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Transfer of pharmacopoeial liquid chromatography reversed-phase methods for determination of related compounds in diclofenac sodium and metamizole sodium from conventional to core-shell column

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Abstract

Core-shell silica particles were developed as a new material for chromatographic stationary phases in order to provide fast and high efficiency separations of small and large molecules and complex samples, at pressures compatible with conventional HPLC equipment. The aim of our work was to show the applicability of the HPLC columns based on a core-shell technology for determination of related substances in diclofenac sodium and in metamizole sodium using the methods described in the corresponding monographs of the European pharmacopoeia. The obtained results have shown that the proposed methods can be successfully transferred on core shell column, with suitable adjustment of injection volume and flow rate. The advantage of using core-shell column is fast and highly efficient separation on conventional HPLC equipment with increased sensitivity of the method and high throughput of the analysis, providing enhanced lab productivity and reduced costs.

Key words: core-shell column, HPLC, diclofenac sodium, metamizole sodium

Introduction

Recent developments in liquid chromatography (LC) are focused in obtaining HPLC columns with higher efficiency and increased resolution in less analysis time. For that purpose, shorter columns packed with smaller particle size (3 μm and sub-2 μm) have been introduced in LC. The small particle diameter improves the separation kinetics and therefore efficiency, but at the expense of increased operating backpressure, requiring specially designed ultra high performance pumping and flow system (Brice et al., 2009; Naijun, Andrew, 2007). Core-shell silica particles were developed as a new material for chromatographic stationary phases in order to provide fast and high efficiency separations of small and large molecules and complex samples, at pressures compatible with conventional HPLC equipment (Guiochon, Gritti, 2011). The solid-core and the well defined porous outer layer provide shorter diffusion paths of the analytes into the stationary phase, which significantly influence the separation parameters of the core-shell columns compared with those in fully porous particles. This particle morphology reduces band broadening and therefore improves separation efficiency (Destefano et al., 2012). When analytes are eluted from the column in narrow chromatographic bands, or in other words in low volume peaks, the sensitivity of the analysis is increased as the solute mass is concentrated into a smaller volume (Pereira, 2012). The columns packed with core-shell particles have been employed in a wide range of applications for analysis and quality control (Kirkland et al., 2013; Ruta et al., 2012). Core-shell

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columns have been mostly used for reversed-phase HPLC, but core-shell particles have been also employed in hydrophilic interaction liquid chromatography (HILIC) and chiral separation (Destefano et al., 2008; Berger 2011; Wu, et al., 2013). These materials have shown superior performance for separating both small molecules (Song et al., 2009; Yang et al., 2011) and larger compounds such as peptides (Schuster et al., 2012) and proteins (Fekete et al., 2012). The benefits of transferring the HPLC methods from columns packed with fully porous silica particles to core-shell columns are in maintaining column performance and minimizing operating difficulties, providing significant time and cost savings (Hayes et al., 2014).

The aim of our work was to show the applicability of the HPLC columns based on a core-shell technology for determination of related substances in diclofenac sodium and in metamizole sodium using the methods described in European pharmacopoeia (Ph.Eur.). The Ph.Eur. monograph for Diclofenac Sodium 01/2008:1002 requires use of conventional end-capped octylsylil silica gel for chromatography R (5 µm) with isocratic elution for determination of related substances. This monograph has been recently updated to version 07/2014:1002, where the method requires use of conventional end-capped octadecylsilyl silica gel for chromatography R (5 µm). In the monograph for Metamizole sodium (01/2008:1346, Ph. Eur.), for determination of related substances, base deactivated octadecylsilyl silica gel for chromatography R (5 µm) is proposed as a stationary phase, while in recently updated monograph version 04/2014:1346 (Metamizole sodium monohydrate) the method has been changed to ultra high performance liquid chromatography, requiring the use of column packed with end-capped octadecylsilyl silica gel for chromatography R (1.8 µm).

In our study we have applied the columns based on core-shell technology for determination of related substances of diclofenac sodium and metamizole sodium using the methods described in the Ph.Eur. monographs 01/2008:1002 and 01/2008:1346 respectively.

Experimental

Chemicals and standards

Diclofenac sodium, diclofenac impurity A, metamizole sodium and metamizole impurity A certified reference standards, were purchased from EDQM (Strasbourg, France). Phosphoric acid (85 %), sodium dihydrogen phosphate, (Ph.Eur., grade for analysis), potassium hydrogen phosphate dibasic (analytical grade) and triethylamine were purchased from Merck, Germany. Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from Carlo Erba Reagents, France. Water R, was obtained with a TKA-LAB Reinstwasser system (Niederelbert, Germany).

