

Causes and Measures for Addressing Ghost Peaks in Reversed Phase HPLC Analysis

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1. The Problem of Ghost Peaks

One of the brain-racking challenges in LC analysis is the presence of ghost peaks (see Fig. 1). Ghost peaks are of unknown origin in a chromatogram, are easily misidentified when they are close to peaks of interest, and can result in quantitative errors when they overlap peaks of interest. Uncertainty in data quality and reliability is of course the result. To make matters worse, considerable time can easily be expended in determining the source of an apparent impurity, even if there is none associated with the compound of interest.

With the increase in UV detector sensitivity that has come about in recent years, detection has become possible at very low concentrations, bringing the problem of ghost peaks into the spotlight. In this report, we focus on the source of ghost peaks in reversed phase analysis using a UV detector, and propose various remedial measures that can be effective.

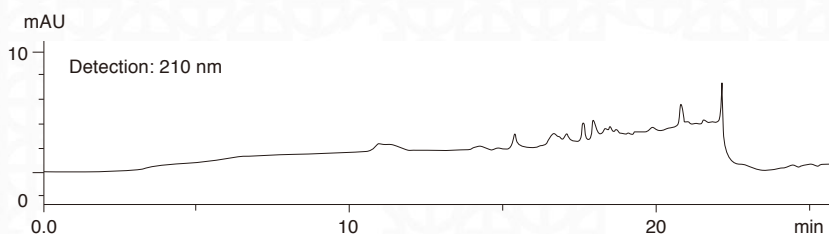


Fig. 1 Example of Ghost Peaks (data provided by Daiichi Sankyo Co., Ltd.)

2. Ghost Peaks in Reversed Phase Analysis

The sources of ghost peaks are typically divided into three categories: those due to impurities other than the target substance, internal contamination of the LC instrument, and impurities in the mobile phase, as shown below.

- (1) Impurities in instrument
- (2) Impurities in sample
- (3) Impurities in mobile phase

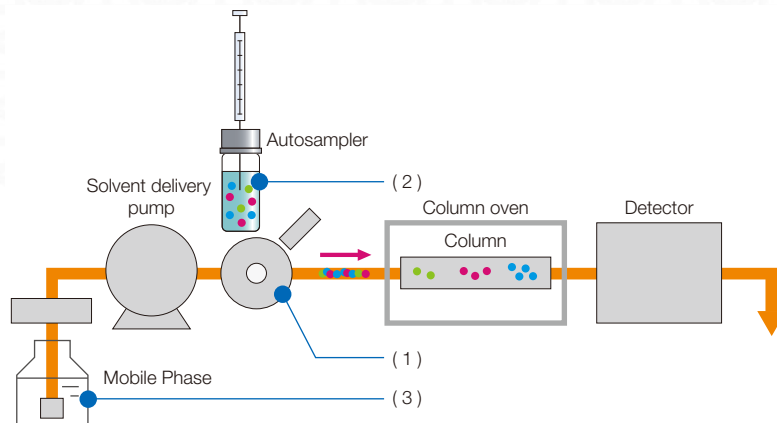


Fig. 2 Sources of Ghost Peaks

2-1. Ghost Peaks Due to Instrument

The most common reason for ghost peaks originating in the instrument is carryover attributable to the autosampler (or manual injector). When the needle is dipped into the vial to aspirate the sample, substances in the sample, which become the source of carryover, are adsorbed to the inside and outside surfaces of the needle. Those substances which are not eliminated even after needle rinsing are carried over to the next analysis, and appear as ghost peaks. Since substances that exhibit particularly strong adsorption will appear even after several blank analyses (analysis in which only mobile phase is injected), it may be difficult to identify the autosampler as the source of the ghost peaks (see Fig. 3).

On the other hand, ghost peaks may also be attributed to instrument contamination other than the case described here (see Section 2-3 for details).

