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CHIRAL STABILITY-INDICATING HPLC METHOD FOR ANALYSIS OF DONEPEZIL IN PHARMACEUTICAL FORMULATIONS

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A stability indicating chiral high-performance liquid chromatographic (HPLC) method was developed and subsequently validated for the separation and simultaneous determination of S-(+)- and R(-)-donepezil hydrochloride (DP) in tablet products. Baseline resolution was achieved by using Chiralcel-OJ-H column with a mobile phase consisted of ethanol-n-hexane- triethylamine (20:80:0.3, v/v/v). The detection wavelength was 268 nm. Arotinolol was chosen as internal standard to guarantee a high level of quantitative performance. Chromatographic peak purity data of DP enantiomers using photodiode array detector indicated no co-eluting peaks with the main peaks of drugs, which demonstrated the specificity of the assay method for their estimation in presence of degradation products. Denepezil enantiomers and their drug products were exposed to thermal, photolytic, hydrolytic and oxidative stress conditions and the stressed samples were analyzed by the proposed method. The described method was linear over the range of 25 - 2500 ng / ml ($r_=$ 0.999) with detection limit of 10 ng/ml for both enantiomers. The recoveries of S-(+)- and R(-)-DP from tablets preparations ranged from 98.0 to 100.5 % and 98.0 to 100.8 %, respectively. The intra-day and inter-day precision and accuracy were evaluated by calculating the % RSD (n = 6) and the % error were found to be in the ranges of 0.58 - 1.29% and -1.07 - 1.04% for both enantiomers, respectively. The proposed method can be useful in the quality control of drug products.

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1. Introduction

Donepezile hydrochloride (DP), (±)-2-[(1-benzylpiperidine-4-yl)ethyl]-5,6 dimethoxyindan-1-one hydrochloride, which is commercially available as Aricept®, is a potent, selective, and reversible acetyl cholinesterase inhibitor both in vivo and in vitro and has been prescribed worldwide for the treatment of Alzheimer's disease [1]. It is the second drug approved by the U.S. Food and Drug Administration for the treatment of mild to moderate dementia of the Alzheimer's type. DP was demonstrated to be a potent and selective inhibitor of brain acetyl cholinesterase with fewer adverse effects than physostigmine and tacrine [2, 3]. It is marketed in tablet form for oral administration.

Pharmaceutical product quality is of vital importance for patient safety. The presence of impurities and potential degradation products can cause changing of chemical, pharmacological and toxicological properties of drugs having significant impact on product quality and safety. Drug stability is considered to be the secure way to ensure delivery of therapeutic values to the patients [4, 5].

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Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and enables recommendation of storage conditions, retest periods, and shelf lives to be established. The two main aspects of drug product that play an important role in shelf life determination are assay of active drug, and degradation products generated ,during the stability study. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines[6] and USP-26[7].

The nature of the stress testing will depend on the individual drug substance and the type of drug product involved. Although stability indicating methods have been reported for assay of various drugs and drug products [8].

Donepezil hydrochloride (DP) is 2,3-Dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]-methyl]-1*H*-inden-1-one hydrochloride)[9] is a potent, selective and reversible acetylcholine esterase inhibitor and has been prescribed worldwide of the treatment of Alzheimer's disease[10]. DP treatment for Down syndrome showed potential improvement of the symptom in non-randomized-controlled trial[11]. Donepezil is administrated in a racemic drug .The donepezil enantiomers have differing extents of inhibition against acetylcholine esterase in vivo and in vitro[10].

Several analytic HPLC methods for determination of donepezil have been applied. Yasui-Furukori et al [12] determined DP in human plasma with ultraviolet absorbance detection. An isocratic HPLC method with fluorescence detection at 390 nm with an excitation at 325 nm for determination of donepezil in human and rat plasma, blood and brain microdialysates samples was developed[13]. High-throughput liquid chromatography / tandem mass spectrometry (LC–MS/MS) method with an automated liquid-liquid extraction was developed for quantitative determination of donepezil in human plasma [14]. Enantioresolution analysis of DP was reported utilizing either avidin column [10] or capillary electrophoresis [15]. Determination of a centrally acting acetylcholine esterase inhibitor DP in rat plasma by liquid chromatography with fluorimetric detection set at 318 / 390 nm was described by Haginaka and Seyama[16]. Moreover, Radwan et al [17] developed stereoselective HPLC assay of donepezil enantiomers with UV detection and its application to pharmacokinetics in rats using Chiralcel OD column.

