

The importance of TDM and the role of herbal medicine in kidney transplant patients



Analysis of biological samples to quantification of analyte concentration


By:

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Analysis of biological samples to quantification of analyte concentration

- Outline

- Quantification of analyte concentration

- Which parameters should be considered in quantification of analyte concentration?

- Accuracy

- Precision

- Calibration curve and linear range

- Common errors in calibration curve

- Selectivity in Bioanalysis

- Extraction method in Bioanalysis

Quantification of analyte concentration

- Therapeutic drug monitoring (TDM)
- Pharmacokinetic studies: bioequivalence studies
- Quantification of toxicants and biomarkers in biological samples
- In Vitro study: Dissolution
- Quality control studies
- ...



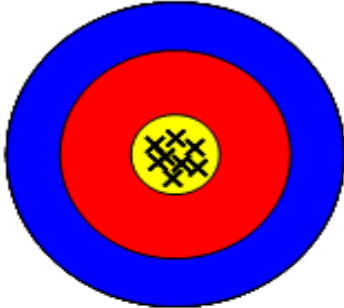
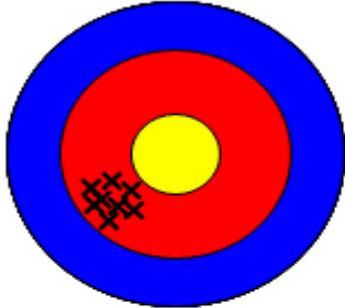

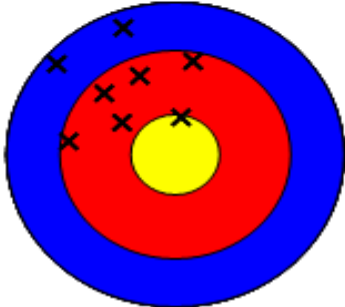


Quantification of analyte concentration

- Classical reactions
- Instrumental analysis methods
- Commercial kits: Immunoassay methods

Which parameters should be considered in quantification of analyte concentration

- Accuracy and Precision

	Accurate	Inaccurate (systematic error)
Precise		
Imprecise (reproducibility error)		

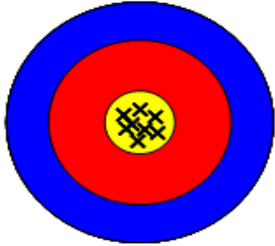
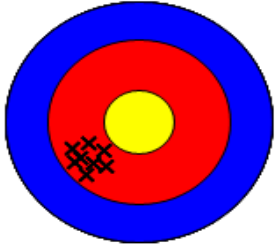

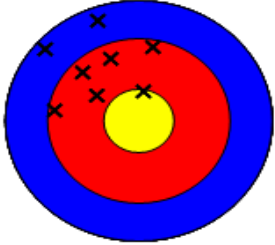


Which parameters should be considered in Quantification of analyte concentration

- Accuracy
- Low accuracy method: acceptable conclusion is not possible
- Comparison with other methods
- Example
- TDM of mycophenolic acid
 - Quantification by a commercial ELISA kits
 - Range of plasma concentration in 100 samples (0.1-0.4 ppm)
 - Plasma concentration in the literature: 1-3.5 ppm

Which parameters should be considered in Quantification of analyte concentration

- Precision

	Accurate	Inaccurate (systematic error)
Precise		
Imprecise (reproducibility error)		

