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# **Ion trap mobility spectrometry—reducing downtime in cleaning validation and verification**

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## Abstract

*Pharmaceutical manufacturers must validate their cleaning processes to ensure compliance with cGMP regulations. Ion trap mobility spectrometry (ITMS) has the advantage of being a specific method for measuring chemical residue with a very fast sampling time—less than one minute per sample—to facilitate faster turnaround during cleaning validation. Minimizing equipment downtime potentially impacts the efficiency and economics of pharmaceutical production.*

## Introduction

The determination of a “clean state” in pharmaceutical manufacturing is crucial to a well-controlled final product for distribution. The US Food and Drug Association (FDA) requires the validation of cleaning methods, and the specificity of their requirements has increased with the expanding base of knowledge surrounding product manufacturing and safety.

Sections in the 1963 GMP<sup>1</sup> and the 1978 cGMP<sup>2</sup> regulations dealt with requirements for cleaning, but as the knowledge around cleaning and cross-contamination increased, so did the scope and depth of the FDA’s regulations. A key point in the development of these regulations was a 1991 lawsuit brought by the FDA against a drug manufacturer alleging flawed manufacturing and distribution processes, quality controls, and testing procedures. The FDA cited the company for use of contaminated water, distribution of mislabeled drugs, and re-distribution of drugs previously returned as defective.<sup>3</sup> Ultimately, the court ruled against the drug manufacturer and halted the distribution of 24 products.<sup>4,5</sup> This decision fundamentally established legal precedent for the FDA to require manufacturers to validate every step of their manufacturing processes.

During and following this case, the FDA issued an internal inspection guideline<sup>6</sup> and the official “Guide to Inspection of Cleaning Processes,”<sup>7</sup> the latter of which serves as the

starting point for most inquiries regarding cleaning validation. Even with the amount of published regulatory guidance available both in print and online, or through organizations specializing in cleaning validation and cGMP regulations, companies still face significant challenges in understanding, documenting, and validating their cleaning processes. Specifically, companies have to develop and document procedures for effectively sampling their equipment and then analyzing those samples so as to produce a measurement that gives, with a high degree of confidence, enough information to denote a “clean” state. Until that clean state is obtained, the manufacturing processes of that particular line or piece of equipment are halted.

The financial impact of equipment downtime is considerable to an industry where exorbitant sums of money are poured into research and development with cycles that can last almost 15 years and cost more than \$800 million per drug,<sup>8</sup> and into the development of manufacturing processes where costs are on par with R&D expenditures.<sup>9,10,11,12,13</sup> With products having a finite period of patent protection and technology advancing at rapid rates, companies must maximize profits from every drug they produce in order to recoup investments in development.

## Current Methodology

Companies have several options for effectively sampling and analyzing the residues present on manufacturing equipment. Rinse sampling and swab (direct) sampling are commonly employed methods for recovering residue off of equipment surfaces, although visible inspection is also widely used as a criterion in conjunction with the other methods. In order to determine the amount of active ingredients in the residue sampled, the pharmaceutical and biotech industries commonly use analytical methods such as high performance liquid chromatography (HPLC) and measurement of total organic carbon (TOC).

In order to sample the residues left on the surfaces of manufacturing equipment after cleaning, several methods can be used. Rinse sampling consists of taking an aliquot of the solution that is passed through the equipment train, and then providing a measurement of the average amount of residue present across the entire train. This remains a valuable process for sampling the parts of a system potentially unreachable for direct sampling, such as the innermost portions of tubes and piping. However, this technique's inherent disadvantage is that it does not provide information about specific areas of the equipment because the resulting measurement could indicate residue evenly distributed across the entire manufacturing train or residue concentrated in a single spot on a single piece of equipment.

As the distribution of residue is important for the potential transfer of contaminants from one batch to the next, the FDA places a good deal of importance on swab sampling, which allows direct sampling of discrete locations throughout the equipment. These locations are usually selected as those toughest to clean and where uneven residue buildup occurs during cleaning.

HPLC remains the most widely used analytical method for cleaning validation and verification in the pharmaceutical industry. This technique has several advantages, including a high level of specificity and the versatility to measure mixtures of different types of residues. HPLC uses the interaction between the compounds in a liquid "mobile" phase and a solid "stationary" phase in order to separate and quantify compounds in a mixture based on a particular physical or chemical property such as molecular charge, size, or polarity. This does not come without associated cost, however, as a single measurement can take up to 60 minutes. The laboratory time associated with the measurement can take anywhere from a few days to two weeks, depending on the laboratory. This waiting period is one of the root causes of downtime in cleaning validation and verification.

