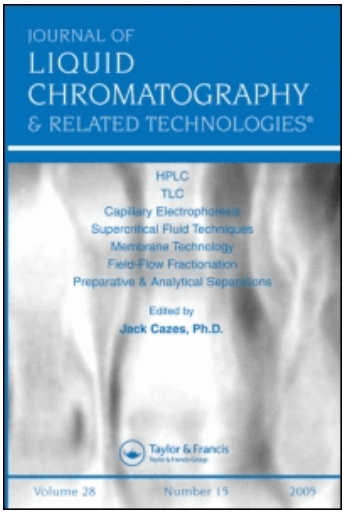

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

EXPERIMENTAL DESIGN STRATEGIES IN LC METHOD DEVELOPMENT AND IN ROBUSTNESS TESTING FOR REVERSIBLE CHOLINESTERASE INHIBITOR RIVASTIGMINE IN PHARMACEUTICAL FORMULATION

Mohammad H. Semreen[a]; Hassan Y. Aboul-Enein[b]
[a] College of Pharmacy, University of Sharjah, Sharjah, United Arab Emirates [b] Pharmaceutical and Medicinal Chemistry Department, National Research Centre, Dokki, Cairo, Egypt

Online publication date: 29 December 2009

To cite this Article Semreen, Mohammad H. and Aboul-Enein, Hassan Y.(2010) 'EXPERIMENTAL DESIGN STRATEGIES IN LC METHOD DEVELOPMENT AND IN ROBUSTNESS TESTING FOR REVERSIBLE CHOLINESTERASE INHIBITOR RIVASTIGMINE IN PHARMACEUTICAL FORMULATION', Journal of Liquid Chromatography & Related Technologies, 33: 2, 191 — 201
To link to this Article: DOI: 10.1080/10826070903439317
URL: http://dx.doi.org/10.1080/10826070903439317

Journal of Liquid Chromatography & Related Technologies, 33:191–201, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903439317

EXPERIMENTAL DESIGN STRATEGIES IN LC METHOD DEVELOPMENT AND IN ROBUSTNESS TESTING FOR REVERSIBLE CHOLINESTERASE INHIBITOR RIVASTIGMINE IN PHARMACEUTICAL FORMULATION

Mohammad H. Semreen[1] and Hassan Y. Aboul-Enein[2]

[1]*College of Pharmacy, University of Sharjah, Sharjah, United Arab Emirates*
[2]*Pharmaceutical and Medicinal Chemistry Department, National Research Centre, Dokki, Cairo, Egypt*

□ *An isocratic, reversed phase liquid chromatographic (RPLC) method was developed for the quantitative determination of rivastigmine hydrogen tartrate, in bulk drugs and in pharmaceutical formulation. The chromatographic separation was achieved on A Vertex Hypersil reversed phase C_{18} column (25 cm × 4.6 mm i.d., particle size 5 μm) using acetonitrile and phosphate buffer. The procedure was validated by linearity (correlation coefficient = 0.9983), accuracy, robustness, and intermediate precision. An experimental design was used during validation to calculate method robustness and intermediate precision. For the robustness test, three factors were considered: percentage v/v of acetonitrile, flow rate, and temperature; an increase in flow rate results in decrease of the concentration of the drug from the expected value, while the percentage of organic modifier and temperature have no effect on the response. For intermediate precision measurement, the considered variables were analyst, equipment, and days. The RSD value (1.10%, n = 24) indicate a good precision.*

Keywords drug analysis, experimental design, high performance liquid chromatography, rivastigmine

INTRODUCTION

Rivastigmine-[(1S)-1-dimethylaminoethyl]phenyl]N-ethyl-N methylcarbamate, Figure 1, is a parasympathomimetic and a reversible cholinesterase inhibitor. Rivastigmine is indicated for the treatment of mild to moderate dementia of the Alzheimer's type. Pathological changes in dementia of the Alzheimer type involve cholinergic neuronal pathways that project from

