

Container Closure Integrity Testing In Lieu Of Sterility by Dye Ingress Method Using Uv-Visible Spectroscopy

Deepikaa.S.K¹, Murugan.M², Jeyapragash.R^{3*}

¹Student- M.Pharmacy- Pharmaceutical Quality Assurance, Sri Ramachandra Faculty of Pharmacy, SRIHER (DU), Porur, Chennai.

²Assistant Professor- Department of Pharmaceutical Chemistry, Sri Ramachandra Faculty of Pharmacy, SRIHER (DU), Porur, Chennai.

³Deputy Manager- Quality Assurance, Steril-Gen Life sciences (P) Ltd., (Madras Pharma Group) Mangalam, Puducherry.

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ABSTRACT: Container Closure Integrity is crucial for ensuring and maintaining the sterility and stability of Pharmaceutical products. This study involves the application of dye ingress method validated using UV- Visible Spectroscopy as an alternative for sterility testing in stability studies. The research emphasizes on developing standard analytical method validation criteria and assessing the effectiveness of the Packaging Integrity Apparatus. The method's sensitivity and reliability in detecting micro leaks is confirmed by performing specificity, limit of detection (LOD), linearity, and precision assessments. The outcomes of this method support to the liable practice of Container Closure Integrity Testing (CCIT) in compliance with regulatory guidelines, ensuring product sterility and quality throughout its shelf life.

Keywords: Container closure Integrity (CCI), Dye Ingress Method, UV- Visible Spectroscopy, Method Validation, Sterility Assurance.

I. CONTAINER CLOSURE INTEGRITY TESTING:

Container closure integrity is a critical quality attribute to maintain the sterility and stability of a drug product throughout its shelf life.^[1, 2] It is also a central aspect of product contamination control. CCIT commonly referred to as leak detection for packaging inspection system to maintain an aseptic barrier against potential contaminations.^[4] This testing method is deterministic, which means that it is less prone to mistakes, repeatable, and produces quantifiable and predictable findings.^[3] Stringent regulations for Container Closure Integrity Testing (CCIT) are enforced by the Food and Drug Administration (FDA) and the United States Pharmacopeia (USP), which are the driving forces behind the safety

evaluation of materials and container closure systems in the US.^[6]

A nationwide outbreak of septicaemias in US hospitals throughout the 1970s was brought on by tainted, inadequately packed infusion supplies. Since then, authorities have paid close attention to CCI and seen it as a critical quality attribute of parenteral medicinal formulations.^[5]

As per the definition provided by the US Food and Drug Administration (US FDA), a packaging system, or container closure system, is the totality of packaging elements that collectively safeguard and contain the dosage form. This includes both primary and secondary packaging components if the latter are meant to offer supplementary protection to the drug product (US FDA, 1999). The European Medicines Agency (EMA) provides a comparable definition, except that immediate packing is used in place of primary packing (EMA, 2005). The US Federal Food, Drug, and Cosmetic Act (US FD&C Act) states that if a drug's container contains any harmful or toxic material, it is deemed adulterated in the US. Furthermore, substances from the container sealing systems could seep into an injectable.^[7]

"Container closure System Integrity in Lieu of Sterility testing as a component of the stability protocol for Sterile Products" was released on 2008 by FDA as a guidance for the industry. This recommends the use of physical container closure Integrity (pCCI) or microbial Container closure Integrity (mCCI) tests in the stability testing program instead of sterility testing. The sterility testing for release cannot be replaced by pCCI or mCCI tests and not all the health authorities have globally emphasis to replace the sterility testing on stability (shelf- life testing) with CCI.

As per 21 CFR part 211.94, the Container closure system should provide adequate protection against external factors during storage and use that could cause deterioration or contamination of the drug product. The standard also describes the standards, testing methods, and specifications for the validation procedures.

This research article aims to evaluate and validate the container closure integrity (CCI) of pharmaceutical packaging systems by utilizing samples and positive controls to make sure the test can identify microscopic leaks as an alternative to sterility testing, using the dye ingress method to ensure product sterility and safeguard against contamination. The ability to identify minor leaks in a container closure system is demonstrated by the Container Closure Integrity Test (CCIT) verification method by using sterile water for Injection vials by employing an Ultraviolet-Visible Spectrometer.

Regulatory Framework and Expectations:

As mentioned above, regulatory expectations and requirements have been spelled out and documented in some international regulatory regulations and guidelines. According to the US FDA (1999), the suitability of a selected container closure system must be established in accordance with protection, safety, compatibility, and performance.^[8] In the aspect of protection against microbial contamination, reactive gases, and moisture, the suitability of CCS is an essential part. To ensure product integrity, this guideline aligns with cGMP requirements and rigorous testing protocols for no leaks and contamination during product manufacturing and distribution. This highlights the importance of validation in CCI when considering transportation and shipping conditions.^[9] WHO recommends CCI as a global health context by addressing specific challenges and complementing other regulatory frameworks.

