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A new HPLC method to determine Donepezil hydrochloride in tablets

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Abstract

A HPLC stability-indicating assay for Donepezil hydrochloride in tablets was developed and validated. Donepezil hydrochloride is a reversible inhibitor of acetylcholinesterase, indicated for the treatment of mild to moderate dementia of the Alzheimer's type. The HPLC method was performed with a reversed phase C18 column, detection at 268 nm and a mixture of methanol, phosphate buffer 0.02 M and triethylamine (50:50:0.5) as mobile phase. Typical retention time for Donepezil was 9 min. The method was statistically validated for linearity, accuracy, precision and selectivity following ICH recommendations. Due to its simplicity and accuracy, the method can be used for routine quality control analysis.

Keywords: Donepezil hydrochloride; Assay; HPLC; Validation

1. Introduction

Donepezil hydrochloride {2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1H-inden-1-one hydrochloride} (DH) is a reversible inhibitor of acetylcholinesterase and it exerts its therapeutic effect by increasing acetylcholine concentrations and enhancing cholinergic function. It is indicated for the treatment of mild to moderate dementia of the Alzheimer's type [1].

All analytical methods found in literature are used to determine DH in human plasma [2–5]. This report describes the development and validation of a stability-indicating method for the assay of DH in tablets.

2. Experimental

2.1. Instrumentation

The HPLC system consisted of a dual-piston reciprocating Spectra Physics pump (Model ISO chromatographic LC pump), a Rheodyne injector (model 7125, CA), a UV-Vis Hewlett-Packard

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detector (model 1050, Japan) and a Hewlett-Packard integrator (series 3395, CA).

2.2. Materials and reagents

Donepezil hydrochloride was donated by Laboratorios Kampel Martian (Buenos Aires, Argentina) and it was recrystallized in our laboratory to obtain a working standard. Solvents were HPLC grade and passed through a 0.45 micron membrane filter.

Two local commercial tablets formulations were analyzed. Both lots contained Donepezil hydrochloride (5 mg) in a matrix of lactose, microcrystalline cellulose, sodium starch glycolate, polyvinylpyrrolidone K90, magnesium stearate and colloidal silicon dioxide.

2.3. Chromatographic conditions

Chromatography was performed on a Microsorb-MV RP-18, 5 μm (Varian, Catalog No. R00862005).

The mobile phase was a mixture of methanol, 0.02 M buffer phosphate and triethylamine (50:50:0.5). Buffer phosphate was prepared by dissolving 13.8 g of monobasic sodium phosphate in 900 ml of water, mixing with 10 ml of triethylamine and adjusting to pH 2.7 ± 0.5 with phosphoric acid. The mobile phase was filtered through a nylon membrane (pore size 0.45 μm) and degassed before use. Chromatography was performed at room temperature using a 1.0 ml/min flow rate and a 15 min run time. In these conditions, DH retention time (t_R) was roughly 9 min. The injection volume was 20 μl and ultraviolet detection was at 268 nm (2 aufs).

2.4. Working solution

A working solution of DH (0.04 mg/ml) was prepared in mobile phase.

2.5. Sample solution

Thirty tablets were weighed and crushed to fine powder. Powder samples, equivalent to 25 mg of DH, were placed in a 25-ml volumetric flask. Mobile phase (20 ml) were added and the mixture was sonicated for 5 min. The mixture was then diluted to 25 ml with mobile phase, thoroughly mixed and filtered through Whatman No 42 paper. Then, 1.0 ml of the filtered preparation was transferred to a 25 ml volumetric flask and diluted to volume with mobile phase. Prior to injection, all the samples were passed through a 0.45 micron membrane filter.

2.6. Sample preparations for assay validation

Six solutions were prepared in mobile phase at concentrations ranging from 10 to 60 μg/ml, to study system linearity response.

System precision was evaluated by performing six consecutive injections of DH standard solution. Method precision was evaluated by six repeated assays of the same lot of two commercial formulations. Accuracy was assessed at 80, 100 and 120% of DH by recovery experiments, applying the assay method to a mixture of matrix components to which known amounts of the standard have been added.