2-2. Ghost Peaks Due to Sample

When there is an impurity in the sample having the same absorption wavelength as the compound of interest, it results in a ghost peak. Since a ghost peak that originates in the sample does not appear in a blank injection, determining the source is relatively easy.

The remedy is to devise a sample preparation procedure to eliminate the impurity, such as an extraction or use of a disposable filter, etc. Further, when the appearance of a ghost peak is due to degradation of the sample, that degradation can be inhibited by using an autosampler with a vial cooling feature.

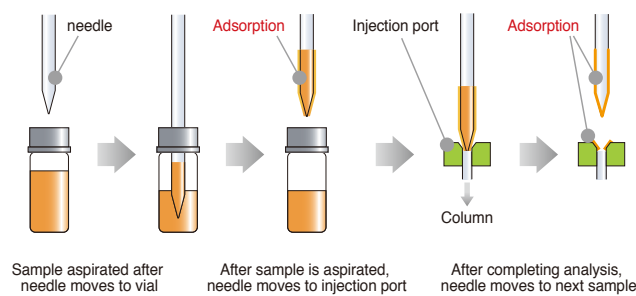
On the other hand, the source of the ghost peak may not be in the sample itself, but rather, from dissolved oxygen in the sample solvent. This is especially noticeable when using a UV detector. Fig. 4 shows a comparison of the results of analysis of the same sample in three different vials, in which oxygen was bubbled through one sample, another in which the vial was uncovered to allow saturation of the sample with air, and another in which helium was bubbled through the sample to remove the dissolved oxygen. From these results, it is clear that the difference in the amount of dissolved oxygen in the mobile phase and sample solution is directly linked to the appearance of the ghost peak.

2-3. Ghost Peaks Due to Mobile Phase

Ghost peaks can also be generated from the mobile phase in various ways, including the following.

- Generation of organic substances in mobile phase due to prolonged use, or dissolution of organic substances from the air in the mobile phase
- Mobile phase vial contamination due to topping off existing mobile phase with new mobile phase over a long period of time instead of preparing a fresh bottle each day or for each set of samples
- Use of contaminated organic solvent and/or water to prepare mobile phase
- Use of contaminated mobile phase reagent

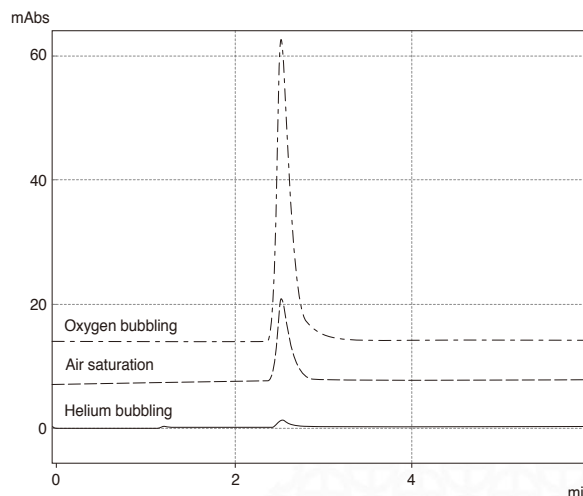
Due to the high consumption of mobile phase and the relatively long periods of time it is used before being replaced, pretreatment measures such as filtering that are typically used for samples are not practical for eliminating contaminants in mobile phase. Furthermore, mobile phase contaminants adhere to the inside of the instrument and tubing, small amounts are inevitably transported to the column along with the mobile phase during the solvent delivery process, and are likely to be detected as ghost peaks.



	Carryover
Blank Test (1st time)	0.0006%
Blank Test (2nd time)	0.0003%
Blank Test (3rd time)	0.0004%

After injecting chlorhexidine (2,000 µg/µL), 3 consecutive blank injections were run.

