Identifying and Collecting Weak UV Absorbance and Low Level Peaks of Interest From a Complex Mixture Using Conditional Logic Purification on Mass and UV Signals

Application Note PHA0411

Keywords

Gilson GX-271 LC/MS Purification System, Fraction Collection, Active Pharmaceutical Ingredient (API), Metoprolol, Verapamil, Chloramphenicol, Doxepin, Sulfamethazine

Introduction

Purification of Active Pharmaceutical Ingredients (APIs) often yields high numbers of fractions, often leading to a bottleneck of fractions to dry-down and subsequently analyze. UV-based fraction collection based on slope or threshold values offers some specificity and reduction in the number of fractions collected compared to basic time or volume based collection. LC/MS-based purification provides the highest specificity as a direct result of target ion collection from the API of interest. This application note will demonstrate the improvement in specificity offered by LC/MS based purification when compared to traditional UV alone using the Gilson GX-271 LC/MS Purification System (see figure 1).

Figure 1. Gilson GX-271 LC/MS Purification System

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Mass based purification offers enhanced selectivity for purifying APIs often resulting in higher purity and fewer fractions to process. There is limited information on the effectiveness of mass based purification to accomplish this goal with samples that closely resemble an API purification run. Typical synthesis steps and reactions often lead to impurities and partial products. In some cases, the compound of interest will not be the predominant compound. This represents challenges in purification with traditional UV based injection and fraction collection. Utilizing a mass signal in conjunction with conditional logic collection parameters and complimentary UV data can provide the selectivity to collect only APIs of interest. This selectivity has shown results that dramatically reduce collected fractions, collecting only the target ions of interest for an efficient purification process versus traditional UV-only based purification.

In this study, API purification was simulated using a mixture of several pharmaceuticals at varying concentration levels. The compound of interest representing the API has a low, relative percent area compared to other compounds present in the same sample solution.

Materials & Methods

Materials

Chemicals and reagents were obtained from various scientific suppliers. All solvents used were HPLC grade or higher. All reagents were ACS grade or better.

Gilson LC/MS Purification System:

- GX-271 Liquid Handler
- Direct Injection Module – 5 mL sample loop
- 322 Pumping System H2 Heads (0.25 – 30 mL/min)
- 155 UV/VIS Detector
- 307 Make Up Pump
- MRA Splitter
- Flexar SQ 300 Single Quadropole Detector
- TRILUTION LC 2.1 SP5 LCMS Software

Purification Column:

Phenomenex - Axia 21.2 mm x 50 mm; Luna 5micron C18 (2), 100 A

Method:

Mobile Phase:

Pump A: 100% Water
Pump B: 100% Methanol

Mobile Phase Gradient:

0.0 min. – 10% B
0.5 min. – 10% B
9.0 min. – 95% B
10.0 min. – 95% B
10.2 min. – 10% B
11.5 min. – 10% B

Makeup Pump:

100% Methanol with 0.1% Formic Acid
0.3 mL/min

Split Ratio:

10,000:1

UV Detector:

Dual wavelength @ 220 nm and 254 nm

MS Detector:

Ionization Mode: Positive
Channel 1: Full Scan 200 – 500 amu
Channel 2: Target m/z 267.3

Mass Spectrometer (MS) & Fraction Collection Parameters:

MS Parameters:

Ionization Mode: Positive
Channel 1: Full Scan 200 – 500 amu
Channel 2: Target (EIC): m/z 267.3
Adduct 1: 1
Adduct 2: 23

Conditional Logic Fraction Collection Parameters:

Primary Conditions: UV 254 nm
Slope Collection: FS =25, BS=25, P.W. = 0.2 AND Statement 10.0 min – 95% B
Secondary Conditions: MS Target Mass 267. 3
Level: ≥ 500,000 TIC

