

Original Paper

Analysis of Acid Dissociation Equilibrium of Bupropion by Capillary Zone Electrophoresis **After Degradation Under Heat**

Toshio TAKAYANAGI^{*1}, Daichi ITOH², Hitoshi MIZUGUCHI¹¹Graduate School of Science and Technology, Tokushima University, 2-1 Minamijousanjima-cho, Tokushima 770-8506, Japan²Graduate School of Advanced Technology and Science, Tokushima University, 2-1 Minamijousanjima-cho, Tokushima 770-8506, Japan

Abstract

An acid dissociation constant (pK_a) of bupropion has been determined in an aqueous solution by capillary zone electrophoresis (CZE). Electrophoretic mobility of bupropion was measured by CZE over its acid-dissociating pH range, and a pK_a value has been determined as 8.75 ± 0.02 (mean \pm standard error, ionic strength: **0.010 mol dm⁻³**). Although bupropion degrades under heated and alkaline conditions and the degraded species from bupropion coexist in the aqueous solution, the pK_a value of bupropion can also be determined by CZE with the residual bupropion without any interference from the degraded species. The pK_a value determined **after** the heat-**degradation** was 8.74 ± 0.02 ; the result agreed well with the one determined with the freshly prepared solution. Utilization of the separation characteristics of CZE has been demonstrated **with bupropion** for the analysis of the fast equilibrium of acid-base reaction **under the coexistence of the degraded species**.

Keywords: Bupropion; Acid dissociation constant; Capillary zone electrophoresis; Heat degradation

1. Introduction

Capillary zone electrophoresis (CZE) is a useful tool to determine **the** acid dissociation constant (pK_a) of pharmaceuticals in an aqueous solution [1,2]. Protonation or deprotonation of any acid-base substances **s** accompanies with the changes in its effective charge with varying pH of the solution, which results in the change in the effective electrophoretic mobility of the substance. The effective electrophoretic mobility was used for the determination of the acid dissociation constant of the substances of interest. Advantages on using CZE for the pK_a determination include: (1) aqueous solution is handled in CZE without any stationary matrix, (2) low concentration of an analyte is acceptable if detected, (3) small sample volume is available, and (4) other substances except the analyte are allowed to coexist owing to the CZE separation [1,2]. Dissociation constant of ropinirole has been determined by CZE in the presence of some impurities [3].

The CZE separation is also beneficial to the analysis of labile substances; degraded species from the labile substance as an analyte can be resolved from the analyte,

and thus the equilibrium is analyzed with the labile analyte by measuring the effective electrophoretic mobility [4,5]. Phenolphthalein is known to be degradable at alkaline aqueous solution. Although the acid-base property of phenolphthalein was difficult to analyze by photometric titration in **an** aqueous solution [6], the pK_a values were determined by CZE even under the degrading conditions by resolving the kinetically degrading species from the equilibrium species of phenolphthalein [4]. Applicability of the CZE analysis has been demonstrated **with** the labile substances with a series of pharmaceutical candidates [5]. Acid dissociation constants of degradable pharmaceuticals of haloperidol [7] and pravastatin [8] have been analyzed by CZE under the coexistence of their degradation products.

Bupropion hydrochloride (**Fig. 1, base form**) is an antidepressant of aminoketone class (Wellbutrin, GlaxoSmithKline) with neurochemical properties different from those of common tricyclic antidepressants and monoamine oxidase inhibitors [9,10]. It works as a dopamine and norepinephrine reuptake inhibitor [11]. It is also licensed to non-nicotine pharmacological therapy for

*Corresponding author: Toshio TAKAYANAGI
Tel: +81-88-656-7409; Fax: +81-88-656-7409
E-mail: toshio.takayanagi@tokushima-u.ac.jp

smoking cessation (Zyban, GlaxoSmithKline) [12]. Metabolism in human plasma [13] and degradation under stressed conditions [14] have been investigated with BUP. It is reported that 3-chlorobenzoic acid and some alcohols are formed by the degradation of bupropion under heated and alkaline conditions [14]. Capillary zone electrophoresis has been used for the chiral resolution of BUP by electrokinetic chromatography by using sulfated- β -cyclodextrin as a chiral selector [15].