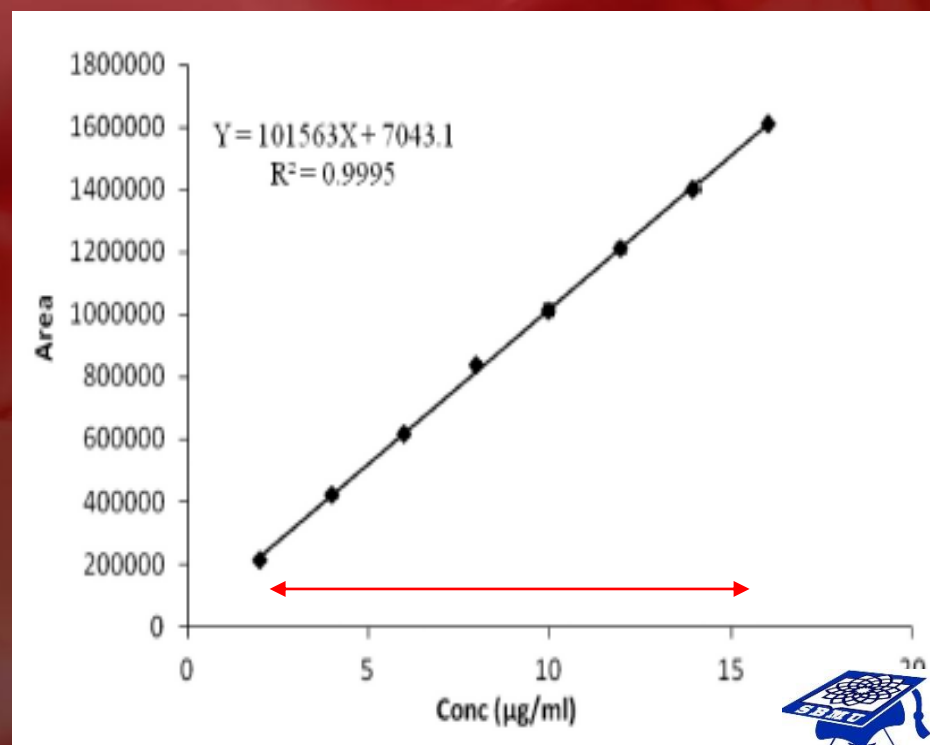
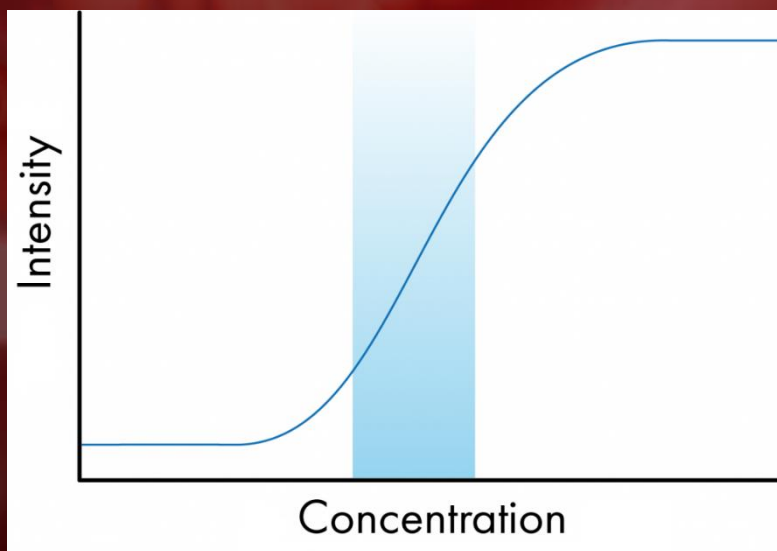
Interventional study

- Low precision of an analytical methods (high SD):
 - Statistical test: insignificant results

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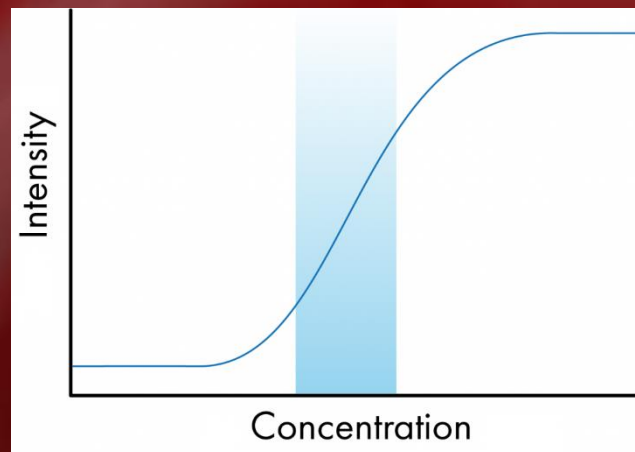
Calibration curve and linear range

- Standard samples: calibration curve
- Correlation coefficient (R^2), Slope, intercept



Wide linear range is an advantages in developing analytical method

- It is an advantages in developing analytical method
- An important issue in bioanalysis



