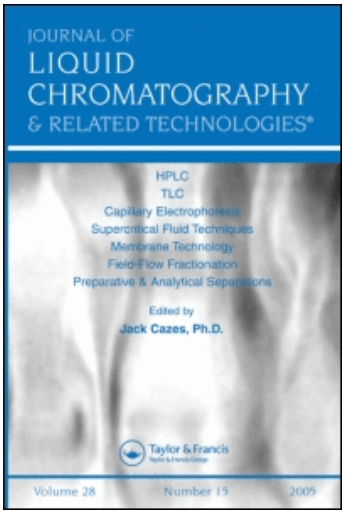

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HPLC Determination of Piracetam in Tablets; Validation of the Method

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Abstract: A simple, rapid, and validated HPLC method has been developed for determination of Piracetam in film coated tablets. A Lichrospher® (100 RP-18 column was used with a mobile phase consisting of methanol–water (5:95). Quantitative evaluation was performed at 215 nm. The HPLC method is selective, precise, and accurate and can be used for routine analysis of the preparations in pharmaceutical industry quality control laboratories.

Keywords: Piracetam, HPLC, Tablets, Validation

INTRODUCTION

Piracetam, 2-(2-Oxopyrrolidin-1-yl) acetamide is a drug that acts on the CNS and is said to protect the cerebral cortex against hypoxia. It is used as an adjunct in the treatment of myoclonus of cortical origin and has also been used in dementia.[1] Piracetam can be used also for the treatment of schizophrenia.[2] This drug is already marketed in Indonesia in present days. No official method was described in the Indonesian Pharmacopoeia[3] and other Pharmacopoeias.[4–7] British Pharmacopoeia 2003[8] described a liquid

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chromatography method for analyzing piracetam as a bulk drug. Determination of piracetam and its metabolites in biological fluids using HPLC methods was reported.[9] The determination of piracetam using a gas chromatographic method with nitrogen-phosphorus detector has been also described.[10] Determination of piracetam impurities using TLC and FTIR spectroscopy were also reported.[11,12]

The aim of this present work was to develop a simple, validated, and rapid HPLC method for routine analysis of piracetam in tablet preparations using an HPLC method.

EXPERIMENTAL

Materials and Reagents

Piracetam (Tianjin Zhongjin,China, Batch no. T20021101: assay: 100.2%, manufacturing date: October 2002; expiry date: October 2005) was of pharmaceutical grade. The substance has identical IR-, UV-spectrum, and a melting point compared to the standard piracetam (Sigma, St. Louis, USA), and was used as received for preparing laboratory-made tablets and standard solutions.

Methanol (Mallinckrodt Baker Inc. Phillipsburg, NJ, USA) was an analytical grade reagent. The solvent and reagent were used without further purification. Excipients for laboratory made film coated tablet preparations (magnesium stearate, Vivastar®, Polyvinylpyrrolidone, Eudragit E100®, isopropyl alcohol, talcum, titanium dioxide, poly ethylene glycol 4000, sunset yellow) were pharmaceutical grade substances.

Laboratory-made film coated tablet preparations were prepared containing five different concentrations of piracetam (640, 720, 800, 880, and 960 mg tablet^{-1}); these were used for accuracy determination.

Commercial Piracetam film coated tablets (CT-1 and CT-2) containing 800 mg tablet^{-1} were purchased in June 2004 from a local pharmacy in Surabaya. The commercial pharmaceutical preparations were produced in Indonesia.

Stock standard solutions were prepared daily by dissolving accurately weighed piracetam (20.0, 30.0, 40.0 mg) in mobile phase (50.0 mL). For a linearity study, various standard solutions were prepared from the stock solutions by dilution with the mobile phase (100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 μg mL^{-1}, and each of these solutions (20 μL) was injected into the HPLC systems. The standard solution was stable at least for 24 hours (at 24 $\pm$ 2°C, room humidity 50 $\pm$ 10%); the result of analysis of the standard solution that was kept for 6 and 24 hours yielded 100 $\pm$ 0.33% and 100 $\pm$ 1.06%, respectively (mean $\pm$ SD, n = 3, compared to the fresh solution).

