

GE
Sensing

Direct Swabbing and Surface Recovery with Ion Trap Mobility Spectrometry

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Ion Trap Mobility Spectrometry has been discussed as a fast and specific technique for the analysis of samples for cleaning validation and verification in the pharmaceutical industry. This study presents data on the use of this technology for “direct swabbing,” or directly sampling and analyzing the equipment of interest. Recovery results from stainless steel surfaces for two different compounds, cefuroxime sodium and pseudoephedrine HCl, are presented. At-line analysis has the potential of greatly improving the efficiency of analyzing cleaning results and improving equipment turnaround.

Introduction

Ion Mobility Spectrometry (IMS) and Ion Trap Mobility Spectrometry (ITMS) are built on the principle of measuring the drift velocity of ions as they are propelled through a “drift gas” at ambient pressure via the force of an electric field.¹ This technology has been in use for over 30 years, primarily applied in detecting trace amounts of narcotics and explosives², and is found at most airports as part of their security screening procedures.

This technology has more recently been applied to the pharmaceutical industry, mainly focusing on applications involving cleaning validation or verification. While the technology has been reviewed³, and specific applications described^{4,5}, the published data to date focused on results generated from extracted solutions rather than from the direct sampling of a surface of interest.

The act of taking a sample directly from the surface of equipment has been termed “direct swabbing,” in that the sample is analyzed directly instead of including the intermediate extraction step. Similar to the use of ITMS in security applications, the advantage of direct swabbing is that it allows the user of the instrumentation to generate results without the need to send samples back to an analytical laboratory. Additionally, the portability of commercially available ITMS instrumentation allows the testing to be completed at-line.

The FDA’s “*Guide to Inspections Validation of Cleaning Processes*” discusses the sampling methods applied to the cleaning process—rinse and swab (direct) sampling—as well as the analytical methods necessary to measure the samples taken. Specifically, these sampling and analytical methods need to be challenged and a “recovery” that describes the effectiveness of the sampling/analytical combination needs to “show that contaminants can be removed from the equipment surface and at what level, i.e. 50% recovery, 90% recovery, etc.”⁶

The guide also discusses cleaning limits, and while it purposefully stays away from tangential description, it puts forth that the limits for a particular compound and process must be “practical, achievable, and verifiable” and that the analytical method used to measure them needs to have the requisite level of sensitivity for these measurements.

The determination of carryover limits for a particular compound has been described using both the maximum allowable dose carried over to the next product batch⁷ as well as use of acute data such as LD50 values (the amount/dose of a substance that produces death in half of the animals tested).^{8,9} It is important to note that the limits referred to in the present study are the maximum allowable amount of residue on the equipment surface as opposed to the limit in the subsequent product or the limit in an analytical sample.

The wide range of potential carryover limits in pharmaceutical cleaning challenges the analytical methods used to measure the limits, regardless of whether the method used is a direct swabbing method or one that relies on extraction and dilution. The analytical method needs to have the appropriate dynamic range to measure the substance at its cleaning limit with an appropriate linear range that ensures the ability to effectively differentiate a passing result from a failure.¹⁰

In this series of experiments, we demonstrate the ability to recover the residues of two compounds from stainless steel surfaces and analyze the results directly using ITMS.

One of the substances selected is cefuroxime sodium, classified as a β -lactam antibiotic with typically very low carryover limits due to potentially severe allergic reactions¹¹ and anaphylactic shock¹² in some cases of ingestion. The second compound is pseudoephedrine HCl, a common decongestant with cleaning limits significantly higher than cefuroxime sodium.¹³ Figure 1 shows the chemical structures of these molecules.

The goals of this experiment are to demonstrate that ITMS can be used in a direct swabbing capacity to generate acceptable recovery levels across a wide range of carryover limits.