In this study, utilization of the CZE determination of pK_a has been demonstrated with the degradable bupropion in an aqueous solution **even after the degradation under heat**. An acid dissociation constant of BUP has been determined with both freshly-prepared and heat-degraded BUP.

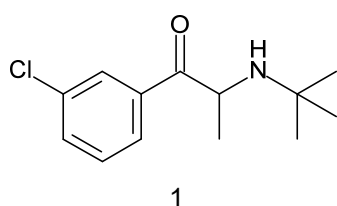


Fig. 1. Structure of bupropion (BUP).

2. Experimental

2.1. Apparatus

An Agilent Technologies ^{3D}CE was used as a capillary electrophoresis system, equipped with a photodiode array detector. A fused-silica capillary purchased from GL Sciences (Tokyo, Japan) was held in a capillary cassette and the cassette was installed in the CE system; the capillary cassette was thermostat at 25°C. The dimensions of the capillary were 64.5 cm in total length, 56 cm in effective length from the injection point to the detection point, and 50 μm in inner diameter. An Agilent Technologies ChemStation software (Ver. B.04.02) was used to control the ^{3D}CE system and to record and analyze the electropherograms.

A Waters LCT Premire XE was used as an LC-MS system. A reversed-phase column of Acquity UPLC BEH C18 (50 mm in length \times 2.1 mm in inner diameter, particle size: 1.7 μm) was attached to the LC-MS system. A mixed solvent of 50%(v/v) methanol in water was used as an eluent. A TOA DKK HM-25G pH meter equipped with a combined glass electrode was used for the pH measurements of the separation buffers after calibrated daily with the standard solutions. A Nitride Semiconductors (Naruto, Tokushima) NS 375 LIM was used as a UV lamp.

2.2. Chemicals

Bupropion hydrochloride was purchased from Tokyo Chemical Industry (Tokyo, Japan). It was dissolved in

purified water at a concentration of $1.0 \times 10^{-3} \text{ mol dm}^{-3}$; it was used after dilution with water. 1-Ethylquinolinium iodide (EtQu^+I^-) and 3-chlorobenzoic acid were also from Tokyo Chemical Industry. Other reagents were of analytical grade. Water used was purified by Milli-Q gradient A10 (Merck-Millipore, Darmstadt, Germany).

Separation buffers were prepared with $0.010 \text{ mol dm}^{-3}$ MES-NaOH (pH 5.5–6.9), $0.010 \text{ mol dm}^{-3}$ HEPES-NaOH (pH 7.1–8.4), or $0.010 \text{ mol dm}^{-3}$ H_3BO_3 -NaOH (pH 8.5–9.3). Ionic strength of the buffers was controlled at $0.010 \text{ mol dm}^{-3}$ by adding appropriate amount of NaCl.

2.3. Procedure for the determination of pK_a by CZE

After the separation capillary was filled with a separation buffer and equilibrated, a sample solution containing bupropion hydrochloride at $2.0 \times 10^{-5} \text{ mol dm}^{-3}$ and 1-ethylquinolinium iodide at $2.0 \times 10^{-5} \text{ mol dm}^{-3}$ was introduced into the capillary by applying pressure (250 mbar s). Ethanol was also added in the sample solution at the concentration of 1.0%(v/v) to monitor the electroosmotic flow (EOF). Then, a separation voltage of 25 kV was applied to the capillary for the zone electrophoresis. An analyte of BUP was photometrically detected at 220 nm. Effective electrophoretic mobility of BUP, $\mu_{\text{eff,Bup}}$, was calculated in an ordinary manner with the migration times of BUP and EOF, and an acid dissociation constant of BUP was determined by a non-linear least-squares analysis with a series of the $\mu_{\text{eff,Bup}}$ values after standardized with the electrophoretic mobility of EtQu^+ . A software of R program (Ver. 3.1.1) was used for the analysis [16].

