Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 850 (2007) 544–547
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Quantification of *l*-stepholidine in rat brain and plasma by high performance liquid chromatography combined with fluorescence detection

John Odontiadis [a], Erin M. MacKenzie [a], Sridhar Natesan [b], David Mamo [b], Shitij Kapur [b], Glen B. Baker [a,*]

[a] *Bebensee Schizophrenia Research Unit and Neurochemical Research Unit, Department of Psychiatry, University of Alberta, Edmonton AB, Canada*
[b] *Centre for Addiction and Mental Health, 250 College St., Toronto ON, Canada*

Received 19 July 2006; accepted 20 November 2006
Available online 11 December 2006

Abstract

A sensitive and reliable assay for the quantification of *l*-stepholidine (SPD) in rat plasma and brain was developed using high performance liquid chromatography (HPLC) combined with fluorescence detection. Brain regions (prefrontal cortex, striatum, and cerebellum) and plasma from rats treated with SPD (10 mg/kg s.c.) 20, 40, 60, or 90 min prior to euthanasia were analyzed for SPD levels. Brain samples were homogenized in ice-cold 0.1 M perchloric acid and centrifuged to remove proteins. The supernatants and diluted plasma samples, to which *O*-desmethylvenlafaxine was added as a process standard, were basified and extracted with ethyl acetate. The organic phase was taken to dryness and the residue taken up in mobile phase. The samples were then injected into an HPLC equipped with a fluorescence detector (excitation and emission wavelengths set at 280 and 320 nm, respectively). The mean recovery of SPD was 74.6%, and reliability studies confirmed the reproducibility of the assay (intra- and inter-assay coefficients of variation of 4.8% and 5.3%, respectively). The assay was readily applicable to the brain and plasma samples obtained from rats injected with SPD as described above; the levels and patterns of disappearance of SPD in brain regions and plasma are shown.

Keywords: *l*-Stepholidine; High performance liquid chromatography; Antipsychotic; Schizophrenia

1. Introduction

l-Stepholidine (SPD; Fig. 1) is a natural compound extracted from the Chinese herb *Stephania intermedica*. SPD has been shown to possess agonist activity at dopamine (DA) D_1 receptors and antagonist activity at D_2 receptors, with higher affinity for the D_1 than the D_2 receptor [1–6]. Given that schizophrenia has been associated with reduced DA activity in the mesocortical system (consisting mainly of D_1 receptors) and increased DA activity in the mesolimbic pathway (consisting mainly of D_2 receptors) [7], SPD is an excellent candidate for a novel antipsychotic agent. Indeed, electrophysiological, biochemical, and behavioural studies in animals have suggested that SPD may possess antipsychotic properties [8,9]. Furthermore, published clinical experience in the Chinese medical literature with the extract suggest that it is well-tolerated in patients suffering from dyskinesia and is at least as efficacious as perphenazine in patients with schizophrenia [10,11]. Clinical trials investigating the therapeutic potential of this natural product in schizophrenia are currently underway in Canada.

Here we describe a rapid and sensitive method for the determination and quantitation of SPD in rat plasma and brain tissue. High performance liquid chromatography (HPLC) combined with fluorescence detection was used to quantify SPD. *O*-Desmethylvenlafaxine (ODV) was used as a process standard. To our knowledge this is the first method described for the extraction and quantitation of SPD from biological samples.

* Corresponding author at: Department of Psychiatry, 1E7.31 Mackenzie Centre, University of Alberta, Edmonton, AB, Canada T6G 2R7.
Tel.: +1 780 492 5994; fax: +1 780 492 6841.
E-mail address: glen.baker@ualberta.ca (G.B. Baker).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.11.039

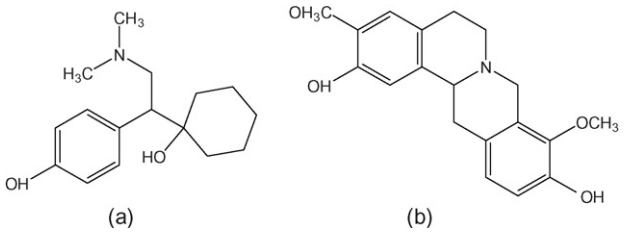

Fig. 1. The chemical structures of (a) *O*-desmethylvenlafaxine and (b) *l*-stepholidine.