Forced degradation studies were performed to provide an indication of the stability-indicating properties and specificity of the procedure. Degradation samples were prepared by transferring $\approx$ 25 mg of DH into 25 ml volumetric flasks. Intentional degradation was attempted using acid, base, hydrogen peroxide and light. After completing degradation treatments, samples were allowed to cool at room temperature and prepared according to assay sample solution, after neutralization when required. Samples were analyzed against a control sample (lacking degradation treatment).

2.7. Procedure

Prior to injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Acceptable results for the number of theoretical plates, tailing factor and precision, calculated using USP 24 equations [6] and detector linearity criteria were required before sample analysis. Quantification was accomplished using the external standard method. Each solution was injected in triplicate

Table 1
Degradation of DH

Condition	Time (h)	% Recovery	RRT* of degradation products
Acid 1 N HC1, ref.	4	98.9	0.26, 0.52, 0.58
Base 1 N NaOH, ref.	4	54.2	0.26, 0.84
H_2O_2 100 vol. ref.	4	51.5	0.26, 0.28, 0.34, 0.39, 0.44, 0.57, 0.63, 0.71
Daylight	24	100	None detected

* RRT, relative retention time; ref. refluxed.

and the R.S.D. was required to remain below 0.7% on DH peak area basis.

3. Results and discussion

3.1. System suitability

A system suitability test was defined based on the results obtained in several representative chromatograms. The column efficiency determined from the analyte peak >2000, the tailing factor <1.7 and the R.S.D. for six injections was $<1.0\%$.

3.2. Selectivity

Using the assay chromatographic conditions described, DH showed degradation products after oxidation, alkaline and acid hydrolysis. The percentages of DH recovered are shown in Table 1. Degradation peaks, where observed and resolved from DH peak (Fig. 1).

Table 2
Linearity data

% w/w	Injected (μg)	Average peak area response	RSD (%)
25	0.21	1313829	0.9
50	0.42	2666984	0.0
75	0.63	3824890	0.3
100	0.84	5211506	0.3
125	1.05	6384827	0.6
150	1.26	7634922	0.4

Slope[a] $= 1.10^6 \pm 148604$
Intercept[b] $= 91599 \pm 121679$

[a] Confidence limits of the slope ($P = 0.05$).
[b] Confidence limits of the intercept ($P = 0.05$).

Neither formulation ingredients nor degradation products interfered with DH quantification. No evidence of interactive degradation products was seen during evaluation. The method was reproduced in regular laboratory temperature (22–25 °C) and selectivity was not affected.

3.3. Linearity

Six solutions containing DH at concentrations ranging from 10 to 60 μg/ml were analyzed. The peak area versus concentration curve proved to be linear (Fig. 2). The regression line equation calculated by least-squares method was $Y = 6 \times 10^6 X + 91599$ with a coefficient of correlation $r = 0.9995$ while intercept values were not significantly different from zero, ($P = 0.05$) (Table 2).

Microsoft Excel software was used to plot the peak areas versus micrograms injected.

3.4. Accuracy

Recovery data obtained from the study of a mixture of DH and matrix components to which known amounts of the standard have been added ranged from 98.7 to 101.4% with a mean value of 100.1% ($n = 9$) and R.S.D. of 0.7 (Table 3).

The mean t value versus the true value with 95% confidence shows that the experimental average was not significantly different from the true value (t_{n-1}; α: 0.05) of 2.36, for 8 dof for each lot.

Method accuracy was demonstrated by plotting the amount of DH found (expressed in milligrams) against the amount present. Linear regression analysis rendered a slope not significantly different from 1 (t-test, $P = 0.05$), an intercept not significantly different from zero (t-test, $P = 0.05$) and $r = 0.998$.

