

# Practical Application of Quality by Design Principles to The Development of an HPLC Assay Method for an API and Impurities

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**A method for the assay of an active pharmaceutical ingredient (API) and five known and unknown impurities was developed in accordance with Quality by Design (QbD) principles. Highly influential separation parameters were simultaneously and systematically studied so that the quality of the HPLC method could be understood, controlled and ensured. Consequently, during routine analysis out of specification (OOS) results can be corrected more effectively.**

## Introduction

The application of Quality by Design and “do it right first time” principles to the development of HPLC methods is notably on the increase, particularly within the field of pharmaceutical analysis.<sup>1–5</sup> A systematic and science-based approach, beginning with predefined goals and focusing on understanding<sup>6</sup> ensures higher quality, more robust and reliable HPLC methods that leads to fewer analysis failures and better method transfer.<sup>7–9</sup>

A key step in guaranteeing the quality of an HPLC method is to investigate the influence of

critical parameters on the selectivity of the separation. A tool commonly employed in such systematic studies is chromatography modelling software that maximizes the information content of a limited amount of experimental data.<sup>10</sup> Optimum run conditions and their tolerance to change can be predicted, visualized and examined within resolution models, considerably reducing the necessary investment in developing a robust set of separation conditions.



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**Experimental**

**Eluents** Eluent A: phosphate buffer 10 mM with different pH values  
 Eluent B: acetonitrile (AN), methanol (MeOH) and ternary mixtures  
 Flow-rate: 0.8 mL/min

**Equipment** Column: YMC (stagma AG, Reinach, Switzerland) ODS-AQ (150 mm × 3 mm, 3 μm)  
 Instrument: Agilent 1200 Quaternary, dwell volume: 1 mL, extracolumn volume: 0.017 mL, injection volume: 5 μL  
 Detection: 243 nm

**Sample** System suitability test mixture solution with four qualified by-products and one unknown, sample concentration: 0.5 mg/5 mL

**Software** Chromatography modelling software: DryLab 4 v.1 (Molnár-Institute, Berlin, Germany)  
 Chromatography data system: Chromeleon 6.8 (Thermo Fisher, Germering, Germany)

**Experiments for modelling** Gradient time ( $t_G$ ):  $t_{G1}$ : 20 min,  $t_{G2}$ : 60 min (10→80 %B)  
 Temperature (T):  $T_1$ : 30 °C,  $T_2$ : 50 °C  
 pH of eluent A (pH):  $pH_1$ : 2.2,  $pH_2$ : 3.0,  $pH_3$ : 3.8  
 Ternary eluent composition ( $t_C$ ):  $t_{C1}$ : AN,  $t_{C2}$ : AN:MeOH (1:1, V:V),  $t_{C3}$ : MeOH  
 Cube A:  $t_G$ -T- $t_C$  at pH 3.0 (12 experimental runs)  
 Cube B:  $t_G$ -T-pH in AN (12 experimental runs)

**Results and Discussion**

The first step in this work was to clearly define method goals; next candidate method conditions were systematically investigated with the aid of chromatography modelling software. Resolution/ design spaces and robustness spaces were graphically depicted and final method conditions with tolerance limits were ascertained. Finally, a comprehensive multifactorial robustness evaluation was performed initially *in silico* and then verified experimentally.

**Method intent:** The aim of this study was the development of a quality, robust HPLC method for the assay of an API and five

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impurities. Baseline separation or better ( $R_{s,crit} \geq 1.5$ ) for all peaks, within a minimum analysis time was required.

**Method design and selection:** The four critical separation parameters gradient time ( $t_G$ ), temperature (T), pH of eluent A (pH) and ternary eluent composition ( $t_C$ ) were selected for systematic evaluation. First the influence on relative retention of  $t_G$ , T and  $t_C$  was investigated in a simultaneous fashion at a fixed value of pH (pH 3.0) by means of a 3D resolution map (Cube A). Once an appropriate ternary eluent composition was determined (acetonitrile), a further 3D resolution map modelling  $t_G$ , T and pH was constructed (Cube B). Parameter settings and ranges were selected in accordance with the software's guidelines and the generic design of experiments (DoE) is shown in Figure 1.

