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9-Fluorenylmethyl chloroformate as a fluorescence-labeling reagent for derivatization of carboxylic acid moiety of sodium valproate using liquid chromatography/tandem mass spectrometry for binding characterization: A human pharmacokinetic study

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ABSTRACT

In High Performance Liquid Chromatographic (HPLC) determination of chemicals with acidic functions, different labeling agents are used to improve sensitivity of the assay. 9-Fluorenylmethyl chloroformate (FMOC-Cl), on the other hand, is a suitable labeling agent, which reacts with both primary and secondary amines and less readily with hydroxyl groups in alkaline conditions. However, the reagent has not been applied in labeling of chemicals with acidic function yet. In this study which is the first report on application of FMOC-Cl in derivatization and analysis of a drug with acidic function, valproic acid (VPA), one of a series of fatty carboxylic acids with anticonvulsant activity, was derivatized using the reagent and quantified in serum samples by HPLC with fluorescence detection. In addition, to document the reaction between the labeling agent and carboxylic acid moiety of the drug, we developed a liquid chromatography–tandem MS/MS (LC–MS/MS) method. Following liquid–liquid extraction, derivatization of the drug and an internal standard was achieved in alkaline medium. The elute was monitored by a fluorescence detector with respective excitation and emission wavelengths of 265 and 315 nm. The present method is more sensitive comparing with other published HPLC procedures for analysis of VPA. The assay is sensitive enough to measure drug levels obtained in human single dose studies with a limit of quantification of 0.01 μg/mL. Also the method is linear over the concentrations range of 0.01–32 μg/mL of VPA in human serum using 100 μL serum sample and 5 μL injection. The coefficient variation values of both inter and intra day analysis were less than 12% and the percentage error was less than 4%. The method performance was studied and the validated procedure applied in a randomized cross-over bioequivalence study of two different VPA preparations in 24 healthy volunteers.

1. Introduction

Fluorescence labeling of carboxylic acids and other compounds with acidic function is accomplished mainly by methoxycoumorin or phenacyl ester derivatives. Most of these agents however, are light-sensitive and/or need multi steps reaction to convert a non-fluorescent compound into a fluorescent product [1]. 9-Fluorenylmethyl chloroformate (FMOC-Cl; Fig. 1A) is an ester, which is used to introduce FMOC group as the FMOC carbamate for protection of amines. Because the fluorenyl group is highly fluorescent, the labeling agent is suitable for analysis of primary and secondary amines in alkaline conditions [2]. FMOC-Cl can also be used in derivatization of chemicals with hydroxyl groups. The resulted FMOC-alcohol has strong fluorescence absorbance and the reagent has been used in analysis of macrolide antibiotics [3,4]. However, application of FMOC-Cl for derivatization and analysis of carboxylic acids has not been reported yet. Valproic acid (2-propylvaleric acid, VPA; Fig. 1B) is an antiepileptic agent which is used in the treatment of various types of epilepsy as well as in the treatment of bipolar disorders, major depression, migraine headaches and schizophrenia [5]. The neurological adverse effects of VPA are dose-dependent thus, its blood level quantification in epileptic patients is recommended. [6]. Therefore, a simple and sensitive analytical method is required for therapeutic drug monitoring (TDM) and to quantify VPA levels in

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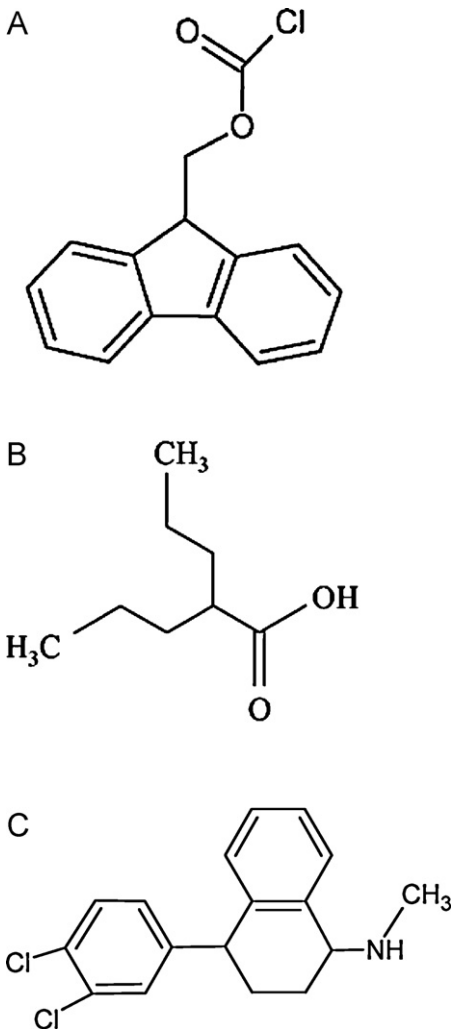

