AMD & AMV and statistical analysis in accordance to ICH Q14 and Q2

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T.ME/ANALYTICALMETHODVALIDATION

ICH HARMONISED GUIDELINE

ANALYTICAL PROCEDURE DEVELOPMENT

Q14

Final Version

Adopted on 1 November 2023

Q14
Document History

Code	History	Date
Q14	Endorsement by the Members of the ICH Assembly under *Step 2* and release for public consultation	24 March 2022
Q14	Adoption by the Regulatory Members of the ICH Assembly under *Step 4*	1 November 2023

General Consideration for Analytical Procedures

- Minimal versus enhanced approaches to analytical procedure development
- Analytical Procedure Lifecycle

General Consideration for Analytical Procedures

The goal of development is to obtain an analytical procedure fit for the intended purpose:

to measure an attribute or attributes of the material with the needed specificity/selectivity, accuracy, precision over the reportable range.

هدف توسعه به دست آوردن یک روش آنالیز متناسب با هدف مورد نظر است:

اندازه گیری یک ویژگی یا ویژگی های ماده به صورت اختصاصی یا انتخابی، با دقت و صحت مورد نیاز در محدوده قابل قبول برای گزارش

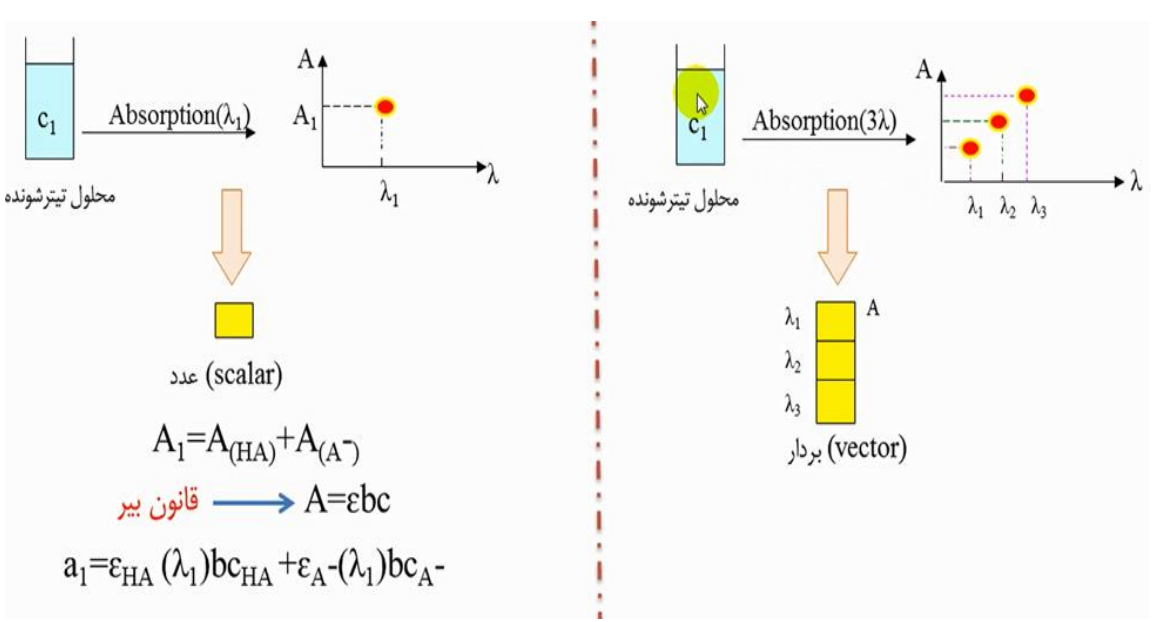
Minimal Approaches

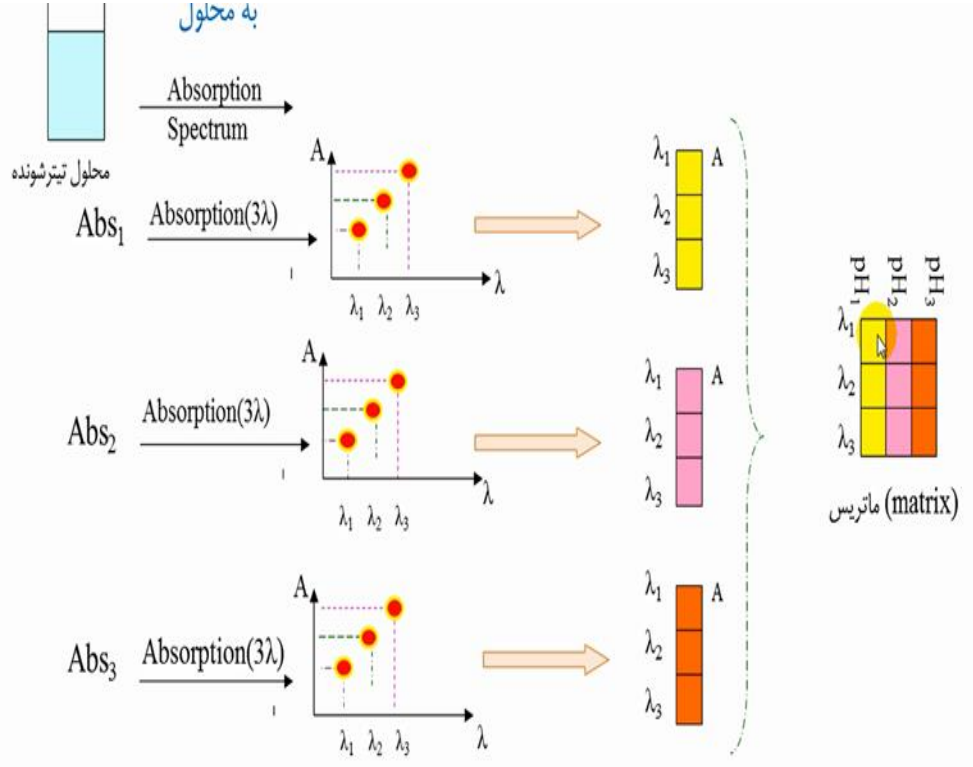
- Selecting an appropriate technology
- Conducting studies to evaluate analytical procedure performance characteristics
- Documenting the analytical procedure including the analytical procedure control strategy

Enhanced Approaches

- Conducting risk assessment
- Conducting uni- or multivariate experiments
- Defining an analytical procedure control strategy including set-points and/or ranges for relevant analytical procedure parameters.

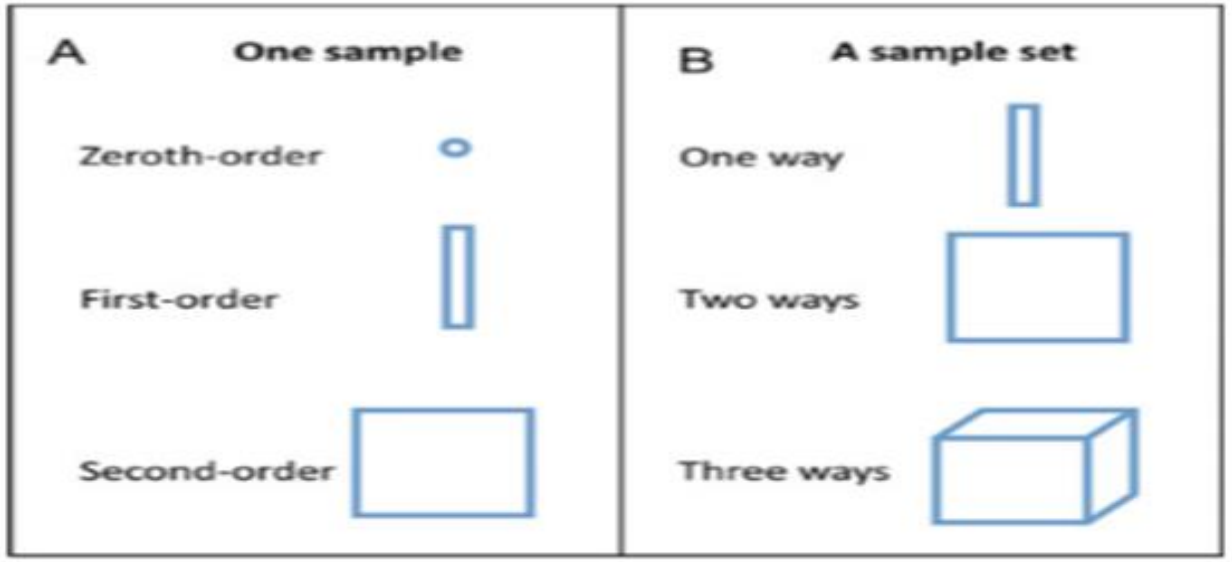
Introduction to Data Science





Orders and Ways

ترتیب یا مرتبه (Order) یک ویژگی داده دستگاهی برای یک نمونه آزمایشی است و way یک ویژگی داده برای یک مجموعه نمونه است. آنها نوع مدل ریاضی را که می توان با داده ها ساخت را مشخص می کنند.



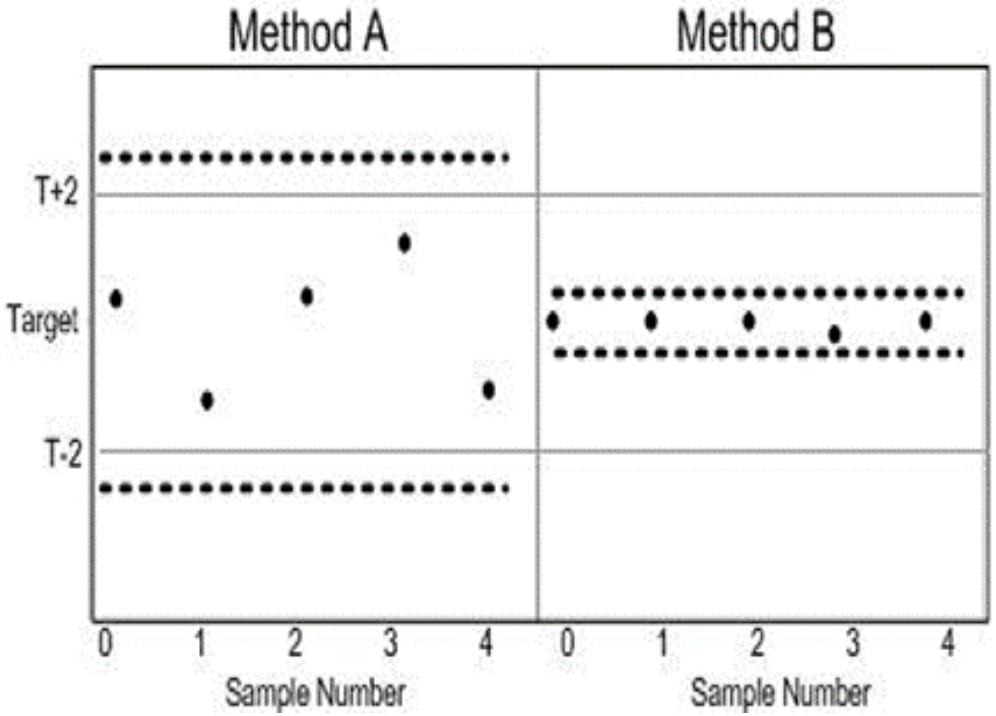
Analytical Target Profile

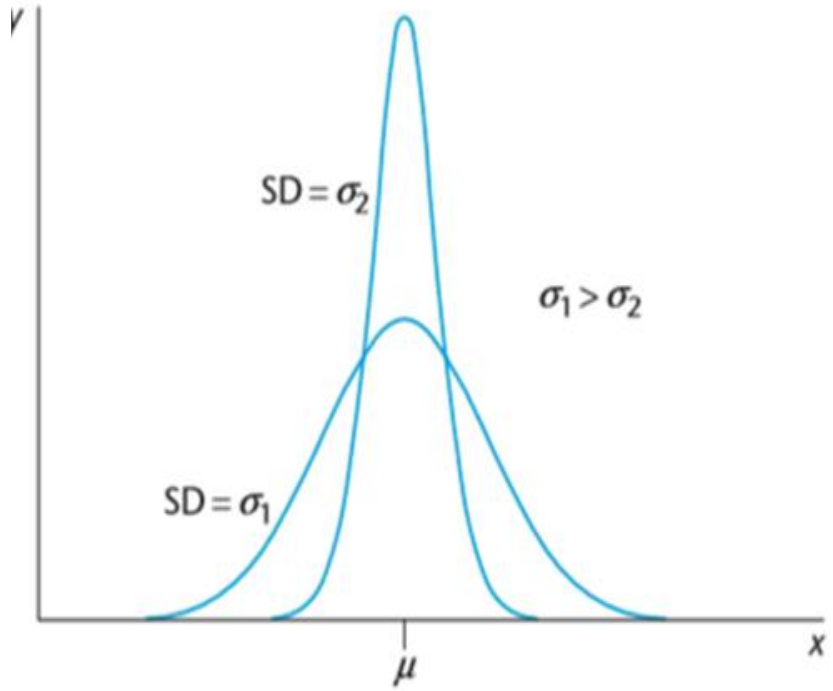
ATP شامل توصیفی از هدف مورد نظر از روش تحلیلی، جزئیات مناسب در مورد ویژگی های محصول که باید اندازه گیری شود و ویژگی های عملکرد مرتبط با معیارهای عملکرد مرتبط است.

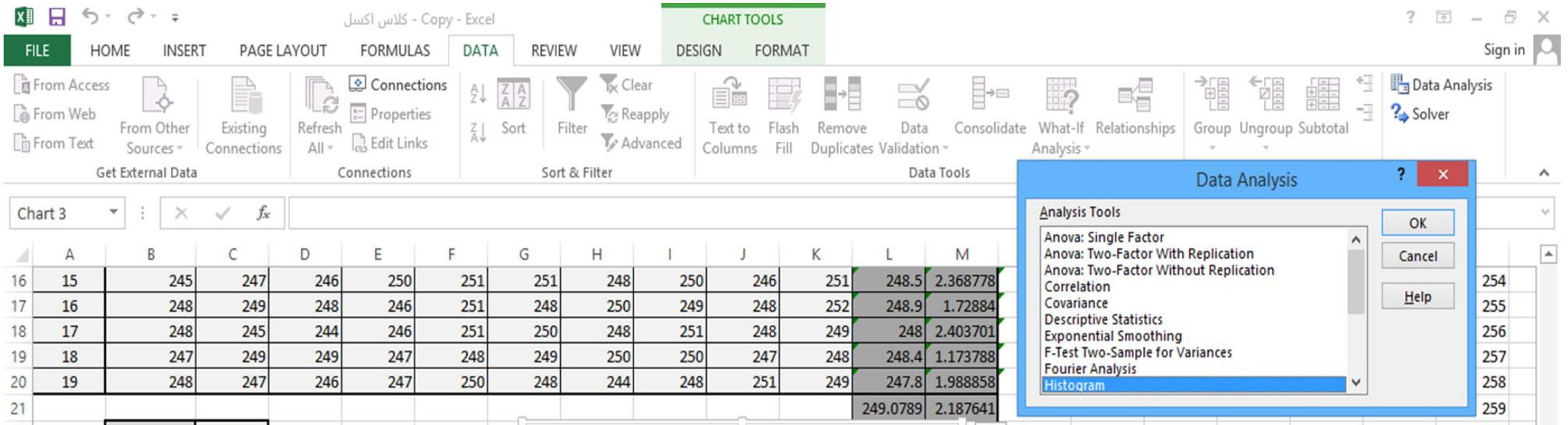
- شامل الزامات اندازه گیری برای یک یا چند ویژگی کیفیت است.
- انتخاب فناوری آنالیز را هدایت می کند.

مستندات رسمی و ارائه ATP اختیاری است اما می تواند ارتباطات نظارتی را بدون توجه به رویکرد توسعه انتخاب شده تسهیل کند.