Key standards and guidelines for CCIT:

- USP <1207>: Package Integrity Evaluation
- USP <1207.1>: Package Integrity testing and test method Selection.
- USP <1207.2>: Package Integrity Leak test technologies
- USP <1207.3>: Package Seal Quality Test Methods
- ASTM F1929: Standard Test Method for Detecting Seal Leaks
- ISO 11607: Packaging for Terminally Sterilized Medical Devices

- FDA “Container Closure System Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products”, 2008

CCIT Testing Strategy Development:

• Dye compatibility:

The product and dye must be compatible to prevent any interaction that could affect the detection accuracy. It should be detected, even it may mixed with the product solution to ensure leak identification.

Commonly used dyes in this method are Methylene blue, Crystal Violet, and Rhodamine B which were selected based on the solubility, detectability, and stability.

• Concentration of Dye:

The concentration of dye for Vial Immersion should be directly proportional to the quantity of the product in the vial to maintain the detection sensitivity.

For Example:

If an analytical method has 0.1 ppm as the Limit of detection (LOD), it should be capable of detecting the 0.1% methylene blue (1 μ L) in a 10 mL Vial. If the same solution enters into more than 10 mL fill, then the diluted concentration may fall under the LOD value, making it unreliable to detect.

• Vacuum and Hold Time Optimization:

To force the dye solution into micro leaks vacuum application is crucial. For effective detection, the vacuum level and hold time should be evaluated carefully as 1- 5 μ m. Vacuum parameters include a vacuum level of 400- 600 mbar negative pressure and a hold time of 10- 30 minutes, followed by an atmospheric period to dye penetration. It is validated by using positive controls with microleaks to determine its sensitivity and reproducibility.

• Validation and Regulatory Considerations:

USP <1207> guidelines are followed for CCIT method validation.

Specificity ensures that there is no interaction between the dye and the product, preventing any interference that could affect the test results. Sensitivity (LOD) should detect the leaks reliably, Linearity should elicit the absorbance that should be directly proportional to the concentration of the selected dye, and Precision

should determine a consistent absorbance of spiked sample solutions.

Dye Ingress Method:

The Dye Ingress method is the most common CCIT type, similar to microbial ingress testing. The containers are inspected visually or with spectroscopy to observe the traces of dye inside the containers after the immersion in dye by applying a controlled vacuum or pressure. (10) It is widely used for its cost-effectiveness, simplicity, and reliability. USP and ISO suggest Methylene blue as the dye used for this method at a

concentration of 0.1% w/v. Different Concentrations of dye and can be prepared according to the requirements. Surfactants(if required) are used to reduce the surface tension of the dye, as they enhance the detection of defects and leaks by improving the wetting property.^[11] The Dye Ingress method is destructive and has a major advantage over the microbial ingress method in that it may be utilized with vials containing bactericidal or bacteriostatic formulations. Thus this method is considered an excellent method for evaluating the container closure integrity.^[12]

II. METHODOLOGY:

Method Description:

Table No.01 Method description

Instrumentation	Shimadzu UV- VIS Spectrophotometer (2600i) or equivalent., Analytical Balance, Sonicator, Package Integrity Tester (Leak Test Apparatus)
Reagents	Methylene Blue, Water
Materials	Needle (#14 gauge), Micropipette (2µm), Sealant, Cotton Sterile water for injection USP, 1 mL filled in 2R Vial Fiolax Clear Tubular Vial USP-I with 13 mm Chloro Butyl Rubber Stopper C1474 and sealed with 13mm Flip off Seals, White Matte Top.
Instrument Condition	Vacuum: 450 mm of Hg Vacuum hold time: 10 minutes Vacuum Release & Hold time: 30 minutes

Analytical Method development

Specificity:

Specificity is the method's ability to measure the interference at the wavelength of Methylene blue in the formulation (SWFI). The specificity shall be demonstrated by measuring the spectrum of solutions, i.e., sterile water for injection USP 1 ml solution (blank) and Methylene blue spiked sample solution.

- Solution Preparation:**

Use sterile water for injection USP 1 ml sample as blank (diluent), prepare methylene blue stock solution at a concentration of 100 µg/ ml, and methylene blue spiked solution from the stock as 2.0 µg/ ml concentration.

