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A COMPREHENSIVE REVIEW: ROLE OF ACETONITRILE AND METHANOL AS MOBILE PHASE SOLVENTS IN RP-HPLC

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ABSTRACT:

The purpose of this review article is to help understand why acetonitrile and methanol are commonly used in RP-HPLC mobile phases. Acetonitrile and methanol are used as commonly in RP- HPLC, this is due the many advantages like good elution strength, cost effectiveness, low viscosity, high polarity, low UV absorbance, purity, broad solubility range, chemical inertness, and eco-friendly. By using these solvents when compared to other solvents like acetone, ethanol isopropanol and n propanol, Tetra hydro furan have the limitations like higher cost, UV transparency, viscosity, limited Solubility. By using the Acetonitrile and methanol we can achieve a effective elution of analytes, separation and isolation techniques and also it highlights how important the solvent fie effective separation and repeatable analysis.

KEYWORDS: Methanol, Acetonitrile, Solvents, RP-HPLC, Elution, Solubility.

INTRODUCTION:

The purpose of this review article is to help understand why acetonitrile and methanol are commonly used in RP-HPLC mobile phases. The most commonly employed analytical method in the pharmaceutical sector today is reversed phase high performance liquid chromatography (RP-HPLC), which is used in almost every phase of the growth and development, discovery, and production of novel pharmaceutical products. Different elution systems, ranging from isocratic to multi-solvent gradient conditions, can be used with HPLC. In RP-HPLC, two-component solvent systems such as methanol–water or acetonitrile–water are most commonly employed. However, in cases where the separation proved challenging, three components. Solvent systems could be a wise option. For the separation of natural products, the most widely utilized three-component solvent system is methanol, acetonitrile, and water. Reversed-phase chromatography's mobile phases are based on a polar solvent, usually water, to which a less polar solvent, like methanol or acetonitrile, is added. In reversed-phase chromatography, acetonitrile (MeCN) and methanol (MeOH) are the most often utilized organic modifiers. Despite the fact that each solvent has pros and cons, one of their main advantages from a chromatographic standpoint is that they provide significantly varying selectivity, which makes them useful for method development. This article will compare the two solvents' various properties, including pressure, elution strength, and UV cut-off. It will also go over how organic eluent selection can be utilized as a tool for method development. Water, a less polar organic solvent (the organic modifier), and additives like buffers, acids, or bases are commonly found in reversed-phase mobile phases. While the organic component of the mobile phase has a higher elution strength in reversed phase, the aqueous component has a weaker analyte elution strength. Analyte retention can thus be managed by varying the amounts of organic and aqueous materials. Although only a few numbers of organic solvents have been utilized often in practice, a variety of organic solvents can be employed as the organic modifier in reversed-phase liquid chromatography.

CRITERIA FOR THE SOLVENTS USING RP-HPLC:

Solvent Purity:

To guarantee low absorbance in UV spectra and the absence of air bubbles, the solvent chosen as a mobile phase in LC should be of HPLC or MS grade and should be filtered and sonicated.

Solvent Viscosity:

To help guarantee an ideal linear velocity across the HPLC column stationary phase, which in turn produces narrower and sharper peaks, the reversed phase mobile phase should have an ideal viscosity. This will increase the analysis's overall sensitivity.

Refractive index:

One important factor to take into account when employing a refractive index detector is the refractive index. To prevent negative peaks when using a RI detector, the mobile phase and sample diluent should ideally have identical refractive indices; in other words, the sample should be produced in the mobile phase.

Solvent Boiling Point:

For better recovery, it is essential to select a solvent with a low boiling point. In LC-MS, in particular, a more volatile solvent will enhance ionization, which will raise MS sensitivity.

Non-Corrosiveness:

The HPLC tubing, pump valves, seals, and other components that are in the flow path must not be corroded by the mobile phase solvent.

Solvent Toxicity:

Use the stabilized solvent and make sure it is not poisonous. Selecting a solvent that is completely miscible with the sample is important.

Solvent Miscibility:

Choose a solvent which is perfectly miscible with the sample. The analyte also must be soluble in it. Otherwise, you will experience sample precipitation at the column inlet frits creating high backpressure, baseline issue, less sensitive peaks for your analyte and peak area and peak shape issues in the chromatogram.

Mobile phase Transparency:

To achieve mobile phase transparency, a mobile phase that transmits at the detection wavelength must be selected.

Elution Strength:

Elution strength is the solvent's ability to elute target analytes from the stationary phase. As the elution strength increases, so does the solvent's efficacy and selectivity. The solvent employed in the mobile phase should have optimal and sufficient elution strength to attain the target analyte's selectivity.

PROPERTIES IN RP-HPLC:

The majority of chromatographers prefer acetonitrile as their mobile phase solvent due to its ideal viscosity, strong elution, and good UV transparency.