Analytical Target Profile







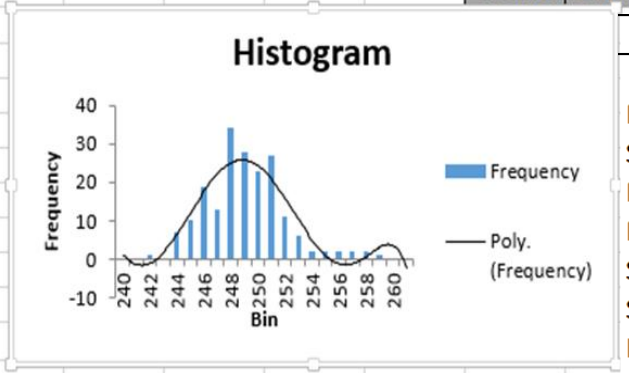
	A	B	C	D	E	F	G	H	I	J	K	L	M
16	15	245	247	246	250	251	251	248	250	246	251	248.5	2.368778
17	16	248	249	248	246	251	248	250	249	248	252	248.9	1.72884
18	17	248	245	244	246	251	250	248	251	248	249	248	2.403701
19	18	247	249	249	247	248	249	250	250	247	248	248.4	1.173788
20	19	248	247	246	247	250	248	244	248	251	249	247.8	1.988858
21												249.0789	2.187641

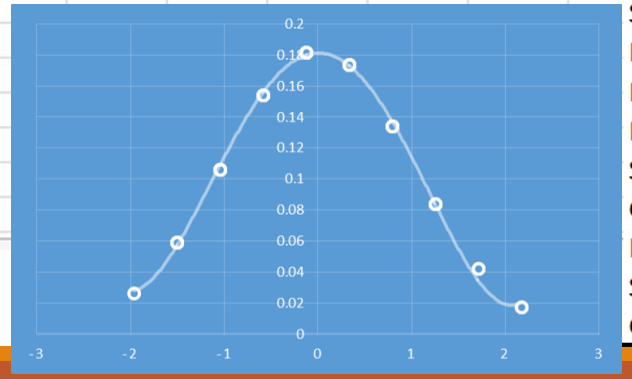
22	n	10
23	K	3
24	CL	249.0789
25	Process SD	2.187641
26	σx-bar	0.691793
27	UCL	251.1543
28	LCL	247.0036
29	USL	269
30	LSL	231
31	α	0.0027
32	ARL	370.3983
33		
34	CP	2.895052
35	CPU	3.035394
36	CPL	2.75471
37	CPK	2.75471
38		

Bin	Frequency
240	0
241	0
242	1
243	0
244	7
245	10
246	19
247	13
248	34
249	28
250	23
251	27
252	11
253	6
254	2

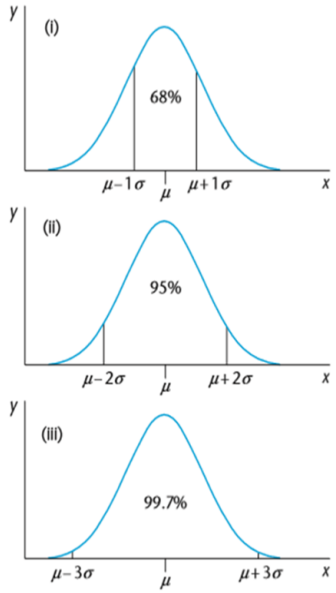
4.150756

38





Column1	
Mean	248.2777778
Standard Error	0.296045054
Median	248
Mode	248
Standard Deviatior	2.175477971
Sample Variance	4.732704403
Kurtosis	-0.470005121
Skewness	0.130958117
Range	9
Minimum	244
Maximum	253
Sum	13407
Count	54
Largest(1)	253
Smallest(1)	244
Confidence Level(9	0.593791182



$$y = \left(\frac{1}{\sigma\sqrt{2\pi}}\right)\exp\left[\left(-\frac{(x-\mu)^2}{2\sigma^2}\right)\right]$$

Knowledge and risk management in Analytical Procedure Development and continual improvement

- Knowledge Management
- Risk Management

Knowledge Management

دانش قبلی به طور صریح یا ضمنی برای اطلاع رسانی تصمیمات در طول توسعه رویه تحلیلی و مدیریت چرخه حیات استفاده می شود.

- دانش درون سازمانی (Internal knowledge) حاصل از توسعه اختصاصی و تجربه آنالیزی یک شرکت
- دانش برون سازمانی(External knowledge) مانند ارجاع به انتشارات علمی و فنی یا اصول علمی تثبیت شده باشد.
- دانش محصول قبلی(Prior product knowledge) نقش مهمی در شناسایی تکنیک های تحلیلی مناسب دارد.

دانش مربوط به روش های آنالیز باید به طور فعال در طول چرخه عمر محصول مدیریت شود

Risk Management

- ارزیابی تأثیر بالقوه پارامترهای روش تحلیلی بر عملکرد روش تحلیلی(ANOVA)
- شناسایی و اولویت بندی پارامترهای روش تحلیلی برای بررسی تجربی(Screening)
- نیاز و میزان نظارت مستمر را به عنوان بخشی از بررسی ریسک اطلاع دهید(Test Verification)

ارتباطات ریسک باید برای حمایت از بهبود مستمر عملکرد رویه تحلیلی در طول چرخه عمر آن استفاده شود.

نتیجه مدیریت ریسک کیفیت باید در بخش‌های مربوطه سیستم کیفیت دارویی متقاضی (PQS) ICH Q10 مستند شود.

ANOVA

با ANOVA برای تجزیه و تحلیل تفاوت‌های میان میانگین‌های گروهی، انتخاب ویژگی بهینه‌سازی و اطمینان از استحکام یک مدل قوی، عمیقاً فرو بروید.

تحلیل واریانس (ANOVA) چیست؟

آنالیز واریانس (ANOVA) یک روش آماری است که برای تجزیه و تحلیل تفاوت بین میانگین‌های گروهی در یک نمونه استفاده می‌شود. این کار را با بررسی میزان واریانس در هر گروه و مقایسه آن با مقدار واریانس بین گروه ها انجام می دهد

P-Value interpretation in ANOVA

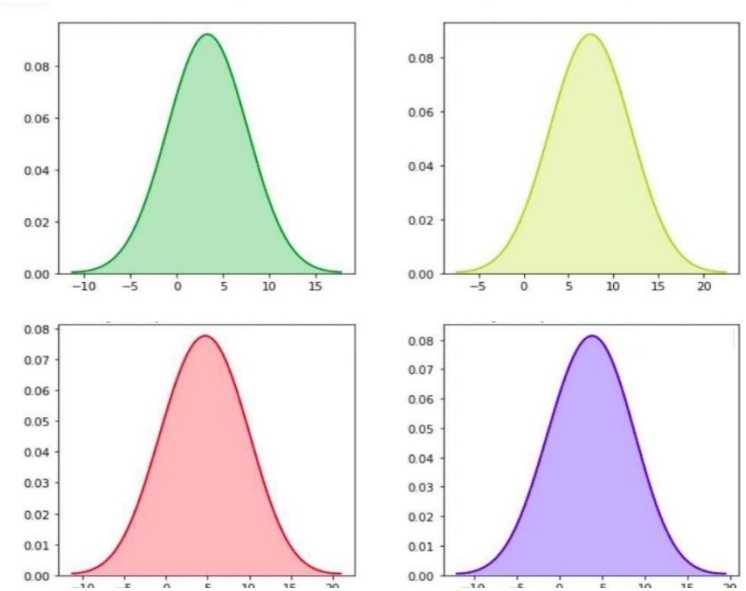
P-value ≤ α : تفاوت بین برخی از میانگین ها از نظر آماری معنی دار است

اگر مقدار p کمتر یا مساوی با سطح معنی‌داری باشد، فرضیه صفر را رد می‌کنید و نتیجه می‌گیرید که همه میانگین‌های جمعیت برابر نیستند. از دانش تخصصی خود برای تعیین اینکه آیا تفاوت ها عملاً قابل توجه هستند یا خیر استفاده کنید. برای اطلاعات بیشتر به بخش آماری و اهمیت عملی بروید.

P-value > α : تفاوت بین میانگین ها از نظر آماری معنی دار نیست

اگر مقدار p بزرگتر از سطح معنی داری باشد، شواهد کافی برای رد فرضیه صفر مبنی بر اینکه میانگین جمعیت همه برابر هستند را ندارید.

ANOVA



Conditions	Replicate measurements	Mean
A Freshly prepared	102, 100, 101	101
B Stored for 1 hour in the dark	101, 101, 104	102
C Stored for 1 hour in subdued light	97, 95, 99	97
D Stored for 1 hour in bright light	90, 92, 94	92
	Overall mean	98

Anova: Single Factor

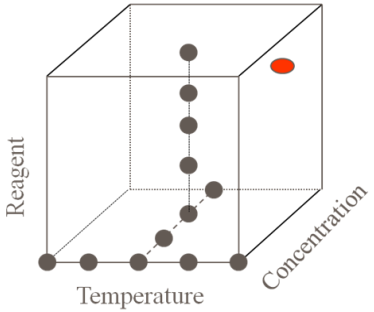
SUMMARY

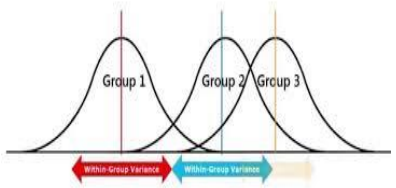
Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	303	101	1
Column 2	3	306	102	3
Column 3	3	291	97	4
Column 4	3	276	92	4

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	186	3	62	20.66667	0.0004	4.066181
Within Groups	24	8	3			
Total	210	11				

تکنیک های غربالگری فاکتور های موثر بر نتایج آنالیز(Screening)



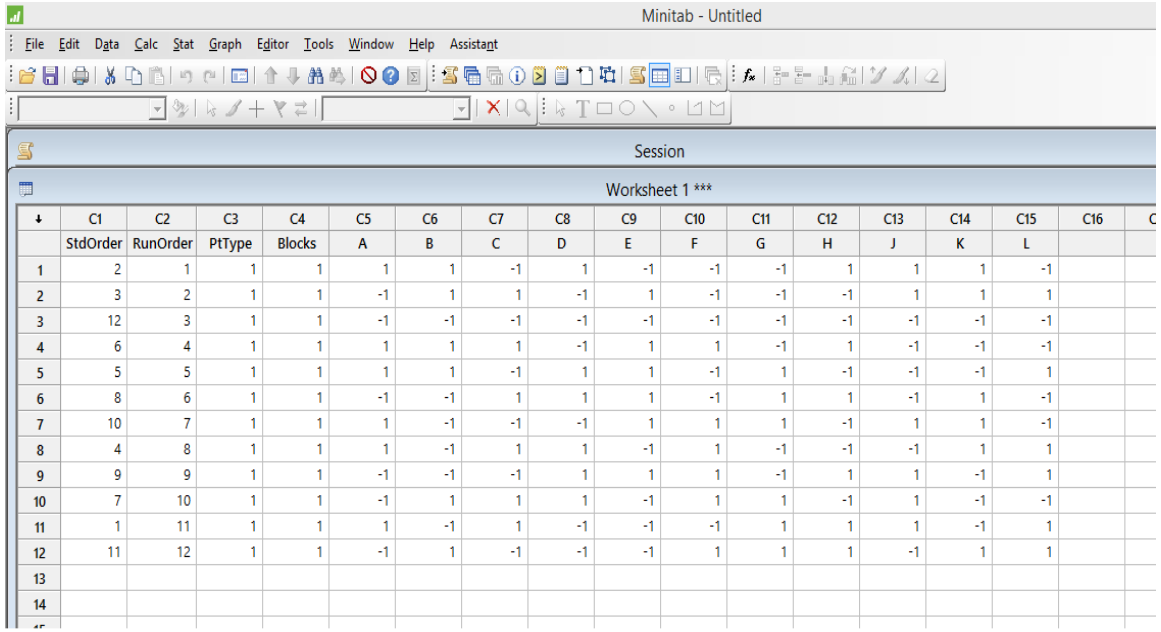


تکنیک های تصادفی سازی(Technique of Randomization)

تحلیل واریانس دو طرفه(Two-Way ANOVA)

طرح یک فاکتور در یک زمان(OFAT (One-Factor-at-a-time design

طرح پلاکت برمن(Plackett-Burman)

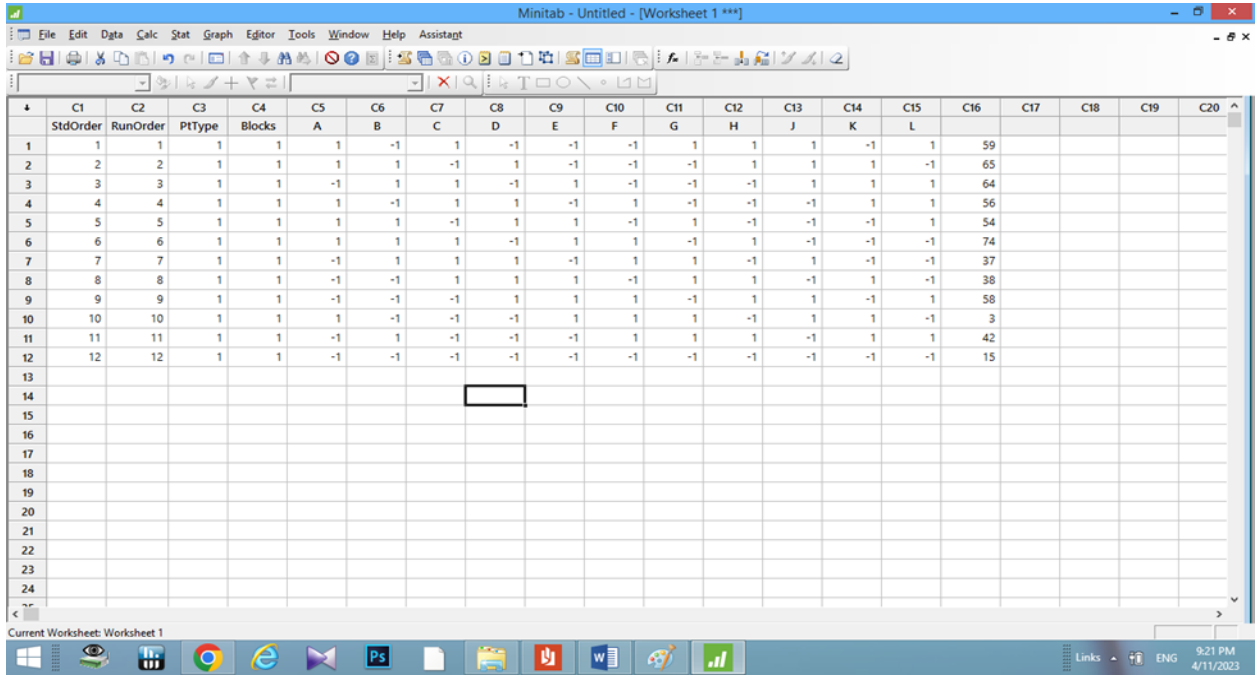


	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16
	StdOrder	RunOrder	PtType	Blocks	A	B	C	D	E	F	G	H	J	K	L	
1	2	1	1	1	1	1	-1	1	-1	-1	-1	1	1	1	-1	
2	3	2	1	1	-1	1	1	-1	1	-1	-1	-1	1	1	1	
3	12	3	1	1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	
4	6	4	1	1	1	1	1	-1	1	1	-1	1	-1	-1	-1	
5	5	5	1	1	1	1	-1	1	1	-1	1	-1	-1	-1	1	
6	8	6	1	1	-1	-1	1	1	1	-1	1	1	-1	1	-1	
7	10	7	1	1	1	-1	-1	-1	1	1	1	-1	1	1	-1	
8	4	8	1	1	1	-1	1	1	-1	1	-1	-1	-1	1	1	
9	9	9	1	1	-1	-1	-1	1	1	1	-1	1	1	-1	1	
10	7	10	1	1	-1	1	1	1	-1	1	1	-1	1	-1	-1	
11	1	11	1	1	1	-1	1	-1	-1	-1	1	1	1	-1	1	
12	11	12	1	1	-1	1	-1	-1	-1	1	1	1	-1	1	1	
13																
14																

طرح پلاکت برمن (Plackett-Burman)

$$A + B \rightarrow C$$

Factor		Low	High
x_1	NaOH (%)	40	50
x_2	Temperature (°C)	80	110
x_3	Nature of catalyst	A	B
x_4	Stirring	Without	With
x_5	Reaction Time (min)	90	210
x_6	Volume of Solvent (ml)	100	200
x_7	Volume of NaOH (ml)	30	60
x_8	Substrate/NaOH ratio (mol/ml)	0.5×10^{-3}	1×10^{-3}
x_9	Catalyst/substrate ratio (mol/ml)	4×10^{-3}	6×10^{-3}
x_{10}	Reagent/substrate ratio (mol/mol)	1	1.25



$$C16 = 47.08 + 4.750\,A + 8.917\,B + 7.583\,C + 4.250\,D + 1.417\,E - 2.083\,F - 8.250\,G + 8.917\,H + 0.5833\,J - 2.417\,K + 8.417\,L$$

