Laboratory Case Study

Significant Costs Savings Achieved Through the Development of a New Analytical Method for Residual API Analysis, with a Kinetex® 1.7 µm Core-Shell UHPLC Column

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> "...an annualised cost saving for the site of €320,000."

Introduction

The pharmaceutical industry has been undergoing extensive changes in recent years, primarily brought upon by the need to reduce operational costs. This is translated into the following challenges in the QC laboratory:

1. Need to reduce equipment costs
2. Need to reduce analysis costs
3. Need to analyse more samples in a shorter time
4. Reduction in laboratory consumables
5. Reduced test complexity within the laboratory (right first time motif)

Presently in the pharmaceutical industry, the majority of HPLC methods typically employ HPLC-columns packed with fully porous 3 µm and 5 µm spherical silica. Compared to the newer fully porous sub-2 µm columns, these older types of columns suffer from certain performance limitations, such as (a) broader peak shapes, (b) reduced sensitivity and (c) limited resolving power leading to the requirement for longer run times.

Combining the latest small sub-2 µm particle size column technology with the newer types of fast LC instruments, which have significantly reduced dead volumes, allow the modern chromatographer to develop faster, more efficient analytical methods, which can deliver significant cost savings into the QC laboratory. This technical note demonstrates one such cost reduction project applied to an analytical method used to analyse for residual API (Active Pharmaceutical Ingredient) from manufacturing surfaces.

The aim of this project was to combine multiple older analytical methods (total 16) which were used for the analysis of residual API's from production surfaces into one single combined method. The challenge for this new project was to develop a new analytical method which is focused on analytical simplicity, speed of analysis, and low detection limits while separating 16 different APIs (mixture of molecules ranging from neutral, basic to organic salts). This type of combined method benefits the QC laboratory, as the following reductions/costs savings can be utilised:

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- Reduction in analysis time (faster run time in combined method)
- Reduction in equipment (only 1 UHPLC needed, only 1 column
- Reduction in solvents (only 2 mobile phases)
- Reduction in number of analysts required
- Reduction in solvent waste and waste disposal costs

This project resulted in an annual cost saving of €250,000 to €350,000 Euros, based upon the reduction in analysts, solvents, wastes, equipment overheads. These savings are greater than the cost in installing a new HPLC or UHPLC into a laboratory and using a new column type not already available on site.

Experimental Conditions

Columns: Kinetex® 1.7 µm and 2.6 C18, 100 x 2.1 mm
Mobile Phase: A: 5 mM Ammonium formate pH 3.25 / Acetonitrile (95:5)
B: 5 mM Ammonium formate pH 3.25 / Acetonitrile (10:90)
Gradient:

Step Number	Time (min)	% A	% B	Curve
1	0.00	70	30	6
2	1.50	50	50	6
3	3.00	43.7	56.3	6
4	5.00	5	95	6
5	6.00	5	95	6
6	6.10	70	30	6

Flow Rate: 0.4 mL/min
Temperature: 50 °C
Detector: PDA 210-200 nm, extracted channels 225 nm and 280 nm
Instrument: Waters® ACQUITY® equipped with PDA
Sample: 1. Antidepressant drug (containing a HCl salt)
2. Hormone therapy #1 (containing a salt)
3. SERM drug (containing a basic functional group)
4. CNS drug (containing basic functional group)
5. PPI drug (containing basic functional group)
6. CNS drug (containing basic functional group)
7. CNS drug (containing basic functional group)
8. Hormone therapy #2 (neutral)
9. Oral contraceptive hormone #1 (neutral)
10. Hormone therapy #3 (neutral)
11. Oral contraceptive hormone #2 (neutral)
12. Hormone therapy #4 (neutral)
13. Oral contraceptive hormone (neutral)
14. Hormone therapy #5 (neutral)
15. Hormone therapy #6 (acetate salt of 14)
16. Immunosuppressant drug (macromolecule, containing basic functional group)
Swab Extraction Procedure: A swab containing residual API from different production surfaces was extracted with 2 mL of a solution containing a mixture of 1:1 acetonitrile/water and shaken for 5 minutes.

Results and Discussion

The APIs (Active Pharmaceutical Ingredients) used in these experiments are a mixture of organic salts, organic bases, and neutral organic compounds. The analysis was performed on a Waters® ACQUITY® under gradient conditions with a flow rate of 0.4 mL/min. Two different UV wavelengths were used for quantification, 225 nm as shown in **Figure 1** and 280 nm as shown in **Figure 2**.