Various stability–indicating methods for determination of DP have been puplished. A specific spectrofluorimetric and derivatives spectrophotometric methods have been developed for assay of DP in presence of its oxidatative degradate in tablet form [18]. Also an isocratic HPLC stability-indicating assay of DP in tablets with C18 column and detection at 268 has been reported[19]. Whereas, Kafkala et al[20] used gradient HPLC technique to separate and quantity DP in presence of it impurities content in oral pharmaceutical formulation.

So far, to our present knowledge, no stability-indicating assay method for the determination of DP enantiomers is available in the literature and keeping into the view of susceptibility of donepzil enantiomers under variety of conditions. It was felt that an HPLC method that separates the drug enantiomers from their degradation products formed under ICH suggestion conditions [9] would be of great interest.

2. Experimental

2.1 Chemicals

Standard racemic donepezil HCl [Lot.No. 17090202] was obtained from [Pfizer PGM, France], S-(+)- and R(-)-enantiomer were purchased from Eisai Co. Ltd. (Tokyo, Japan). The internal standard arotinolol gifts from Sumitomo Pharmaceutical Co. (Osaka, Japan). n-Hexane and ethanol (HPLC grade) were purchased from BDH Chemicals (Poole, UK). Triethylamine was purchased from Fisher Scientific (Pittsburgh, PA, USA). The tablets were purchased from local market.

2.2 Chromatographic conditions

The HPLC system used was a Shimadzu HPLC (Kyoto, Japan) consisting of an LC-10 AT VP pump, a SPD-10A VP UV-Vis spectrophotometric detector, and a SCL-10A VP system controller. Data collection and integration was accomplished using LG computer. The analytical column used was a Chiralcel-OJ-H column (250 × 4.6 mm i.d. (MA, USA). The mobile phase consisted of ethanol-n-hexane- triethylamine (20:80:0.3, v/v/v), filtered through a Millipore filter membrane 0.45 μm from Nylon, Millipore (Yonezawa, Japan) Separation was carried out isocratically at ambient temperature (25 ± 1°C), at flow-rate of 1.0 ml/min, with UV set at 268 nm. The injection volume was 100 μl.

2.3 Preparation of standard stock solutions

Stock solutions containing 1 mg/ml of individual S-(+)- and R-(-)- DP hydrochloride were prepared in methanol on a free-base basis and corrected for salt and purity. The internal standard (IS) arotinolol was prepared in methanol to give a concentration of 1 mg/ml. The solutions were stable for at least three weeks if kept in the refrigerator.

2.4 Forced degradation studies

In order to establish whether the analytical method and the assay were stability-indicating, pure active pharmaceutical ingredient (API) of both DP enantiomers were stressed under various conditions to conduct forced degradation studies[21, 22]. As the two enantiomers are freely soluble and stable in methanol, so methanol was used as a co-solvent in all the forced degradation studies. All solution prepared for use in forced degradation studies, were prepared by dissolving API in small volume of methanol and later diluted with aqueous hydrogen peroxide, distilled water, aqueous hydrochloric acid or aqueous sodium hydroxide, to achieve a concentration of 1000 ng/ml of both enantiomers. Photo-degradation studies were performed in methanol. The solutions were exposed to sunlight during the daytime for 10 days. The resultant solutions were analysed every day, control samples which were protected from light with aluminum foil were also placed in the day light concurrently. For thermal stress, samples of drug substances were placed in a controlled-temperature oven at 60 ± 2°C for 10 days. And the resultant solution analysed every day.

2.5 Preparation of standard solutions of tablets

Two commercially formulations (Aricept® tablets) labeled to contain 5 or 10 mg DP were analyzed. Twenty tablets of each formulation were weighed. The tablets were ground to homogenous powder. A portion of the powder equivalent to one tablet content of DP was transferred into a 25 ml-volumetric flask. About 20 ml methanol was added the mixture was sonicated for 15 min. The flask was made up to volume with methanol. Aliquots of the solution was transferred to 20 ml volumetric flasks and made up to volume with mobile phase to yield concentration for each of enantiomers in the range of linearity previously described. The solution were filtered through millipore membrane filter (0.2 um) before injection.

