KNAUER

Application Note

► Separation of clindamycin phosphate and process impurities

Category	Pharmaceutical analysis
Matrix	Drugs
Method	UHPLC
Keywords	Antibiotics, process impurities, quality control
Analytes	Clindamycin phosphate, lincomycin, clindamycin
ID	**VPH0006N**, 01/11

PLATIN blue

Summary

In this application note an isocratic UHPLC separation of clindamycin phosphate from its precursor product lincomycin and impurities like clindamycin is presented. The method uses a sub-2 µm BlueOrchid C18A column and can be carried out in less than 3 minutes. The high speed and reliability of the method make it well-suited for routine analysis in drug-manufacturing quality control.

Introduction

Clindamycin phosphate is a lincosamide antibiotic used for the treatment of serious infections caused by susceptible anaerobic bacteria and strains of streptococci, pneumococci and staphylococci. Clindamycin phosphate products are especially prescribed in case of penicillin-allergic patients and can be used in a wide field of diseases including lower respiratory tract infections, lung abscess, skin and skin structure infections, gynecological infections, intra-abdominal infections and bone and joint infections.[1] This long list underlines the high relevance of clindamycin phosphate as a pharma product and the importance of its quality control. According to the European Pharmacopoeia[2], quality control has to be carried out by an HPLC method. To achieve the requested resolution of 6.0 between the peaks of clindamycin phosphate and clindamycin hydrochloride, analyses using conventional HPLC methods require more than 20 minutes per run. This is not advantageous for routine analyses.

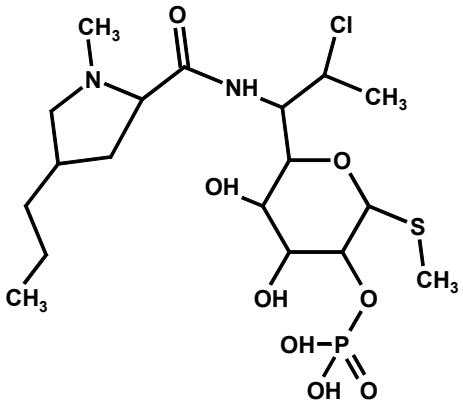
In this application note, a UHPLC method is presented that reduces the analysis time of clindamycin phosphate quality control to less than 3 minutes per sample, easily meeting the requested resolution. By applying a PLATINblue UHPLC system in combination with a BlueOrchid C18A column, the time per analysis can be reduced to one sixth compared to the method suggested by the European Pharmacopoeia.[2]

Experimental preparation of standard solution

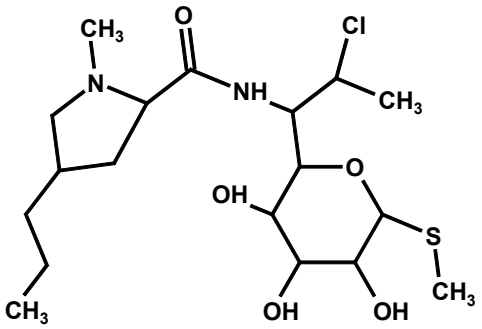
All standard solutions were prepared as described in the European Pharmacopoeia[2] using the mobile phase consisting of 20 % acetonitrile and 80 % potassium dihydrogen phosphate 13.6 g/l adjusted to pH 2.5 with phosphoric acid as solvent. For the reference solution A, 75 mg of clindamycin phosphate were dissolved and diluted to 25 ml. For the reference solution B, 5 mg of lincomycin and 15 mg of clindamycin were dissolved in 5 ml of reference solution A and diluted to 100 ml with the mobile phase. At last, 1 ml of reference solution A was diluted to 100 ml to create reference solution C.