Fig. 1. Chemical structures of (A) Valproate (VPA), (B) 9-Fluorenylmethyl chloroformate (FMOC-Cl) and (C) the I.S. Sertraline.

single dose pharmacokinetic studies. To quantify blood levels of the drug several analytical methods including GC [7–12], LC–MS [13–18], capillary electrophoresis [19] and HPLC [19–26] have been reported. Direct assay of the drug using HPLC and conventional UV detection at 210 nm has been reported [20]. However, due to weak UV absorbance of the drug the system should be set at highest sensitivity. Furthermore high background noises are provided at this wavelength, which makes difficult to obtain a stable baseline. To improve sensitivity and suitability of analysis derivatization technique has been reported using different labeling agents including 4-bromomethyl-6,7-dimethoxycoumarin (BrMMC) [21], 6,7-methylenedioxy-1-methyl-2-oxo-1,2dihydroquinoxaline-3-ylpropionohydrazide [22], 2-(2-naphthoxy)ethyl2-(piperidino) ethanesulfonate (NOEPES) [23] and 4-bromophenacyl bromide [24]. Recently *N*-(1-Naphthyl) ethylenediamine has been used as new regent for derivatization of VPA in human plasma [25]. This method however, is tedious, needs multi steps extraction procedure and long reaction time (1 h, 85 °C). Derivatization of VPA with NOEPES needs a toxic solvent (toluene) and long reaction time (1 h 95 °C). BrMMC derivative is light sensitive and the reaction should be done in a dark medium. In other published methods low sensitivity of the assay and/or long reaction time of derivatization have been reported [26,19].

Present study is the first report on application of FMOC-Cl in labeling of a chemical with carboxylic acid function in which the reagent has been used for derivatization and analysis of VPA an antiepileptic agent with the acidic group. Furthermore, in this study an LC–MS/MS method is described and applied to document the reaction between FMOC-Cl and carboxylic acid moiety of the drug. The new method was validated and used in a randomized cross-over bioequivalence study of two different VPA preparations in 24 healthy volunteers.

2. Materials and methods

2.1. Chemicals

VPA (purity 99.5%), sertraline (I.S.; Fig. 1C) and FMOC-Cl were from Sigma (St. Louis, MO, USA). HPLC grade acetonitril and methanol as well as diethyl ether and hydrocloridric acid were purchased from Merck (Darmstand, Germany). All reagents were of the maximum available purity and were used without further purification. Water was glass-double distilled and further purified for HPLC with a Maxima purification system (USF ELGA, England).

2.2. Standard solutions

A stock solution of VPA and the I.S. (1000 μg/mL) were prepared in acetonitrile. Working solutions of VPA (0.1–320 μg/mL) and the I.S. (20 μg/mL) were prepared by dilution of the appropriate volumes in acetonitrile. Solutions of FMOC-Cl (500 μg/mL) and HCl (0.1 M) were prepared in acetonitrile and distilled water, respectively. A borate buffer (0.05 M) was prepared in water and adjusted to pH 9.3 with 0.05 M sodium hydroxide solution. All solutions stored at 4 °C and were stable for at least 4 weeks.

2.3. Instrumentation

2.3.1. HPLC system

The HPLC system used consisted of two pumps of Shimadzu LC-10A solvent delivery system, a system controller (SCL 10AD), a spectroflurometric detector (RF-551) operated at excitation and emission wavelengths of 265 and 315 nm, respectively, a column oven (CTO-10A), a degasser (DGU-3A) and a data processor (C-R4A) all from Shimadzu, Kyoto, Japan. The analytical column was a Shim pack CLC-ODS (Shimadzu, Kyoto, Japan), 150 mm × 4.6 mm I.D., 5 μm particle size, which was protected by a Shim-pack G-ODS guard column (1 cm × 4.0 mm I.D., 5 μm particle size). A mixture of acetonitril and distilled water (78:22) was used as the mobile phase. The column oven temperature was set at 50 °C and the mobile phase was filtered, degassed and pumped at a flow rate of 1.7 mL/min.

