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The new HPLC methodology for the chiral separation of tamsulosin enantiomers on amylose tris(3,5-dimethylphenylcarbamate) stationary phase ☆

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ABSTRACT

New chromatographic method for the enantioseparation of (*R*,*S*)-tamsulosin and the determination of (*R*)- and (*S*)-tamsulosin was developed with the aid of amylose tris(3,5-dimethylphenylcarbamate) stationary phase. The method was compared to the known procedure for tamsulosin enantioseparation on cellulose tris(3,5-dimethylphenyl carbamate). Careful validation of both methods allowed to prove advantages of the new procedure: significantly better resolution as well as twice better sensitivity. The method was applied to quantification of (*R*)- and (*S*)-tamsulosin contents in prolonged release Apo-Tamis 0.4 mg hard capsules (Apotex Europe B.V) and Omnic Ocas 0.4 mg coated tablets (Astellas).

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1. Introduction

Tamsulosin, a sympatholytic agent, is α_1-receptor antagonist used in the symptomatic treatment of benign prostatic hyperplasia (BPH) [1]. Tamsulosin is one of the first selective α_1 prostate receptor specific blockers. Benign prostatic hyperplasia is a common disease with an estimated 50% of men by age 50 years having evidence of it, with the treatment including α_1 receptor blocking. Tests on rabbits and prostate specimens showed that the (*R*)-tamsulosin enantiomer (Fig. 1) is 50–140 times more active than (*S*)-enantiomer and the desired sympatholytic properties (α_1-receptor antagonist) are demonstrated for (*R*)-enantiomer, and (*S*)-enantiomer does not show such activity [2]. Chemically, tamsulosin is 5-[(2*R*)-2-[[2-(2-ethoxy phenoxy)ethyl]amino]propyl]–2-methoxy benzene sulfonamide hydrochloride. In commercial preparations tamsulosin occurs as a pure (*R*)-enantiomer.

Tamsulosin enantioseparation has previously been performed with the aid of capillary electrophoresis with the use of cyclodextrines as chiral selectors [3–6] as well as liquid chromatography with the use of crown ether [7] or cellulose carbamate [8] chiral stationary phases. Separation on cellulose stationary phase with water-acetonitrile containing potassium hexafluorophosphate mobile phase offered baseline resolution of $R_S = 1.10$ [8,9].

In the present paper we report the new method for chromatographic separation and quantification of tamsulosin enantiomers on chiral amylose tris(3,5-dimethylphenyl carbamate) stationary phase, offering better resolution and sensitivity, than these recommended previously. The new procedure was successfully applied for the determination of tamsulosin in medicinal products: Apo-Tamis (Apotex Europe B.V.) and Omnic Ocas (Astellas).

2. Material and methods

2.1. Material

Standard tamsulosin hydrochloride preparation (*R*-isomer)-Tamsulosini hydrochloridum from WS Zentiva No. 435 (99.7%), and *S*-(+) Tamsulosin hydrochloride (tamsulosin hydrochloride impurity) from Gedeon Richter.

Medicinal products with prolonged release containing 0.4 mg tamsulosin: Apo-Tamis hard capsules supplied by Apotex Europe B.V., and Omnic Ocas coated tablets supplied by Astellas.

2.2. Apparatus

Two chromatographic set-ups were used in this study: Shimadzu LC with CBM-10A controller, CTO-10A oven, UV–vis

☆ Dedicated to Professor Yuri A. Zolotov on the occasion of his 80th birthday.

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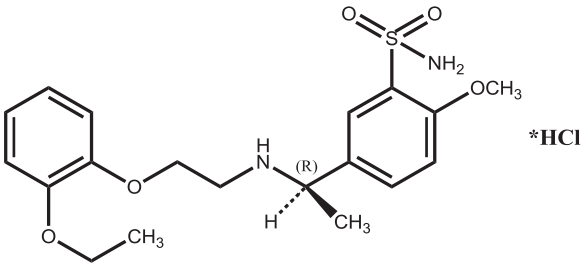

Fig. 1. (*R*)-Tamsulosin hydrochloride.

SPD-10A detector, LC-10AT pump, SIL-10A autosampler and LC-10 software, as well as a Dionex Ultimate 3000LC, Ultimate 3000pump, UV–vis Ultimate 3000detector, Ultimate 3000autosampler, Ultimate 3000thermostat and Chromeleon software.