1. **Acetonitrile (CH_3CN):**

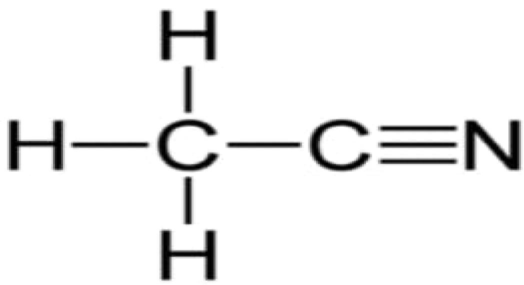

Fig.no: 01

Chemical name: Acetonitrile

IUPAC name: Acetonitrile

Synonyms: Methyl cyanide, Cyanomethane, Ethanenitrile

Solubility: Miscible with water, ethanol, acetone, chloroform, ether

Viscosity: ~0.37 cP at 25 °C

Boiling point: 81.6 °C

Melting point: −45 °C

Relative humidity effect: Hygroscopic (absorbs moisture from air, but not strongly like methanol)

Density: 0.786 g/cm³ at 20 °C

Refractive index: 1.344 at 20 °C

Appearance: Colourless liquid with ether-like odour

Elution strength (RP-HPLC, relative to hexane = 0): ~0.65

Parameter	Requirements	Factors
Boling point	Sufficiently High	To prevent formation of vapour bubbles interfering work of pump valves and detectors; to minimize changes of eluent composition due to low boiling component evaporation.

Density	Sufficiently High	Denser eluents demand low pressure height over the pump entry, turbulent flow type is less typical for them.
Viscosity	Low	In low-viscosity eluents diffusion and mass-exchange run more efficiently, pump working pressure is less.
Absorption in UV Spectrum Area	Low	Visible absorption of an eluent worsens spectrophotometric detector sensitivity.
Refractive Index	Low	High refraction index worsens refractor-metric detector sensitivity.
Chemical Stability	High	In order not to use stabilizers, to avoid retention time drift.
Chemical Inertness	High	To avoid interaction with analyte, to avoid sorbent and sorbate modification.
Purity	Lack of volatile and Non- volatile admixtures	To prevent hindrances in the work of detectors and columns.
Cost	Low	To decrease expenses for analysis.
Miscibility with Buffer	High	To avoid eluent break, buffer precipitation and hindrances when detecting.
Eluotropic Strength	Sufficiently High	To reduce time for analysis.
Solubilizing Capacity	High	To prevent analyte precipitation in chromatographic tract.
Toxicity	Low	To provide work safety.

2. **Methanol (CH_3OH):**

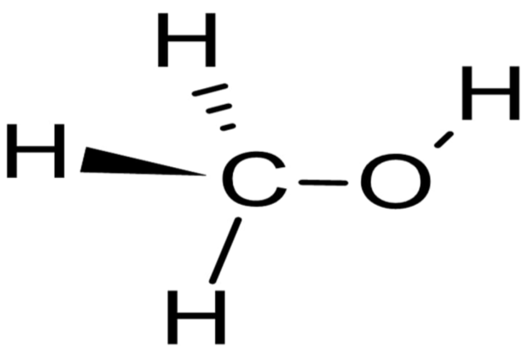

Fig.no: 02

Chemical name: Methanol

IUPAC name: Methanol

Synonyms: Methyl alcohol, Wood alcohol, Carbinol

Solubility: Miscible with water and most organic solvents

Viscosity: ~0.59 cP at 25 °C

Boiling point: 64.7 °C

Melting point: −97.6 °C

Relative humidity effect: Strongly hygroscopic (absorbs water readily from air)

Density: 0.791 g/cm³ at 20 °C

Refractive index: 1.328 at 20 °C

Appearance: Colourless liquid with slight alcoholic odour

Elution strength (RP-HPLC, relative to hexane = 0): ~0.73

Parameter	Requirement	Factors
Boling point	Sufficiently High	Prevents vapor bubble formation in pump valves/detectors; avoids eluent composition change due to evaporation.

Density	Sufficiently High	Denser eluents cause lower pressure height over pump entry; less turbulent flow.
Viscosity	Low	Low viscosity improves diffusion and mass-exchange; reduces pump pressure.
Absorption in UV Spectrum Area	Low	Prevents interference with spectrophotometric UV detection.
Refractive Index	Low	High refractive index would worsen refractometric detector sensitivity.
Chemical Stability	High	Stable solvents prevent retention time drift; no stabilizers needed.
Chemical Inertness	High	Avoids interaction with analyte and column sorbent/sorbate modification.
Purity	Lack of volatile and Non- volatile admixtures	No volatile or non-volatile admixtures. Prevents interference in detector and column performance.
Cost	Low	Reduces expenses in analysis.
Miscibility with Buffer	High	Ensures no eluent break or buffer precipitation; avoids detector hindrance.
Eluotropic Strength	Sufficiently High	Determines solvent ability to shorten analysis time (weaker than ACN).
Solubilizing Capacity	High	Prevents analyte precipitation in chromatographic system.
Toxicity	Low	Lower toxicity improves lab safety (methanol is moderately toxic).