Experimental sample preparation

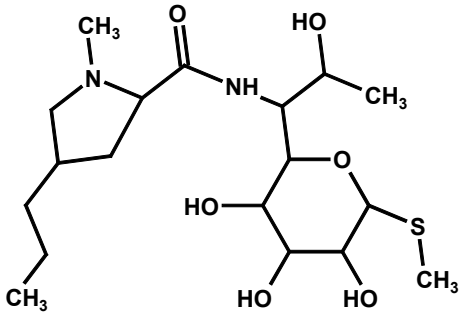
For a test solution, 75 mg of a clindamycin phosphate sample including different process impurities were dissolved in the mobile phase and diluted to 25 ml.



Clindamycin phosphate



Clindamycin



Lincomycin

Figure 1

Chemical Structures

Method parameters

Column	BlueOrchid C18A, 1.8 µm, 100 x 2 mm
Eluent A	Buffer (13.6 g/l KH_2PO_4) adjusted to pH 2.5 with phosphoric acid
Eluent B	Acetonitrile
Gradient	Isocratic, 80 % A, 20 % B
Flow rate	0.7 ml/min
Injection volume	5 µl
Column temperature	30 °C
System pressure	approx. 870 bar
Detection	UV at 210 nm (50 Hz, 10 mm cell, 2 µl)
Run time	3 min

Results

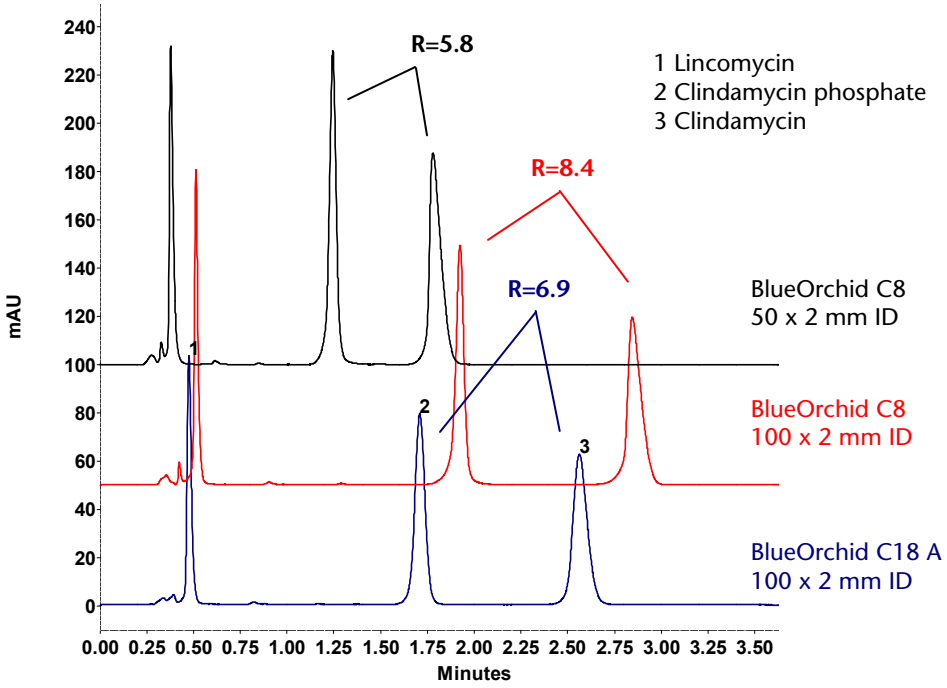


Fig. 2

Chromatograms of reference solution B, comparison of different types of BlueOrchid columns