2.3. Columns, reagents, chromatographic conditions

Chiral separations were performed using 4.6 × 250 mm, 5 μm, columns: Chiralcel OD-H (cellulose tris(3,5-dimethylphenyl carbamate)) and Chiralpak AD-H (amylose tris(3,5-dimethylphenyl carbamate)) from Daicel Chemical Industries Ltd.

n-Hexane 95% HPLC (Labscan Ltd), ethanol 99.5%, analytical (POCH), ethanol 96%, analytical (POCH), triethylamine, analytical (Sigma-Aldrich), ethanolamine, Reagent Plus ≥ 99% (Sigma-Aldrich).

2.3.1. Cellulose tris(3,5-dimethylphenyl carbamate) column

Mobile phase: hexane–isopropyl alcohol–triethylamine (80:20:0.2, v/v/v), flow rate 1.0 ml/min, detection UV, 279 nm, t=25 °C. For the preparation of calibration curves 96% ethanol solutions containing 1–100 μg/ml of the analyte were applied (injection volume 20 μl).

2.3.2. Amylose tris(3,5-dimethylphenyl carbamate) column

Mobile phase: hexane–ethanol–ethanolamine (80:20:0.2, v/v/v), flow rate 1.2 ml/min, detector UV, 279 nm, t=25 °C. Preparation of a calibration curve was identical as described above.

2.4. Analysis of medicinal preparations Apo-Tamis 0.4 mg capsules and Omnic Ocas prolonged release 0.4 mg coated tablets

2.4.1. Extraction of tamsulosin from apo-Tamis and Omnic-Ocas pharmaceutical preparations

Six Apo-Tamis capsule contents or six Omnic-Ocas tablets were carefully ground. Accurate weight amount of each preparation corresponding to one dose (130 mg for Apo-Tamis or 245 mg of Omnic-Ocas) was placed into 25 ml volumetric flask and filled with ethanol (it should be noted that bigger amount of pharmaceutical formulations subsequently resulted in sticky suspensions, not ready to filter). The samples were sonicated for 90 min.

2.4.2. Determination of analyte content and precision of the method

The suspensions prepared as above were filtered and injected onto a column (injection volume 20 μl, n=6). Determined (*R*)-tamsulosin content was 16.0 ± 0.2 μg/ml.

2.4.3. Determination of analyte recovery within the content range of 80%–120% (according to ICH validation of analytical procedures [10])

Portions of 15 ml of the suspension prepared according to Section 2.4.1. (corresponding to 0.24 mg of active substance) were transferred into nine volumetric flasks (50 ml). 0.4 mg of tamsulosin HCl standard was poured into the first three flasks, 0.6 mg into subsequent three flasks and 0.75 mg into the last three flasks.

Table 1
Influence of flow rate on precision of peak areas measurements and selectivity.

Flow rate (ml/min)	Peak area stability, RSD		Resolution, R_s
	(*R*)-tamsulosin (%)	(*S*)-tamsulosin (%)	
1.0	1.07	1.70	7.93
1.2	1.54	1.75	7.51
1.4	1.47	1.37	7.21

The flasks were filled with ethanol, sonicated for approx. 10 min, and filtered. The solutions of following concentrations were used:

First three flasks: 0.24 mg + 0.4 mg = ca. 0.64 mg/50 ml i.e. 12.8 μg/ml (80% dose, with respect to the primary 16 μg/ml solution).
Subsequent three flasks: 0.24 mg + 0.6 mg[1] = ca. 0.84 mg/50 ml i.e. 16.8 μg/ml (100% dose).
Last three flasks: 0.24 mg + 0.75 mg[1] = ca. 0.99 mg/50 ml i.e. 19.8 μg/ml (118% dose).

2.4.4. Intra-day precision (repeatability)

Solutions of (*R*)-tamsulosin hydrochloride in 96% ethanol (containing 16 μl/ml of tamsulosin) were injected onto a column within the same day (injection volume 20 μl). Precisions of peak areas measurements (RSD = 1.09%) and retention times (RSD = 0.12%) were calculated (n=6).

2.4.5. Inter-day precision (intermediate precision)

Solutions of (*R*)-tamsulosin prepared as in Section 2.4.4. were injected onto a column within three consecutive days. Precisions of peak areas measurements (RSD = 0.75%) and retention times (RSD = 0.19%) were determined (n=3 for each day).

2.4.6. Sample stability

Solutions of (*R*)-tamsulosin prepared as in Section 2.4.4. were injected onto a column within 72 h (sampling times 0 h, 24 h, 48 h, 72 h). Precision of peak areas measurements (RSD = 1.44%) were calculated (n=2 for each sampling time).

2.4.7. Influence of flow rate on the precision of peak areas measurements and selectivity

Solutions of (*R*)- and (*S*)-tamsulosin hydrochloride in 96% ethanol (containing 16 μl/ml of free tamsulosin each) were injected onto a column. Applied flow rates: 1.0 ml/min, 1.2 ml/min, 1.4 ml/min (injection volume 20 μl, n=6 for each flow rate). Precisions of peak areas measurements as well as selectivity were calculated (Table 1).