2. Experimental

2.1. Materials

SPD was purchased from Calbiochem (La Jolla, CA, USA) and ODV was a gift from Wyeth Pharmaceuticals (Madison, NJ, USA). Methanol, acetonitrile, ethyl acetate, potassium carbonate, sodium phosphate (monobasic, anhydrous), hydrochloric acid, sulfuric acid, and perchloric acid were purchased from Fisher Scientific Canada (Mississauga, ON, Canada). All chemicals were of HPLC or reagent grade.

2.2. Standards

A stock solution of SPD for analysis was made by dissolving SPD in 1.0 M sulfuric acid in methanol, and ODV was dissolved in 2.7 M hydrochloric acid in methanol. Stocks were protected from light and stored at −80 °C, and were thawed prior to use. New stock solutions were prepared every 3 months.

2.3. Dosing, sample preparation and extraction

SPD was dissolved in 30% dimethylformamide in distilled water, and acidified with 2% glacial acetic acid. Male Sprague-Dawley rats weighing approximately 300–320 g received subcutaneous bolus injections of SPD at a dose of 10 mg/kg, and were euthanized 20, 40, 60, or 90 min after drug injection. Brains were removed immediately and the prefrontal cortex, striatum, and cerebellum were dissected out, flash-frozen, and stored at −80 °C until the analysis was conducted. Trunk blood was also collected, and plasma isolated by centrifugation and stored at −80 °C.

At the time of analysis, each brain region sample was thawed on ice, weighed, and homogenized in 5 volumes of ice-cold 0.1 M perchloric acid. Homogenates were then vortexed and centrifuged at 12,000 × *g* for 10 min. The supernatants were retained and diluted 40× with distilled water. ODV (25 ng) was added to 250 μl of the supernatant from each sample. SPD and ODV were then isolated by solvent extraction by shaking on a vortex mixer for 3 min with 1.5 ml ethyl acetate after addition of 100 μl of 25% potassium carbonate. Samples were centrifuged at 3000 × *g* for 5 min, and the organic phase was transferred to a glass tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 μl mobile phase and 45 μl was injected onto the HPLC analytical column. Standard curves were prepared in naïve (untreated) brain homogenate containing 7 SPD concentrations (0 to 100 ng/g) and a constant ODV concentration (25 ng) as a process standard.

Plasma samples were thawed on ice, vortexed, and diluted by a factor of 40 (i.e. 40 μl plasma was added to 1560 μl distilled water). Diluted plasma (250 μl) was used for the assay in the same procedure described for the brain supernatant. Standard curves were prepared in diluted naïve plasma containing 8 SPD concentrations (0 to 200 ng/ml) and a constant ODV concentration (25 ng) as a process standard.

2.4. Chromatography

HPLC analysis was conducted using a Waters Alliance system (Waters Corporation, Milford, MA) and a Shimadzu fluorescence detector (Shimadzu Scientific Instruments, Columbia, MD). The excitation and emission wavelengths were set at 280 and 320 nm, respectively. A 5 μm (spherical, pore size 125 A°) Waters X-Terra Rp C_{18} column (4.6 × 150 mm) and a μBondapak C_{18} guard column (10 μm, irregular, pore size 125 A°) were used and maintained at 30 °C. The mobile phase consisted of filtered 70% 30 mM sodium phosphate (pH 4.5) and 30% acetonitrile. The flow rate was set at 0.3 ml/min.

3. Results and discussion

3.1. Standard curve and validation data

Curves were generated by plotting the ratio of the area under the curves of SPD:ODV versus the concentration of SPD. The curves were linear, with r^2 values above 0.99 obtained consistently ($r^2 = 0.9965 \pm 0.0020$, mean ± SD for 6 individual curves). The method was shown to be linear up to 1000 ng/ml, but for the purposes of the analysis described here, it was not necessary to go above 200 ng/ml. The mean standard curve slopes and intercepts were 0.0572 ± 0.0036 and -0.04827, respectively.