TOC is commonly used in biotech applications. The technique is not a specific method. It measures the total amount of organic carbon present in a sample and cannot differentiate the sources of carbon to identify how much was due to any single molecular entity. The way to ensure that the lack of specificity does not account for underestimating the presence of a particular compound is to assume that all of the carbon measured by this technique is attributed to the "worst case" product in the mixture. Using this strategy, TOC has been accepted as a valid analytical method for cleaning.

The main advantage is that the technology gives a very fast, simple "go/no-go" output in a total carbon level. It is remarkably useful in biotech cleaning applications where denatured proteins rarely can be identified as the "active" product in a drug after the completed cleaning process. The lack of specificity, though, presents problems in instances requiring a specific measurement technique. Samples measured by this technique must be soluble in water, as organic solvents contribute to the total carbon measurement. Finally, while attributing all of the carbon measured to the active is a means of the technique's acceptance, this fundamentally lowers the threshold limits for a clean state, and may cause false negative cleaning results.

## **Ion trap mobility spectrometry**

Ion trap mobility spectrometry (ITMS) technology has the potential to widely impact cleaning validation and verification in the pharmaceutical industry. ITMS can identify compounds in a mixture and determine their quantity, through measuring the time of flight of ionized molecules down a drift tube.

The key underlying principle of ITMS is that the average velocity ( $V$ ) of an ion in a drift tube scales linearly with an applied electric field ( $E$ ). This linear scaling factor is referred to as the "ion mobility" constant ( $K$ ).  $K$  can also be adjusted for standard temperature and pressure conditions ( $K_o$ ).

$$V_{avg} = KE \quad \text{and} \quad K_o = K(273/T)(P/760)$$

This relationship is the foundation for more complicated equations involving correcting for temperature and pressure, the mass of the ion in question and that of the gas in the drift tube, and the effective collision cross section of the molecule, among other parameters.

Instrumentation for ITMS essentially comprises sample introduction, ionization, a drift tube with an electric field, and a detector. The Kaye Validator® ITMS instrument (Figure 1) represents such an example. This device performs ion trap mobility spectrometry, in which the use of an “ion trap” mechanism allows ions to “build up” in a trapped area and then be periodically released into the drift tube for measurement.

The goal of this technique is to identify the



Figure 1. Kaye Validator ITMS.

compounds of interest in samples taken from equipment after cleaning and determine whether the amount of the compound detected is below the threshold limit for cleanliness. The technology has been proven in the security industry, which uses ITMS technology for detection of trace amounts of active compounds in narcotics and explosives.

Figure 2 shows a schematic of the Kaye Validator® ITMS instrument. The instrument contains a desorber that heats a sample in order to put it into a vapor phase. The vapor passes through a silicon membrane, which keeps environmental contaminants out of the system. Beyond the membrane is an air loop with two regenerative dryers maintaining a state of very low humidity.

Once introduced into the air loop, the vapor passes into the sample chamber, where the molecules are bombarded with electrons from the radioactive (Ni-63) source, creating positive and negative ion species. The “ion trap” gates the samples, and opening and closing pulses at 33Hz allow samples to enter the drift tube where they enter an electric field of 1000V and fly towards the detector.

The polarity of the electric field oscillates in conjunction with the ion trap, alternatively sending positive and negatively charged ions down the drift tube. This allows the Kaye Validator® ITMS to display information on

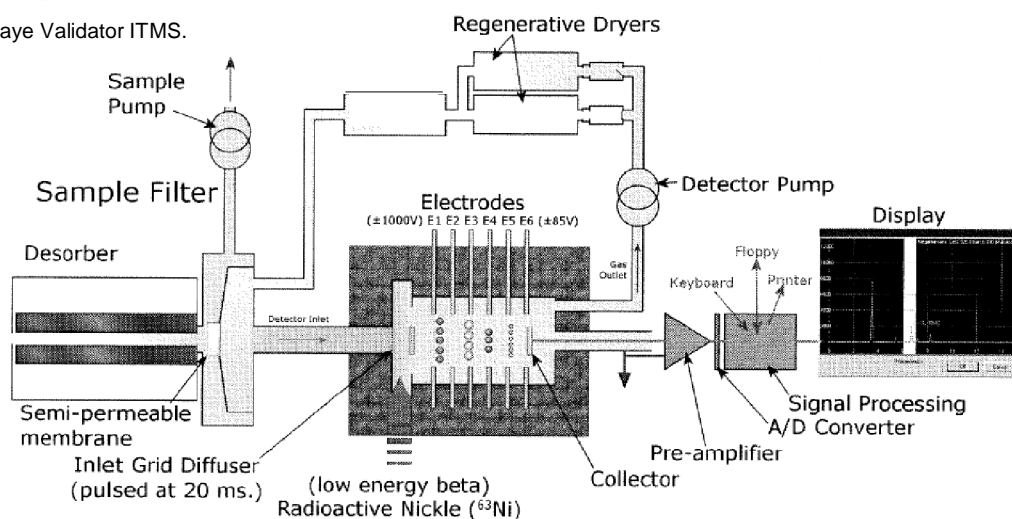
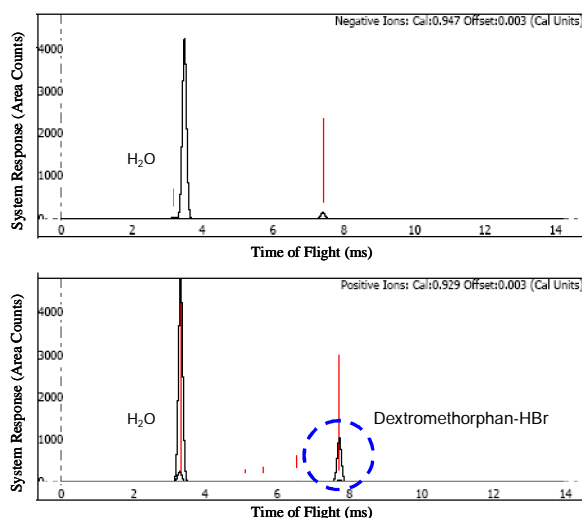


