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Analytical Method Development and Validation for the Estimation of Nicotine Content in Various Tobacco Grades by RP-HPLC

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Abstract **Review Article**

A simple, precise, rapid, selective, and economic reversed phase high- performance liquid chromatography (RP-HPLC) method has been developed and validated for the estimation of nicotine content in various tobacco grades in laboratory prepared mixture on a Phenomenex C18 (250×4.6mm i.d) chromatographic column equilibrated with mobile phase containing Methanol/potassium dihydrogen ortho phosphate buffer. Experimental conditions such as organic phase ratio, flow rate, selection of wavelength, etc. were critically studied and the optimum conditions were selected. Efficient chromatographic separation was achieved with mobile phase containing combination of Methanol & potassium dihydrogen ortho phosphate buffer in ratio of 50:50v/v at flow rate of 1.0 ml/min and eluent was monitored at 260 nm. The sample was injected by using a 20µl fixed loop, and the total run time was 6 min. The retention time for Nicotine was 2.797 min. The method was linear in the range of 25µg/ml to 125µg/ml for Nicotine. The proposed method was successfully applied to the analysis of Nicotine in laboratory prepared mixtures. The developed method was validated according to ICH guidelines. Regression coefficient was 0.9996 with asymmetry of NMT 2 and percentage recovery was 99.70%. LOD and LOQ values were 0.41 and 1.23 respectively and method was found to be satisfactory also estimation of nicotine content in various tobacco grades was obtained. This validated HPLC procedure is economic, sensitive, user-friendly & less time consuming than other chromatographic procedures.

Keywords: RP-HPLC, Nicotine, Tobacco Grades, Chromatographic Separation, Laboratory Prepared Mixtures and Validation.

INTRODUCTION

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compound. The substance may be a single compound or a mixture of compounds and it may be inany of the dosage form.

Quality assurance (QA) plays a central role in determining the safety and efficacy of medicines. Highly specific and sensitive analytical techniques hold the key to the design, development, standardization and quality control of medicinal products. They are equally important in pharmacokinetics and in drug metabolism studies, both of which are fundamental to the assessment of bioavailability and the duration of clinical response.

The present Study is aimed to develop a new, simple, fast, rapid, accurate, efficient, reproducible, RP-HPLC method for the estimation of nicotine content in various tobacco grades as per ICH guidelines.

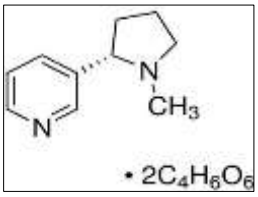
Objectives:

1. The analytical method for the estimation of nicotine content in various tobacco grades will be developed by RP-HPLC method by optimizing the chromatographic conditions and validated.
2. To develop a new, simple, rapid, sensitive, accurate, reproducible, and economical analytical method for the determination of

nicotine content in various tobacco grades by RP-HPLC method.

3. The developed method is validated according to ICH guidelines for various parameters specified in ICH guidelines.

Drug Profile of Nicotine:
Chemical Structure:



Synonyms: Tobacco, Baccy, Nightshade.
Biological Source: It is derived from the plant Nicotiana Tabacum.
Chemical Name: 3-[2s] (1-methylpyrrolidin-2-yl) pyridine
Empirical Formula: C10H14N2 .2C4H6O6
Melting Point: -79 ºC (-110 ºF)
Boiling Point: 247º C (477 ºF)
Molecular weight: 162.12 gm/mol
Density: 1.01 gm/cm3
PKa: 3.2, 9.2

Category:

Anti-craving Agents, Central Nervous System Agents, Autonomic drugs, Nicotinic Agonists, Ganglionic Stimulant, Vasoconstrictive, Phytotoxin.

Description:

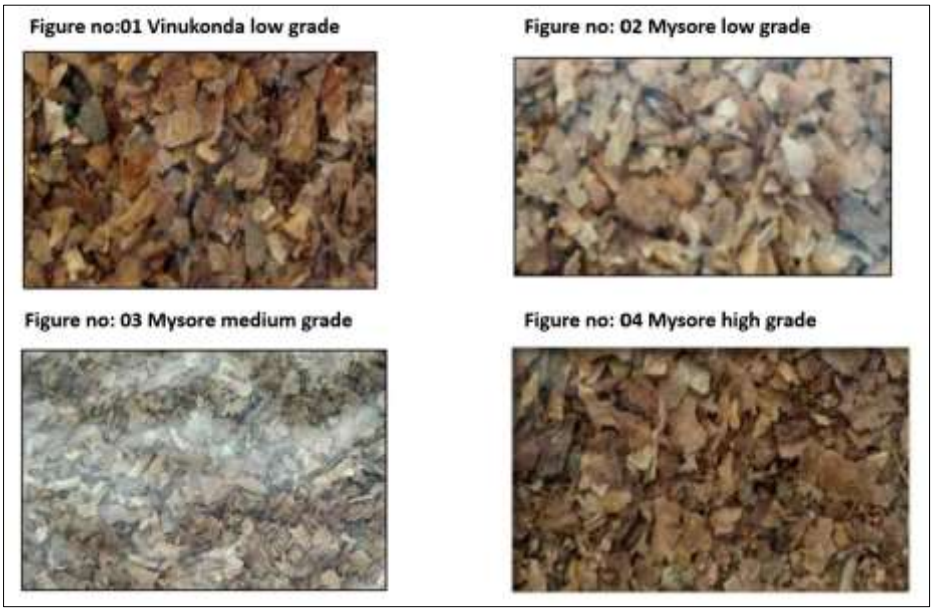
Nicotine is highly toxic alkaloid. It is the prototypical agonist at nicotinic cholinergic receptors where it dramatically stimulates neurons and ultimately blocks synaptic transmission. Nicotine is also important medically because of its presence in tobacco smoke.

Chemistry:

Nicotine is a hygroscopic, oily liquid that is miscible with water in its base form. As a nitrogenous base, nicotine forms salts with acids that are usually solid and water soluble, for example nicotine sulfate which, being a solid, is easier to handle in its use as an insecticide. Its flash point is 95°C and its auto-ignition temperature is 244°C.

Storage Condition: Store below 40°C, protect cartridges from light

Different Tobacco grades from different areas in South India:



Method Development: It was done by changing various columns, mobile phase ratios, buffers and its P^H etc.

Initial Chromatographic Conditions:

Several trials were performed for selection of column and mobile phase for the Estimation of Nicotine content in various Tobacco grades (vinukonda low grade burley, mysore (low, high, medium).

Preparation of Buffer:

Accurately weighed 6.24g of sodium dihydrogen ortho phosphate was taken in a 1000 ml volumetric flask. 0.68 ml of OPA was added and volume was made up to the mark with double distilled water.

Chromatographic Condition for Trail-01:

- **Preparation of Mobile phase:** Prepared mixture of buffer and acetonitrile was taken in the ratio of 75: 25 % v/v.

- **Chromatographic Conditions:**
  - Column : Agilent C_{18} coloum 5µm (4.6x250mm)
  - Mobile phase: Phosphate buffer: Acetonitrile (75:25%v/v)
  - Flow rate: 1.0 ml/min
  - Detection wavelength: 260nm
  - Injection Volume: 10µg/ml
  - Temperature: ambient
  - Run time: 10 mins

Observation: Extra peak was detected and Asymmetry for peak was more.
Reason: May be mobile phase composition was not suitable.

Chromatographic Condition for Trail-02:

- **Preparation of Mobile phase:** Prepared mixture of buffer and Acetonitrile was taken in the ratio of 65: 35% v/v as mobile phase.

- **Chromatographic Conditions:**
  - Column: Agilent C_{18} coloum 5µm(4.6x250mm)
  - Mobile phase : Phosphate buffer : Acetonitrile (65:35%v/v)
  - Flow rate: 1.0 ml/min
  - Detection wavelength : 263nm
  - Injection Volume : 10µg/ml
  - Temperature: ambient
  - Run time: 10 min

Observation: Asymmetry for Peak was more, Extra peaks were detected and Peak efficiency was less.
Reason: May be column and mobile phase composition was not suitable.

Chromatographic Condition for Trail-03:

- **Preparation of Buffer:** Accurately weighed 2.72g of potassium dihydrogen phosphate was taken in a 1000 ml volumetric flask about 0.1 ml of OPA was added and volume was made up to the mark with double distilled water.

- **Preparation of Mobile Phase:**
  - Prepared mixture of potassium dihydrogen ortho phosphate buffer and acetonitrile was taken in the ratio of 70:30% v/v as mobile phase.

- **Chromatographic Conditions:**
  - Column: Agilent C_{18} column 5µm (4.6x250mm)
  - Mobile phase: phosphate buffer : methanol (70:30%v/v)
  - Flow rate : 1.0 ml/min
  - Detection wavelength : 260 nm
  - Injection Volume : 10 µg/ml
  - Temperature : Ambient
  - Run time : 6min.

Observation: The peak was not resolved.
Reason: May be column and mobile phase composition was not suitable and injection volume was less.

Chromatographic Condition for Trail-04:
Preparation of Mobile Phase: Prepared mixture of potassium dihydrogen ortho phosphate buffer and methanol in the ratio of 20:80% v/v was used.

