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Short communication

Investigations of artifact peaks in sensitive high-performance liquid chromatography methods

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Abstract

Chromatograms of sensitive HPLC methods often show disturbing peaks, which mostly can be traced back easily. But sometimes such peaks are difficult to reproduce and therefore hard to trace. This paper describes two cases where artifact peaks arose due to contamination by the septum and by the sampling equipment, respectively, leading to misinterpretation of impurities and erroneous quantification. Furthermore, troubleshooting and ways to overcome these deficiencies are outlined.

Keywords: Band profiles; Artifact peaks; Troubleshooting

1. Introduction

Analytical methods are developed and validated within the Analytical Development Section of our Institute to support development and evaluation of new pharmaceutical products. Determination of impurities in both drug substances and products as well as for the analysis of cleaning validation samples are mainly performed by sensitive HPLC methods [1]. The resulting chromatograms often show artifact peaks, which mostly can be traced back to impurities derived from mobile phases, buffers, or dirty glassware [2–4]. In some cases such extra peaks also originate from other sources. They are often difficult to reproduce and therefore hard to trace [5]. Two cases in which such artifact peaks were detected and successfully tracked down are described.

2. Experimental

The results described in the first case were obtained using a Hewlett-Packard 1090 HPLC System and a Waters Symmetry C_{18} column (5 μm particle size, 150×4.6 mm). The system dwell volume was 0.64 min. The mobile phase was a mixture of acetonitrile (HPLC grade) and 10 m*M* phosphate buffer (pH 6.0). Solvent A contained 40% and solvent B 80% acetonitrile. The column was operated at 40°C with a 1 ml/min flow-rate and UV absorbance detection at 220 nm. After injection of the sample (50 μl) the column was eluted using solvent A for 20 min, followed by a linear gradient of 0–100% solvent B within 20 min and a 20 min hold. The system equilibrium time between runs was 15 min. Solvent A was also used as sample solvent. Sample solution contained approximately 2 mg/ml drug substance (Fig. 1). After the first injection the sample vial was vigorously shaken provoking contact between the pierced vial septum and the sample solution. The second injection is illustrated in Fig. 2.

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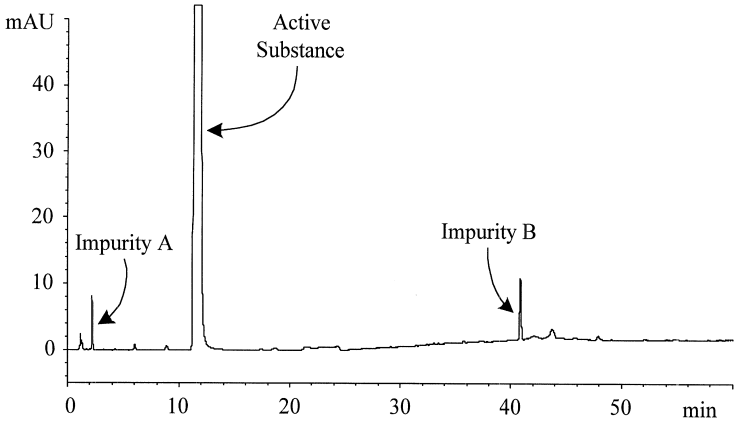

Fig. 1. Typical chromatogram obtained during the determination of impurities and degradation products in a drug substance.

Moreover, two different types of vial septa (PTFE–rubber and PTFE–silicon–PTFE, respectively) were screened for potentially extractable compounds. Vial septa from both types were placed into vials. Then, the vials were filled with 1 ml sample solvent and allowed to stand at room temperature for 15 min. The resulting solutions were analyzed (Figs. 3 and 4).

