

Using Ion Mobility Spectrometry for Cleaning Verification in Pharmaceutical Manufacturing

Reno Debono,* Stephen Stefanou, Matt Davis, and Gaurav Walia



The use of ion mobility spectrometry (IMS) for cleaning verification has the potential to save millions of dollars by substantially reducing the time required to verify the cleaning of pharmaceutical production facilities. The technique's analysis requires ~30 s/sample compared with 15–30 min/sample using high-performance liquid chromatography, thus showing the potential to be 30 to 60 times faster. Once implemented in a production facility, the method could save one to two days of production time per turnaround.

Reno Debono, PhD, is an R&D manager at Barringer Instruments Inc., 30 Technology Dr., Warren, NJ 07059, tel. 908.222.9100 ext. 3017, fax 908.222.1557, rdebono@bii.barringer.com. Stephen Stefanou is a scientist III, Matt Davis is a project leader, and Gaurav Walia is a group leader at Forest Laboratories, Farmingdale, NY.

*To whom all correspondence should be addressed.

Validation of cleaning processes has long played a critical role in pharmaceutical manufacturing. FDA requires that firms provide written documentation detailing the cleaning processes used for various pieces of equipment as well as how the cleaning processes are validated. Validation requires the development of a cleaning verification method with the typical detection limits of either ~10 ppm or biological activity levels of 1/1000 of the normal therapeutic dose.

Two methods of sampling the surface of equipment after cleaning are direct-surface and rinse sampling. The advantages of the direct-surface method are that it can reach hard-to-clean areas and it allows insoluble residues to be sampled. However, rinse sampling can evaluate a larger surface area as well as areas that are not accessible with a swab.

Many pharmaceutical companies currently use high-performance liquid chromatography (HPLC) to evaluate samples collected from production machines. Scientists prepare a mobile phase and diluents and set up the instrument. The next step is sample preparation, which requires 60–90 min for each sample collected from a production site. Most pharmaceutical companies use an HPLC system with an autosampler that allows sample analysis to take place unattended. Cleaning pharmaceutical equipment, collecting and preparing samples for HPLC analysis, acquiring data, and processing can require up to two days. Production then could resume at the beginning of the third day, resulting in two days of lost production time.

Ion mobility spectrometry

The amount of time spent on the cleaning verification process introduces delays into critical development programs. Ion mobility spectrometry (IMS) offers an alternative to the lengthy, traditional cleaning verification process. The technique has high sensitivity and is easy to use, which has allowed it to be widely implemented in airports to detect narcotics and explosives, for example (see www.barringer.com).

IMS detects trace quantities of specific compounds by vaporizing and ionizing a sample and measuring the characteristic speed at which the ions move through air at atmospheric

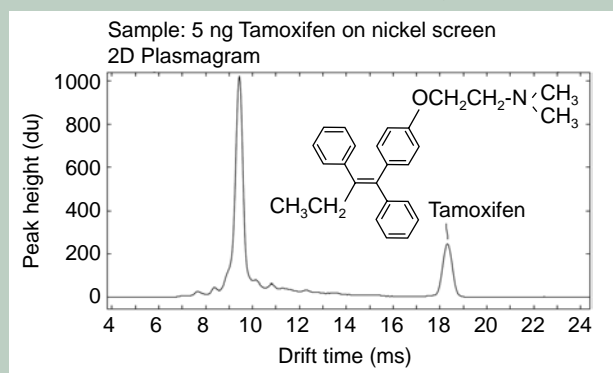
IMS operations

Solid or liquid samples are directly introduced to the analyzer by thermal desorption. Resulting vapors are selectively ionized in a controlled chemical ionization environment (ionization region) to produce molecular ions or molecular ion clusters. The instrument used (IonScan-LS, Barringer Instruments, Warren, NJ) can measure both positive and negative ions.