Chromatographic Conditions

HPLC separation was performed on Agilent 1100 and Agilent 1200 LC Systems (used as a conventional HPLC with pressures up to 400 bar). ChemStation software, Version A.10.02 (for experiments conducted on Agilent 1100) and Version B.04.03 (for experiments conducted on Agilent 1200) was used for data acquisition and instrument control.

Method for related substances in diclofenac sodium

The separation was performed on Poroshell 120 EC C8 50 x 4.6 mm, 2.7 µm (Agilent) using a mixture of 34 volumes of a solution containing 0.5 g/L phosphoric acid and 0.8 g/L sodium dihydrogen phosphate R, adjusted to pH 2.5 with phosphoric acid R and 66 volumes of methanol R as a mobile phase. The column temperature was 25°C. Flow rate was 1 mL/min. Injection volume was 3 µL. UV detection was performed at 254 nm.

System suitability solution for diclofenac sodium

Reference solution b, containing diclofenac sodium (5 µg/mL) and diclofenac impurity A (5 µg/mL), prepared as described in Ph.Eur. monograph for Diclofenac Sodium 01/2008:1002 was used as system suitability solution.

Method for related substances in metamizole sodium

The separation was performed on Kinetex XB-C18, 50 mm x 2.1 mm, 5 µm (Phenomenex, Inc), using a mixture of 28 volumes of methanol R and 72 volumes of a buffer solution containing 1000 volumes of a 6.0 g/L solution of sodium dyhidrogen phosphate R and 1 volume of triethylamine R, pH 7.0 (adjusted with strong sodium hydroxide solution R) as a mobile phase. The column temperature was 25°C. Flow rate was 0.2 mL/min. Injection volume was 0.5 µL. UV detection was performed at 254 nm.

System suitability solution for metamizole sodium

Reference solution e, containing metamizole sodium (0.3 mg/mL) and metamizole impurity A (0.4 mg/mL) prepared as described in Ph.Eur. monograph for Metamizole sodium 01/2008:1346 was used as system suitability solution.

Results and Discussion

Two methods described in European pharmacopoeia (method for determination of related substances in diclofenac sodium and method for determination of related substances in metamizole sodium) were transferred from the conventional fully porous column, to column based on core-shell technology. The suitability of the core-shell columns was evaluated by comparing the obtained results for system suitability parameters against the chromatogra-

phic system requirements according to Ph.Eur. (*2.2.46 Chromatographic separation techniques*). This chapter also limits the extent to which the various parameters of a chromatographic test may be adjusted to satisfy the system suitability criteria without fundamentally modifying the methods. According to these requirements the injection volume may be decreased, provided detection and repeatability of the peak(s) to be determined are satisfactory and no increase is permitted, and the flow rate may be adjusted (± 50 per cent). Regarding the column parameters there is no change of the identity of the substituent of the stationary phase permitted (e.g. no replacement of C18 by C8) and the particle size may be reduced maximum 50 per cent, but it cannot be increased. The length of the column and the internal diameter may be adjusted ± 70 per cent and ± 25 per cent respectively.

Method for related substances in diclofenac sodium

The method for determination of related substances in diclofenac sodium described in the Ph. Eur. monograph 01/2008:1002, was transferred from conventional end-capped octylsylil silica gel for chromatography R (0.25 m x 4.6 mm; 5 µm) on core-shell column (C8, 50 mm x 4.6 mm; 2.7 µm). All method modifications, except the column length, were within the limits of Ph.Eur. (2.2.46). Reducing the column internal diameter facilitates sensitivity improvements and shorter columns can often deliver the required resolution (Pereira, 2012). The reduction of column size results in reduction of column volume, which required scaling down the injection volume from 20 µL to 5 µL and 3 µL (Figure 1).

Satisfactory system suitability requirements of the transferred method were obtained with the injection volume of the sample of 5 µL and 3 µL in total analysis time of 5 minutes (1.6 times of the retention time of diclofenac, Rt = 2.9 min). The requirement in Ph.Eur monograph for Diclofenac sodium (01/2008:1002) for the resolution (Rs) between the peaks from diclofenac sodium and impurity A (at least 6.5) was fulfilled (Rs = 10.6 for volume of injection 5 µL and Rs =12.2 for volume of injection 3 µL). The obtained values from system suitability tests (Table 1) for number of theoretical plates (N), retention factor (k'), resolution (Rs), symmetry factor (As) and relative standard deviation (RSD %), indicate on a satisfactory column efficiency and adequate performance of the chromatographic system.

