | 5.2 | 47.4 |

| Actual Concentration (pg/mL) | Number of Values | Mean | Standard Deviation | %CV | %Accuracy |
|---|---|---|---|---|---|
| 5 | 3 of 3 | 5.08E+00 | 1.64E-01 | 3.2 | 101.6 |
| 10 | 3 of 3 | 9.71E+00 | 6.50E-01 | 6.7 | 97.1 |
| 50 | 3 of 3 | 9.71E+00 | 1.38E+00 | 2.9 | 96.9 |
| 100 | 3 of 3 | 9.71E+00 | 7.61E+00 | 7.4 | 103.4 |
| 500 | 3 of 3 | 9.71E+00 | 1.64E+01 | 3.1 | 104.9 |
| 1000 | 3 of 3 | 9.71E+00 | 2.09E+01 | 2.1 | 100.5 |
| 5000 | 3 of 3 | 9.71E+00 | 1.74E+02 | 3.3 | 104.0 |
| 10000 | 3 of 3 | 9.71E+00 | 1.79E+02 | 1.7 | 103.7 |
| 50000 | 3 of 3 | 9.71E+00 | 1.14E+03 | 2.3 | 99.0 |
| 100000 | 3 of 3 | 9.71E+00 | 3.67E+03 | 4.1 | 89.8 |
| 250000 | 3 of 3 | 9.71E+00 | 2.42E+04 | 9.7 | 99.3 |

**Figure 2. Comparison of Calibration Curves and Statistics for Budesonide.** Superior precision at the LLOQ and greater linear dynamic range was achieved using the SignalFinder™ Algorithm (right) compared to conventional integration (left).

## Methods

***Sample Preparation:*** A standard curve of budesonide was prepared in Sprague-Dawley rat whole blood over a concentration range of 5 – 250,000 pg/mL. A 50 µL aliquot was spotted on a DBS card (ID Biologics 226). After drying overnight at room temperature, two 8 mm punches were made from each sample. Punches were sonicated in 250 µL methanol for 60 minutes. After centrifugation, 200 µL was decanted, dried down and reconstituted in 100 µL 50/50 methanol / water.

***Chromatography:*** Separation was performed using a Waters ACQUITY UPLC system coupled to an AB SCIEX QTRAP® 5500 system. Sample volume of 20 µL was injected on a 1.7 µm BEH C-18 column (2.1 x 50 mm). A gradient of water (with 0.1% ammonium hydroxide) and methanol was used as shown in Table 1 at a 0.6 mL/min flow rate[2]. Column was heated to 60°C and total run time was 2.5 minutes.

**Table 1. Chromatography Method.** HPLC gradient conditions with total run time of 2.5 minutes.

| Time (min) | Flow Rate (ml) | %A | %B |
|---|---|---|---|
| 0 | 0.6 | 95 | 5 |
| 0.5 | 0.6 | 95 | 5 |
| 1 | 0.6 | 0 | 100 |
| 1.5 | 0.6 | 0 | 100 |
| 2.0 | 0.6 | 95 | 5 |
| 2.5 | 0.6 | 95 | 5 |

***Mass Spectrometry:*** A QTRAP® 5500 system was operated in ESI mode using a Turbo V™ source. The MRM transitions of 431.3 **à** 323.2, and 501.2 **à** 313.2 were monitored for budesonide and fluticasone propionate (internal standard), respectively.

***Data Analysis:*** MultiQuant™ Software 2.0 was used for all quantitative data processing. The SignalFinder™ Algorithm with software saturation correction was applied as described previously[1] and the standard MQ4 algorithm was used as the benchmark for comparison.

## Broad Dynamic Range

The reconstituted DBS samples were analyzed and the calibration curve was evaluated. A lower limit of quantification (LLOQ) of 5 pg/mL was achieved for budesonide in the DBS matrix. In addition, a linear calibration curve range of 5 pg/mL to 250 ng/mL was demonstrated representing 4.5 orders of magnitude of linear dynamic range (Figure 2). This remarkable result was achieved due the high sensitivity of the QTRAP 5500 system and the innovative data processing performed using the SignalFinder integration algorithm in MultiQuant™ software.