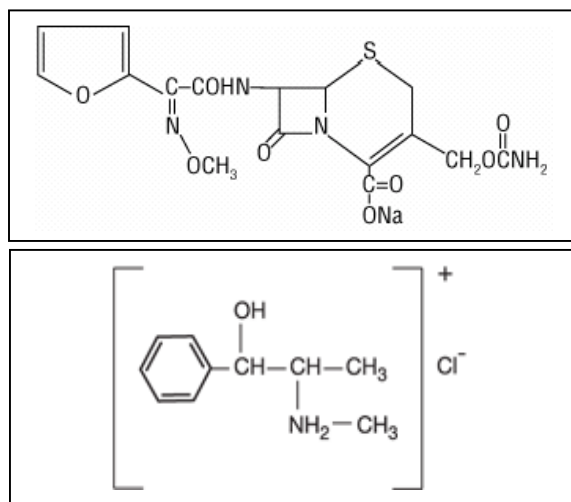


Figure 1. Molecular structures of cefuroxime sodium (top) and pseudoephedrine HCl (bottom). The molecular weights of these compounds are 446.4 and 201.7, respectively.

Materials and Methods

This experiment used the Kaye Validator® ITMS for sample measurement. Samples were prepared using USP-grade cefuroxime sodium and pseudoephedrine, with dilutions being prepared in methanol.

Instrument settings for the cefuroxime testing were: desorber and detector temperatures of 249°C and 205°C, respectively, with a scan time of 60 seconds (15 samples acquired per second, integrated over the full scan time). NH_3 and dichloromethane were present as a dopant

in the drift gas; and gas flow settings were 250cc/minute in both the sample and detector flow.

For the pseudoephedrine testing, the desorber and detector temperatures were 249°C and 205°C, respectively. Scan time was set to 180 seconds with five samples acquired per second. No dopant was present in the drift gas (atmospheric air). Gas flow settings were 500cc/minute in the sample flow chamber and 250cc/minute on the detector flow.

The swabs used were a specialized polyimide material manufactured for use with the Kaye Validator ITMS. Stainless steel (316) coupons with a #7 finish (GlobePharma) were used during the swab recovery studies. Further details on the methodology used during the experiment are provided in the results section.

Results and Discussion

This experimentation included analysis of compounds to determine their time of flight (TOF), generation of calibration curves and determination of the linear ranges, and finally measurements of samples taken directly from the steel coupons in order to determine our recovery percentage. As ITMS uses the time of flight as a metric of identifying a molecule, the first stage of our experimentation was to determine the time of flight for both cefuroxime and pseudoephedrine.

Determining Time of Flight

In order to determine the quantitative response of the system, it is important to determine the time of flight (TOF) for the molecule in question. The instrument used for this study has the ability to collect data for both positive and negative ions within a single measurement. This brings several potential advantages—among them the ability to detect multiple ion species regardless of the charge on the “preferred” ion state in a single scan (a “single mode” instrument would require two separate measurements).

Additionally, as there is no need to switch modes in the instrumentation, the Validator ITMS eliminates re-equilibration time

associated with switching modes, shortening the amount of time necessary to develop a method for a particular substance.

Using samples of the pure API dissolved in methanol, aliquots were spiked directly onto the swabs used in the instrument, the swabs were analyzed, and the resulting peaks were recorded. In addition, measurements were taken on (A) swabs without any substance present, (B) swabs that were spiked with 100 μ l of methanol and allowed to dry, and (C) with the instrument having no swab inserted, in order to account for our background peaks. Finally, we took a very small sample of the dry API powder swiped directly onto the swab. This would highlight any differences seen due to interactions with the solvent.

The time of flight for cefuroxime sodium was determined to be a positive ion complex at 7.790ms, with the time of flight for pseudoephedrine determined to be a positive ion complex at 5.885ms. Representative plasmagrams (similar to a chromatogram in HPLC) with locations of the representative API peaks as well as the locations of the drift gas peak and common fragments in the cefuroxime data are shown in Figure 2.