- Procedure:**

Scan the sample for a spectrum of the following solution into the UV-visible spectrophotometer against a blank (as such, sterile water for injection USP 1 ml) solution sample solution. Scan from 400nm to 800nm and record the wavelength maxima using a 1 cm cell/cuvette.

Limit of Detection:

- Solution Preparation:**

Use sterile water for injection USP 1 ml sample as blank (diluent), LOD stock solution at a concentration of 100 µg/ ml, and LOD solution at a concentration of 0.1 ppm of methylene blue from the stock solution.

- Procedure:**

Measure the absorbance of the above solutions at the wavelength maxima for all the LOD solutions individually in a UV-visible spectrophotometer against a blank solution (diluent).

Linearity:

The linearity of the analytical method is its ability to elicit test results that are directly proportional to the concentration of the methylene blue taken for test within a given range. Linearity shall be performed from the range lower level (LOD) to higher level (4.0 ppm)

- **Solution Preparation:**

Use sterile water for injection USP 1 ml sample as blank (diluent), Stock solution-1 at a concentration of 100 µg/ ml was prepared, followed by stock solution-2, and 3 are diluted to 10 µg/ ml and 1 µg/ ml, respectively. From stock 3, 0.2 ppm, 0.5 ppm, and 1.0 ppm concentration solutions were diluted, and from stock 2, 2.0 ppm, and 4.0 ppm concentration solutions were prepared.

- **Procedure:**

Measure the Absorbance of the Linearity solutions at wavelength maxima on a UV-spectrophotometer against solution from diluent as blank.

Method Precision:

Perform the CCIT as per the method procedure and determine the absorbance of six spiked test solution preparations and calculate the %RSD. The repeatability of the method shall be established by using six spiked sample preparations.

- **Solution Preparation:**

Methylene blue stock solution is prepared at a concentration of 1 ppm. This methylene blue spiked solution was prepared by diluting 1 mL of stock solution to 10 mL, achieving 0.1 ppm concentration. LOD solution and LOD stock solution were prepared at a concentration of 1 ppm and 0.1 ppm respectively.

- **Procedure:**

Measure the Absorbance of the solution at 400 to 800 nm for all 5 test samples and LOD solution individually in a UV-visible spectrophotometer with a 2 mm cuvette against a blank solution

Method to Validate the Packaging integrity Apparatus

Sample Preparation: Use 15 vials of Sterile Water for Injection USP, 1 mL as a test sample.

Note: The sample size was determined based on the number of crimping heads in the filling machine.



Fig.01 Calibrated Micropipette



Fig.02 Sample Vials with Flip off seal

Positive Control Preparation:

Take three vials of sterile water for injection USP, 1 mL – (Vial Size – 2R) for positive control, and shall be intentionally damaged to simulate small leaks. Remove the flip from the flip-off seal of the vial and insert a needle (about #21 gauge) through the rubber stopper. Then carefully insert a 2 µm µTip™ Micropipette inside the vial. Cautions to be taken care not to damage the tip of the micropipette. Apply sealant around the glass micropipette.



Fig.03 Positive Control Vials

Needle control:

The needle control study ensures that the leak happened only through a micropipette, not by other routes in positive control. Take a vial of sterile water for injection USP, 1 mL – (Vial Size – 2R) remove the flip from the flip-off seal of the vial which shall be intentionally damaged to simulate small leaks by inserting a needle (about #21 gauge) through the stopper. Remove the needle and apply the sealant which is used to apply for positive control over the simulated leak.



Fig.04 Negative Control Vials

Preparation of blank (negative control):

Take a vial of sterile water for injection USP, 1 ml – (vial size – 2R), and use the vial as such without treating it with methylene blue solution for the analysis.

Procedure for dye ingress method

Ensure the desiccator of the leak test apparatus is clean and dry. Remove the top lid of the desiccator and fill one-third of the desiccator with 0.1 % methylene blue solution (1 g of methylene blue powder dissolved in 1000 mL of purified water). Place five (5) sample vials, one positive control vial, and a needle control vial prepared. Ensure entire vials including micropipette (in case of positive control) are immersed and access to the dye should not be blocked by the air pocket. Place the perforated plate of the package integrity tester and then close the package integrity tester with the lid tightly and apply the vacuum to 450 mm/Hg. Maintain the vacuum pressure of 450 mm/Hg for 10 minutes. Release the vacuum and leave the vials in the dye solution at atmospheric pressure for not less than 30 minutes. Remove the vials from the dye solution, and wipe externally with a lint-free cloth.



Fig.05 Package Integrity Tester (Desiccator)

III. RESULTS AND DISCUSSION

Specificity:

The UV spectra of methylene blue spiked solution show the maxima at about 663.5 nm at an absorbance of 0.485. It shows that the method is specific to detect the presence of methylene blue.