Chromatographic conditions:
Column: Phenomenex C18 (250×4.6 mm, 5.0 µm)
Mobile phase: phosphate buffer: methanol (20:80%v/v)
Flow rate: 1.0 ml/min
Detection wavelength: 260nm
Injection Volume: 25 µl/ml
Temperature: ambient
Run time: 10min.

Observation: The peak was not resolved
Reason : May be the mobile phase composition was not suitable.

Chromatographic Condition for Trail 05:

- **Preparation of Mobile phase:** Prepared mixture of potassium dihydrogen ortho phosphate buffer and methanol in the ratio of 55:45% v/v was used.

- **Chromatographic Conditions:**
  - Column : Phenomenex C_{18} (250×4.6 mm, 5.0 µm)
  - Mobile phase : phosphate buffer : methanol (55:45% v/v)
  - Flow rate : 0.8 ml/min
  - Detection wavelength : 260 nm
  - Injection Volume : 25µg/ml
  - Temperature : ambient
  - Run time : 5min.

Observation: Retention time was more, Asymmetry was within limit and theoretical plate count was found to be satisfactory.
Reason: May be flow rate will be change.

Optimized Method Trail 06:

- Accurately weighed 2.72g of potassium dihydrogen phosphate was taken in a 1000 ml volumetric flask about 0.1 ml of OPA was added and volume was made up to the mark with double distilled water.

Preparation of Mobile Phase: Prepared mixture of potassium dihydrogen ortho phosphate

- buffer and methanol in the ratio of 50:50% v/v was used as mobile phase.

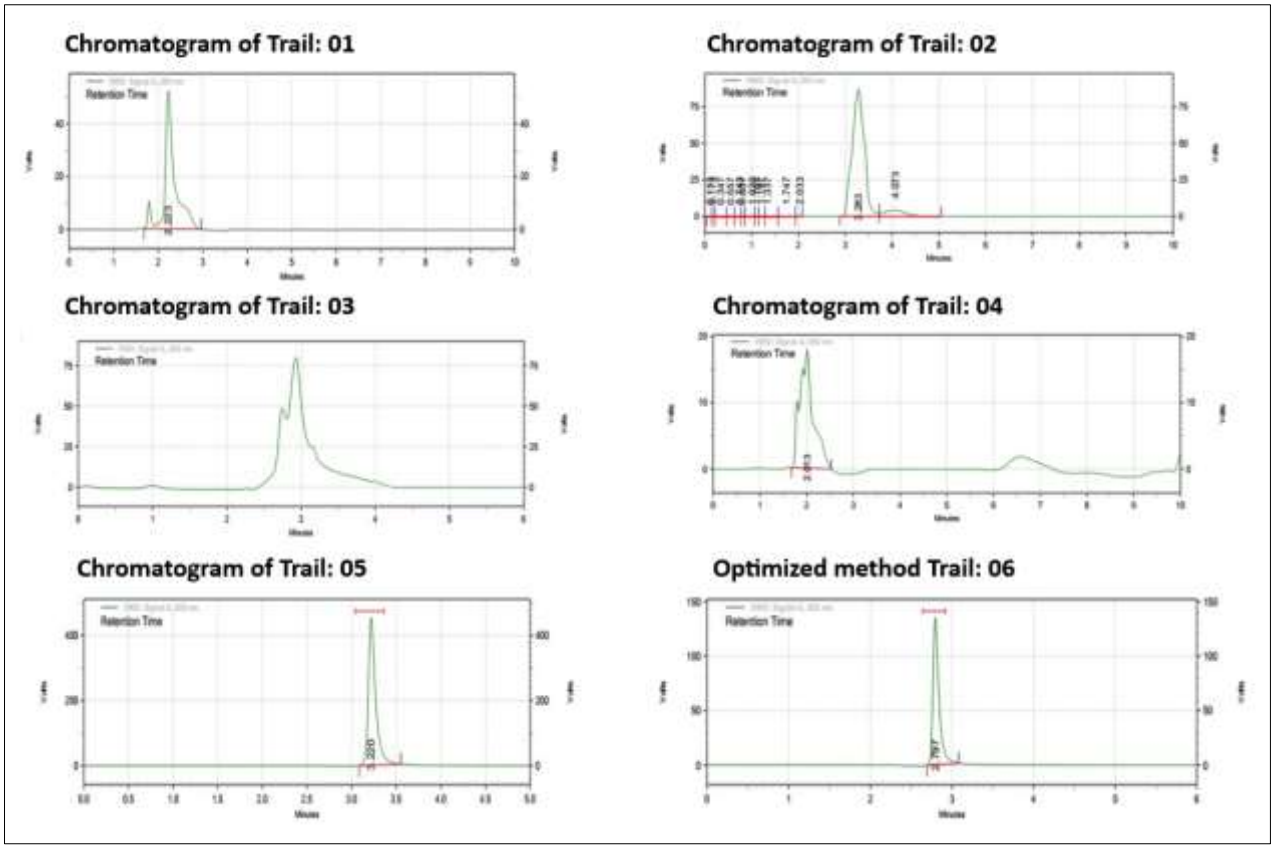
Optimized Chromatographic conditions:

- Column : Phenomenex C_{18} (250×4.6 mm, 5.0 µm)

- Mobile phase : Potassium dihydrogen ortho phosphate buffer : methanol (50:50 %v/v)
- Flow rate : 1.0 ml/min
- Detection wavelength : 260 nm
- Injection Volume : 25 µg/ml
- Temperature : ambient
- Run time : 6 min.