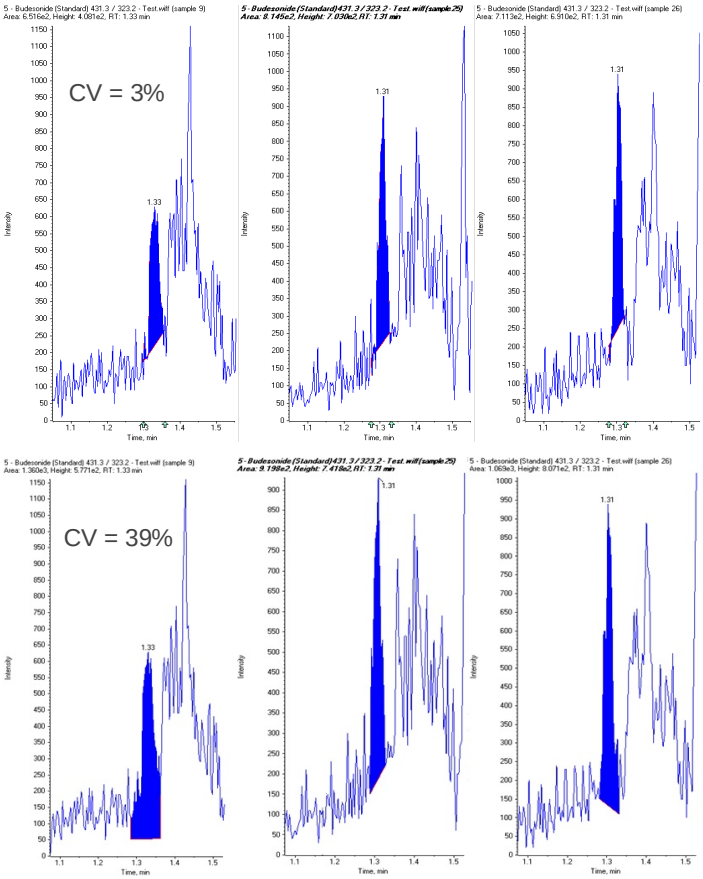



**Figure 3. Improving Integration Consistency can Result in a Lower LLOQ.** Integration of the 5 pg/mL standard (in triplicate) using SignalFinder™ Algorithm (top) resulted in significantly higher precision when compared to conventional integration (bottom).

## Peak Integration Consistency

The SignalFinder™ Algorithm demonstrated very consistent integration, even for weak or poorly resolved peaks (Figure 3) without having to adjust integration parameters. A CV of 3% was achieved at the LLOQ of 5 pg/mL, without any manual adjustment to the integration parameters. By using advanced peak modeling, the algorithm can better detect where the peak starts and ends. The model is automatically constructed based on an actual analyte peak in the batch being analyzed. The integrated peak area is calculated from the actual raw data and not the model.

When a conventional integration algorithm was used for the same data set, consistent integration at the LLOQ could not be achieved without having to resort to manual integration. The lack of integration consistency resulted in a CV of 39.2% at 5 pg/mL and 20.6% at 10 pg/mL. Using conventional integration, the LLOQ would have been 50 pg/mL, due to poor precision (CV greater than 20%) for the two lowest calibration points (Figure 2).

The combination of the high sensitivity of the QTRAP® 5500 system with advanced peak modeling resulted in achieving a very low limit of quantification of 5 pg/mL for budesonide in DBS matrix.

## Extension of Linear Range

The analysis of budesonide poses an additional challenge in assay linear range. A $C_{max}$ of 2,432 pg/mL was reported[3] following administration of a standard dose of 1.2 mg. At the same time, this compound has a short half-life resulting in rapid elimination. Therefore, the widest possible assay range is desired to assess the pharmacokinetics.

In conventional plasma assays, dilutions are easily performed by diluting the sample in blank plasma prior to extraction when the concentration is outside the assay range. The integrity of the dilution is demonstrated by diluting a quality control sample in the same manner and extracting it in the same batch.

This approach is not possible in a dried blood sample as it cannot be diluted prior to extraction. Instead, the dilution is performed after extraction with a blank DBS extract. No option is available for dilution prior to extraction. If an online direct elution method is used to extract the DBS sample, this problem becomes even more difficult as the extract is not available for an offline dilution with a blank extract.