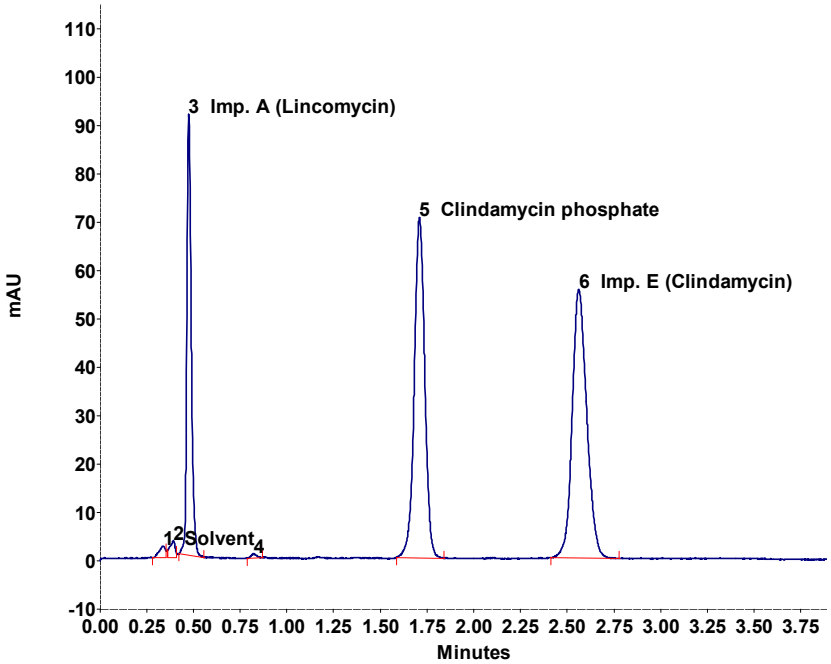


Fig. 3

Chromatogramm of reference solution B on the chosen BlueOrchid C18A column

Pk #	Name	Retention time	Area	Resolution (USP)	S/N (ASTM)	Asym-metry
1	Solvent	0.338	6423	0.0	4.5	0.000
3	Imp. A (Lincomycin)	0.475	60519	0.0	368.0	1.300
5	Clindamycin phopsphate	1.710	237372	17.1	377.8	0.988
6	Imp. E (Clindamycin)	2.562	259800	6.9	373.4	1.130
Totals			**634850**			

Results (continued)

During method development, three different column types were tested: BlueOrchid C8 50 x 2 mm, BlueOrchid C8 100 x 2 mm and BlueOrchid C18 A 100 x 2 mm. With the chosen parameters, all three columns achieved a baseline separation of the target compound and its impurities (fig. 2). The 50 x 2 mm C8 column showed a resolution value of 5.8 between clindamycin phosphate and clindamycin. According to the European Pharmacopoeia[2] the resolution has to be at least 6.0, which suggests applying a 100 x 2 mm C8 column. This column reached a resolution value of 8.4. Although the BlueOrchid C18A column achieved only a resolution of 6.9, it was chosen for further method development, because of its lower retention times.