Plackett-Burman Design

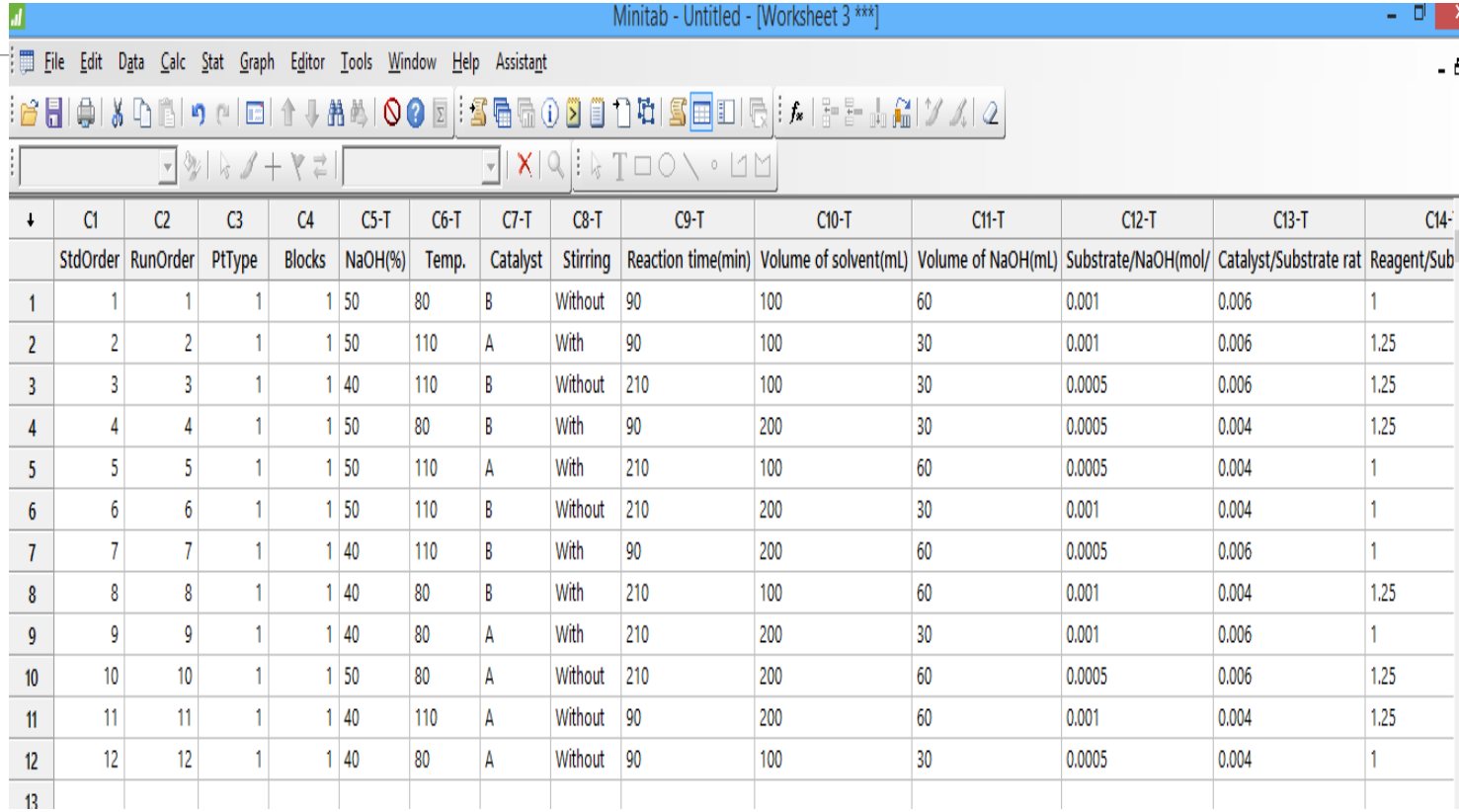
Factors: 11 Replicates: 1

Base runs: 12 Total runs: 12

Base blocks: 1 Total blocks: 1

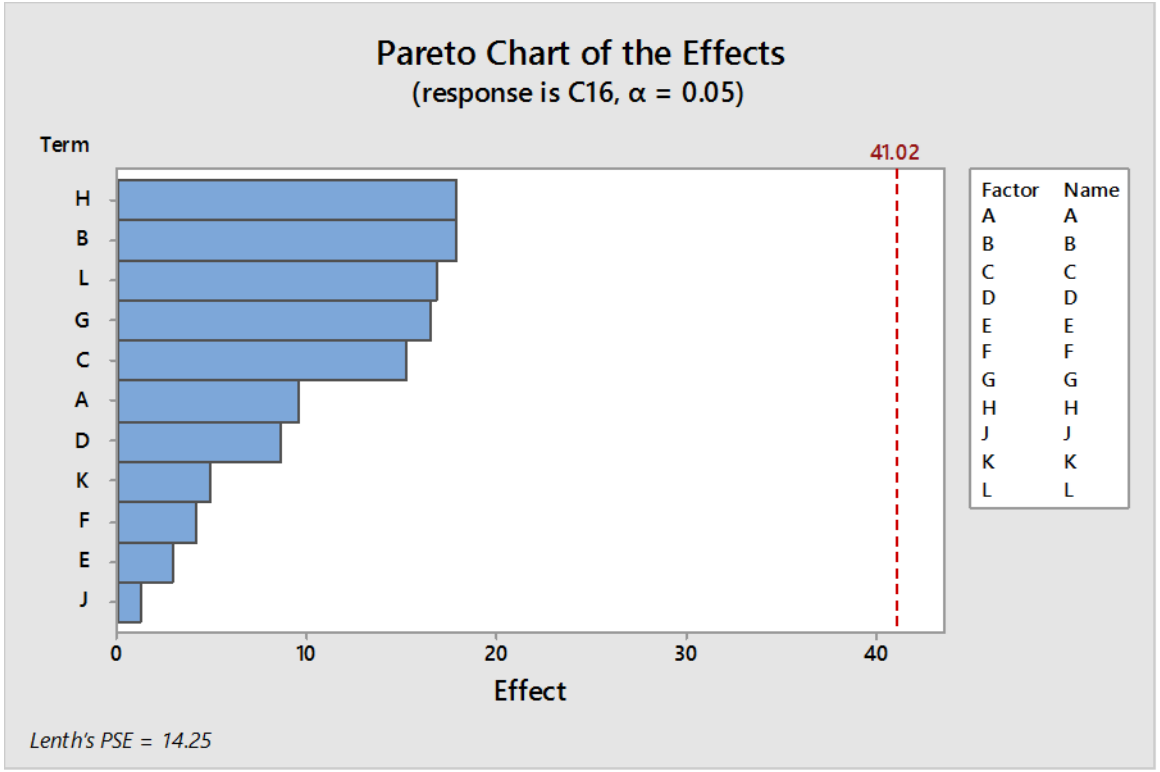
Design Table

Run	Blk	A	B	C	D	E	F	G	H	J	K	L
1	1	+	−	+	−	−	−	+	+	+	−	+
2	1	+	+	−	+	−	−	−	+	+	+	−
3	1	−	+	+	−	+	−	−	−	+	+	+
4	1	+	−	+	+	−	+	−	−	−	+	+
5	1	+	+	−	+	+	−	+	−	−	−	+
6	1	+	+	+	−	+	+	−	+	−	−	−
7	1	−	+	+	+	−	+	+	−	+	−	−
8	1	−	−	+	+	+	−	+	+	−	+	−
9	1	−	−	−	+	+	+	−	+	+	−	+
10	1	+	−	−	−	+	+	+	−	+	+	−
11	1	−	+	−	−	−	+	+	+	−	+	+
12	1	−	−	−	−	−	−	−	−	−	−	−



	C1	C2	C3	C4	C5-T	C6-T	C7-T	C8-T	C9-T	C10-T	C11-T	C12-T	C13-T	C14-
	StdOrder	RunOrder	PtType	Blocks	NaOH(%)	Temp.	Catalyst	Stirring	Reaction time(min)	Volume of solvent(mL)	Volume of NaOH(mL)	Substrate/NaOH(mol/	Catalyst/Substrate rat	Reagent/Sub
1	1	1	1	1	50	80	B	Without	90	100	60	0.001	0.006	1
2	2	2	1	1	50	110	A	With	90	100	30	0.001	0.006	1.25
3	3	3	1	1	40	110	B	Without	210	100	30	0.0005	0.006	1.25
4	4	4	1	1	50	80	B	With	90	200	30	0.0005	0.004	1.25
5	5	5	1	1	50	110	A	With	210	100	60	0.0005	0.004	1
6	6	6	1	1	50	110	B	Without	210	200	30	0.001	0.004	1
7	7	7	1	1	40	110	B	With	90	200	60	0.0005	0.006	1
8	8	8	1	1	40	80	B	With	210	100	60	0.001	0.004	1.25
9	9	9	1	1	40	80	A	With	210	200	30	0.001	0.006	1
10	10	10	1	1	50	80	A	Without	210	200	60	0.0005	0.006	1.25
11	11	11	1	1	40	110	A	Without	90	200	60	0.001	0.004	1.25
12	12	12	1	1	40	80	A	Without	90	100	30	0.0005	0.004	1
13														

Pareto Chart



Evaluation of robustness and parameters ranges of analytical procedures

- Robustness
- Analytical procedure parameter range

Robustness

استحکام یک روش آنالیز معیاری از ظرفیت آن برای برآورده کردن معیارهای عملکرد مورد انتظار در طول استفاده عادی است.

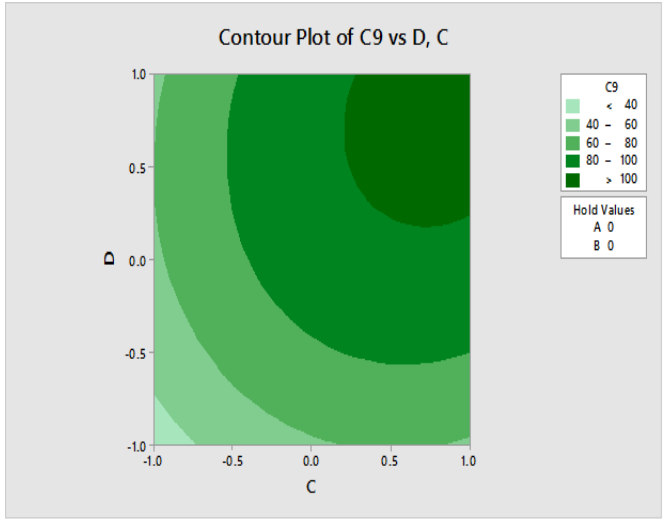
استحکام با تغییرات عمدی **فاکتورهای روش آنالیز** آزمایش می‌شود

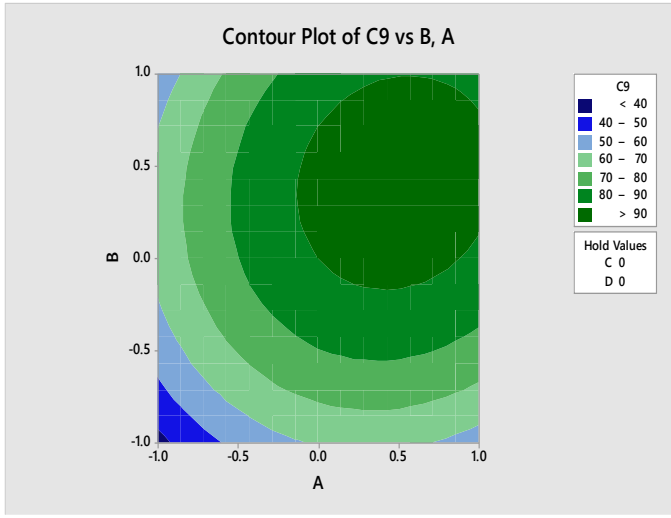
دانش زمینه ای و ارزیابی ریسک می‌تواند انتخاب پارامترهایی را برای بررسی در طول مطالعه استحکام نشان دهد.

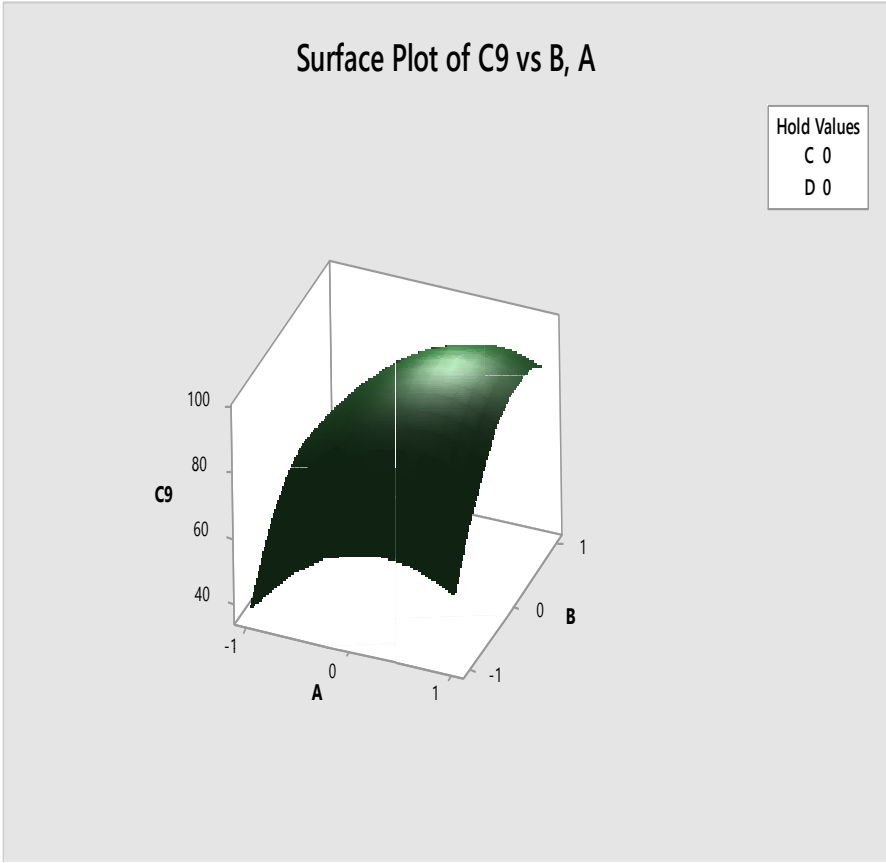
آن پارامترهایی که احتمالاً بر عملکرد رویه در طول دوره استفاده مورد نظر تأثیر می‌گذارند باید مورد مطالعه قرار گیرند.

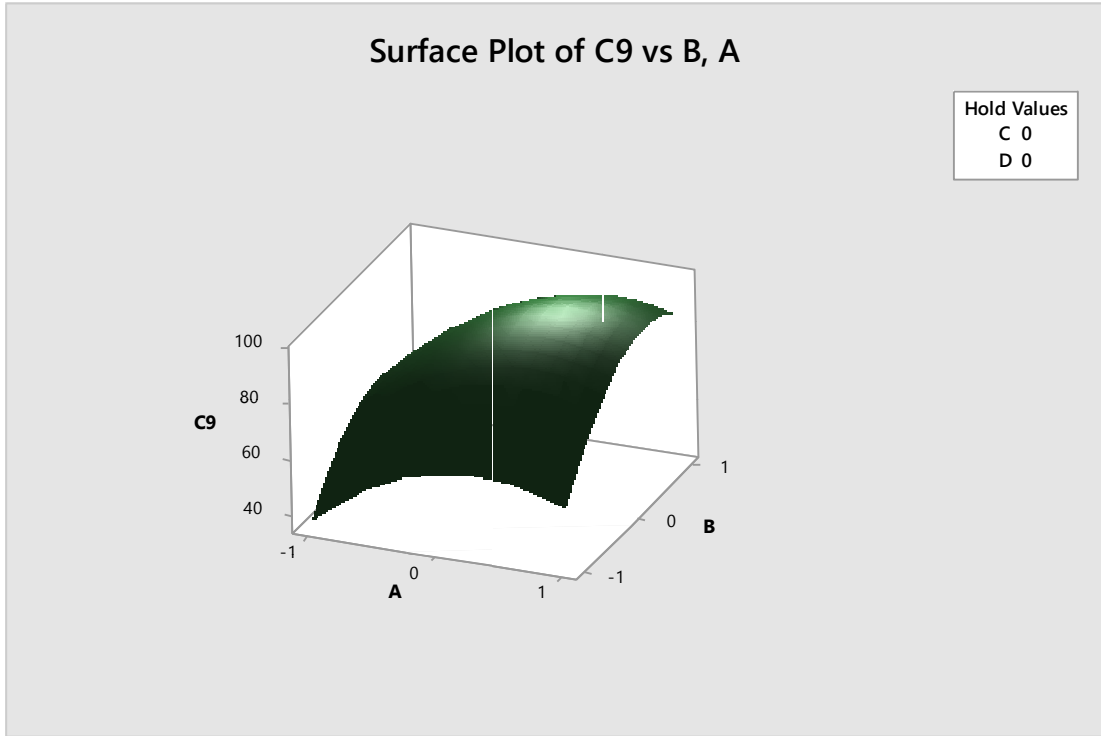
برای اکثر روش ها، ارزیابی استحکام در طول توسعه انجام می شود. اگر ارزیابی استحکام قبلاً در طول توسعه انجام شده باشد، نیازی به تکرار آن در طول اعتبارسنجی نیست

نتیجه ارزیابی استحکام باید مستند شده و همچنین در استراتژی کنترل روش تحلیلی منعکس شود.


