Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 670–675
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Optimization and validation of a dissolution test for selegiline hydrochloride tablets by a novel rapid HPLC assay using a monolithic stationary phase

Paraskevas D. Tzanavaras [a,*], Demetrius G. Themelis [a], Anastasia Zotou [a], John Stratis [a], Bo Karlberg [b]

[a] *Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece*
[b] *Department of Analytical Chemistry, Stockholm University, SE-10691 Stockholm, Sweden*

Received 7 September 2007; received in revised form 7 November 2007; accepted 20 November 2007
Available online 4 December 2007

Abstract

The present study reports the optimization and validation of a dissolution test for selegiline·HCl tablets using a new high-performance liquid chromatographic (HPLC) method. Rapid separation of the analyte from sample matrix was achieved in less than 60 s using a Cromolith® RP-18e monolithic column using UV detection at 220 nm. Thorough validation of the assay based on pre-defined criteria included linearity, LOD/LOQ, accuracy, precision, selectivity and ruggedness. The dissolution test was optimized in terms of dissolution medium, basket (type I)/paddle (type II) agitation and rotation speed. Its ruggedness was also validated. The presented analytical and dissolution procedures are currently being applied in the quality and stability control of Cosmopril® tablets (5 mg/tablet selegiline·HCl, Cosmopharm Ltd., Korinthos, Greece).

Keywords: Selegiline hydrochloride; Dissolution; High-performance liquid chromatography; Monolithic column; Pharmaceutical analysis

1. Introduction

Selegiline hydrochloride, also commonly referred to in the clinical and pharmacological literature as L-deprenyl, is an acetylenic derivative of phenethylamine. It acts as an irreversible inhibitor of monoamine oxidase (MAO), an intracellular enzyme associated with the outer membrane of mitochondria. This is considered to be of primary importance in the treatment of Parkinson's disease, although the mechanisms accounting for selegiline's beneficial adjunctive action are not fully understood [1]. Studies have shown that selegiline can also slow the progression of Alzheimer's disease [2] and that it might be a useful solution to smoking addiction, since it reduces the intensity of nicotine cravings [3]. Since selegiline was the first MAO inhibitor to be approved for the treatment of Parkinson's disease, concerns were raised about the safety of the drug. These concerns were especially based on previously reported side-effects of non-selective MAO inhibitors. The contacted studies proved that selegiline is well-tolerated when given alone, although minor symptoms such as nausea, headaches and dizziness have been reported during monotherapy. More severe effects – including mortality – have been reported when selegiline and levodopa or pethidine were administered together. Thus, the concomitant use of these drugs is not recommended [4].

Drug absorption after oral administration of a solid dosage form depends on the release of the active ingredient from the formulation, its dissolution under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, in vitro dissolution may be relevant to the prediction of in vivo performance [5–7]. It is therefore widely accepted that dissolution testing is a very important tool in the pharmaceutical industry for providing valuable information to both formulation teams that design new products and quality control scientists for ensuring lot-to-lot quality and consistency within pre-defined specification criteria [8,9].

A key determinant of the reliability of results of dissolution tests is the validity of the analytical assay used to determine the active pharmaceutical ingredient in test samples. The ideal method intended for this purpose should be adequately sensitive, selective against pharmaceutical excipients, robust and

* Corresponding author. Fax: +30 2310 997719.
E-mail address: ptzanava@chem.auth.gr (P.D. Tzanavaras).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.11.039

rapid in order to allow the analysis of the large number of samples generated from dissolution experiments. A very attractive approach that fulfils the above-mentioned demands is HPLC using columns with monolithic stationary phases. Such columns, which have become commercially available recently [10,11], allow efficient separations at higher flow rates and lower back-pressures than conventional particulate-based columns. Such systems have already proven to have useful features for a number of applications in the pharmaceutical industry [12–14].

