KNAUER

Application Note

► High speed analysis of paracetamol and its process impurities

Category	Pharmaceutical analysis
Matrix	Drugs
Method	UHPLC
Keywords	Quality control, analgesic and antipyretic drug, industrial by-products, acetaminophen
Analytes	Paracetamol
ID	**VPH3**, 12/08, updated 01/10

PLATIN blue

Summary

This application note describes a gradient method using a sub-2 µm column for the simultaneous determination of nine process-related impurities and one degradation product of paracetamol in less than 2 minutes. The high speed and reliability of the method make it well-suited for routine analysis in drug-manufacturing quality control.

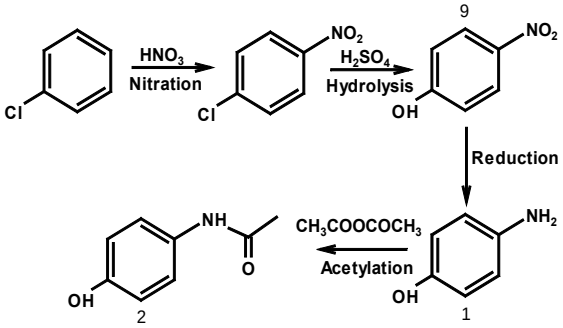
Introduction

Paracetamol is a major ingredient in numerous medications due to its analgesic and antipyretic properties. During its synthesis (Fig. 1), a total of 10 process-related impurities are observed (Fig. 2). Several HPLC applications have been developed for the monitoring of these impurities [1-2], including the European Pharmacopoeia which has adopted an isocratic HPLC method using a silica-based C8 column with 5 µm particle size, requiring a run time of 45 min [3]. By using a gradient method and standard HPLC instrumentation, the analysis can be reduced to 7 min [4].

To remain competitive however, pharmaceutical laboratories require even faster methods with higher efficiency and rapid resolution. These priorities have led to the development of stable sub-2 µm columns which can meet these demands. Compared to 5 µm columns, sub-2 µm columns offer shorter analysis times, improvements in resolution, sensitivity and peak capacity.

Fig. 1

Synthetic process of paracetamol

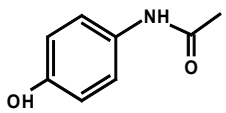


Experimental sample preparation

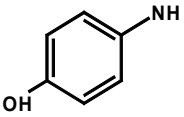
Paracetamol derivatives can be extracted from simple matrices such as drug formulations (after homogenization) with water in an ultrasonic bath. Only 100 mg of the total sample are transferred into a 100 ml volumetric flask. Approximately 80 ml of a 50% water-methanol mixture were added and the sample was stirred. After 20 min treatment in an ultrasonic bath, the sample solution was cooled down and the volume adjusted to 100 ml with water. Before injection the sample was filtered through a 0.45 µm syringe filter.

Experimental preparation of standard solution

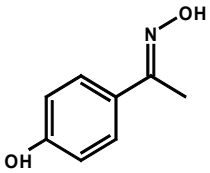
All standard solutions were prepared with methanol. 0.2 g of every substance to be examined was dissolved in 10 ml methanol. 1 ml of the test solution was diluted to 50 ml using mobile phase. 5 ml of this solution were diluted to 100 ml with mobile phase. The final concentration of each compound in the standard was 0.020 mg/ml.



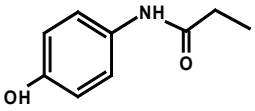
paracetamol (N-(4-hydroxyphenyl)acetamide



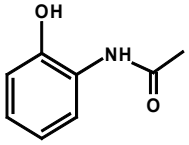
4-aminophenol



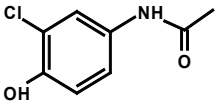
1-(4-hydroxyphenyl)ethanone oxime



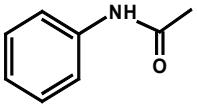
N-(4-hydroxyphenyl)-propanamide



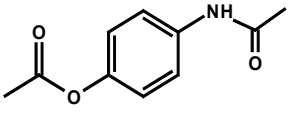
N-(2-hydroxyphenyl)-acetamide



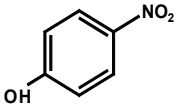
N-(3-chloro-4-hydroxyphenyl)acetamide



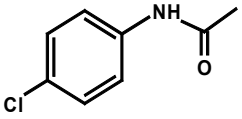
N-phenylacetamide



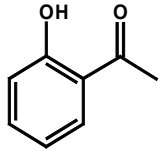
4-(acetylamino)phenyl acetate



4-nitrophenol



N-(4-chlorophenyl)-acetamide



1-(2-hydroxyphenyl)-ethanone

Fig. 2

Chemical structures

Paracetamol and its process impurities are based on a hydroxy-phenyl structure.

