# Ph. Eur. Monograph 0049: Paracetamol Related Substances on Core-Shell Kinetex® 5 µm C18 column

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## Overview

N-(4-hydroxyphenyl) acetamide, commonly referred to as paracetamol, is one of the most familiar analgesics and antipyretic therapeutics in today's drug market.

For this report, we focused on the complex related substances profile of paracetamol as identified in the Ph. Eur. monograph 0049 published in supplement 10.7 in October 2021. This monograph is based on Pharmeuropa draft monograph (PA/PH/Exp. 10A/T (19) 136 ANP – 32.1)[1], which was already investigated in our TN-1274[2] technical note.

## Experimental

The experiments were performed on an Agilent® 1290 binary UHPLC system equipped with a UV-VIS detection set at 254 nm (no reference wavelength was utilized). Analytical reference standards for paracetamol, paracetamol impurity K (4-Aminophenol) and impurity J ((4-Chloroacetanilide) were obtained from Sigma-Aldrich® (St. Louis, Missouri, USA) and evaluated with the Kinetex 5 µm C18 column (Phenomenex, Torrance, California, USA).

## LC Conditions

**Column:** Kinetex 5 µm C18
**Dimension:** 150 x 4.6 mm
**Part No.:** 00F-4601-E0
**Mobile Phase:** A: 1.7 g $KH_2PO_4$ + 1.8 g $K_2HPO_4$ in 1000 mL water
B: Methanol

**Gradient:**

| Time(min) | %B |
|---|---|
| 0.0 | 5 |
| 2.3 | 5 |
| 15.2 | 10 |
| 29.6 | 10 |
| 58.4 | 34 |
| 60.8 | 34 |

**Flow Rate:** 1.5 mL/min
**Injection:** 50 µL
**Column Temperature:** 30 °C
**System:** Agilent 1290 binary UHPLC system
**Detection:** UV @ 254 nm

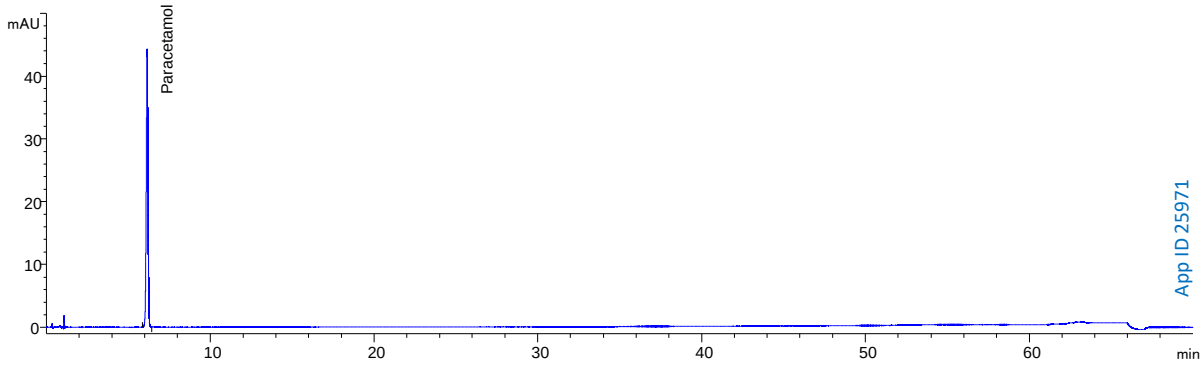
| Solution | Step 1 | Step 2 | Step 3 | Step 4 | Final Conc. |
|---|---|---|---|---|---|
| Test Solution | 50.0 mg paracetamol | dissolve with 0.75 mL methanol | dilute to 5.0 mL with water | | 10 mg/mL paracetamol |
| Ref a | dilute 1.0 mL Test Solution to 100.0 mL with solvent mixture | dilute 1.0 mL of that solution to 20.0 mL with solvent mixture | | | 5 µg/mL paracetamol |
| Ref b | 5.0 mg of imp J | dissolve with 25 mL of methanol | dilute to 250.0 mL with solvent mixture | dilute 1.0 mL of that solution to 200.0 mL with solvent mixture | 0.1 µg/mL imp J |
| Ref c | 5.0 mg of imp K | dissolve with solvent mixture | dilute to 100.0 mL with solvent mixture | dilute 1.0 mL of that solution to 10.0 mL with solvent mixture | 5 µg/mL imp K |
| Ref d | dilute 1.0 mL Ref c to 10.0 mL with solvent mixture | | | | 0.5 µg/mL imp K |
| Ref e (system suitability) | 1 mL Ref a | + 1 mL Ref c | dilute to 10 mL with solvent mixture | | 0.5 µg/mL paracetamol<br>0.5 µg/mL imp K |

## LC Conditions (continued)

The dwell volume of the HPLC system used for the development was 1.13 mL. Therefore, we adjusted the isocratic hold according to Ph. Eur. Chapter 2.2.46[3] to reflect the dwell volume of 150 µL of the Agilent® 1290 binary UHPLC system used for this study (for details see TN-1274[2]).