Contour Plot of C9 vs D, C
C9
< 40
40 – 60
60 – 80
80 – 100
> 100
Hold Values
A 0
B 0
D
C
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0


Contour Plot of C9 vs B, A
C9
< 40
40 – 50
50 – 60
60 – 70
70 – 80
80 – 90
> 90
Hold Values
C 0
D 0
B
A
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0


Surface Plot of C9 vs B, A
Hold Values
C 0
D 0
C9
100
80
60
40
A
-1
0
1
B
1
0
-1


Surface Plot of C9 vs B, A
Hold Values
C 0
D 0
C9
100
80
60
40
A
-1
0
1
B
-1
0
1

Analytical procedure control strategy

- Established conditions for analytical procedures

- EC ها را می توان از ارزیابی ریسک، دانش قبلی و نتایج حاصل از آزمایش های تک متغیره و/یا چند متغیره شناسایی کرد.

- با یک رویکرد حداقلی، ماهیت و وسعت ECها به رویکرد توسعه، پیچیدگی روش تحلیلی و چگونگی تأثیر پارامترها و سایر عوامل بر عملکرد روش تحلیلی بستگی دارد.

- با یک رویکرد پیشرفته، باید درک بیشتری از الزامات اندازه گیری، مناسب بودن فناوری های موجود و رابطه بین پارامترهای روش تحلیلی و عملکرد وجود داشته باشد.

Established conditions for analytical procedures

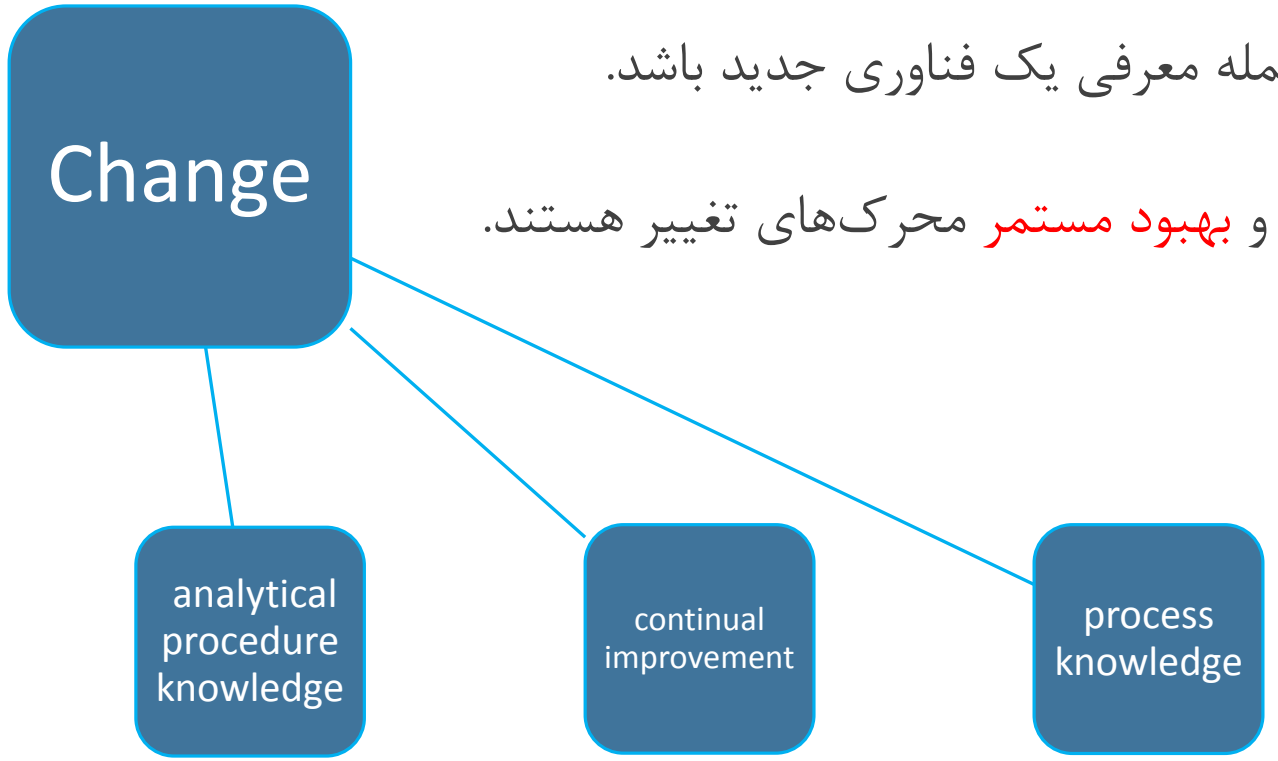
EC ها می توانند شامل موارد زیر باشند:

- Performance characteristics and associated criteria
  - ویژگی های عملکرد و معیارهای مرتبط به عنوان مثال، شامل ATP
- Analytical procedure principle
  - اصل روش آنالیز(به عنوان مثال، مبنای فیزیکوشیمیایی یا تکنولوژی خاص).
- SST and sample suitability assessment criteria
  - معیارهای ارزیابی شایستگی SST و نمونه.
- Set points and/or ranges for one or more analytical procedure parameters
  - نقاط و/یا محدوده را برای یک یا چند پارامتر رویه تحلیلی تنظیم کنید.

Lifecycle management and post approval changes of analytical procedures

تغییرات در روش های تحلیلی می تواند در طول چرخه عمر محصول رخ دهد و می تواند شامل اصلاح رویه های موجود یا جایگزینی کامل از جمله معرفی یک فناوری جدید باشد.

دانش فرآیند، دانش فرآیند تحلیلی و بهبود مستمر محرک‌های تغییر هستند.



Examples of analytical procedure change evaluation

Risk Factor: Extent of change

Change of analytical procedure principle (physicochemical/biochemical basis)

Bridging strategy:

Full validation of new procedure And **Comparative analysis of representative samples and reference materials.** And/or Demonstration that the analytical procedure's ability to discriminate between acceptable and non-acceptable results remains comparable

Evidence of the suitability of a new procedure:

Analytical procedure performance characteristics are evaluated and criteria are met after the change And Results are comparable after change or differences are acceptable and potential impact on specification evaluate

Examples of analytical procedure change evaluation

Risk Factor: Extent of change

Change within same analytical procedure principle

Bridging strategy:

Partial or full revalidation of the analytical procedure performance characteristics affected by the change And, as appropriate **Comparative analysis of representative samples and reference materials** And/or Demonstration that the analytical procedure's ability to discriminate between acceptable and non-acceptable results remains comparable

Evidence of the suitability of a new procedure

Analytical procedure attributes are evaluated and criteria are met after change And, as appropriate Results are comparable after change or differences are acceptable and potential impact on specification evaluated

Examples of analytical procedure change evaluation

Risk Factor: Extent of change

Transfer of analytical procedure to a different site with no change in procedure itself

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Bridging strategy:

Partial or full revalidation of the analytical procedure performance characteristics and/or **Comparative analysis of representative samples and reference materials** or Justification for not performing additional transfer experiments

Evidence of the suitability of a new procedure

Analytical procedure attributes are evaluated and criteria are met after change And/or Results are comparable

ICH HARMONISED GUIDELINE

VALIDATION OF ANALYTICAL PROCEDURES

Q2(R2)

Final Version

Adopted on 1 November 2023

ICH HARMONISED GUIDELINE

Q2(R2)
Document History

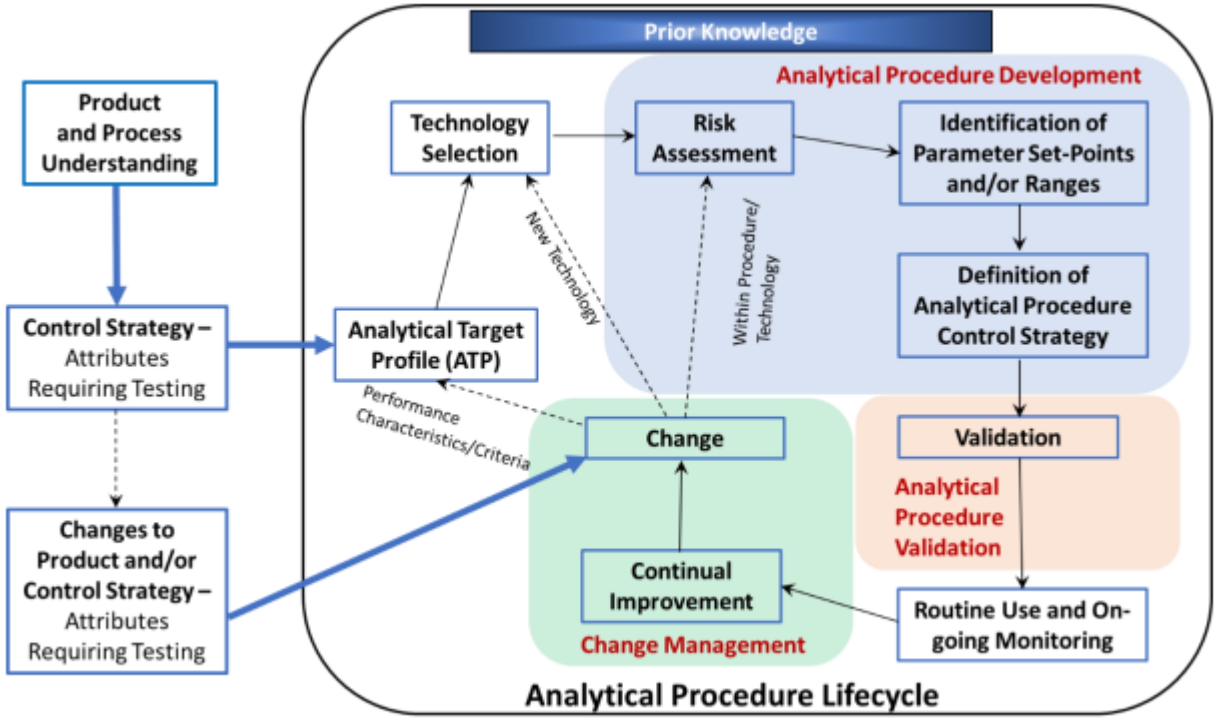
Code	History	Date
Q2	Approval by the Steering Committee under *Step 2* and release for public consultation.	26 October 1993
Q2A	Approval by the Steering Committee under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	27 October 1994
Q2B	Approval by the Steering Committee under *Step 2* and release for public consultation.	29 November 1995
Q2B	Approval by the Steering Committee under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	6 November 1996
Q2(R1)	The parent guideline is now renamed Q2(R1) as the guideline Q2B on methodology has been incorporated to the parent guideline. The new title is "Validation of Analytical Procedures: Text and Methodology".	November 2005
Q2(R2)	Complete revision of guideline to include more recent application of analytical procedures and to align content with *Q14*. Endorsement by the Members of the ICH Assembly under *Step 2* and release for public consultation.	24 March 2022
Q2(R2)	Adoption by the Regulatory Members of the ICH Assembly under *Step 4*.	1 November 2023
Q2(R2)	Error Correction to Table 5: Dissolution with HPLC as product performance test for an immediate release dosage form Reportable Range Linearity formulae on page 25; Correction to Tables 6-11 on pages 26-32.	30 November 2023

Analytical Procedure Validation Study

A validation study is designed to provide sufficient evidence that the analytical procedure meets its objectives

یک مطالعه اعتبار سنجی برای ارائه شواهد کافی مبنی بر اینکه روش آنالیز، اهداف خود را برآورده می کند، طراحی شده است.

The analytical procedure lifecycle



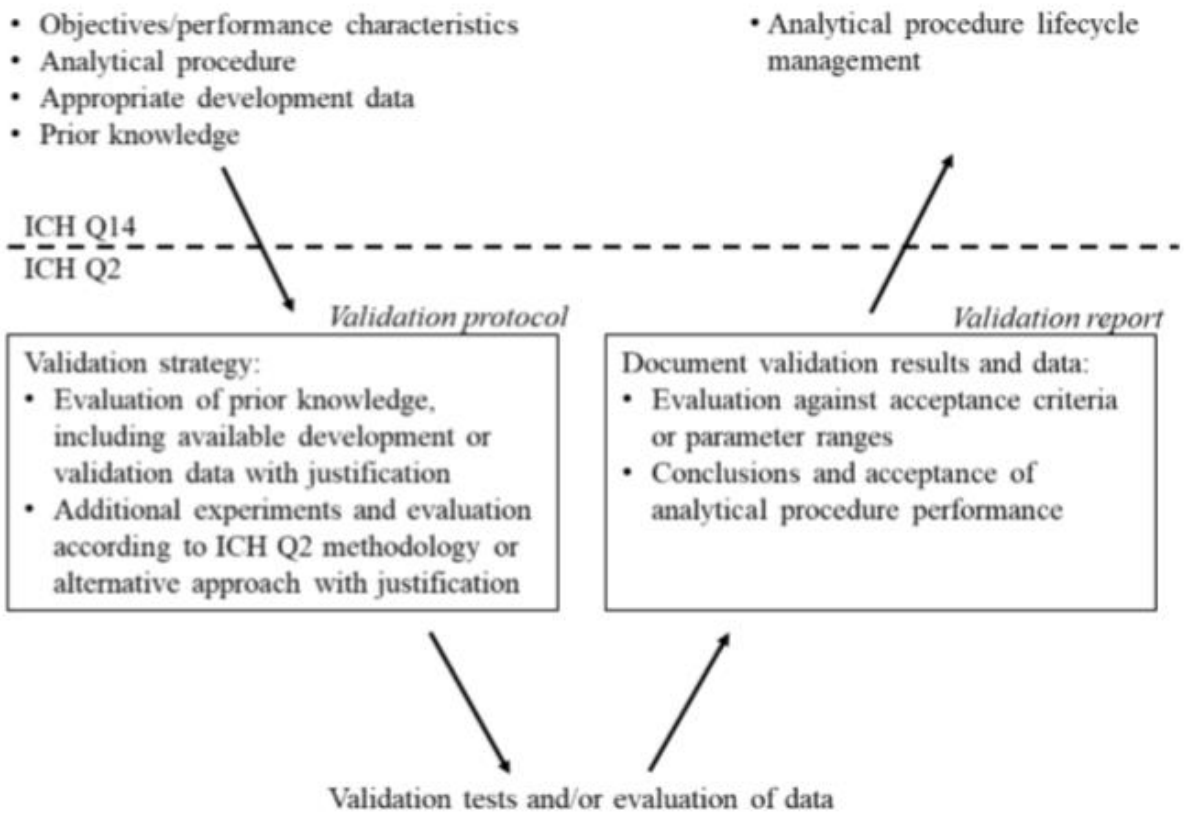
GENERAL CONSIDERATIONS FOR ANALYTICAL PROCEDURE VALIDATION

Analytical Procedure Validation Study	Validation During the Lifecycle of an Analytical Procedure	Reportable Range	Demonstration of Stability-Indicating Properties	Considerations for Multivariate Analytical Procedures
• Validation Tests • performance characteristics • Analytical Procedure Objective Determination	• Changes	• specification	• Samples spiked with target analytes and known interferences • Samples that have been exposed to various physical and chemical stress conditions • Actual product samples that are either aged or have been stored under stressed conditions	• In the first phase, model development consists of calibration and internal testing. Calibration data are used to create the calibration model • In the second phase, model validation, a validation set with independent samples is used for validation of the model

