

# A SYSTEM FOR THE RAPID DEVELOPMENT OF ANALYTICAL CHROMATOGRAPHY METHODS WITHIN THE PHARMACEUTICAL INDUSTRY

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## INTRODUCTION

The modern pharmaceutical industry is facing ever increasing demands to work on larger R&D portfolios, at the same time regulatory authorities and our own business needs are demanding more information in support of projects and submissions. Further industry drivers are dictating that rather than simply growing the organisation, we instead establish more efficient and productive ways of working. Within AstraZeneca Process R&D (PR&D) the development of analytical methods to support developing the synthetic routes by which drugs are manufactured consumes a significant amount of resource and (due to the nature of the work) many of the methods are only used a few times. Traditional chromatographic method development can be both time consuming and labour intensive and is therefore not an effective approach to take for much of the work that we do. We have therefore actively designed ways of speeding up the method development process. This poster describes the system that we have introduced to develop Gas Chromatography methods during the early stages of project development and how the recent utilisation of InertCap columns has significantly enhanced the capabilities of this system.

## SUMMARY OF IMPLEMENTED SYSTEM

An Agilent 6890 GC was fitted with two narrow bore columns of differing phase types and a series of pre-programmed methods (refer Figure 3) and sequences are used to simultaneously screen samples on both columns. An assessment of the resulting chromatograms is performed and a second list of methods can then be selected and run if a satisfactory separation has not been achieved. As the system is fully automated multiple samples can be treated in this manner and it is possible to run a number of method development experiments simultaneously. Since the introduction of these systems, there has been a significant increase in the numbers of GC methods being developed but the corresponding time taken to do this has been dramatically reduced. In addition less experienced analysts are now able to produce methods that they would previously have not been able to do without assistance.

## SYSTEM DEVELOPMENT - METHOD TRANSFER TO SHORT NARROW BORE COLUMNS

The initial work described in this poster was actually performed about 3 years ago and focussed on proving that the fast GC developments being reported in literature and application notes could be practically transferred into our laboratories. Since the Process R&D laboratory at that time primarily used columns with 0.25 - 0.32 mm i.d.'s and ca 30 m lengths, the first steps were to demonstrate that the narrow bore columns would not only reduce the time in which a separation could be achieved, but that it would be repeatable and robust. Using Grob mixes, it was demonstrated for three separate columns (1, 17 and 1701) that software translations repeatedly resulted in comparable separations to the traditional methods but at greatly reduced times (ca 30 mins reduced to ca 8 mins). Example chromatograms are shown in Figures 1 and 2.

Figure 1: Grob mix on 30m x 0.32 mm column

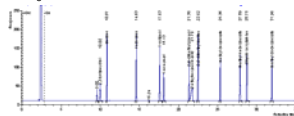
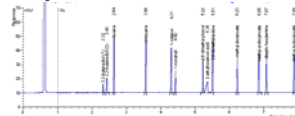


Figure 2: Grob mix on 10m x 0.1mm column



## SYSTEM DEVELOPMENT - INSTRUMENTATION AND SELECTION OF COLUMNS

The second phase of the work was to select which stationary phases needed to be incorporated into the system and following on from that, what instrument configurations and hardware would be required to establish them in a usable system. For this, real samples from AstraZeneca's pharmaceutical projects were screened along with Grob test mixes on the three stationary phases already tested. Evaluation of the data from this set of experiments showed that as expected all three columns gave different overall selectivities, however it also showed that using a 1 alongside a 17 or 1701 column resulted in as many satisfactory separations as by using all three stationary phases is that the use of a third stationary phase was not actually necessary. Demonstrating that the system could use just two columns was a very significant step, since the standard GC platform within PR&D (Agilent 6890's) could be operated with two columns simultaneously installed without any major modification. The use of more than two columns would have either required the use of some column switching technology or more than one instrument, both of which would have complicated the system from a users perspective.

It was decided to use the 17 phase alongside the 1 in preference to the 1701 since it had demonstrated better peak shapes for some of the acidic and basic compounds that had been tested and these are commonly encountered within the pharmaceutical industry.

## SYSTEM DEVELOPMENT - CHROMATOPHIC CONDITIONS

The strategy for selection of the other chromatographic conditions was based traditional gas chromatographic method development. The start point is a standard set of chromatographic conditions, followed by iterative changes to parameters based on observed results. In this system however all the methods are pre-programmed and are named according to the effect which they are designed to have on the chromatography (rather than by which instrument parameters have been altered). This was so that the system could be readily understood by users with a more limited understanding of Gas Chromatography as it was also an aim of the project to make the development of GC methods a more accessible activity. For this, five different methods were programmed into the instrument (refer Figure 3), consisting of the initial standard method and four second tier options for use in cases that require further method development (these are for early eluting compounds, late eluting compounds, injector degradation and on column degradation).

Figure 3: Pre-programmed chromatographic conditions

Column	Initial Method (1)	Early Eluters (2)	Late Eluters (3)	Injector (4)	Column Deg (5)
Column	Ether 1 or 17; dimensions 10 m x 0.1 mm i.d. x 0.1 µm				
Head pressure	312 Kpa	312 Kpa	312 Kpa	312 Kpa	400 Kpa
Oven Temperature	60°C to 300°C 2 min	60°C to 300°C 15 min	60°C to 300°C 4 min	60°C to 300°C 2 min	120°C to 300°C 2 min
Injector Temperature	250 °C	250 °C	250 °C	200 °C	250 °C
Injection Volume	1 µl	1 µl	1 µl	1 µl	1 µl
Detection	FID @ 300 °C	FID @ 300 °C	FID @ 300 °C	FID @ 300 °C	FID @ 300 °C
Split Flow	50 ml/min	50 ml/min	37 ml/min	50 ml/min	56 ml/min

## MASS SPEC

The identity of impurities and reaction byproducts is a key element in better understanding the chemical processes under investigation within PR&D. Therefore GC/MS systems (EI and CI) have also been set up to complement the systems described above. In cases where there is a need to identify peaks observed in a standard Gas Chromatographic analysis, identical methods are readily available on the GC/MS. These systems have in fact now become so popular with our customers that they are in many cases the initial starting point in their analytical investigations.

