A Preliminary Investigation Of Ghost Peaks In A Reversed-Phase Gradient HPLC System

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Introduction

This study involves a reversed-phase gradient HPLC related substances (impurity) analytical method, for a drug product. These types of analyses are of crucial importance because the data they supply is such that it might be part of qualifier information used by regulatory organisations to determine whether marketing authorisations applications are to be accepted or not.

Problems were observed in this method as ghost peaks were coinciding with the retention time of the active pharmaceutical ingredient (API) and also appearing at relative retention times of probable related substances of the API, thus making the quantification of such impurities difficult. *Ghost peaks* is the most common recently used term for non-reproducible, random and uncontrollable peaks, for which several terms were coined in the past decades including spurious peaks, vacant peaks, eigen peaks and system peaks.[1] They are more important in reversed-phase gradient analytical systems which tend to be more able to concentrate any contaminants present in the system as the organic component of the mobile phase varies (increases).

This investigation involved a preliminary analysis of the situation, the primary aim being the determination of the source of the ghost peaks should their source be one and consistent, and hence the eradication of peaks by taking limited precautionary measures thus avoiding the re-development and/or re-validation of the method.

Methodology

The general methodology followed consisted of individual investigations, each addressing one or a few issues that were possibly giving rise to the appearance of ghost peaks on chromatograms. In general, each investigation followed upon the results of previous ones such that conclusions reached earlier were acted upon, the system improved and the new investigations carried out on the improved system so as to attempt the achievement of a ghost peak-free chromatogram system for the specific reversed-phase gradient HPLC method. Several aspects of the chromatograph itself, the buffer preparation method and the materials used for buffer solution preparation were investigated at different instances and in different manners.

Initial protocols were set according to an already implemented and validated related substances method for a particular drug product, with amendments. The purposes of these amendments were to promote the practicality of the investigation by cutting down on variability factors. For these reasons, mobile phase solvent B was adjusted from 70:30 acetonitrile: buffer solution to 100% acetonitrile solution.

The general methods of analysis employed changing certain variables and comparing the outcome in chromatograms before and after the change was implemented using paired t-test or repeated measures ANOVA. Towards the end of the study, the mode of analysis was modified in that comparisons were done within the same investigation using different conditions in order to improve the comparability.

Results

Figure 1: The initial situation of the ghost peak problem on the particular chromatographic system being utilised.

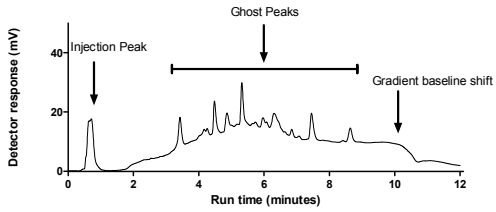


Figure 2: Comparison of peaks in gradient runs carried out with water instead of buffer and the normal buffer solution.

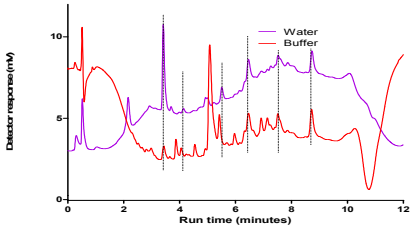


Figure 3: Microbiological testing results using two different media (TSA and R2A) for the buffer solutions and respective water samples.

Analysed liquid	5 days after testing	
	TSA	R2A
Water sample 1	confluent growth	confluent growth
Water sample 2	confluent growth	confluent growth
Water sample 3	confluent growth	confluent growth
Buffer #23	No growth	48 colonies
Buffer #24	No growth	No growth
Buffer #25	No growth	No growth

Figure 4: Gradient runs performed using water, instead of buffer solution, arising taken from two different sources: Milli-Q system water and HPLC-grade water.

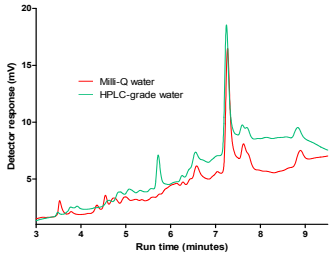


Figure 5: Gradient runs performed using buffer solutions prepared from two different water sources: Milli-Q system water and HPLC-grade water.

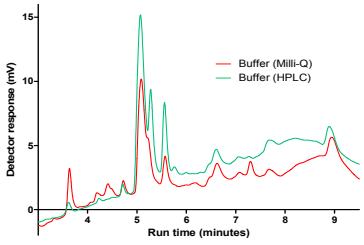
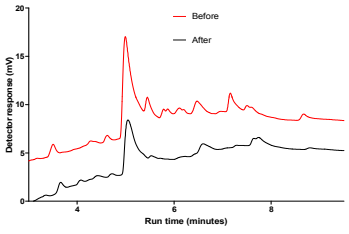


Figure 6: Two chromatograms for two gradient runs performed using the same buffer solution before and after system sanitisation.



Conclusions

Results indicated that the ghost peaks were not all due to microbial contamination and probably, this factor contributed only partially. It appeared that chemical and, possibly, dust contamination were important contributors. There were, in fact, no differences between results obtained from buffer solutions kept in amber and clear reagent bottles (a difference would be expected in case of microbial growth). In fact, microbiological testing indicated that buffer solutions sustain very little, if any, microbial growth at all. On the other hand, water samples showed severe microbial growth, when tested a week after sampling and containment in amber reagent bottles. Therefore, all these outcomes indicated that any microbial growth was possibly occurring in water, at least during the period before usage, which growth was not sustained by the buffer due to the high pH but cell fragments reminiscent of that growth remain even after filtration due to size issues. Particles from the air could also be acting as contaminants when they find their way into buffer solutions while the latter are being used as part of MP solvents.

The chromatograph is possibly another contributor of ghost peaks. Results showed that the suction filters may be chromatograph components that contaminate the system possibly by holding back material but as solvent moves through them, some of this material (e.g. metabolites) is eluted. It appeared that the column is not a contributor at all, this being evidenced by similar ghost peak patterns attained with a replacement identical column. The in-line filters, auto-sampler and injector also appear not to be culprits in contaminating the system. Measures that appeared to improve the system were system sanitisation and general precautions which included thorough cleaning of reagent bottles, the preparation of buffer solution in clean glass reagent bottles rather than plastic containers, frequent replacement of the seal wash solution, the use of propan-2-ol directly from its original container rather than from wash bottles to avoid possible contamination with plasticiser, and complete replacement of acetonitrile solvent when it finishes (to avoid accumulation of dust and microbial particles settling from the air in this solvent).

References

[1] S. Williams, Journal of Chromatography A, **1052** (2004) 1-11.