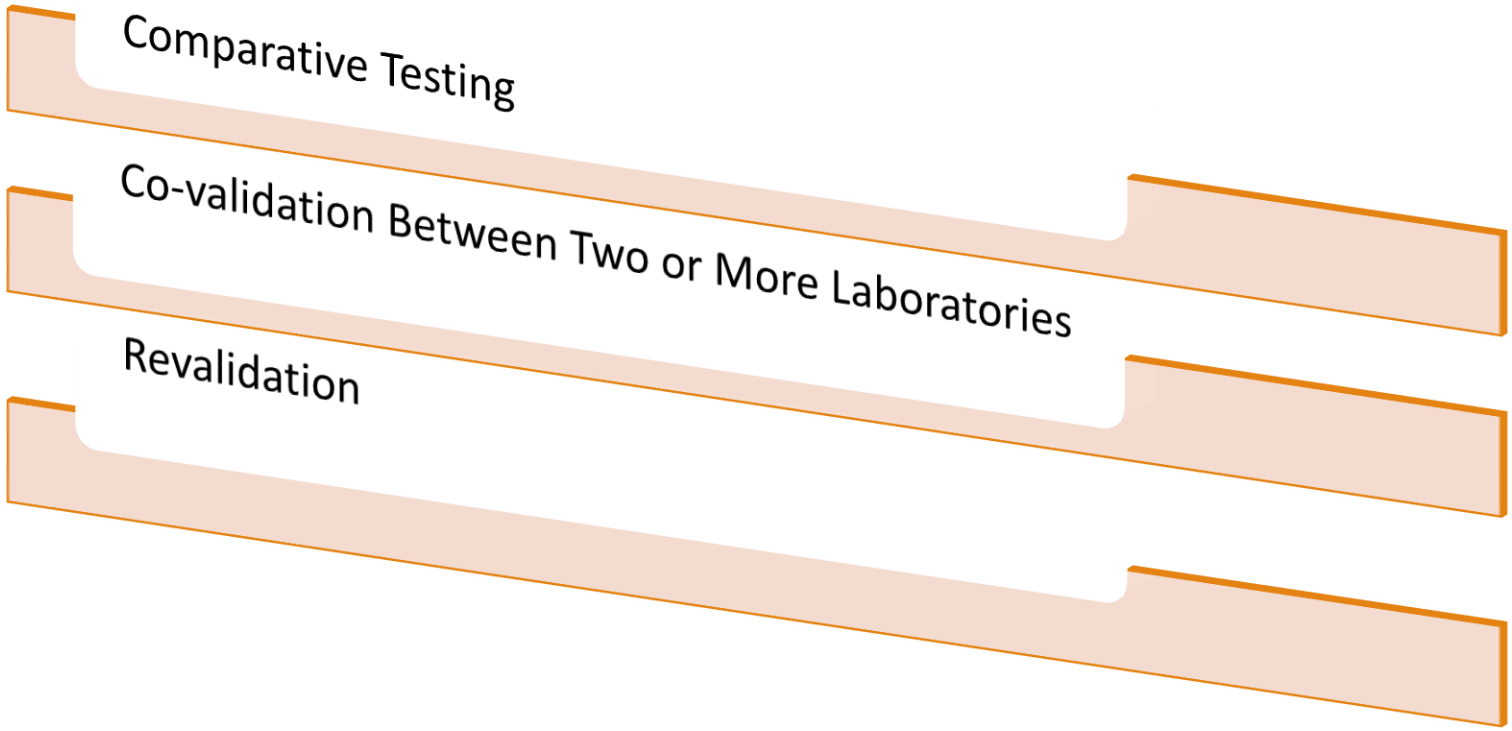
Typical performance characteristics and related validation tests for measured quality attributes

Measured Quality Attribute / Analytical Procedure Performance Characteristics to be Demonstrated (2)	IDENTITY	IMPURITY (PURITY) Other quantitative measurements (1)		ASSAY Content or potency / Other quantitative measurements (1)
		Quantitative Test	Limit Test	
Specificity (3) Specificity Test	+	+	+	+
Range Response (*Calibration Model*)	-	+	-	+
Lower Range Limit	-	QL†	DL	-
Accuracy (4) Accuracy Test	-	+	-	+
Precision (4) Repeatability Test	-	+	-	+
Intermediate Precision Test	-	+ (5)	-	+ (5)

Validation study design and evaluation



TRANSFER OF ANALYTICAL PROCEDURES



TRANSFER OF ANALYTICAL PROCEDURES

Statistical Analysis

significance test

این رویکرد آزمایش می کند که آیا تفاوت بین دو نتیجه قابل توجه است یا اینکه آیا می توان آن را صرفاً با تغییرات تصادفی محاسبه کرد

در ایجاد یک آزمون معناداری، ما در حال آزمایش صحت فرضیه ای هستیم که به عنوان فرضیه صفر(Null Hypothesis) شناخته می شود و اغلب با H0نشان داده می شود.

فرضیه صفر بیان می دارد که روش آنالیز در معرض خطای سیستماتیک نیست.

از عبارت nullبه این معنی استفاده می شود که تفاوتی بین مقادیر مشاهده شده و شناخته شده به غیر از تغییرات تصادفی وجود ندارد.

با کاهش احتمال تصادفی بودن وقوع اختلاف مشاهده شده، احتمال صحت فرضیه صفر کاهش می یابد.

معمولاً اگر احتمال وقوع چنین تفاوتی به طور تصادفی کمتر از ۱ در ۲۰ (یعنی 0.05یا ۵٪) باشد، فرضیه صفر رد می شود. در چنین حالتی گفته می شود که تفاوت در سطح P=0.05یا ۵٪ معنی دار است.

TRANSFER OF ANALYTICAL PROCEDURES

Statistical Analysis

significance test

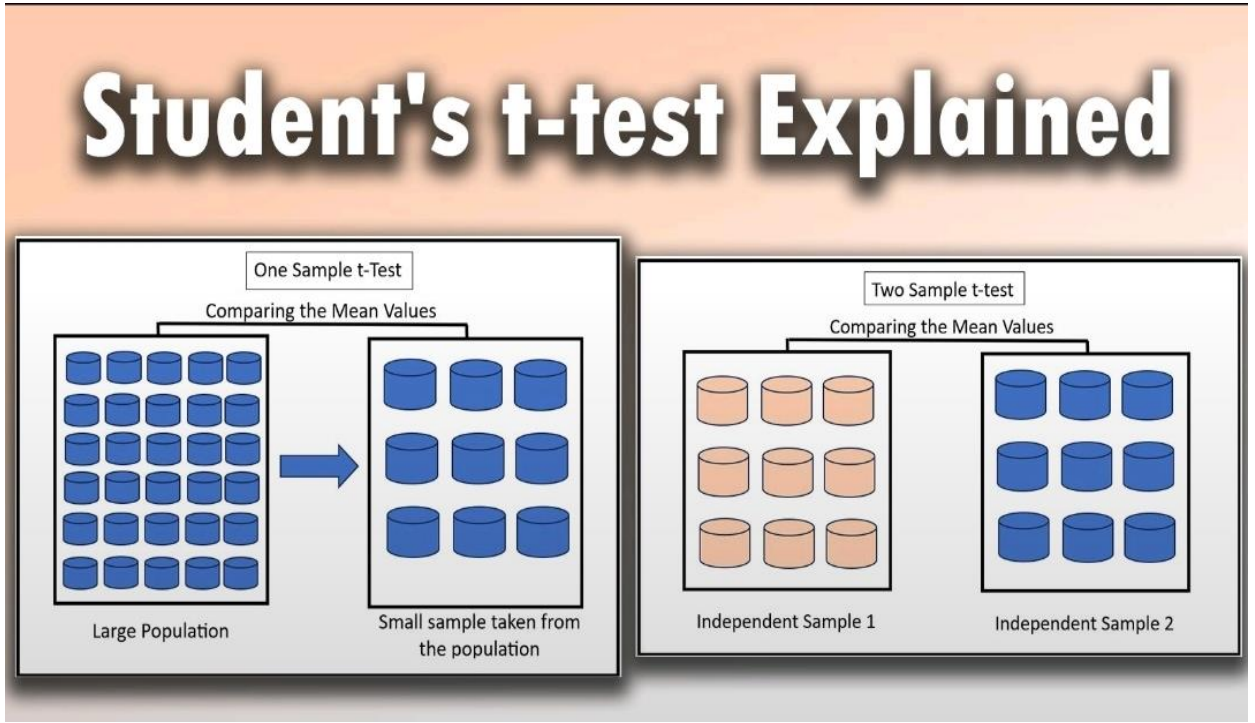
T-student test

$$t = (\bar{x} - \mu)\sqrt{n}/s$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_1}}}$$

$$t = (\bar{x}_1 - \bar{x}_2)/s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

$$t = \bar{d}\sqrt{n}/s_d$$



TRANSFER OF ANALYTICAL PROCEDURES

Statistical Analysis

significance test

Normal	Romatoied
1.84	2.81
1.92	4.06
1.94	3.62
1.92	3.27
1.85	3.27
1.91	3.76
2.07	

$$With\ the\ number\ of\ degree\ of\ freedom = \frac{\left(\frac{s_1^2}{n_1}+\frac{s_2^2}{n_2}\right)^2}{\frac{s_1^4}{n_1^2(n_1-1)}+\frac{s_2^4}{n_2^2(n_2-1)}}$$

UV	FTIR
84.63	83.15
84.38	83.72
84.08	83.74
84.41	84.2
83.82	83.92
83.55	84.16
83.92	84.02
83.69	83.6
84.06	84.13
84.03	84.24

$$t = \bar{d}\sqrt{n}/s_d$$

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	1.921428571	3.465
Variance	0.005714286	0.19403
Observations	7	6
Hypothesized Mean	0	
df	5	
t Stat	-8.477239299	
P(T<=t) one-tail	0.000187645	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.000375291	
t Critical two-tail	2.570581836	

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	84.057	83.888
Variance	0.113912222	0.116662222
Observations	10	10
Pearson Correlation	-0.421258103	
Hypothesized Mean Dif	0	
df	9	
t Stat	0.933575015	
P(T<=t) one-tail	0.18744557	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.374891141	
t Critical two-tail	2.262157163	

TRANSFER OF ANALYTICAL PROCEDURES

Statistical Analysis

significance test

Normal	Romatoied
1.84	2.81
1.92	4.06
1.94	3.62
1.92	3.27
1.85	3.27
1.91	3.76
2.07	

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	7	13.45	1.921429	0.005714
Column 2	6	20.79	3.465	0.19403

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.697672	1	7.697672	84.30046	1.72E-06	4.844336
Within Groups	1.004436	11	0.091312			
Total	8.702108	12				

time1	time2
59	59
56	59
56	59
59	58
57	55
55	57

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	57	57.83333333
Variance	2.8	2.566666667
Observations	6	6
Pearson Correlatic	0.149209419	
Hypothesized Mea	0	
df	5	
t Stat	-0.9552009	
P(T<=t) one-tail	0.191671665	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.383343331	
t Critical two-tail	2.570581836	

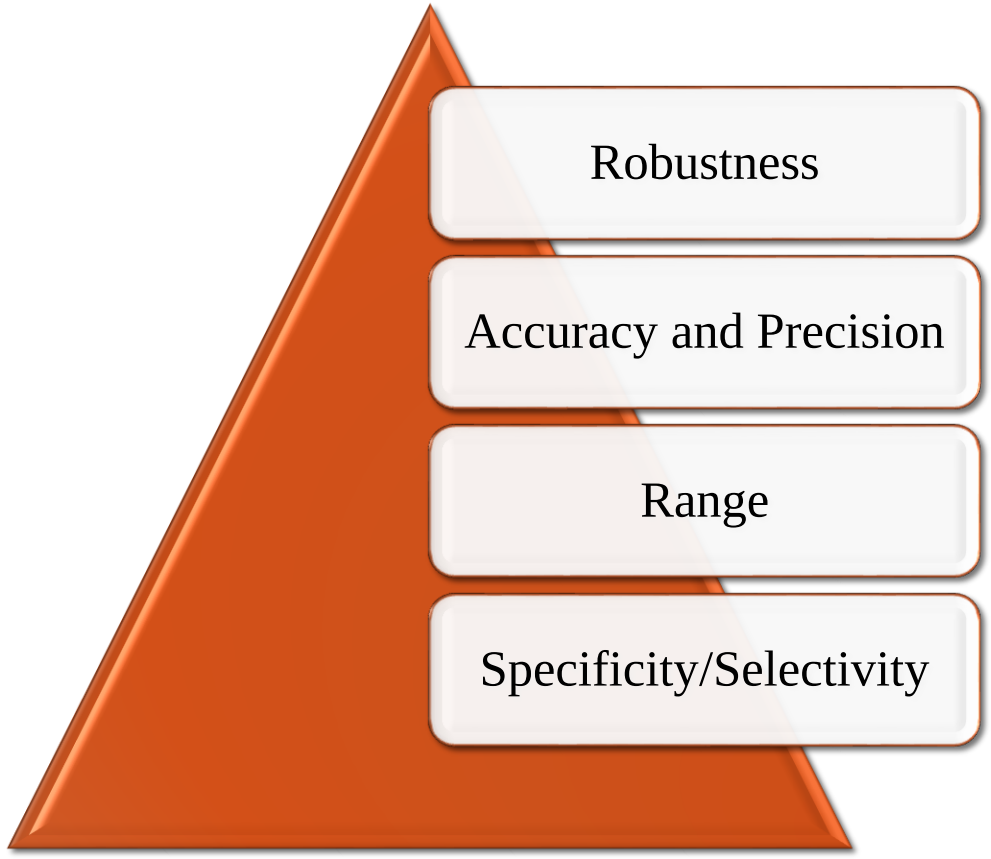
Reportable Range

The required reportable range is typically derived from the specification and depends on the intended use of the procedure. The reportable range is confirmed by demonstrating that the analytical procedure provides results with acceptable response, accuracy and precision. The reportable range should be inclusive of the upper and lower specification or reporting limits, as applicable.

محدوده قابل گزارش مورد نیاز معمولاً از مشخصات مشتق شده است و به استفاده مورد نظر از روش بستگی دارد. محدوده قابل گزارش با نشان دادن اینکه روش آنالیز نتایجی را با پاسخ، دقت و دقت قابل قبول ارائه می دهد تأیید می شود. محدوده قابل گزارش باید شامل مشخصات بالا و پایین یا محدودیت های گزارش، در صورت لزوم باشد.

Use of analytical procedure	Low end of reportable range	High end of reportable range
Assay of a product (1)	80% of declared content or 80% of lower specification acceptance criterion	120% of declared content or 120% of the upper specification acceptance criterion
Potency	Lowest specification acceptance criterion -20%	Highest specification acceptance criterion +20%
Content uniformity	70% of declared content	130% of declared content
Dissolution: Immediate release • one point specification • multiple point specification Modified release	 Q - 45% of the lowest strength Lower limit of reportable range (as justified by the specification) or QL, as appropriate. Lower limit of reportable range (as justified by the specification) or QL, as appropriate.	 130% of declared content of the highest strength
Impurity (1)	Reporting threshold	120% of specification acceptance criterion
Purity (as area %)	80% of lower specification acceptance criterion	Upper specification acceptance criterion or 100%

VALIDATION TESTS, METHODOLOGY AND EVALUATION



Specificity/Selectivity

- **General Considerations**

- Absence of Interference (e.g., impurities, degradation products, related substances, matrices, or other components likely to be present)
- Orthogonal Procedure Comparison (Other valid technique, Correlation matrix)
- Technology Inherent Justification (e.g., resolution of isotopes in mass spectrometry, chemical shifts in NMR spectroscopy)

Correlation

4x3 double

	1	2	3	4
1	0.2120	0.3990	0.1900	
2	0.0720	0.1330	0.1550	
3	0.0360	0.0630	0.2130	
4	0.0780	0.1410	0.2730	
5				

3x3 double

	1	2	3
1	1	1.0000	-0.1985
2	1.0000	1	-0.2080
3	-0.1985	-0.2080	1
4			
5			

Specificity/Selectivity

❑Recommended Data

- Identification

✓ confirmed by obtaining positive results comparable to a reference material using samples containing the analyte, along with negative results from samples which do not contain the analyte.

✓should be applied to materials structurally similar to or closely related to the analyte to confirm that a positive result is not obtained

✓انتخاب چنین مواد بالقوه تداخلی باید بر اساس قضاوت علمی با در نظر گرفتن تداخلاتی باشد که ممکن است رخ دهد.

Specificity/Selectivity

- Assay, Purity and Impurity Test(s)

✓For separation techniques: (e.g., for critical separations in chromatography, specificity can be demonstrated by the resolution of the two components which elute closest to each other)

✓For non-separation techniques (e.g., bioassay, ELISA, qPCR), specificity can be demonstrated through the use of reference materials or other suitably characterised materials to confirm the absence of interference in relation to the analyte.

در صورتی که یک رویه واحد خاص یا به اندازه کافی انتخابی در نظر گرفته نشود، باید از یک رویه اضافی برای اطمینان از تبعیض کافی استفاده شود. به عنوان مثال، در جایی که از تیتراسیون برای سنجش رهاسازی یک ماده دارویی استفاده می شود، ممکن است ترکیبی از سنجش و آزمایش مناسب برای ناخالصی ها استفاده شود.