Sample Extraction

Twenty film coated tablets were each weighed and their mean was determined. After homogenizing the powder, an equivalent weight of *ca.* 1/50 tablet (equivalent to 15.0 mg piracetam) was transferred into a 25.0 mL volumetric flask containing about 10 mL of the mobile phase, ultrasonicated for 15 min and diluted to 25.0 mL with the mobile phase. The solution was filtered through 0.45 μm Durapore™, membrane filters (Milipore, Ireland) before injection to HPLC apparatus (20 μL).

Chromatography

The HPLC systems used in this work was comprised of a Shimadzu HPLC-2010C (Kyoto, Japan) system equipped with auto-injector, and Chromatography Data System Shimadzu Class-VP version 6.12; a Hitachi L-6200 (Tokyo, Japan) intelligent pump equipped with a Hitachi LC organizer and dynamic mixer mode 655A, a Hitachi L-4500 photo diode array detector (DAD detector), a 20 μL Rheodyne 7125 injector (for selectivity and precision studies); an Agilent 1100 HPLC Series (Waldbronn, Germany) equipped with a photo diode array detector (DAD), auto-sampler and Agilent Chem Station Plus for LC 2002 was used for inter-laboratory precision study. The analysis was carried out on a LiChrospher 100 RP-18 (5 μm; Cat. 1.50983, E. Merck, Darmstadt, Germany) with flow rate of 1.2 mL min^{-1}.

As mobile phase, a mixture of methanol–water (5:95,v/v) that was modified from published[9] work was used. The mobile phase was prepared daily, and filtered through 0.45 μm Ultipor N™ (Pall, Washington, USA) filters and ultrasonicated for 30 min before use.

To confirm the purity and identity of the analyte peak, the eluent was also monitored using a DAD detector in the range of 210–400 nm; all qualitative data evaluation (identification, purity check) were performed on a Hitachi model D-6500 chromatography data station software, DAD system manager. Routine quantitation was performed at 215 nm via peak areas with linear regression, using at least three points of external calibration. The quantitative calculations were performed using software Chromatography Data System Shimadzu Class-VP version 6.12, or Agilent Chem Station Plus for LC 2002, or a Hitachi model D-6500 chromatography data station software, DAD system manager.

Validation

The method was validated for linearity, detection limit (DL), quantitation limit (QL), accuracy, robustness, and range by the modified method of

Funk et al.[13] and Kromidas.[14] The selectivity of the method was proven by identification and purity checks of the analyte peaks. In order to assure the selectivity of the method, forced degradation studies using HCl, NaOH and H_2O_2 were performed on ca. 500.0 mg powdered laboratory made film coated tablets (equivalent to a half film coated tablet). Five point accuracy studies (80–120% of the expected value) were performed on the laboratory-made film coated tablet preparations. A standard addition method (30% of the label claim) accuracy study was performed on the two different commercial film coated tablets. The precision (repeatability, intra-, and inter-laboratory studies) was evaluated by analyzing six different extract aliquots from laboratory-made tablets containing 80.0, 100.0, and 120.0% piracetam film coated tablets on different days by different analysts, and HPLC equipment in two laboratories.

RESULTS AND DISCUSSION

Extract of the excipients of the laboratory-made tablets showed no peak. All HPLC chromatograms of the extracts of laboratory-made tablets showed a single peak of piracetam. To confirm the identity and purity of the analyte peaks a DAD detector was used. Figure 1 showed the typical HPLC chromatogram of piracetam, its UV spectrum, and the contour plot of the HPLC chromatogram. The λ of piracetam at 215 nm was then selected for further work, and quantitative evaluation. All the UV-spectra of the analyte peaks showed good correlation to the standard peak ($r > 0.99$). Purity checks of

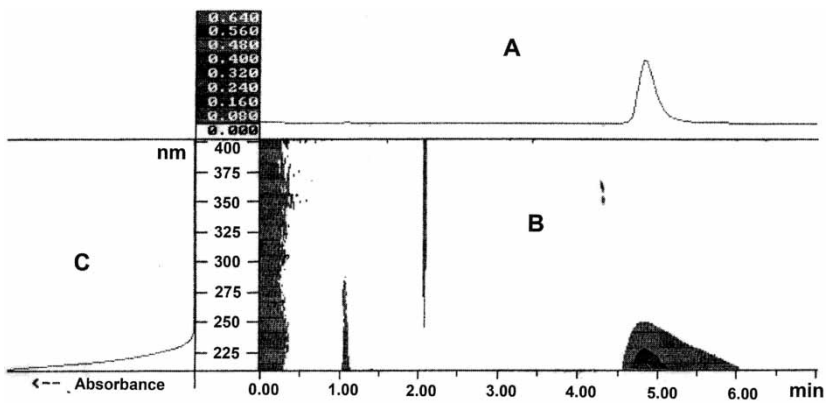

Figure 1. Typical HPLC chromatogram of piracetam at 215 nm (A), its contour plot (B), and UV spectrum (C). Mobile phase: methanol-water (5:95,v/v), flow rate of 1.2 mL min^{-1}, injection volume 20 μL; Column: LiChrospher 100 RP-18 (5 μm; Cat. 1.50983, E. Merck, Germany); detector: DAD Hitachi L-4500.4.

