Science Together

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Inject, collect, repeat - Stacked injection made easy

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SUMMARY

Stacked injections are an easy way to increase productivity and improve efficiency in preparative chromatography. With this approach multiple injections are nested in one batch run for a maximum throughput and minimum downtimes between peak collections. This technique saves time as well as solvent. The fraction collection is automated for ease of use and best reliability.

INTRODUCTION

For preparative applications solvent consumption is oftentimes high whereas obtaining a decent yield is rather challenging. A way to improve both are stacked injections. Using this method system downtimes can be minimized thus leading to a more efficient overall purification process. The main aim of running stacked injections is to use the column bed more efficiently by reducing non elution phases, whilst retaining peak purity. Stacked injections can be an alternative as well if a column overload with target compound is desired but not possible without coelution of impurities or matrix compounds. The sample is loaded in such volumes, that sufficient target compound resolution is still achieved. Usually the yield would be minimal, but via stacked injection multiples of the actual sample volume are injected in one long run. For this method high robustness is needed, especially regarding retention time shifts. With a well-designed method injection cycles can be repeated almost indefinitely which allows a semi-continuous process. Large batches of sample can be automatically processed without losing product purity. A requirement for stacked injections is a short isocratic method with all peaks eluting in close proximity. Ideally the time frame between injection and target peak is free of impurities. Consequently an impurity eluting with a long delay after the target peak makes stacked injections challenging if not impossible. Technical requirements for stacked injection are an automated sample injection system (sample pump and automated injection valve with sample loop or VariLoop), and a fractionation system (multi position valve or fraction collector). If solvent recycling is desired, a multi position valve for fractionation is necessary. Manually programming stacked injection methods is oftentimes time-consuming and complicated. The PurityChrom software includes a stacked injection function which was used in the described application (see materials and methods). It provides a tool for a dynamic method writing, making it easy to adjust cycle times and cycle numbers.

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RESULTS

For the determination of the cycle-time (time until the first peak occurs) and to test the robustness several repetitive single runs with the established isocratic method were performed **(Fig 1)**. From those measurements the delay time between injection and the first peak was determined, as well as the time for the signal to return to baseline after the last eluting peak. Parameters for automated fractionation (threshold, peak windows) were derived from these results as well. Once those parameters were determined stacked injection mode was established easily with the PurityChrom software. The result for a run with three stacked injections is shown in **Fig 2**. In a doubled total runtime, a trifold sample volume was processed without losing any performance compared to two single batch injections **(Fig 1)**. The use of an automated fractionation system eliminated the need of continuous human system surveillance whereas solvent recycling during the delay time made it possible to save on overall cost for a run.

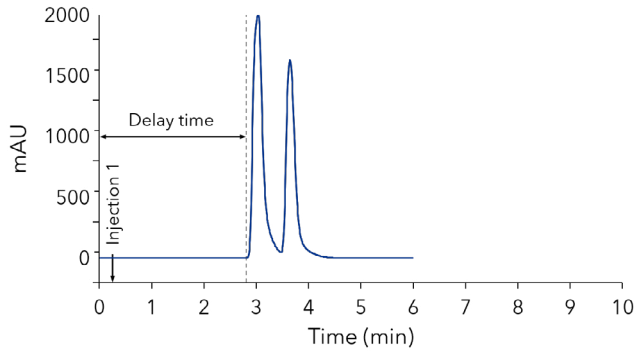


Fig. 1 Chromatogram of single injection; 500 µL injection volume; flowrate 25 mL/min; eluent 25/75 ethanol/water (v/v); ambient temperature.