2.6 Validation

Validation of the optimized method was done with respect to various parameters ,as required under ICH guidelines (18) using a set of calibration standards ranging in concentration from 25-2500 ng/ml, and sets of three standards quality control (QC) at concentration of 75 ng/ml (QC-low),1250 ng/ml (QC-intermediate) and 2000 ng/ml (QC-high) for each enantiomer.

2.7 Linearity

Aliquot volumes of the final solution of S-(+)- and R-(-)-DP were transferred to a series of 10 ml volumetric flasks to produce solutions covering the concentration range of 25 - 2500 ng/ml for each enantiomer, respectively. A volume equivalent to 500 ng/ml of arotinolol was added to each flask and the solution was diluted to 10 ml with methanol. Calibration standards of each concentration were analyzed in triplicate. Calibration curves of DP enantiomers were constructed using normalized drug/internal standard peak area ratio versus nominal concentrations of the analyte. Least squares linear regression analysis of the data gave slope, intercept and correlation coefficient data. From this data a first order polymonial model was selected for each analyte.

2.8 Recovery

A 100 µl of the selected assay solutions were injected into the HPLC system and the chromatograms recorded. The nominal contents of the drug in each solution were calculated from the linear regression equations. The percent recovery and the percent RSD were calculated.

2.9 Specificity

The specificity of the assay was checked by analyzing the stability study samples. The chromatograms of standard DP solutions were compared with chromatograms obtained by analyzing the stability study samples.

2.10 Precision and accuracy

The within-run and between-run precision (reported as %RSD) and accuracy (reported as % error) of the assay were determined by assaying three QC samples in triplicate over a period of 3 days. The concentration represented the entire range of the calibration curve. The regression equations were used to determine the concentration in QC samples.

2.11 Limit of detection and limit of quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined as 3 and 10 times the baseline noise, respectively (20)[23].

3. Results and discussion

3.1 Optimization of the chromatographic conditions

The chemical structures of S-(+)-DP, R-(-)-DP and artinolol (IS) are shown in Fig. 1. The HPLC method carried out in this study, aimed at developing a chromatographic system, capable of eluting and resolving DP enantiomers from their degradation products. The preliminary investigations were directed toward the effect of various factors on the system. The factors assessed include, the type of column and the composition of mobile phase. The samples were initially analyzed using a mobile phase consisting of isopropanol-hexane (20:80 v/v). Under this condition, no separation of DP enantiomers was achieved. Partial separation was observed when triethylamine was added to the mobile phase. In order to improve the peak shape, ethanol was used instead of isopropanol. Various percentages of ethanol, n-hexane and triethylamine were tested to achieve the optimum separation of the two enantiomers. Lowering the ethanol contents resulted in an increase in the chromatographic run. In the optimized method, the typical retention times of the internal standard, S-(+)-donepezil and R-(-)-donepezil were 7.0 ± 0.34 , 10.6 ± 0.44 and 14.4 ± 0.48 min as shown in Fig. 2. The system suitability parameters including retention time (t_r), separation factor (α), retention factor (k), resolution, are shown in Table I.

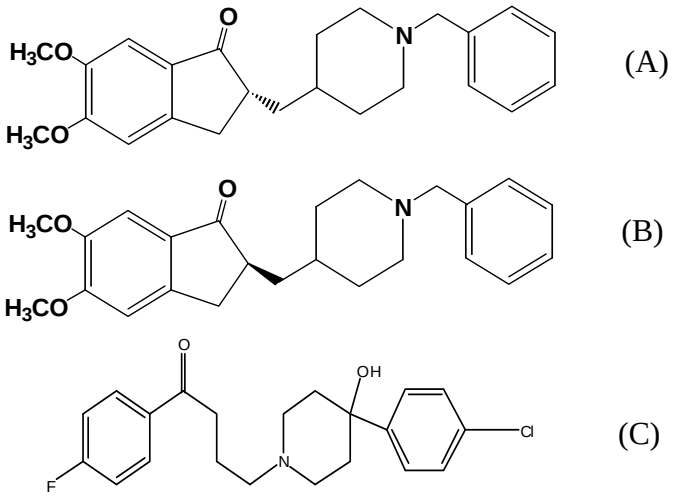

Fig. 1. The chemical structure of (A), S-(+)-donepezil (B), R-(-)-donepezil and (C) arotinolol (IS).