Several analytical techniques have been applied to the analysis of selegiline·HCl in various matrices. HPLC methods with UV detection for pharmaceutical applications [15,16] employ conventional particulate-based columns such as Spherisorb (150 mm × 4.6 mm i.d., 3 μm) at a flow rate of 1.0 ml min^{-1} [16] or LiChrocart (250 mm × 4 mm i.d., 5 μm) at 0.2–0.8 ml min^{-1} resulting in analyte retention times in the range of 20–80 min depending on the flow rate [15]. LC–MS bioanalytical assays of matrices such as human hair [17] and human plasma [18] offer highly sensitive procedures with detection limits being in the sub-ppb area. However, such sophisticated instrumentation is not attractive to routine pharmaceutical analysis, while the range of the calibration curves is narrow for most quality control applications, ranging up to 40 ng ml^{-1} [17] and 20 ng ml^{-1} [18]. Capillary electrophoresis (CE) has proven a valuable tool for chiral separations of racemic mixtures of selegiline using cyclodextrin modifiers [19,20]. No quantitative analytical and validation data are included, while the analysis time exceeds 20 min in both cases [19,20]. An indirect enzymatic assay with fluorescence detection offers a very low detection limit of 0.25 ng ml^{-1}, but requires several time-consuming steps, including a 15 min long incubation period per sample [21]. Finally, a GC–MS approach reported by Patrick et al. is highly sensitive but with limited linearity (0–6 ng ml^{-1}), while synthesis of a deuterated internal standard is required [22].

To the best of our knowledge this is the first reported application of a monolithic stationary phase for the HPLC determination of selegiline·HCl. The present work has two main objectives. The first is to report a reliable and validated HPLC assay for the high-throughput determination of selegiline·HCl intended for pharmaceutical applications. The second objective is to apply the assay to the optimization and validation of a dissolution test for selegiline·HCl containing tablets intended for use in their routine quality and stability control in the pharmaceutical industry.

2. Experimental

2.1. Reagents and materials

All reagents were of analytical grade and were provided by Merck (Darmstadt, Germany), unless otherwise stated.

Water and acetonitrile (ACN) used in this work were of HPLC grade. The mobile phase consisted of 50:50 (v/v) ACN/phosphate buffer (3.7 mM KH_2PO_4 and 4.4 mM $K_2HPO_4 \cdot 3H_2O$, pH 7.0). The mobile phase was filtered under vacuum through 0.45 μm nylon membrane filters (Whatman) and degassed ultrasonically for 30 min prior to use.

Selegiline·HCl reference standard (lot no. 0208H194-14, assay: 98.9%) was provided by Siegfried (Switzerland). The standard stock solution of selegiline·HCl ($\gamma = 1000$ mg l^{-1}) was prepared by dissolution of the appropriate amount of the analyte (ca. 50 mg accurately weighed) in 50 ml of a 1:1 water/ACN mixture. This solution was kept refrigerated and protected from light. Working solutions were prepared by diluting the stock solution as appropriate in either doubly de-ionized water or phosphate buffer depending on the dissolution medium used.

Pharmaceutical grade excipients for preparing the placebo mixture and tablets used in accuracy and selectivity studies (lactose monohydrate, maize starch, polyvidone, talc and magnesium stearate) were obtained from domestic suppliers.

All dissolution media used in this study (water, phosphate buffer and HCl solution) were degassed under vacuum prior to use and stored at 35 °C to minimize re-aeration.

Selegiline·HCl tablets manufactured by Cosmopharm Ltd. (Cosmopril®, 5 mg/tablet, lot 012) were used in all cases for validation purposes. The nominal composition of each tablet is 5 mg selegiline·HCl, 84 mg lactose monohydrate, 46 mg maize starch, 9 mg polyvidone, 3 mg talc and 3 mg magnesium stearate.

2.2. Instrumentation

The HPLC equipment used was an HP 1100 system (Agilent Technologies, USA), comprising a quaternary pump (G1311A), a vacuum degasser (G1322A), a column thermostat (G1316A), an autosampler (G1313A) and a DAD spectrophotometric detector (G1315A). Chromatographic parameters such as peak areas, retention times, theoretical plates, etc. were calculated using the Chem Station® software.

A Chromolith® RP-18e monolithic column (100 mm × 4.6 mm i.d., Merck) was used throughout this study, while the mobile phase was filtered using a Schleicher and Schuell (Germany) vacuum filtration system.