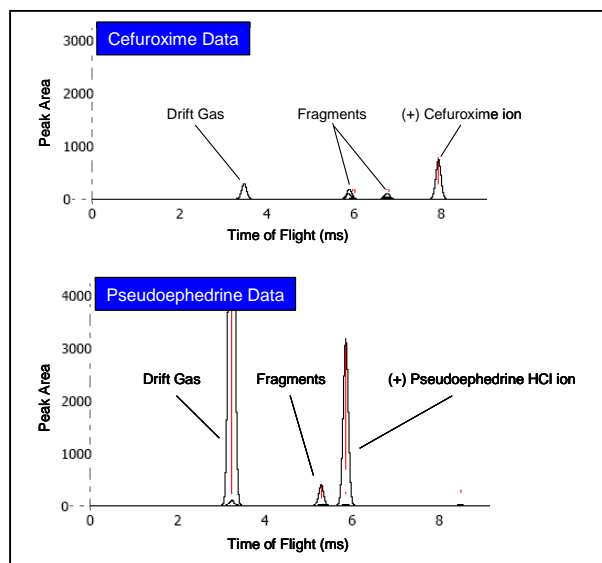


Figure 2. Plasmagram of cefuroxime (top) and pseudoephedrine (bottom) measurements. Positive ion data is shown, indicating the primary ion complex, drift gas and fragments.

For the remainder of the analysis, cefuroxime was identified as a positive ion with a time of flight of 7.790 \pm 0.04ms; pseudoephedrine was identified as a positive ion with a time of flight of 5.885ms \pm 0.04ms. No instances of a peak potentially associated with the main cefuroxime ion or pseudoephedrine ion occurred outside these windows of detection.

Determining Quantitative Response

After determining the time of flight for each API, the quantitative instrument response for each compound and the linear range were determined. The carryover limits for cefuroxime and pseudoephedrine used in this experimentation are 1 μ g and 20 μ g per 25cm², respectively. Figure 3 shows the instrument response curve for both cefuroxime and pseudoephedrine. The parameters of the instrumentation were adjusted in order to establish the appropriate linear range for each compound (described previously).

Cefuroxime Data

The cefuroxime measurements encompass sample amounts between 250ng and 3 μ g. As the instrument was able to give a repeatable response at 250ng that can be used for quantification, and cefuroxime was detectable at sample amounts lower than 250ng, for the purposes of this experiment 250ng is considered the limit of quantification (LOQ) and it is assumed that the limit of detection (LOD) is below 250ng.

For the purposes of this experiment, the linear range is considered to be between 500ng and 1.5 μ g, values corresponding to 50% and 150% of the carryover limit, respectively. This is a greater tolerance than called for normally, as cefuroxime's low carryover limits appropriate a wider window of measurement.

Additionally, 500ng is twice the value of the limit of quantification and more than twice the level of the limit of detection. The R² value for the linear range of this calibration curve is >0.95.