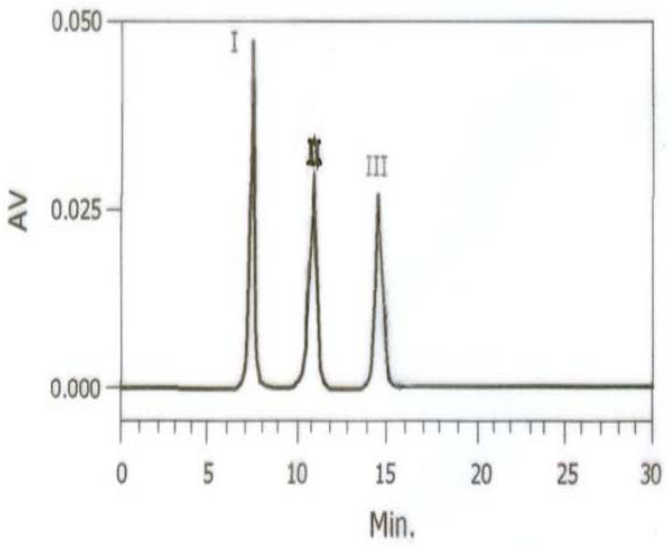

Fig. 2. Chromatogram of (I) arotinolol (IS), 5 µg/ml, (II) S-(+)-DP, 1000 ng/ml and (III) R-(-)-DP, 1000 ng/ml

Table 1. Chromatographic parameter data for donepezil enantiomers and internal standard (mean ± SD, n = 3).

Analyte	R_s[a]	k[b]	α[c]	t_R[d]
Arotinolol	-[e]	2.89±0.190	-[e]	7.00 ± 0.34
S-(+)-donepezil	1.93	4.91±0.20	1.69	10.64±0.44
R-(-)-donepezil	3.25	7.00±0.290	1.43	14.41±0.48

[a] Resolution factor, calculated as $R_s = (t_2-t_1)/0.5(w_1+w_2)$. Where t_2 and t_1 are the retention of second and first peaks, w_1 and w_2 are the peak width of first and second peaks.
[b] Capacity factor, calculated as $T_R - T_o / T_o$
[c] Separation factor, calculated as k_2/k_1
[d] Retention time
[e] Not calculated

3.2 Forced degradation studies

The HPLC studies of samples obtained on stress testing of DP enantiomers under different conditions suggested the following degradation behaviors (Table II). Complete degradation of S-(+)- and R-(-)-donepezil were found with either 1N HCl or 1N NaOH. Since, 0.1N of acid or alkali was used; the degradation studied was followed after 0, 10, 20, 30, 60, 90, 120, 240 min. and after 48 h. Through analyzed of the degraded samples against control sample by proposed HPLC technique. DP enantiomers were found to be stable under acidic condition up to 240 min. Only around 8% of the DP enantiomers was degraded through 48h and the main analytes were seen at 10.60 and 14.40 min during HPLC analysis (Fig.3,b). Whereas, alkaline stress conditions resulted in 14% decomposition of the DP enantiomers (Fig 3,c) as compared to the standard solution of the drug (Fig.3,a). The drug enantiomers were complete degraded when utilized 30 % H_2O_2 but falling by 28% when used 3% H_2O_2 through 48 hr. (Fig.3,d). Under heat stress condition (60 ^oC) S-(+)- and R-(-)-donepezil were found to be largely unstable and falling by 60% (Fig.3,f). Moreover, slightly degradation was observed on exposure of solid drug powder to light (Fig.3,e). The linear regression analysis of DP enantiomers in pure solution was constructed by plotting the peak area ratio of each enantiomer to the internal standard (*y*) versus analyte concentration in ng/ml (*x*).