Figure 2. Kaye Validator ITMS schematic.

both positive and negative ions after each scanning session without needing to switch modes. The detector at the end of the tube measures the time of flight for each ionized species.

## Identification

The identification of molecules in a mixture is based on the molecule's time of flight (TOF), directly proportional to the molecule's effective collision cross section, essentially related to the size of the molecule. The molecule's time of flight becomes its "fingerprint" that will not change, regardless of the other molecules present in the system, given that the physical characteristics of the instrumentation (e.g. voltage of the electric field, length of the drift tube) remain constant.



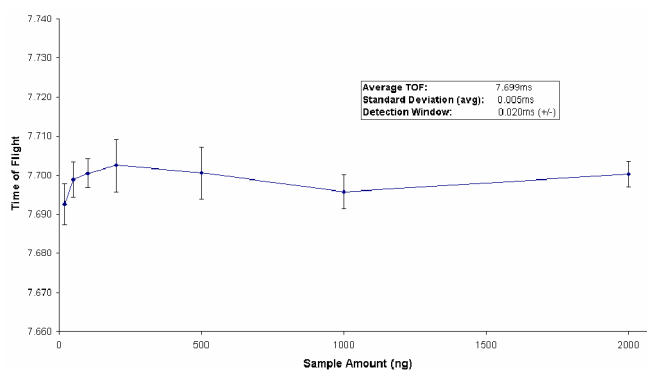
**Figure 3. Plasmagram display for dextromethorphan.** The API peak is discernable at 7.698ms in the positive ion data (bottom plot).

Figure 3 shows the two-dimensional output of the system after data collection, known as a plasmagram. This plot shows the data from both positive (bottom) and negative (top) ions in the system. The water in the system is displayed as a prominent peak on both the positive and negative data, and is easily identifiable and should not be construed as an "unknown" peak during method development. While regenerative dryers serve to keep the

inner air loop as dry as possible, there are always trace amounts of water vapor present.

The API peak—dextromethorphan hydrobromide, a common cough medication—is easily identifiable in this case with a TOF of 7.698ms in the positive ion data.

Note that there is a small peak present in the negative ion data. This is from a negative ion complex of the API. It is common for molecules to form both negative and positive ion peaks, and for one of those peaks to be formed preferentially. In the case of dextromethorphan, the positive peak is preferential and is used for calibration and quantification.



**Figure 4. Peak stability with increasing amounts of sample.**

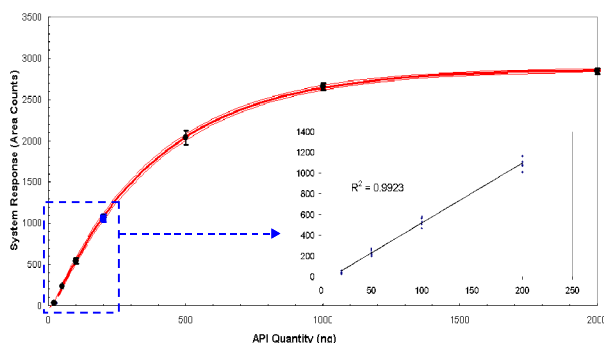
Figure 4 shows that the peak location is extremely stable, and does not trend with sample concentration. The standard deviation on the TOF for the compound is only 5 $\mu$ s, meaning that a detection algorithm that included a measurement window of +/- 20 $\mu$ s would be sufficient to encompass nearly four standard deviations ( $4\sigma$ ) of the potential peak variation. This level of detection is robust, and will be able to identify the API in question regardless of the other constituents in a given mixture.