Dissolution experiments were carried out using a Distek Premiere 5100 system equipped with a programmable auto-sampler.

2.3. HPLC analytical procedure

Twenty microliters of each sample and standard to be analyzed were injected into the monolithic column via the autosampler of the HPLC system. The flow rate was set at 3 ml min^{-1}, the column temperature at 25 °C, and detection at 220 nm. Under these conditions each separation cycle was completed in 60 s. Peak areas was used for signal evaluation, while each standard was injected in triplicate and each sample in dublicate.

2.4. Dissolution experiments

For each dissolution experiment, 12 selegiline·HCl-containing tablets were weighed and placed in the dissolution apparatus in batches of six. The temperature of the dissolution medium (volume 500 ml in all cases) was set at 37.0 ± 0.5 °C, and the rotation speed of the baskets or paddles was pre-set according to the experimental matrix. Sample aliquots (ca. 7 ml)

were withdrawn automatically by the autosampler and filtered in-line through 45 μm PTFE disc-filters. No additional pretreatment was required prior to HPLC analysis. Dissolution profiles were constructed at 5, 10, 20, 30 and 60 min in all cases.

3. Results and discussion

3.1. Validation of the HPLC assay

The parameters to be validated for the HPLC assay were according to the ICH guidelines [23] and were based on the following acceptance criteria:

1. Linearity (5–150% and $n = 8$): percent residuals should be within the range ±3.0%.
2. Within-day precision: the R.S.D. derived from repetitive injections of synthetic samples (100% level and $n = 8$) at the beginning, middle and end of a working day should not exceed 2.0%.
3. Day-to-day precision: the R.S.D. of the slopes of aqueous calibration curves (5–150% and $n = 8$) obtained within eight consecutive days should not exceed 5.0%.
4. Accuracy: the recoveries from the analysis of synthetic samples (10, 25, 50, 75, 100 and 120% level) should be within the range 97.5–102.5% in all cases.
5. Selectivity: injection of placebo mixture should reveal no interfering peaks.
6. Ruggedness: deliberate variations of HPLC parameters (ACN fraction, flow rate and pH of mobile phase, column temperature and injection volume) within the range ±3.0% should cause relative errors of less than ±5.0% in analyses of synthetic samples at the 100% level.

3.1.1. Linearity, LOD and LOQ

The nominal concentration of selegiline·HCl after the dissolution experiments – assuming quantitative dissolution – is ca. 10 mg l^{-1} (5 mg selegiline·HCl per tablet in 500 ml of dissolution medium). In order to bracket effectively the above-mentioned concentration, linearity was validated in the range of 0.5–15.0 mg l^{-1}, corresponding to 5–150% of the target value, using eight calibration points ($n = 8$). A linear regression equation was obtained with a regression coefficient, r, of 0.9999:

$$A = 9.56\,(\pm 0.07) \times \gamma(\text{selegiline} \cdot \text{HCl}) - 0.54\,(\pm 0.70)$$

The validity of the regression line was examined using the residuals test. In all cases, the percent residuals were in the range from −2.1 to +1.3%, meeting the ±3% acceptance criterion.

The detection (LOD) and quantitation (LOQ) limits of the assay were determined based on the S/N criteria and were found to be 0.030 mg l^{-1} (S/N = 3) and 0.100 mg l^{-1} (S/N = 10), respectively. The estimated LOQ value corresponds to 1% of the target concentration verifying the suitability of the developed assay for dissolution studies of the selected formulation. Precision experiments at the LOQ level using synthetic samples yielded an acceptable R.S.D. of 2.3% ($n = 12$).