3. Results and discussions

3.1. Determination of **an** acid dissociation constant of BUP by CZE

Bupropion with its protonated form, HBup^+ , dissociates a proton to form a neutral Bup in an aqueous solution as in equilibrium (1) with its acid dissociation constant, K_a . The acid dissociation constant is written as in Eq. (2).



$$K_a = \frac{[\text{H}^+][\text{Bup}]}{[\text{HBup}^+]} \quad (2)$$

The acid-base equilibrium is sufficiently fast against the separation time of CZE. Thus the effective electrophoretic mobility of BUP, $\mu_{\text{eff,Bup}}$, is contributed from the two species of HBup^+ and Bup, and it is written as in Eq. (3).

$$\mu_{\text{eff,Bup}} = \frac{[\text{H}^+]}{[\text{H}^+] + K_a} \mu_{\text{ep,HBup}^+} + \frac{K_a}{[\text{H}^+] + K_a} \mu_{\text{ep,Bup}} \quad (3)$$

where $\mu_{ep,HBup}$ and $\mu_{ep,Bup}$ are the electrophoretic mobility of $HBup^+$ and Bup species, respectively. Since Bup is neutral, a value of $\mu_{ep,Bup}$ in Eq. (3) was set as zero in the analysis. A series of pairs of $[H^+]$ and $\mu_{eff,BUP}$ were input in Eq. (3) and values of K_a and $\mu_{ep,HBup}$ were optimized by a non-linear least-squares analysis [17].

In this study, the acid dissociation constant of $HBup^+$ has firstly been determined through the electrophoretic mobility of BUP over its dissociable pH range. Typical electropherograms are shown in Fig. 2. The migration time of BUP is shorter than that of EOF at the weakly acidic to neutral pH region, which suggests the cationic BUP in an aqueous solution. It is getting closer to that of EOF with increasing pH of the separation buffer. The result is interpreted as the acid dissociation equilibrium of $HBup^+$, as written in Eq. (1). Changes in the effective electrophoretic mobility of BUP are shown in Fig. 3. The value of $\mu_{eff,Bup}$ was standardized with that of $EtQu^+$, μ_{EtQu} , since $EtQu^+$ is a monocation over the pH range and the electrophoretic mobility of $EtQu^+$ is thus a certain value. The standardized value, $\mu_{eff,Bup}/\mu_{EtQu}$, was used for the analysis of the K_a value. As is noticed in the electropherograms in Fig. 2, it can also be noticed in Fig. 3 that the positive electrophoretic mobility has been observed with BUP at the weakly acidic pH region. The $\mu_{eff,Bup}/\mu_{EtQu}$ value decreases with increasing pH of the separation buffer by the deprotonation from $HBup^+$. An acid dissociation constant of $pK_a = 8.75 \pm 0.02$ (mean \pm standard error) was determined by the analysis. A curve in

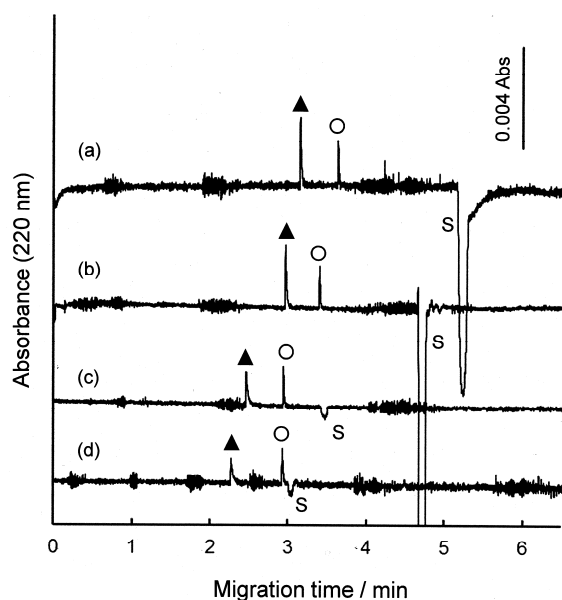


Fig. 2. Electropherograms of bupropion with changing pH of the separation buffer. Signals: \circ , BUP; \blacktriangle , $EtQu^+$. S, EOF. pH of the separation buffers: (a), 6.22; (b), 7.08; (c), 8.50; (d), 9.60. The CZE conditions are written in the text.