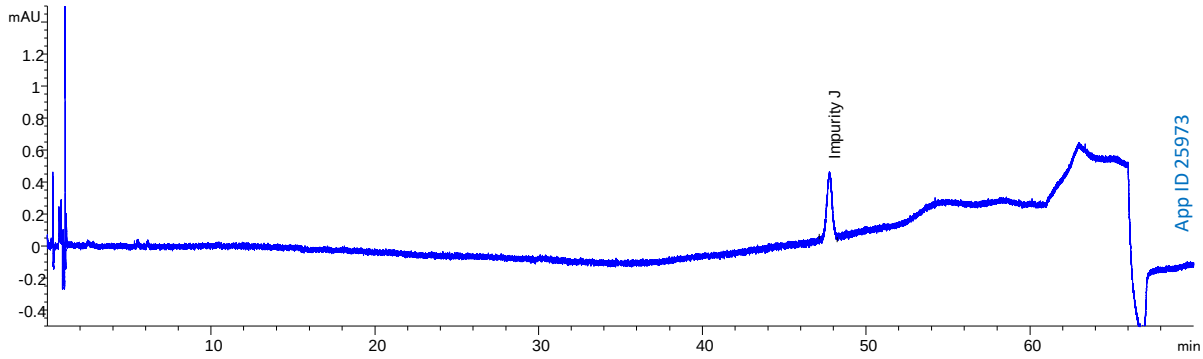
## Chromatograms and Data

**Figure 1.** Reference solution (a) on Kinetex® 5 µm C18




| # | Analyte | $t_R$ (min) | Area | Height | Width | Area % | Symmetry |
|---|---|---|---|---|---|---|---|
| 1 | Paracetamol | 6.148 | 303.9 | 44.2 | 0.1057 | 100.0 | 1.023 |

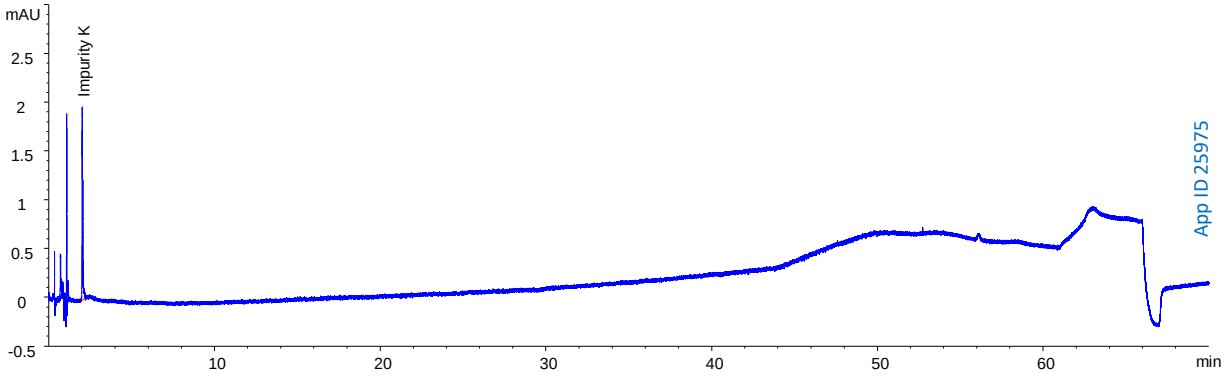
**Figure 2.** Reference solution (b) on Kinetex 5 µm C18




| # | Analyte | $t_R$ (min) | Area | Height | Width | Area % | Symmetry |
|---|---|---|---|---|---|---|---|
| 1 | Impurity J | 47.755 | 9.6 | 0.42 | 0.3834 | 100.0 | 0.864 |