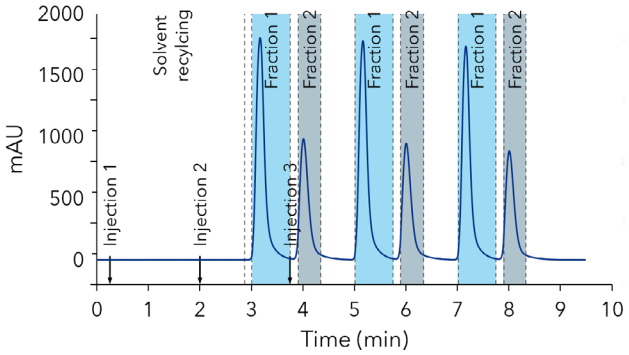


Fig. 2 Chromatogram of three stacked injections; 500 µL injection volume per injection; flowrate 25 mL/min; eluent 25/75 ethanol/water (v/v); ambient temperature; automated fractionation.

SAMPLE PREPARATIONS

An exemplary sample mixture of 2 mg/mL caffeine and 12 mg/mL paracetamol (acetaminophen) was prepared in 25/75 ethanol/water (v/v) and filtered through a 0.45 µm RC-membrane (regenerated cellulose).

CONCLUSION

The shown example separation showed that the development and application of stacked injections for purification purposes is straightforward and easy with PurityChrom. All injection times are calculated by the software once the cycle time is determined. With the automated fractionation and solvent recycling, a highly autonomous process can be achieved. Even challenging fractionations could be automated with a combination of threshold and peak window functions. Thus, large sample volumes can be processed efficiently without losing product purity. The overall runtime is shortened drastically compared to single batch runs, since in a stacked injection process the delay before the first peak is used already for the next injection. Consequently, time and solvent are saved even if solvent recycling is not an option.

MATERIALS AND METHODS

A preparative LC system as described in **Tab 4** was used. The method parameters are shown in **Tab 1** to **Tab 3**. The flow cell was attached as close to the column exit as possible using the fiber optics adapter kit, decreasing any detection delays. The method of stacked injection was realized using the PurityChrom Software. The stacked injection functionality of the autosampler control file is used for an automatic injection by a sample pump via an injection valve **(Fig 3)**. Critical parameters like cycle time and number of injections were transferred to the stacked injection function of PurityChrom **(Fig 4)**. When a run was started the autosampler control file loads the time control file (method file) where all remaining method parameters are defined. The overall runtime is automatically extended by the autosampler control file, depending on the number of chosen injections. The Flow chart is shown in **Fig 3**. As depicted, the sample is pumped with the sample pump in a circle, filling the sample loop constantly. On an injection event the content of the sample loop is directed into the main flow by switching the injection valve to the inject position. Until the first peak is occurring, the solvent is recycled. This is achieved using one position of the 12 port multi position valve for that purpose, in which the outlet is used to retransfer noncontaminated solvent into the solvent bottle. A second position is used for the waste, which is not recycled. Two more valve positions are defined for the fractionation of the respective target compounds. Every time a target peak appears in an injection cycle it is pooled in one fraction **(Fig 1)**. The automation is realized using a combination of PurityChrom's peak sampling and threshold function.