Correspondence: Professor Hassan Y. Aboul-Enein, Pharmaceutical and Medicinal Chemistry Department, National Research Centre, Dokki, Cairo 12311, Egypt. E-mail: enein@gawab.com; Dr. Mohammad H. Semreen, College of Pharmacy, University of Sharjah, P. O. Box 27272, United Arab Emirates Sharjah, UAE. E-mail: msemreen@sharjah.ac.ae

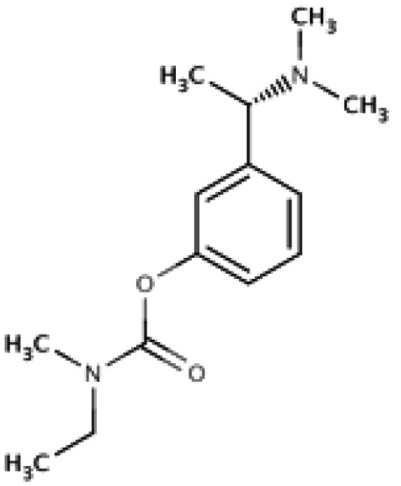

FIGURE 1 Chemical structure of rivastigmine.

the basal forebrain to the cerebral cortex and hippocampus. These pathways are thought to be intricately involved in memory, attention, learning, and other cognitive processes.[1,2] While the precise mechanism of rivastigmine action is unknown, it is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by cholinesterase. If this proposed mechanism is correct, the rivastigmine effect may lessen as the disease process advances and fewer cholinergic neurons remain functionally intact.[3]

Few HPLC methods were reported in the literature for the quantitative determination of Rivastigmine and its major metabolite with atmospheric pressure chemical ionization tandem mass spectroscopy[4]and for the determination of the dissociation constants of basic acetyl cholinesterase inhibitors[5] and reversed phase with UV detection.[6]

A gas chromatographic-mass spectrometric (GC-MS) method which was developed in house and validated according to the guidelines for analytical method establishment was used to determine rivastigmine and its major metabolite in toxicokinetic, preclinical studies with a limit of quantitation (LOQ) of 0.200 ng mL^{-1}.[7]

This paper describes a rapid and sensitive HPLC determination method with UV detection, useful for routine control of revastigmine in pharmaceutical formulation and in pharmacokinetic studies. The method was validated by linearity, accuracy, precision, and robustness. An experimental design was used during validation to evaluate method robustness and for the determination of intermediate precision.

EXPERIMENTAL

Apparatus

Different HPLC systems were used at the two laboratories involved in this study. The specifics are provided below.

Lab. A: the HPLC 1 apparatus was a Merck Hitachi chromatographic system pump (L-6200A) equipped with a suptumless injector (Rheodyne 7725). An UV detector (L-4000A) was used. Peak area integration was performed using a chromatographic data system (PE NELSON 1022 HPLC system manager program). A Vertex Hypersil reversed-phase C_{18} column (25 cm × 4.6 mm i.d., particle size 5 μm) was utilized.

Lab. B: the HPLC 2 apparatus, a Waters chromatographic system pump (Waters 510 HPLC) equipped with a septumless injection (Rheodyne 7725), and a UV detector (Waters 486 Tunable Absorbance Detector) was used.

Peak area integration was performed using a chromatographic data system (Waters 746 Data Model). A Vertex Hypersil reversed-phase C_{18} column (25 cm × 4.6 mm i.d., particle size 5 μm) was used.

The experimental design and statistical analysis of the data was performed, by Nemrod software[8] (LPRAI, Marseille, France).

Reagents

Acetonitrile HPLC grade was purchased from Scharlau (Barcelona, Spain). Water used in the mobile phase was deionized, distilled, and filtered through a 0.45 μm Millipore filter (Sartorius, Germany) under vacuum before use.

Di-sodium hydrogen phosphate (Panreac, Barcelona, Spain) was analytical grade. Exelon® 1.5 mg capsules and rivastigmine reference standard was supplied by the Quality Control Laboratories, Ministry of Health, (Amman – Jordan).

Preparation of Standard/Sample Solutions

The preparation of standard/sample solutions was prepared by weighing the required amount of rivastigmine into a 100 mL volumetric flask. The substances were dissolved and diluted to volume with the mobile phase.

