

# Characterization, Principle and Solution of Solvent Effect in Reversed Phase Chromatography

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**Abstract:** *In the ultraviolet-visible spectrophotometry, high performance liquid chromatography and gas chromatography, the term "solvent effect" has different meanings. In this paper only high performance liquid chromatography is included. The commonly used solvent effects in reversed-phase chromatography, such as normal phase chromatography, ion chromatography, hydrophilic interaction chromatography, etc. can refer to the analytical methods and related principles of this paper.*

**Keywords:** Solvent effect, reversed phase chromatography, Characterization

## 1. The concept of solvent effect

The currently accepted understanding of the solvent effect is the phenomenon that the solvent strength of the diluent is greater than that of the mobile phase. If the sample is dissolved in pure acetonitrile and injected into an acetonitrile-water (18:82) reversed phase system, the result of peak bifurcation or tailing will result. However, this concept does not explain other chromatographic behavioral anomalies caused by diluents in practice, such as the main peak anomaly of the dissolution sample of the pH 6.8 dissolution medium in the dissolution curve study, or the peak bifurcation after SDS assisted dissolution.[1] In the case where the solvent strength of the diluent is not necessarily greater than the mobile phase, but the chromatographic behavior is abnormal.

Since many chromatographic behavioral anomalies caused by diluents cannot be explained by the current solvent effects, I believe it is necessary to re-explain the concept of solvent effects: solvent effects are due to the specific components in the test sample in the diluent. The adverse effects of differences in the state of the mobile phase on chromatographic behavior.[1] If this adverse effect is more serious, it may cause obvious chromatographic behavior abnormalities, such as peak bifurcation, tailing, and the like.

## 2. The characterization of the solvent effect

Solvent effect is an adverse effect on chromatographic behavior, the degree of influence is different, and the characterization of chromatographic behavior is also different; the lighter effect may have no obvious abnormality of chromatographic behavior, such as unobservable peak broadening, etc.[2] The possible performance of the grades is that the peaks are significantly broadened and the column efficiency is low. The more serious performances are that the peak retention time of the specific components is unstable, the peak shape is abnormal, and the response value is unstable.

Peak-type anomalies are the most intuitive and most common manifestations of solvent effects, such as peak bifurcation, tailing, and leading edge. Unstable retention time and unstable response values are also common manifestations, and may also be accompanied by peak-type

anomalies; the solvent effect that is more difficult to detect is that the peak shape is normal, but the response value fluctuates greatly. Reasons for system suitability such as instrument failure, such as peak symmetry found during method development, but low column efficiency and large continuous RSD, it is recommended to assess the risk of solvent effects.

The solvent effect has certain rules, such as the peak of the component with small retention time is easy to occur, the peak shape of the component with the peak of the multi-component chromatogram is better, and the injection volume can be reduced, etc., in practice, According to the law, a preliminary judgment is made as to whether the abnormal chromatographic behavior is a solvent effect.

## 3. The principle of solvent effect and its solution

As described above, the solvent effect is an adverse effect of the difference in the state of the specific component in the diluent and the mobile phase on the chromatographic behavior, and the difference mainly includes the elution ability, the ionization state, the ion association, the interconversion equilibrium, and the like.

### a) Elution ability

This is the current general understanding that the above-mentioned "pure acetonitrile dissolved sample, injected into the mobile phase of acetonitrile-water (18:82) in the reversed phase system, will result in peak bifurcation or tailing".[3] If the diluent has a higher elution capacity than the mobile phase, it may cause a solvent effect.

The solution is to adjust the diluent or mobile phase so that the elution ability of the two is close to or the diluent has a lower elution ability than the mobile phase. In general, in order to avoid the solvent effect caused by the elution ability, it is safer in reversed-phase chromatography that the organic ratio in the diluent is lower than that of the mobile phase or, if necessary, slightly higher than the mobile phase, but the solvent should be evaluated. The risk of the effect, such as judging according to the rules mentioned above.

**b) Ionization Status**

Many of the active ingredients of the drug can be ionized, such as amlodipine besylate, penicillin potassium, etc. Most of these compounds are not strong electrolytes and can only be partially ionized; that is, part of the ionization equilibrium is the ion state and the other part is the molecular state. The two states are different in the distribution of the stationary phase and the mobile phase. In a reversed-phase system, the molecular state is more tightly bound to the stationary phase, and the retention is stronger; the ionic state is more inclined to the mobile phase, and the retention is weaker.