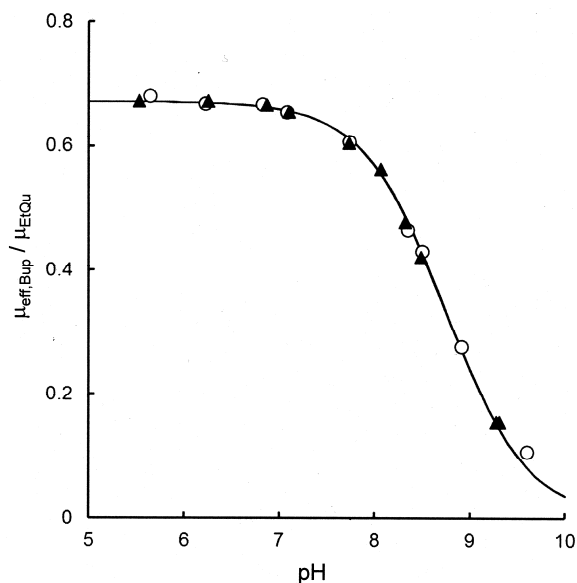


Fig. 3. Changes in the effective electrophoretic mobility of BUP with pH. \circ , Freshly prepared BUP; \blacktriangle , degraded BUP under $70^\circ C$ for 7 days. The CZE conditions and the degradation conditions are written in the text.

Fig. 3 is the simulated result with the optimized pK_a and $\mu_{eff,Bup}/\mu_{EtQu}$ values. The pK_a value determined in this study is somewhat different from the reported values; 7.0 [18,19], 7.2 [20], 7.4 [21] and 7.9 [9,10]. It is not certain if the reported values could have excluded the interferences from the degraded species. In CZE, the electrophoretic mobility is measured in an aqueous solution without any interference from the coexisting substances including the degraded species and a drastic change in electrophoretic mobility accompanies with the acid dissociation equilibrium, the pK_a value determined in this study would be a reliable one compared with the reported values.

3.2. Degradation of BUP

It has been reported that BUP is degradable under several conditions [14]. In this study, five stressed conditions of acidic ($0.010 \text{ mol dm}^{-3} \text{ HCl}$), alkaline ($0.010 \text{ mol dm}^{-3} \text{ NaOH}$), UV light radiation (375 nm, 1.4 W; the bupropion solution in a glass vial was irradiated with the light on the top of the window of the lamp housing), heat ($70^\circ C$), and oxidative ($1.0\%(\text{v/v}) \text{ H}_2\text{O}_2$) were examined. An aliquot of $1.0 \times 10^{-3} \text{ mol dm}^{-3}$ aqueous BUP solution was exposed to one of the stressed conditions, and a small portion of it was periodically sampled over several days and analyzed by CZE after 10 times dilution with the purified water.

The residual amount of BUP was quantified by CZE, where the peak height of BUP was standardized with that of $5.0 \times 10^{-4} \text{ mol dm}^{-3} \text{ EtQu}^+$ for the quantification. The results are shown in Fig. 4; bupropion gradually decomposed under heated or alkaline conditions as

previously reported [14]. The residual amount of BUP was about 50% under heated for 8 days and about 10% under alkaline for 7 days.