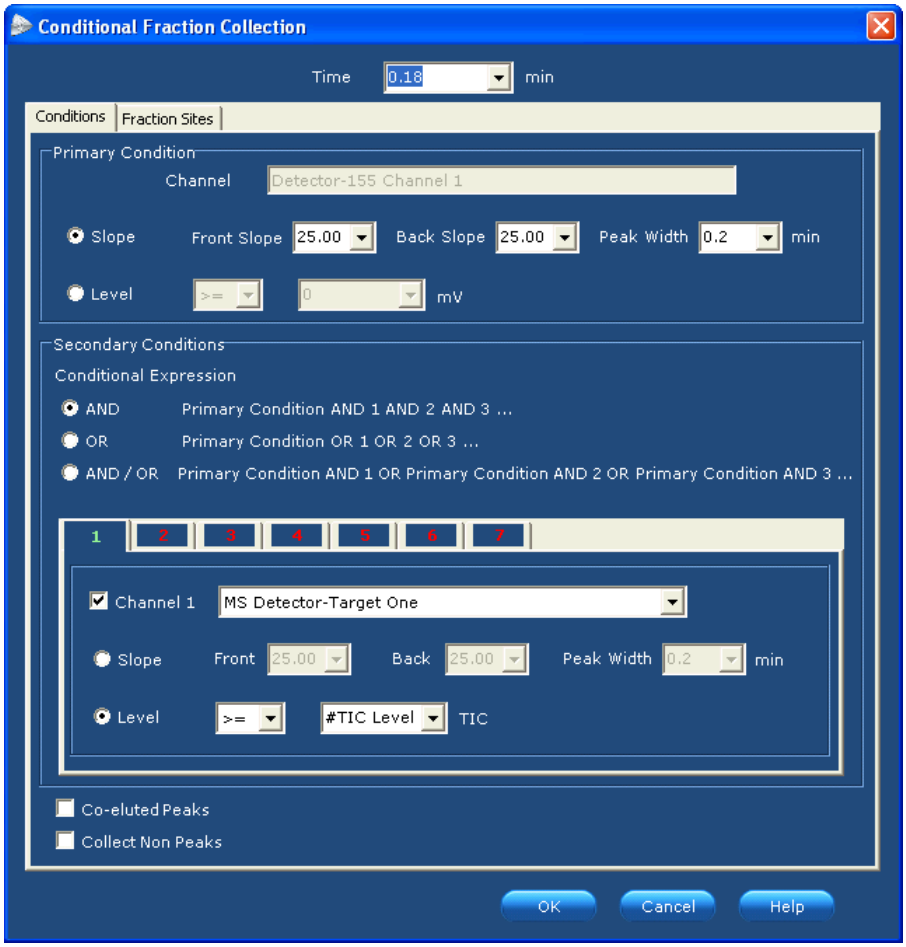


Figure 2. Gilson TRILUTION® LC Conditional Fraction Collection Task Properties

Methods – Sample Preparation

Sample Solution:

40 mg/mL stock solutions of four compounds (Metoprolol, Verapamil, Chloramphenicol, Doxepin, and Sulfamethazine) were mixed together in Dimethyl Sulfoxide (DMSO), resulting in ~8 mg/mL of each compound. The injection volume was 2000 uL.

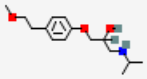
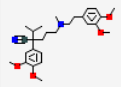
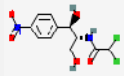
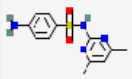
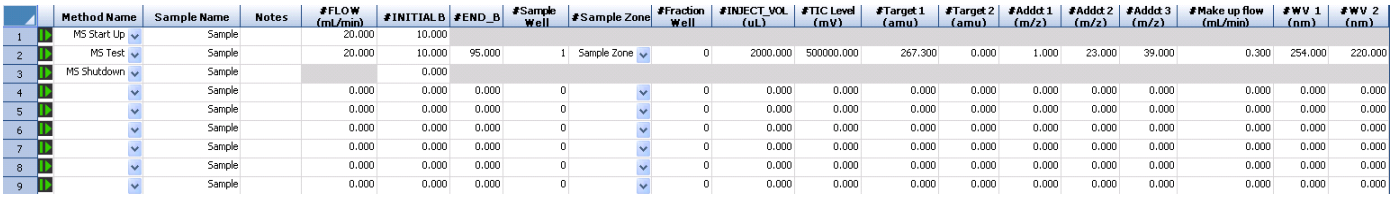
Compound	Molecular Weight	Chemical Structure
Metoprolol	267.36	
Verapamil	454.60	
Chloramphenicol	323.13	
Doxepin	279.37	
Sulfamethazine	278.33	

Table 1. Compound Solutions and Corresponding Molecular Weights

The flexibility of TRILUTION® LC System Software is used to establish method variables values in the run screen, allowing changes to be made that impact the method gradient, flow rate, injection volume, fraction collection mass targets and adducts, etc. The variables and values used are indicated below. A start up method initiates the system conditions and flow rate prior to the injection and fraction collection method. The stop method automatically stops the system flow rate and turns off the detector lamp.