Method parameters

Column	BlueOrchid 120-1.8 C18, 100 x 2 mm		
Eluent A	acetonitrile		
Eluent B	phosphate buffer (pH 3.7)		
Gradient	**Time [min]**	**% A**	**% B**
	0.00	13	87
	0.30	13	87
	2.00	70	30
	2.50	70	30
Flow rate	0.8 ml/min		
Injection volume	1 µl		
Column temperature	50 °C		
System pressure	approx. 870 bar		
Detection	UV at 245 nm (100 Hz, 0.005 s)		
Run time	3 min		

Results

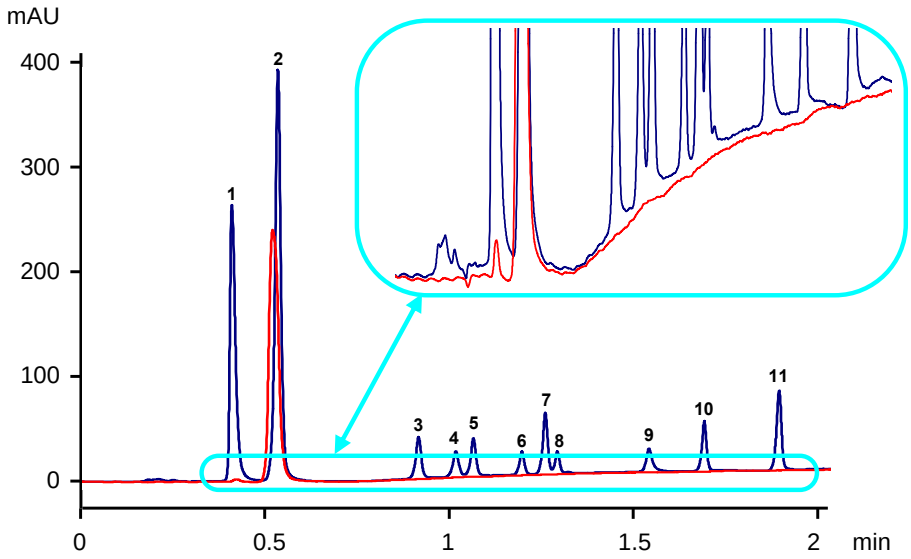


Fig. 3

Separation of paracetamol mixtures (standard = blue, pharmaceutical formulation = red)

Using a KNAUER PLATINblue UHPLC system and a BlueOrchid C18 1.8 µm column, paracetamol and ten impurities were successfully separated in under 2 min (Fig. 3), more than 3x faster than the conventional HPLC gradient method [4] and more than 15x faster than the isocratic method proposed by the European Pharmacopeia. Moreover, the UHPLC method required only one-fifth of the sample volume and eluent consumption per sample was reduced by more than 80%.
The analysis of the pharmaceutical formulation clearly showed the trace amount (0.02% w/w) of the degradation product 4-aminophenol (Peak 1)
The limit of detection (LOD) for all compounds was in the range of 0.01-0.08 µg/ml. Retention time reproducibility of the UHPLC method was in the range of 0.1 - 0.7% RSD (n = 5).

Method performance

Limit of detection	0.01 – 0.08 µg/ml range (S/N = 3)
Linearity (r^2)	0.999885-0.99993
Linearity range	0.1 to 100 ng
Retention time precision*	< 1 % RSD
Peak area precision*	< 3 % RSD

*repeatability calculated over 5 replicate runs

Conclusion

The high speed analysis of paracetamol and its related process impurities in a pharmaceutical formulation illustrates how analytical labs can benefit from sub-2 µm columns like BlueOrchid in combination with a UHPLC system like PLATINblue, in terms of faster separations, higher resolution, higher sensitivity and reduced mobile phase consumption.

References

[1] Nageswara Rao R.; Narasaraju A. Analytical Science, Vol. 22, 287-292 (2006)

[2] Nageralli B.S.; Seetharamappa J.; Gowda B.G.; Melwanki M. Journal of Chromatography B, Vol. 798, Number 1, 49-54 (2003)

[3] European Pharmacopoeia Monograph Paracetamol 01/2005:0049 / 2.2.29

[4] KNAUER, Applications Journal, V7801, 07/2008, 79, at www.knauer.net

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Physical properties of recommended column

Stationary phase	BlueOrchid 120-1.8 C18
USP code	L1
Pore size	120 Å
Pore volume	0.98 ml/g
Specific surface area	320 m^2/g
Particle size	1.8 µm
Form	spherical
Surface area	320 m^2/g
% C	19
Endcapping	yes
Dimensions	100 x 2 mm
Order number	10BF181BOE

Recommended instrumentation

The high speed analysis was performed on a KNAUER high pressure gradient PLATINblue system, equipped with two pumps P-1, degasser unit M-1, autosampler AS-1, column oven, and detector MW-1.

Description	Order No.
PLATINblue Pump P-1 (2x), incl. pump head	A60013
PLATINblue Degasser M-1	A60501
SmartMix 100	A5350
PLATINblue Autosampler AS-1	A63500
Column Oven	A0585
PLATINblue Detector MW-1	A61031
3 mm flow cell	A4042
ChromGate software	A1456

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