The results described in the second case were obtained using a Hewlett-Packard 1090 HPLC System and a Shandon Hypersil BDS column (3 μm particle size, 100×4.6 mm). The system dwell volume was 0.49 min. The mobile phase was a mixture of 0.02 *M* tetrabutylammonium hydrogensulphate buffer–acetonitrile (HPLC grade) (30:70). The column was operated at ambient temperature with a 2 ml/min flow-rate and UV absorbance detection at 263 nm. The injection volume was 50 μl. A mixture of methanol–water (50:50) was used for both sample solvent and extraction solution. The standard solution was prepared to obtain a concentration of about 25 μg/ml (Fig. 5). The extraction of the samples was carried out by shaking the swabs in 5 ml of extraction solution for 20 min (Fig. 6). A part of a glove used for sampling was extracted also (Fig. 7). Additionally, several other glove types consisting of different materials, with or without coating were analyzed using the

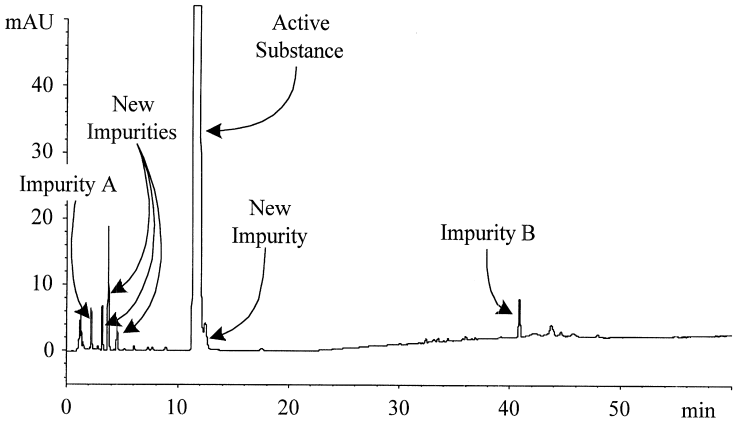

Fig. 2. Chromatogram of the second injection of a drug substance sample showing new impurities.

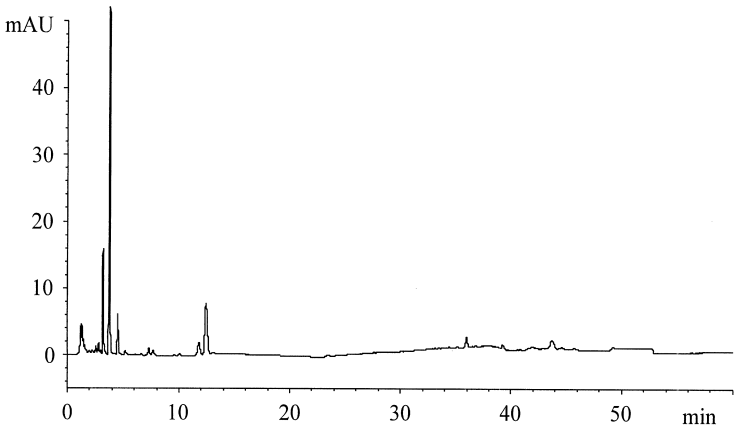

Fig. 3. Chromatogram of an extract of a PTFE–rubber vial septum originally used in this method.

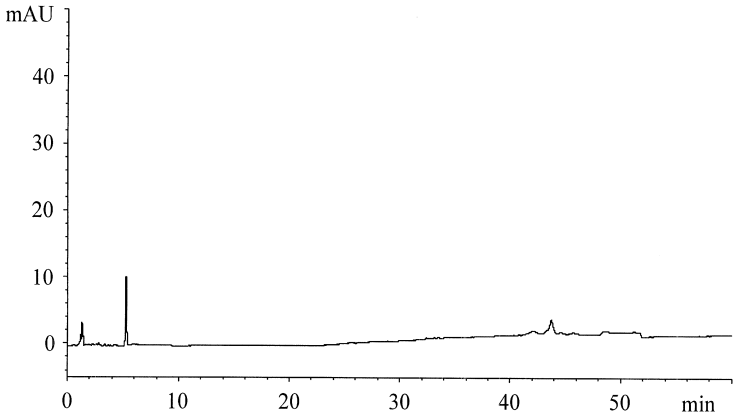

Fig. 4. Chromatogram of an extract of a PTFE–silicon–PTFE vial septum showing one small impurity peak only.