- Dilution factor: In bioanalysis should be considered

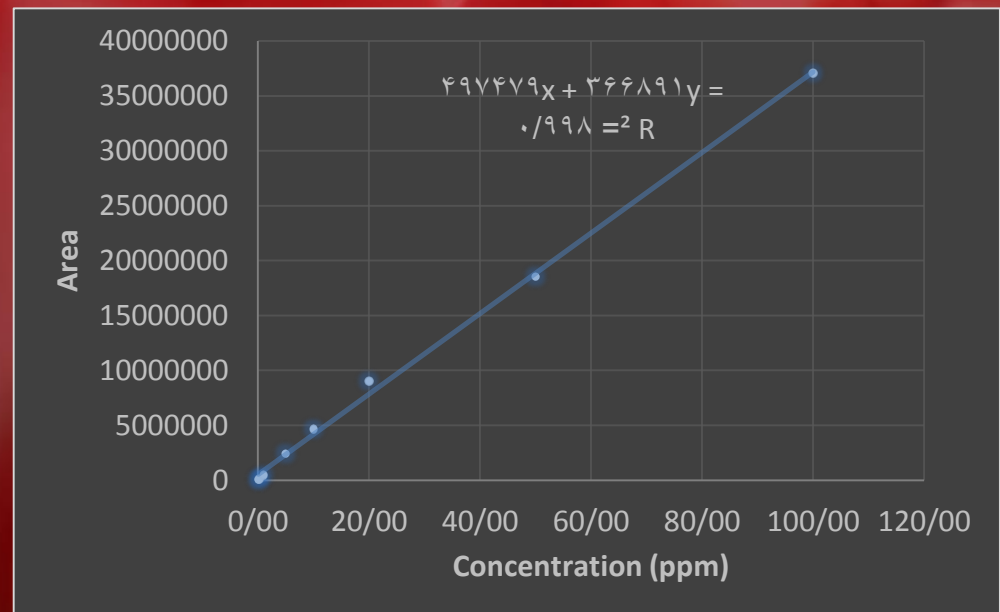


Common errors in calibration curve

- Beware of R^2 !
- Beware of intercept!
- Beware of type of chart for plotting of calibration curve
- LOD and LOQ or LLOQ
- Calibration curve for quantification of endogenous compounds

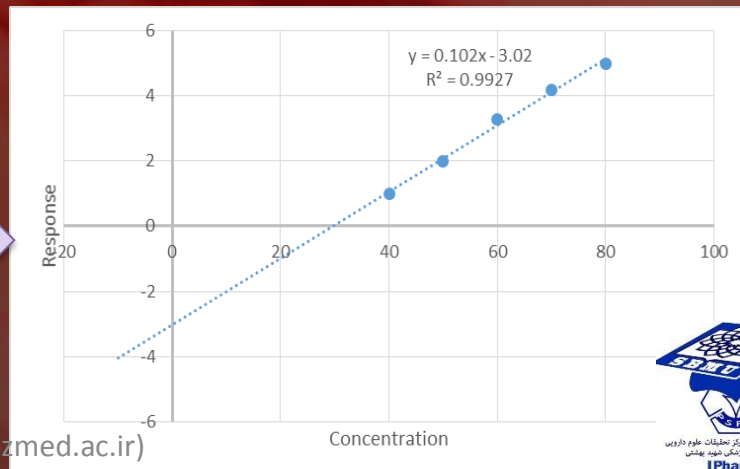
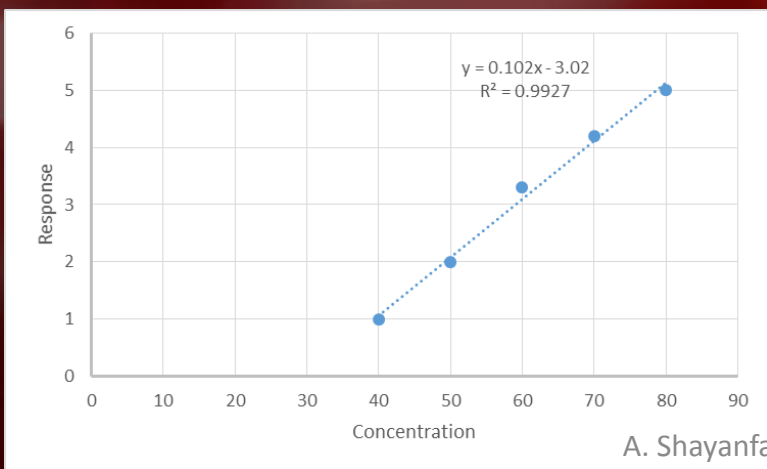
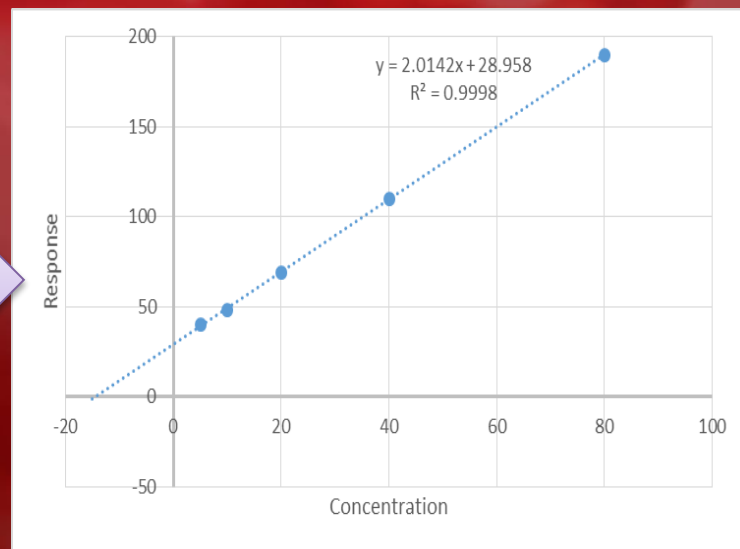
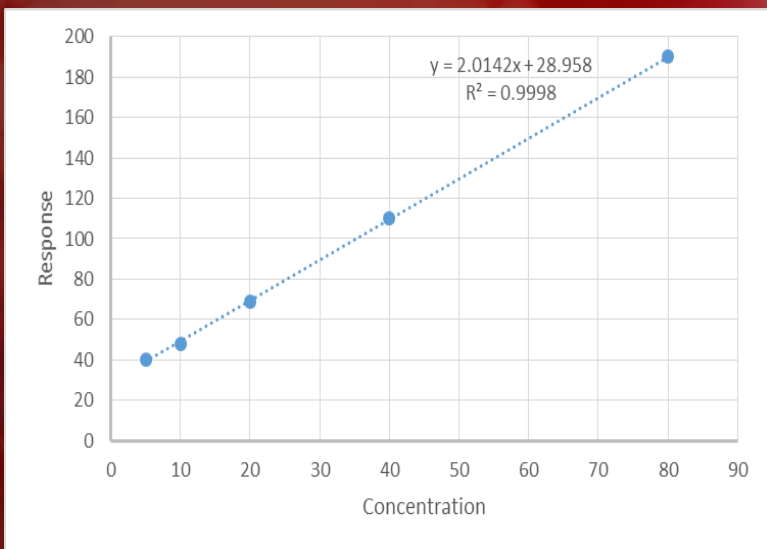
Beware R²