the analyte peaks showed that all the peaks were pure ($r > 0.99$). This showed the proposed HPLC method is sufficiently selective. The relative standard deviation (RSD) of its retention time (R_t) data from this work (except of the robustness) were 4.4% ($n = 136$). The asymmetry factor (at 10% peak height) yielded relatively good values (1.37–1.85; $n = 136$).

Using this HPLC system, the linearity of piracetam was achieved in the range of 100 to 1000 μg mL^{-1} (linear regression line equation: $Y = 261443 + 11356\ X$, $n = 10$, relative process standard deviation V_{XO}[13] = 2.39%, $r = 0.9992$, injection volume 20 μL). The calculated testing-value X_p (for $p = 0.05$),[13] and the RSD of response factor (RF) were satisfactory (58 μg mL^{-1} and 4.1%, respectively). ANOVA regression-test for linearity testing of the regression line showed calculated F-value (4764.6, for $p < 0.0001$), standard error of the intercept (S_a) and slope (S_b) were 102082 ($p > 0.01$) and 164.5 ($p = 0.000$). The plots of the residuals against the quantities of the analyte confirmed the linearity of the basic calibration graph (data not shown). The residuals were distributed at random around the regression line; neither trend nor uni-directional tendency was found. This basic linear calibration curve showed variance homogeneity over the whole range. The calculated parameter PW[14] was 1.32; this PW-value is less than the F_{table}-value (6.99; for $f_1 = 7$, $f_2 = 7$; $p < 0.01$). The data of the linear regression calibration curves, those used in this present work for accuracy, precision, degradation, and stability studies were summarized in Table 1. All data showed satisfactory results (S_a, S_b, r, $F_{calculated}$, and RSD of RF).

Although the validation parameters DL and QL (quantitation limit) were not required for the assay of active ingredient(s) in tablets,[4] those parameters were also determined in this present work. These parameters might also be able to be used for other purposes (e.g., for *in vitro* bio-equivalence-, stability-studies, *etc.*). DL was determined by making a linear regression of relatively low concentration of piracetam (10 to 90 μg mL^{-1}, injection volume 20 μL) according to the method of Funk et al.[13] The calculated equation of the regression line was $Y = -14393 + 12791.5\ X$ ($n = 9$; $V_{XO} = 2.73\%$; $r = 0.9989$; RSD of RF = 3.7%). ANOVA calculated F-value = 3201.9 ($p < 0.0001$), $S_a = 12720$ ($p = 0.295$), $S_b = 226$ ($p = 0.000$). The calculated of testing-value X_p[13] (for $p = 0.05$) was 6.30 μg mL^{-1}. In this case the value of DL = X_p.[13] According to Carr and Wahlich,[15] the value of the QL could be estimated at 3 times of the DL-value (18.9 μg mL^{-1} for injection volume 20 μL).

Table 2 demonstrates the high accuracy as revealed by the percentage of mean recovery data (98.8–100.4%). To prove whether systemic errors did not occur, a linear regression of recovery curve of $\mathbf{X_f}$ (concentration of the analyte measured by the proposed method) against $\mathbf{X_c}$ (nominal concentration of the analyte) of the laboratory-made tablets was constructed.[13] The confidence interval data ($p = 0.05$) of the intercept {VB(a_f)} and slope {VB(b_f)}

Table 1. Linear regression data of the calibration curves used in the present work