3.1.2. Precision (repeatability and reproducibility)

The repeatability (within-day precision) of the proposed HPLC assay was validated by consecutively injecting and analyzing eight synthetic samples containing 10 mg l^{-1} selegiline·HCl (100% level) at the beginning, middle and end of a working day. In order to prepare the synthetic samples, a placebo mixture (all excipients excluding the active ingredient) was prepared according to the manufacturing protocol of the tablets, as mentioned in Section 2.1. Accurately weighed amounts of the placebo were dispersed via ultrasonication in standard solutions of the analyte at the concentration levels mentioned above. The placebo concentration was fixed at 0.6 mg ml^{-1} (at least 2-fold higher than the theoretically expected in real dissolution samples). The synthetic samples were ultrasonicated for 15 min, filtered through 0.45 μm disposable syringe filters (Whatman) and injected in the HPLC system via the autosampler. The calculated mean relative standard deviation (R.S.D.) was 0.75% ($n = 8 \times 3$).

Reproducibility (day-to-day precision) was validated by constructing eight calibration curves – within a period of eight consecutive days – for the concentration ranges mentioned in the previous section (5–150% selegiline·HCl, eight calibration curves × eight concentration levels). The experimental results confirmed the reproducibility of the HPLC assay since the relative standard deviation of the slopes over the test period was 2.11% ($n = 8$).

3.1.3. Accuracy studies

The accuracy of the developed HPLC method was validated by analyzing synthetic samples – prepared as described in the previous section – at six selegiline·HCl concentration levels, namely 1.0, 2.5, 5.0, 7.5, 10.0 and 12.0 mg l^{-1}, covering the range of 10–120% of the target concentration. Aqueous calibration curves were used in all cases for recoveries calculation. Both within and day-to-day accuracy were evaluated by performing the analyses on two consecutive days. The experimental

Table 1
Accuracy of the HPLC assay

Synthetic sample[a]	Selegiline·HCl added (mg l^{-1})	Recovery (±S.D.) (%)[b]
Day I		
S1	1.0	99.2 (±0.7)
S2	2.5	99.4 (±0.6)
S3	5.0	99.4 (±0.7)
S4	7.5	101.0 (±0.5)
S5	10.0	100.8 (±0.8)
S6	12.0	99.0 (±0.7)
Day II		
S7	5.0	99.6 (±0.4)
S8	10.0	100.5 (±0.9)
S9	15.0	100.9 (±1.1)
S10	400.0	101.6 (±0.8)
S11	500.0	101.1 (±0.4)
S12	600.0	99.0 (±0.5)

[a] Containing 0.6 mg ml^{-1} placebo mixture.
[b] Mean of three analyses.

results are presented in Table 1. The percent recoveries met the acceptance criteria in all cases ranging between 99.0 and 101.6%.

3.1.4. Selectivity studies

The selectivity of the HPLC assay against the pharmaceutical excipients used in the manufacturing process of the formulations was initially validated as follows: An amount of 30 mg of the placebo mixture prepared as described previously was dispersed in 10 ml of 1:1 water/ACN. The resulting suspensions were ultrasonically agitated for 15 min and filtered prior to injection into the HPLC system (note that the placebo concentration used was 10-fold higher than the theoretically expected concentration in real dissolution samples). No interfering peaks were observed at the expected retention time of the active ingredient (Fig. 1).

The selectivity of the assay was further validated at the optimal dissolution conditions using "placebo tablets" manufactured in an identical manner to selegiline-containing ones by replacing the active ingredient with lactose. The "placebo tablets" were subjected to dissolution test and sampling was carried out at 5, 10, 20, 30, 60 and 180 min. No interfering peaks were observed after HPLC analysis of 12 "placebo tablets".