The degraded solution of BUP heated at 70°C for 11 days was analyzed by the LC-MS to examine the degradation products. After the degraded solution was diluted 100 times, an aliquot of 20 μL of the solution was introduced into the LC-MS. Negative masses of 154.99 and 156.99 with a retention time at 0.74 min were detected. Both of the masses correspond to 3-chlorobenzoate ion (3-CIBz⁻, Fig. 5, 2), and it is supposed to be formed from BUP, as has been reported [14]. Positive masses of 184.05 and 186.05 with a retention time at 0.94 min were also detected by the LC-MS. They would be 1-(3-chlorophenyl)-2-hydroxy-1-propanone (Fig. 5, 3), as was also reported [14].

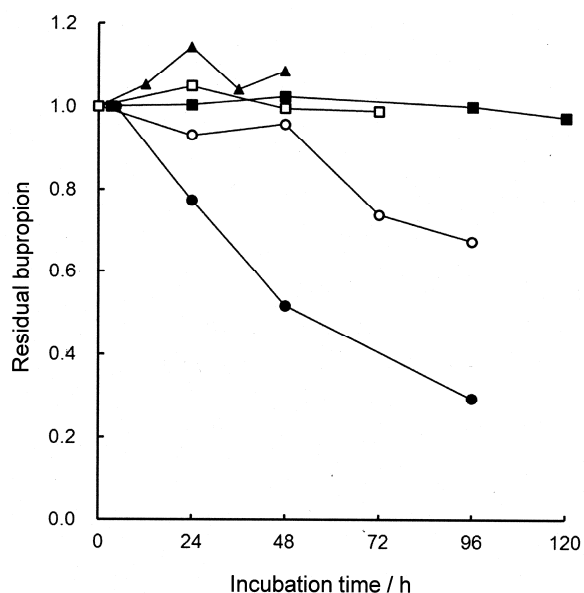


Fig. 4. Degradation profile of BUP under some stressed conditions. Residual BUP was quantified by CZE, where $5.0 \times 10^{-4} \text{ mol dm}^{-3}$ EtQu⁺ was used as a quantification standard with the peak height. Degradation conditions: ○, heated at 70°C; ●, alkaline with $0.010 \text{ mol dm}^{-3}$ NaOH; ▲, acidic with $0.010 \text{ mol dm}^{-3}$ HCl; □, UV light irradiation; ■, oxidative with 1.0%(v/v) H₂O₂.

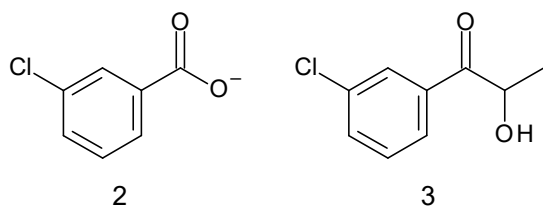


Fig. 5. Degraded species from bupropion reported [14]. The two species were also detected in this study by LC-MS.

The degraded species from BUP was verified by CZE; the results are shown in Fig. 6. A CZE signal corresponding to an anionic species is detected at 5.9 min with the degraded solution. When 3-chlorobenzoate is spiked to the degraded solution, the CZE signal at 5.9 min increased. Therefore, the degraded species is also verified as 3-CIBz⁻. However, a degraded species of 3 was not detected by CZE, because it is neutral.