Calibration Curve

Five solutions at five different concentrations were prepared by dissolving the amount of rivastigmine in mobile phase. The final concentrations

of rivastigmine were 0.400, 0.488, 0.556, 0.648, 0.772 mg mL^{-1}, respectively. Before injecting solutions, the column was equilibrated for at least 30 min with the mobile phase. Six determinations were carried out for each solution. Peak areas were recorded for all the solutions. The correlation graph was constructed by plotting the peak areas obtained at the optimum wavelength of detection versus the injected amounts.

Chromatographic Conditions

The mobile phase was a mixture of acetonitrile and 0.01 M di-sodium hydrogen phosphate pH 8.4, (70:30, v/v). The flow rate was 1.3 mL min^{-1}. The UV detector wavelength was set at 214 nm and an attenuation of 2.5 a.u.f.s was used.

RESULTS AND DISCUSSION

A chromatogram of rivastigmine is shown in Figure 2. The substance is well resolved with retention time of 5.89 min. by using the selected chromatographic conditions. The method was validated statistically for its linearity, accuracy, robustness, and precision.

Linearity

The linearity of peak area response versus concentrations was studied from 0.400 to 0.772 mg mL^{-1} for rivastigmine. A linear response was observed over the examined concentration range. Table 1 summarizes the correlation coefficient, slope, and intercept.

Accuracy and Repeatability

Accuracy was studied using simulated preparations at three different concentrations, corresponding to 0.400, 0.556, and 0.772 mg mL^{-1} of rivastigmine. Recovery data obtained were within the range 99.15–100.35% and RSD was 0.53% (Table 2), satisfying the acceptance criteria for the study.

Repeatability

The system repeatability was assessed from twelve replicate injections of a sample solution of rivastigmine at the analytical concentration of ~0.772 mg mL^{-1}. The RSD% found was 0.42% for the active ingredient (Table 3).

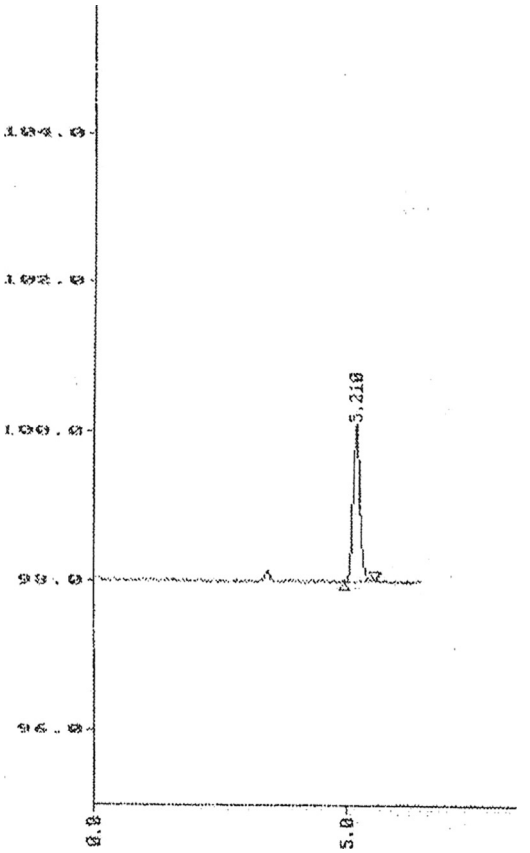

FIGURE 2 Chromatogram of a solution containing rivastigmine. Chromatographic conditions are described in Experimental section.

Robustness Testing

As defined by the ICH, The robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variations in method parameters.[9] Table 4 shows the optimized chromatographic conditions and the variations deliberately made. In order to study the

TABLE 1 Calibration Curve of Rivastigmine

Concentration ($mg\,mL^{-1}$)	Area	Slope	Intercept	r^2
0.400	16373	417.92	−392.21	0.9983
0.488	19702			
0.556	23222			
0.648	26573			
0.772	31861			

TABLE 2 Accuracy and Recovery for Rivastigmine

Concentration ($mg\,mL^{-1}$)	n	Recovery (%)	RSD (%)
0.400	4	99.86	0.32
0.556	4	100.35	0.78
0.772	4	99.15	0.51
Mean		99.79	0.54

simultaneous variation of the factors on the considered responses, a multivariate approach using a design of the experiments is recommended in robustness testing.[10]