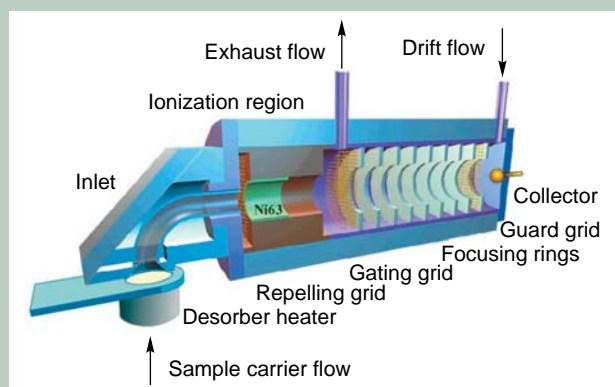
Ions are gated by an electronic shutter into a drift tube, where they collide with neutral gas molecules at atmospheric pressure before striking the collector and generating an electric current (see Sidebar figure 1). Ions are separated according to their size and shape on the basis of drift time in the 3–50-ms range.

Larger ions have longer drift times than smaller ions as a result of their larger cross-sectional areas. Accurate identification is based on the detection of peaks within ± 0.04 ms of their expected positions.

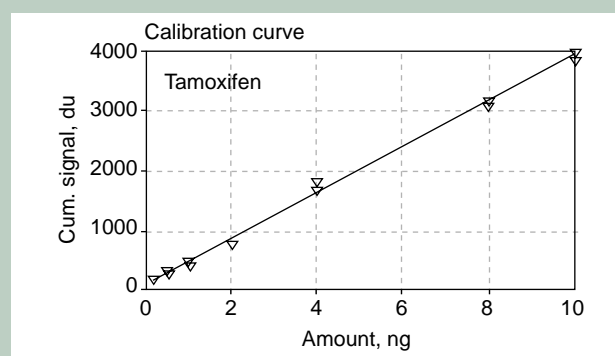
A typical report characterizing ion mobility for cleaning method development is shown in the example of Tamoxifen in Sidebar figures 2 and 3.



Sidebar figure 2: Ion mobility spectrum for Tamoxifen.



Sidebar figure 1: Diagram of the IonScan-LS.



Sidebar figure 3: Calibration curve for Tamoxifen.

Table I: IonScan data acquisition settings.

Analysis delay (s) following start of desorption	0.1
Scan period (ms)	20.0
Shutter grid width (ms)	0.2
Maximum analysis duration (s)	8.0
Maximum number of segments per analysis	20.0
Number of co-added scans per segment	20.0
Analysis duration (s)	8.0
Number of segments per analysis	20.0
Delay (ms) before start of sampling for each scan	1.0
Sampling period (s)	50.0
Number of sample points per scan	379.0
Duration (ms) of background reference	0.3

Table II: Temperature and flow-rate settings.

Drift heater (°C)	233
Inlet heater (°C)	285
Desorber heater (°C)	300
Drift flow (cm ³ /min)	300
Stand-by flow (cm ³ /min)	75

pressure in an electric field (see sidebar “IMS operations”). The IonScan-LS detection tool (Barringer Instruments) can be configured so that it requires very little skill on the part of the user. IMS can convert cleaning verification into a simi-

larly fast and simple process that can be deployed on the manufacturing floor. Such a process change could eliminate the need to tie up resources in the quality control department and simultaneously free up scarce and valuable production resources.

Methods development

Before IMS can be implemented for cleaning verification, a formalized and validated method that can consistently detect whether the limits on the active batch-to-batch carryover have been exceeded is required. Essentially, methods development is intended to challenge the specificity and sensitivity of an instrument in combination with the sampling method to detect specific residual contaminants. Typically, ion peaks observed in IMS have drift times ranging from 10 to 30 ms. In the IonScan-LS, compounds are identified by detecting expected peaks that are within ~ 0.04 ms. In addition, an internal calibration substance of known ion mobility is used to ensure that the instrument is working properly. When a substance of interest is detected, the software triggers an alarm to alert the operator. This article will describe experiments that used the IonScan-LS for cleaning validation and will include the results.