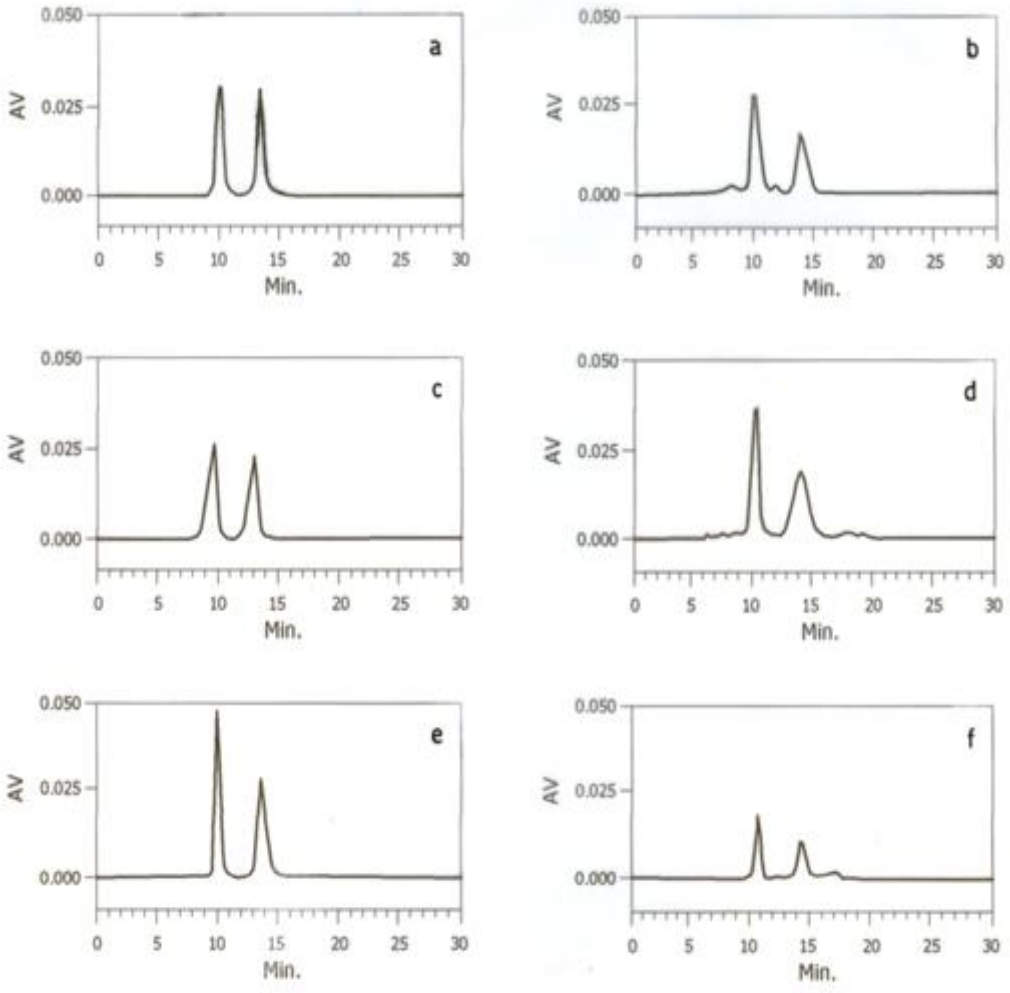

Fig. 3. Typical HPLC chromatograms of DP enantiomers, (a) pure bulk sample, (b) in 0.1 N HCl after 48 h, (c) in 0.1 N NaOH after 48 h, (d) in 3% H_2O_2 after 48 h. (e) under UV light after 10 d, (f) at 60°C after 10 d.

Table 2. Summary of forced degradation results.

Stress condition	Time	% Assay of remaining active substance	
		S-(+)-DP	R-(-)-DP
Acid hydrolysis 0.1 N HCl at RT	48 h	93.590%	92.980%
Base hydrolysis 0.1 Na OH at RT	48 h	86.380%	85.240%
Oxidation 3% H_2O_2 at RT	48 h	77.00%	76.30%
Light (photolytic degradation)	10 d	98.29%	97.96%
Thermal (60°C bulk drug)	10 d	40.00%	39.50%

3.3 Validation

3.3.1 Linearity and sensitivity

The calibration curves were linear in the range of 25 – 2500 ng/ml, with a correlation coefficient (r) of 0.999 for both enantiomers (Table III). A typical calibration curve has the regression equation of $y = 0.2643\ x - 0.0047$ for S-(+)-DP and $y = 0.2616\ x - 0.0045$ for R-(-)-DP. The limit of detection (LOD) and the limit of quantitation (LOQ) for each enantiomer were 10 ng/ml and 25 ng/ml, respectively (Table 3).