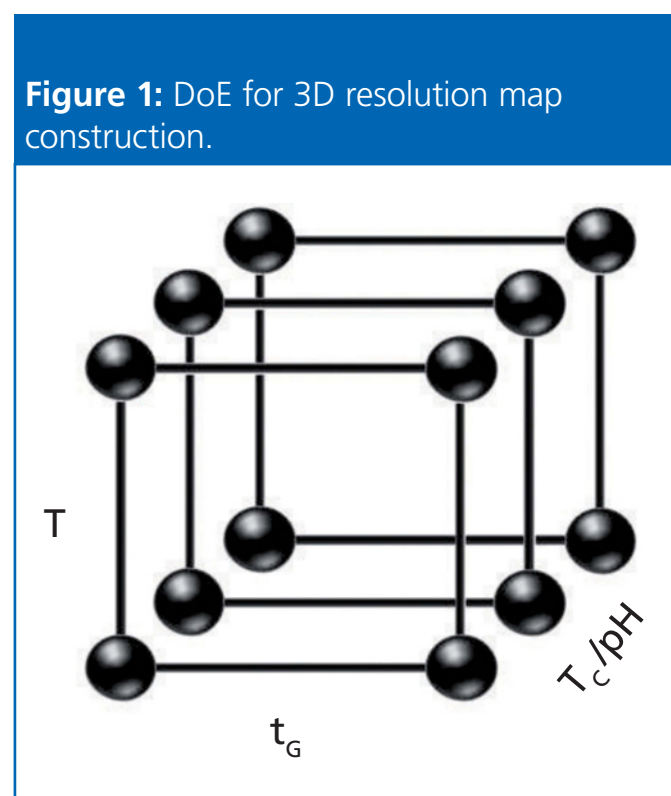
Resolution models map the critical resolution for each combination of the study parameters (i.e.,  $t_G$ , T, pH,  $t_C$ ). The value of the critical resolution ( $R_{s,crit}$ ) is represented in colour so that warm colours show large  $R_{s,crit}$  values and cold colours show low values corresponding to inefficient separations. Specifically, in red regions the resolution is baseline or above ( $R_{s,crit} \geq 1.5$ ) and dark blue lines signalize peak overlaps ( $R_{s,crit} = 0$ ). Models are calculated from retention data and experimental conditions and can be fine tuned further by entering peak width data and/or tailing factors, though this was not done in this study.

The resulting resolution models for Cube A and Cube B are shown in Figure 2 (a) and (b) respectively.

By removing all working points (i.e., combinations of measured parameters) with a critical resolution below the threshold of 1.5 ( $R_{s,crit} < 1.5$ ) from the resolution maps, robustness regions were identified and the robustness of the separation was visualized as red irregular geometric bodies. The robustness space for Cube A and Cube B are shown in Videos 1 and 2.

[Click here for Video 1.](#)

Video 1: Dynamic view of Cube A robustness



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space. Red regions represent robust above baseline separations.

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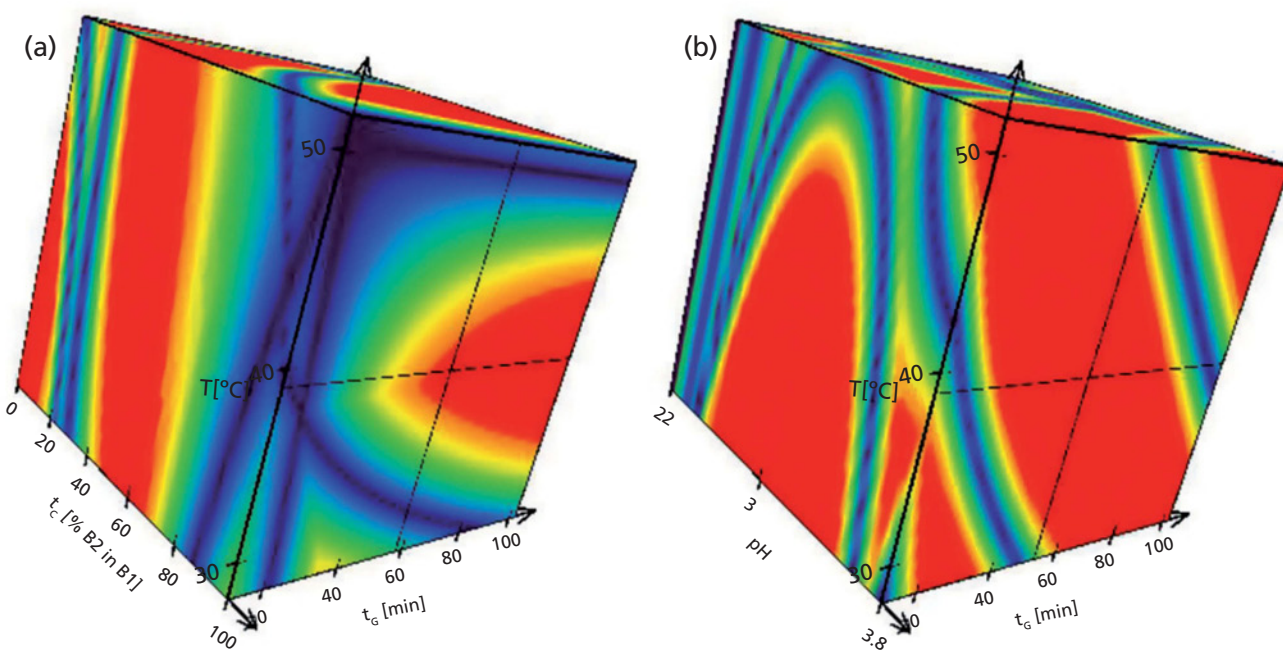
Video 2: Dynamic view of Cube B robustness space. Red regions represent robust above baseline separations.