Experimental. The development process began by collecting samples, which consisted of swabbing a predefined surface area after exposure to product X, the active pharmaceutical ingredient (API) that was selected for these experiments. Both positive and negative control samples were used in the study. A positive control sample is a clean swab that is fortified with a known

Table III: Order of analyses for system suitability.

Number of Injections	Solution of Analysis	System Suitability Requirement
1	<i>n</i> -hexane (IMS blank)	1. The plasmagram does not exhibit any significant peaks at the drift time of the analyte.
1	Negative control sample	See Requirement 1.
1	Blank swab sample	See Requirement 1.
5	Standard solution 1 (S1)	2. System correlation coefficient (R^2) is ≥ 0.97 .
5	Standard solution 2 (S2)	
5	Standard solution 3 (S3)	
5	Standard solution 4 (S4)	
5	Standard solution 5 (S5)	
1	<i>n</i> -hexane (IMS blank)	See Requirement 1.
1	Positive control sample	3. Recovery of the positive control sample is 80.0–120.0% (compared with the calibration curve).
1	<i>n</i> -hexane (IMS blank)	See Requirement 1.

drug is detected and quantified by the IMS instrument. Quantitation was accomplished using an external standard calibration curve ranging from 0.1 to 1.6 ng.

Experiment procedure. The following procedure, specific to product X, is atypical in length. Most chemical substances traditionally require a simpler and significantly more abbreviated sample preparation.

Materials and methods. ACS reagent-grade materials and reagents were used. Deionized water was used as a diluent unless otherwise indicated. The nylon swabs used were TWTX1004 (Alpha-Wipe, Texwipe, Upper Saddle River, NJ). Positive-mode filter disks (6813410, Barringer Instruments) were used as

Table IV: Linearity and positive swab calculations for product X.*

Trial	Cumulative Amplitude (ng)				
	0.1	0.2	0.4	0.8	1.6
1	179.0	337.0	604.0	1209.0	2222.0
2	151.0	352.0	621.0	1150.0	2169.0
3	152.0	377.0	701.0	1098.0	2060.0
4	157.0	304.0	699.0	1192.0	2144.0
5	168.0	348.0	623.0	1122.0	2321.0
Average	161.0	344.0	650.0	1154.0	2183.0
% RSD	7.39	7.72	7.17	4.03	4.43

* Samples are calculated using a linear curve.

Table V: Recovery of product X.

(+) Swab	Cumulative Amplitude	Amount (ng)	True Value (ng)	% Recovery
1	722	0.49	0.5	97.4
2	693	0.47	0.5	93.1
3	712	0.48	0.5	95.9
4	830	0.57	0.5	113.7
5	776	0.53	0.5	105.6
6	637	0.42	0.5	84.6
Average	728	0.49	0.5	98.4

amount of the API, and a negative control sample is a clean swab only. Both positive and negative control samples undergo the same preparation procedure as the traditional HPLC cleaning verification samples.

General swab preparation and extraction procedure. Each swab is placed in a specified volume of water and agitated to dissolve any material on the swab. The analyte then is extracted from the water phase with the solvent hexane. Two microliters of hexane with the dissolved sample solution is injected onto the surface of a PTFE-mesh filter. (Solvents are chosen on the basis of specific API requirements.) After the solvent has evaporated, the

sample disks for the Ion Scan-LS. Product X was used as a reference standard. The IonScan-LS instrument parameters are shown in Tables I and II. The maximum analysis duration was 12 s, and the scan period was 20 ms. A 5- μ L syringe was used, and the injection volume was 2 μ L.

Standard solutions were prepared containing 0.05 μ g/mL, 0.1 μ g/mL, 0.2 μ g/mL, 0.4 μ g/mL, and 0.8 μ g/mL of product X. Each solution was extracted by the extraction procedure mentioned subsequently (see "Extraction procedure" section).