3. Results and discussion

The methods for the separation and determination of (*R*) and (*S*)-tamsulosin with use of amylose tris(3,5-dimethylphenyl carbamate) and cellulose tris(3,5-dimethylphenyl carbamate) stationary phases were validated and compared (Table 2).

In agreement with the reported data [8], resolution (R_S) of 1.10 was achieved on column with cellulose tris(3,5-dimethylphenyl carbamate) (Chiralcel OD-H) stationary phase. It should be noted that employing of hexane–isopropyl alcohol–triethylamine mobile phase (as compared to used in [8] water-acetonitrile containing potassium hexafluorophosphate mobile phase) did not improve the resolution on that stationary phase. The elution order was *R* enantiomer before *S* (Fig. 2A).

On amylose tris(3,5-dimethylphenyl carbamate) (Chiralpak AD-H) the resolution R_S of (*R*,*S*)-tamsulosin was 7.93. The elution

Table 2
Comparison of the methods for the determination of (*R*)- and (*S*)-tamsulosin with the use of amylose tris(3,5-dimethylphenylcarbamate) and cellulose tris(3,5-dimethylphenyl carbamate) stationary phases.

	Amylose tris(3,5-dimethylphenyl carbamate)		Cellulose tris(3,5-dimethylphenyl carbamate)	
	(*R*)-tamsulosin	(*S*)-tamsulosin	(*R*)-tamsulosin	(*S*)-tamsulosin
Retention time (min)	28.4	16.8	29.5	34.4
Calibration graph	$(0.2052 \pm 0.0063)X + (-0.0533 \pm 0.2898)$	$(0.1973 \pm 0.0100)X + (-0.2252 \pm 0.4684)$	$(7342 \pm 257)X + (-4313 \pm 12549)$	$(8870 \pm 65)X + (-3714 \pm 3217)$
Linearity range (μg/ml)	0.5–100	0.5–100	1–100	1–100
Correlation coefficient, R_2	0.9988	0.9967	0.9987	0.9999
Limit of detection (μg/ml)	0.2	0.2	0.3	0.3
Limit of quantification (μg/ml)	0.6	0.6	1.0	1.0

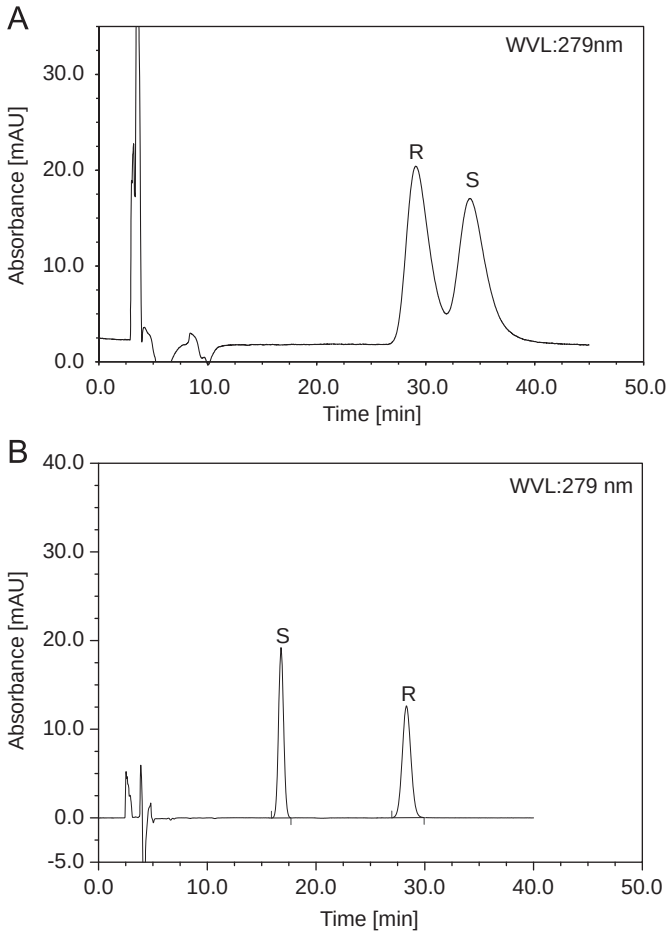

Fig. 2. Chromatograms of (*R*,*S*)-tamsulosin hydrochloride. A: cellulose tris(3,5-dimethylphenylcarbamate); Chiralcel OD-H, 250 × 4.6 mm, 5 μm; mobile phase: hexane–isopropyl alcohol–triethylamine, 80:20:0.2 v/v/v; flow rate 1.0 ml/min, λ=279 nm. B: amylose tris(3,5-dimethylphenylcarbamate); Chiralpak AD-H 250 × 4.6 mm, 5 μm; mobile phase: hexane–ethanol–ethanolamine 80:20:0.2, v/v/v; flow rate 1.2 ml/min; λ=279 nm.