A response surface method was carried out to obtain more information and to investigate the behavior of the responses around the nominal values of the factors. Response surface methodology (RSM) has the following advantages: (a) to allow a complete study where all interaction effects are estimated; (b) to give an accurate description of an experimental region around a center of interest with validity of interpolation.[11–15] Generally, the large number of experiments required by standard designs applied in RSM discourage their use in the validation procedure. However, if an analytical method is fast and requires the testing of a few factors (three or less), a good choice for robustness testing may be the central composite design (CCD), widely employed because of its high efficiency with respect to the number of runs required. A CCD in k factors requires 2^k factorial runs, $2k$ axial experiments, symmetrically spaced at $\pm\alpha$ along each variable axis, and at least one center point. Three to five center repetitions are generally carried out in order to know the experimental error variance and to test the productive validity of the model.[18]

TABLE 3 Repeatability of the Method

Trial	Actual Concentration	Recovery (%)
1	0.759	98.39
2	0.761	98.65
3	0.766	99.30
4	0.763	98.89
5	0.770	99.82
6	0.766	99.30
7	0.764	99.00
8	0.765	99.13
9	0.765	99.12
10	0.766	99.34
11	0.761	98.58
12	0.768	99.53
Mean	0.764	99.08%
R.S.D.	0.41%	0.42%

TABLE 4 Chromatographic Conditions and Range Investigated During Robustness Testing

Variable	Optimized Value	Range Investigated
Mobile phase CH_3CN/Phosphate, buffer, pH 8.4	70/30 v/v	65/35–75/25 v/v
Flow rate ($mL\,min^{-1}$)	1.3	1.1–1.5
Temperature (°C)	25	23–27

In order to study the variables at no more than three levels (−1, 0, +1), the design used in robustness testing of revastigmine is a face-centered design (FCD) with $\alpha = \pm 1$.[14] Three factors were considered: percentage of acetonitrile (x_1); flow rate $mL\,min^{-1}$ (x_2); temperature°C (x_3). The experimental domain of the selected variables is reported in Table 4. The ranges examined were small deviations from the method settings and the considered responses were the obtained drug concentration $mg\ mL^{-1}$(Y).

A three-factor FCD requires 18 experiments, including four replicates of the center point. The experimental plan and the corresponding response are reported in Table 5. All experiments were performed in randomized order to minimize the effects of uncontrolled factors that may introduce a bias on the response.

A classical second-degree model with a cubic experimental domain was postulated. Experimental results were computed by Nemrod software.[8] The coefficients of the second order polynomial model were estimated

TABLE 5 Experimental Plan for Robustness Testing and Obtained Responses

No. Exp	Random	ACN (%)	Flow ($mL\,min^{-1}$)	Temp. (°C)	Found Conc. ($mg\,mL^{-1}$)
1	5	65	1.1	23	0.589
2	3	75	1.1	23	0.605
3	12	65	1.5	23	0.611
4	11	75	1.5	23	0.603
5	17	65	1.1	27	0.602
6	1	75	1.1	27	0.597
7	6	65	1.5	27	0.601
8	13	75	1.5	27	0.607
9	7	65	1.3	25	0.610
10	2	75	1.3	25	0.598
11	18	70	1.1	25	0.589
12	10	70	1.5	25	0.604
13	16	70	1.3	23	0.607
14	8	70	1.3	27	0.602
15	14	70	1.3	25	0.602
16	9	70	1.3	25	0.601
17	15	70	1.3	25	0.598
18	4	70	1.3	25	0.602

by the least squares regression. The equation model for Y (found concentration) was as follows:

$$Y = 0.47097 + 0.0003x_1 - 0.1173x_2 - 0.0041x_3 + 0.02267x_1^2 - 0.02433x_2^2 + 0.03367x_3^2 - 0.01225x_1x_2 - 0.0060x_1x_3 - 0.0125x_2x_3$$

Only the factor flow rate (x_2) was significant for the regression model assumed. The method was validated by the analysis of variance (ANOVA). The statistical analysis showed that the model represents the phenomenon quite well and the variation of the response was correctly related to the variation of the factors, showing good agreement between experimental and predicted values.