2.3.2. LC–MS/MS system

An Agilent 1200 series LC system consisting of a quaternary delivery pump, a thermostated column compartment, a degasser (Agilent Technologies, Germany) and a Rheodyne 7725i manual injector valve with a 20 μL sample loop (Cotati, CA, USA) was used. The mass analysis was performed with an Agilent 6410 Triple Quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) which was run by Agilent MassHunter Workstation data acquisition system B.01.03. Ionization was achieved using electrospray ionization (ESI) in the negative mode with the capillary voltage 4000 V. Nitrogen was used as nebulizer gas with nebulizer pressure of 40 psi and source temperature of 100 °C. Drying gas (nitrogen) was heated to 300 °C and delivered at a flow-rate of 10 L/min. Fragmentor voltage and collision energy for all analytes were 135 and 5 V, respectively and dwell time was 200 ms. Separation of FMOC derivative of VPA was carried out isocratically on a MZ PerfectSil target C18 column (125.0 mm ×4.0 mm I.D., 5 μm) using a C18 guard column (MZ-Anaysentechnik GmbH, Germany). The column temperature was maintained at 38 °C and a mixture of

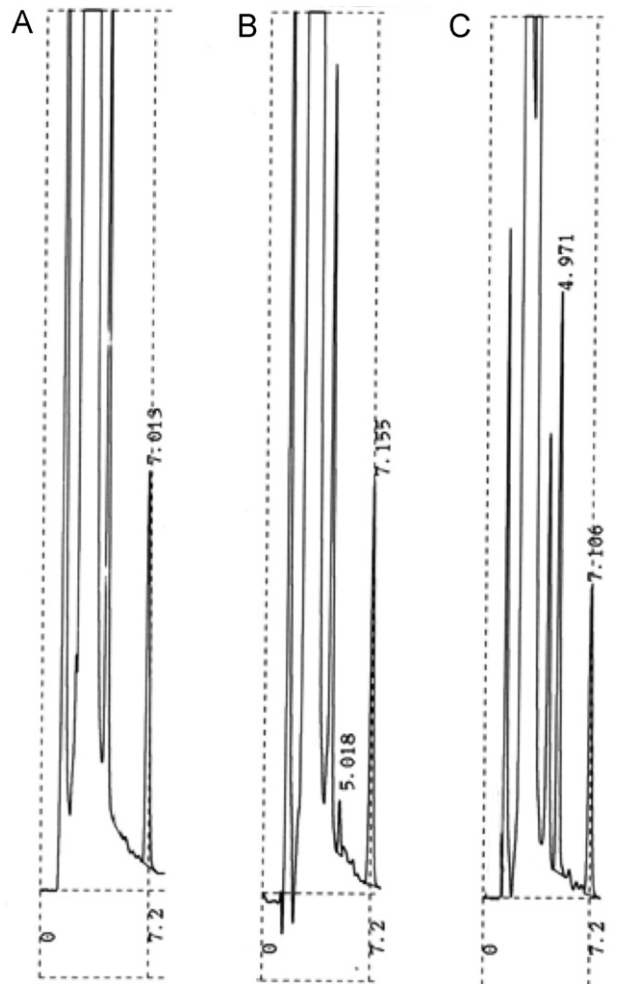

Fig. 2. Typical chromatograms of (A) human blank serum containing sertraline as the I.S. (B) human blank serum spiked with 0.01 μg/mL VPA and the I.S., and (C) serum samples collected from a healthy volunteers 5 h after a single oral administration of 250 mg VPA. Peaks eluted at 5.0 and 7.1 min correspond to VPA and the I.S., respectively.

water (containing formic acid 0.5 mL/L) and methanol (85:15, v/v) was eluted as mobile phase at a flow rate of 0.6 mL/min.

2.4. Extraction procedure and derivatization

Serum samples were stored at −40 °C until assay and frozen samples thawed in water at 37 °C. Aliquots of blank, calibration standard or unknown human serum samples (100 μL) were pipetted into separate Eppendorf tubes, containing 50 μL of the working I.S. solution. The samples were mixed with 25 μL of the HCL (0.1 M) solution and extracted with 1 mL of diethyl ether. After vortex mixing for 15 s, separation of the organic phase and its evaporation at 40 °C· the residue was reconstituted in 125 μL of the FMOC-Cl solution. Following addition of 25 μL borate buffer (0.05 M; pH 9.3) and brief mixing, the samples were kept at 60 °C for 10 min and then a volume of 5 μL of the reaction mixture was injected onto the HPLC or LC–MS/MS systems.

2.5. Optimization of the derivatization conditions

Quality control samples prepared with the drug working solutions to make low (0.02 μg/mL), medium (1.0 μg/mL) and high (10.0 μg/mL) concentrations of VPA were used to optimize derivatization of the drug with FMOC-Cl, while the I.S. was reacted with the reagent at the concentration of 20 μg/mL. Concentrations of the FMOC-Cl solutions were optimized in the range of 100–1000 μg/mL. pH and concentrations of the buffer solutions ranging from 6 to 12 and 0.01 to 0.5 M respectively, were tested to obtain optimal conditions. The polarity of the reaction solution was tested using various organic solvents–water proportions, ranging from 1:1 to 10:1, the reaction was allowed to proceed in a water bath at different temperatures ranging from 40 to 80 °C and times between the ranges of 5 and 25 min.