C (mg/L)	Area
0.10	56123
0.20	114024
1.00	456123
5.00	2435621
10.00	4659231
20.00	9023487
50.00	18532145
100.00	37054932



Check Accuracy!

Beware of intercept!



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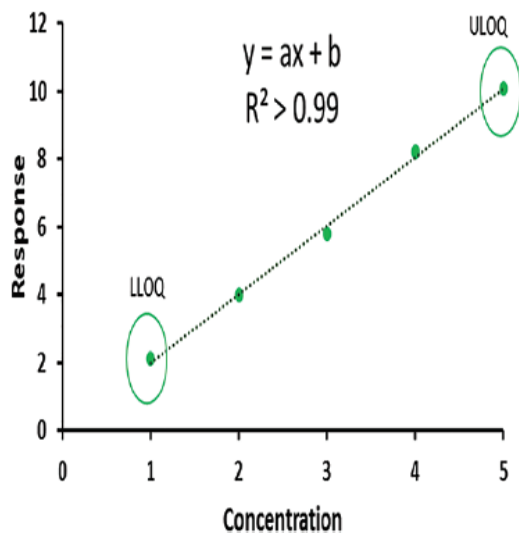
Developing New Criteria for Validity Evaluation of Analytical Methods

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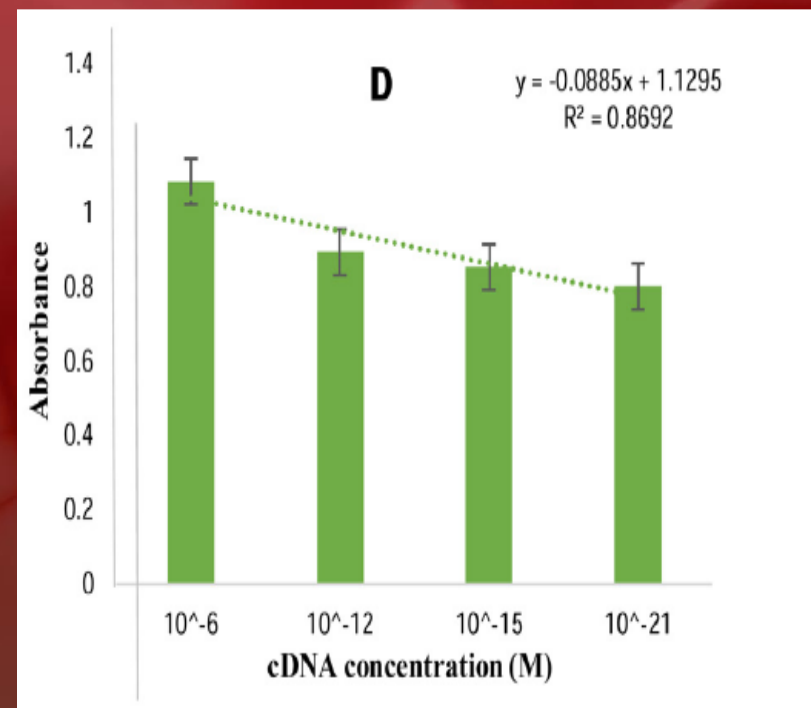
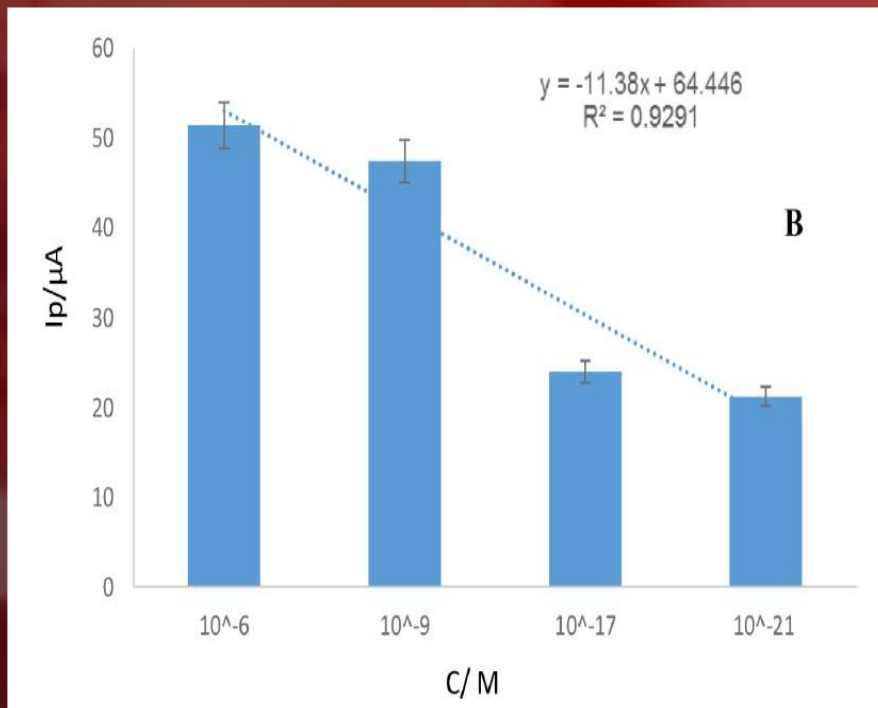
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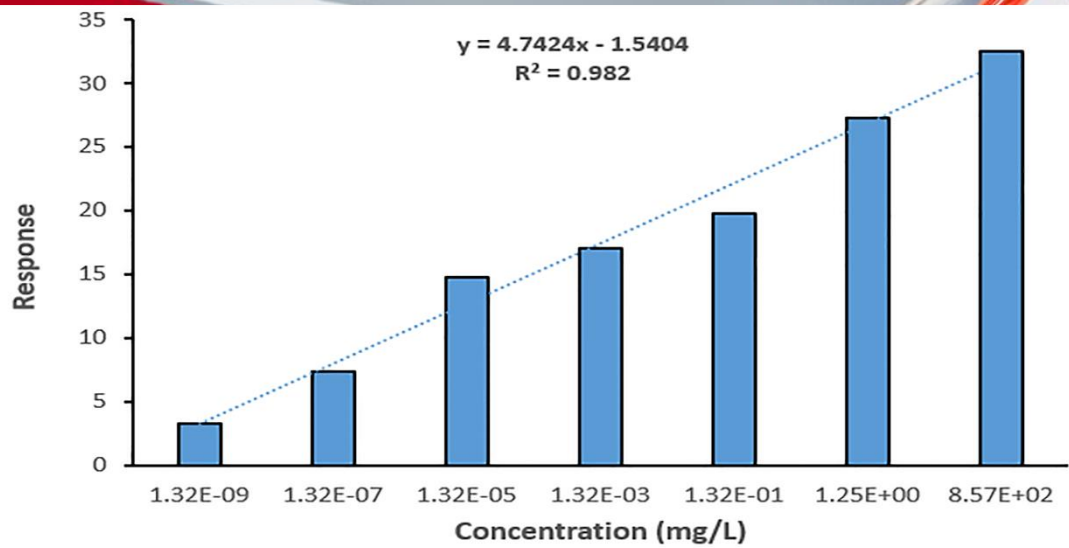
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$$\text{Ratio} = \frac{((a \times \text{ULOQ} + b) / (a \times \text{LLOQ} + b))}{(\text{ULOQ} / \text{LLOQ})}$$