Specificity/Selectivity

- Assay, Purity and Impurity Test(s)

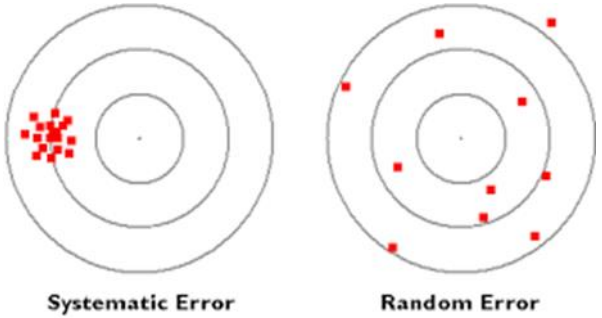
  - Impurities or related substances are available or can be intentionally created:

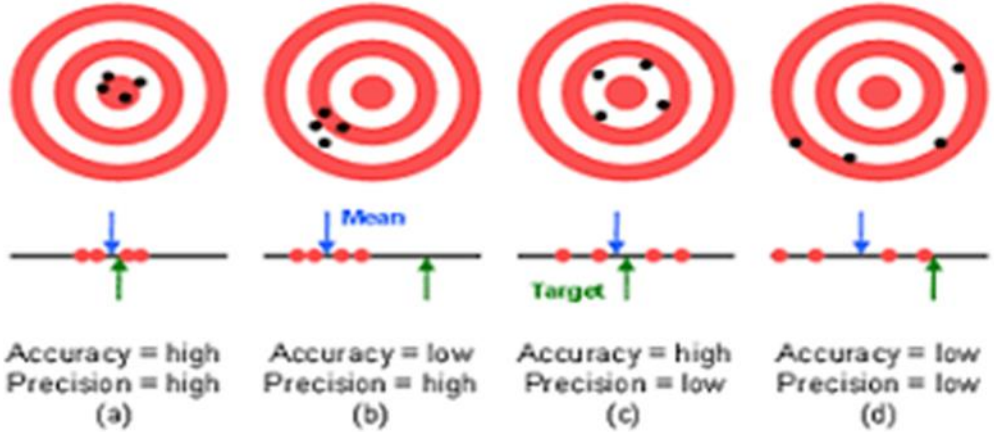
    performed by spiking product

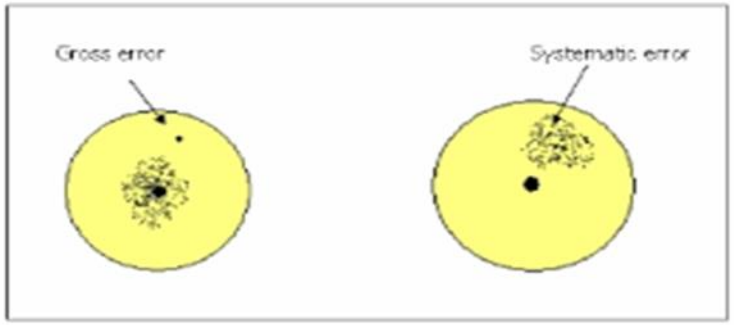
  - Impurities or related substances are not available:

    comparing the test results of samples containing typical impurities, related substances or degradation products with an orthogonal procedure(Other Analytical Technique).

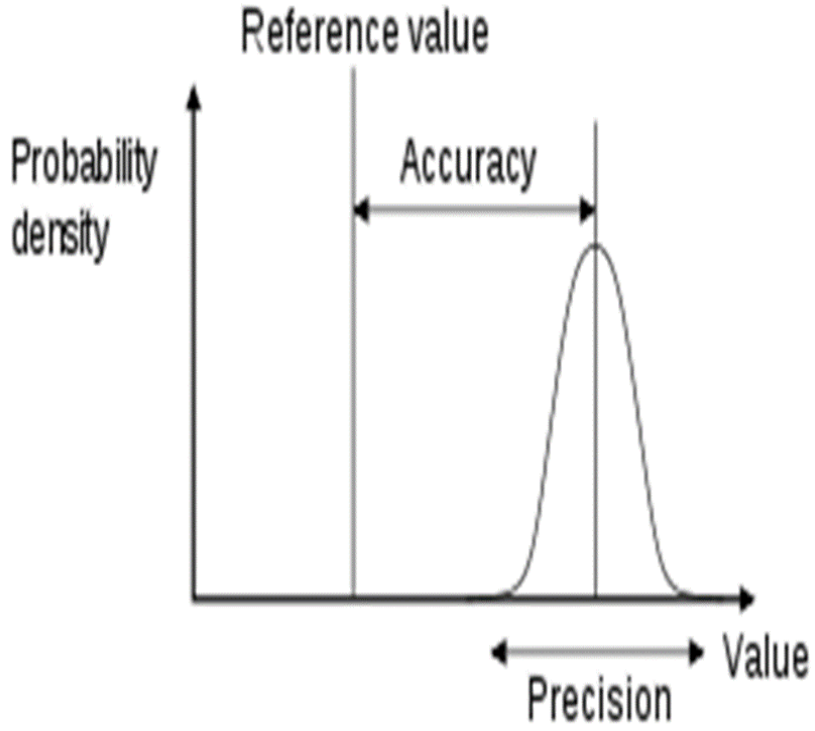
Accuracy and Precision

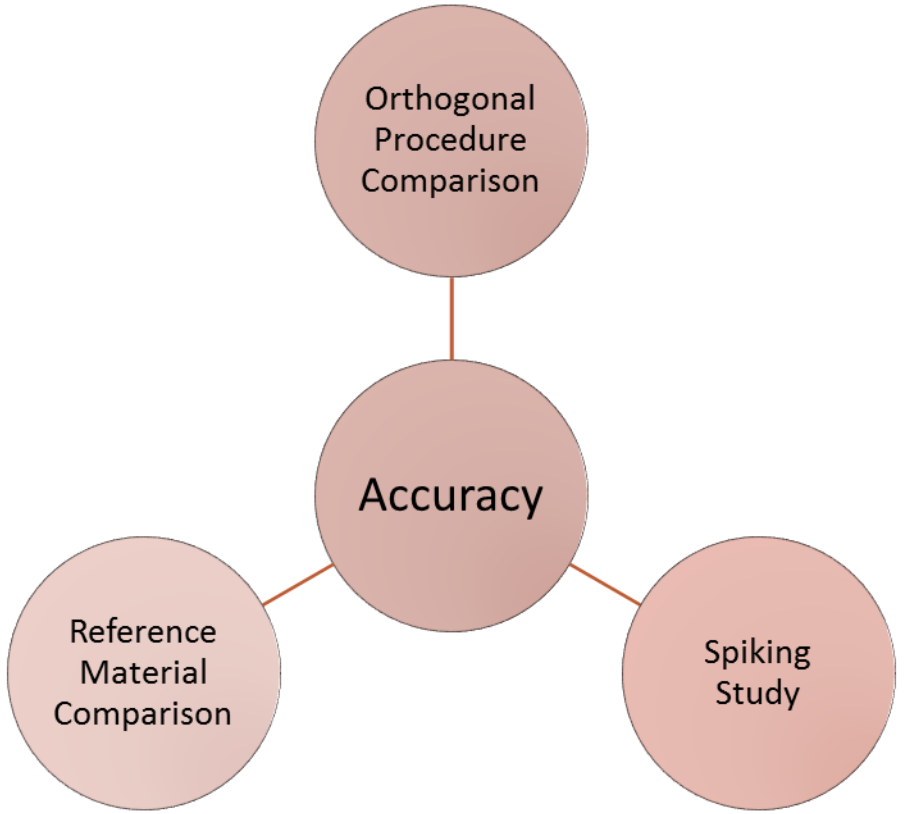






Accuracy


Reference value
Probability density
Accuracy
Precision
Value


Orthogonal Procedure Comparison
Accuracy
Reference Material Comparison
Spiking Study

Accuracy Recommended Data

Accuracy should be assessed using an appropriate number of determinations and concentration levels covering the reportable range (e.g., 3 concentrations/3 replicates each of the full analytical procedure)

Accuracy should be reported as the mean percent recovery of a known added amount of analyte in the sample or as the difference between the mean and the accepted true value, together with an appropriate 100(1-α) % confidence interval (or justified alternative statistical interval). The observed interval should be compatible with the corresponding accuracy acceptance criteria, unless otherwise justified.

- $\beta \pm t_{1-\alpha:n-1} \times S/\sqrt{n}$

Accuracy Data Analysis

Sr.No.	Conc.Level(%)	Result(mg/g)	Group Mean
1	50	992.1	992.00
2	50	988.43	
3	50	991.90	
4	100	987.22	990.11
5	100	997.5	
6	100	993	
7	150	992.4	993.04
8	150	993.67	
9	150	987.76	

Accuracy Data Analysis

Anova: Single Factor

SUMMARY

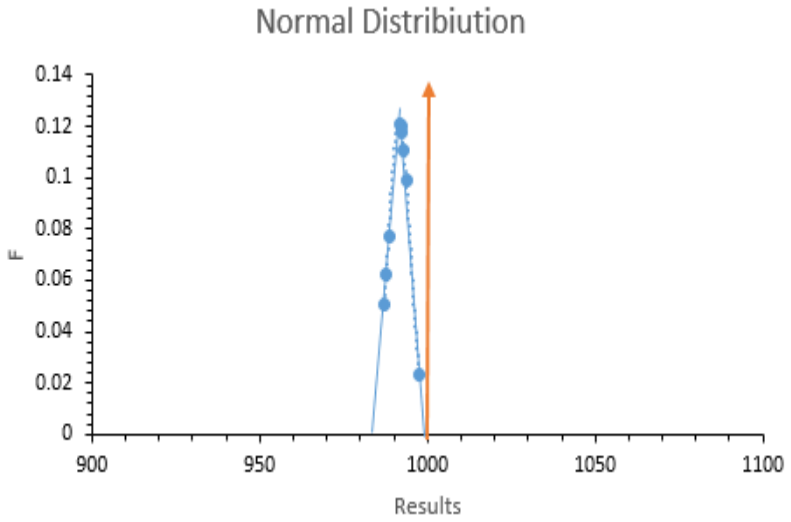
Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	2972.43	990.81	4.2583
Column 2	3	2977.72	992.5733333	26.55613
Column 3	3	2973.83	991.2766667	9.678433

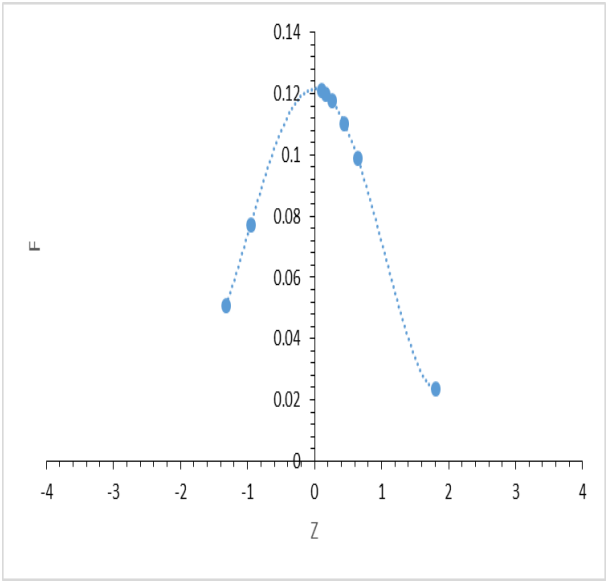
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.008466667	2	2.504233333	0.185531	0.835253	5.143253
Within Groups	80.98573333	6	13.49762222			
Total	85.9942	8				

Accuracy Data Analysis

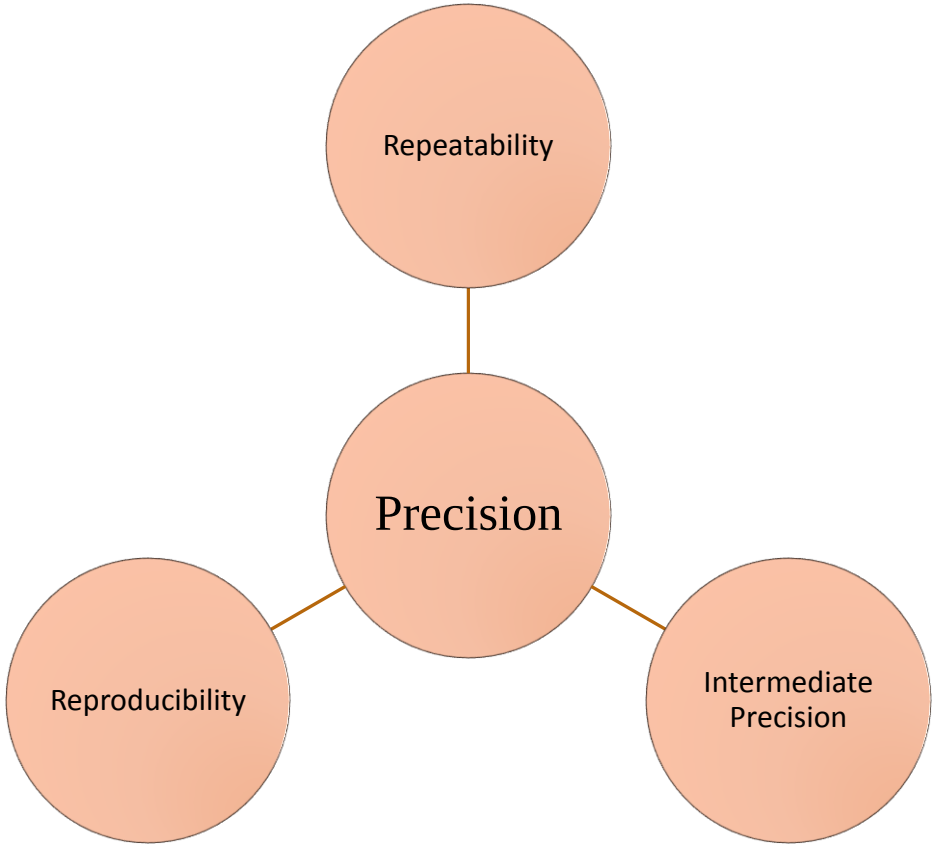
Column1	
Mean	991.5533333
Standard Error	1.092869566
Median	992.1
Mode	#N/A
Standard Deviation	3.278608699
Sample Variance	10.749275
Kurtosis	-0.096380388
Skewness	0.260803757
Range	10.28
Minimum	987.22
Maximum	997.5
Sum	8923.98
Count	9
Largest(1)	997.5
Smallest(1)	987.22
Confidence Level(95.0%)	2.520161739





- $\beta \pm t_{1-\alpha:n-1} \times S/\sqrt{n}$

Precision


Repeatability
Precision
Reproducibility
Intermediate Precision

Precision Recommended Data

The standard deviation, relative standard deviation (coefficient of variation), and an appropriate 100(1-α) % confidence interval (or justified alternative statistical interval) should be reported.

The observed interval should be compatible with the corresponding precision acceptance criteria, unless otherwise justified.

Precision Data Analysis

$$\bar{x} = 1/n \sum x_i$$

$$S = \sqrt{\sum (x_i - \bar{x})/(n-1)}$$

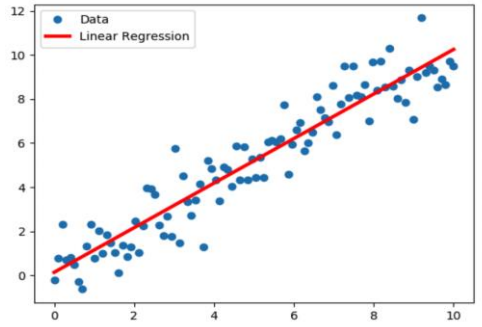
$$Coefficient\ of\ variation\ (CV) = relative\ standard\ deviation\ (RSD)$$
$$= 100 \times s/\bar{x}$$

- **One-Way ANOVA**

Response

- Linear Response $\eta = \beta_0 + \beta_1 x$
- Non-linear Response $y = \beta_0 + \beta_1 x + \beta_2 x^2 + \varepsilon$
- Multivariate Calibration

Straight Line Regression and Calibration



Regression analysis is used to study the relationship between two or more variables

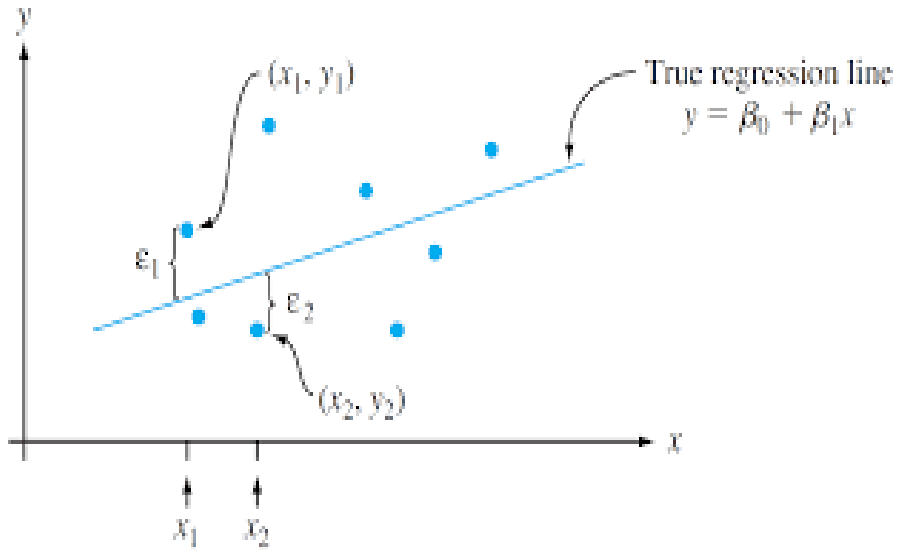
Model I regression assume that the independent variable is not subject to error. An important application of *Model I regression* analysis is calibration where an instrumental response is related to the known concentration of the analyte in calibration standard

If both variables are subject to error, *Model II regression* techniques, which take into account the error associated with both variables, must be applied. This is the case, for example, in method-comparison studies where there are measurement errors in both methods.