order was *S* enantiomer before *R* (Fig. 2B). On amylose stationary phase both limit of detection (LOD) and limit of quantification (LOQ) were lower than on cellulose phase (Table 1). LOD and LOQ were estimated as the amounts for which signal-to-noise ratios were $S/N > 3$ and ≥ 10, respectively. The developed method was validated with respect to peak areas and retention times precision. Additionally influence of different flow rates (1.0–1.4 ml/min) on the precision of peak areas measurements and selectivity, sample stability within 72 h, intra-day precision for six different injections and inter-day precision for 3 consecutive days have been determined (robustness). It appeared that the method was characterized with good repeatability (RSD for peak areas and retention times were 1.09% and 0.12%, respectively), high intra- and inter-day precision (respective RSD values for peak areas 1.09% and 1.74% and for retention times 0.12% and 0.19%). Changing flow rate within $\pm$0.2 ml/min did not much influence resolution factor ($\Delta R_s < 5.6\%$).

(*R*)-tamsulosin content was determined in two medicinal products (Apo-Tamis and Omnic Ocas)—Table 3. In both formulations the analyte was retained at polymer matrix responsible for the active substance slow release. It was therefore necessary to develop a method enabling quantitative active substance recovery. The best method proved to be 90 min sonication and the extraction of active substance with ethanol (25 ml). An (*R*)-tamsulosin content in Apo-Tamis 0.4 mg capsules and Omnic

Table 3
(*R*)-tamsulosin determination in two medicinal products: Apo-Tamis and Omnica Ocas.

	Amylose tris(3,5-dimethylphenyl carbamate)		Cellulose tris(3,5-dimethylphenyl carbamate)	
	Apo-Tamis 0.4 mg	Omnic Ocas 0.4 mg	Apo-Tamis 0.4 mg	Omnic Ocas 0.4 mg
Content[a] (mg)	0.409 ± 0.003	0.388 ± 0.004	0.401 ± 0.008	0.391 ± 0.003
RSD (content) (%)	0.74	0.91	2.01	0.84
Recovery[b] (%)	97.66 ± 0.86	97.74 ± 1.13	97.81 ± 1.00	98.82 ± 1.25
RSD (recovery) (%)	1.15	1.51	1.33	1.64

n measurements with the confidence level 0.05.
[a] $n=6$.
[b] $n=9$.

Ocas 0.4 mg tablets was found to lie between 0.388 and 0.409 mg with RSD between 0.91% and 2.01%. Recovery measured in 9 tests within the range of 80%–120% (according to the International Convention of Harmonization rules [10]) was found to be between 97.66% and 98.82% with RSD 1.16–1.64% (Table 2). No (*S*)-tamsulosin enantiomer in the amount $\geq 0.2\%$ was found in both medicinal products within the available analysis conditions.

4. Conclusions

New chromatographic method for the enantiodifferentiation of (*R*,*S*)-tamsulosin and the determination of (*R*)- and (*S*)-tamsulosin contents was developed with the aid of amylose tris(3,5-dimethylphenylcarbamate) stationary phase. The method was compared to the known procedure of Tamsulosin enantioseparation on cellulose tris(3,5-dimethylphenyl carbamate) [8]. The new method offers much better resolution ($R_S=7.93$, as compared to 1.10, Fig. 1). Moreover, it is twice more sensitive than the previously recommended one [8].

Both methods were employed for the quantification of (*R*)- and (*S*)-tamsulosin content in prolonged release Apo-Tamis 0.4 mg hard capsules (Apotex Europe B.V) and Omnic Ocas 0.4 mg coated tablets (Astellas). The developed procedures are characterized by high precision, accuracy and recovery ($>97\%$) in spite of difficulties related to the tamsulosin recovery from pharmaceutical formulation's polymeric matrix. The results showed that (*R*)-tamsulosin (as compared to (*S*)-enantiomer) formed more stable solute-stationary phase complex on amylose tris(3,5-dimethylphenylcarbamate) than on cellulose tris(3,5-dimethylphenylcarbamate) stationary phase. Since the tested stationary phases differ in macromolecular structure-linear for cellulose and helical for amylase-the difference (with other parameters being similar-substituent type on cellulose and amylose molecules, chromatographic conditions) could be considered as the cause for the varying enantioselectivities and the difference in the solute-stationary phase complex stabilities of both phases [11].

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