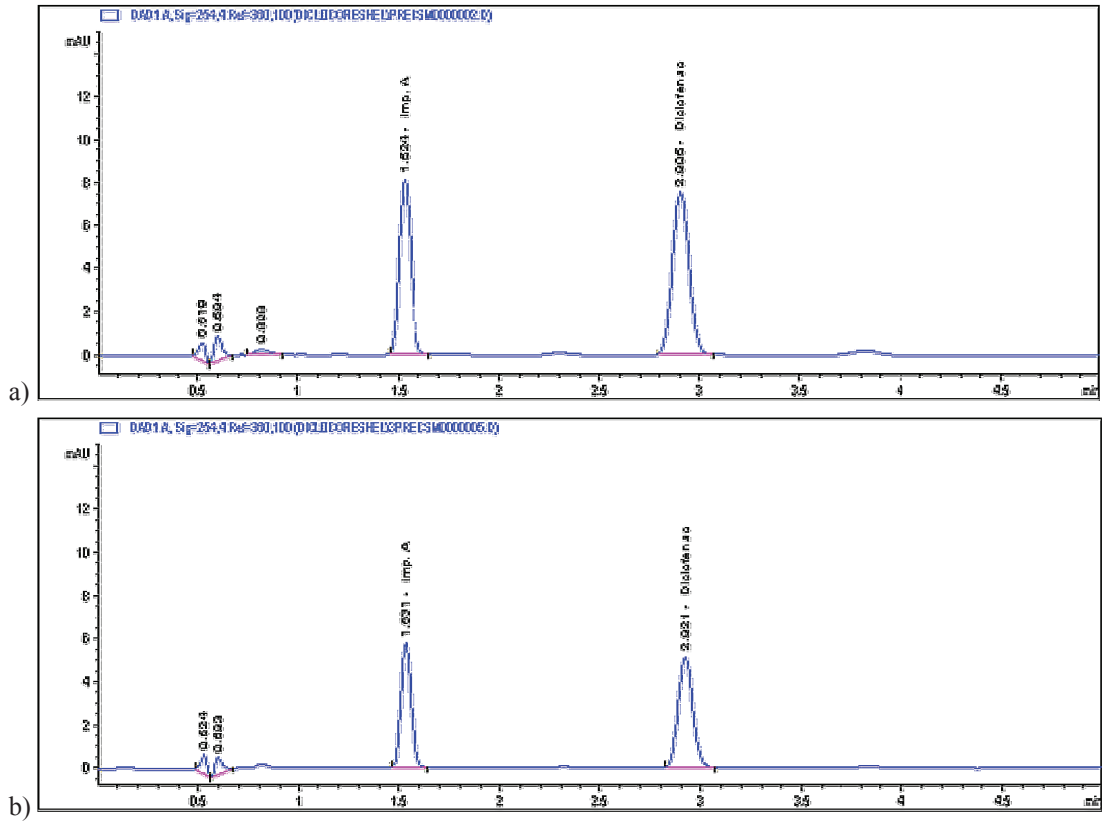

Fig 1. Chromatogram of System suitability solution for diclofenac sodium, a) injection volume 5 µL, b) injection volume 3 µL, obtained using core-shell column (C8).

Table 1. System suitability values for the method for related substances of diclofenac sodium obtained using core-shell column (C8)

System suitability parameter	Imp.A	Diclofenac	Imp.A	Diclofenac
	V inj. = 5 µL		V inj. = 3 µL	
Rt (min)*	1.524	2.905	1.531	2.921
k'	2.056	4.826	2.071	4.859
As	0.90	0.93	0.90	0.92
N	3074	5872	4225	7563
Rs (Ph.Eur. Rs ≥ 6.5)	10.5		12.2	
RSD (%)				
Rt*	0.03 %	0.05 %	0.06 %	0.06 %
Peak area*	0.27 %	0.27 %	0.29 %	0.32 %

*n = 6.

The limit of detection (DL) and limit of quantification (QL) were determined on the basis of standard deviation (SD) of the response and the slope, obtained from regression analysis of relationship between the response (peak area) and the concentration of diclofenac sodium in concentration range from 0.2 µg/mL to 5 µg/mL (0.2, 0.5, 1, 2, 3, and 5 µg/mL). The obtained values for limit of detection, DL = 0.05 µg/mL (volume of injection 5 µL) and DL = 0.03 µg/mL (volume of injection 3 µL) and for the limit of quantification, QL = 0.17 µg/mL (volume of injection 5 µL) and QL = 0.08 µg/mL (volume of injection 3 µL) are below the disregard limit (0.5 µg/mL) given in the Ph.Eur. monograph, indicating on satisfactory sensitivity of the method.