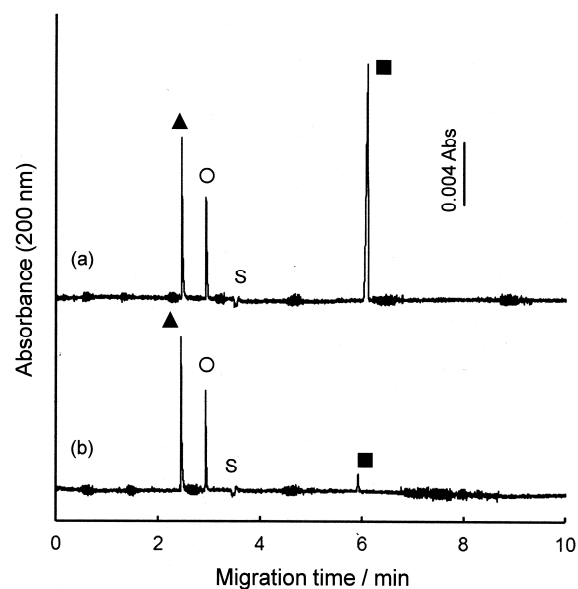


Fig. 6. Electropherograms of degraded BUP for the qualitative analysis of the degradation product. Separation buffer, H₃BO₃-NaOH (pH 8.49). Detection wavelength, 200 nm. Signals: ○, BUP; ▲, EtQu⁺; ■, a degradation product (3-CIBz⁻). S, EOF. Other conditions are written in the text. (a) degraded BUP under 70°C for 8 days spiked with $1.0 \times 10^{-4} \text{ mol dm}^{-3}$ 3-chlorobenzoic acid; (b) degraded BUP under 70°C for 8 days. The degraded solution was diluted by 10 times and measured.

3.3. Determination of an acid dissociation constant of BUP after heat-degradation

It is noticed in Fig. 6 that the residual BUP is still detected with the degraded BUP solution. The residual bupropion is resolved from the degraded species. Therefore, the acid dissociation equilibrium of BUP can be analyzed with the residual BUP by CZE through the measurements of its electrophoretic mobility, even in the presence of the degraded species.

The acid dissociation constant of BUP was also determined with the degraded BUP solutions heated at 70°C for 7 days. Since CZE includes electrophoretic separation, the degraded species would be resolved from BUP and they would not interfere with the BUP signal. Typical electropherograms are shown in Fig. 7. Difference in the electropherograms between freshly-prepared (Fig. 2) and heat-degraded (Fig. 7) solutions is that the relative signal

height of BUP against a standard of EtQu⁺ is smaller with the degraded solution. However, the CZE signals corresponding to BUP is still detected with the degraded solution. Signals corresponding to the degradation product of 3-ClBz⁻ are also detected in Fig. 7. Effective electrophoretic mobility of BUP was also plotted as a function of pH; the results are also shown in Fig. 3. Analysis of the changes in the effective electrophoretic mobility gave a pK_a value of 8.74±0.02. The pK_a value determined with the degraded solution is almost identical to the one determined with the freshly prepared solution.

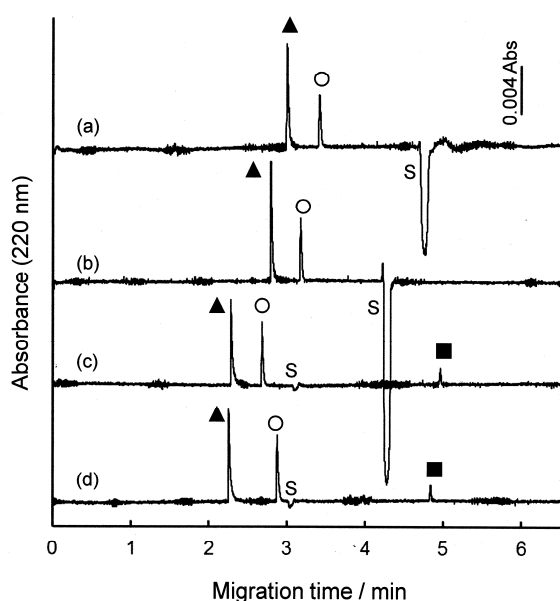


Fig. 7. Electropherograms of the degraded BUP with changing pH of the separation buffer. The degradation conditions are written in the text. pH of the separation buffer: (a), 6.25; (b), 7.10; (c), 8.49; (d), 9.28. ○, BUP; ▲, EtQu⁺; ■, 3-ClBz⁻ as a degradation product from BUP.