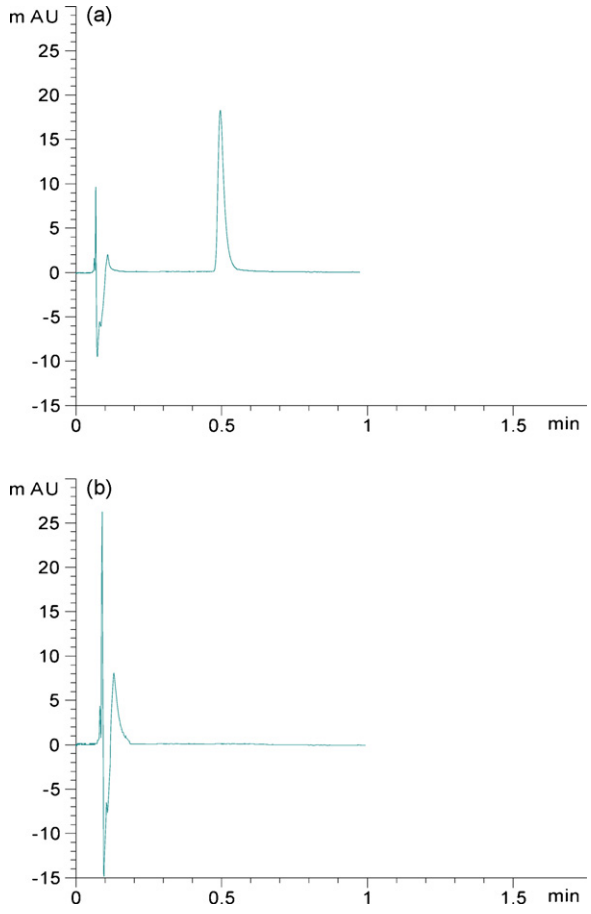

Fig. 1. (a) Representative chromatogram of selegiline·HCl at the 100% level under the experimental conditions described in Section 2.3 and (b) chromatogram of placebo for selectivity studies.

Table 2
Ruggedness study of the HPLC assay

Chromatographic parameters	Percent recovery[b,c] (±S.D.)
Optimal conditions[a]	99.3 (±0.5)
Variation of the mobile phase flow rate	
$Q = 3.09$ ml min^{-1}	97.9 (±0.6)
$Q = 2.91$ ml min^{-1}	102.8 (±0.8)
Variation of the ACN:water ratio	
51.5:48.5 (v/v)	98.1 (±0.5)
48.5:51.5 (v/v)	101.2 (±0.7)
Variation of the mobile phase pH	
pH 6.8	99.6 (±0.6)
pH 7.2	99.0 (±0.4)
Variation of column temperature	
$T = 24$ °C	98.8 (±0.9)
$T = 26$ °C	99.2 (±0.7)
Variation of the injection volume	
$V = 19.4$ μl	97.1 (±0.6)
$V = 20.6$ μl	103.4 (±1.1)

[a] For experimental details see Section 2.3.
[b] Each synthetic sample contains 10 mg l^{-1} selegiline·HCl and 0.6 mg ml^{-1} placebo.
[c] Percent recovery according to the calibration curve obtained under optimal conditions (mean of three injections ± standard deviation).

3.1.5. Ruggedness of the proposed HPLC method

The experimental results of the ruggedness study are summarized in Table 2. Critical chemical and instrumental chromatographic parameters such as the composition and flow rate of the mobile phase, pH, column temperature and sample injection volume were deliberately varied in the range of ±3.0% compared to their optimal values. Recovery values obtained using synthetic samples at the 100% level confirmed the ruggedness of the HPLC assay, since the obtained values were within the acceptance limits (95.0–105.0%) in all cases.

3.2. Evaluation and optimization of the dissolution test

The dissolution test was optimized in terms of dissolution medium, paddle (type II)/basket (type I) agitation and rotation speed.

Initial dissolution experiments were carried out under the following experimental conditions: 500 ml H_2O as the dissolution medium, medium temperature of 37.0 ± 0.5 °C, rotation speed of baskets 50 rpm. To construct the dissolution profile, sampling was performed at 5, 10, 20, 30 and 60 min, while 12 tablets, in batches of six, were used in all cases. A typical dissolution profile obtained under these experimental conditions is depicted in Fig. 2a.

3.2.1. Study of the dissolution medium

Although selegiline·HCl is a water-soluble compound, pure water is not consider as an "ideal" dissolution medium, mainly due to its extremely low capacity against potential pH variations during the dissolution tests, especially in the case of salts. Additionally, water is not considered a physiologically relevant medium as it is not representative of the gastrointestinal