Table 3. Validation parameters for the determination of donepezil enantiomers using the proposed method.

Parameters	S-(+)-donepezil	R-(-)-donepezil
Concentration range ng/ml	25 – 2500	25 - 2500
Intercept (a)	0.0047	0.0045
Slope (b)	0.2643	0.2616
Correlation coefficient (r)	0.999	0.999
$S_{y/x}$	0.0024	0.0023
S_b	0.0009	0.0009
LOQ (ng/ml)[a]	25	25
LOD (ng/ml)[a]	10	10

[a] Average of six determinations

3.3.2 Accuracy and precision

The results of the statistical analysis of the experimental data, such as the slopes, the intercepts and the correlation coefficients obtained by the least squares treatment of the results along with standard deviation of the slopes and intercepts on the ordinate and the standard deviation of the residuals were shown in Table 3. The within-day precision and accuracy (n = 6) as expressed by percentage RSD and percentage error were 0.66-1.24% and 0.40-0.80, respectively, for S-(+)-DP, 1.29-1.76 and 0.48-0.93% for R-(-)-DP, respectively. The between-day precision and accuracy (n = 6) expressed by percentage RSD and percentage error were 0.56-2.15 and 1.04-2.50%, respectively for S-(-)-DP and 0.85-1.27 and 0.08-1.50 % for R-(-)-DP, respectively, (Table IV).

Table 4. Accuracy and precision data for donepezil HCl enantiomers.

Analyte	Actual concentration (ng/ml)	Experimental concentration (ng/ml)	Error %	RSD %
Within-day[a] S-(+)-donepezil	75	74.40 ±0.96	-0.80	1.29
	1250	1240 ± 12.56	-0.80	1.01
	2000	1990.50±11.49	0.48	0.58
R-(-)-donepezil	75	74.30 ±0.96	-0.93	1.29
	1250	1243.33±12.86	-0.53	1.03
	2000	1993.67±11.06	-0.32	0.55
Between-day[b] S-(+)-donepezil	75	74.20 ± 0.95	-1.07	1.28
	1250	1261.67±12.13	1.04	0.96
	2000	2005 ±11.50	0.25	0.57
R-(-)-donepezil	75	74.40 ±0.94	-0.80	1.26
	1250	1240 ±12.46	-0.80	1.00
	2000	2003 ±11.67	0.15	0.58

[a,b] Mean ± SD based on n = 6.

Table 5. Determination of the DP enantiomers content in pharmaceutical dosage forms.

Dosage forms	Parameter			
	Actual concentration (ng/ml)	Experimental concentration (ng/ml)	Content %	RSD %
Aricept® 5 mg[a]				
	500	490±19.50	98.00%	3.98
S-(+)-donepezil	1000	990±13.80	99.00%	1.39
	2500	2510±6.87	100.40%	0.27
	500	490±19.50	98.00%	3.98
R-(-)-donepezil	1000	980±13.45	98.00%	1.37
	2500	2520±6.75	100.80%	0.27
Aricept® 10 mg[b]				
S-(+)-donepezil	500	495±19.35	99.00%	3.40
	1000	995±13.65	99.50%	1.37
	2500	2511±6.72	100.44%	0.27
R-(-)-donepezil	500	493±19.38	98.60%	3.93
	1000	985±13.50	98.50%	1.37
	2500	2520±6.75	100.80%	0.27

[a,b] Products of Pfizer PGM, France. [Lot. No. 17090202]

3.3.3 Application of the proposed method

The validity of the method developed here was applied to various concentrations taken from the pharmaceutical formulations (Aricept 5 mg & 10 mg tablets) for determining their content of DP enantiomers. The values of the overall drug percentage recoveries and the %RSD values of S-(+)- and R-(-)-DP are presented in Table V, indicating that these values are acceptable and the method is accurate and precise.