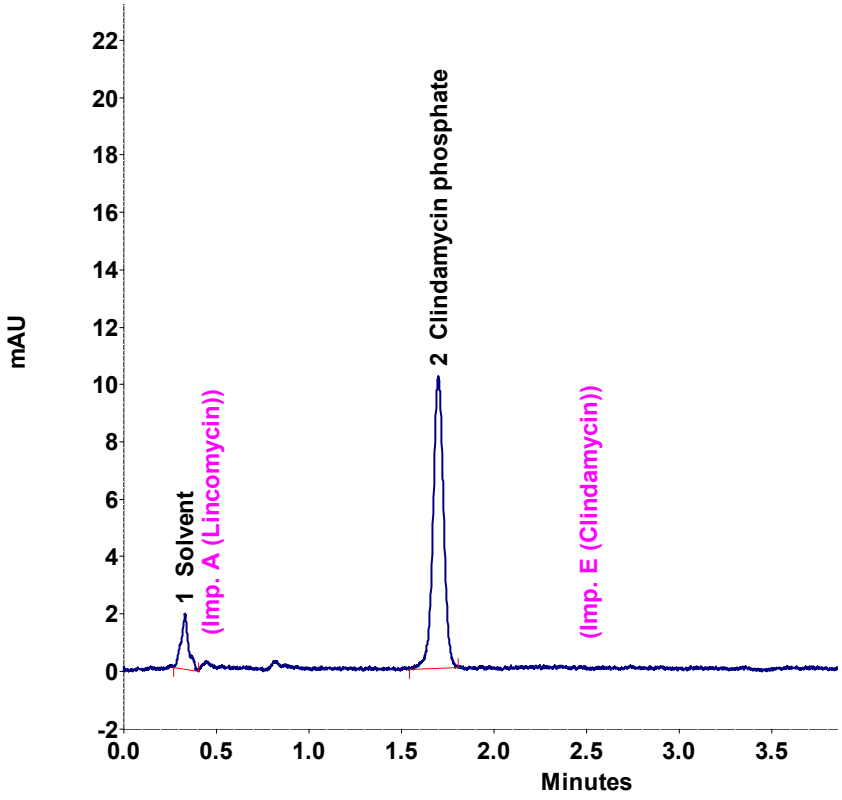


Fig. 4

Chromatogram of reference solution C separated on the chosen BlueOrchid C18A column

Report for reference solution C

Pk #	Name	Retention time	Area	Resolution (USP)	S/N (ASTM)	Asym-metry
1	Solvent	0.331	7110	0.0	8.7	1.106
	Imp. A (Lincomycin)					
2	Clindamycin phopsphate	1.700	43227	0.0	136.4	0.982
	Imp. E (Clindamycin)					
Total			**50337**			

Results (continued)