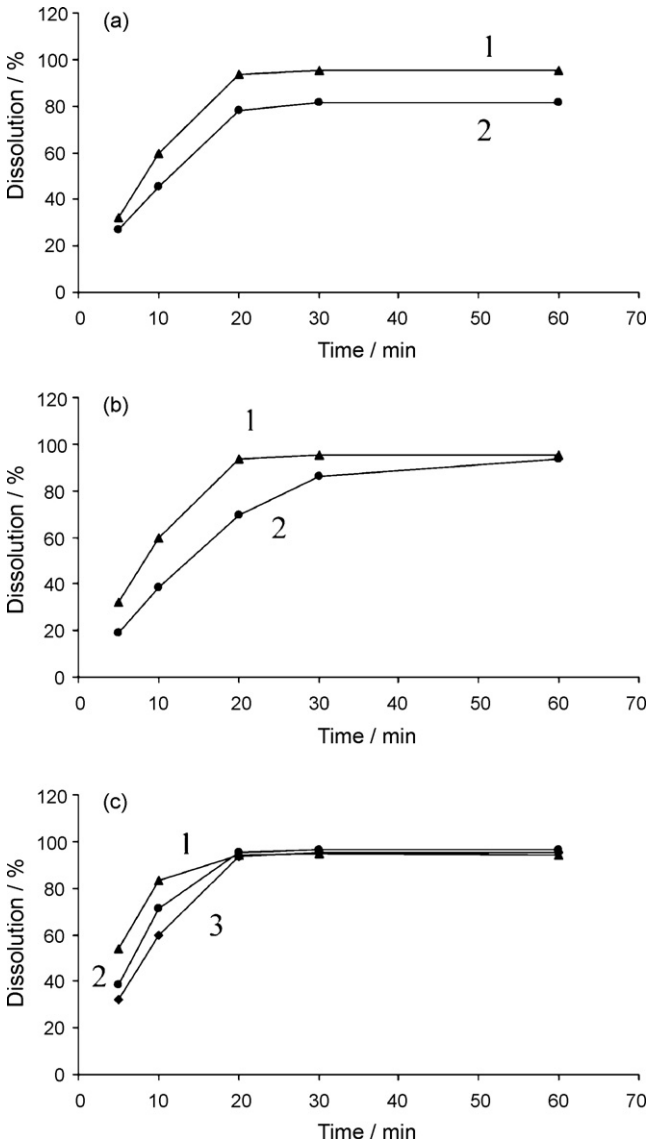

Fig. 2. Optimization of the dissolution test: (a) effect of dissolution medium: (1) = phosphate buffer (0.1 mol l^{-1}, pH 6.8), (2) = water (b) basket vs. paddle: (1) = basket at 50 rpm, (2) = paddle at 50 rpm, (c) effect of baskets' rotation speed: (1) = 100 rpm, (2) = 75 rpm and (3) = 50 rpm.

environment. For this reason, the FDA has undertaken a study in order to evaluate alternative solutions for QC purposes of pharmaceutical products currently having water as the media [24]. Two alternative media that are frequently employed in such studies are 0.1 mol l^{-1} HCl, to simulate gastric fluid conditions and 0.1 mol l^{-1} phosphate buffer (pH 6.8) to simulate intestinal conditions in terms of pH.

Under the experimental conditions described above, no detectable amounts of the analyte dissolved when HCl was used. On the other hand, as can be seen in Fig. 2a, the dissolution rate was significantly improved when phosphate buffer (pH 6.8) was used rather than water.

Additional experiments were carried out in order to evaluate the stability of selegiline·HCl in the selected medium [25]. Accurately weighed amounts of the API were dissolved in the phosphate buffer at final mass concentrations of 2, 5 and 10 mg l^{-1}. The resulting solutions were incubated in a water-bath at 37 °C under continuous stirring at 50 rpm. Sampling and HPLC analysis was carried out at $t = 0$, 15, 30, 60 and 120 min. No instability of the analyte was observed, since the R.S.D.s of the obtained peak areas were <1.7% in all cases ($n = 3 \times 5$ at each concentration level).

3.2.2. Basket (type I) versus paddle (type II) agitation

Using phosphate buffer as the dissolution medium, experiments were carried out to compare the performance of basket versus paddle agitation, rotating at 50 rpm in both cases. The experimental results presented in Fig. 2b show that the dissolution rate was faster when baskets were used, and the dissolution profiles tended to coincide only at 60 min. Basket (type I) agitation was therefore selected for further studies.