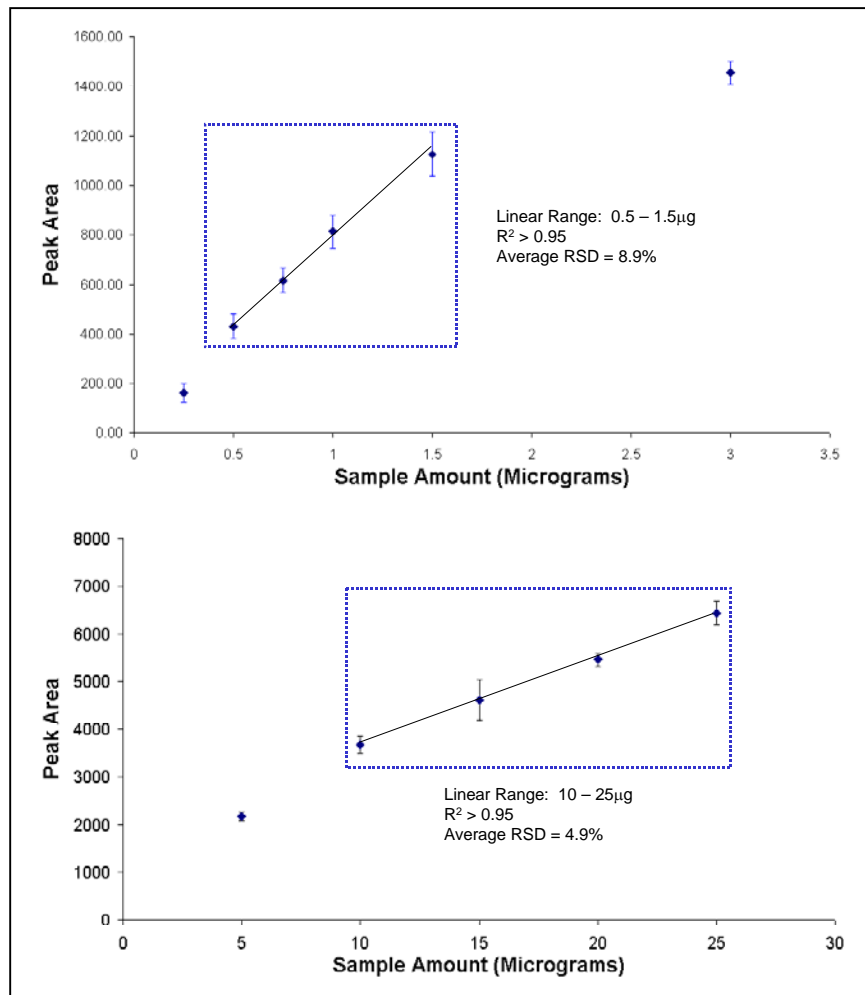


Figure 3. Quantitative response of cefuroxime sodium (top) and pseudoephedrine HCl (bottom) in the ITMS instrument. Data shown is average value at each sample amount with error bars representing one standard deviation from the mean. R^2 values were determined using a scatter plot encompassing all of the data in the linear range.

For the purposes of recovery, the spiked samples represent 100% recovery for the API. This was validated with two sets of measurements: (1) measuring for any residual cefuroxime on traps containing 1.5µg and 3µg after they had been sampled for the calibration curve; and (2) measuring a sample of five glass fiber traps coated with polytetrafluoroethylene (PTFE) that were placed underneath the sample traps as they were spiked with cefuroxime. Both sets of measurements failed to show any presence of residual cefuroxime.

Pseudoephedrine Data

The pseudoephedrine measurements encompass sample amounts between 5µg and 25µg. The limits of detection and quantification with these instrument settings are well below 5µg, and the lower bound of the linear range (10µg) is therefore greater than twice the amount of both the LOD and LOQ. The linear range of 10-25µg encompasses more than +/-25% of the carryover limit of 20µg. Again, the R^2 value for

the linear range of this calibration curve is >0.95, and the tests mentioned above for validating 100% recovery of the spiked samples were performed as described previously.

Measurements of swabs after they had been sampled produced no trace of pseudoephedrine. Measurement of the PTFE traps placed underneath the 20µg sample yielded trace amounts (under 100 instrument counts, representing under 100ng of pseudoephedrine) in two out of five samples. As this represents less than 0.4% of the total sample, the spiked samples are considered to be representative of 100% recovery for this experiment.

Swab Recovery

Swabbing was performed on 316 stainless steel coupons with a #7 finish, in an area of 25cm². Aliquots of each sample were spiked onto the coupons and allowed to dry before swabbing. Material used for swabbing is a specialized polyimide material developed for use with the Kaye Validator ITMS instrument. The swabs have a specific “sampling area” that comprises the area of the swab that is fully sampled by the instrument.

This area was wet with 200µl of methanol and, using a PTFE barrier between the swabber’s finger and the swabbing material, the trap was applied to the surface and

swabbing commenced with overlapping vertical strokes across the surface. The swabber performed eight strokes in a vertical motion, followed by eight overlapping strokes in a horizontal motion. Figure 4 shows these motions, as well as the use of the PTFE barrier.

After swabbing, the traps were allowed to dry and were measured with the ITMS system. The areas for the API peaks were recorded and the amount of API present determined through the equation generated by the linear fits of the data shown in Figure 3. Table 1 shows the calculated recovery percentages for each level.