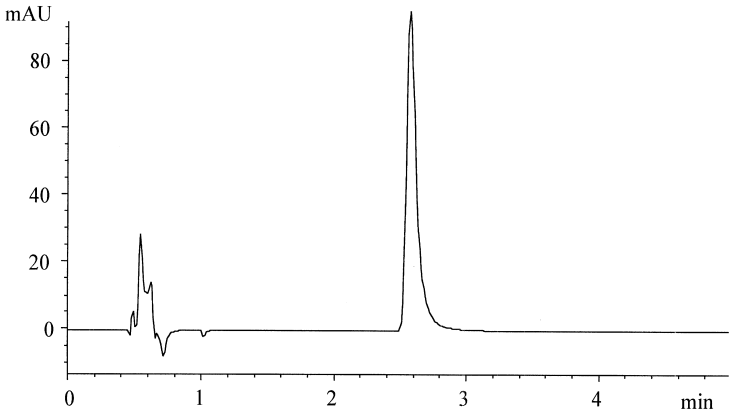

Fig. 5. Chromatogram of a reference standard solution.

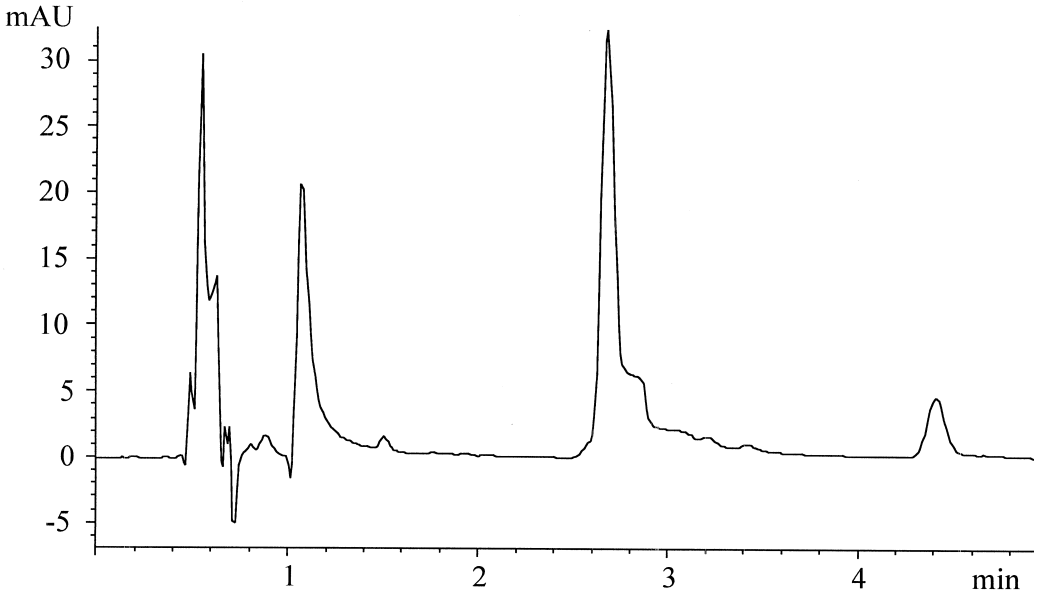

Fig. 6. Chromatogram of a cleaning validation sample.

same procedure. Fig. 8 shows a selection of the glove types examined.

3. Results and discussion

The first case illustrates the examination of several unexpected small peaks showing up during the determination of impurities in a new drug substance. These peaks only appeared in a few chromatograms and in second or later injections. Fig. 1 shows the chromatogram obtained from the first injection of a sample. In addition to the active substance peak, two impurity peaks are present in small amounts (0.04% and 0.09%, respectively). Fig. 2 illustrates the second injection of the same sample after the vial was vigorously shaken, showing the appearance of some new impurities. It was supposed that these impurities