The interpretation of the results has to start from the analysis of the whole model equation rather than from the analysis of the single coefficient; it is important, for the response surface study to consider also the factors whose coefficients are statistically non-significant. For this reason the analysis of the response surface plot is necessary.

As shown in Figures 3(a–c), the analysis produces three-dimensional graphs by plotting the response model against two of the factors, while the third is held constant at a specified level, usually the proposed optimum. Figure 3a shows a graphical representation of the response surface for the variation of percentage of acetonitrile (x_1) and flow rate (x_2), while the temperature (x_3) is maintained constant at its optimum of 25°C. An increase in the flow rate results in a decrease of the found amount concentration (Y), while the percentage of organic modifier had no important effect on the response. Analogous interpretation may be derived by examining Figure 3b that plots the factors flow rate (x_2) versus temperature (x_3). In Figure 3c, where the factor flow rate is maintained constant, the method can be considered robust for the studied experimental response.

In conclusion, the analysis of response surface confirms that Y is not robust for factor x_2, accordingly, a precautionary statement should be included in the analytical procedure for this factor.

Intermediate Precision

The intermediate precision is a measure of precision between repeatability and reproducibility and it should be established according to the circumstances under which the procedure is intended to be used.[16,17] The analyst should establish the effects of random events on the precision of the analytical procedure. The intermediate precision is obtained when

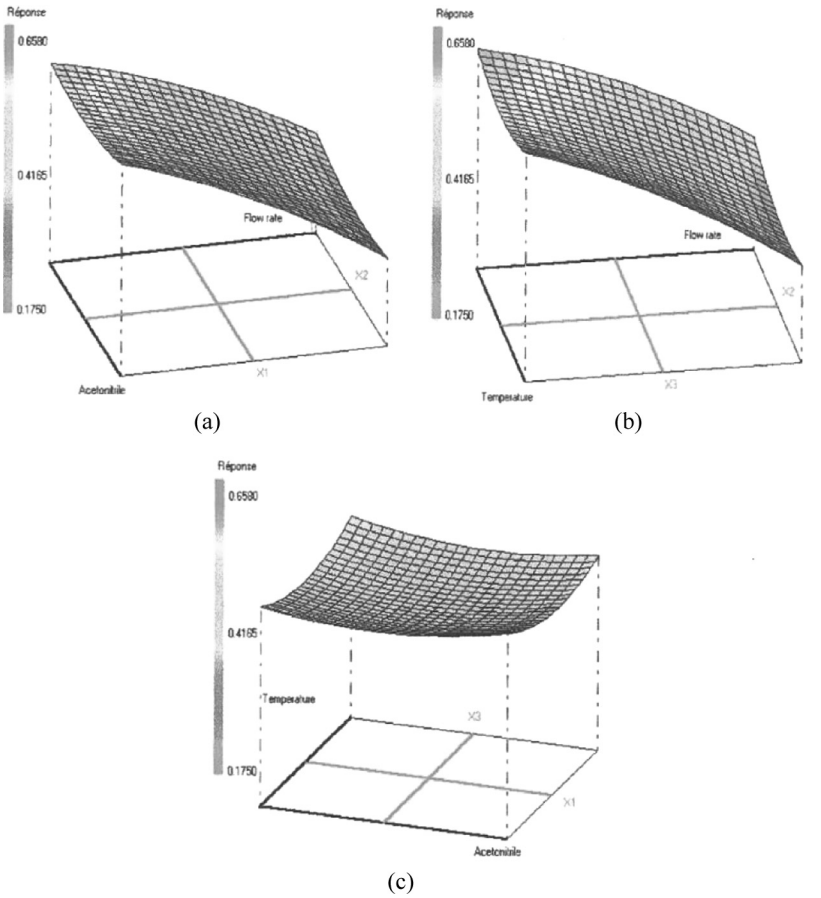

FIGURE 3 Three dimensional plot of the response surface for Y (found concentration). (a) Variation of the response Y as a function of x_1 (% acetonitrile) and x_2 (flow rate); fixed factor x_3(temperature) = 25°C. (b) Variation of the response Y as a function of x_2 (flow rate) and x_3 (temperature); fixed factor x_1 (% acetonitrile) = 70% v/v. (c) Variation of the response Y as a function of x_1 (% acetonitrile) and x_3 (temperature); fixed factor: x_2 (flow rate) = 1.3 mL/min.