Fig. 3 Carryover



Peaks generated after placing the sample in the vial, and bubbling with oxygen, air and helium, respectively

Fig. 4 Dissolved Oxygen in Sample Solvent

In reversed phase analysis using gradient elution, such substances that cause ghost peaks are retained in the column at the start of analysis, and are then eluted as the organic solvent ratio increases, making separation very difficult when they exhibit behavior similar to that of the compound of interest.

Thus, it is difficult to specify the cause when ghost peaks are of mobile phase origin, and furthermore, it is difficult to address the problem by making changes to the method.

3. Utilizing the Ghost Trap DS

When identifying the cause of ghost peaks in the mobile phase as described in Section 2-3 is difficult, and drastic prevention measures are problematic, trapping of the causative substances is an effective means of suppressing the appearance of the ghost peaks.

Fig. 5 and Table 1 show and describe the Ghost Trap DS cartridges that can be used to reduce or eliminate ghost peaks that are due to the mobile phase. Installing the Ghost Trap DS in the mobile phase flow line provides for adsorption of the substances responsible for the ghost peaks, especially when conducting gradient elution with reversed phase analysis and a UV detector. Ghost peaks are prevented from appearing because the mobile phase is sent to the column after removal of the problematic substances.

The Ghost Trap DS can be connected at various positions in the flow line (see Fig. 6) according to the LC system configuration and the analysis application. Fig. 7 shows a comparison of the chromatographic results using the connection positions indicated in (a) to (e).



Fig. 5 Ghost Trap DS Modules

- (a) Ghost Trap DS not used
- (b) Positioned after solvent delivery pump A (aqueous solution pump)
- (c) Positioned after solvent delivery pump B (organic solvent pump)
- (d) Positioned before gradient mixer
- (e) Positioned after gradient mixer

The results clearly indicate that under these conditions, many ghost peaks originate in the pump A flow line (most likely from the mobile phase rather than some contamination in pump A). It is also clear that there are slight differences in the ghost peak pattern when the Ghost Trap DS is connected before or after the gradient mixer, indicating that the presence of ghost peaks can also be due to contamination of the mixer. Since the Ghost Trap DS can also be connected downstream of the gradient mixer, more certain removal of ghost peaks can be expected, as shown in Fig. 7 (e).

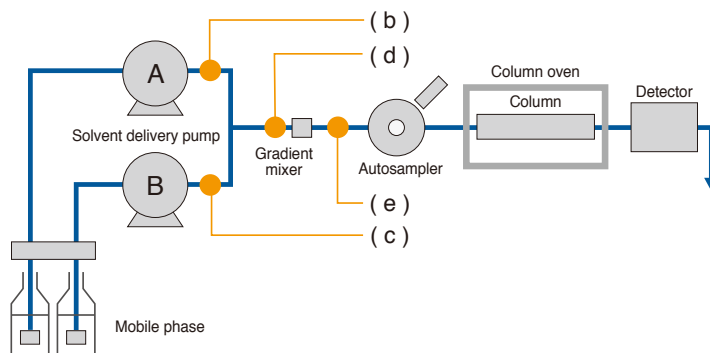


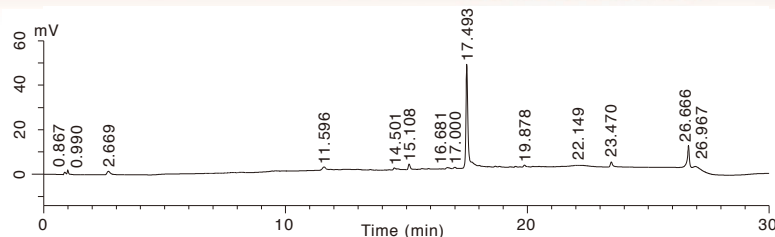
Fig. 6 Ghost Trap DS Test Connection Positions