HPLC[a]	Intercept (standard error)	Slope (standard error)	r	F_{cal} (ANOVA)	RSD of RF (%)	n	Type of study
1[e]	1414266 (739318; p = 0.307)	22246 (1188; p = 0.03)	0.9986	350.1 (p = 0.033)	4.4	3[b]	Stability
1	385245 (75377; p = 0.123)	11352 (121; p = 0.007)	0.9999	8770 (p = 0.007)	2.1	3[b]	Precision
1	388577 (69957; p = 0.113)	11414 (112; p = 0.006)	0.9999	10293 (p = 0.006)	2.2	3[b]	Precision
1	396224 (69236; p = 0.110)	11374 (111; p = 0.006)	0.9999	10436 (p = 0.006)	1.9	3[b]	Precision
1	311324 (100529; p = 0.199)	11497 (161; p = 0.009)	0.9999	5057 (p = 0.009)	0.7	3[b]	Precision
1	16766 (86931; p = 0.305)	12031 (139; p = 0.007)	0.9999	7406 (p = 0.007)	0.8	4[c]	Accuracy
1	108873 (23149; p = 0.47)	11913 (315; p = 0.001)	0.9993	1430 (p = 0.001)	1.6	4[b]	Degradation
2	−134129 (200720; p = 0.69)	9454 (396; p = 0.02)	0.9990	569b (p = 0.027)	1.0	3[d]	Precision
2	−363341 (223969; p = 0.35)	10805 (518; p = 0.03)	0.9989	434.4 (p = 0.03)	4.8	3[d]	Precision
3[e]	69.508 (203; p = 0.79)	12.32 (0.32; p = 0.016)	0.9999	14377 (p = 0.016)	Nd[f]	3[c]	Precision

[a]1: HPLC Shimadzu 2010C, 2: Hitachi L-6200, 3: Agilent 1100 series.
[b]Range 400–800 μg mL^{-1}.
[c]200–1000 μg mL^{-1}.
[d]200–600 μg mL^{-1}.
[e]Using different lot of HPLC-column.
[f]Not determined.

Table 2. Results from determination of the accuracy-studies of the laboratory-made (LM) and commercial preparations

	Sample		
	LM-tablet	CT-1	CT-2
Amount found[a] (mean ± SD)[e]	—	98.77 ± 0.24	101.49 ± 0.70
Amount added[a]	—	34.22	33.07
% Recovery (mean ± SD)	99. 6 ± 1.5[d]	100.38 ± 0.17[e]	98.77 ± 0.51[e]
Recovery curve[b]	$X_f = 17.695 + 0.965\ X_c$	—	—
$V_{B(af)}$[c]	17.695 ± 48.242	—	—
V^c_{B9bf}	0.965 ± 0.078	—	—

[a]% of label claim.

[b]X_f and X_c are, respectively, the measured and nominal concentration of the analyte ($\mu g\ mL^{-1}$; injection volume 20 μL).

[c]For $p = 0.05$.

[d]$n = 10$.

[e]$n = 3$.

from the recovery curve did not reveal the occurrence of constant- and proportional-systematic errors.

All the RSD values of the repeatability, intermediate precision, and interlaboratory study evaluations were less than 2% (see Table 3), and the calculation by using David-, Dixon-, and Neumann-Tests[14] showed satisfactory results (data not shown). All the standard deviation (SD) data (not shown) of the precision studies yielded values below the permitted maximum

Table 3. Results from evaluation of precision of laboratory-made tablets

	RSD value (%, n = 6)		
Measurement	Piracetam 640 mg $tablet^{-1}$	Piracetam 800 mg $tablet^{-1}$	Piracetam 960 mg $tablet^{-1}$
1[a]	1.66	1.07	1.14
2[a]	1.50	1.92	1.34
3[a]	1.96	1.76	1.01
4[b]	Nd[c]	0.95	1.13

[a]Each measurement was performed by a different analyst on the different days, and Equipments within one laboratory.

[b]Measurement was performed in the different laboratory.

[c]Not determined.

Table 4. Robustness evaluation of the proposes HPLC method

Mobile phase (methanol–water)	R_t (min) (n = 3)[c]	RF[a] (n = 3)[c]	AF[b] (n = 3)[c]
4:96	5.085	8.267×10^{-5}	1.85
5:95[d]	4.559	8.443×10^{-5}	1.72
6:94	4.425	8.227×10^{-5}	1.76
Mean ± SD	4.690 ± 0.349	$8.312 \times 10^{-5} \pm 1.15 \times 10^{-6}$	1.78 ± 0.07
Flow of mobile phase (mL min^{-1})			
1.1	4.964	1297.533	1.73
1.2[d]	4.559	1319.027	1.72
1.3	4.250	1368.877	1.81
Mean ± SD	4.591 ± 0.358	1328 ± 36.599	1.75 ± 0.05

[a]Response factor.
[b]Asymmetry factor at 10% of peak height.
[c]The presented values were mean of 3 replications.
[d]The mobile phase composition and flow rate that used in this present work.

standard deviation as reported by Ermer[16] (2.43 for specification range 95–105%, basic lower limit 99%, n = 6). The measurements were performed within two laboratories on the different days by different analysts and by using different HPLC equipment. These results demonstrated that the accuracy and precision of the proposed method were satisfactory in the range of 80 to 120% of the expected value.

