# A Study For The Method Transfer Of Acetylsalicylic Acid From HPLC To RRLC

D. Borg [1], H. Frey [2] and C. Farrugia [1]

[1] Department of Chemistry, University of Malta, Msida MSD2080, Malta

[2] Development Division, Actavis Ltd., Bulebel Industrial Estate, Zejtun ZTN3000, Malta

## Introduction

The increasing demand for greater pharmaceutical throughput, as well as the need to develop cost effective and faster analysis has prompted the development of advanced forms of HPLC. Other drivers include the worldwide acetonitrile shortage and stricter regulations on waste generation and disposal. The Rapid Resolution Liquid Chromatography (RRLC) is a fast LC system that is designed to tolerate high back pressures and accommodate columns of smaller particle size and dimensions. Its design incorporates a minimal delay volume which is crucial when using small particle sizes to avoid band broadening. Consequently the final result is a method of significantly shorter run time with less solvent consumption and waste generation. Therefore companies are investing in instruments, such as the RRLC, in order to develop methods that are faster and cost effective in terms of money, time and resources. New analytical methods are being developed on the RRLC and already existing HPLC methods are being geometrically transferred onto the smaller columns of the RRLC. However prior to performing method transfers onto new instruments, it needs to be established whether the experimental parameters affect the results in the same way on both instruments. Hence, this study represented an investigation to determine whether methods could be transferred simply from a 'standard' HPLC with standard columns to a RRLC system, or else whether methods required to be optimised and revalidated on transferring.

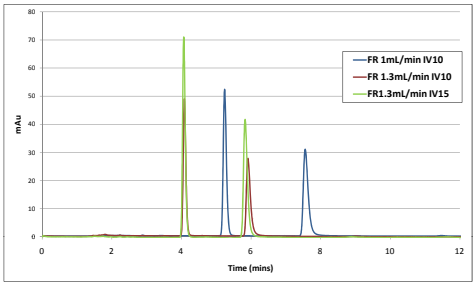
## Methodology

The starting point of this study was the European Pharmacopoeia reference method for the test for related substances of Aspirin, using the standard column, Phenomenex Luna C18 250 × 4.6 mm with a particle size of 5 µm. HPLC analyses were carried out on an Agilent 1100 series chromatograph, while the RRLC analyses were carried out on an Agilent 1200 series (SL) chromatograph. Both instruments were equipped with a diode array detector. The general methodology consisted of varying analytical parameters on both instruments in order to determine which parameters behave differently and therefore need to be properly investigated when an HPLC method is transferred to RRLC or vice versa. The parameters that were varied included flow rate, injection volume and temperature. Flow rate was varied between 0.5 and 2.0 mL/min at 25, 40 and 55°C with an injection volume of 10 µL, and injection volume was varied between 5 and 20 µL at 10, 25, 40 and 55°C with a flow rate of 1 mL/min. Subsequently, two parameters were varied simultaneously in order to determine whether concurrent or combined effects were present: injection volume and flow rate were varied between 5 and 15 µL, and 0.5 and 1.5 mL/min, respectively, at temperatures of 25 and 40°C. The chromatograms obtained were analysed for retention times of the main analyte peaks, area, theoretical plates, resolution between the salicylic acid and acetylsalicylic acid peaks, asymmetry, width, width at 50% height, height, capacity factor and dead time. The data obtained was subjected to statistical tests using SPSS and GraphPad Prism 5.00 for Windows.

## Results and Discussion

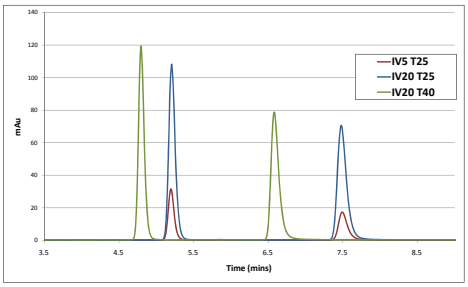
The peak area decreased on increasing the flow rate for both HPLC and RRLC and at all the temperatures investigated. The decrease in area was reinforced on increasing the temperature. Moreover increasing the temperature also reduced the solvent's density hence facilitating the flow of mobile phase through the column. However this effect was not as prominent as that of changing the flow rate. The peak area was also affected by the injection volume. On increasing the injection volume, the peak width and the peak area increased proportionally. It was also noticed that the slopes obtained for the various temperatures were significantly different implying that the temperature also influenced the peak area. On increasing the injection volume, the band of solute molecules at the start of the column increased. Consequently the molecules diffused outwardly and lagged behind and a broader peak width was observed. The height of the peak was also observed to increase on increasing the injection volume. Conversely, on increasing the temperature, the diffusion of the molecules increased and the band broadening that was observed on increasing the injection volume was somewhat decreased. Temperature affected the peak area to a lesser extent than the injection volume. The rate of increase of the peak area on using larger injection volumes decreased on going to higher flow rates.

***Figure 1:*** Effect of changing the flow rate (FR) and injection volume (IV) on the RRLC Reference B solution.




The retention times obtained between HPLC and RRLC were significantly different at 40°C. This discrepancy may be due to inaccurate control of column temperature. In HPLC, when the column temperature is set at temperatures higher than of the mobile phase, which is at room temperature, then the column attains a temperature gradient profile rather than having a uniform temperature. The thermostat of the RRLC is more advanced than that of HPLC and hence is expected to control the temperature within the column more efficiently. It was also observed that overall the resolution decreased on going from HPLC to RRLC. This difference was observed mostly at 25 and 40°C. This difference may be attributed to the higher pressures of the RRLC that was speeding the solute molecules through the column hence decreasing the elution time and consequently the resolution. It was expected that the resolution obtained by a RRLC would be higher due to the smaller delay volume of the instrument that would otherwise decrease the band broadening and as a result improve the resolution. However this effect was probably counteracted by the higher pressure of the RRLC that was more effectual than the influence of a shorter delay volume due to the presence of a guard column and the large volume of the column that was used which increased the delay volume significantly.

***Figure 2:*** Effect of changing the injection volume (IV) and temperature (T) on the RRLC Reference B solution.




Furthermore, it resulted that the injection volume was not a significant factor for HPLC but it was quite significant for RRLC. When the data obtained by the two instruments was compared at 25°C, it resulted that the injection volume, flow rate and instrumentation were all significant factors that contributed to the change in theoretical plates. Also the interaction between the injection volume and the instrumentation was significant. In general, it was seen that the mean theoretical plates were lower for RRLC than for HPLC at different temperatures at flow rates. This may be due to the higher back pressure of the RRLC that was increasing the extent of longitudinal diffusion and thus increasing the extent of band broadening. Furthermore, since the retention times obtained by the two instruments were significantly different, than it is to be expected that the theoretical plates are also significantly different since the two parameters are directly proportional to each other.

## Conclusions

Increasing the temperature offered many advantages that included reduced retention times, shorter analysis run time and less consumption of mobile phase. However it also resulted in certain disadvantages, that included possibility of sample degradation and smaller capacity factors, efficiency and resolution. It is important to keep the column dimensions in mind when monitoring the effect of chromatographic parameters since columns with different dimensions and particle sizes affect the chromatogram in a distinct way. It was also concluded that by using the large column, the benefits of RRLC were not fully exploited.

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