

Validation of Qualitative Identification Methods for Botanicals: Issues and Approaches

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Identification of Botanicals by Chemical Methods



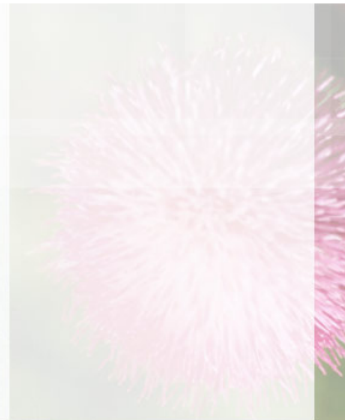
- Many DS manufacturers use extracts of botanicals in their products.
- New GMPs for DS require manufacturers to perform at least one identity test on all ingredients.
- Use of extracts precludes macroscopic and/or microscopic identification.
- As a result, chemical test methods must be used to verify the identity of the botanical.
 - TLC
 - HPLC
 - GC



The Problem



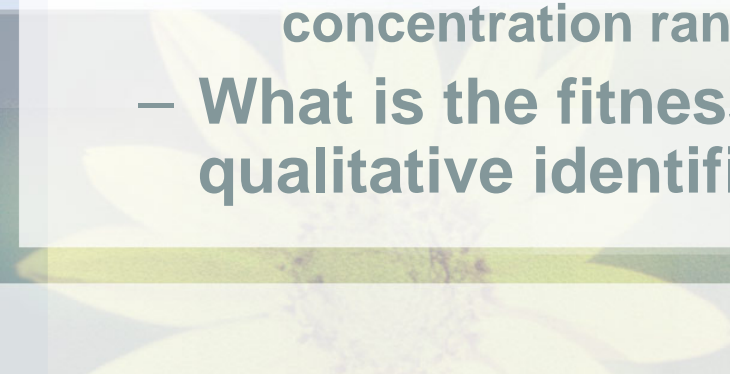
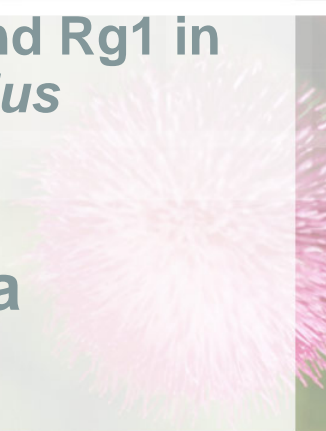
- How do we ensure that the chemical test method we are using to identify the botanical is accurate and reproducible?
 - The series of experiments performed to demonstrate the accuracy and reproducibility of a method is known as “Validation.”



Fitness for Purpose



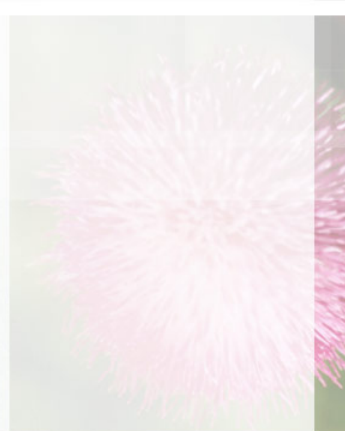
- In order to validate any method (quantitative or qualitative), we must know what the *fitness for purpose* of the method is.
 - Answers the question “What is the method supposed to do?”
 - Example of fitness for purpose statement for a quantitative method:
 - “The method will be able to quantify the ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1 in *Panax ginseng* and *Panax quinquefolius* botanical raw materials, extracts, at a concentration range of ...”
 - What is the fitness for purpose of a qualitative identification method?



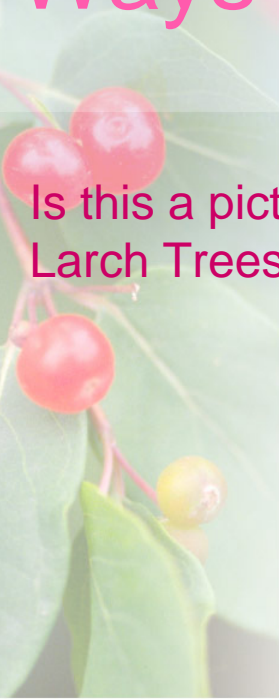
How to Identify Trees from Quite a Ways Away



Is this a picture of Larch Trees?