Table 1
Inter and intra-day precision and accuracy for determination of VPA in human serum by the HPLC method. (Accuracy has been calculated as a percentage of the nominal concentration.)

Known concentration (μg/mL)	Concentration found (mean ± SD)	Precision (%)	Accuracy (%mean deviation)
Inter-day			
0.02	0.021 ± 0.0011	7.7	3.9
1.0	0.997 ± 0.033	3.4	−0.3
10.0	10.02 ± 0.18	1.8	0.18
Intra-days			
0.02	0.022 ± 0.0025	11.3	3.0
1.0	0.997 ± 0.048	4.8	−0.3
10.0	10.1 ± 0.17	1.6	1.0

2.6. Method validation

The calibration curves were prepared over a concentration range of 0.01–32 μg/mL at nine levels by spiking the appropriate amounts of the standard solutions in 100 μL pooled human blank serum obtained from the normal subjects. After evaporation of 100 μL from the each working solutions under a gentle stream of nitrogen at 50 °C, the residues were reconstituted in 100 μL of drug-free human serum, mixed for 10 s on a vortex mixer and subjected extraction, derivatization and analysis as described above. The linearity of the method was checked in the same day ($n = 6$) and in six consecutive days. Calibration curves were obtained using a weighted regression with a weighting factor of $1/(\text{concentration})^2$ by linear least-squares regression analysis plotting of peak-area ratio (VPA to I.S.) vs. the drug concentrations.

Intra and inter-day variations were calculated by repeated analysis ($n = 6$) of different concentrations of VPA in a single analytical run and in ten analytical runs performed on different days, respectively using the quality control samples. The limits of detection (LOD) and quantification (LOQ) were defined as the concentrations of drug giving a signal-to-noise ratio of 3:1 and the lowest serum concentration that quantified with a coefficient of variation of less than 20%, respectively.

The specificity of the method was examined by the presence of disturbing endogenous peaks in twenty-four human serum samples from different volunteers. These samples were pretreated according to the sample preparation procedure except for the addition of the I.S. Several potentially co-administrated drugs with VPA including acetaminophen, phenytoin, phenobarbital carbamazepine, lamotrigine, zonisamide, topiramate, primidone, gabapentin, vigabatrin ethosuximide, clonazepam, diazepam, theophylline, etidronate, gentamicin, baclofen, topiramate, erythromycin, propranolol, clarithromycin and azithromycin was examined in selectivity study of the assay.

Average recoveries of the extraction procedure for both VPA and the I.S. were estimated by comparing the peak areas obtained from derivatization of an extracted spiked blank sample with those obtained from derivatization of the similarly treated standard. Stability of the quality control serum samples was subjected to short-term (12 h, 25 °C) at room temperature, three freeze/thaw (−40 to 25 °C) cycles and long-term (30 days, −40 °C).

2.7. Application of the method

The present method was applied in a randomized crossover bioequivalence study of two different VPA preparations in twenty-four male healthy volunteers. Written informed consent was obtained from the subjects and Ethics Committee of Kermanshah University of Medical Sciences approved the study protocol. After an overnight fasting, all the volunteers received a single oral dose of 250 mg VPA from either test or reference preparations on two working days separated by a wash-out period of three weeks. Blood sampling was carried out at suitable intervals up to 24 h. Analysis of the drug and calculation of pharmacokinetic parameters including maximum concentration (C_{max}), area under the concentration time curve from zero to the time of last sampling ($AUC0–t$) and area under the concentration time curve from zero to infinity ($AUC0–\infty$) were performed using Lagran pharmacokinetics software (Faculty of pharmacy, University of Alberta, Edmonton, Canada). Student's t-test was used for statistical analysis of the data and statistical significance was defined at the level of $p < 0.05$.

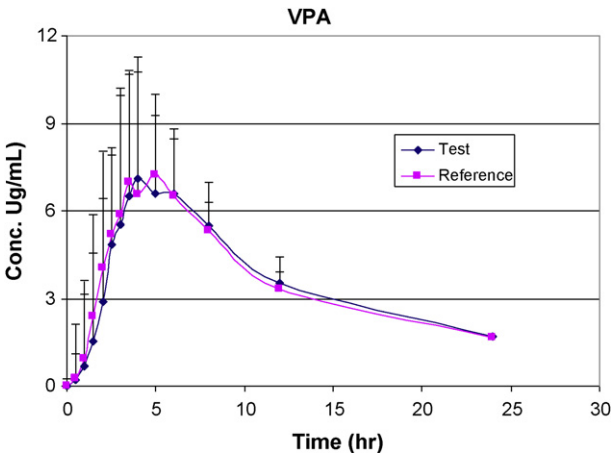

Fig. 3. Mean serum concentrations vs. time profiles of VPA for two different preparations in 24 human volunteers after administration of a single 250 mg oral dose.