	Method Name	Sample Name	Notes	#FLOW (mL/min)	#INITIAL B	#END_B	#Sample Well	#Sample Zone	#Fraction Well	#INJECT_VOL (uL)	#TIC Level (mV)	#Target 1 (amu)	#Target 2 (amu)	#Addct 1 (m/z)	#Addct 2 (m/z)	#Addct 3 (m/z)	#Make up flow (mL/min)	#WV 1 (nm)	#WV 2 (nm)
1	MS Start Up	Sample		20.000	10.000														
2	MS Test	Sample		20.000	10.000	95.000	1	Sample Zone	0	2000.000	500000.000	267.300	0.000	1.000	23.000	39.000	0.300	254.000	220.000
3	MS Shutdown	Sample			0.000														
4		Sample		0.000	0.000	0.000	0		0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5		Sample		0.000	0.000	0.000	0		0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6		Sample		0.000	0.000	0.000	0		0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7		Sample		0.000	0.000	0.000	0		0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8		Sample		0.000	0.000	0.000	0		0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9		Sample		0.000	0.000	0.000	0		0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Figure 3. TRILUTION LC Run Screen Sample List Displaying Method Variables

Variable Name	Value	Units
#FLOW	20	mL/min
#INITIAL B	10	%
#END_B	95	%
#Sample Well	1	NA
#Sample Zone	Sample Zone	NA
#Fraction Well	0	NA
#INJECT_VOL	2000	µL
#TIC Level	500000	mV
#Target 1	267.30	amu
#Target 2	0.00	amu
#Addct 1	1	amu
#Addct 2	23	amu
#Addct 3	39	amu
#Make up flow	0.3	mL/min
#WV 1	254	nm
#WV 2	220	nm

Table 2. Method Variables and Values Used in TRILUTION LC (see Figure 3)

Results

Performing sample injection and subsequent fraction collection using just UV and slope based collection criteria would produce seven different fractions, each of which would need to be subdivided into 2 or 3 fractions. The total number of resulting fractions using this collection method would be 20 tubes containing about 15 to 20 mL each. Using the Fraction Simulator tool within TRILUTION LC, fraction collection can be predicted and tested to provide a 'best fit' method for efficient fraction collection.