Imagine a situation where if the sample is a basic compound, the diluent is pH 6.8 buffered salt solution (the dissolution curve study is more common), and the mobile phase is a phosphate solution of pH 3.0 - the organic phase system, which has a very high probability A solvent effect that causes significant peak anomalies. Since the ionization state of the sample in the pH 6.8 buffered saline solution is much lower than the phosphate solution of pH 3.0, it takes a period of time to buffer the ionization state from the diluent to the mobile phase during the mixing of the test solution with the mobile phase. The state, if for some reason (the diluent is buffer salt, the mobile phase buffer is weak, etc.), the time is longer, and the peak of the component to be tested may have a significant peak abnormality.

There are generally four solutions for solvent effects in this case: replacing the diluent to make the ionization state of the analyte in the diluent consistent or close to the mobile phase, reducing the injection volume, adding counter ions to the mobile phase, or increasing the mobile phase. Buffering ability. See the "[Guidelines for the Selection of Buffer Salts for Method Development](#)" for the buffering capacity.

**c) Ion Association**

This is often the case in tests where the solubility of the analyte is poor in the presence of sodium lauryl sulfate in the diluent, the peak of the component to be tested may be severely deformed, or the retention time fluctuates greatly. If the analyte is a basic compound, the reason is that the cation of the analyte in the ionic state forms an ion association with the lauryl sulfate anion, and the substance remains in the reversed phase chromatography with respect to the object to be tested. Enhanced; the association is not present in the mobile phase, and if the mobile phase eliminates the effect of the association for a longer period of time, a significant solvent effect is produced.

The simple method is to replace the diluent, such as the diluent can not be replaced, can also change the mobile phase, such as adding the counter ion of the analyte in the mobile phase, also forming an ion association, reducing the analyte and the mobile phase in the diluent the difference.

**d) Interchange balance**

For analytes having a tautomeric structure in solution, such as keto-enolinterconversion, a significant solvent effect may also result if the tautomeric state differs greatly between the diluent and the mobile phase. Keto-enol-type interconversions can be found in the topic of typical special structural compounds that should be noted in the

development of HPLC methods: keto-enol-type interconversion .

**4. Summary**

The solvent effect caused by the above difference is caused by the large difference between the distribution coefficient of the stationary phase and the diluent and the distribution coefficient of the stationary phase-mobile phase. It is the result of the three-party game of stationary phase, diluent and mobile phase. . Therefore, when assessing the risk of solvent effects, it should be combined with the specific situation of the three parties to assess the strength of the interaction between the three parties. The method not mentioned above to solve the solvent effect is to change the type of stationary phase, such as replacing the C18 column with a CN column, because the difference in partition coefficient caused by the difference in state between the diluent and the mobile phase is smaller than the C18 column in the CN column.

That is to say, the effect of eliminating or reducing the solvent effect can be started from the three aspects of diluent, mobile phase and stationary phase, the diluent is close to the mobile phase, the amount of diluent is reduced, or the mobile phase is close to the nature of the diluent. Enhance the ability of the mobile phase to change the state of the analyte; or change the type of stationary phase to reduce the effect of differences in the state of the component to be tested on chromatographic behavior.

Other situations not listed in this paper can also be used to assess the risk of solvent effects in the way of thinking in this paper.

**5. The special case of some solvent effects derived from the concept of thinner**

The diluent described herein is all components except the test substance in the test solution, and is not simply understood as a solvent for dissolving the test sample. The evaluation of the solvent effect cannot evaluate only the difference between the state of the analyte in the solvent for dissolution and the state of the mobile phase, but should be evaluated in combination with other components in the solution.

It is envisaged that if a method for detecting the residual trifluoroacetic acid in a basic compound is developed, it is likely that the peak form of trifluoroacetic acid alone is normal, and the peak of trifluoroacetic acid is significantly abnormal after mixing with the basic compound. Combined with the above related content of ion association, we can easily judge. However, if the diluent in the detection method is understood only from the solvent for dissolution, the cause of the problem cannot be found.

Therefore, when detecting the content of certain impurities in the test sample, especially the high concentration test solution, it is very likely that the test sample participates in the formation of the solvent effect, and the principle is analyzed with reference to the above relevant contents.

The peaks of the individual components in the relevant substance detection process are normal, and the peaks of the peak-type anomalies in the mixed solution can also be understood as other components participating in the formation of the solvent effect.

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