3. Results and discussion

3.1. Derivatization conditions

The derivatization of carboxylic acid moiety of VPA using FMOC-Cl as the labeling agent appeared to be highly dependent on pH of the buffer solution. The reaction can therefore efficiently proceed over the pH range of 8.5–11.5 and the optimal pH for the reaction was found to be 9.3. Maximal yield of the derivative was obtained with a FMOC-Cl solution of 500 μg/mL, a reaction temperature of 60 °C for 10 min and a reaction medium consisting of buffer-acetonitrile (1:4, v/v).

3.2. Method validation

Typical chromatograms of human blank serum and human blank serum spiked with VPA (0.01 μg/mL) and the I.S. are shown in Fig. 2A and B, respectively. VPA and the I.S. were eluted with respective retention times of 5.0 and 7.2 min. No endogenous substance from the serum components was eluted in the retention times of the drug or the I.S. Fig. 2C shows the chromatogram of serum sample obtained at 5 h after a single oral dose of 250 mg VPA from a healthy volunteer. In selectivity study none of the tested drugs was interfered with analysis of VPA under described conditions. The limits of detection and quantification were estimated to be 0.004 and 0.01 μg/mL, respectively using a volume of 100 μL serum sample and 5 μL injection. The method was linear over the concentration range of 0.01–32 μg/mL using line-fit plot in regression analysis with the correlation coefficients of equal to or better than 0.997. Intra and inter-day reproducibility for calibration curves were determined on the same day in replicate ($n = 6$) and on different days ($n = 6$) respectively, using same pooled serum sample. The intra-day average slope of the fitted straight lines was 0.408 ± 0.024 μg/mL (C.V. = 5.9%) and the mean intercept of the calibration curves was 0.02 ± 0.003 (C.V. = 15%). The corresponding mean (±SD) coefficient of the linear regression analysis was 0.996 ± 0.010 (C.V. = 0.1%). For calibration curves prepared on different days, the mean ± SD of results were as follows: slope 0.501 ± 0.021 μg/mL (C.V. = 4.2%), coefficient of the linear regression analysis = 0.997 ± 0.011 (C.V. = 0.1%) and intercept = 0.026 ± 0.004 (C.V. = 15.3%). The inter and intra-day accuracy and precision data are shown in Table 1. The coefficient of variation values for both inter and intra day analysis was less than 12% whereas the percentage error was less than 4.0%. Stock solutions of