How to Identify Trees from Quite a Ways Away

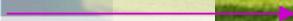


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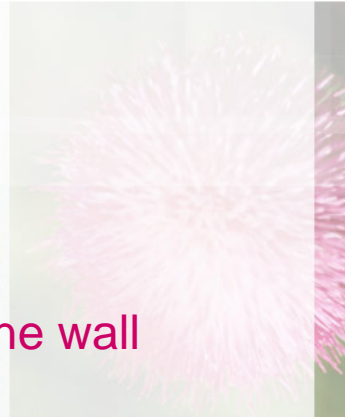


Clouds

Grass



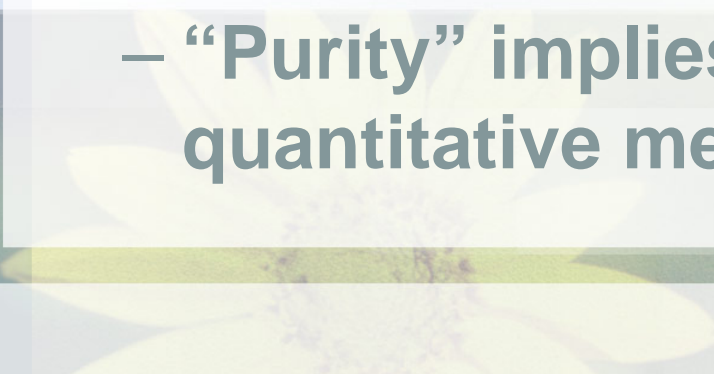
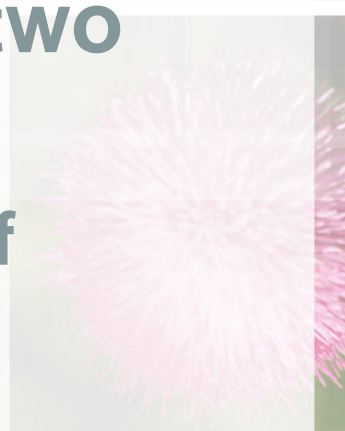
Stone wall



Fitness for Purpose



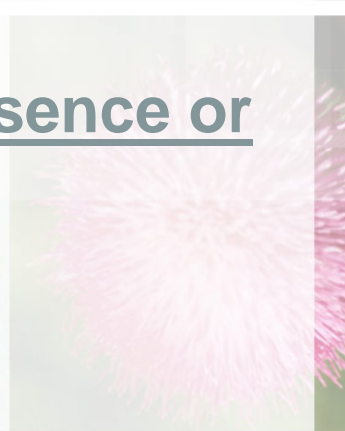
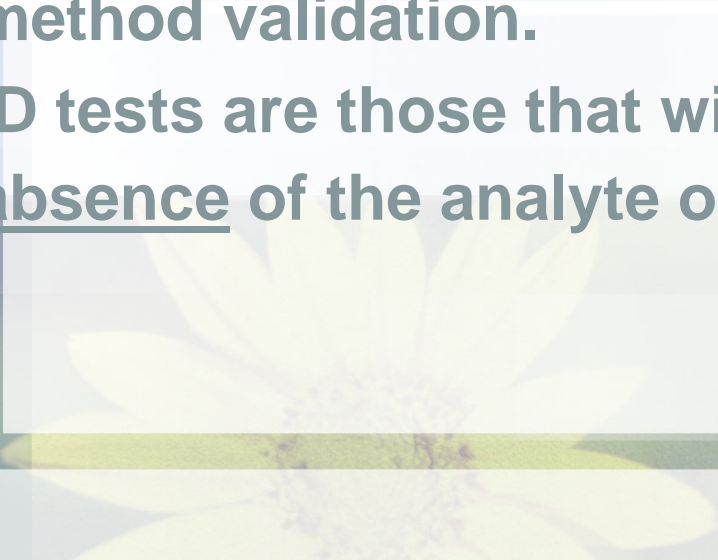
- **Botanicals are never 100% pure**
 - Dirt/filth
 - “Weeds”
 - Insects
 - Pesticides/herbicides/heavy metals
 - Other related species
- **“Identity” and “Purity” are two separate specifications.**
 - “Purity” implies some type of quantitative method