Cefuroxime Recovery Data		
Amount on Coupon (n)	Mean Amount Recovered	Recovery %
1.5 Micrograms (n = 7)	1.02 Micrograms	67.2%
1 Microgram (n = 11)	660 Nanograms	65.6%
500 Nanograms (n = 7)	310 Nanograms	62%
Average Swab Recovery		65.1%
Swab Recovery RSD%		17.4%
Pseudoephedrine Recovery Data		
Amount on Coupon (n)	Mean Amount Recovered	Recovery %
15 Micrograms (n = 8)	13.34 Micrograms	88.9%
20 Micrograms (n = 8)	17.07 Micrograms	85.3%
Average Swab Recovery		87.1%
Swab Recovery RSD%		14.2%

Table 1. Recovery data for cefuroxime and pseudoephedrine

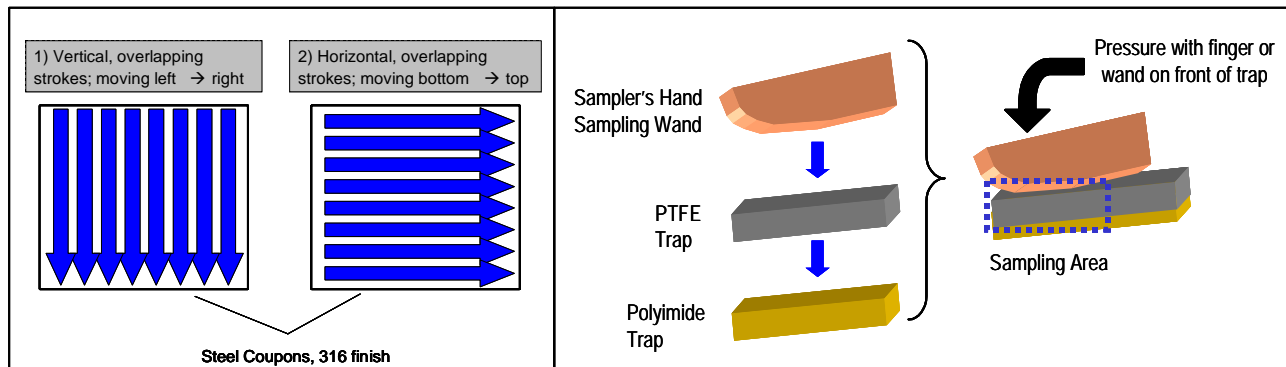


Figure 4. Swabbing motion on the steel coupons (left), where strokes are initiated in a vertical direction and are then followed by strokes in a horizontal direction. Diagram (right) shows the use of a PTFE barrier to prevent contamination in between the trap and the sampler.

Conclusions

These data show a recovery percentage of greater than 65% and strong repeatability, with an RSD of 17.4% for cefuroxime and a recovery percentage of greater than 87% for pseudoephedrine with an RSD of below 15%. Additionally, the recovery percentages at varying levels of sample for this experiment are consistent. These data demonstrate the desired result of this experimentation, namely that it is possible to repeatably generate acceptable recovery of residues and measure the samples directly using ITMS.

While this experiment shows the feasibility of the technique, the method itself has the potential to be improved so that higher recoveries are possible. Potential alterations in the pressure and speed of the swabbing, the orientation or the “leading edge” used with each swabbing stroke, and the amount of solvent used could lead to higher recovery percentages.

This study demonstrates the feasibility of using the Kaye Validator ITMS for the direct sampling of equipment in the pharmaceutical industry. While cleaning validation and verification of equipment involves increased layers of complexity—one of them being the different types of surfaces that are likely to be encountered during cleaning—the Validator ITMS demonstrates the ability to produce acceptable levels of recovery and repeatability with a technique that is far faster than the technology currently used by most of the industry.

Given the high costs associated with manufacturing in pharmaceuticals, as well as the push for greater process understanding through PAT, the implementation of ITMS as a fast, specific analytical technology for at-line measurements has the potential to deliver substantial improvements in cleaning analysis and monitoring efficiency.

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