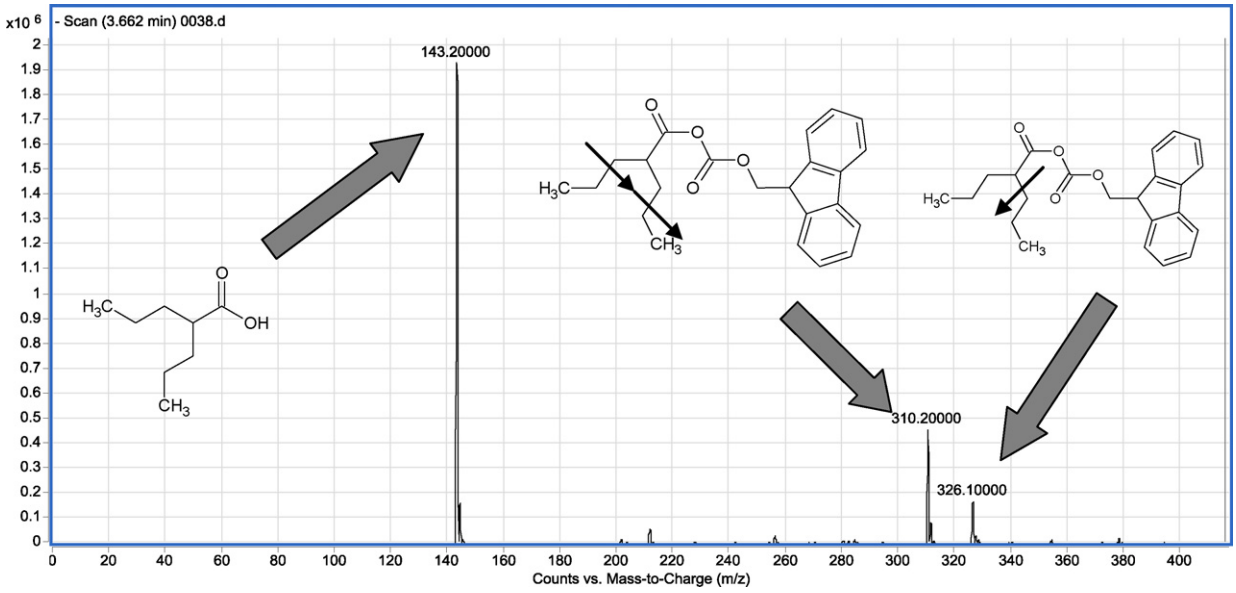

Fig. 4. In source CAD mass spectrum of VPA after derivatization with FMOC-Cl at 135 V fragmentor voltage.

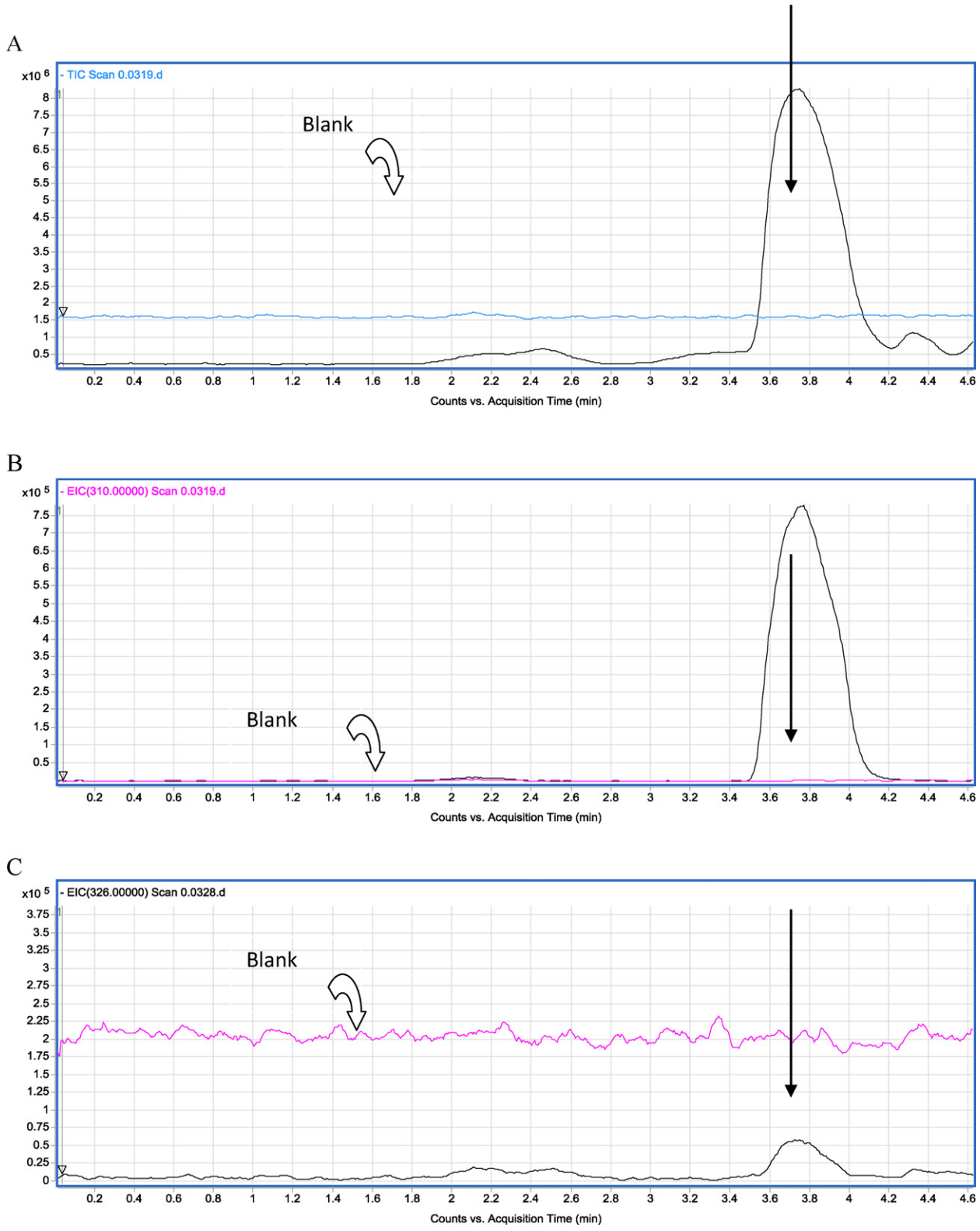

Fig. 5. (A) Total Ion Chromatograms (TIC) of derivatized blank and VPA (1 μg/mL) and their Extracted Ion Chromatograms (EIC) at (B) $m/z = 310$ and (C) m/z 326.