Fitness for Purpose



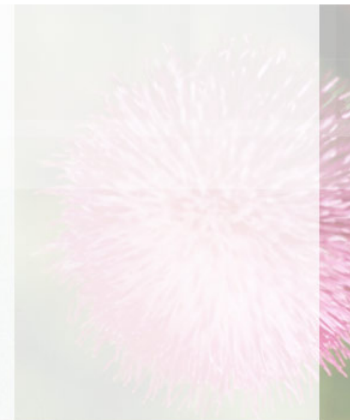
- Recommendation of AOAC Qualitative Study Protocol Task Group (April, 2006)
 - There are two conditions (1) identifying an adulterant (e.g. Allergen in corn) and (2) identifying the bulk matrix (the corn)
 - If the scope of the method includes adulterants, then that should be covered in method development and made clear in the protocol for method validation.
 - ID tests are those that will detect the presence or absence of the analyte or botanical.



Validation of ID Methods



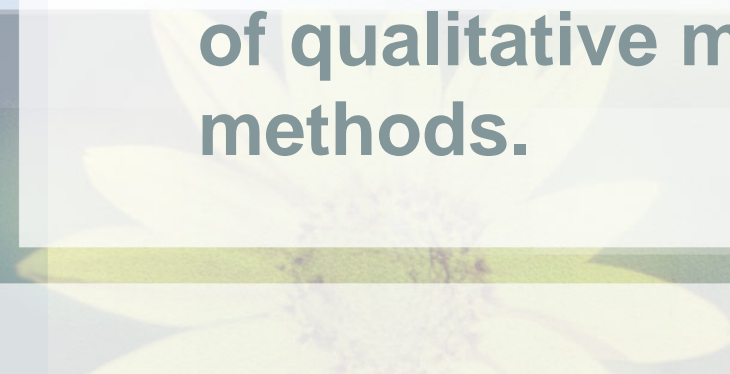
- Many well-established guidelines for validation of quantitative methods.
 - AOAC
 - USP
 - ICH
- Reliability of quantitative methods measure by:
 - Accuracy (% recovery)
 - Precision (% RSD)
 - Linearity/Range
 - LOD/LOQ
 - Selectivity
 - Ruggedness



Validation of ID Methods



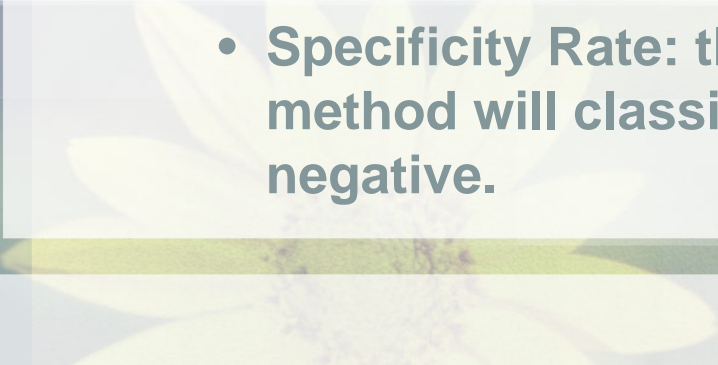
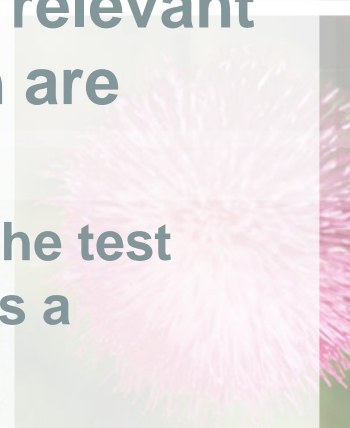
- **What characteristics do we test to determine the reliability of qualitative ID methods?**
 - Qualitative ID methods do not report results as numbers, but as “yes/no” or “presence/absence.”
 - What, if any, kind of statistics can we perform on this type of result?
 - Concepts borrowed from validation of qualitative microbiological ID methods.



Validation of ID Methods



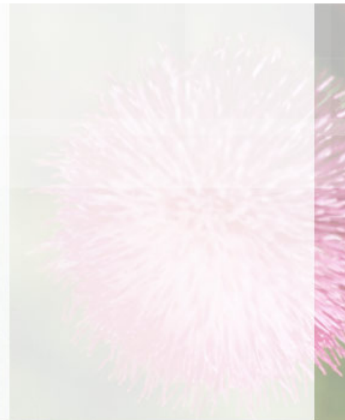
- **Concepts from Microbiological Method Validation**
 - **Sensitivity (Inclusivity):** the ability of the method to detect the target analyte from a wide range of strains.
 - **Sensitivity Rate:** the probability that the test method will classify a true positive as a positive.
 - **Specificity (Exclusivity):** the lack of interference in the method from a relevant range of non-target strains, which are potentially cross-reactive.
 - **Specificity Rate:** the probability that the test method will classify a true negative as a negative.