Table 1 Ghost Trap DS Modules

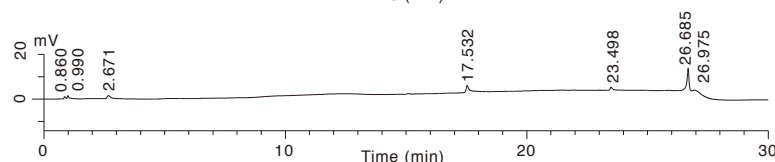
Part Name	P/N	Description (Quantity)	Size	Internal Volume	Pressure Tolerance
Ghost Trap DS	228-59921-91	Cartridges (2)	30 mmL. x 7.6 mmI.D.	700 µL (Approx.)	35 MPa
	228-59921-92	Cartridges (2) Holder (1)			
	228-59921-93	Cartridges (2)	20 mmL. x 4.0 mmI.D.	150 µL (Approx.)	
	228-59921-94	Cartridges (2) Holder (1)			

- (Note)
- When this product is connected after the gradient mixer or after the pump confluence, the system delay volume calculation must include both the mixer volume and the Ghost Trap cartridge volume when conducting gradient analysis.
 - When a mass spectrometer is used as the detector, bleed noise may be generated due to this product.
 - In analysis using ion pair reagents, the ion pair reagents may be retained in this product, thereby influencing retention times and peak shapes.
 - When using this product, prior to connecting the analytical column, be sure to thoroughly rinse the trap beforehand with mobile phase (at close to the final organic concentration when conducting gradient analysis).
 - Please be aware that all impurities cannot be removed with the Ghost Trap DS, but a significant reduction in the presence of ghost peaks can be expected, as shown in Fig. 7.

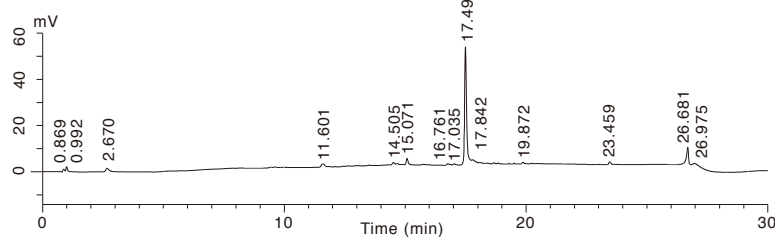
(a) Ghost Trap DS not used



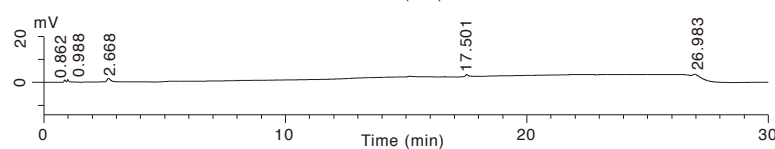
(b) Positioned after solvent delivery pump A



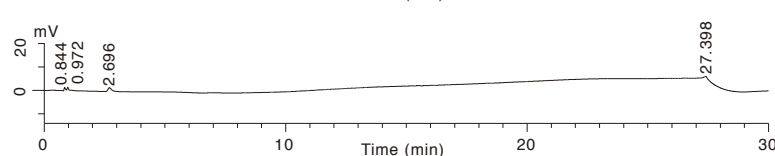
(c) Positioned after solvent delivery pump B



(d) Positioned before gradient mixer



(e) Positioned after gradient mixer



Analytical column : ODS series column
Mobile phase : A; 25 mmol/L (Potassium)phosphate buffer(pH 4.0)/ Acetonitrile = 9 / 1 (vol/vol)
B; Water / Acetonitrile = 1 / 9 (vol/vol)
Flow rate : 0.65 mL/min
Oven temperature : 45°C
Detector : UV210mm

Fig. 7 Example of Ghost Peak Removal Using Ghost Trap DS (data provided by Daiichi Sankyo Co., Ltd.)

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SHIMADZU CORPORATION. International Marketing Division

3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan

Phone: 81(3)3219-5641 Fax: 81(3)3219-5710

URL <http://www.shimadzu.com>