VPA and the I.S. were stable for 3 and 60 days respectively, when stored at 4 °C and the derivatized solutions were found to be stable (>95%) for about 2 h. After 60 days, the concentrations of the drug in serum stored at −40 °C and following 3 thaw–freeze cycles were found to be 99% from the initial values. The mean recoveries of VPA and the I.S. from serum were 92 ± 3% and 90 ± 3%, respectively.

3.3. LC–MS/MS study of the reaction

FMOC-Cl efficiently reacts with carboxylic acid moiety of VPA in alkaline medium and comparing to previously reported methods the new technique is simple, rapid (10 min reaction time), more sensitive and needs low serum volume (100 μL). Reaction of

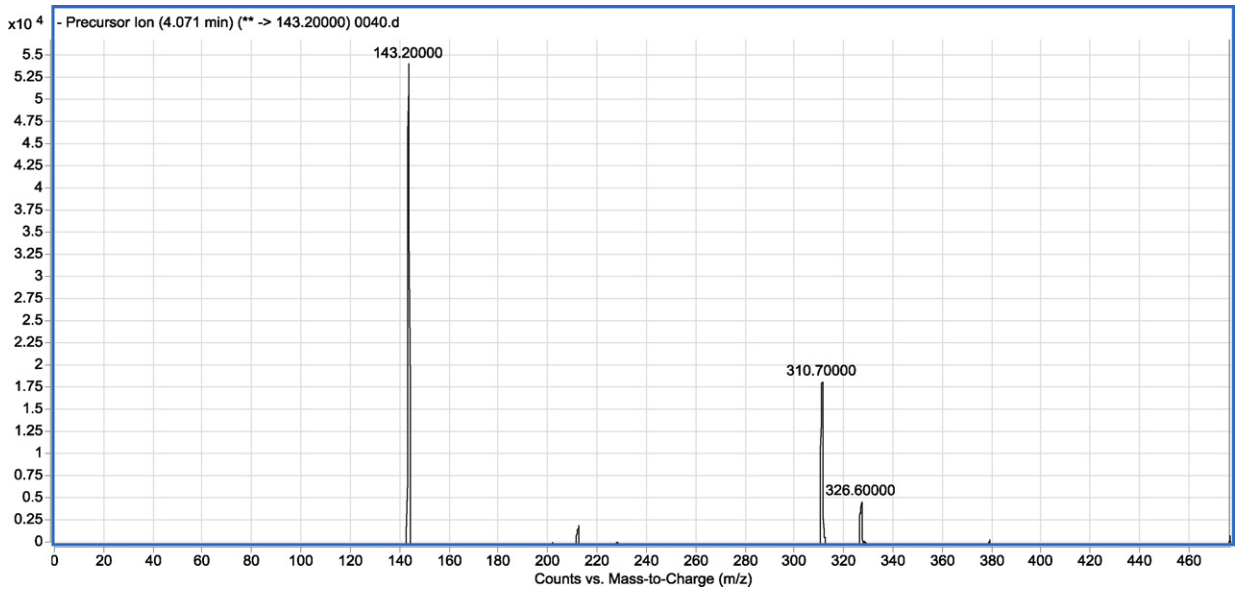

Fig. 6. Precursor ion spectrum of *m*/*z* 143 corresponding to molecular weight of VPA in negative mode.

VPA with FMOC-Cl was studied by LC–MS/MS technique using full scan, ion product scan and precursor scan modes in both positive and negative electrospray ionization. The negative mode produces stronger mass response hens, acquisition of mass spectrometry data were made in the negative ESI. The mass parameters were optimized to obtain the highest molecular ion abundance of the analytes. The maximum ion intensity were achieved when the source temperature, ion spray current and fragmentor voltage were set at 300 °C, 4000 V, and 135 V, respectively. In full scan mode, the negative ion mass spectrum of free VPA produced a molecular ion by hydride abstraction ($[M-H]^-$) at *m*/*z* 143.2 corresponding to molecular weight of the drug. After derivatization however, the mass spectra of eluting derivatives appeared to be complicated. While the major ions observed were not the molecular ions of the derivatives as expected, several molecular ions including free VPA and unknown adducts were appeared at different *m*/*z* values indicating in-source fragmentation of the resulted derivative. Thus the FMOC-VPA parent ion (*m*/*z* 366 after loss of HCl) was not seen and two intense stable ions were appeared at *m*/*z* 326 and 310. It seems dominant fragmentation paths of the analyte are loss of a propyl or two ethyl groups producing ions at *m*/*z* 326 and 310, respectively (Fig. 4).