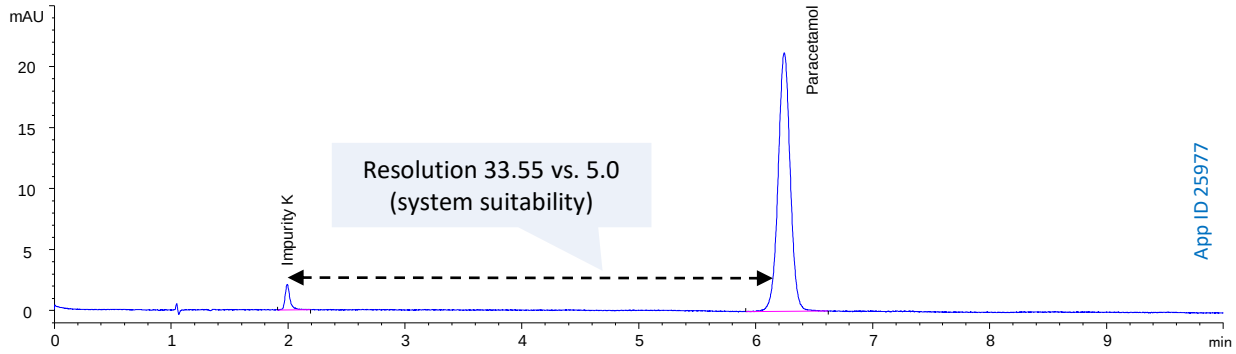
## Chromatograms and data (continued)

**Figure 3.** Reference solution (d) on Kinetex® 5 µm C18




| # | Analyte | $t_R$ (min) | Area | Height | Width | Area % | Symmetry |
|---|---|---|---|---|---|---|---|
| 1 | Impurity K | 2.026 | 5.4 | 2 | 0.0409 | 100.0 | 0.73 |

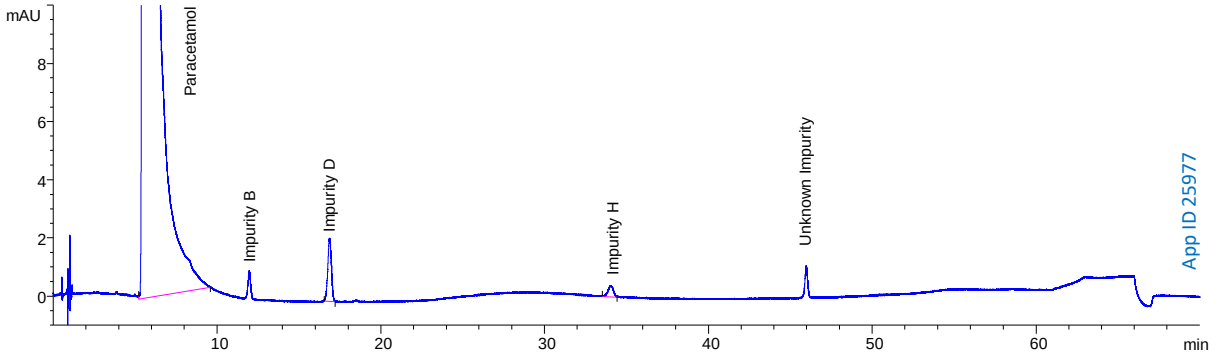
**Figure 4.** Reference solution (e) on Kinetex 5 µm C18 (system suitability requirement R ≥ 5.0)




| # | Analyte | $t_R$ (min) | Resolution |
|---|---|---|---|
| 1 | Impurity K | 2.01 | |
| 2 | Paracetamol | 6.24 | 33.55 |

## Chromatograms and data (continued)

**Figure 5.** Test solution on Kinetex® 5 µm C18




| # | Analyte | $t_R$ (min) | Area | Height | Width | Area % | Symmetry |
|---|---|---|---|---|---|---|---|
| 1 | Paracetamol | 5.890 | 156222.4 | 3892.5 | 0.6689 | 99.966 | 1.683 |
| 2 | Impurity B | 11.972 | 10.7 | 9.6E-1 | 0.1852 | 0.007 | 0.947 |
| 3 | Impurity D | 16.872 | 33.2 | 2.2 | 0.1808 | 0.021 | 0.957 |
| 4 | Impurity H | 34.05 | 8.8 | 3.9E-1 | 0.3783 | 0.006 | 1.158 |

The details on the peak identification are described in our technical note TN-1274[2].

