

Spinsolve

Column Chromatography

Column chromatography is a universally used technique in chemistry laboratories in which compounds are purified from mixtures on the basis of some physicochemical property. Thin-layer chromatography (TLC) is the traditional method of determining the correct solvent system in which to perform column chromatography, and analyzing the elution profile once the procedure has started. Small samples ($\sim 1 \mu\text{L}$) of each fraction are sequentially spotted onto a plate of the same stationary phase medium as used in the column.

We demonstrate an alternative procedure to TLC, employing direct analysis of the eluents using ^1H NMR spectroscopy.



A mixture of benzyl alcohol, benzaldehyde, methyl benzoate and ibuprofen (Figure 1) was purified on silica gel using dichloromethane as the mobile phase. Test tube fractions were collected at 3 minute intervals, from which 0.5 mL aliquots were analysed directly using the Spinsolve (Figure 2).

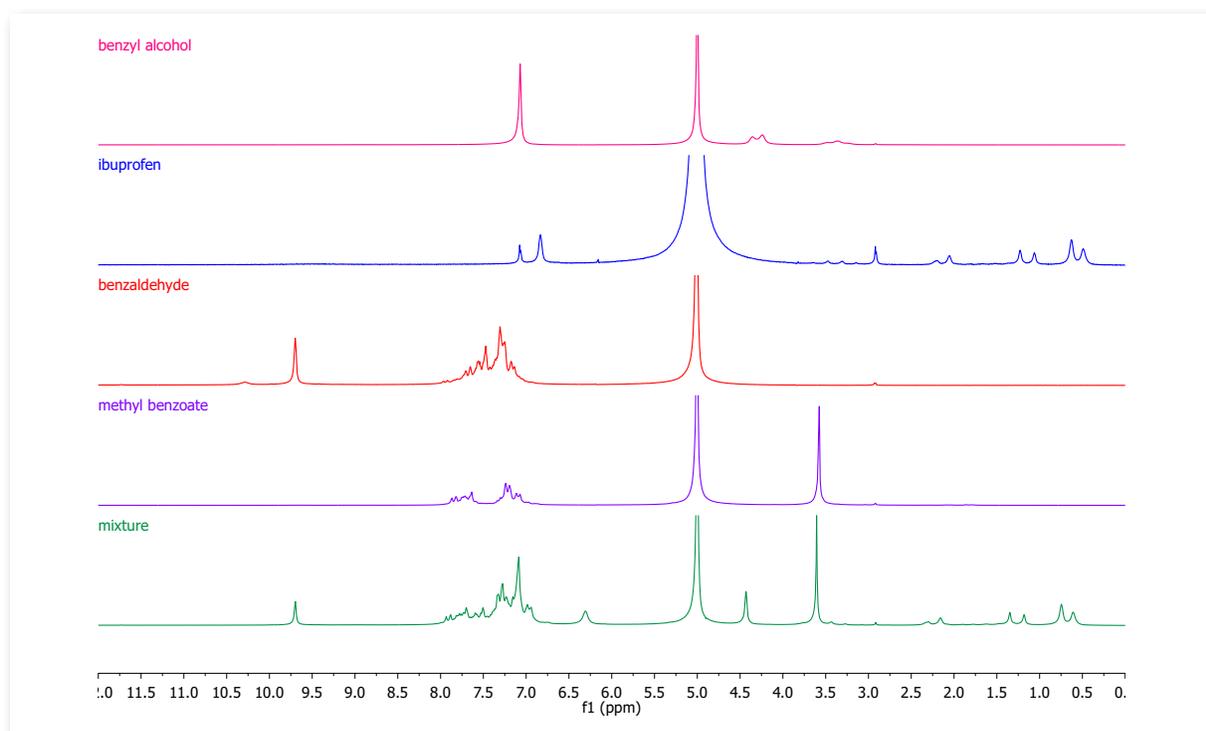
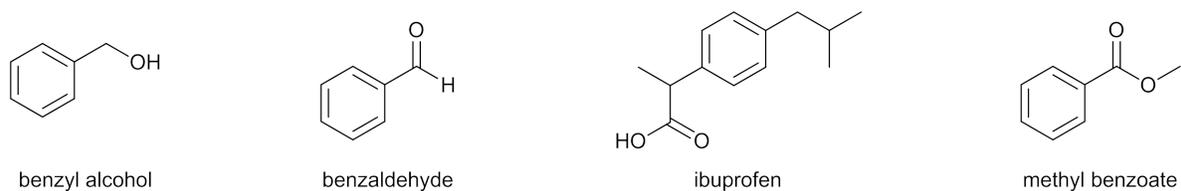


Figure 1: 1D proton NMR spectra of the mixture to be purified and the constituent compounds in dichloromethane, obtained using the Spinsolve.