Validation of ID Methods



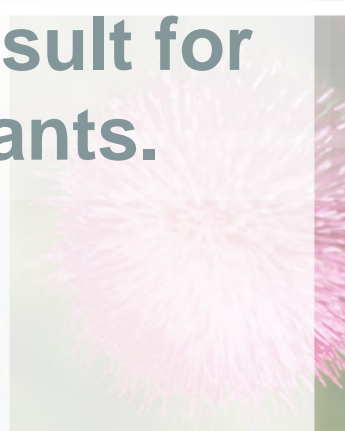
- **Concepts from Microbiological Method Validation**
 - **False Negative Rate:** the probability that the method will classify a true positive as a negative.
 - **False Positive Rate:** the probability that the method will classify a true negative as a positive.



Validation of ID Methods



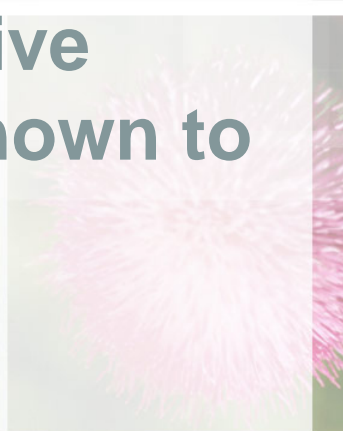
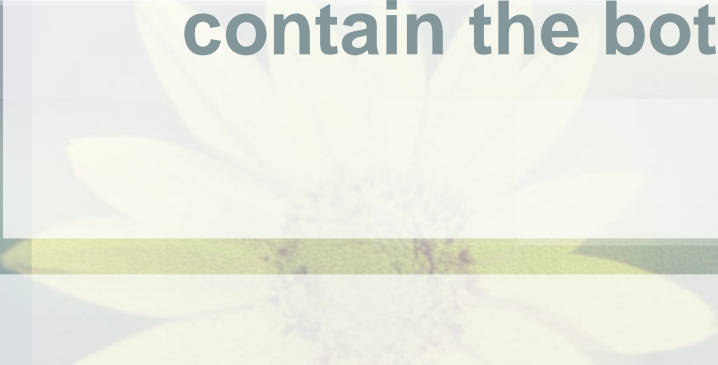
- **Application to TLC identification of botanical extracts.**
 - **Sensitivity is the ability of the method to detect the botanical in the presence of carriers and/or known adulterants.**
 - **Specificity is the ability of the method to yield a negative result for known contaminants/adulterants.**



Validation of ID Methods



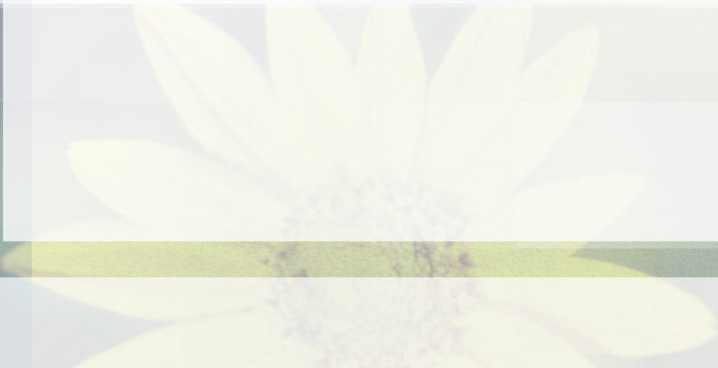
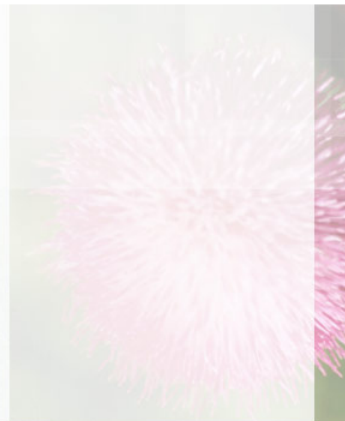
- **Application to TLC identification of botanical extracts.**
 - False positive rate is the probability the method will yield a positive result for the botanical when the material is a known contaminant or adulterant.
 - False negative rate is the probability the method will yield a negative result for a material that is known to contain the botanical.