**Figure 6.** Batch-to-batch reproducibility analysis of reference solution (e) on Kinetex 5 µm C18

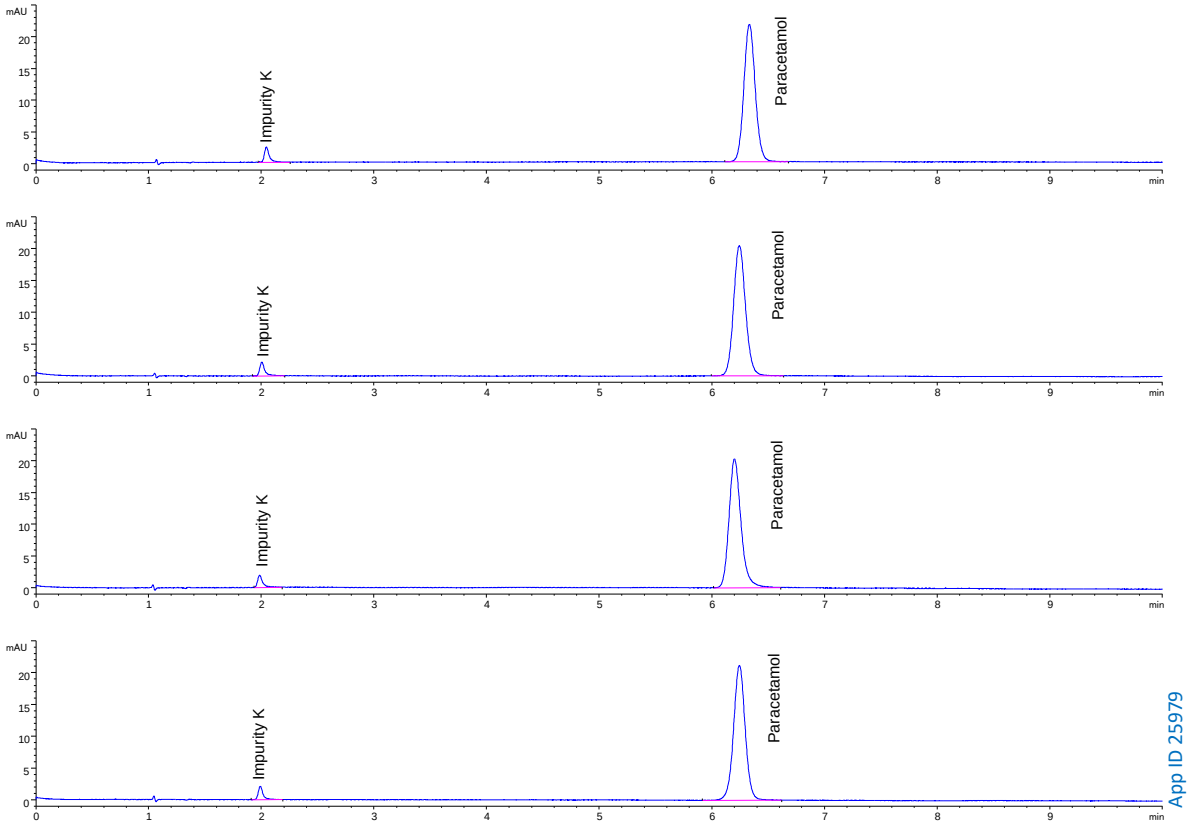


**Table 1.** Reproducibility data reference solution (e) on 4 different batches of Kinetex® 5 µm C18

| Batch | $t_R$ Impurity K (min) | $t_R$ Paracetamol (min) | Resolution |
|---|---|---|---|
| 5701-0070 | 2.05 | 6.33 | 33.23 |
| 5701-0075 | 2.01 | 6.24 | 32.20 |
| 5701-0077 | 1.99 | 6.20 | 32.27 |
| 5701-0079 | 1.99 | 6.24 | 33.55 |

## Conclusion

The above experiments show Kinetex 5 µm C18 is suitable under the conditions outlined in the monograph for paracetamol and even gives increased resolution for the system suitability solution, reference solution (e) (**Figure 4**). With the Kinetex 5 µm C18 column we also demonstrated batch-to-batch reproducibility (**Table 1**, retention times and resolution) across multiple (4) batches. Therefore, Kinetex 5 µm C18 is a reliable solution for the analysis of paracetamol in routine laboratories following the Ph. Eur. regulations. Please also refer to our technical note TN-1274[2] discussing the draft of the monograph for paracetamol. The changes in elution order for 3 impurities (I, J, and L) observed during the work has been communicated to the EDQM. As a result the elution order for these impurities has been corrected with the release of the new monograph.

## References

1. Paracetamol monograph draft (PA/PH/Exp. 10A/T (19) 136ANP published in Pharmeuropa 32.1 (01/2020)
2. Zeshan Aqeel, Dirk Hansen, and Heiko Behr (2020) TN-1274 European Pharmacopoeia Paracetamol Monograph Draft Method: Achieving Improved Sensitivity, Resolution, and Separation for Paracetamol and All 14 Related Impurities using Kinetex® 5 µm C18 Core-Shell Columns
3. European Pharmacopoeia; Supplement 10 – Chapter 2.2.46 Chromatographic Separation Techniques.

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