Experiments showed that the mean extraction recovery of SPD was 74.6%, and the LOQ and LODs were 1 and 0.3 ng/ml, respectively. Intra-assay variability, calculated by extracting 6 samples of the same concentration (10 ng/ml) and comparing the analyte-to-process standard ratio, yielded a coefficient of variation of 4.8%. Inter-assay reliability, calculated by comparing the analyte-to-process standard ratio of 10 ng/ml of SPD from 3 curves from different days, yielded a coefficient of variation of 5.3%.

The retention times for SPD and ODV were 11.3 and 10.4 min, respectively. There was not complete baseline separation, but the sensitivity of the method was still sufficient to provide routine analysis of SPD in the brain and plasma samples under investigation in the study described here. Venlafaxine itself could also be used as process standard but increased the time required for analysis. Several gradients were tested but did not result in improved separation. Chromatographs of naïve and SPD-treated plasma and cerebellum samples are shown in Fig. 2, along with a chromatogram of two spiked standards in naïve plasma.

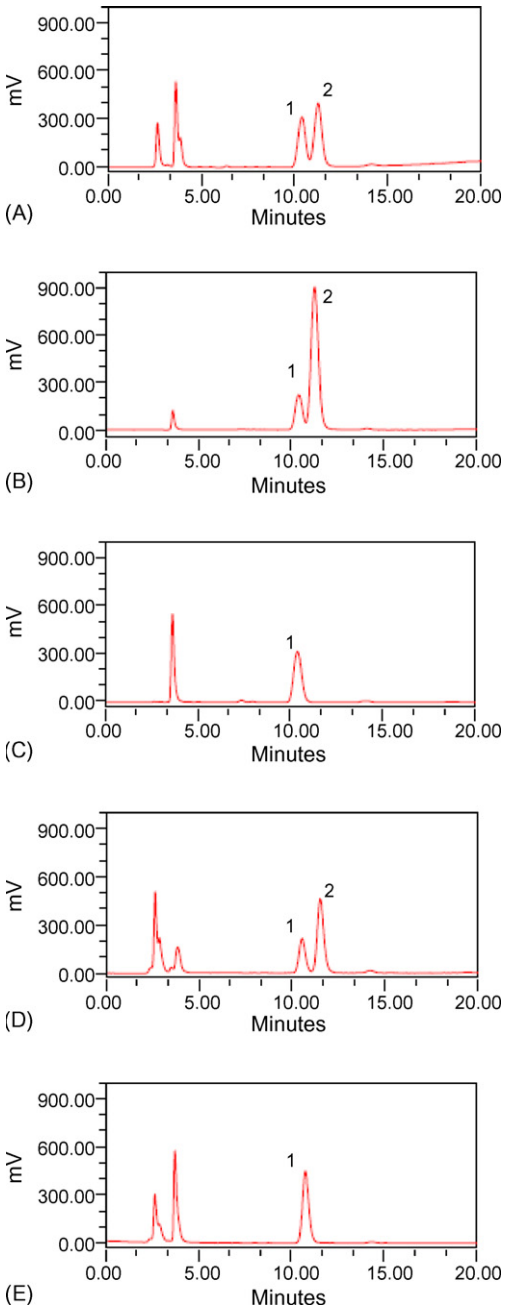

Fig. 2. Typical chromatographs of (A) naïve plasma spiked with 25 ng of the process standard ODV (1) and 100 ng SPD (2); (B) plasma from a rat euthanized 20 min after SPD injection (10 mg/kg s.c.); (C) plasma of untreated rat spiked with ODV; (D) cerebellum of rat euthanized 40 min after SPD injection; (E) cerebellum of untreated rat spiked with ODV. There were no interfering peaks at the same retention time as ODV in the naïve samples.

3.2. Method application to rat brain and plasma

This method was used to determine the SPD concentration in the prefrontal cortex, striatum, cerebellum, and plasma of rats 20, 40, 60, or 90 min after subcutaneous administration of SPD (10 mg/kg s.c.). These results are summarized in Fig. 3 and demonstrated that the patterns of disappearance of SPD in brain regions and plasma were similar.

