药明康德
WuXi AppTec

Comparison between Magnetic Bead and Membrane Immunoaffinity Purification Methods for the Measurement of Monoclonal Antibody in Rat Serum

Zhiyu Li, Zhiren Yu, Feifei Cui, Weiqun Cao, Lili Xing, Xin Zhang, and Yi Tao

Introduction

Protein quantification by liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become an increasingly popular field for pharmacokinetic study in the drug discovery phase. Traditional method development for one protein using a ligand binding assay usually requires 2-3 months. By using LC-MS/MS techniques, development times can be reduced to one week. However, high interference using the standard surrogate peptide approach for LC-MS/MS based large molecule quantitation leads to long separation times and unsatisfactory lower limit of quantitation (LLOQ).

In order to reduce interference and improve the sensitivity of protein quantitation by LC-MS/MS, researchers will undergo an immunoaffinity purification (IP) step to purify the target protein from matrices. One method for IP is the use of magnetic beads coupled to a capture reagent. This method is reliable, but time-consuming.

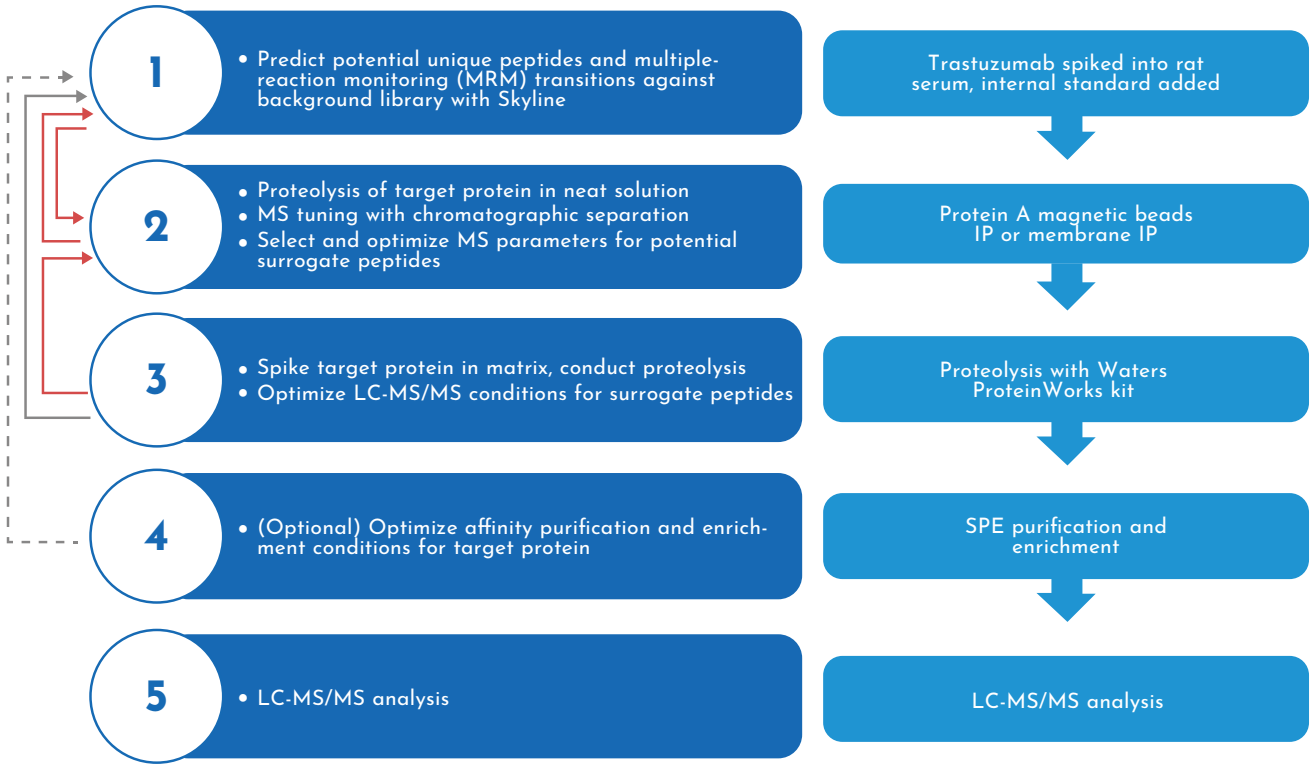
Membrane immunoaffinity, which takes decidedly less time than magnetic beads, has emerged as a novel material with potential to replace the magnetic bead method.

