# Tips: LC-MSD as a platform for inhalation product development

## *Choosing a testing facility with LC-MSD capability can save significant amounts of time and money for pulmonary delivery product development projects*

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According to an estimate produced by the Boston Consulting Group in 2001, development of a new drug cost nearly $900 million, of which more than $150 million was attributable to the chemistry component alone. Given that costs at the time were rising at nearly 7.5% above general price inflation, it's not unreasonable to guess that chemistry costs are now well above $200 million, and an average of 12 to 17 years elapses between the discovery of a drug and its approval, when it can start generating revenue. For inhalation products, analytical development undoubtedly costs more than for solid dosage and other drug forms due to additional testing necessary for the delivery systems. With development costs that high, finding an analytical testing facility that employs fast liquid chromatography coupled with a mass-selective detector (LC-MSD) instead of HPLC to analyze samples can save several months worth of time and millions of dollars.

For inhalation products, quantifying the active ingredient of a drug requires numerous cascade impaction tests since one of the most important physicochemical parameters influencing the deposition of medications in the lung is the particle size distribution of the drug formulation. A single Andersen cascade impactor (ACI) experiment generates 12 samples for analysis; taking 2 injections per sample plus injections of standards, each experiment requires 28 injections. An IND filing stability study alone can require over 13,000 injections, and the total for a new product development can exceed 20,000 (see Table 1). Each

**Table 1**

**Analytical testing requirements**

| | One ACI | Day activity analysis | Formulation Screening | Short Stability Study | IND Filling Stability Study |
|---|---|---|---|---|---|
| Number of ACIs | 1 | 12 | 40 | 288 | 480 |
| Number of samples | 12 | 144 | 480 | 3456 | 5760 |
| Number of Injections | 28 | 336 | 1120 | 8064 | 13440 |

can tested for ACI is also tested for dose uniformity, and dose uniformity tests generate 10 samples per can.

Using LC-MSD instead of HPLC to assay the samples can cut run times by as much as 90%, significantly cutting development time and cost. A typical HPLC-UV method averages a run time of 10 minutes, compared to an average run time of 1 to 2 minutes for LC-MSD. This speed means that you can get the results of a day activity analysis in half a day instead of waiting almost 2 1/2 days and that the time spent on an IND filing stability study can be cut from 93 days to 19 (see Table 2).

**Table 2**

**Analysis time comparison between LC and LC-MSD methods.**

| | LC | LC-MS |
|---|---|---|
| One ACI | 4.7 hrs | 0.9 hrs |
| Day activity analysis | 56 hrs | 11.2 hrs |
| Formulation Screening | 7.8 days | 1.5 days |
| Short Stability Study | 56 days | 11.2 days |
| IND Filling Stability Study | 93.3 days | 18.7 days |

In addition, LC-MSD performs well for ACI particle size distribution testing, even for very small amounts of product, improving accuracy in line with regulatory guidelines. Because the amount of drug tested can skew the results because of variation from actuation to actuation, the most accurate and representative results for ACI particle size distribution testing are obtained by using the smallest quantity of drug possible.

Using the patient dose for the particle sizing tests would give the most accurate results, but samples generated in unit dose experiments typically contain very low concentrations of drug, often as little as 1 to 200 ng/mL, and HPLC-UV does not have the sensitivity necessary for that small a quantity of drug. An MS with a single quadrupole analyzer has sensitivity 1000 times higher than that of a UV detector.

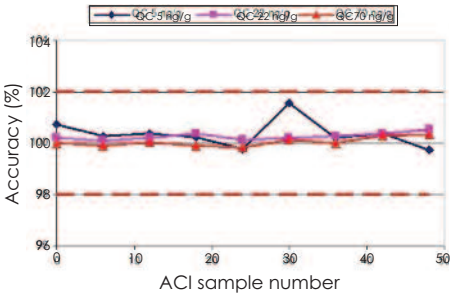
Due to its operating principle, MSD also provides significantly higher selectivity than UV detection. A mass spectrometer detects molecules according to their mass-to-charge ratio (m/z), and, while two compounds can have similar retention characteristics with a fast-LC method, making them difficult to quantify accurately on an HPLC-UV chromatogram, it is unlikely they have the same mass spectra. With different mass-to-charge values, the chromatograms exhibit no overlap, making the compounds easy to distinguish.

