

## Sample Recovery:

*The significance of sample recovery in the preparative sector now sits in the flash environment.*



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Geoff has worked within the preparative instrument and chromatography consumable sector for over 25 years.

The significance of sample recovery is critical to leading Pharma and CRO who are managing expensive samples and tight budgets. For a chemist running infrequent numbers of purifications, it can be frequently overlooked. If you feed 40 grams of sample into your purification system and you are only receiving a total return of 30 grams of material post purification 25% of your initial sample is lost to the process.

This might not be important if you are only performing a crude clean-up of a sample with nominal commercial value. However, industry and academia are inherently faced with diverse application demands. At some stage you are going to want to run a purification where your sample is expensive, at this point you want as much of it back as possible and you don't want to be reliant on third party time constraints.

A relative novice can now take the maximum amount of sample to the next stage of their project. How? Utilising the improved purification power of an Interchim 20 bar system running in conjunction with their 15 micron chromatography media and maximizing purification potential using Intersoft® X and Genius AI.

Recovery rate is determined by numerous factors, the most critical being pump capability, system fluidics and software management of the purification process. Significant secondary factors include columns adopted and solvent.

All Interchim generation 5 systems display a recovery rate of >95%. That is unprecedented.

A key focus for the US with Interchim Gen4 systems has been sample recovery in the preparative sector (250 bar systems) using dedicated stainless steel prep columns with MS detection. Within this environment we have been able to demonstrate and achieve 95 - 98% sample recovery, a level not typically seen within competitor systems. A key factor for our recovery percentage is the systems reliance on fluidics rather than software delay. Recovery capability is well demonstrated in one of my favorite applications, the purification of caffeine, and is frequently adopted by my clients to verify the performance of their systems.

### Genentech California (US Pharma) system recovery analysis over time using caffeine

System: Gen 4 4250 (250 Bar)

Detection: Integral UV, iELS, MS

Date	User	Standard	Injected (mg)	Recovery %	Comments
24th July 17	AG	Flavone	24.6	91	New Installation
24th July 17	AG	Flavone	25.9	91	New Column/ Guard Column
28th March 18	AG	Flavone	27.6	99.2	
28th March 18	AG	Flavone	28.9	99.7	
28th March 18	AG	Flavone	31.7	99.3	
25th April 18	AG	Flavone	33.9	98.2	
25th April 18	AG	Flavone	29.2	98.2	
15th June 18	AG	Flavone	27.8	97.5	
15th June 18	AG	Flavone	32.4	97.5	
15th June 18	AG	Flavone	32.9	98.2	

More in depth discussion, information and MS specific test procedures are available upon request.

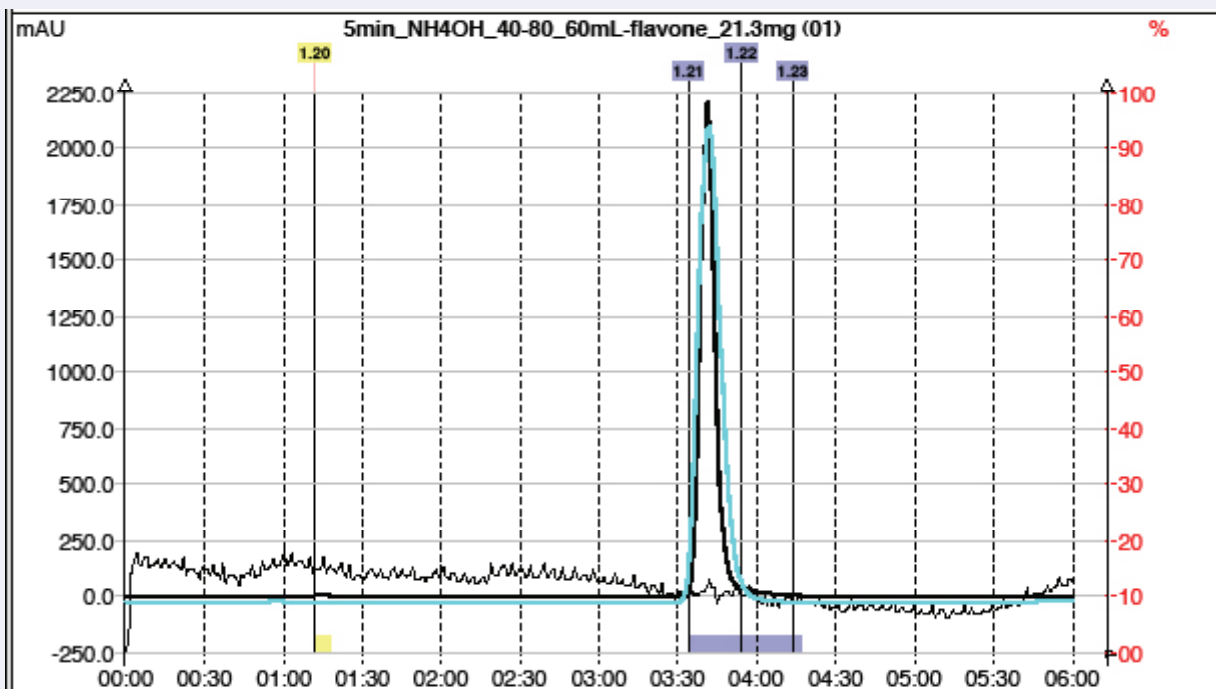


Cheshire Sciences



User: Client, US Pharma  
 System: Interchim 4250 250 Bar Preparative system  
 Detection: UV, (system integrated)  
 iELS, (system integrated)  
 MS with interface, (stand alone)

Sample: Flavone 21.3 mg  
 Column: Gemini 10 micron NX-C18 100x30mm + Guard 75.0g (3626psi)  
 Solvent A: -  
 Solvent B: Water + 0.1% Ammonium Hydroxide  
 Solvent C: Acetonitrile  
 Solvent D: -  
 Channel 1: UV 600: SIG1 >254nm  
 Channel 2: UV500: SIG2 >220nm  
 Channel 3: ELSD >35°



Peak Tracking

25 mL		25 mL		25 mL					
20		21		22		23			
RackSet #1		RackSet #1		RackSet #1		RackSet #2		RackSet #2	

Collection Table

Rack	Pos.	Fraction	Peak	RSet	Coll.	Volume	Area	%Area	Start Time	End Time
1	20	001	001	1	1	5.6	0.1	0.3 %	00:01:12	00:01:18
1	21/23	002/004	002	1	1	42.6	15.5	101.6 %	00:03:34	00:04:17
-	-	-	-	-	-	314.8	-0.3	-2.0 %	00:00:00	00:00:00

Elution Steps

N°	Time	Flow Rate	%B	%C
01	00 s	60.0	60	40
02	45 s	60.0	60	40
03	04:00	60.0	20	80
04	04:10	60.0	05	95
05	05:00	60.0	05	95
06	05:15	60.0	60	40
07	06:00	60.0	60	40

Detection Steps

N°	Time	Parameter	Collect	Threshold	F1
01	00 s	UV600:SIG1 ==> 254 nm	Yes	5	1
		UV600:SIG2 ==> 220 nm	No	0	1
		ELSD ==> 35°	No	10	5

Collection Steps

N°	Time	Local	Volume	Mode	Action
01	00 s	Yes	20.0	Threshold	None