Observation: Peak was completely resolved, retention time was less and Peak shape was good.

Discussion: Nicotine was eluted at 2.797min with good resolution and Asymmetry. Plate count and tailing factor was satisfactory. So this method was considered as optimized and validated.



Method Validation:

Method validation is the process of proving that an analytical method is accepted for its intended purpose. For pharmaceutical methods, guidelines from the Unites States Pharmacopoeia (USP), International Conference on Harmonization (ICH) and the Food and Drug Administration (FDA) provides a frame work for performing such validations. Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results, when used by different operators employing the same equipment in the same or different laboratories.

DEFINITION

"Validation is the process of providing documented evidence that the method does what it is intended to do." In other words, the process of method validation ensures that the proposed analytical methodology is accurate, specific, reproducible, and rugged for its intended use. FDA defines validation as "establish of the documented evidence which provides as high degree of assurance that a specific process will consistently produce a product of predetermined specifications and quantity attributes".

The objective of validation is to form a basis for written procedure for Production and Process control, which are designed to assure that the drug products have the Identity, Quality and Purity.

For method validation, these specifications are listed in USP Chapter <1225>, and can be referred to as the "Eight Steps of Method Validation," as shown in figure below. These terms are referred to as "**analytical performance parameters**", or sometimes as "analytical figures of merit." Most of these terms are familiar and are used daily in the laboratory.

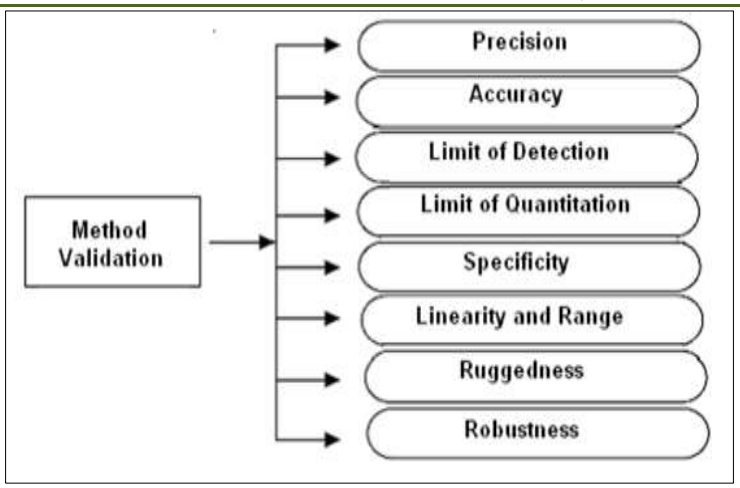


Figure 5: The USP Eight Steps of Method Validation

CONCLUSION

A simple, sensitive, precise and specific Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for Estimation of Nicotine content in various Tobacco grades. The separation was performed on Phenomenex C18 (250×4.6mm i.d) chromatographic column. The mobile phase consists mixture of methanol and potassium dihydrogen ortho-phosphate buffer (50:50). The flow rate was kept at 1.0 mL/ min and detection was performed at 260 nm. According to ICH guidelines, system suitability parameters constitute integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. The obtained parameters. The linear response was observed in the range of 25µg/ml to 125µg/ml for Nicotine standard. The proposed method had adequate specificity for estimation of nicotine content in various tobacco grades. The percentage recoveries were found to be within limits of acceptance criteria between the ranges of 98 % to 102 %. System precision, method precision and intermediate precision were found to be within limits and method was found to be robustness. The results of assay showed good results. Summary of validation parameters is shown in Table. The method was validated statistically and was applied successfully for estimation of Nicotine content in various Tobacco grades.

The Developed and validated method for the Estimation of Nicotine content in various Tobacco grades was found to be simple, precise, accurate and rapid. The mobile phase is simple to prepare and economical. The proposed method was simple and did not involve laborious time-consuming sample preparation. Short run time and the possibility of analysis of large number of samples. Hence, the method was easily and conveniently adopted for routine analysis of assay of Nicotine content estimation in various Tobacco grades. All the Validation Parameters were accepted within the limited as per ICH guidelines. Therefore, the method is suitable for its intended use.

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