A number of different potential working points fulfilling the method goals can be observed within the robustness spaces. From these, the final working point  $t_G$ : 15 min,  $T$ : 33 °C,  $t_C$ : AN, pH 3.0 was selected due to the

reasonable size of its surrounding robust region and its relatively short run time. A comparison between the predicted chromatogram and experimental verification under these conditions is given in Table 1.

**Method evaluation:** The selected working point was next subjected to a further multifactorial robustness evaluation. First, the tolerance to changes in additional experimental parameters was investigated *in silico* by studying the influence of six factors simultaneously, namely  $t_G$ ,  $T$ , pH, flow-rate, start %B and end %B. This was

**Figure 2:** 3D resolution maps for (a) cube A modelling  $t_G$ ,  $T$ ,  $t_C$ ,  $R_{S,crit}$  at constant pH (pH = 3.0) and (b) cube B modelling  $t_G$ ,  $T$ , pH,  $R_{S,crit}$  at constant  $t_C$  ( $t_C$  = AN).







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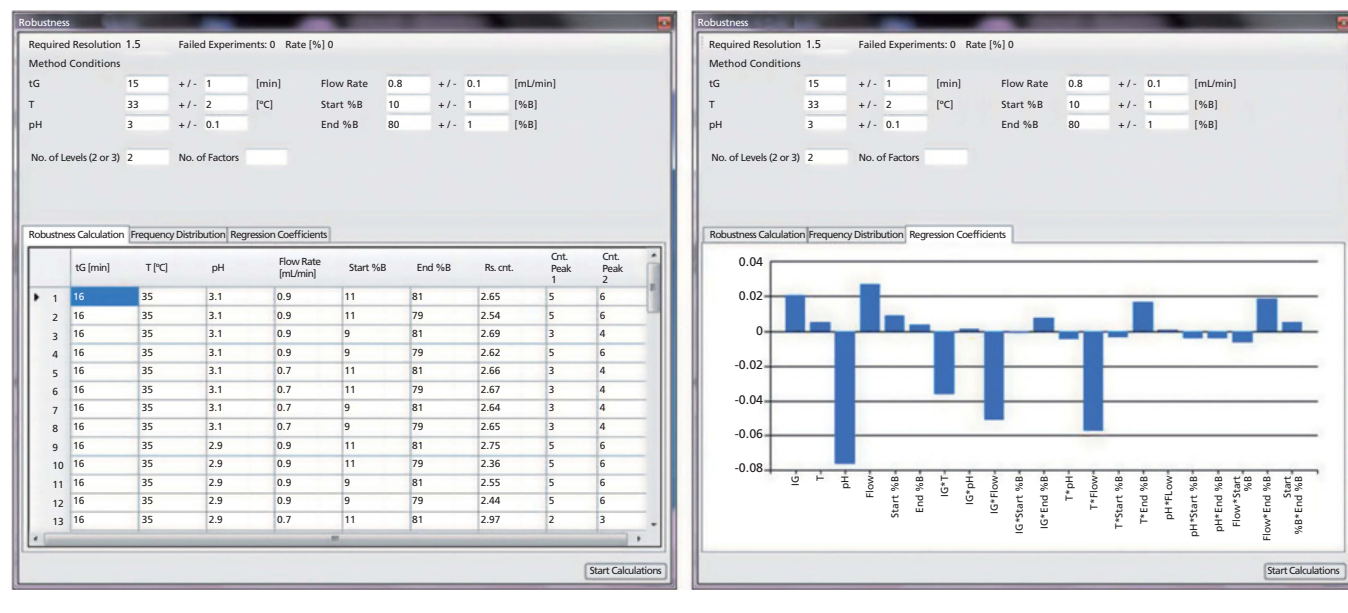