the assay is performed by multiple analysts, using multiple instruments, on multiple days, in one laboratory.[9]

In order to study these effects simultaneously, a multivariate approach was used. The considered variables included analyst (1 and 2), equipment (HPLC1 and HPLC2), and days (1 and 2) as shown in Table 6. The considered response was the found drug amount ($mg\,mL^{-1}$). A linear model ($y = b_0 + b_1x_1 + b_2x_2 + b_3x_3$) was postulated and a full factorial design 2^3 was employed to estimate the model coefficients. Each experiment was repeated three times in order to evaluate the experimental error variance. The analyses were carried out in a randomized order according to the experimental plan reported in Table 6.[15]

TABLE 6 Experimental Plan for Intermediate Precision and Obtained Responses

No. Exp	Random	Analyst	Instrument	Day 2	Found Conc. ($mg\,mL^{-1}$)
1	11	Analyst 2	HPLC 2	Day 2	0.610
2	4	Analyst 2	HPLC 2	Day 2	0.590
3	7	Analyst 2	HPLC 2	Day 2	0.611
4	13	Analyst 1	HPLC 2	Day 2	0.605
5	15	Analyst 1	HPLC 2	Day 2	0.597
6	2	Analyst 1	HPLC 2	Day 2	0.602
7	8	Analyst 2	HPLC 1	Day 2	0.598
8	20	Analyst 2	HPLC 1	Day 2	0.596
9	3	Analyst 2	HPLC 1	Day 2	0.602
10	16	Analyst 1	HPLC 1	Day 2	0.601
11	10	Analyst 1	HPLC 1	Day 2	0.597
12	12	Analyst 1	HPLC 1	Day 2	0.600
13	9	Analyst 2	HPLC 2	Day 1	0.603
14	23	Analyst 2	HPLC 2	Day 1	0.590
15	1	Analyst 2	HPLC 2	Day 1	0.589
16	18	Analyst 1	HPLC 2	Day 1	0.601
17	22	Analyst 1	HPLC 2	Day 1	0.611
18	5	Analyst 1	HPLC 2	Day 1	0.597
19	21	Analyst 2	HPLC 1	Day 1	0.606
20	19	Analyst 2	HPLC 1	Day 1	0.608
21	24	Analyst 2	HPLC 1	Day 1	0.599
22	17	Analyst 1	HPLC 1	Day 1	0.590
23	6	Analyst 1	HPLC 1	Day 1	0.604
24	14	Analyst 1	HPLC 1	Day 1	0.607

CONCLUSION

A RPLC method has been developed and vaildated for quantitative determination of rivastigmine hydrogen tartrate. The proposed method is rapid, precise, accurate, and selective. The developed method is stability indicating and can be used for assessing the stability of rivastigmine hydrogen tartrate as bulk drug and can be conveniently applied for the determination of rivastigmine hydrogen tartrate in quality control laboratories.

ACKNOWLEDGMENTS

One of the authors (M. H. Samreen) would like to thank the College of Research and Graduate Studies at the University of Sharjah-UAE for their generous financial assistance (Research Grant 091106). Also a special thanks to the Ministry of Health Quality Control Laboratory, Amman, Jordan, for providing Exelon® 1.5 mg capsules and rivastigmine reference standard.