Validation of ID Methods



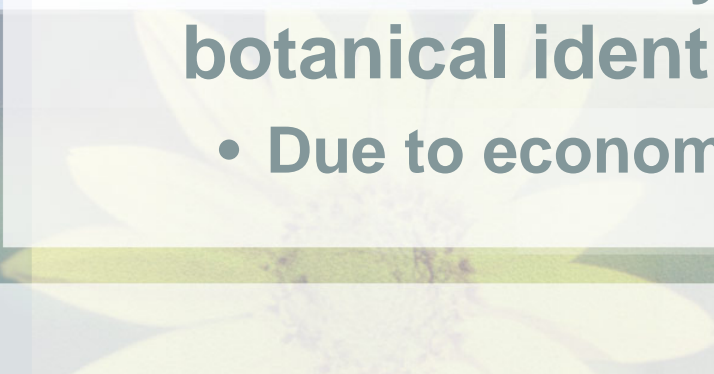
- **Requirements to determine performance characteristics:**
 - **Authentic reference materials**
 - botanical of interest
 - likely contaminants/adulterants
 - **Samples must include definite positives, definite negatives, and a range of known adulterants.**



Validation of ID Methods



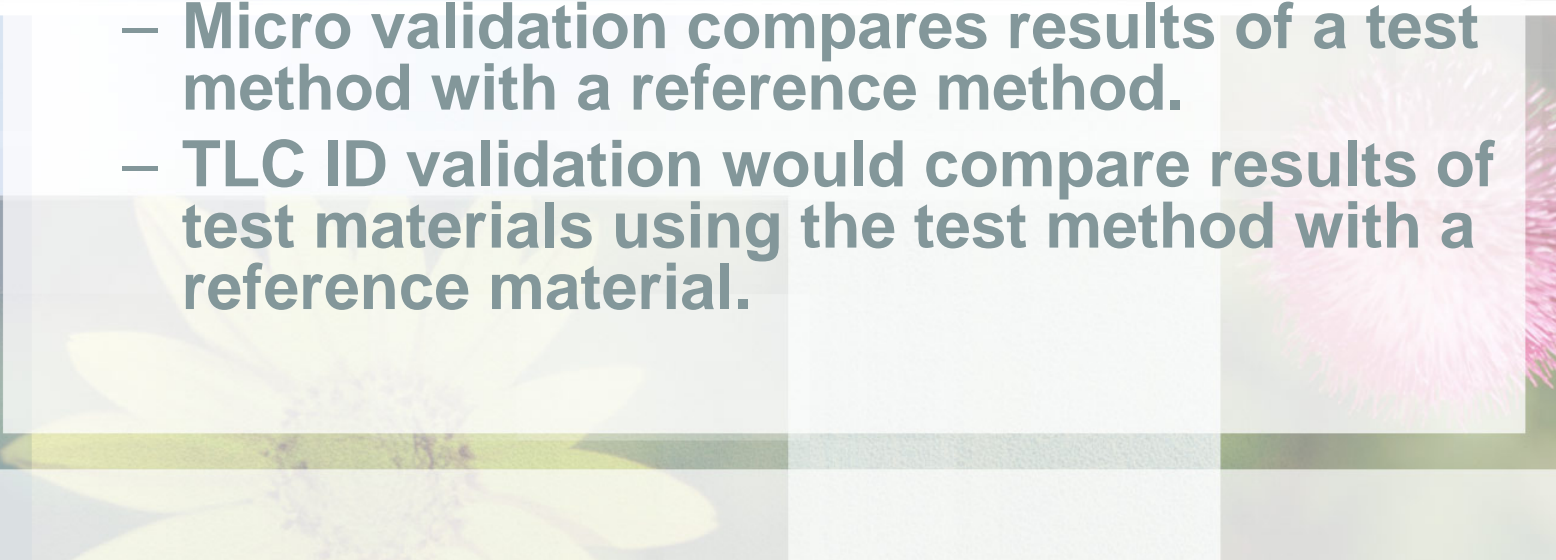
- **Differences between validating microbiological ID methods and TLC ID methods of botanicals:**
 - Sensitivity rate and false negative rate are usually most critical for micro methods
 - Due to safety issues
 - Selectivity rate and false positive rate are usually most critical for botanical identification methods
 - Due to economic adulteration



