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Development of an LC-MS/MS method for the characterization of liposomes used in drug delivery

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Introduction

The use of liposomes as drug delivery systems offers several advantages compared to free drug administration, such as their non-toxic composition and available surface modifications, which can lead to improved longevity and site targeting.[1,2] For liposomes to behave as expected upon administration, their formulation must be meticulously planned. Thus, it is imperative to study the final composition of loaded liposomes and compare them to the intended design. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) is ideal for such characterization.

Objectives:

- To develop an LC-MS/MS method for the quantification of both drug and lipids within a liposome
- To use this method to compare the composition of empty and loaded liposomes

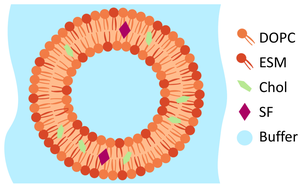


Figure 1. Scheme of sorafenib (SF)-loaded liposome.

Method

Preparation of Liposomes

Adapted from Ip et al.[3] Lipid films prepared with 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), egg sphingomyelin (ESM), cholesterol (Chol) and sorafenib (SF) at 2:2:1:0.5 molar ratios (if empty, no SF added). Once rehydrated with 1x PBS, 100 nm unilamellar vesicles were prepared using the Avanti® Mini Extruder. Liposomes were purified from free drug by removing supernatant after centrifugation at 10,000 rpm for 20 mins.

Characterization of Liposomes

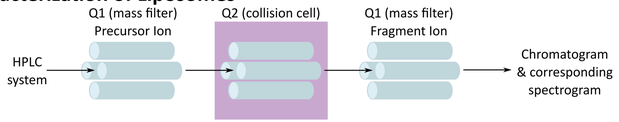


Figure 2. Scheme of a triple-quadrupole LC-MS/MS instrument.

- SCIEX API 4000 system with a Higgins Analytical PROTO 200 C4 5µm column
- Method developed by Loryn Arnett and Matthew Forbes used as a starting point (A: 0.1% formic acid, B: 50/50 acetonitrile to isopropanol)
- Mobile phase gradient and run time were modified for new protocol

Synthesis of Cholesterol Derivative

Adapted from Liebisch et al.[4] Cholesteryl acetate synthesized from cholesterol and acetyl chloride.

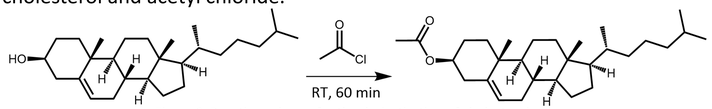


Figure 3. Reaction scheme for the derivatization of cholesterol.

Results and Discussion

Optimization of MS/MS Parameters

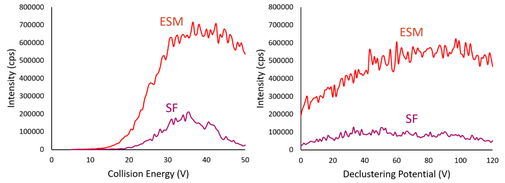


Figure 4. Collision energy (CE; left) and declustering potential (DP; right) voltage ramps for optimization.

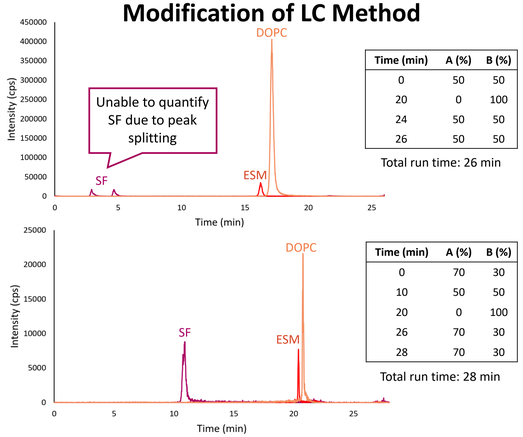
- Precursor (Q1) and fragment (Q3) ions identified
- CE and DP optimized to maximize ionization by choosing voltages with highest signal intensity

Table 1. Optimized multiple reaction monitoring (MRM) parameters.