Straight line regression

The use of a calibration line for determining the concentration of an analyte in a sample is an important application of straight line regression

$$\eta = \beta_0 + \beta_1 x$$

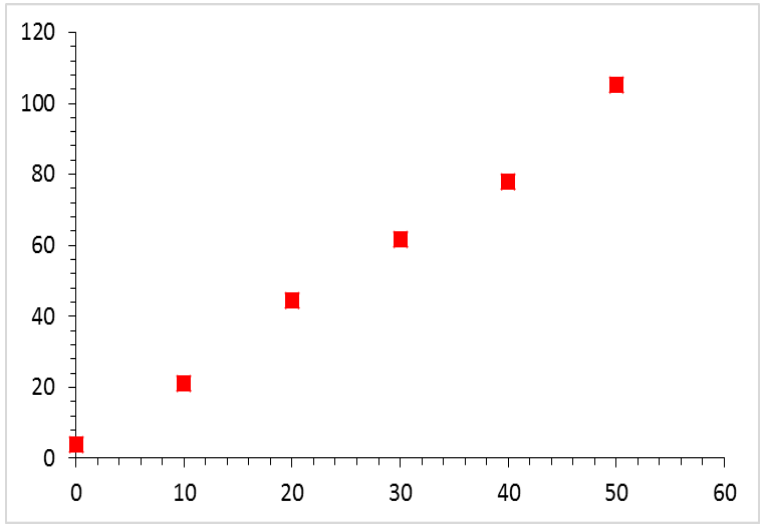


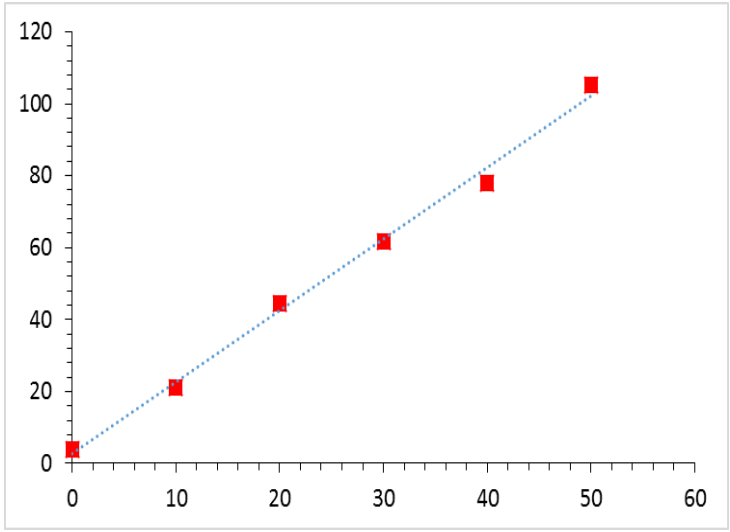
Example

$$\eta = \beta_0 + \beta_1 x$$

$$y_i = \beta_0 + \beta_1 x_i + \varepsilon_i$$

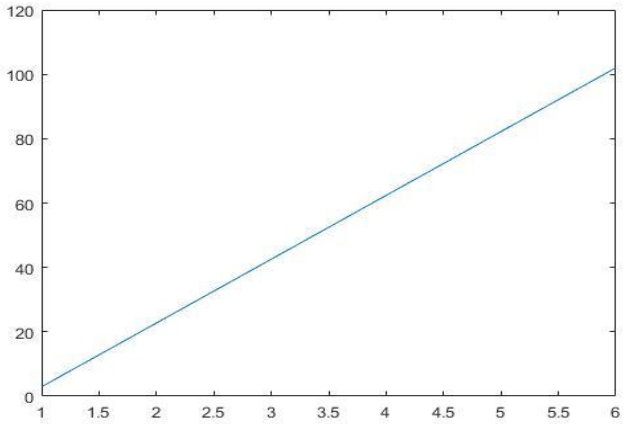
i	xi(ng/mL)	yi(I)
1	0	4.0
2	10	21.1
3	20	44.6
4	30	61.8
5	40	78.0
6	50	105.2

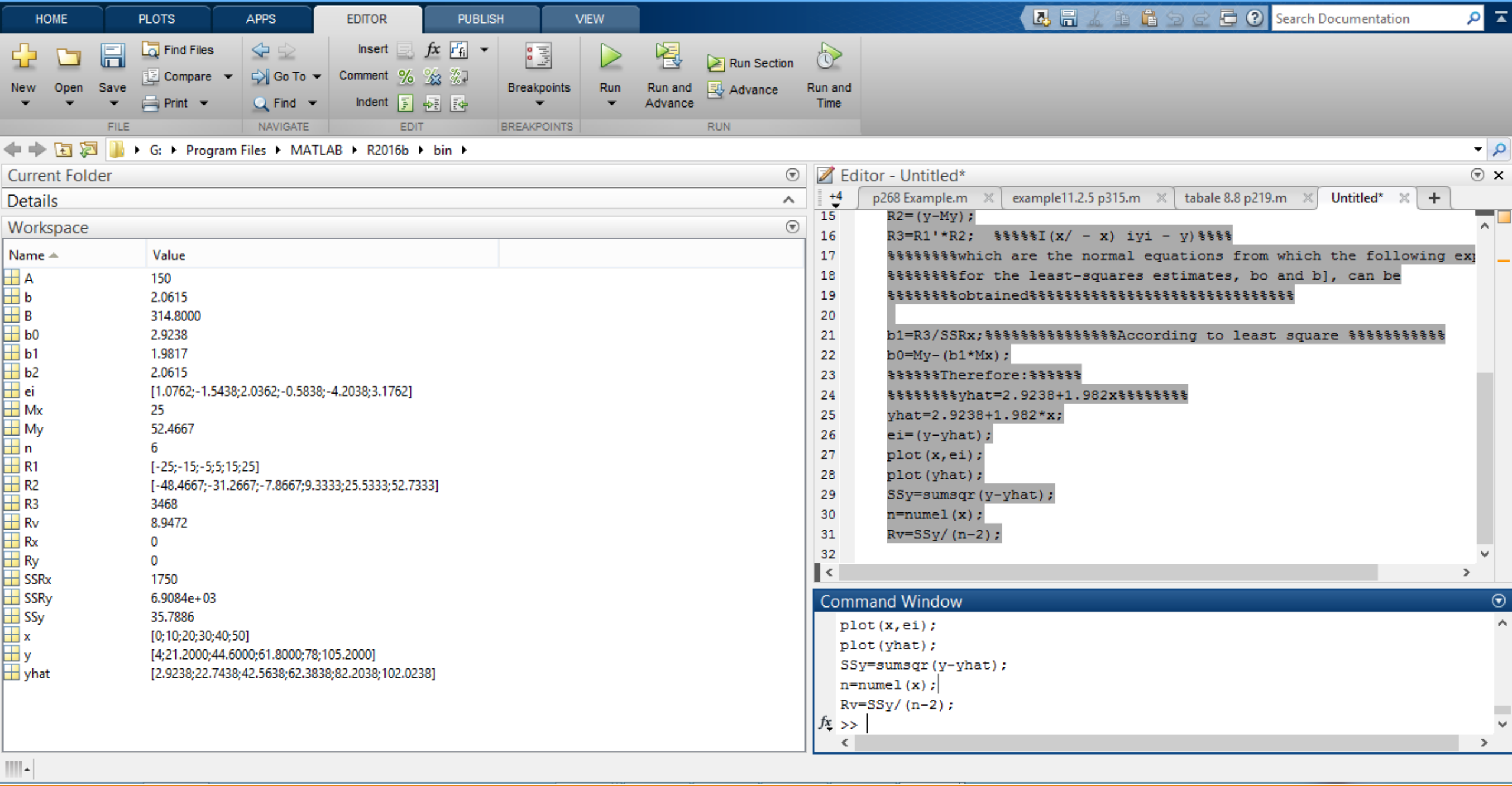




```
x=[0.0;10;20;30;40;50];
y=[4.0;21.2;44.6;61.8;78.0;105.2];
plot(x,y);
b=inv(x'*x)*x'*y;
b2=x\y;
A=sum(x);
B=sum(y);
Mx=mean(x);
My=mean(y);
SSRx=sumsqr(x-Mx);
SSRy=sumsqr(y-My);
Rx=sum(x-Mx);
Ry=sum(y-My);
R1=(x-Mx);
R2=(y-My);
R3=R1'*R2;   %%%%%I(x/ - x) iyi - y)%%%%
%%%%%%%%%which are the normal equations from which the following expressions
%%%%%%%%%for the least-squares estimates, bo and b], can be
%%%%%%%%%obtained%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

b1=R3/SSRx;%%%%%%%%%%%%%%%%According to least square %%%%%%%%%%%
b0=My-(b1*Mx);
%%%%%%%Therefore:%%%%%%
%%%%%%%%%yhat=2.9238+1.982x%%%%%%%%%
yhat=2.9238+1.982*x;
ei=(y-yhat);
plot(x,ei);
plot(yhat);
SSy=sumsqr(y-yhat);
n=numel(x);
Rv=SSy/(n-2);
```

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Current Folder
Details
Workspace
Name
Value
A 150
b 2.0615
B 314.8000
b0 2.9238
b1 1.9817
b2 2.0615
ei [1.0762;-1.5438;2.0362;-0.5838;-4.2038;3.1762]
Mx 25
My 52.4667
n 6
R1 [-25;-15;-5;5;15;25]
R2 [-48.4667;-31.2667;-7.8667;9.3333;25.5333;52.7333]
R3 3468
Rv 8.9472
Rx 0
Ry 0
SSRx 1750
SSRy 6.9084e+03
SSy 35.7886
x [0;10;20;30;40;50]
y [4;21.2000;44.6000;61.8000;78;105.2000]
yhat [2.9238;22.7438;42.5638;62.3838;82.2038;102.0238]
Editor - Untitled*
p268 Example.m
example11.2.5 p315.m
tabale 8.8 p219.m
Untitled*
15 R2=(y-My);
16 R3=R1'*R2; %%%%%I(x/ - x) iyi - y)%%%%
17 %%%%%%%%which are the normal equations from which the following exp
18 %%%%%%%%for the least-squares estimates, bo and b], can be
19 %%%%%%%%obtained%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
20
21 b1=R3/SSRx;%%%%%%%%%%%%%%%According to least square %%%%%%%%%%
22 b0=My-(b1*Mx);
23 %%%%%%Therefore:%%%%%%
24 %%%%%%%%yhat=2.9238+1.982x%%%%%%%%
25 yhat=2.9238+1.982*x;
26 ei=(y-yhat);
27 plot(x,ei);
28 plot(yhat);
29 SSy=sumsqr(y-yhat);
30 n=numel(x);
31 Rv=SSy/(n-2);
32
Command Window
plot(x,ei);
plot(yhat);
SSy=sumsqr(y-yhat);
n=numel(x);
Rv=SSy/(n-2);
>>

According to the least square calculations in matlab we'll have:

B1=1.9817

B0=2.9242

And $\hat{y} = 2.924 + 1.982x$

According to the least square calculations in Excel(Regression data analysis) we'll have:

And $\hat{y} = 2.886 + 1.982x$

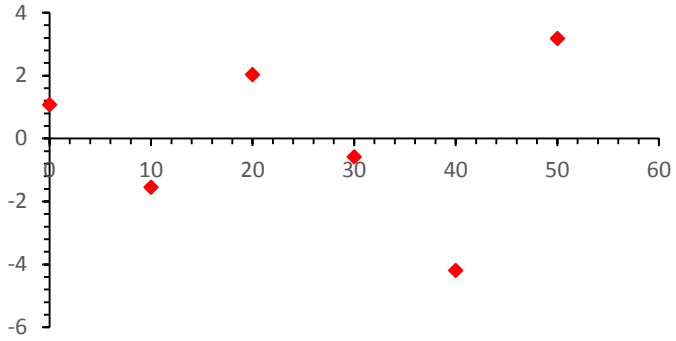
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.885714286	2.174357361	1.327157319	0.255142006	-3.151269565	8.922698137	-3.151269565	8.922698137
X Variable 1	1.982571429	0.07181666	27.60600992	1.02411E-05	1.783176414	2.181966443	1.783176414	2.181966443

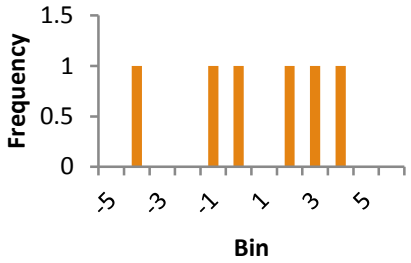
x_i	y_i	y_i-hat	$e_i(y_i-y_i\text{-hat})$	e_i^2
0	4	2.92	1.08	1.1664
10	21.2	22.74	-1.54	2.3716
20	44.6	42.56	2.04	4.1616
30	61.8	62.38	-0.58	0.3364
40	78	82.19	-4.19	17.5561
50	105.2	102.01	3.19	10.1761
Sum of error			0.00	
Sum of square error				35.7682
n				6
Degree of freedom(n-2)				4
S_e^2				8.94
Standard error				2.99

Regression Statistics	
Multiple R	0.99738593
R Square	0.994778694
Adjusted R Square	0.993473367
Standard Error	3.004306433
Observations	6

In the least-squares method the following assumptions concerning the residuals are made:

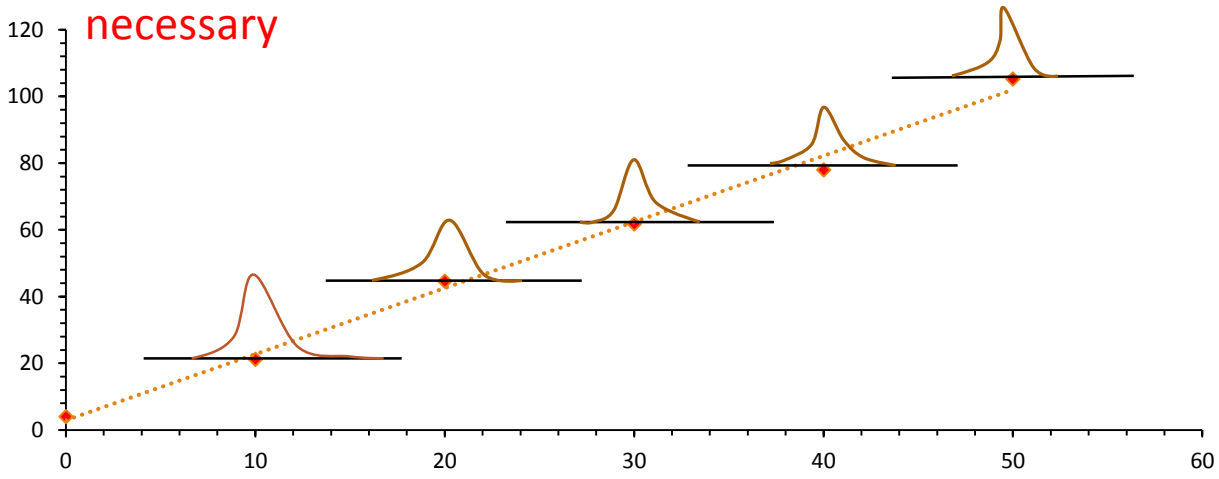
- for each x_i, the residuals e_i are from a population that is normally distributed with mean zero





- the e_i are independent
- they all have the same variance σ^2

To check homoscedasticity, replicate measurements are necessary



The regression procedure involves several steps:

- Selection of a model
- Establishment of the experimental design
- Estimation of the parameters of the model
- Validation of the model
- Computation of confidence intervals