Validation of ID Methods



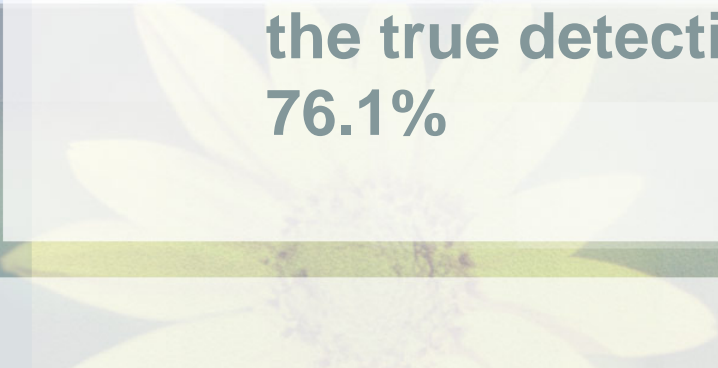
- **Differences between validating microbiological ID methods and TLC ID methods of botanicals:**
 - Micro methods are usually looking at very low levels of analyte (the bugs) in a bulk matrix.
 - Identification of botanical extracts is usually dealing with identification of the bulk material as opposed to trace contaminants.
 - Micro validation compares results of a test method with a reference method.
 - TLC ID validation would compare results of test materials using the test method with a reference material.



Validation of ID Methods



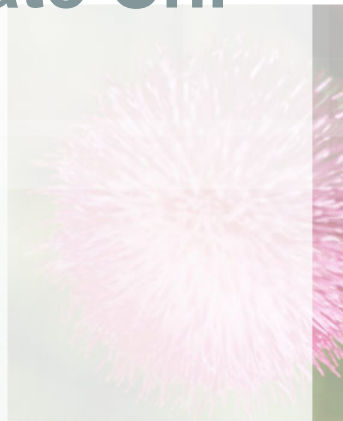
- **How many materials needed?**
 - AOAC task group recommended at least 10 known positives and 10 known negatives.
 - If 100% of the samples are identified correctly, there is 95% confidence that the true detection rate is not lower than 90.5%
 - If 90% of the samples are identified correctly, there is 95% confidence that the true detection rate is not lower than 76.1%



Validation of ID Methods



- **How many analysts needed?**
 - For a single laboratory, at least 3 analysts must independently test the sample materials.
- **Statistical Treatment:**
 - McNemar test used to calculate Chi-squared value.

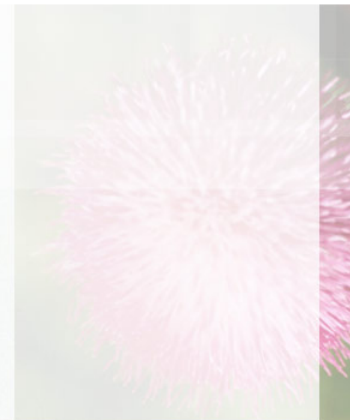


Validation of ID Methods



Status of Test Samples	Test Result		Total
	Positive	Negative	
Positive	N_{11}	N_{12}	$N_{1.}$
Negative	N_{21}	N_{22}	$N_{2.}$
Total	$N_{.1}$	$N_{.2}$	N

- **Chi-square (χ^2) = $(|N_{12} - N_{21}|)^2 / (N_{12} + N_{21})$**
- **Sensitivity Rate = $N_{11}/N_{1.}$**
- **Specificity Rate = $N_{22}/N_{2.}$**
- **False Negative Rate = $N_{12}/N_{1.}$**
- **False Positive Rate = $N_{21}/N_{2.}$**



Conclusions



- There currently are no accepted guidelines for the validation of qualitative TLC methods for the identification of botanicals.
- Validation guidelines used for quantitative methods are generally inappropriate for validating qualitative ID methods.
- Identity methods can only determine the presence or absence of a botanical/analyte, and say nothing about the purity.



Conclusions

- **Adapting guidelines for validation of qualitative microbiological methods for use with botanical identification can provide much useful information about the reliability of the method.**
 - **Specificity**
 - **Sensitivity**
 - **False positive rate**
 - **False Negative rate**