A reduced run time of 6 minutes compared to the 10 to 20 minutes in the original method was achieved. The critical pair for separation was compounds 5 and 6, as the closest eluting peaks. However, molecules 5 and 6 are not in the same class of drug and would not routinely be manufactured on the same equipment set and hence seen in the same sample. Therefore complete resolution was not required.

It is noted that the sharper peak shapes obtained with the sub-2 µm core shell technology (Kinetex® C18, 1.7 µm 100 x 2.1 mm) is vital in achieving adequate separation with the shortest possible run time. When the larger particle size of 2.6 µm was used (Kinetex C18, 2.6 µm 100 x 2.1 mm), inferior separation resulted for the following pairs: 4-5, 8-9 and 11-12 (refer to **Figures 3** and **4**). This is due to the reduced efficiency of the larger 2.6 µm particle size yielding broader peaks.

The recovery of the residual APIs from the swab over different surface types was shown to be greater than 90 % for all actives. The method was shown to be robust according to ICH (International Conference on Harmonisation of Techical Requirements for Registration of Pharmaceuticals for Human Use) guidelines.

Conclusion

A new analytical method was developed which is capable of analysing 16 different API residues from production surfaces. It has been shown that the 1.7 µm Kinetex 100 x 2.1 mm column was capable of resolving 16 different chemical entities with a 6 minute run time. This new analytical method will be used to replace 16 older methods thereby facilitating an annualised cost saving for the site of €320,000.

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Figure 1.
Sample Chromatogram at 225 nm using a Kinetex®, C18, 1.7 µm, 100 x 2.1 mm

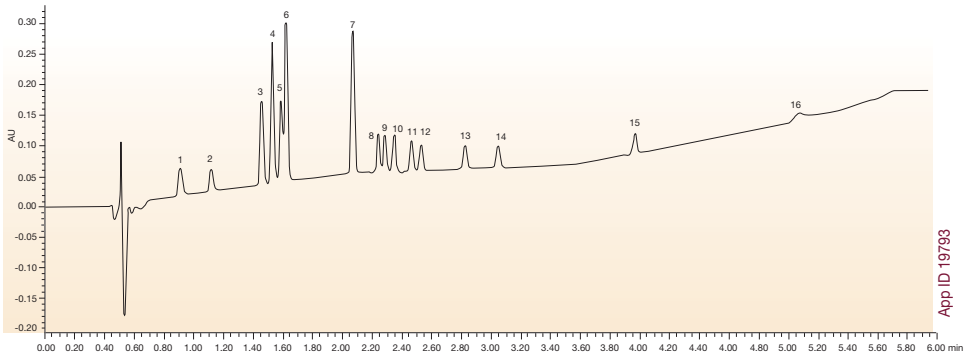


Figure 2.
Sample Chromatogram at 280 nm using a Kinetex, C18, 1.7 µm, 100 x 2.1 mm

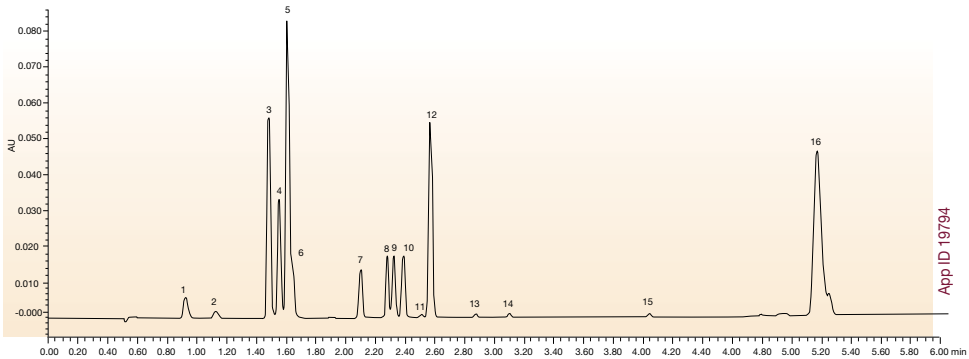


Figure 3.
Sample Chromatogram at 225 nm using a Kinetex, C18, 2.6 µm, 100 x 2.1 mm