3.2.3. Effect of the baskets' rotation speed

The effect of the rotation speed of the baskets on the dissolution profile of selegiline·HCl containing tablets was examined at 50, 75 and 100 rpm. Increasing the rotation speed resulted in faster dissolution kinetics in the timeframe of 5–20 min, although the profiles coincided thereafter (Fig. 2c). Therefore the rotation speed of 100 rpm was selected for further experiments.

3.3. Ruggedness of the dissolution test

The ruggedness of the dissolution test was validated by deliberately introducing small variations, in the range of ±3% compared to the optimal values, to critical parameters including the pH of the dissolution medium (6.6–7.0), temperature (36.0–38.0 °C) and basket rotation speed (97.0–103.0 rpm). The obtained dissolution profiles (12 tablets in all cases) were compared to those obtained under the optimal conditions by calculated similarity factors (f_2) derived by the following equation [8]:

$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \right) \Sigma_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where n is the number of time points, R_t the dissolution value of the reference (pre-change) batch at time t and T_t is the dissolution value of the test (post-change) batch at time t. Generally, f_2 values greater than 50 (50–100) indicate similarity or equivalence of the compared dissolution profiles.

The experimental results are presented in Table 3. The values of the similarity factor ranged between 79 and 88 in all cases, confirming the ruggedness of the dissolution test.

3.4. Study of the effect of batch-to-batch variations

The effect of batch-to-batch variations on the dissolution behavior of the formulation can be related to the discriminating ability of the dissolution test. This was evaluated by deliberately varying some parameters of the manufacturing process and the formulation characteristics on pilot scale batches of ca. 1000 tablets each. These parameters were the mesh size of the granule prior to tableting, the equipment used for tableting and the

Table 3
Ruggedness study of the dissolution test

Dissolution parameters	Similarity factor (f_2)
Optimal conditions[a]	100
Variation of the dissolution medium pH	
pH 6.6	82
pH 7.0	88
Variation of the baskets' rotation speed	
97 rpm	84
103 rpm	88
Variation of column temperature	
$T = 36.0\,^{\circ}C$	79
$T = 38.0\,^{\circ}C$	85

[a] For experimental details see Section 3.2.

resistance to crushing (hardness) of the final product. The nominal composition and mass of the tablets were kept constant in all cases.

(i) Two lots of granules were prepared in terms of mesh size (Gr1 and Gr2). In Gr1 more than 85% of the material ranged between 125 and 500 μm, while in Gr2 more than 85% of the granule ranged between 500 and 1000 μm. No statistically different dissolution profiles were observed, since the values of the similarity factor were >70 in all cases.
(ii) The effect of the manufacturing equipment was evaluated by using two different tableting machines, namely KILIAN E150 (KILIAN+ CO. GmbH, Germany) and Fette Exacta 21 (Wilhelm Fette GmbH, Germany). The experimental values of the f_2 factor suggested similarity of the dissolution behavior of the formulations tested (f_2 = 76–93 in all cases).
(iii) The effect of the hardness of the tablets, expressed as resistance to crushing, was examined in the range of 20–150 N. It should be noted that the product specifications on this parameter for market release are 50–100 N. The experiments showed statistically similar dissolution profiles of the formulation in the range 20–120 N. Higher hardness values resulted in slower dissolution kinetics in the range of 5–20 min. However, in all cases the tablets met the dissolution specification for market release (>80% in 20 min).

4. Conclusions

A dissolution test for selegiline·HCl containing tablets has been optimized and validated. All necessary analyses were carried out by an ultra-fast HPLC method that has been developed and validated for this purpose. To the best of our knowledge this is the first HPLC assay using a monolithic stationary phase for this active pharmaceutical compound. The ability of the monolithic column, used throughout this study, to operate effectively at elevated flow rates enabled separation/detection cycles to be completed within 60 s. Thorough validation demonstrated the accuracy, precision, selectivity and ruggedness of the analytical method. The developed, validated HPLC assay/dissolution scheme has been successfully applied for over 12 months, to date, in the on-going stability and manufacturing control of selegiline·HCl-containing formulations, i.e. lots 0 1 0, 0 1 1 and 0 1 2 of Cosmopril® Tabs (5 mg selegiline·HCl/tab, Cosmopharm Ltd., Korinthos, Greece).

