# Fast Reversed Phase HPLC of Analgesics

## Application Note


### Author

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### Introduction

One of the most common analgesics is aspirin (4-acetosalicylic acid), which is synthesized by acetylation of salicylic acid (2-hydroxybenzoic acid). The anti-inflammatory action of aspirin is derived from its ability to acetylate the enzyme prostaglandin synthase so preventing the synthesis of the prostaglandins, which promote inflammation. The by-product of this reaction is salcylic acid. It is therefore essential that aspirin products or studies to investigate the action of aspirin quantify both the aspirin and the salicylic acid. A second analgesic, phenacetin, is often incorporated in aspirin-based preparations. However, in such preparations, there are difficulties in quantifying the relative proportions of the two active ingredients. The HPLC method used would normally be based on a UV detector but the extinction coefficients are very different for the two compounds because phenacetin has a much stronger UV chromophore.

A simple isocratic HPLC method using a highly retentive polymeric reversed phase column, PLRP-S 100Å, has been developed that is capable of resolving aspirin, salicylic acid and phenacetin in under 10 minutes. PLRP-S columns are robust enough to be stable at pH 1-14 and cope with vigorous clean up procedures and aggressive eluents. The Agilent evaporative light scattering detector is ideal for analgesics because its response is independent of the UV properties of analytes.


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### Conditions

Column: PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
Eluent: 0.1% TFA, 50% Water, 50% ACN
Flow Rate: 0.5 mL/min
Detection: Agilent ELSD (neb=95 °C, evap=50 °C, gas=1.0 SLM)

## Results and Discussion

The disproportionate response obtained using a UV detector is illustrated in Figure 1a, which shows the response for equal amounts of aspirin and phenacetin when monitored at 280 nm. A more uniform response is clearly obtained when the Agilent ELSD is employed (Figure 1b).

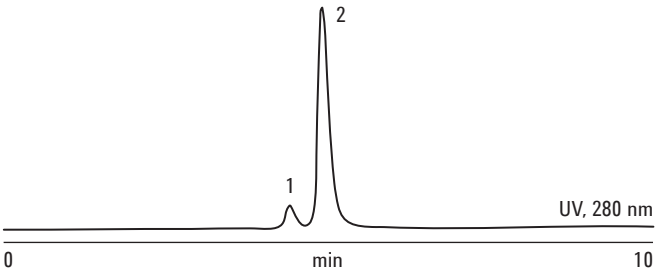


***Figure 1a. Separation of aspirin (1) and phenacetin (2) using a UV detector.***

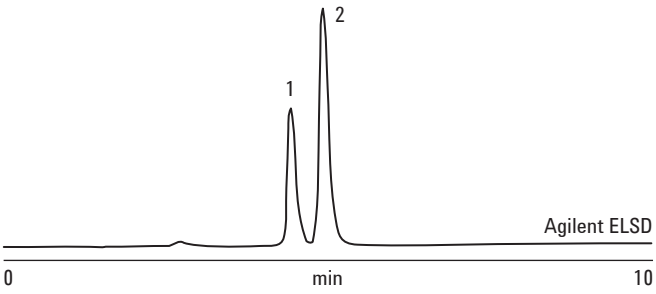


***Figure 1b. Separation of aspirin (1) and phenacetin (2) using the Agilent evaporative light scattering detector.***

Figure 2 shows the relatively similar responses from all three analgesics when the Agilent ELS detector was used.

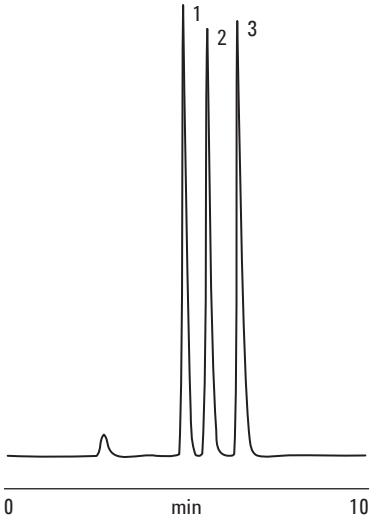


***Figure 2. Separation of aspirin (1), phenacetin (2) and salicyclic acid (3) using the Agilent ELSD.***

## Conclusion

Coupling a PLRP-S column with the Agilent ELS detector provides an ideal system for the separation of analgesics, where resolution would otherwise be problematic because of the presence of compounds with strong UV chromatophores. As a single column, PLRP-S operates across the entire range of HPLC eluents. It is chemically stable and physically robust, and so it is possible to switch between organic modifiers, such as ACN and tetrahydrofuran, and eluent pH 0 to 14.

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