## ACIDS, BASES AND INERTCAP COLUMNS

Despite the significant benefits over traditional method development approaches for projects in early phase, the analysis of acidic and basic compounds generally has remained problematic and we have therefore looked on the constant look out for possible solutions.

It should be noted when considering the following that the success rate of the system is far higher overall than for the specific range of acidic and basic compounds used in this test.

AstraZeneca became aware of the InertCap range of columns from GL Sciences, who kindly offered to supply a small number of columns for us to evaluate and since the exact dimensions we required were not standard catalogue items, 10 m x 0.1 mm x 0.1 µm columns were specifically manufactured. Although GL sciences have (for understandable reasons) not been prepared to divulge the exact nature of InertCap columns, they offer an equivalent to all the common stationary phases (including 1 and 17). If the claims of improved performance for acids and bases held up with our compounds, then this type of analysis could potentially be achieved without any additional complexity being added into the method development system.

In order to evaluate this, an otherwise identical system to that previously described was set up with an InertCap-1 and an InertCap-17 and range of 16 different compounds and test samples run on both for direct comparison. The range of compounds selected consisted of 4 with acidic properties, 11 with basic properties and one neutral; they were primarily a mixture of real development samples however 2 probes were also taken from the InertCap literature.

A basic assessment of the resulting peak shapes (refer Figures 4 and 5) clearly shows that whilst there were some acceptable results using the original columns and not every problematic compound has been solved by using the Inertcap columns; there is a significantly higher chance of obtaining a good peak shape when using InertCap columns than when using the columns originally selected.

Figure 4: Peak shape comparison of Original 1 Column with InertCap 1

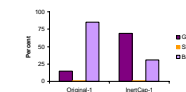


Figure 5: Peak shape comparison of Original 17 Column with InertCap 17

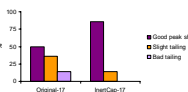


Figure 6: Method success rate of original System

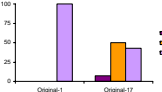
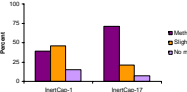
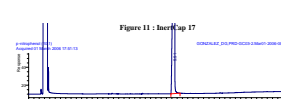
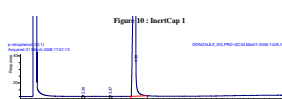
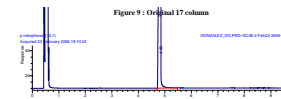
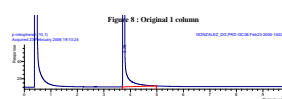


Figure 7: Method success rate of InertCap System



Another assessment was performed, that determined whether or not a satisfactory analytical method had been achieved (refer Figures 6 and 7). Although this is a very similar assessment to that above, a good analytical method must consider factors others than just the tailing of the main component peak and in this assessment these additional considerations have been included.

Example chromatograms are shown (refer Figures 8-11), in which the improvement in peak shape between the original columns and the InertCaps are clearly observed.



Although these improvements for the analysis of acids and bases were very exciting, it was important that the InertCap columns were equivalent to the standard 1 and 17 phases in all other aspects. To test this the capacity factors of the columns were compared to those of the InertCap columns (refer Figures 12 and 13); this clearly shows that the selectivities are very comparable and certainly for our purposes could be considered equivalent.

Figure 12: Capacity Factor Comparison of 1 Columns

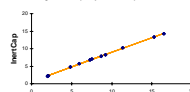
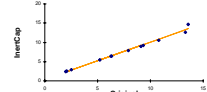


Figure 13: Capacity Factor Comparison of 17 Columns



## CONCLUSION AND SUMMARY

- The development of Capillary GC methods using a generic method development process has significant advantages within the pharmaceutical industry, particularly during the very early phases of projects.
- Chromatographic conditions that are fit for purpose can be achieved within a fraction of the time compared to traditionally used approaches.
- Since the introduction of this system within AstraZeneca Process R&D, more Gas Chromatographic methods are being developed due to the increased ease and speed with which analysts can carry out this activity.
- It is essential as projects progress and the methods become used frequently, that they are further evolved to ensure robustness. As the projects reach later stages of development, the additional resources required for this are considered to give acceptable return.
- In line with the gas chromatography in general, the system which was initially established showed some shortcomings for the analysis acidic and basic compounds.
- GL Sciences have a range of Inertcap columns that for the range of acidic and basic compounds that we tested gave superior performance whilst maintaining comparable selectivities to the columns that were originally used in the system.

## FUTURE WORK

- Further developments to the system are continuously being sought and we believe that there are opportunities to introduce the use of method development software into the overall process.
- A similar approach for chiral GC analysis is currently in the initial feasibility stage, however it is believed that this may require the use of some column switching technology since more columns will be required to achieve the required range of selectivities.

## REFERENCES

- Development of Fast GC for Analytical Laboratory Use (Final Year Student Project), Morgan Whiting, Tony Shephard.
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- Assessment of InertCap Columns, Diana Gonzalez (Internal AstraZeneca Report)
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