ITMS: Applications in At-Line Cleaning Validation and Verification

Ion Trap Mobility Spectrometry (ITMS) offers a fast, specific analytical technology for at-line measurements, with the potential to deliver substantial improvements in cleaning analysis and monitoring efficiency.

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Ion Trap Mobility Spectrometry (ITMS) represents a fast and specific technique for the analysis of samples for cleaning validation and verification in the pharmaceutical industry. Furthermore, at-line analysis has the potential for greatly improving the efficiency of analysing cleaning results and improving equipment turn-around. This article reviews a study on the use of this technology for ‘direct swabbing,’ or directly sampling and analysing the equipment of interest. Recovery results from stainless steel surfaces for two different compounds, cefuroxime sodium and pseudoephedrine HCl, are presented.

ITMS APPLICATIONS

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). The technology has been in use for over 30 years, primarily applied in detecting trace amounts of narcotics and explosives (2), and is found at most airports as part of their security screening procedures.

More recently, ITMS has found application in the pharmaceutical industry, mainly focusing on cleaning validation and verification. While the technology has been reviewed (3) and specific applications described (4,5), the data published to date has focused on results generated from extracted solutions, rather than 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 analysed directly instead of via an intermediate extraction step. Similar to the use of ITMS in security applications, the advantage of direct swabbing is that it allows the user 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, that is 50% recovery, 90% recovery, etc" (6).

The guide also discusses cleaning limits, and while purposefully staying 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 batch (7), as well as the 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).

EXPERIMENTATION

In this series of experiments, we demonstrated the ability to recover the residues of two compounds from stainless steel surfaces and analyse the results directly using ITMS. One of the substances selected was cefuroxime sodium, classified as a β-lactam antibiotic with typically very low carryover limits due to potentially severe allergic reactions (11) and anaphylactic shock (12) in some cases of ingestion. The second compound was pseudoephedrine HCl, a common decongestant with cleaning limits significantly higher than cefuroxime sodium (12). The chemical structures of these molecules are shown in Figure 1.

The goals of this experiment were to demonstrate that ITMS can be used in a direct swabbing capacity to generate acceptable recovery levels across a wide range of carryover limits. The experiment used the Kaye Validator® ITMS for sample measurement, and samples were prepared using USP-grade cefuroxime sodium and pseudoephedrine, with dilutions being prepared in methanol.

The 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 recovery percentage. As ITMS uses the TOF as a metric for identifying a molecule, the first stage of the study was to determine the TOF for both cefuroxime and pseudoephedrine.

Determining Time of Flight

The instrument used for the determination of time of flight 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 (active pharmaceutical ingredient) dissolved in methanol, aliquots were spiked directly onto the swabs used in the instrument, the swabs were then analysed and the resulting peaks recorded. In addition, measurements were taken on: (a) swabs without any substance present; (b) swabs that were spiked with 100µl of methanol and allowed to dry; and (c) with the instrument having no swab inserted, in order to account for background peaks. Finally, a very small sample of the dry API powder was 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 7.790 ms, with the time of flight for pseudoephedrine determined to be a positive ion complex at 5.885 ms. 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.

For the remainder of the analysis, cefuroxime was identified as a positive ion with a time of flight of 7.790 ± 0.04 ms; pseudoephedrine was identified as a positive ion with a time of flight of 5.885 ms ± 0.04 ms. 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 were 1µg and 20µg per 25 cm², 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 encompassed sample amounts between 250ng and 3µg. As the instrument was able to give a repeatable response at 250ng that could be used for quantification, and cefuroxime was detectable at sample amounts lower than 250ng, for the purposes of this experiment 250ng was considered the limit of quantification (LOQ) and it was assumed...
that the limit of detection (LOD) was below 250ng. The linear range was considered to be between 500ng and 1.5µg, 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 was twice the value of the limit of quantification and more than twice the level of the limit of detection. The R^2 value for the linear range of this calibration curve was >0.95.

For the purposes of recovery, the spiked samples represented 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 fibre 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 encompassed sample amounts between 5µg and 25µg. The limits of detection and quantification with these instrument settings were well below 5µg, and the lower bound of the linear range (10µg) was therefore greater than twice the amount of both the LOD and LOQ. The linear range of 10-25µg encompassed more than +/-25% of the carryover limit of 20µg. Again, the R^2 value for the linear range of this calibration curve was >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 were 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. The material used for swabbing was a specialised 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.

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 (see page 94) shows the calculated recovery percentages for each level.

**CONCLUSION**

These data show a recovery percentage of greater than 65% and strong repeatability, with a relative standard deviation (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 were consistent. These data demonstrated 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 demonstrated 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 pharmaceutical manufacture, as well as the push for greater process understanding through the FDA’s PAT initiative, 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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References

13. Cleaning limits provided in private communications

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<th>Amount on coupon (e)</th>
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Table 1: Cefuroxime recovery and pseudoephedrine recovery data

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