## Quantification

Unlike the security application where the identification of trace amounts of an active compound is sufficient to raise an alarm, the use of ITMS in pharmaceutical cleaning must have the ability to definitively verify that a

given compound is below the established carryover limit from batch to batch.

Figure 5 shows a typical calibration response curve for a pure active ingredient, dextromethorphan. This figure shows a logarithmic response to increasing concentration grounded in the chemistry of sample ionization.<sup>13</sup> While the response is not linear, it remains possible to approximate the relationship at lower concentrations with a linear function. The inset of Figure 5 shows a linear fit of the response between 20 and 200ng of sample with the resultant R<sup>2</sup> value (0.9923).



**Figure 5.** System response/calibration curve for dextromethorphan. Linear range from 20-200ng shown in inset.

Describing the system response/calibration curve for a particular substance allows determination of the system's limits of detection (repeatability of detection of low amounts of sample) and quantification (ability to quantify the amount of sample in the higher regions of the response curve). While the instrument will still show a repeatable signal at the quantification limit, small changes in intensity are associated with wide swings in sample amount for a given substance. These limits, and the relative standard deviation of the measurements within the quantification range, may be impacted by several factors including the solvent used for dilution, the measurement integration time, the temperature of the desorber, and other factors common to analytical measurements.

The optimal combination of experimental factors, once achieved through developmental

experimentation, should produce a robust measurement procedure that will be useful in practice. In the case of dextromethorphan, a small amount of method development, considering only the solvent used and the volume used in the sample, shows a wide range of quantification (20ng – 1757ng), and a very low relative standard deviation (1.6% at 1000ng). These parameters offer a very wide range of measurements and a level of precision that would be widely acceptable as an analytical method in the field.

## Direct Swabbing

While ITMS proves to be a robust analytical technique, its greatest advantages are the ability to measure samples very quickly and its potential for at-line use with “direct swabbing.” Unlike HPLC, measurements with an ITMS system most often take between 10 seconds and one minute. The ability to turn around results this quickly is a boon in itself, but what sets ITMS apart is that its method of sample introduction allows swab samples to be inserted directly into the instrument with no dilution or extraction. Importantly, the instrument also has the ability to accept samples that have been diluted/extracted if the expected amount of residue from direct swabbing exceeds the system's detection capacity.

The security industry heavily uses the direct swabbing method and its application to pharmaceutical cleaning validation and verification can potentially affect drastic reductions in downtime currently experienced with HPLC. Additionally, direct swabbing offers a specific method that is as fast as TOC in providing results.

## Conclusions

Ion trap mobility spectrometry provides an important technological innovation—a fast, specific method for quantifying residues after cleaning with the potential to drastically impact the downtime experienced in manufacturing due to cleaning validation and verification. With the escalating costs of health care

putting pressure on the price of therapy, and in turn putting pressure on the profit margins of the companies producing those therapies, companies are increasingly searching for innovative means of reducing their costs of production and improving their bottom line. Through the reduction of equipment downtime and the shortening of lab analysis cycles associated with cleaning, ITMS has the potential to drastically affect the economic efficiencies of manufacturing and cleaning validation in the pharmaceutical industry.

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<sup>1</sup> FDA GMP regulations, Section 133—Drugs; Current Good Manufacturing Practice In Manufacture, Processing, Packaging, or Holding. G.P. Larrick. FR DOC 63-6336, June 19, 1963

<sup>2</sup> FDA Dept. of Health and Human Services, Subchapter C (Drugs, General) Part 211: Current Good Manufacturing Practices for Finished Pharmaceuticals. 43 FR 45077, Sept. 29, 1978

<sup>3</sup> Moses, Jonathan M. *The Wall Street Journal*, Jun 15, 1992. Page B5

<sup>4</sup> United States vs. Barr Laboratories, 812 F. Supp. 458 (DNJ, 1993)

<sup>5</sup> Medical Marketing and Media. March 1993; Volume 28 (3). Page 8

<sup>6</sup> FDA. Mid Atlantic Region Inspection Guide: Cleaning Validation. July 28, 1992

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<sup>10</sup> Staff Reporter. Pharmaceuticals Trouble in the Making: Sloppy manufacturing comes under fire, but inefficiency is the real problem. *The Economist*. August 29, 2002

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<sup>13</sup> Eicman and Karpas. Ion Mobility Spectrometry. 2<sup>nd</sup> Edition, Taylor and Francis Group (CRC Press). Boca Raton, FL. 2005. Pages 92-98

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