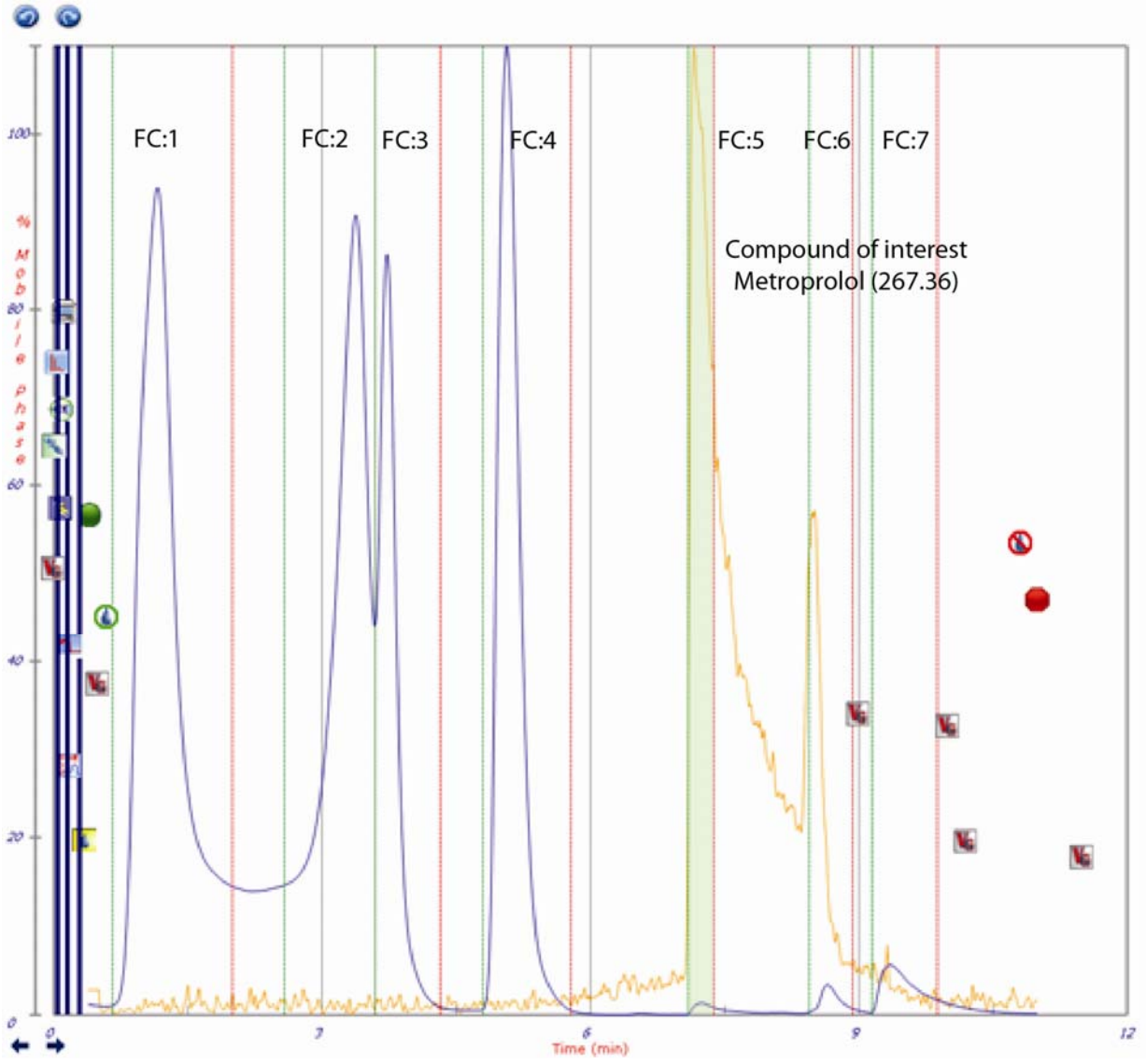


Figure 4. Fraction Simulation Using UV and Slope Collection Mode

The simulated fraction collection for the API of interest (Metoprolol) is in relatively low amount compared to the other impurities or peaks of non-interest. The same compound mixture was purified with conditional logic collection utilizing both a UV wavelength of 254 nm and a target mass of 267.36 (Metoprolol). The Metoprolol target mass channel showed tailing. As a result, the next UV peak was eluted while the target mass of 268.2 [M+H] was still present, which caused a second fraction to be collected. The gradient conditions could be modified to increase the separation and prevent the second fraction. The gradient conditions could be modified to increase the separation and prevent the second fraction. The spectrum also showed the formation of sodium adduct at 290.3 [M+Na]. Increasing the make-up flow rate to 0.5 mL/min was attempted to reduce the amount of tailing with minimal improvement.

Figure 5. Gilson TRILUTION® LC Software Conditional Logic Fraction Collection Purification Based on UV and MS Signals – Fraction Tracking Function

The Metoprolol peak (see figure 4, peak at 6.823 minutes) only represented 0.125% of the total peak area. Purification using Conditional Logic Fraction Collection based on UV and MS signals, the number of fractions were reduced to only two fractions.

Sample Table

Injection Number	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Sample Name	Fraction Site(s)
2	Chloramphenicol	0.767	5484120.8781	32.234	120.713	New Mix	
2	Sulfamethazine	2.988	6870992.4011	40.385	112.655	New Mix	
2	Doxepin	4.673	4310787.5011	25.337	150.053	New Mix	
2	Metroprolol	6.823	21246.6674	0.125	1.241	New Mix	Fraction Zone-1
2	Verapamil	8.257	80284.1664	0.472	3.931	New Mix	Fraction Zone-2
2	1	8.949	246174.582	1.447	6.798	New Mix	