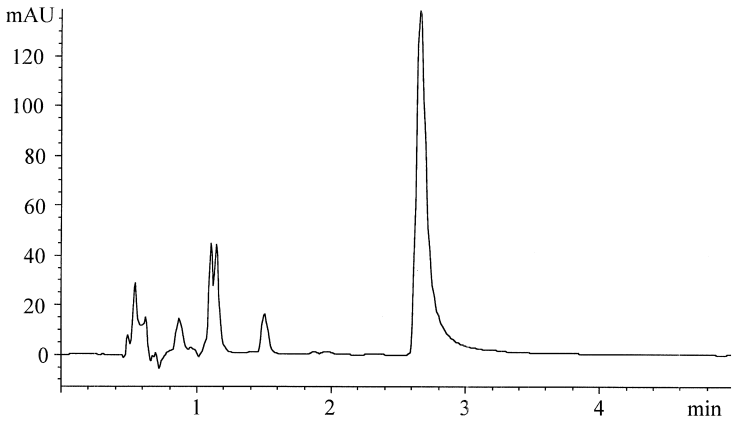

Fig. 7. Chromatogram of an extract from a latex glove used for sampling.

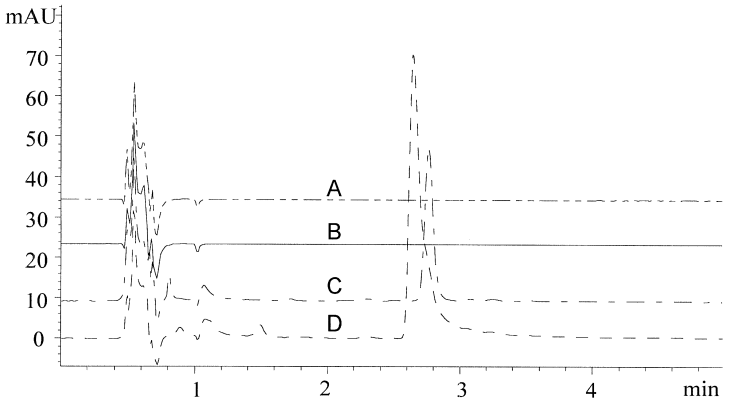

Fig. 8. Chromatograms of extracts from different glove types (A and B: polyethylene gloves without coating; C and D: latex gloves with coating).

were brought into the vials by the injection needle or that the metal injection needle was initiating degradation of the drug substance. By injecting blank solutions frequently, shaking the sample vials between single injections and by analyzing an extract of the vial septum (Fig. 3), the origin of the artifact peaks was traced back to the rubber of the septum used. If a sample is injected just once, the rubber is protected by a PTFE layer, which separates the rubber from the sample. Once this layer is pierced, a direct contact between the rubber and the sample solution and consequently contamination of the sample might occur (sample solvent: 40% acetonitrile in phosphate buffer). This problem can be avoided by either using single injections or inert types of vial septa. Fig. 4 illustrates the chromatogram of the extract of a rather inert type of vial septum (PTFE–silicon–PTFE), showing one small peak only.

In the second case, a cleaning validation process was carried out after production of a pharmaceutical product. Different parts of the manufacturing equipment were swabbed and the swabs prepared for analysis. Fig. 5 illustrates a chromatogram obtained from a reference standard solution. A typical chromatogram of a cleaning validation sample is presented in Fig. 6 showing an interfering peak at the retention time of the active ingredient, making a correct quantification impossible. Because this peak had not appeared during validation of the analytical method, it was concluded that it originated from contamination of either the chromatographic system or the sample. The examination of possible contamination sources demonstrated that the peak derived from latex gloves used during the sampling procedure (Fig. 7). Consequently, several glove types were examined for extractable components causing potential interfering peaks. Fig. 8 shows a selection of glove types examined. Some glove types (type A and B) consisting of polyethylene without coating material were suitable for sampling. Due to the results of these experiments other gloves (e.g. C and D) consisting of latex with coating were excluded from use in the sampling procedure. These results yielded in the implementation of specific glove types into the analytical method.

4. Conclusion

These examples show the importance of a thorough validation of sensitive HPLC methods, also considering possible contamination sources such as sampling procedure and equipment, respectively. However, if artifact peaks appear in a HPLC method, the following procedure may help to overcome these problems. To start with, try to exclude sources which are not responsible for the artifact peaks. Next, learn more about the peaks and try to reproduce them. Finally, find an alternative sampling procedure to avoid artifact peaks or modify chromatographic parameters to achieve separation.

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