The obtained results have shown that using the core-shell column for determination of related substances in diclofenac sodium, satisfactory method performance are obtained in total analysis time from 5 to 7 minutes. The updated method for determination of related substances in diclofenac sodium in Ph. Eur. monograph 07/2014:1002, could be also transferred from conventional end-capped octadecylsilyl silica gel for chromatography R (0.25 m x 4,6 mm; 5 µm) on a suitable core-shell column (C18), in order to shorten the analysis time (25 min according to monograph 07/2014:1002, Ph.Eur.).

Method for related substances in metamizole sodium

The method for determination of related substances in metamizole sodium described in the Ph. Eur. monograph 01/2008:1002, was transferred from conventional base deactivated octadecylsilyl silica gel for chromatography R (0.25 m x 4.6 mm; 5 µm) on core-shell column (XB-C18, 50 mm x 2.1 mm, 5 µm). The modification of the method parameters regarding the column dimensions were outside of the limits of Ph.Eur. (2.2.46). Reduction of the column dimensions has the direct benefit on sensitivity and reduces the analysis time (Pereira, 2012). Due to the reduction of column size the injection volume was decreased from 10 µL to 0.5 µL (Figure 2). The adjustment of the method inclu-ded also reduction of the flow rate from 1 mL/min to 0.2 mL/min, which is within the limits of Ph.Eur. (2.2.46), of nece-ssary adjustment of the flow rate, when column dimensions are changed (minimum flow rate is 0.1 mL/min).

Reduction of the injection volume to 1 µL did not gave satisfactory separation (Rs = 2.29) of metamizole from impurity A (Ph.Eur., monograph limit is Rs ≥ 2.5). Satisfactory system suitability requirements were obtained with the injection volume of 0.5 µL in total analysis time of 9 minutes (4.5 times of the retention time of metamizole, Rt = 2.15) and the value for resolution (Rs) between metamizole and impurity A (Rs = 3.11) fulfills the requirement of the Ph.Eur monograph (Rs ≥ 2.5). The obtained values from system suitability tests (Table 2) for number of theoretical plates (N), retention factor (k'), resolution (Rs), symmetry factor (As) and relative standard deviation (RSD %), indicate on a satisfactory column efficiency and adequate performance of the chromatographic system.

Table 2. System suitability values for the method for related substances of metamizole sodium obtained using core-shell column (C18)

System suitability parameter	Imp.A	Metamizole
Rt*	1.401	2.148
k'	1698	3.135
N	531	1315
Rs (Ph.Eur. Rs ≥ 2.5)	3.11	
RSD		
Rt*	0.16 %	0.26 %
Peak area*	0.53 %	0.55 %

*n = 6.

The limit of detection (DL) and limit of quantification (QL) were determined on the basis of standard deviation (SD) of the response and the slope, obtained from the regression analysis of relationship between the response (peak area) and the concentration of metamizole sodium in concentration range from 5 µg/mL to 50 µg/mL (5, 10, 20, 25, 30, and 50 µg/mL). The obtained values for limit of detection, DL = 0.33 µg/mL and for the limit of quantification, QL = 0.99 µg/mL are below the disregard limit (1.25 µg/mL) given in the Ph.Eur. monograph, indicating on satisfactory sensitivity of the method.

The updated monograph 04/2014:1346 of Metamizole sodium monohydrate, for the method for determination of related substances requires an end-capped octadecylsilyl silica gel for chromatography R (50 mm x 4.6 mm; 1.8 µm). For this type of column, the use of ultra high performance liquid chromatography system is necessary. Ac-