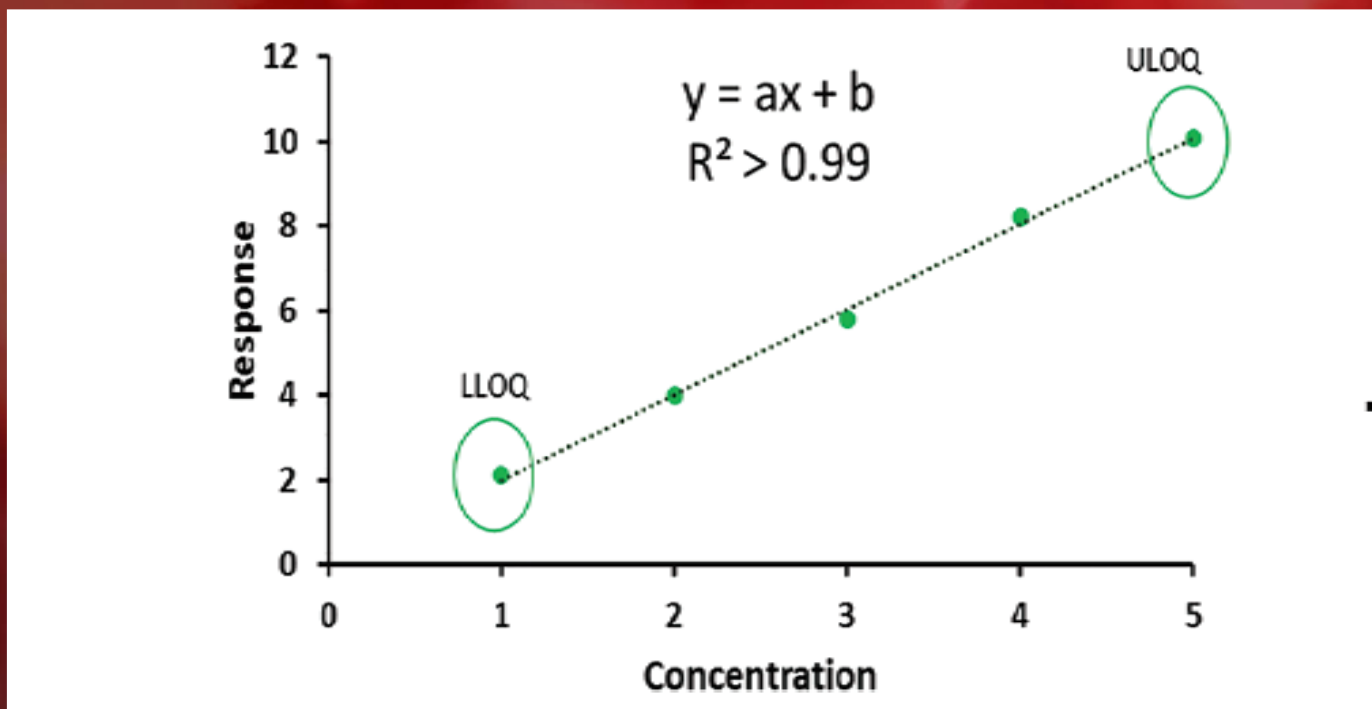
Beware of type of chart for plotting of calibration curve





(a)

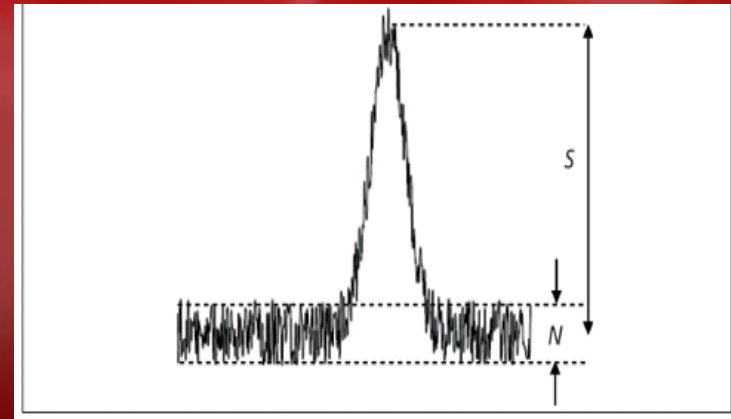
LOD and LOQ or LLOQ



- LOD and LOQ: Various methods calculation

LOD and LOQ

- Visual evaluation



- Based on calibration curve:
- $LOD=3*S/Slope$, $LOQ=10*S/Slope$
- S: Standards deviation blank or calibration curve

EXTENDED ABSTRACT

Are LOD and LOQ Reliable Parameters for Sensitivity Evaluation of Spectroscopic Methods?