Comparative Drawbacks of Different Solvents in RP-HPLC Compared to Methanol and Acetonitrile:

Acetonitrile is without a doubt the best organic solvent since it has a very low UV cutoff for enhanced UV-visible detection sensitivity and generates the lowest system backpressure in water mixes. Methanol is another popular organic solvent that is far less expensive than acetonitrile, as well as having a similar elutropic strength and a relatively low UV absorbance. The main drawback of methanol is that, especially when used with small particle size HPLC columns, it can result in backpressures that exceed a number of HPLC system limits.

Ethanol:

Ethanol is generally not recommended since it results in abnormally high backpressures in water mixtures. However, ethanol is sometimes preferred in completed product production methods to avoid residual solvents.

ISO AND n-PROPANOL:

Because of their relatively significant elution strength, iso- and n-propanol are most commonly used in column cleaning at modest flow rates because they also generate large backpressures. These are commonly used when proteins are still intact since they are effective solubilizers.

Tetrahydrofuran:

Tetrahydrofuran is less commonly used because of its much higher cost, even though its elution strength is similar to that of n-propanol. However, it can be used as a cleaning or regeneration solvent on materials that are fatty or highly pigmented.

Propylene carbonate:

Propylene carbonate (PC), a carbonate ester derived from propylene glycol, was proposed as a possible ACN replacement in RP-HPLC. This polar aprotic solvent is mostly used as a reactive intermediate or as an inert solvent in a number of industries. It is used as a solvent for medications and cosmetics applied topically, as well as for paint removal, degreasing, and cleaning purposes.

The two primary drawbacks of PC clearly preclude a direct translation from ACN to PC in RP-HPLC. The primary drawback of PC is its poor water solubility, which is why a third solvent, such as MeOH or EtOH, is usually added. The latter is the best choice due to its green features. PC has a high density and viscosity, which causes high-pressure drops in the chromatographic system. The backpressure will increase even more if EtOH is used as the ternary solvent.

Acetone:

Acetone is an even more eco-friendly alternative to ACN in RP-HPLC applications due to its low toxicity and biodegradability. Acetone and ACN have similar physicochemical characteristics in terms of their solubility and miscibility with other solvents.

Acetone's high UV cut-off, which surpasses 340 nm and limits its use in UV detection, is its main drawback when compared to ACN. Furthermore, because acetone is so volatile, pumping it is difficult.

However, the new generation of aerosol-based LC detectors and the quick surge in popularity of MS-based LC detectors have expanded the alternatives for employing acetone in RP-HPLC. However, the options for using acetone in RP-HPLC have increased due to the rapid rise in popularity of MS-based LC detectors and the new generation of aerosol-based LC detectors.

ADVANTAGE OF METHANOL IN RP-HPLC:

Low UV Absorbance:

Given its low UV absorption properties, it is performs well in light-sensitive tests.

Broad Solubility Range:

With methanol's wide array of compounds, it becomes all the more simpler gradient and isocratic separations.

Gradient stability:

It offers stable and accurate quantification of analytes in methods such as the gradient HPLC.

Cost-effectiveness:

It is relatively less expensive than other solvents.

Environmentally friendly:

This solvent's low levels of toxicity and biodegradability make it more environmentally friendly.

ADVANTAGE OF ACETONITRILE IN RP-HPLC:

Acetonitrile (ACN) is one of the most popular HPLC solvents due to its versatility and favourable properties:

High polarity:

It can dissolve a wide range of analytes, from polar to moderately non-polar.

Miscibility with water:

It allows for the creation of mobile phases with varying strengths, enabling fine-tuning of the separation.

Low viscosity:

It flows easily through the column, ensuring efficient separation.

Low UV absorbance:

It minimizes interference with UV detection, allowing for sensitive analysis of analytes.

Solvent Strength and Purity:

Acetonitrile is a perfect solvent for HPLC applications due to its excellent purity levels and broad dissolution range. Its low viscosity makes it easier for it to move through the chromatographic system, which guarantees the best possible analyte separation.

Chemical Inertness:

Acetonitrile's chemical inertness is a major factor in its appeal in HPLC. Acetonitrile interacts with analytes and stationary phases very little, in contrast to other solvents like methanol or water. This improves the dependability of analytical data and guarantees repeatable outcomes.

Evaporative Properties:

The effectiveness of the separation process in HPLC is directly impacted by the solvent's rate of evaporation. Shorter analytical times without sacrificing resolution are made possible by acetonitrile's quick evaporation due to its relatively low boiling point.

Compatibility with Detectors:

Acetonitrile also has the benefit of being compatible with a number of detection methods used in HPLC, including mass spectrometry (MS) and ultraviolet (UV). It's a great option for sensitive analyte detection because of its low UV cutoff and low background noise.

CONCLUSION:

From this review article, one can understand the importance of solvent selection and can also understand why the Acetonitrile and methanol is mostly commonly used in RP HPLC for the effective elution, separation, isolation of anlytes and also can compare the importance of Acetonitrile and methanol in cost effectiveness with UV transparency and high elution strength.

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