Compound	Q1 (m/z)	Q3 (m/z)	Adduct	DP (V)	CE (V)
DOPC*	786.8	184.0	$(M+H)^+$	110	45
ESM	703.7	184.0	$(M+H)^+$	80	38
SF	465.2	269.9	$(M+H)^+$	50	36

*optimized by authors of original method

Modification of LC Method



Time (min)	A (%)	B (%)
0	50	50
20	0	100
24	50	50
26	50	50

Total run time: 26 min

Time (min)	A (%)	B (%)
0	70	30
10	50	50
20	0	100
26	70	30
28	70	30

Total run time: 28 min

Figure 5. Original (top) and modified (bottom) LC methods with corresponding mobile phase gradients (A: 0.1% formic acid, B: 50/50 acetonitrile to isopropanol).

- Elution of SF peak was optimized by modifying mobile phase gradient and total run time

Challenges with Cholesterol

- Cholesterol not efficiently ionized with electrospray ionization (ESI) source → must derivatize
- Cholesteryl acetate synthesized due to ability to form $(M+NH_4)^+$ adducts with positive ESI if ammonium acetate incorporated into mobile phase[4]
  - *Issue: no noticeable $(M+NH_4)^+$ peak was observed*
- Ran without ammonium acetate in mobile phase
  - *$(M+Na)^+$ and $(2M+Na)^+$ observed*
  - *Issue: fragment ion more prevalent than precursor ions*

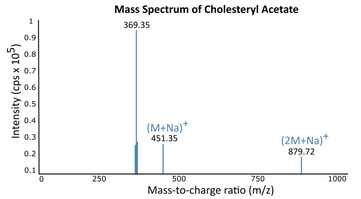


Figure 6. Mass spectrum of cholesteryl acetate showing only prominent peaks (greater than 1 x 10^4 cps).

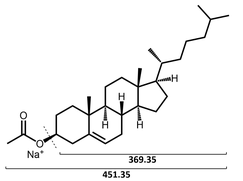


Figure 7. Potential precursor and fragment ions for cholesteryl acetate MRM parameters.

Quantification of Liposome Components

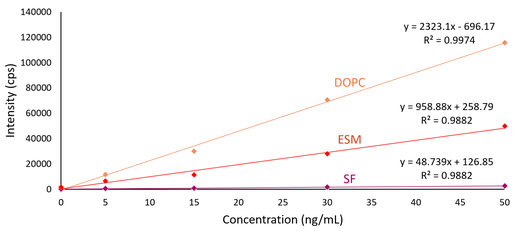


Figure 8. Calibration plots for optimized compounds with corresponding regression line equations and coefficients of determination (R^2).

Table 2. Standard error of regression, limit of detection (LOD) and limit of quantification (LOQ) of all calibration lines.

Compound	$S_{y/x}$	LOD (ng/mL)	LOQ (ng/mL)
DOPC	2776	3.6	12.0
ESM	2462	7.7	25.7
SF	125	7.7	25.6

Preliminary results:

- Empty liposomes have same molar ratio as intended formulation
- SF-loaded liposomes yield varying results likely due to ineffective centrifugation method → must optimize separation of free drug

Conclusion

- Effective LC-MS/MS method was developed for the simultaneous characterization of lipids and a hydrophobic drug in liposomes
- Precursor and fragment ions for a cholesterol derivative have been identified for its inclusion in the method
- The potential uses of this method include the investigation of: effects of drug loading on ratio of liposome components, drug encapsulation efficiency, drug release kinetics, etc.

Future Work

- Optimization of cholesteryl acetate MS/MS parameters: set $(M+Na)^+$ adduct as precursor ion, and lower CE to enhance its signal and prevent fragmentation
- Study effect of drug loading on component ratio: use dialysis to separate SF-loaded liposomes from free drug in solution and compare results to lipid ratio of unloaded vesicles

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