In the current study, we performed thorough testing on the robustness of membrane affinity versus magnetic beads by using both methods to analyze rat pharmacokinetics of the monoclonal antibody (mAB) trastuzumab samples.

Experimental Design

We compared the robustness of two sample IP pretreatment procedures, magnetic bead and membrane IP. Following the pretreatment procedures, the workflow consisted of a tryptic digestion process using a Waters ProteinWorks™ kit, SPE purification enrichment and LC-MS/MS analysis. The general workflow is illustrated in Figure 1.

The steps for the magnetic bead immunoaffinity purification and the membrane immunoaffinity purification process are included below. The membrane immunoaffinity purification separation process is streamlined, simplified and significantly reduces the IP sample pretreatment time to under 15 minutes.



Scheme 1. General method development workflow

Scheme 2. Sample treatment workflow

Figure 1: General workflow of method development and sample treatment

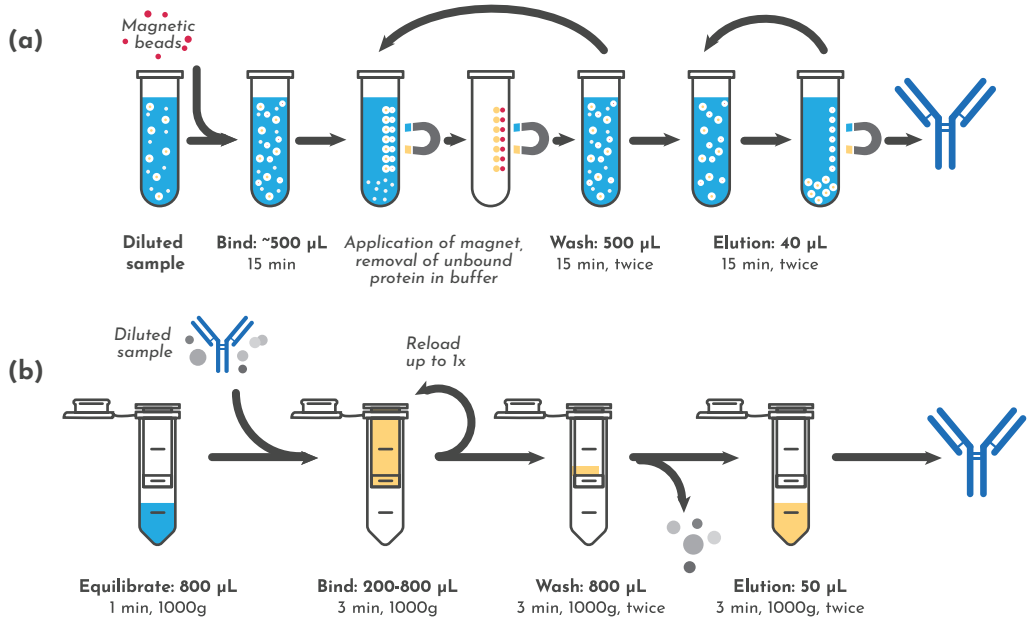


Figure 2: (a) Magnetic bead immunoaffinity purification and (b) the membrane immunoaffinity purification process

Approach	Std. curve range (µg/mL)	Weighting	Linear fit (r^2)	MRM transition
Magnetic Bead	0.05 ~ 50	$1/X^2$	0.9928	485.3/721.3
Membrane	0.05 ~ 50	$1/X^2$	0.9954	485.3/721.3

Table 1. Linear dynamic range and std. curve statistics with different IP methods