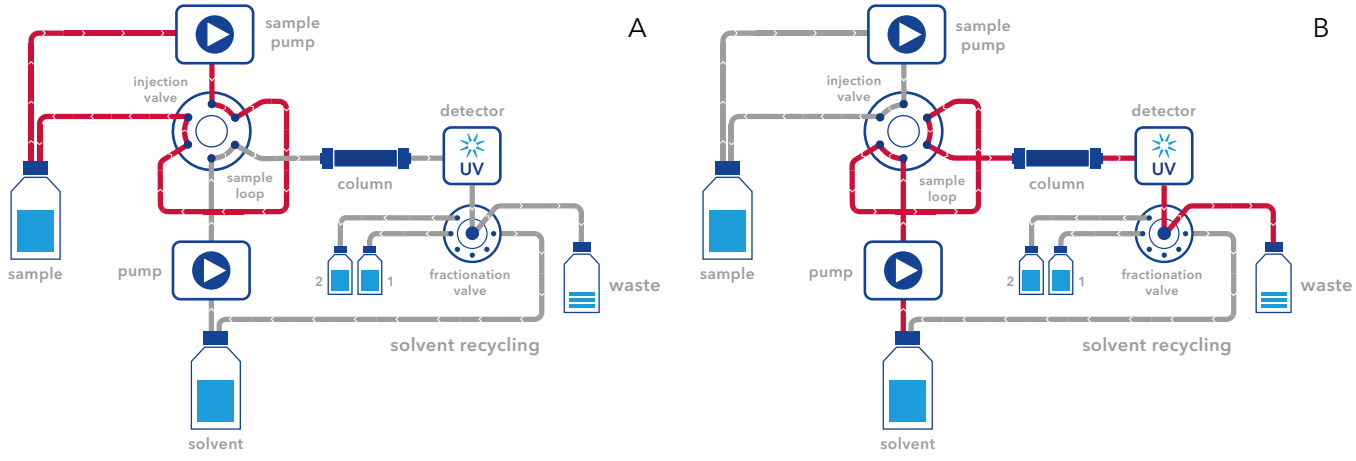


Fig. 3 System flowpath. The sample pump puts the sample in a circle to fill the sample loop constantly (A), before it is directed into the main flow (B).

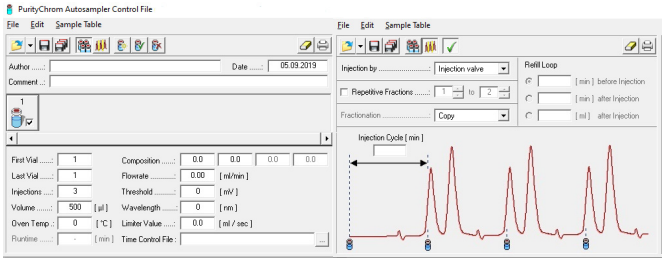
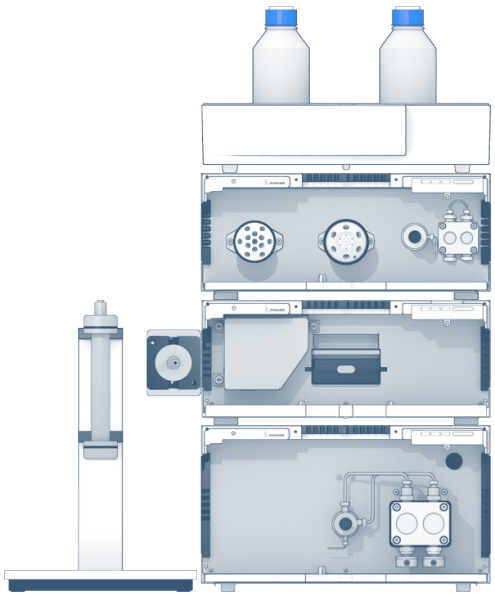


Fig. 4 PurityChrom: Stacked Injection Function

MATERIALS AND METHODS

Tab. 1 Method parameters

Column temperature	ambient
Mode	Reversed phase
Injection volume	500 µL
Injection mode	Full loop; stacked or single
Detection	UV 273
Data rate	2 Hz



Tab. 2 Pump

Eluent	25/75 ethanol/water (v/v)
Gradient	isocratic
Flow rate	25 mL/min
Run time	variable

Tab. 3 Sample pump

Eluent	sample
Gradient	isocratic
Flow rate	2 mL/min
Run time	variable

Tab. 4 System configuration

Instrument	Description	Article No.
Pump	AZURA P 2.1L 250 ml pumphead, sst	APE20LA
Detector	AZURA MWD 2.1L	ADB01
Flow Cell	2 mm path length 1/8", 200 bar	A4079
Detector accessory	Fibre Optics Adapter Kit	AMKX8KIT
Assistant	AZURA ASM 2.1L Left: 12 port MPV, 1/8", sst Middle: 6 port, 2 position injecti-on, 1/16", sst Right: P4.1S 50 ml pumphead, sst	AYFAEEBR
Column	Eurospher II 100-20/45 C18, Column 250x20 mm ID	25JE181E2X
Software	PurityChrom Version 5.9.96	A2650