REFERENCES

1. Cutler, N.R.; Sramek, J.J. Review of the generation of Alzheimer's disease therapeutics: challenges for drug development. Prog. Neuro-Psychopharmacol. Biol. Psychiat. **2001**, *25*, 27–57.
2. Braak, H.; Braak, E. Pathology of Alzheimer's Disease, in *Neurodegenerative Diseases*; Calne, D.B., Ed.; W.B. Sanders: Philadelphia, 1994, 565–613.
3. Bar-On, P.; Millard, C.B.; Harel, M.; Dvir, H.; Enz, A.; Sussman, J.L.; Silman, I. Kinetic and structural studies on the interaction of cholinesterases with the anti-Alzheimer drug rivastigmine. Biochemistry **2002**, *41*, 3555–3564.
4. Pommier, F.; Frigola, R. Quantitative determination of rivastigmine and its major metabolite in human plasma by liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry. J. Chromatogr. B **2003**, *784*, 301–313.
5. Mallikarjuna, R.B.; Srinivasu, M.K.; Kumar, P.K.; Bhradwaj, N.; Pavi, R.; Mohakhud, P.K.; Reddy, G.O.; Kumar, P.R. A stability indicating LC method for rivastigmine hydrogen tartrate. J. Pharm. Biomed. Anal. **2005**, *37*, 57–63.
6. Bartolini, M.; Bertucci, C.; Gotti, R.; Tumiatti, V.; Cavalli, A.; Recanatini, M.; Andrisano, V. Determination of the dissociation constants (pKa) of basic acetylcholinesterase inhibitors by reversed-phase liquid chromatography. J. Chromatogr. A **2002**, *958*, 59–67.
7. Shaa, Y.; Dengb, C.; Liua, Z.; Huanga, T.; Yanga, B.; Duan, G. Headspace solid-phase microextraction and capillary gas chromatographic-mass spectrometric determination of rivastigmine in canine plasma samples. J. Chromatogr. B **2004**, *806*, 59–67.
8. Nemrod, W.; Mathieu, D.; Nony, J.; Phan-Tan-Luu, R. LPRAI SARL, Marseilles, F-13331. France.
9. International Conference on Harmonisation, Topic Q2B, Validation of Analytical Methods: Methodology. The Third International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Yokohama, Japan, 1997.
10. Vander Heyden, V.; Nijhuis, A.; Smeyers-Verbeke, J.; Vandeginste, B.G.M.; Massart, D.L. Guidance for robustness/ruggedness tests in method validation. J. Pharm. Biomed. Anal. **2001**, *24*, 723–753.
11. Fabre, H. Robustness testing in liquid chromatography and capillary electrophoresis. J. Pharm. Biomed. Anal. **1996**, *14*, 1125–1132.
12. Ragonese, R.; Mulholland, M.; Kalman, J. Full and fractionated experimental designs for robustness testing in the high-performance liquid chromatographic analysis of codeine phosphate, pseudoephedrine hydrochloride and chlorpheniramine maleate in a pharmaceutical preparation. J. Chromatogr. A **2000**, *870*, 45–51.
13. Pinzautia, S.; Gratteria, P.; Furlanetto, S.; Muraa, P.; Dreassib, E.; Phan-Tan-Luuc, R. Experimental design in the development of voltammetric method for the assay of omeprazole. J. Pharm. Biomed. Anal. **1996**, *14*, 881–889.
14. Furlanetto, S.; Pinzauti, S.; Gratteri, P.; La Porta, E.; Calzeroni, G. Experimental design strategies in the optimization and robustness testing of adsorptive stripping voltammetric conditions for kynurenic determination. J. Pharm. Biomed. Anal. **1997**, *15*, 1585–1594.
15. Ermera, J.; Plossb, H.J. Validation in pharmaceutical analysis Part II: central importance of precision to establish acceptance criteria and for verifying and improving the quality of analytical data. J. Pharm. Biomed. Anal. **2005**, *29*, 859–870.
16. Abu Abdoun, I.I.; Semreen, M.; Qato, M. Development and validation of HPLC method for the analysis of vardenafil hydrochloride. Int. J. Chem. **2007**, *17*, 259–267.
17. Srinubabu, G.; Raju, Ch.A.I.; Sarath, N.; Kiran Kumarc, P.; Seshagiri Raob, J.V.L.N. Development and validation of a HPLC method for the determination of voriconazole in pharmaceutical formulation using an experimental design. Talanta **2007**, *71*, 1424–1429.
18. Lewis, G.A.; Mathieu, D.; Phan-Tan-Luu, R. *Pharmaceutical Experimental Design*; Marcel Dekker: New York, 1999.