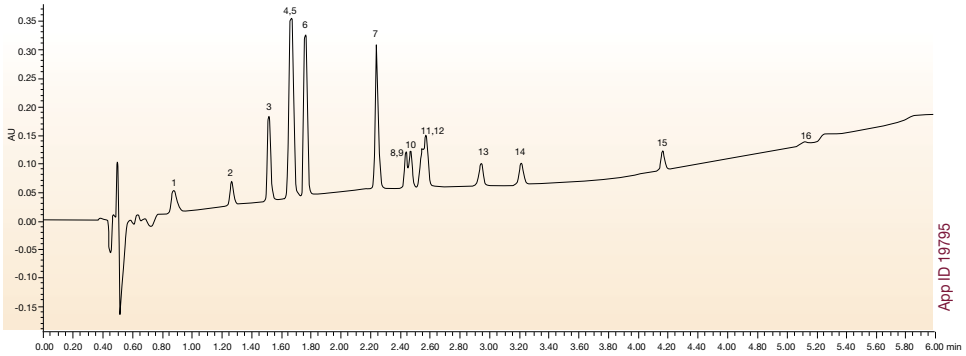
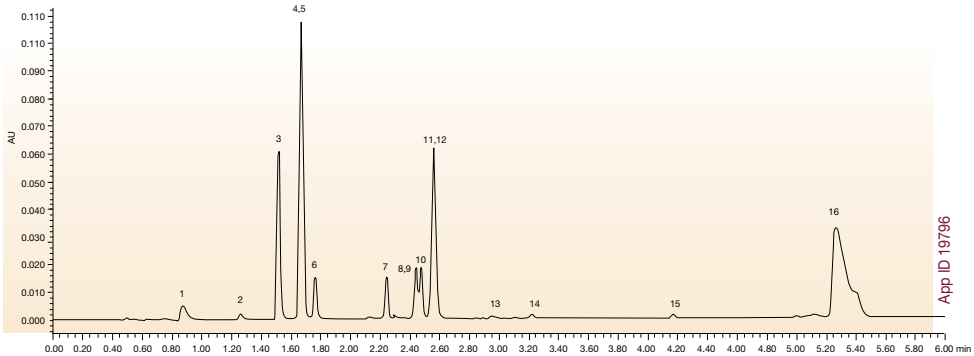


Figure 4.
Sample Chromatogram at 280 nm using a Kinetex, C18, 2.6 µm, 100 x 2.1 mm



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Kinetex® Ordering Information

2.6 µm Analytical Columns (mm)

	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	KrudKatcher™ Ultra In-Line Filter* /3pk
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AF0-8497
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AF0-8497
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AF0-8497
PFP	00A-4477-E0	00B-4477-E0	00C-4477-E0	00D-4477-E0	00F-4477-E0	AF0-8497
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	AF0-8497

2.6 µm MidBore™ Columns (mm)

	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	KrudKatcher Ultra In-Line Filter* /3pk
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	—	AF0-8497
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AF0-8497
C8	00A-4497-Y0	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	—	AF0-8497
PFP	00A-4477-Y0	00B-4477-Y0	00C-4477-Y0	00D-4477-Y0	00F-4477-Y0	AF0-8497
HILIC	00A-4461-Y0	—	—	—	00F-4461-Y0	AF0-8497

2.6 µm Minibore Columns (mm)

	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	KrudKatcher Ultra In-Line Filter* /3pk
XB-C18	00A-4496-AN	00B-4496-AN	00D-4496-AN	00F-4496-AN	AF0-8497
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AF0-8497
C8	00A-4497-AN	00B-4497-AN	00D-4497-AN	00F-4497-AN	AF0-8497
PFP	00A-4477-AN	00B-4477-AN	00D-4477-AN	00F-4477-AN	AF0-8497
HILIC	—	00B-4461-AN	00D-4461-AN	00F-4461-AN	AF0-8497

1.7 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1	KrudKatcher Ultra In-Line Filter* /3pk
XB-C18	00B-4498-AN	00D-4498-AN	00F-4498-AN	AF0-8497
C18	00B-4475-AN	00D-4475-AN	00F-4475-AN	AF0-8497
C8	00B-4499-AN	00D-4499-AN	00F-4499-AN	AF0-8497
PFP	00B-4476-AN	00D-4476-AN	00F-4476-AN	AF0-8497
HILIC	00B-4474-AN	—	—	AF0-8497

*KrudKatcher Ultra requires 5/16 in. wrench. Wrench not provided.

guarantee

If Kinetex analytical columns do not provide at least an equivalent separation as compared to competing column of the same particle size, simular phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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