IP Method	Sample ID	Nominal Conc. (µg/mL)	Intra-Day (Within-run)						Inter-day (Between-run)	
			Day 1		Day 1		Day 1			
			Bias%*	CV%**	Bias%*	CV%**	Bias%*	CV%**	Bias%*	CV%**
Magnetic Bead	LLOQ	0.05	-0.4	8.2	-11.4	14.7	-11.4	19.7	-0.1	17.4
	Low QC	0.15	-3.1	1.8	-10.0	9.2	-10.7	10.5	-0.8	11.9
	Mid QC	20	-8.5	5.4	-0.7	12.1	-12.5	12.4	1.6	13.4
	High QC	40	-4.4	3.2	-1.1	7.2	-7.0	8.1	1.2	7.8
	Dilute QC	80	-12	4.6	---	---	---	---	---	---
Membrane	LLOQ	0.05	4.0	9.4	-6.4	14.1	-4.0	10.6	-2.2	19.3
	Low QC	0.15	-2.7	11.9	-0.7	4.9	-1.3	10.8	-2.0	9.1
	Mid QC	20	1.5	8.3	8.5	9.1	-2.5	4.6	2.5	8.5
	High QC	40	2.8	2.6	1.8	4.8	-4.0	8.1	-1.0	5.9
	Dilute QC	80	11.5	9.5	---	---	---	---	---	---

*Within-run and between-run %Bias must be less than or equal to 15.0%, except for LLOQ, which was 20.0%.
**Within-run and between-run %CV must be within ± 15.0%, except for LLOQ, which was ± 20.0%.

Table 2. Intra- and inter-day accuracy and precision of FTISADTSK quality control samples

Captureum Protein A plates were loaded with various volumes of trastuzumab at a concentration of 50 µg/mL. The signal for FTISADTSK peptide showed a linear relationship with sample volume for sample amount between 2.5 µL and 100 µL. Henceforth, 30 µL of rat serum was used. As shown in Table 1, linear dynamic range and standard deviation curve statistics for signature peptide were used to quantify trastuzumab in rat serum. As shown in Table 2, satisfactory chromatogram was obtained and precision was determined through the analysis of six replicates of QC samples at four different levels.

First, the linerarity, sensitivity, accuracy, precision, matrix effect, carryover and selectivity over the range of 0.05-50 µg/mL were validated. Once validated, researchers applied the purification methods to a study with male Sprague-Dawley rats to further evaluate the pharmacokinetics of trastuzumab. The cohort of Sprague-Dawley rats were dosed with trastuzumab at a concentration level of 10 mg/kg. Sample IP processing workflows for the magnetic bead approach, and the membrane approach are shown in Figure 2(a), and Figure 2(b), respectively.

Serum samples were collected by tail-snip at: predose, 0.5 hr, 2 hr, 6 hr, 24 hr, 2 days, 3 days, 6 days, 8 days, 10 days, 14 days, 17 days, 21 days, 24 days, 28 days, 35 days, 42 days, 49 days, 56 days, and 63 days, processed into serum, split into 2 samples, and then stored frozen at below -60 degrees Celsius before analysis.

Results and Discussion

After subcutaneous (SC) dosing at 10mg/kg, concentrations of the signature peptide FTISADTSK were used to measure the pharmacokinetic profile of trastuzumab, as shown in Figure 3.

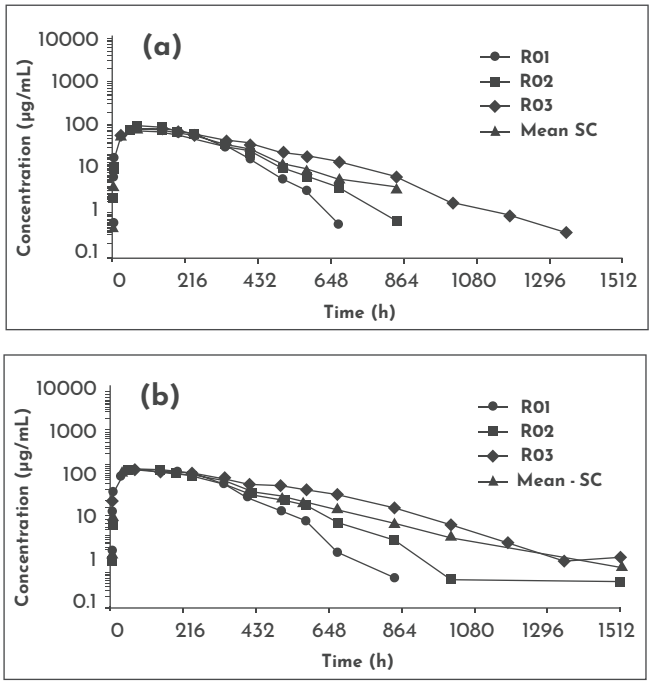


Figure 3: (a) Serum concentration of trastuzumab after SC dosing at 10 mg/kg, determined by signature peptide FTISADTSK, from magnetic bead IP , and (b) membrane IP.