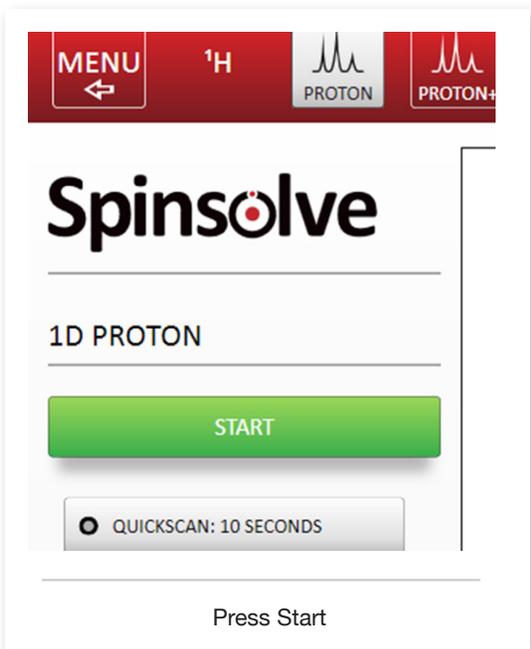
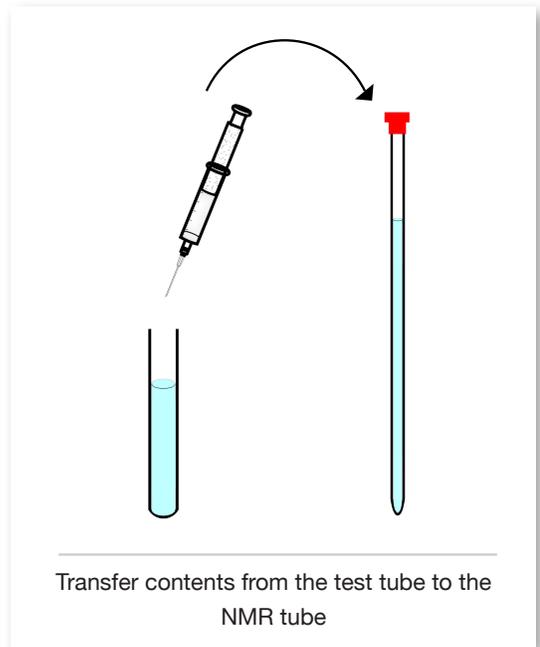
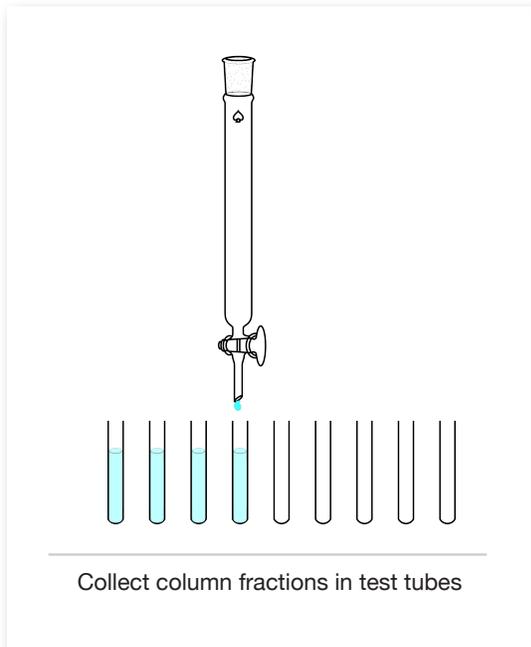


Figure 2: Process for acquiring spectra of chromatography fractions.