LC-MSD offers a particular advantage over HPLC for testing combination products, which present a difficulty for HPLC because the compounds require different mobile phase elution compositions. For example, Salmeterol is eluted with a composition of 35:65 acetonitrile:water, while fluticasone propionate is

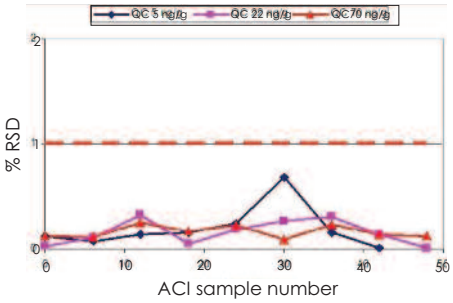
**Figure 1**

**Assay accuracy and precision**

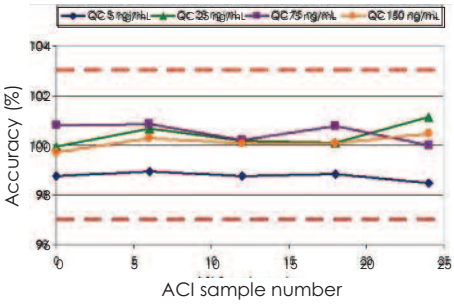
(a) Albuterol assay accuracy



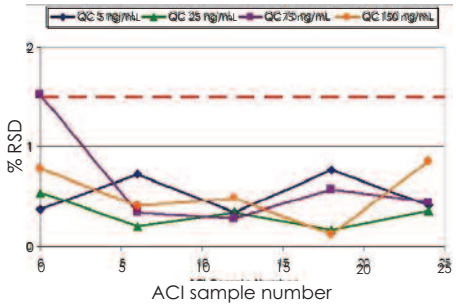

(b) Albuterol assay inter-injection precision (n=3)




(c) BDP assay accuracy




(d) BDP assay inter-injection precision (n=3)



eluted with 70:30 acetonitrile:water. Assaying both compounds with HPLC requires the application of a sharp gradient of the mobile phase composition, which causes the baseline to drift and makes integration of the peaks difficult. This problem does not occur using LC-MSD, therefore combination products can be assayed more quickly and easily, saving time and money.

Experience has shown that validating a method using LC-MSD takes no more time than validating an HPLC-UV method, and LC-MSD performs just as well as HPLC- UV in terms of both inter-injection precision and accuracy. Inter-injection precision, a measure of reproducibility measured by performing several injections of the same sample, should be less than 2% relative standard deviation (RSD), a standard that LC-MS meets easily (Figure 1). Accuracy is determined by taking a ratio of the theoretical concentration and the calculated concentration of the quality control (QC) standard and should be in the range of 97.0-103.0%. LC-MSD performs well within that range over the entire linear range of concentration for common asthma medications (Figure 1).

In order to compensate for drift in the signal over time, LC-MSD requires the use of an internal standard, ideally a deuterated analog of the drug being studied, to achieve the highest possible accuracy. For common asthma medications, deuterated analogs are readily available, and the cost of a custom deuterated analog for a novel compound is unlikely to exceed $60,000 per gram. One gram supplies enough material for all testing necessary during a development project, and the cost is easily outweighed by the savings provided by LC-MSD. Even without an internal standard, an accuracy of 95-105% can be achieved, which is good enough for the early stages of a development project. So, while novel compounds might require commissioning the synthesis of a deuterated analog, a process that can take several months, the first stages of the development process can still move forward.

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