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accomplished without the need for additional experimentation by using simulated data from created models to perform full factorial robustness evaluations, registering responses

including critical resolution ( $R_{S,crit}$ ) and critical peak pairs. The simulated robustness data was also displayed in a regression coefficient plot (Figure 3), showing that, for the studied

**Figure 3:** From left to right: robustness evaluation and regression coefficients plot.

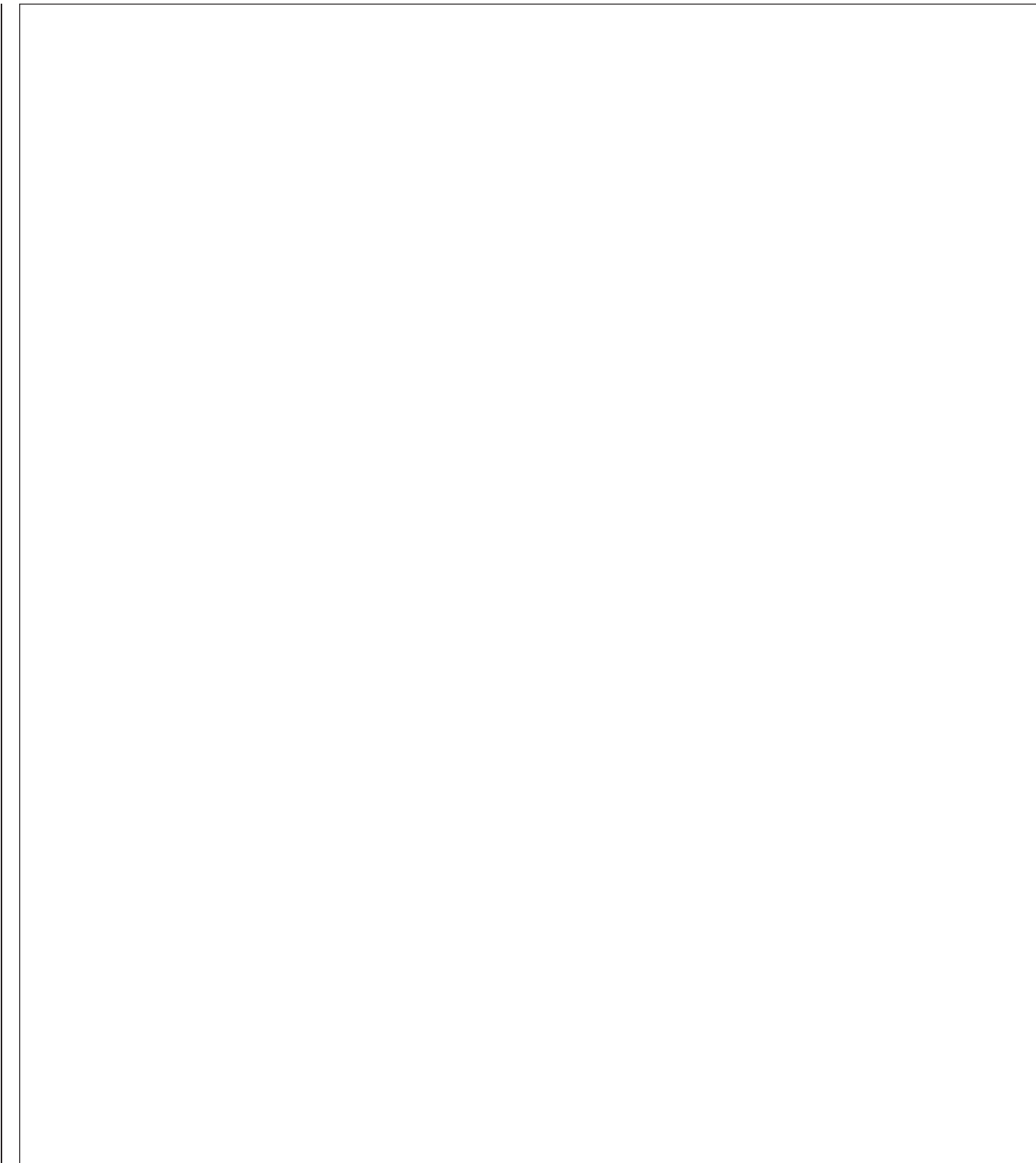


**Table 1:** Comparison of prediction and experimental retention times for the final working point.

| Peak Name  | Retention Time (min) |              |                         | % Error <sup>b</sup> |
|------------|----------------------|--------------|-------------------------|----------------------|
|            | Prediction           | Experimental | Difference <sup>a</sup> |                      |
| Unknown    | 8.18                 | 8.25         | 0.07                    | 0.86                 |
| 501        | 9.44                 | 9.46         | 0.02                    | 0.21                 |
| Main (API) | 9.78                 | 9.80         | 0.02                    | 0.20                 |
| 502        | 10.08                | 10.08        | 0.00                    | 0.00                 |
| 512        | 10.52                | 10.52        | 0.00                    | 0.00                 |
| 374        | 10.95                | 11.05        | 0.10                    | 0.91                 |

<sup>a</sup> Difference = Experimental–Prediction

<sup>b</sup> % Error = [(Experimental–Prediction)/Prediction]×100



tolerance windows, the factor most strongly influencing resolution is the pH of the eluent A.

Once the working point and tolerances were approved within the software, an experimental study summarized in Table 2 was effectuated to confirm results.

The final experimental parameter settings and tolerances for this assay were  $t_G$ : 15 ± 1 min, T: 33 ± 2 °C,  $t_C$ : AN and pH: 3.1 ± 0.1.

With the aid of the software we can visually observe that for the pH another value other than 3.0 would be better selected: The value of 3.1. At this pH both neighbouring peaks of the API (MAIN) are moving away from the API peak. If the pH value is 2.9, both peaks are moving towards the API peak. Furthermore, we can observe