A positive control sample was prepared by placing a swab into a clean 250-mL conical flask and pipetting a known amount of product X onto the swab. A negative control sample was prepared by placing a swab into a clean 250-mL conical flask. Then 50 mL of diluent was pipetted into each flask. Both control samples were mechanically shaken for 15 min. The extracts were filtered through 0.45-mm nylon filters, and the first 2 mL of filtrate from each sample was discarded. The filtrate was extracted by the extraction procedure (see "Extraction procedure" section).

Experimental samples (supplied by manufacturing) were prepared by pipetting 50 mL of diluent into each 250-mL conical flask containing a swab. The flasks were sealed with stoppers and were

shaken mechanically for 15 min. The extract was filtered through a 0.45- μ m nylon filter, and approximately the first 2 mL of filtrate was discarded. The filtrate was extracted by the following extraction procedure.

Extraction procedure. Extraction of the samples and standard solutions was performed by pipetting 10 mL of each solution into a 50-mL scintillation vial with a screw cap and adding 5 g of sodium chloride, 5 mL (1 N) of sodium hydroxide solution, and 10 mL of *n*-hexane to each vial. Each mixture was shaken mechanically for 30 min. Then 2 mL of the *n*-hexane phase was decanted into a separate vial for analysis.

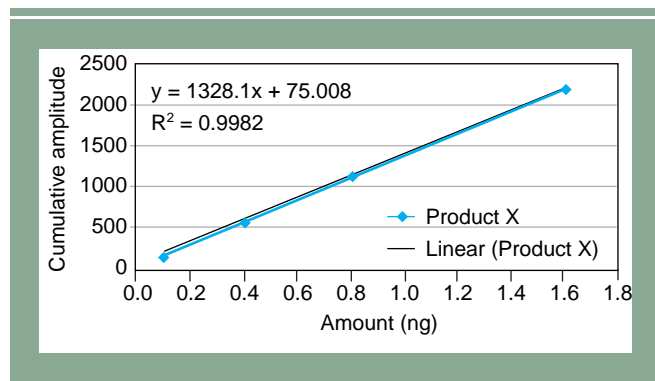


Figure 1: Calibration curve for product X.

For each sample analysis the instrument was equilibrated for at least 2 h, and the program for the compound of interest was loaded. Then 2 μ L of the extracted sample preparation was placed onto the sample disk (PTFE-mesh filter) for the Ion-Scan-LS. The instrument analysis began by sliding the stage holding the sample disk over the desorber, which heated the sample to 300 °C. The desorption temperature was selected on the basis of the chemical structure, melting point, and boiling point of the compounds. A stream of air moving at 300 mL/min was used as a carrier gas to transport the thermally volatilized material into a reaction chamber where a 15 mCu Ni63 radioactive source ionized the compound. The ions were gated into a drift chamber and were accelerated toward the collector electrode. Upon reaching the collector electrode, each group of ions registered on a plasmagram that measured the responses of the groups and the amount of time (in milliseconds) that each group required to travel through the chamber. The cumulative amplitude and the maximum peak amplitude of the analyte were determined for the standard and sample solutions. System suitability was demonstrated by the analyses listed in Table III.

From the cumulative amplitude measured for each of the 25 readings of standard solutions S1–S5 (see Table IV), a linear regression was performed with the equation shown in Figure 1 to determine the slope and formula of the calibration curve. A plot of the results also is shown in Figure 1.

Sample analysis and results

The positive control swab samples were analyzed six times, and the recoveries of product X from the positive swab samples ranged from ~85–114% (see Table V).

Conclusion

IMS is a versatile technique that can identify and quantify a wide range of molecules. IMS accurately verified the cleaning process for product X. The entire process required only 20 s to obtain a sample analysis result. Rapid sample analysis can lead to a significant reduction in cycle time, making it possible to complete both the cleaning and verification processes in <4 h. The overall benefits of using IMS for cleaning verification include the increased use of valuable production equipment and the potential to reduce the lead time of the drug development process. IMS is an efficient technology that likely will play a substantial role in the future of cleaning verification in pharmaceutical manufacturing. **PT**