

# The Effect of Micro-organisms on Ghost Peaks in a Reversed-Phase HPLC System

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## Introduction

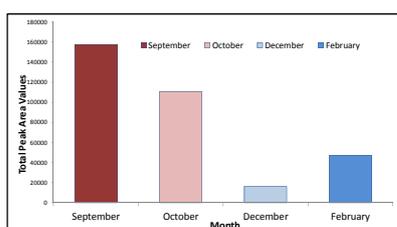
This study involved the analysis of a reversed-phase gradient HPLC related substances method that has been observed to give rise to ghost peaks, which tend to obstruct chromatogram results. The interference of ghost peaks in a related substances analysis is more complicated than in an assay determination. An assay determination chromatogram results in one large peak (API) and many other smaller peaks that do not effect the analysis. On the other hand the important peaks in a related substances determination are much smaller and most notably often have a similar peak height to ghost peaks, which might effect any interpretation of results. Through previous investigations<sup>1</sup> carried out on ghost peaks in a reversed phase gradient HPLC related substances method, some light has been shed on the true nature and source of these peaks, which tend to be contaminants in the form of microorganisms that are of airborne origin. The objective of this study was therefore to devise a suitable HPLC-friendly chemical sanitization method in an attempt to achieve a ghost peak free chromatogram.

## Methodology

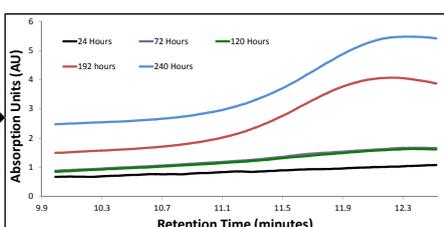
The protocol that was studied throughout the investigations was an existing validated related substances method for the drug product, Pramipexole. This method was chosen due to the production of problematic peaks, which interfere with the analysis of important peaks. The HPLC system used for this study was a LC-10VP Shimadzu model, fitted with a Waters Sunfire C18 column having an internal diameter of 3 mm, a length of 100 mm and a particle size of 3.5 µm. A set of individual investigations were adopted, each of which was thought to promote the formation of ghost peaks in chromatograms. Each separate investigation was set-up so as to improve on observations made in the previous investigations by analysing the individual results. Various factors were studied, ranging from buffer quality and age, time of production and other physical components of the HPLC that are in direct contact with the mobile phase. The factors having the largest contribution towards ghost peaks was established by analyzing the peak area values produced from individual chromatograms at a wavelength of 260 nm in a UV-Vis spectrophotometer.

## Results

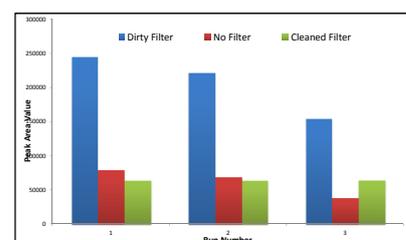
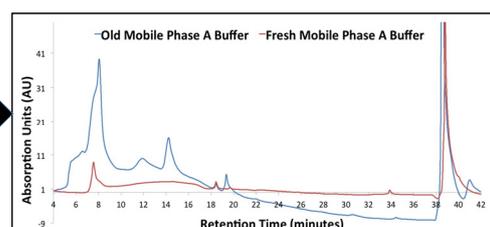
**Figure 1:** Bar graph showing the percentage peak area contributed by the summer period to being much more than the winter months.



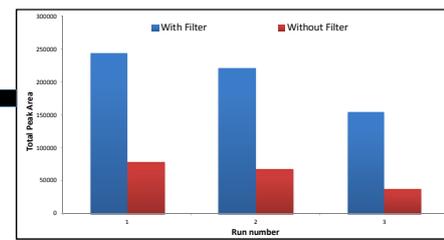
**Figure 2:** Five superimposed chromatograms at a particular focus on the 11.0 – 12.5 minute time range (API peak retention time), where the peak area was seen to increase with time.



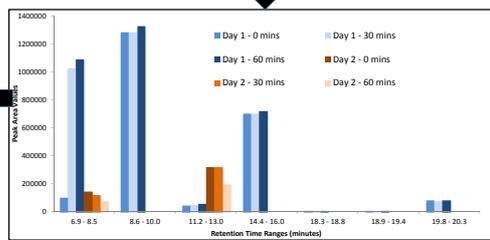
**Figure 3:** Two superimposed graphs showing the lack of contamination of an HPLC system by particulate matter.



**Figure 6:** Contamination further decreased when a sanitization procedure was employed.



**Figure 5:** The peak area values decreased significantly in the absence of a suction filter.



**Figure 4:** Accumulation was seen to decrease drastically when a low flow of a high organic solvent is allowed to pass through the system for a stipulated amount of time.

## Conclusions

The results indicated that microbial contamination, probably of airborne origin, was a main source of ghost peaks. This problem appeared to be augmented during the summer period, especially if the air in the room was very humid. Accumulation inside an HPLC system does tend to occur on the stationary phase of the column especially if sequences are separated by more than twenty-four hours. Accumulation is usually dependant on the method employed and occurs when a high percentage of aqueous buffer is flowing through the system. Results indicated that when a highly hydrophobic solvent was passed through the system, most of the accumulated organic matter eluted off, as ghost peaks tend to decrease to a considerable amount when it is allowed to flow through the system. Finally, suction filters seemed to be a very important source of contamination, especially if present inside an aqueous buffer. Results showed that when the filter was removed, the ghost peaks were reduced significantly, however, it is imperative for sanitisation of the filters to take place. This can be performed by means of sodium hypochlorite and propan-2-ol. Although the latter is not an essential step it may still be applied as a precautionary measure.



**Figure 7:** The metal-solvent suction filters that were used to inject solvent from a mobile phase reagent bottle and into the HPLC system.

## References

- Williams, S. (2004). Ghost peaks in reversed-phase gradient HPLC: a review and update. *Journal of Chromatography A*. **2004**, 1052 1-11.

## Acknowledgements

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