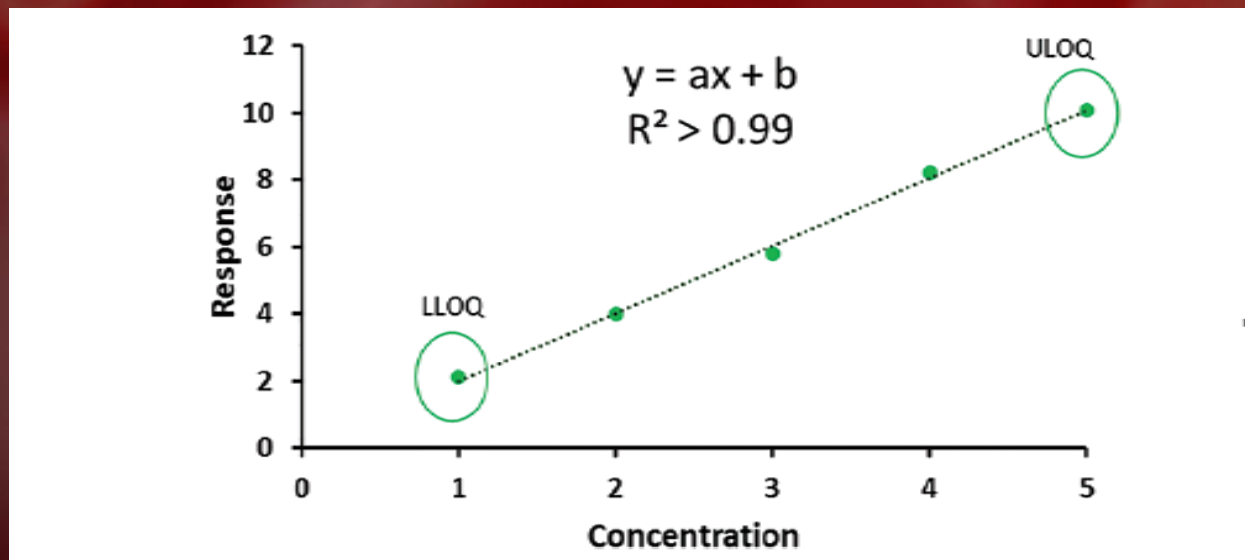
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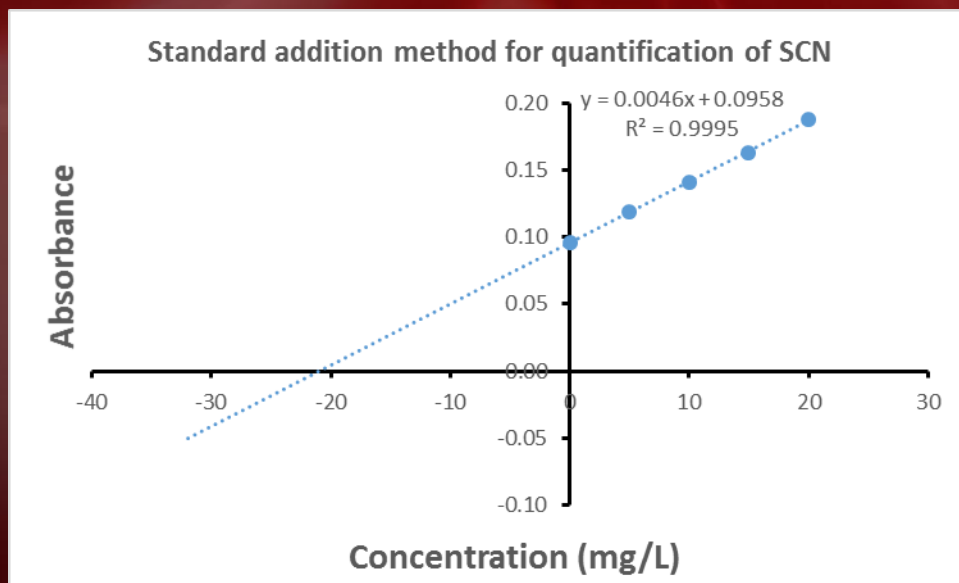
Tabriz University of Medical Sciences, Pharmaceutical Analysis Research Center and Faculty of Pharmacy, Tabriz, Iran

FDA validation: Precision and accuracy <20%-linear range



Calibration curve for quantification of endogenous compounds

- Calibration curve: in the same matrix
- Endogenous compounds: Q10 or SCN
- Standard addition method





Selectivity in Bioanalysis

- Various plasma sample

Developed method should be checked at least six plasma sample.