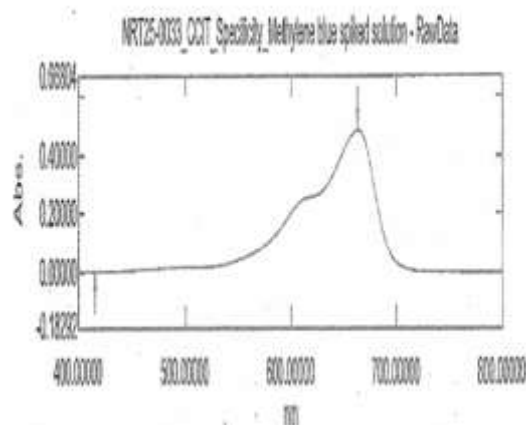


Fig.06 Absorption of Specificity

Linearity:

The table shows that the correlation coefficient (r) is 0.999, and the regression coefficient (r^2) is 0.998. This confirms that the correlation coefficient (r) and regression coefficient (r^2) meet the predefined acceptance criteria. Hence, the method is linear.

Table No.02 Linearity

S.No.	Levels	% level	Concentration in µg/ml	Absorbance
1.	L1	LOD	0.1007	0.018
2.	L2	0.2	0.2014	0.037
3.	L3	0.5	0.5036	0.104
4.	L4	1.0	1.0072	0.206
5.	L5	2.0	2.0144	0.455
6.	L6	4.0	4.0288	0.864
7.	Slope			0.217
8.	Y-intercept			-0.00402
9.	Coefficient of Correlation			0.999
10.	Coefficient of regression (r^2)			0.998
11.	Deviation of Y- Y-intercept from 100% standard concentration			-1.95

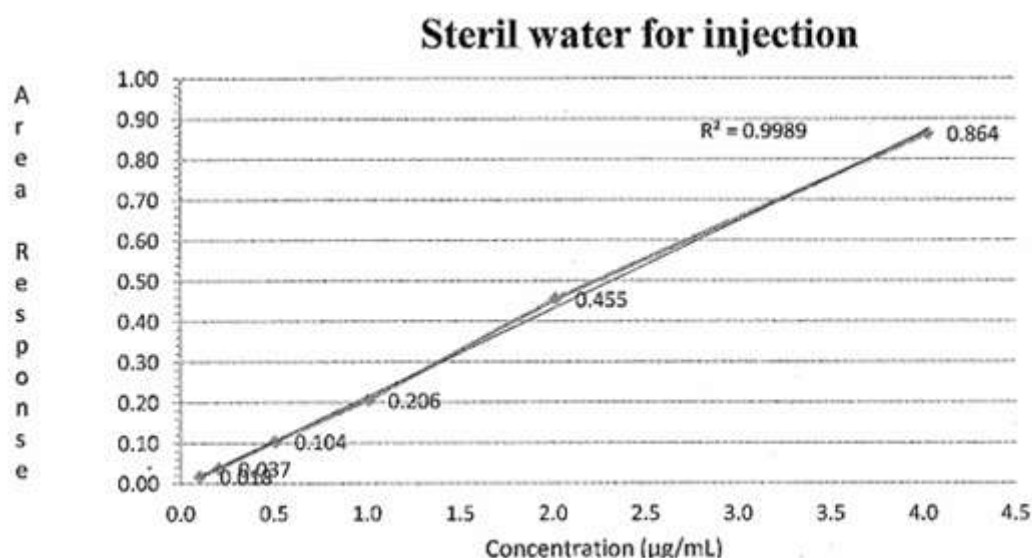


Fig No. 07 Linearity Graph for Concentration vs. Absorbance

Limit of Detection:

- **Observations:**

Table No.04 Limit of detection

S. No.	Sample Analysed	Absorbance
1.	Blank	0.0001
2.	Precision 0.1 ppm	0.0184
3.	Precision 0.1 ppm	0.0170
4.	Precision 0.1 ppm	0.0169
5.	Precision 0.1 ppm	0.0167
6.	Precision 0.1 ppm	0.0166
7.	Precision 0.1 ppm	0.0167

Analytical Results:

Table No.05 Analytical results from validated method

S. No	Vials For Analysis	Result (Absorbance)	Acceptance Criteria
1.	LOD Solution	0.022	➤ The presence of methylene blue should be detected (Conc.: $\approx$ 0.1 ppm of Methylene Blue)
2.	Blank (Negative control)	0.000	➤ Methylene blue should be absent in Blank.
Run # 1			
3.	Test Sample # 1	0.001	➤ All the test Samples of Sterile water for injection USP, 1 mL – (Vial Size – 2R) subjected to dye ingress test should not show absorbance greater than LOD solution absorbance. ➤ If the absorbance of the Test sample solution is less than LOD solution absorbance. ➤ If the absorbance of the Test sample solution is equal to or more than LOD solution absorbance.
	Test Sample # 2	0.001	
	Test Sample # 3	0.001	
	Test Sample # 4	0.001	
	Test Sample # 5	0.001	
4.	Positive Control # 1	2.236	➤ The presence of methylene blue should be detected.
5.	Needle control	0.001	➤ The test sample of needle control vial subjected to dye ingress test should not show absorbance greater than LOD solution absorbance.

S. No	Vials For Analysis	Result (Absorbance)	Acceptance Criteria
Run # 2			
1.	Test Sample # 1	0.000	➤ All the test Samples of Sterile water for injection USP, 1 mL – (Vial Size – 2R) subjected to dye ingress test should not show absorbance greater than LOD solution absorbance. ➤ If the absorbance of the Test sample solution is less than LOD solution absorbance. ➤ If the absorbance of the Test sample solution is equal to or more than LOD solution absorbance.
	Test Sample # 2	0.000	
	Test Sample # 3	0.000	
	Test Sample # 4	0.000	
	Test Sample # 5	0.000	
2.	Positive Control # 2	2.234	➤ The presence of methylene blue should be detected

S. No	Vials For Analysis	Result (Absorbance)	Acceptance Criteria
Run # 3			
1.	Test Sample # 1	0.000	➤ All the test Samples of Sterile water for injection USP, 1 mL – (Vial Size – 2R) subjected to dye ingress test should not show absorbance greater than LOD solution absorbance. ➤ If the absorbance of the Test sample solution is less than LOD solution absorbance, Report the results as “Pass”. ➤ If the absorbance of the Test sample solution is equal to or more than LOD solution absorbance, Report the results as “Fail”
	Test Sample # 2	0.000	
	Test Sample # 3	0.000	
	Test Sample # 4	0.000	
	Test Sample # 5	0.000	
2.	Positive Control # 3	2.228	➤ The presence of methylene blue should be detected

Summary:

Table No.06 Validation Summary

Verification parameter	Acceptance criteria	Results
Specificity	UV spectra of the Methylene Blue Spiked Solution should exhibit maxima at about 664 nm± 5 nm.	UV spectra of Methylene blue spiked solution exhibit maxima at 663.5 nm (Absorbance: 0.553)
	No Absorbance should be observed in the Blank	No absorbance was observed in the blank.

LOD (Sensitivity)	The presence of methylene blue should be detected.	The presence of methylene blue was detected.
Linearity	Correlation coefficient (r) shall be NLT 0.995 and regression coefficient (r ²) shall be NLT 0.990	Correlation coefficient (r) is 0.999 and regression coefficient (r ²) is 0.998
	The % Y intercept should be $\pm 5.0\%$ of the active response at 100% concentration.	The % Y intercept is -1.95 of the active Response at 100% concentration.
Method precision	UV spectra of the methylene blue spiked solution should exhibit maxima 664 nm $\pm$ 2 nm.	UV spectra of the Methylene Blue Spiked solution maxima at 663.5 nm.

IV. CONCLUSION:

Based on the review of data generated during the execution of Container Closure Integrity test by dye ingress method using positive control along with a sample to ensure the test method is capable of detecting small leaks that resulted in complies with the acceptance criteria for Blank (Negative control), Needle Control, Positive Control, and Test samples. Hence, it is concluded that the Container Closure Integrity test by dye ingress method is practical to deduct a small leak in sterile water for injection USP, 1 mL – (Vial Size – 2R) and the same testing method recommended for routine testing.

Revalidation Criteria:

- **Fill volume change:** Revalidate the CCIT method to assess the integrity of different fill volumes and in the worst case scenarios.
- **Change in Rubber Stopper:** Seal integrity should be assessed with new stopper through leak testing and compatibility evaluation.
- **Change in Crimping Machine:** Crimp seal uniformity and its integrity is assessed using CCIT and Visual Inspection.
- **Other Changes:** Requalification of CCIT method for container type, sterilization method, storage conditions or test method changes.

Author Contribution:

Deepikaa.S.K: Validation, formal Analysis, Investigation, Writing- Original draft,
Murugan.M: writing- Review& editing,
Jeyapragash.R- Conceptualization, Methodology, Resources, Supervision, Project Administration,

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Conflict of Interest: The authors claim that there is no conflict of Interest.

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