4. Conclusions

In this study, an acid dissociation constant of bupropion has been determined after the heat-degradation. Even though BUP is degraded by heat, the acid dissociation constant was successfully determined with the residual BUP without any interference from the degraded species. It is demonstrated in this study that the CZE analysis is useful to such acid-base equilibria as including the degradation reaction and the degradation products.

Acknowledgement

This study was supported by the Grant-in-Aid for Scientific Research Program (KAKENHI) of the Japan Society for the Promotion of Sciences (JSPS), Grant Number 26410154. The authors are grateful to Dr. Fujinaga (Tokushima University) and Dr. Ueta (Tokushima

University) for the LC-MS measurements.

References

- [1] Poole, S. K.; Patel, S.; Dehring, K.; Workman, H.; Poole, C. F. *J. Chromatogr. A* **2004**, *1037*, 445-454.
- [2] Nowak, P.; Woźniakiewicz, M.; Kościelniak, P. *J. Chromatogr. A* **2015**, *1377*, 1-12.
- [3] Coufal, P.; Štulík, K.; Claessens, H. A.; Hardy, M. J.; Webb, M. *J. Chromatogr. B* **1998**, *720*, 197-204.
- [4] Takayanagi, T.; Motomizu, S. *Chem. Lett.* **2001**, 14-15.
- [5] Örnkvist, E.; Linusson, A.; Folestad, S. *J. Pharm. Biomed. Anal.* **2003**, *33*, 379-391.
- [6] Tamura, Z.; Abe, S.; Ito, K.; Maeda, M. *Anal. Sci.* **1996**, *12*, 927-930.
- [7] Shimakami, N.; Yabutani, T.; Takayanagi, T. *Bunseki Kagaku*, **2014**, *63*, 643-648.
- [8] Takayanagi, T.; Amiya, M.; Shimakami, N.; Yabutani, T. *Anal. Sci.* **2015**, *31*, 1193-1196.
- [9] Bryant, S. G.; Guernsey, B. G.; Ingram, N. B. *Clin. Pharm.* **1983**, *2*, 525-537.
- [10] Dufresne, R. L.; Weber, S. S.; Becker, R. E. *Drug Intel. Clin. Pharm.* **1984**, *18*, 957-964.
- [11] Stahl, S. M.; Pradko, J. F.; Haight, B. R.; Modell, J. G.; Rockett, C. B.; Learned-Coughlin, S. *Prim. Care Companion J. Clin. Psychiatry* **2004**, *6*, 159-166.
- [12] Slemmer, J. E.; Martin, B. R.; Damaj, M. I. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 321-327.
- [13] Loboz, K. K.; Gross, A. S.; Ray, J.; McLachlan, A. J. *J. Chromatogr. B* **2005**, *823*, 115-121.
- [14] O'Byrne, P. M.; Williams, R.; Walsh, J. J.; Gilmer, J. F. *J. Pharm. Biomed. Anal.* **2010**, *53*, 376-381.
- [15] Castro-Puyana, M.; Ángeles García, M.; Luisa Marina, M. *J. Chromatogr. B* **2008**, *875*, 260-265.
- [16] The R Project for Statistical Computing, <http://www.r-project.org/>.
- [17] Takayanagi, T. *Bunseki Kagaku* **2015**, *64*, 105-116.
- [18] Lemke, T. L.; Williams, D. A. *Foye's Principles of Medicinal Chemistry*, 6th ed; Lippincott Williams & Wilkins, **2007**, Appendix A, p. 1344.
- [19] Ghafourian, T.; Barzegar-Jalali, M.; Hakimiha, N.; Cronin, M. T. D. *J. Pharm. Pharmacol.* **2004**, *56*, 339-350.
- [20] Shardlow, C. E.; Generaux, G. T.; MacLauchlin, C. C.; Pons, N.; Skordos, K. W.; Bloomer, J. C. *Drug Metab. Dispos.* **2011**, *39*, 2076-2084.
- [21] Myung, S.-W.; Yoon, S.-H.; Kim, M. *Analyst* **2003**, *128*, 1443-1446.