Different plasma matrix of patient: Thalassemia

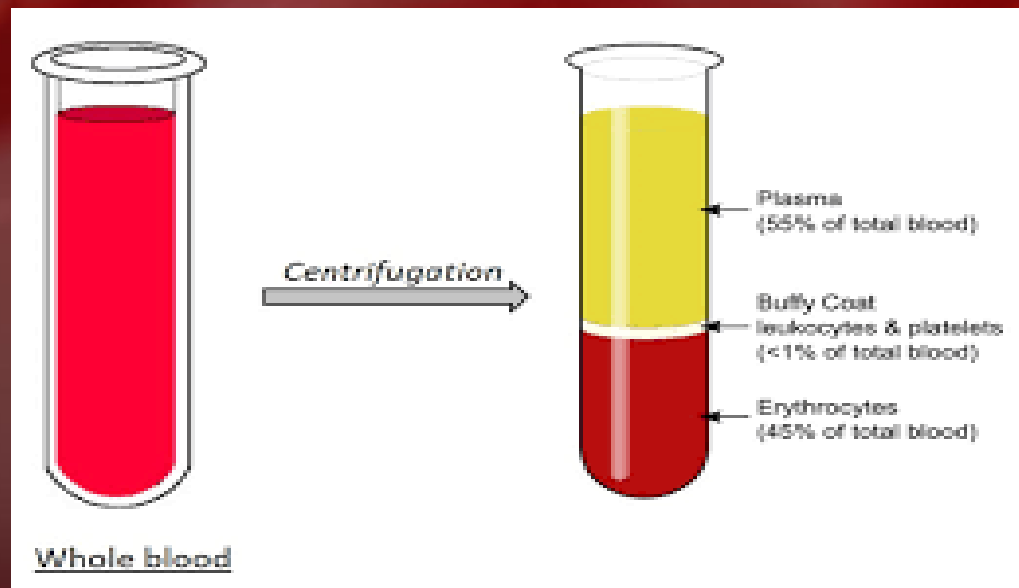
- Co-administrated drugs:

Quantification of indoxyl sulfate in real sample of CKD patient:

Metoprolol, carvedilol, atenolol, propranolol, bisprolol, nephrovit, atorvastatin, amolodipine, dilitizem, verapamil, nifidipine, furosemide, sprinolactone, minoxidil, gemfibrozil, losartan, valsartan, erythropoietin, prazosin, clonidine, nitroglycerin, folic acid, calcium carbonate

Extraction method in Bioanalysis

- Protein precipitation and extraction:
 - Preconcentration and clean up





Extraction method in Bioanalysis

- Simple extraction method (high number of real samples)
- Cost: Commercial solid phase extraction
- Nanoparticles for extraction!



Beware of complex extraction method!

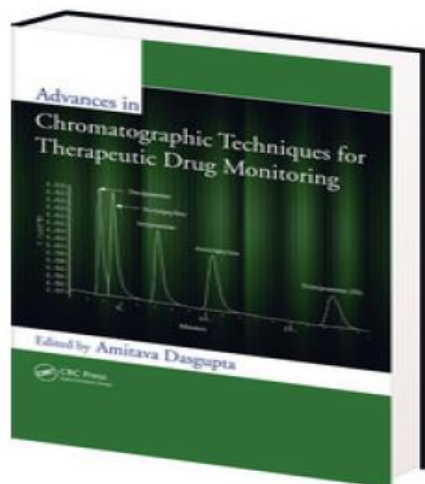
- Complex analytical methods are a good choice for publication of article in high quality journal.
- But the most of them are not applicable for real samples



Conclusion

- Quantification of analyte concentration
- Which parameters should be considered in quantification of analyte concentration?
 - Accuracy
 - Precision
 - Calibration curve and linear range
 - Common errors in calibration curve
- Selectivity in Bioanalysis
- Extraction method in Bioanalysis

References for further study



Advances in Chromatographic Techniques for Therapeutic Drug Monitoring

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Editorial



Analytical Method Validation: The Importance for Pharmaceutical Analysis

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Analytical methods play significant role in many branches such as, food production, natural product analysis, environmental analysis, pharmaceutical and biomedical analysis, and life sciences, etc. In order to reach reliable, accurate and repeatable data validated analytical methods need to achieve this aim.¹⁻³

Validation is the key factor in controlling the reliability of a method that is determined by validation results, where specificity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ), sensitivity and applicability are reported. Validated analytical methods play a major role in achieving the quality and safety of the final product especially in pharmaceutical industry.

- Linearity
- Range
- Limit of detection (LOD)
- Limit of quantification (LOQ)
- Accuracy
- Precision
 - Repeatability
 - Intermediate precision
 - Reproducibility
- Robustness
- Ruggedness
- Stability
- Applicability



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