Validation of the model

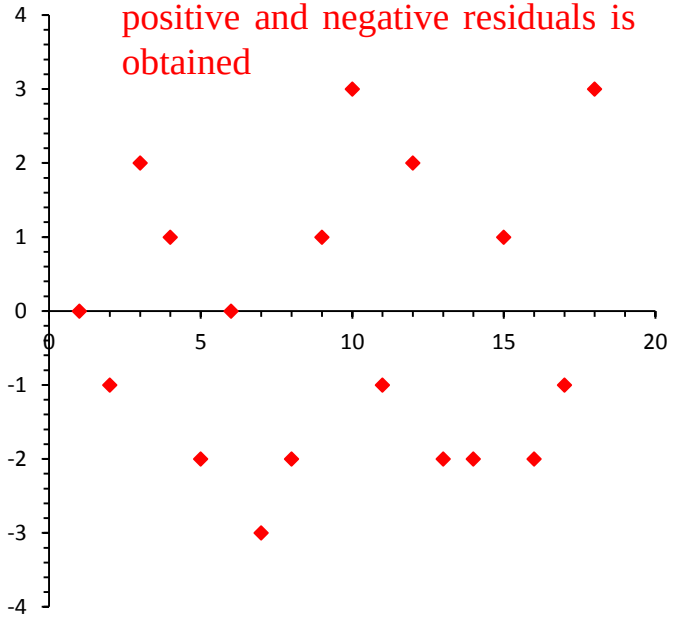
- Analysis of the residuals
- Analysis of variance

Analysis of the residuals

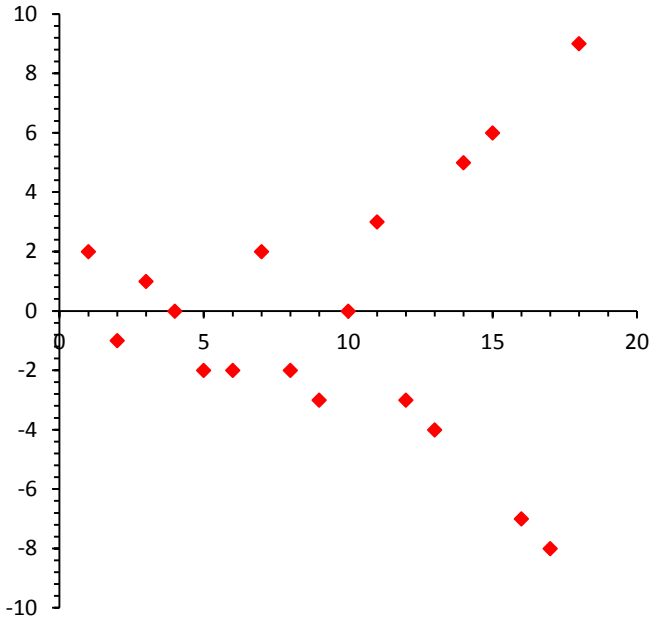
Useful information can also be obtained from a residuals plot where the residuals e_i are plotted against $\hat{y}_i$ or against x_i . It is recommended that such a plot is obtained whenever one needs to validate the model. Since no tests are involved, some experience may be necessary for the interpretation of these plots

Examples of residual plots

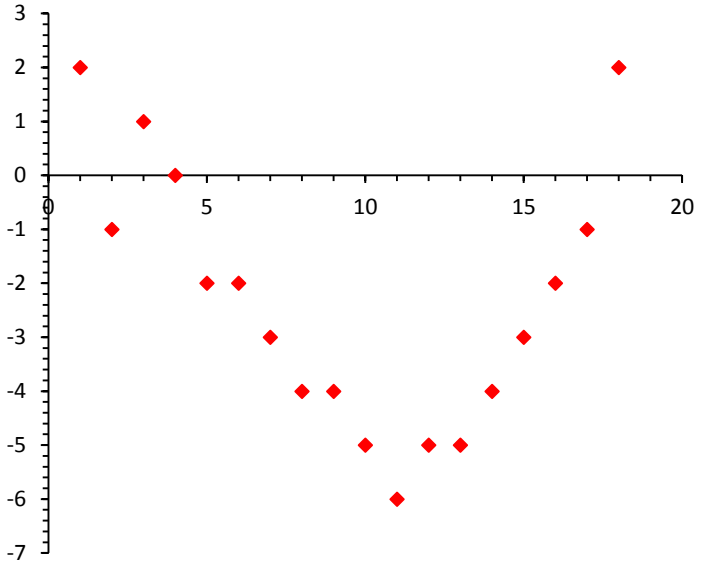
indicates no abnormality: the residuals are randomly scattered within a horizontal band with a number of positive residuals which is approximately equal to the number of negative residuals. Moreover, a random sequence of positive and negative residuals is obtained



the condition of homoscedasticity is not fulfilled: the scatter of the residuals increases with y. This indicates that the precision of the measurements over the concentration range considered is not constant



The U-shaped residuals plot is the result of fitting a straight line to data which are better represented by a curve. There is a lack of fit with the straight line model



Analysis of variance

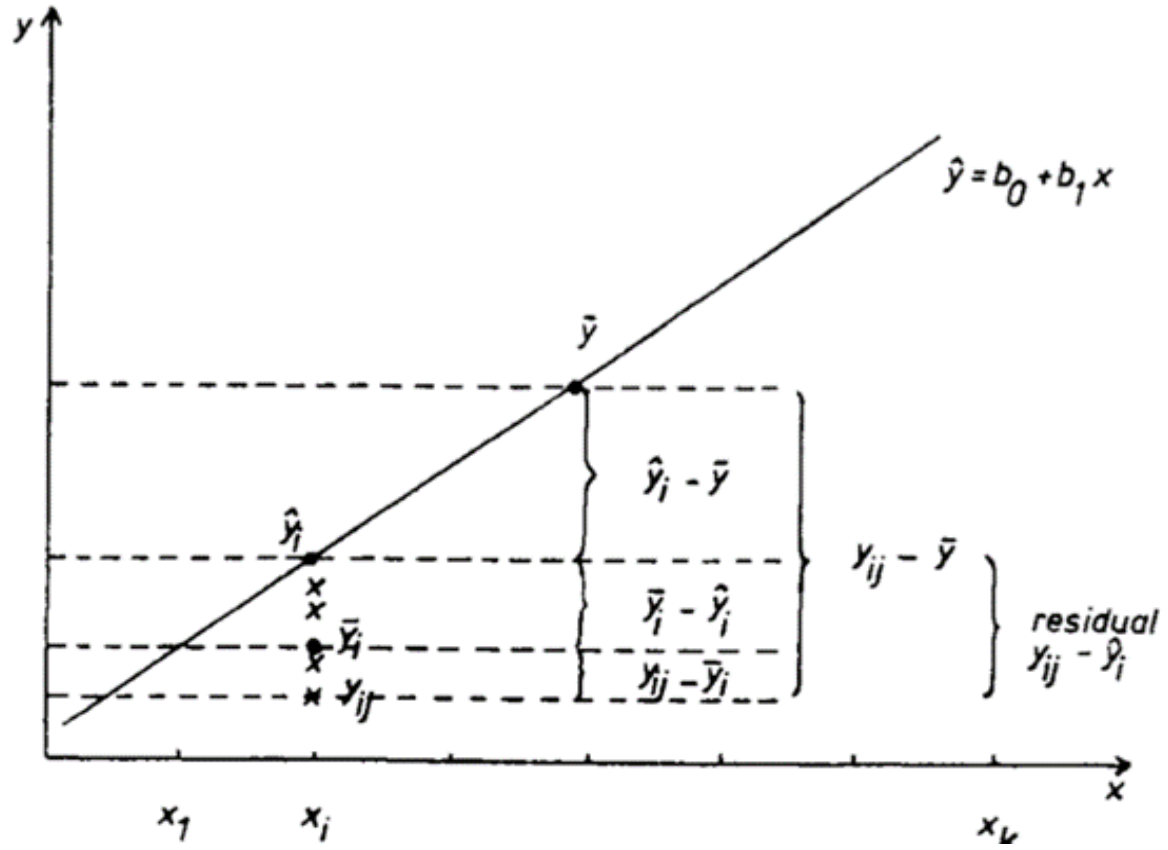
تجزیه و تحلیل واریانس (ANOVA) می تواند برای تشخیص عدم تناسب(lack of fit) در یک رگرسیون استفاده شود تا بررسی شود که آیا مدل انتخاب شده درست است یا خیر. بنابراین اندازه گیری های تکراری مورد نیاز است.

تغییرات کل مقادیر yنسبت به مقدار میانگین، $\bar{y}$، همانطور که با مجموع مجموع مربع ها(Sum of Squares total)، SS_Tتوضیح داده می شود، با رابطه زیر محاسبه می شود:

$$SS_T = \sum_{i=1}^{k}\sum_{j=1}^{n_i}(y_{ij} - \bar{y})^2$$

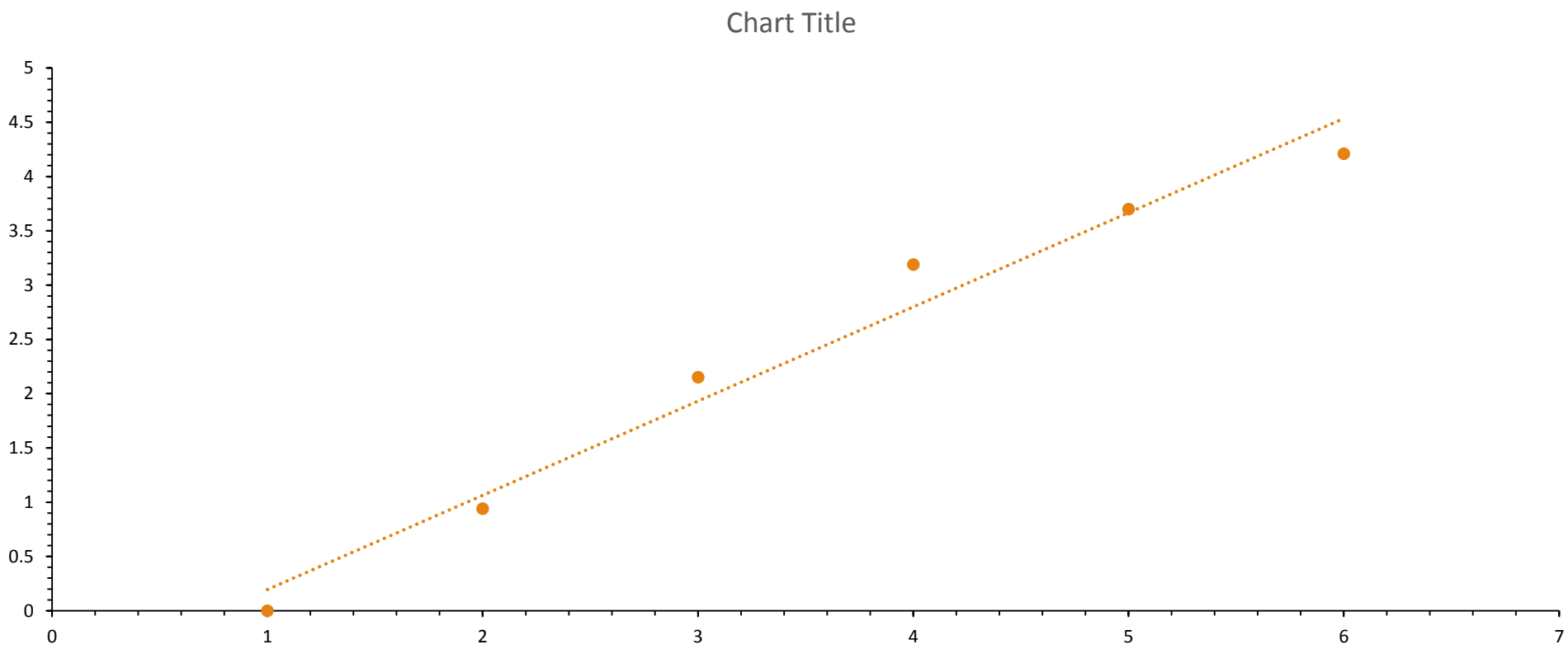
با آنالیز واریانس این مجموع مجذورات به منابع مختلف تغییرات تقسیم می شود.

$$(y_{ij} - \bar{y}) = (y_{ij} - \bar{y}_i) + (\bar{y}_i - \hat{y}_i) + (\hat{y}_i - \bar{y})$$


y
$\hat{y} = b_0 + b_1 x$
$\bar{y}$
$\hat{y}_i - \bar{y}$
$\hat{y}_i$
$y_{ij} - \bar{y}$
$\bar{y}_i - \hat{y}_i$
residual
$y_{ij} - \hat{y}_i$
$\bar{y}_i$
$y_{ij} - \bar{y}_i$
y_{ij}
x
x_1
x_i
x_k

Sr.No.	Y_{ij}	Y_{ij}	Sum Y_{ij}	Y_i-bar	Y-hat
1	0	0	0	0	0.265
2	0.98	0.9	1.88	0.94	1.114
3	2.1	2.2	4.3	2.15	1.963
4	3.16	3.22	6.38	3.19	2.812
5	3.68	3.72	7.4	3.7	3.661
6	4.15	4.27	8.42	4.21	4.509
mean	2.345	2.385	4.73	2.365	2.387333333

$k = 6$ $n = \sum n_i = 11$ $\hat{y} = 0.265 + 0.849x$ $\bar{y} = 2.58$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7

$$SS_T = \sum\sum(y_{ij} - \bar{y})^2 = (0 - 2.36)^2 + (0.98 - 2.36)^2 + (0.90 - 2.36)^2 + \cdots + (4.27 - 2.36)^2 = 20.99$$

$$SS_{PE} = \sum\sum(y_{ij} - \bar{y}_i)^2 = (0.98 - 0.94)^2 + (0.9 - 0.94)^2 + (2.1 - 2.15)^2 + (2.2 - 2.15)^2 + \cdots + (4.27 - 4.21)^2 = 0.018$$

$$SS_{REG} = \sum n_i(\hat{y}_i - \bar{y})^2 = 20.31$$

$$SS_{LOF} = 20.99 - 0.018 - 20.31 = 0.616$$

$$MS_{LOF} = \frac{0.616}{4} = 0.1655$$

$$MS_{PE} = 0.018/5 = 0.0036$$

$$F = \frac{MS_{LOF}}{MS_{PE}} = 45.97$$

F = MS_{LOF}/MS_{PE} = 46.11 is much larger than $F_{o.o5;4,5}$ = 5.19, the lack of fit term is highly significant and consequently the straight line model is not adequate to describe the relationship between y and x.

v_2	v_1												
	1	2	3	4	5	6	7	8	9	10	12	15	20
1	161.4	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5	241.9	243.9	245.9	248.0
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45
3	10.13	9.552	9.277	9.117	9.013	8.941	8.887	8.845	8.812	8.786	8.745	8.703	8.660
4	7.709	6.944	6.591	6.388	6.256	6.163	6.094	6.041	5.999	5.964	5.912	5.858	5.803
5	6.608	5.786	5.409	5.192	5.050	4.950	4.876	4.818	4.772	4.735	4.678	4.619	4.558
6	5.987	5.143	4.757	4.534	4.387	4.284	4.207	4.147	4.099	4.060	4.000	3.938	3.874
7	5.591	4.737	4.347	4.120	3.972	3.866	3.787	3.726	3.677	3.637	3.575	3.511	3.445
8	5.318	4.459	4.066	3.838	3.687	3.581	3.500	3.438	3.388	3.347	3.284	3.218	3.150
9	5.117	4.256	3.863	3.633	3.482	3.374	3.293	3.230	3.179	3.137	3.073	3.006	2.936
10	4.965	4.103	3.708	3.478	3.326	3.217	3.135	3.072	3.020	2.978	2.913	2.845	2.774
11	4.844	3.982	3.587	3.357	3.204	3.095	3.012	2.948	2.896	2.854	2.788	2.719	2.646
12	4.747	3.885	3.490	3.259	3.106	2.996	2.913	2.849	2.796	2.753	2.687	2.617	2.544
13	4.667	3.806	3.411	3.179	3.025	2.915	2.832	2.767	2.714	2.671	2.604	2.533	2.459
14	4.600	3.739	3.344	3.112	2.958	2.848	2.764	2.699	2.646	2.602	2.534	2.463	2.388
15	4.543	3.682	3.287	3.056	2.901	2.790	2.707	2.641	2.588	2.544	2.475	2.403	2.328
16	4.494	3.634	3.239	3.007	2.852	2.741	2.657	2.591	2.538	2.494	2.425	2.352	2.276
17	4.451	3.592	3.197	2.965	2.810	2.699	2.614	2.548	2.494	2.450	2.381	2.308	2.230
18	4.414	3.555	3.160	2.928	2.773	2.661	2.577	2.510	2.456	2.412	2.342	2.269	2.191
19	4.381	3.522	3.127	2.895	2.740	2.628	2.544	2.477	2.423	2.378	2.308	2.234	2.155
20	4.351	3.493	3.098	2.866	2.711	2.599	2.514	2.447	2.393	2.348	2.278	2.203	2.124

v_1 = number of degrees of freedom of the numerator; v_2 = number of degrees of freedom of the denominator.

The End

Thank you for your attention

May 2025-Tehran,IRAN

References

ICH Q10 Pharmaceutical Quality System

ICH Q14 Analytical Procedure Development

ICH M4Q The Common Technical Document For The Registration Of Pharmaceuticals For Human Use