4. Conclusion

A highly specific stability-indicating chiral HPLC assay method was developed for the quantition of donepezil enantiomerrs in presence of their degradation products.The enantioseparation was carried out by the use of cellulose – based Chiralcel OJ-H column. The total run time for the developed method is 15 min, which allows processing of over 96 samples per day. This method has provided good sensitivity and excellent precision and reproducibility.

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References

[1] H. Sugimoto, Y. Tsuchiya, H. Sugumi, K. Higurashi, N.Karibe, Y. Iimura, A. Sasaki, Y. Kawakami, T. Nakamura, S. Araki, Y. Yamanishi, K. Yamatsu, K, J. Med.Chem. **33**, 1880(1990).

[2] T. Kosasa, Y. Kuriya, K.Matsui, Y. Yamanishi, (2000) Europ. J. Pharmacol. **389**, 173(2000).

[3] T. Yoshida, S. Ha-Kawa, K. Nobuhara, M. Yoshimura, T.Minami, A. Nakadaira, M. Suzuki, H. Oda, T. Kinoshita, S. Sawada, Intern. Congress Series **1232**, 745, Vols. 1 and 2(2002).

[4] S. Ahuja, K.M. Alsante, (ed.), Handbook of Isolation and Characterization of Impurities in Pharmaceuticals, Academic Press, San Diego, USA, **2003**

[5] FDA. Guidance for Industry: Impurities in Drug Product, Draf guidance, Center for Drug Evaluation and Research (CDER), (1998).

[6] ICH, October, Q1A Stability Testing of New Drug Substances and Products, in proceeding of the International conference on Harmonization, Geneva, Switzerland. (1993)**.**

[7] The united states pharmacopoeia, 26th ed., US, pharmacopoeial convention, Rockville, MD, USA. **2003**, p. 1151

[8] M. Bakshi, B. Singh, A. Singh, S. Singh, J. Pharm. Biomed. Anal. **26,** 891(2**001)**

[9] The Merck Index, An Encyclopedia of Chemicals, Drugs and Biological, 13th ed., Merck & Co. Inc. , (2001).

[10] K. Matsui, Y. Oda, H. Nakata, T. Yoshimura J. Chromatogr. B **729**, 147(1999).

[11] T. Lott, K. Osann, E. Doran, L. Nelson, Arch. Neurol. **59**, 1133(2002).

[12] N. Yasui-Furukori, R.Furuya, T.Takahata, T. Tateishi J. Chromatogr. B **768**, 261 **(**2002).

[13] K. Nakashima, K. Itoh, M. Kono, N. Nakashima, M. Wada, J. Pharm. Biomed. Anal. **41**, 201, (2006)

[14] Apostolou, C., Dotsikas, Y., Kousoulos, C. , L. Loukas J. Chromatogr. B **848,** 239 (2007).

[15] R. Gotti, V. Cavrini, R.Pomponio , V. Andrisano , J. Pharm. Biomed. Anal. **24**, 863 (2001).

[16] J. Haginaka, C. Seyama, J. Chromatogr. **577**, 95 (1992**)**.

[17] M. Radwan, H. Abdine, B. AL-Quadeb, and H. Aboul-Enein, J. Chromatogr. Biomed. Appl. **830**, 114 (2006)**.**

[18] S. Abbas, Y. Fayez , L. Abdel Fattah Chem. Pharm. Bull. **54**, 1447 (2006).

[19] H. Pappa, R. Farru, P. Vilanova, M. Palacios and M. Pizzorno, J. Pharm. Biomed. Anal., **27**, 177(2002).

[20] S. Kafkala, S. Matthaiou, P. Alexaki, M. Abatzis, A. Bartzeliotis , M. Katsiabani, J. Chromatogr. A **1189**, 392 , (**2008)**.

[21] ICH, March. Q2B Validation of Analytical Procedures: Methodology, in: proceeding of the International conference on Harmonization, Geneva, Switzerland, (1996).

[22] ICH, Guidance on Analytical Method Validation, proceedings of International conference on Quality for the Pharmaceutical Industry, Toronto, Canada, (2002).

[23] J.N. Miller , J. Miller (ed.), Statistics and Chemometrics for Analytical Chemistry, 5th, Pearson Education Ltd, England, (2005)**.**