in a visual way in DryLab that other factors do not influence the selectivity considerably. This opens up new possibilities to deal with out of specification results more flexibly, as we can clearly see what has to be done to correct an OOS case. With regards to judging robustness issues, we have to look at the peak movements and understand which factors provoke which changes in critical peak positions, i.e., in critical resolution.

### Summary

Quality by Design and “do it right first time” principles were successfully employed to develop a robust HPLC assay method in a transparent and understandable way with the assistance of chromatography modelling tools and an appropriate workflow.

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**Table 2:** Experimental robustness evaluation. (a) Parameter settings and levels used in the experimental robustness study.

|          |             | -   | 0   | +   |
|----------|-------------|-----|-----|-----|
| <b>A</b> | $t_G$ (min) | 14  | 15  | 16  |
| <b>B</b> | T (°C)      | 31  | 33  | 35  |
| <b>C</b> | pH          | 2.9 | 3.0 | 3.1 |

**2(b)** DoE design table (randomized).

|          | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|----------|---|---|---|---|---|---|---|---|---|----|----|----|
| <b>A</b> | 0 | 0 | - | + | 0 | - | 0 | + | + | -  | -  | +  |
| <b>B</b> | 0 | 0 | + | - | 0 | - | 0 | - | + | -  | +  | +  |
| <b>C</b> | 0 | 0 | + | - | 0 | + | 0 | + | - | -  | -  | +  |



2(c) Results.

| Exp. | t <sub>g</sub> (min) | T (°C) | pH  | Experimental chromatogram |
|------|----------------------|--------|-----|---------------------------|
| 1    | 15                   | 33     | 3.0 |                           |
| 2    | 15                   | 33     | 3.0 |                           |
| 3    | 14                   | 35     | 3.1 |                           |
| 4    | 16                   | 31     | 2.9 |                           |
| 5    | 15                   | 33     | 3.0 |                           |
| 6    | 14                   | 31     | 3.1 |                           |



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## 2(c) Results. (Continued...)

| Exp. | t <sub>g</sub> (min) | T (°C) | pH  | Experimental chromatogram |
|------|----------------------|--------|-----|---------------------------|
| 7    | 15                   | 33     | 3.0 |                           |
| 8    | 16                   | 31     | 3.1 |                           |
| 9    | 16                   | 35     | 2.9 |                           |
| 10   | 14                   | 31     | 2.9 |                           |
| 11   | 14                   | 35     | 2.9 |                           |
| 12   | 16                   | 35     | 3.1 |                           |

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