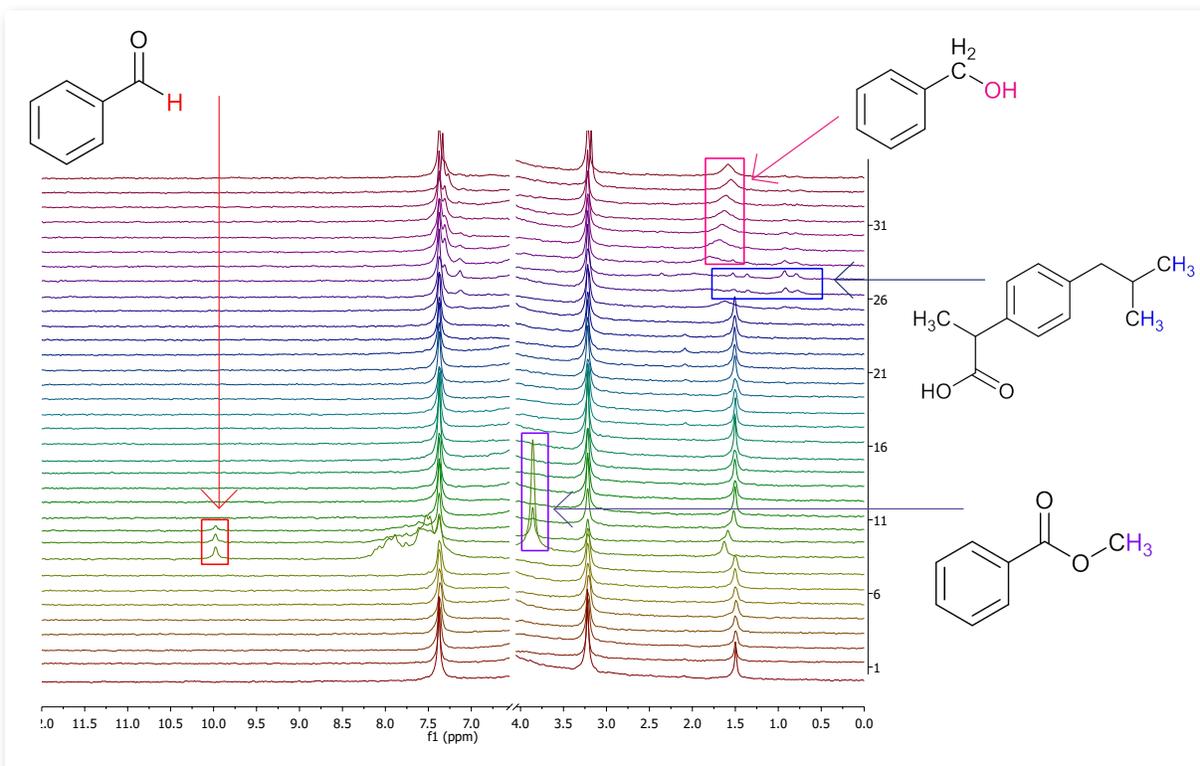


Figure 3: Elution profile of silica gel flash column. Samples were taken directly from test tubes (obtained using the Spinsolve in dichloromethane).



Figure 4: Silica TLC plate of the mixture run with dichloromethane and visualized with UV light.

From Figure 3 it can be seen that benzaldehyde and methyl benzoate co-elute (fractions 9-11), followed by ibuprofen (fractions 26-28) and benzyl alcohol (fractions 29-35). Using NMR to monitor the chromatography has several advantages that traditional TLC doesn't offer. Firstly, the TLC plate does not allow the determination that benzaldehyde and methyl benzoate co-eluted (Figure 4), unless each of the pure compounds were analysed separately which is not possible in all situations, for example if both compounds were the product of a reaction. Secondly, proton NMR can not only be used to determine when compounds are eluting, as TLC does, but it also gives structural information about what is eluting. In this set of compounds, each has a characteristic functional group that is not present on any of the other molecules, thus the appearance of this

functionality in the 1D proton NMR spectrum allows the compound to be identified easily. These compounds, however, are structurally similar and can therefore not be distinguished on a TLC plate by acid charring or UV development.

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Monitoring column chromatography in this way would not be achievable on a high field instrument for a number of reasons. One is that a high field instrument requires a deuterated solvent to lock to the sample which introduces significant cost. It also takes significantly longer to run each sample (it takes time to shim and lock on every sample) such that one column would take a day to collect the 1D proton data for. The proton NMR spectra in Figure 3 took 10 seconds to acquire each spectrum, and with minimal sample preparation between spectra, all 35 samples took less than 15 minutes to acquire. It was estimated that the time to prepare NMR samples from the fractions was comparable to preparing but not running a TLC plate.

Related Applications

The Spinsolve benchtop NMR system solves many of the issues associated with hyphenated NMR techniques, such as LC-NMR (the most common). Currently, LC-NMR systems use a deuterated solvent for the chromatography so that the sample can be analysed by a high field NMR spectrometer. This introduces a significant cost, not only the expense of running a high field instrument but also the cost of deuterated solvents required. The Spinsolve does not require deuterated solvents and is a fraction of the cost of a high field instrument to run. Combining the Spinsolve with an LC set-up would provide a more automated system than that described in this note.

Conclusion

We have demonstrated here how the Spinsolve can be used to monitor flash column chromatography. Using NMR spectroscopy to analyse the elution profile provides advantages over the traditional TLC method as you gain information about when compounds are eluting and something about its structure. In this experiment, each compound was able to be identified by a characteristic peak in the 1D proton NMR despite being structurally related. Chromatography monitoring by NMR is possible using a Spinsolve system as it does not require deuterated solvent to lock the sample and the spectra are acquired significantly quicker than on a high field instrument.

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