Figure 6. Gilson TRILUTION® LC Software Sample Report Table

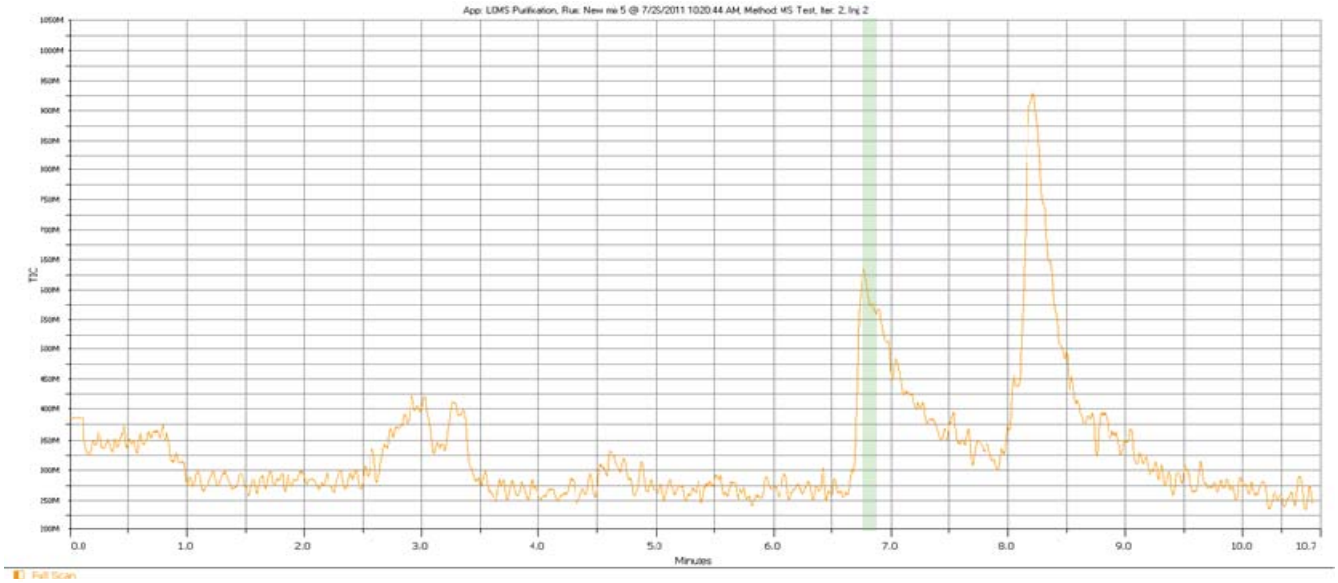


Figure 7. TRILUTION LC Full MS Scan Spectra of Sample Injection

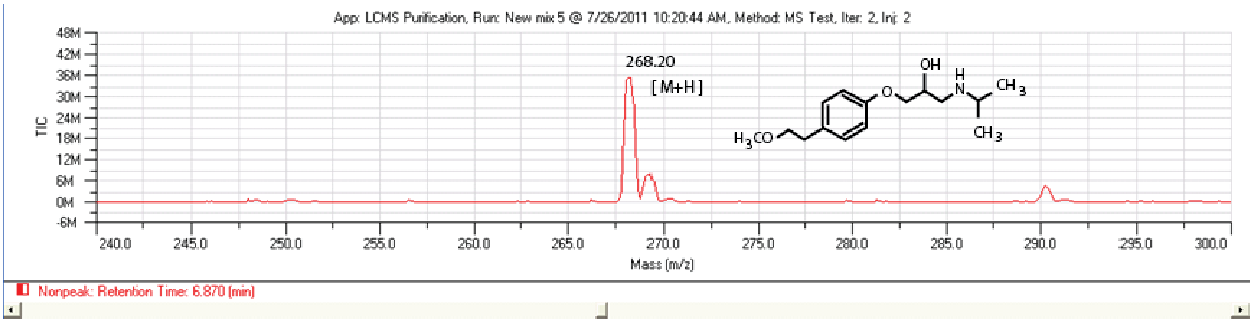


Figure 8. TRILUTION® LC Sampled Spectra for Metoprolol (Compound of Interest)

Summary

UV based purification is an effective means of obtaining pure compound of interest; however, there are limitations with compound specificity. These limitations lead to large numbers of fractions obtained, many of which do not contain the compound of interest. Post fraction collection processing includes dry down, and often re-injection onto LC/MS for compound verification.

Combining UV and LC/MS for purification provides the capabilities to reduce the number of fractions collected and thus reduction in post processing time as a result of positive confirmation of the compound at collection. Conditional logic (UV AND MS signals) properly applied to a complex mixture is shown to provide very good selectivity for fraction collection even if the target compound is present at very low relative amounts.

References

Chemical structures of compounds from PubChem Public Chemical Database (www.pubchem.ncbi.nlm.nih.gov)