The magnetic bead approach and membrane approach produced comparable validation parameters including linearity, intra-, inter-day accuracy and precision, carryover, dilution integrity, matrix effect, specificity, and selectivity. Researchers reported similar pharmacokinetic parameters as shown in Table 3.

IP Method	Magnetic Beads			Membrane		
PK Parameters	Mean	SD	CV (%)	Mean	SD	CV (%)
C_{max} (µg/mL)	86.1	5.65	6.56	71.9	5.06	7.03
T_{max} (h)	64.0	13.9	21.7	96.0	41.6	43.4
$T_{1/2}$ (h)	89.8	34.9	38.9	104	43.7	42.2
$AUC_{0\text{-}last}$ (µg.h/mL)	27612	5172	18.7	23881	4604	19.3

Table 3. PK parameters of a 10 mg/kg dose of trastuzumab as determined by two IP aproaches in rat serum

Conclusion

In this investigation, two validated strategies for protein immunoaffinity purification were compared with trastuzumab as a model system. Both methods offered the same LLOQ of 0.05 µg/mL, and satisfactory chromatogram. The main differences can be attributed to the sample processing time.

Specifically, the sample IP processing time for a 96-well plate with the magnetic bead method was 3-4 hours in comparison to the 10-20 minutes by the membrane approach. Furthermore, the magnetic bead method was more prone to operational pipetting errors.

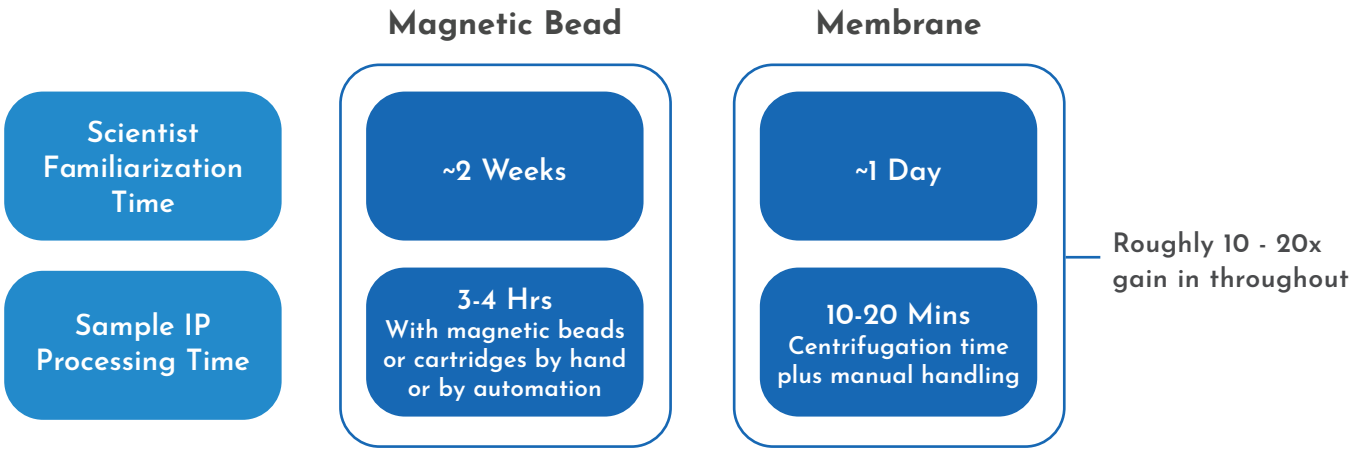


Figure 4. Comparison of familiarization time and sample processing time between the magnetic bead approach and the membrane approach

WuXi AppTec is working to revolutionize bioresearch by advancing discovery efforts and delivering the high-quality data clients need. By working closely with chemistry, biology and pharmacology for effective lead selection during discovery, our team is able to provide expertise in diverse compound classes with knowledge on a range of species, strains, matrices and microsampling techniques.

药明康德
WuXi AppTec

Contact Us to Learn More

labtesting.wuxiapptec.com | info_ltd@wuxiapptec.com