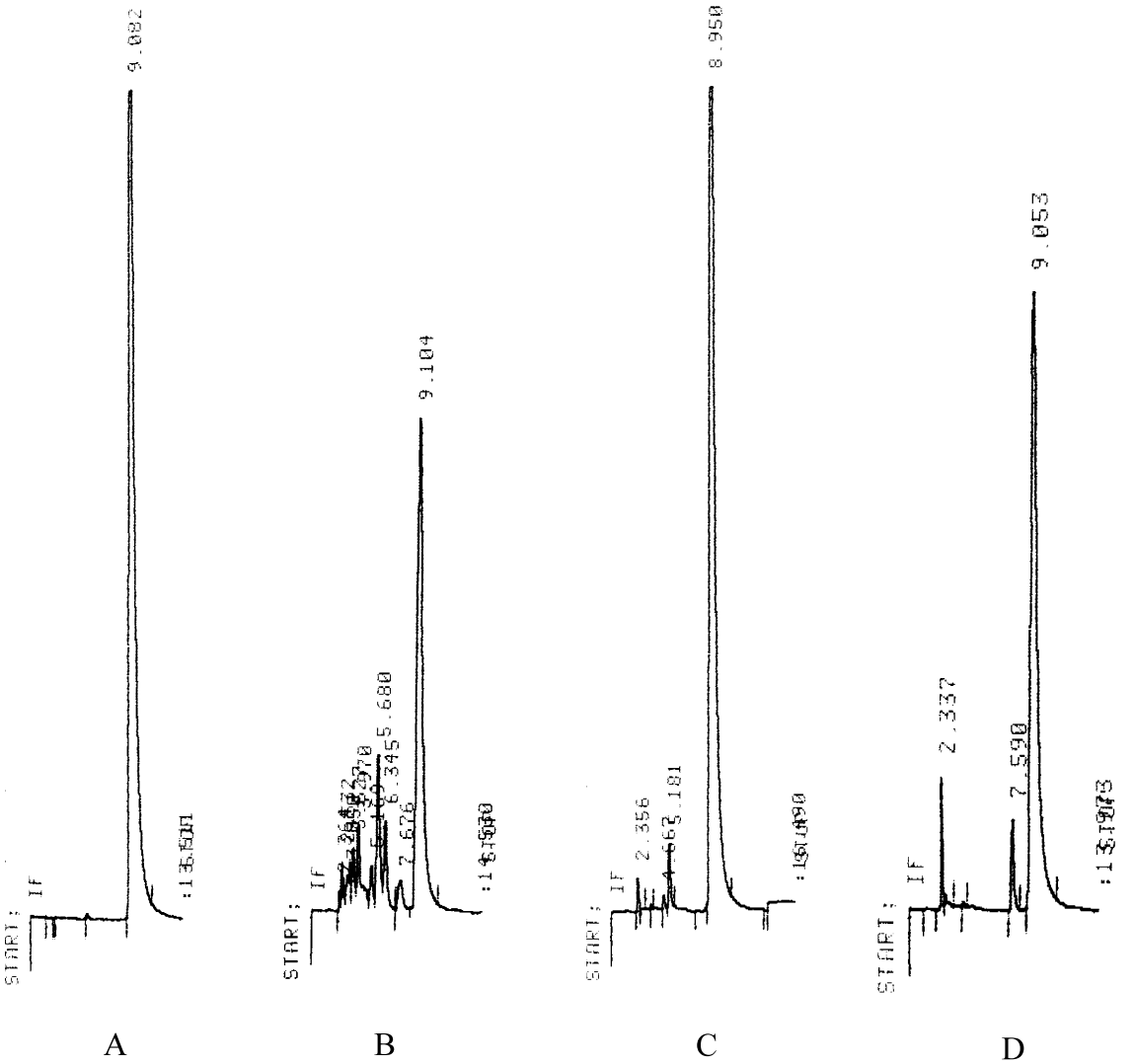

Fig. 1. Chromatograms of Donepezil standard (A); oxidative degradation (B); acid degradation (C); alcaline degradation (D).

DH recovery achieved shows that there was no interference from the excipients present in the tablets.