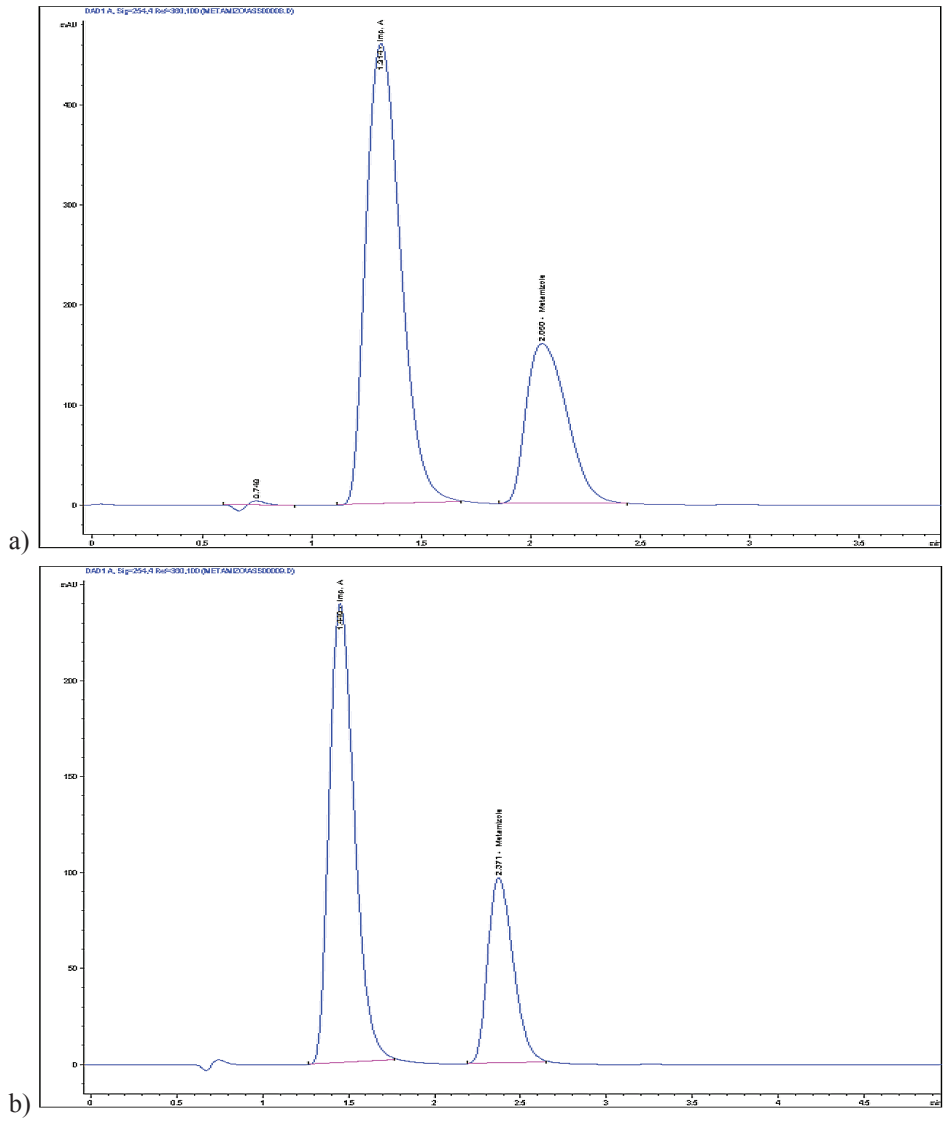

Fig 2. Chromatogram of System suitability solution for metamizole sodium: a) injection volume 1 µL, b) injection volume 0.5 µL, obtained using core-shell column (C18).

cording to the monograph, the analysis times of the method should be around 9 minutes (4.5 times of the retention time of metamizole with Rt around 2 min), which is the same as the analysis time obtained using core-shell column. The advantage of using the column based on core-shell technology is in obtaining comparable and satisfactory column performance on conventional HPLC equipment, available in almost any laboratory.

Conclusion

The results have shown that the pharmacopoeial methods for determination of related substances in diclofenac sodium and in metamizole sodium are successfully transferred on core-shell column, with suitable adjustment of injection volume and flow rate. The adjusted methods using core-shell columns fulfill the Ph. Eur. requirements for system suitability and achieved significant improvements in efficiency performance, resolution and sensitivity.

The advantage of using core-shell column is fast and highly efficient separation on conventional HPLC system with increased sensitivity and throughput of the method, providing enhanced lab productivity and reduced costs.

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Резиме

Трансфер на фармакопејски методи за определување на онечистувања базирани на реверзно-фазна течна хроматографија од конвенционална на core-shell колона

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Клучни зборови: core-shell колона, HPLC, диклофенак натриум, метамизол натриум

Core-shell силика честичките, се развиени како нов материјал за хроматографски стационарни фази, со цел обезбедување на високо ефективно разделување на мали и големи молекули, како и на комплексни смеси со притисоци компатибилни со конвенционална HPLC опрема. Целта на овој труд беше да се покаже применливоста на HPLC колоните базирани на core-shell технологија за определување на сродни супстанции на диклофенак натриум и на метамизол натриум со примена на методите опишани во соодветните монографии во Европската фармакопеја. Резултатите покажаа дека предложените методи може успешно да се трансферираат на *core-shell* колона со соодветно подесување на волуменот на инјектирање и протокот на мобилната фаза. Предноста на употребата на core-shell колона е брзо и ефикасно разделување на конвенционален HPLC систем, со зголемена осетливост на методот и висока ефикасност на анализата, овозможувајќи зголемена продуктивност на лабораторијата и намалување на трошоците.