In order to evaluate the robustness of the proposed method, the influence of small variation on mobile phase composition and flow rate on the values of R_t, response factor (RF), and asymmetry factor (AF) were evaluated. Table 4 indicated that the small variations that were indicated above relatively unaffected the selected parameters.

Table 5. Results of forced degradation studies of laboratory-made tablets

Storage condition	Time	% Recovery of piracetam[b] (mean ± SD, n = 3)
NaOH 1N[a]	16 hours at 80°C	73.9 ± 3.47
NaOH 2 N[a]	16 hours at 80°C	59.45 ± 3.71
HCl 1N[a]	16 hours at 80°C	80.76 ± 2.58
HCl 2N[a]	16 hours at 80°C	47.76 ± 0.80
H_2O_2 15%[a]	16 hours at 80°C	52.40 ± 5.38

[a]5 drops was added into 500 mg of powdered LM tablets.
[b]Purity and identity checks of the analyte peaks yielded good values (using a Hitachi model D-6500 chromatography data station software, DAD system manager, r > 0.99).

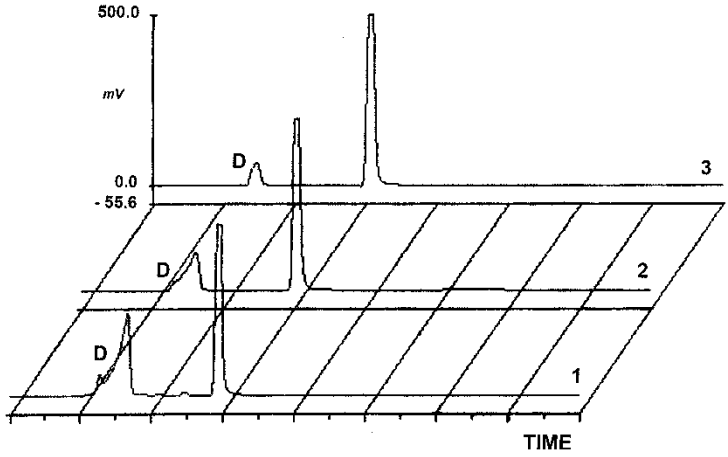

Figure 2. HPLC chromatogram (at 215 nm) of stressed samples, by using H_2O_2 (1), NaOH 1N (2), HCL 1N (3). For detailed information see text and Table 5. D: Degradation product(s), their UV spectra were presented on Figure 3.

Table 5 showed that although the recovery of the piracetam was reduced (47–80%) in stressed samples, purity and identity check of the analyte peaks using DAD detector yielded good values (>0.99), this showed that all the peaks were still pure and identical with the standard. This proved that the analyte peak was not interfered by the degradation products (see Figure 2). The UV spectra of the degradation products showed very similar spectra compared to the UV spectrum of piracetam (Figure 3).

The present work showed that the proposed HPLC method is suitable for the routine analysis of products of similar composition in the pharmaceutical industry quality control laboratories.

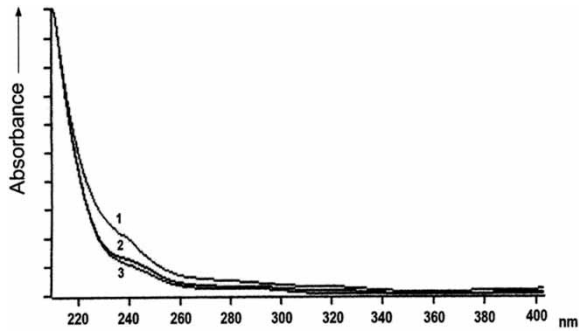

Figure 3. The UV spectra of the degradation product(s) using stressed samples with NaOH 1N (1), HCl 1N (2), H_2O_2 (3). For detailed information see text and Table 5.

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