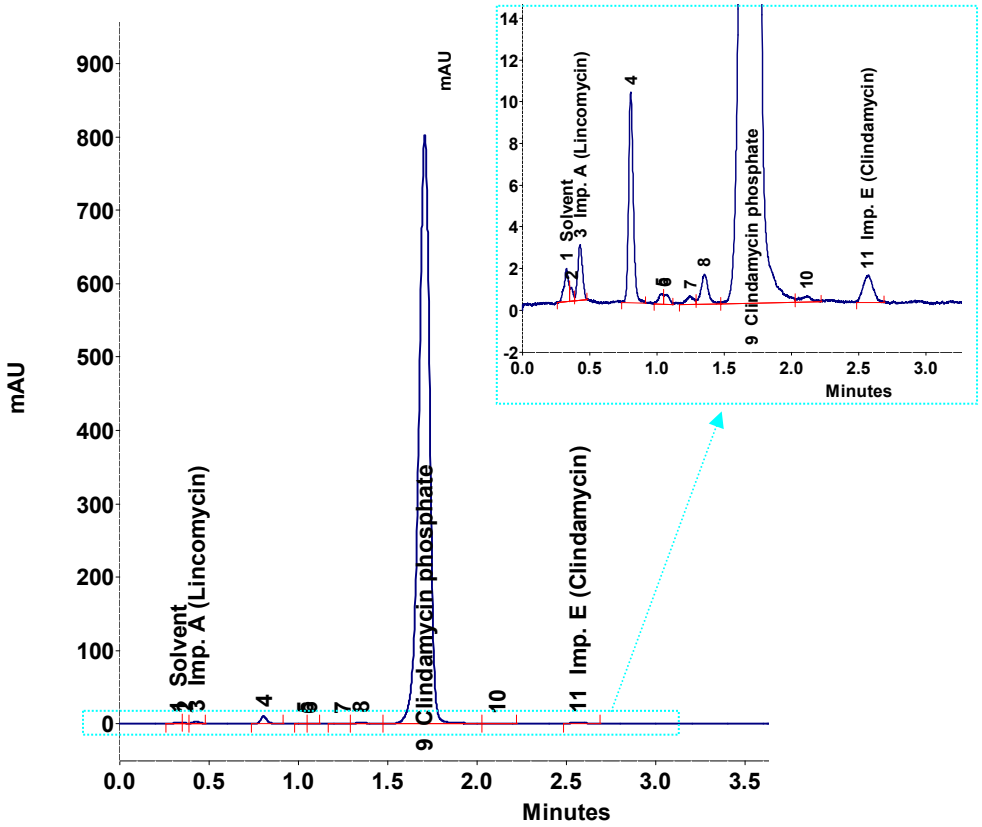


Fig. 5

Chromatogram of a sample of clindamycin phosphate including impurities

Pk #	Name	Retentions-zeit	Fläche	Auflösung (USP)	S/N (ASTM)	Asym-metrie
1	Solvent	0.330	5943		26.8	
2	n.n.	0.367	504		8.9	
3	Imp. A (Lincomycin)	0.430	7410	0,0	27.6	1.100
4	n.n.	0,806	24570		142.8	1.171
5	n.n.	1.040	754		6.7	
6	n.n.	1.061	890		6.7	
7	n.n.	1.247	1456		11.25	0.808
8	n.n.	1.353	5239		23.8	1.118
9	Clindamycin phopsphate	1.708	3469070	14,5	10962	0.873
10	n.n.	2.115	480		4.1	0.939
11	Imp. E (Clindamycin)	2.565	6853	6,9	22.4	1.251
Total			**3523349**			

Fig. 5 clearly shows that the method is sensitive enough to detect a number of different impurities in the real sample. The method presented in this application note allows also in this case for the baseline separation of clindamycin phosphate with good resolution values

The reproducibility of the method was calculated for 10 consecutive runs for reference solution B. The relative standard deviation was determined at 0.279% for the clindamycin peak (t_R=2.565 min). Peak area deviation was calculated at 0.474 %.

Results (continued)

With a KNAUER PLATINblue UHPLC system and a BlueOrchid C18A 1.8 µm column, clindamycin phosphate and two major impurities were successfully separated in less than three minutes, which is more than 6 times faster than the conventional HPLC method. By optimizing the HPLC method defined by the European Pharmacopoeia, the requested resolution could easily be reached. Moreover, the UHPLC method required only ¼ of the sample volume and eluent consumption per sample was reduced by more than 92 %. If the requested resolution according to the European Pharmacopoeia[2] is not required, a shorter C8 column can be used, allowing for additional savings of time and eluent.

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Conclusion

The high speed analysis of clindamycin phosphate and its related process impurities illustrates how quality control can benefit from sub-2 µm BlueOrchid columns in combination with a PLATINblue UHPLC system, in terms of faster separations, higher resolution, and higher sensitivity as well as by reduced mobile phase consumption. Compared with an optimized HPLC method using 5 µm particles in a 4.6 mm ID column, this method saves 92 % of the eluent and is 85 % faster. All these facts make the method well-suited for routine on-line analyses in drug-manufacturing quality control.

References

1. RxList : The Internet Drug Index, http://www.rxlist.com/cleocin-iv-drug.htm#ids (08.12.2010)
2. European Pharmacopoeia; 6 edition (July 23, 2007), pages 1570 – 1571

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Physical properties of recommended column

BlueOrchid C18 A is a polar endcapped phase for alternative C18 selectivity. For the analysis of very polar compounds it can be necessary to use 100% aqueous eluent. BlueOrchid C18 A was specially developed for such hydrophobic and polar interactions. Due to the narrow particle size distribution, the column back pressure of all BlueOrchid columns is lower than other high speed column materials on the market.

Stationary phase	BlueOrchid C18A 1.8 µm
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	12
Endcapping	hydrophilic
Dimensions	100 x 2 mm
Order number	10BI184BOE

Recommended Instrumentation

This application requires the PLATINblue binary high pressure gradient UHPLC system equipped with degasser, autosampler, column thermostat and PDA detector. Other configurations are also available. Please contact KNAUER to configure a system that is perfect for your needs.

Description	Order No.
PLATINblue HP system with PDA	A69420
PLATINblue Pump P-1, incl. 5ml pump head	
PLATINblue Pump-P1, incl. 5ml pump head and degasser	
HPG SmartMix 100	
PLATINblue Autosampler AS-1	
PLATINblue Column Thermostat T-1	
PLATINblue Detector PDA-1	
PDA flow cell (10 mm, 2 µl)	
ChromGate Software with PDA license	
PLATINblue UHPLC method converter	
PLATINblue stainless steel capillary kit	

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