As a result of these difficulties, DBS bioanalysis requires the widest possible linear dynamic range. The peak modeling approach offers yet another benefit in the form of peak saturation

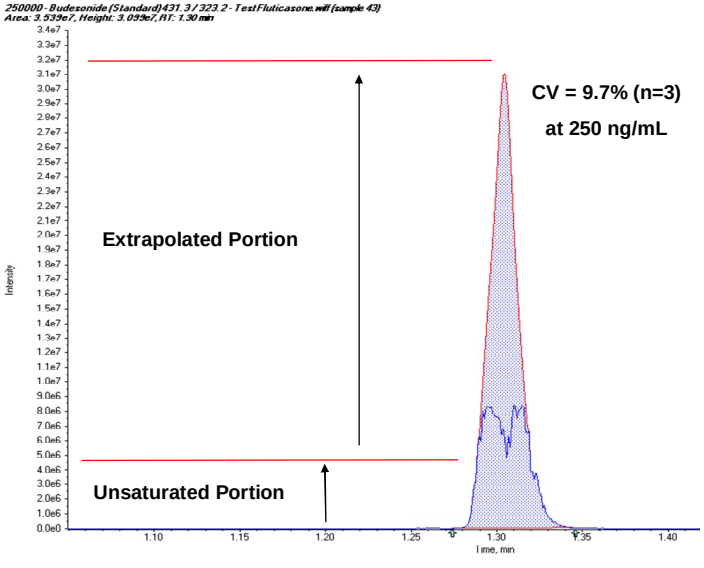



**Figure 4. Successful Correction of Detector Saturation using SignalFinder™ Algorithm at the Upper Limit of Quantification.** This figure shows saturation correction of the budesonide analyte peak in the highest calibrator at 250 ng/mL. Original peak (blue trace) has saturated the detector. The peak is extrapolated (red trace) using the unsaturated portion and the peak model, with a CV of 9.7% (n=3).

correction of a chromatographic peak to extend the linear range of the assay.

Figure 1 describes how a chromatographic peak can be corrected for detector saturation. A saturated peak consists of a saturated portion (above the saturation threshold of the detector) and an unsaturated portion below the saturation threshold. Using an unsaturated peak as a model, the algorithm can extrapolate the correct peak profile based on the unsaturated portion.

In this example, the SignalFinder™ Algorithm was used to correct for detector saturation and extend the top end of the calibration curve. This innovative approach for extending assay range resulted in increasing the upper limit of quantification (ULOQ) to 250 ng/mL, compared with 50 ng/mL without using saturation correction (Figure 2).

Saturation correction was performed reproducibly (Figure 4) at the ULOQ of 250 ng/mL with a CV of less than 10% across three replicates. Obtaining a sufficient number of points across the unsaturated portion of the chromatographic peak is critical for accurate and reproducible saturation correction. Therefore, the ability to perform fast MRM with short dwell times to obtain enough data points, especially across a narrow UHPLC type peak, is essential for the success of this approach. The fast scan speed of the QTRAP® 5500 system and excellent performance at short dwell times enables success.

## Conclusions

- High sensitivity quantification of inhalation drugs in dried blood spots at the low pg/mL range is feasible using the combination of the QTRAP® 5500 system and the SignalFinder™ Algorithm.
- SignalFinder Algorithm performs consistent integration even for weak or poorly resolved peaks, without having to adjust parameters or resort to manual integration.
- Application of saturation correction increased the upper limit of quantification by five fold, reducing or eliminating the need to perform dilutions on DBS extracts.


## Acknowledgements

The authors would like to acknowledge Dr. Neil Spooner who proposed the application of saturation correction to address dilution integrity in DBS analysis and Dr. Gordana Ivosev who developed the SignalFinder Algorithm.


## References


1. MultiQuant™ Software 2.0 with the SignalFinder Algorithm, AB SCIEX Technical Note, Publication 1060010-01.
2. J. Mether *et al*, application note, Waters, 2009.
3. T. W. Harrison and A. E. Tattersfield; *Thorax* 2003 58: 258-260.
4. To download a trial version of MultiQuant™ software please visit http://www.absciex.com/multiquant





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