Acknowledgements

The authors would like to thank Ms. A. Verdoukas and Mrs. Th. Balloma (Production Department, Cosmopharm Ltd., Korinthos, Greece) for their kind technical assistance and useful comments.

References

[1] http://www.selegiline.com.
[2] J. Knoll, Pharmacol. Toxicol. 82 (1998) 57–66.
[3] Science Daily, URL: http://www.sciencedaily.com.
[4] E.H. Heinonen, V. Myllyla, Drug Saf. 19 (1998) 11–22.
[5] F. Gudrun, Drug Inf. J. 35 (2001) 865–874.
[6] P.D. Tzanavaras, A. Verdoukas, T. Balloma, J. Pharm. Biomed. Anal. 41 (2006) 437–441.
[7] J.S. Space, A.M. Opio, B. Nickerson, H. Jiang, M. Dumond, M. Berry, J. Pharm. Biomed. Anal. 44 (2007) 1064–1071.
[8] Food and Drug Administration: Guidance for Industry. Dissolution Testing of Immediate Release Solid Oral Dosage Forms, US Department of Health and Human Services/Food and Drug Administration/Center for Drug Evaluation and Research, Rockville, MD, 1997.
[9] M. Siewert, J. Dressman, C.K. Brown, V.P. Shah, AAPS Pharm. Sci. Technol. 4 (2003) (Article 7).
[10] K. Cabrera, D. Lubda, H.-M. Eggenweiler, H. Minakuchi, K. Nakanishi, J. High Resol. Chromatogr. 23 (2000) 93–102.
[11] K. Nakanishi, N. Soga, U.S. Patent No. 5,624,875, 1997.
[12] P.D. Tzanavaras, D.G. Themelis, J. Pharm. Biomed. Anal. 43 (2007) 1483–1487.
[13] P.D. Tzanavaras, D.G. Themelis, J. Pharm. Biomed. Anal. 43 (2007) 1526–1530.
[14] S. El Deeb, L. Preu, H. Watzig, J. Pharm. Biomed. Anal. 44 (2007) 85–95.
[15] K. Lemr, D. Jirovsky, J. Sevcik, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 3173–3191.
[16] N. Beaulier, T.D. Cyr, S.J. Graham, E.G. Lovering, J. Assoc. Off. Anal. Chem. 74 (1991) 453–455.
[17] K. Nishida, S. Itoh, N. Inone, K. Kudo, N. Ikeda, J. Anal. Toxicol. 30 (2006) 232–237.
[18] M.H. Slawson, J.L. Taccogno, R.L. Foltz, D.E. Moody, J. Anal. Toxicol. 26 (2002) 430–437.
[19] K. Sevcik, Z. Stransky, B.A. Ingelse, K. Lemr, J. Pharm. Biomed. Anal. 14 (1996) 1089–1094.
[20] E. Szoko, K. Magyar, J. Chromatogr. A 709 (1995) 157–162.
[21] I. Mahmood, S. Neau, W.D. Mason, J. Pharm. Biomed. Anal. 12 (1994) 895–899.
[22] K.S. Patrick, B.L. Nguyen, J.D. McCallister, J. Chromatogr. 121 (1992) 254–258.
[23] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (I.C.H.), Q2/R1 Validation of analytical procedures. Text and methodology, 1995.
[24] C. Noory, N. Tran, L. Ouderkirk, S. Brown, J. Perry, J. Lopez, M. Colon, M. Faberlle, K. Henry, J. Rorberg, S.N. Ali, V. Shah, Dissolut. Technol. 6 (1999) 6.
[25] Federation International Pharmaceutical (F.I.P.), F.I.P. guidelines for dissolution testing of solid oral products. Joint report of the section for official laboratories and medicines control services and the section of industrial pharmacists of the F.I.P., 1997.