3.5. Precision

Precision was considered at two levels according to ICH recommendations [7]: repeatability and intermediate precision. Repeatability was evaluated by analyzing six replicate injections of DH reference solution, giving a R.S.D. of 0.5%. Intermediate precision was determined by carrying out two accuracy assays on the same lot of the commercial formulations the same day, by the same operator with different equipment (Table 4). The *t*-test comparing the two sample means with 95% confidence for 10 dof showed that both results were not significantly different. In addition, two lots of commercial formulations were assayed 1 week apart by two different operators with the same equipment (Table 5). For each assay, the results were as follows: mean values 101.3 and 100.1%, S.D. 0.5 and 0.7 and R.S.D. 0.6 and 0.7%. Test *F* comparing the two sample S.D. with 95% confidence for 11 dof showed that both results were not significantly different.

Table 3
Recovery analysis

% w/w	Amount present (mg)	Amount found (mg)	Recovered (%)	Average recovered ($n = 2$)	RSD (%)
80	20.8	21.1	101.4		
	20.5	20.5	100.1	100.8	0.7
	20.6	20.8	100.8		
100	24.3	24.3	100.1		
	26.5	26.3	99.5	99.5	0.7
	25.3	25.0	98.7		
120	30.3	30.0	99.0		
	28.5	28.7	101.0	100.0	1.0
	29.1	29.1	99.0		
Means ($n = 9$)				100.1	0.7

Accuracy acceptance criteria, 98.0–102.0; precision acceptance criteria, 3% within each level.

Table 4
Precision of the assay method

Equipment 1			Equipment 2		
Sample No.	DH (%)	R.S.D. (%)	Sample No.	DH (%)	R.S.D. (%)
1	99.9	0.2	1	99.8	0.2
2	99.1	0.3	2	99.0	0.3
3	100.3	0.1	3	100.1	0.1
4	101.0	0.1	4	98.9	0.1
5	99.6	0.1	5	99.8	0.1
6	100.8	0.4	6	98.5	0.4
Mean	100.1	0.7	Mean	99.3	0.6

Table 5
Precision of the assay method

Lot 1			Lot 2		
Sample No.	DH (%)	R.S.D. (%)	Sample No.	DH (%)	R.S.D. (%)
1	100.6	0.2	1	99.9	0.2
2	100.9	0.2	2	99.1	0.3
3	101.8	0.1	3	100.3	0.1
4	101.2	0.1	4	101.0	0.1
5	101.9	0.1	5	99.6	0.1
6	101.5	0.1	6	100.8	0.4
Mean	101.3	0.6	Mean	100.1	0.7

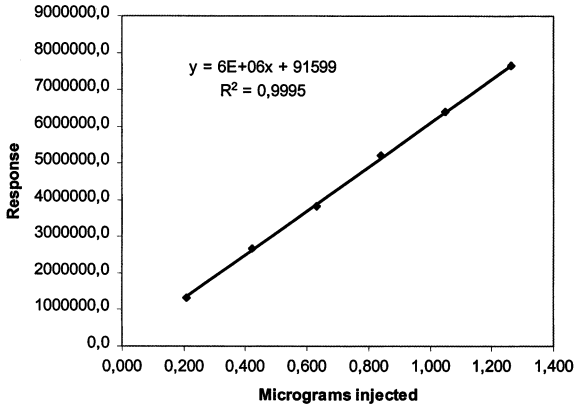

Fig. 2. Linearity.

3.6. Stability of sample preparation

Sample solution injected after 1 week failed to show any appreciable change.

4. Conclusions

The linearity of DH peak area responses was demonstrated from 25 to 150% of the 0.04 mg/ml working analytical concentration by a correlation coefficient of 0.9995. The precision of DH chromatographic response was calculated from six replicate injections of the same solution prepared at the nominal analytical concentration and showed a R.S.D. of 0.5%.

According to recovery studies performed at 80, 100 and 120% of the analytical concentration, the extraction of the active component was shown to be quantitative. Selectivity was demonstrated showing that the DH peak was free of interference from degradation products, indicating that the proposed method can be used in a stability assay. The proposed RP-HPLC method is simple, precise, rapid and selective for the determination of DH and may be employed for its assay in dosage formulations.

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