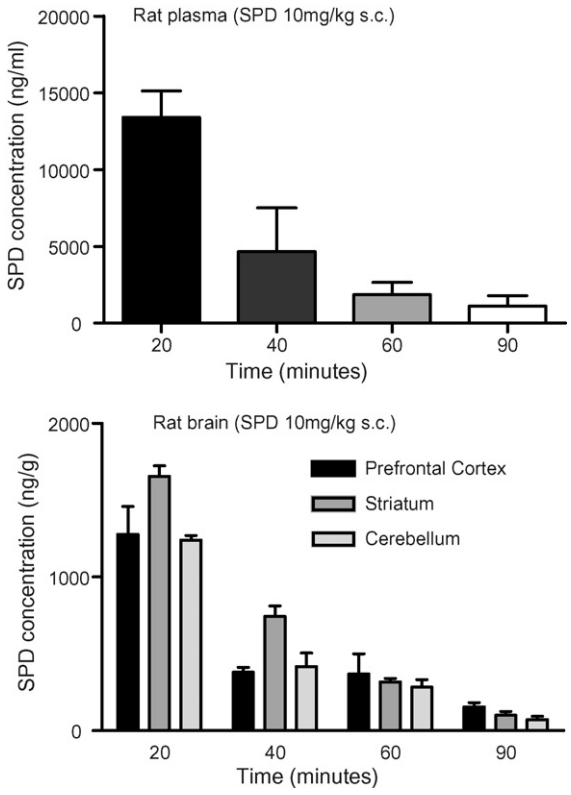

Fig. 3. Concentrations of SPD in rat plasma and brain at 20, 40, 60, and 90 min following drug injection. Values represent means ± standard error of the mean ($n = 5$).

4. Conclusions

To our knowledge, this is the first analytical method described for the extraction of SPD from biological samples. This procedure requires minimal work-up and will now be applied to studies in human subjects taking SPD. A potential drawback to this method for use in the clinical population is that ODV is an active metabolite of venlafaxine, a frequently-prescribed antidepressant drug; therefore in patients taking both venlafaxine and stepholidine, an alternate process standard would have to be used.

Acknowledgements

Funds were provided by the Stanley Medical Research Institute (SMRI), the Abraham and Freda Berger Memorial Fund, and the Canada Research Chair and Canada Foundation for Innovation programs. EMM gratefully acknowledges studentship support from the Alberta Heritage Foundation for Medical Research and the Faculty of Medicine and Dentistry 75th Anniversary Fund. The authors are indebted to Gail Rauw for her expert technical assistance.

References

[1] S.X. Xu, L.P. Yu, Y.R. Han, Y. Chen, G.Z. Jin, Zhongguo Yao Li Xue Bao 10 (1989) 104.
[2] Z.T. Zhu, Y. Fu, G.Y. Hu, G.Z. Jin, Sheng Li Xue Bao 52 (2000) 123.

[3] Z.T. Zhu, Y. Fu, G.Y. Hu, G.Z. Jin, Life Sci. 67 (2000) 1265.
[4] Z.J. Dong, X. Guo, L.J. Chen, Y.F. Han, G.Z. Jin, Life Sci. 61 (1997) 465.
[5] Z.J. Dong, L.J. Chen, G.Z. Jin, I. Creese, Biochem. Pharmacol. 54 (1997) 227.
[6] L.L. Zou, J. Liu, G.Z. Jin, Biochem. Pharmacol. 54 (1997) 233.
[7] A. Abi-Dargham, H. Moore, Neuroscientist 9 (2003) 404.
[8] X.X. Zhang, B.C. Sun, G.Z. Jin, Sci. China 40 (1997) 531.
[9] Y.Q. Mo, X.L. Jin, Y.T. Chen, G.Z. Jin, W.X. Shi, Neuropsychopharmacology 30 (2005) 261.
[10] N. Cai, Zhonghua Shen Jing Jing Shen Ke Za Zhi 21 (1988) 281.
[11] D.C. Wu, X.Z. Xing, W. Wang, H. Xie, Y. Wang, X. Wang, J. Yin, Q. Sun, Z. Cong, G.Z. Jin, Chin. J. New Drugs Clin. Rem. 22 (2003) 155.