Total Ion Chromatograms (TIC) of derivatized blank and VPA (1 μg/mL) samples have been compared in Fig. 5A, and their Extracted Ion Chromatograms (EIC) at *m*/*z* = 310 and 326 are presented in Fig. 5B and C, respectively. These figures show that the stable ions appeared at *m*/*z* 326 and 310 are not present in the blank samples. To investigate correlation between these ions and derivatization of the drug and to characterize chemical structure of the resulted derivative we switched from full scan mode to application of collision induced dissociation (CID) in either product ion scan or precursor ion scan modes.

The *m*/*z* values of 326 and 310 were selected as the precursor ions and product ion spectra, were obtained between *m*/*z* values of 10 and 400, separately using different collision energy ranging from 1 to 10 eV. CID of the both precursors generate main product ion at *m*/*z* 143 corresponding to molecular weight of free VPA. Further characterization of the VPA-FMOC adduct using the precursor ion acquisition technique shows when the *m*/*z* value of 143 is selected as product ion and precursor ion spectra obtained between *m*/*z* values of 143 and 1680, using different collision energy ranging from 1 to 10 eV, most intense ions appeared at *m*/*z* 310 and 326 (Fig. 6). Therefore, our data document derivatization of carboxylic acid moiety of VPA.

Table 2
Mean (SD) pharmacokinetic parameters of two VPA preparations after single oral administration of 250 mg in 24 human volunteers. t_{max}: time to maximum concentration, C_{max}: maximum concentration, AUC: area under the curve, $t_{1/2}$: elimination half life.

Parameter	Prep.		
	Test	Reference	*p* value[a]
C_{max} (μg/mL)	10.8 (4.1)	10.5 (4.3)	NS
*AUC*0–24 (μg h/mL)	89.2 (31.2)	86.4 (25.0)	NS
*AUC*0–∞ (μg h/mL)	119.1 (50.5)	111.2 (36.4)	NS
$t_{1/2}$ (h)	10.8 (4.0)	10.0 (3.2)	NS
t_{max} (h)	4.0 (1.0)	4.3 (2.1)	NS

[a] $P < 0.05$.

3.4. Application of the method

The present method has successfully been used to quantify the drug in a randomized cross-over bioequivalence study following single oral administration of two different VPA preparations in 24 healthy volunteers. Typical serum concentration–time profile and the resulted pharmacokinetic parameters of the drug have been shown in Fig. 3 and Table 2, respectively.

4. Conclusion

Although many chemicals have been used for fluorescence labeling of carboxylic acids, this is the first report in which FMOC-Cl has been applied in derivatization of a drug with carboxylic acid as a functional group. The most advantages of our method are simplicity of operation, rapidity of the assay, higher sensitivity using lower sample volume and its potential application in analysis of other chemicals with carboxylic acid group, which makes it attractive for analysis of chemicals with acidic functions.

References

[1] T. Fukushima, N. Usui, T. Santa, K. Imai, J. Pharm. Biomed. Anal. 30 (2003) 1655.
[2] S. Einarsson, B. Josefsson, S. Lagerkvist, J. Chromatogr. 282 (1983) 609.
[3] Gh. Bahrami, Sh. Mirzaeei, A. Kiani, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 820 (2005) 277.

[4] Gh. Bahrami, B. Mohammadi, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 850 (2007) 417.
[5] R.J. Porter, B.S. Meldrum, in: B.G. Katzung (Ed.), Basic and Clinical Pharmacology, McGraw-Hill, New York, NY, 2011, p. 399.
[6] W. Löscher, CNS Drugs 16 (2002) 669.
[7] M.A. Farajzadeh, Kh. Farhadi, A.A. Matin, P. Hashemi, A. Jouyban, Anal. Sci. 25 (2009) 875.
[8] R. Nishioka, M. Takeuchi, S. Kawai, M. Nakamura, K. Kondo, J. Chromatogr. 342 (1985) 89.
[9] M. Krogh, K. Johansen, F. Tønnesen, K.E. Rasmussen, J. Chromatogr. B: Biomed. Appl. 673 (1995) 299.
[10] P. Shahdousti, A. Mohammadi, N. Alizadeh, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 850 (2007) 128.
[11] E. Gaetani, C.F. Laureri, M. Vitto, J. Pharm. Biomed. Anal. 10 (1992) 193.
[12] D. Yu, J.D. Gordon, J. Zheng, S.K. Panesar, K.W. Riggs, D.W. Rurak, F.S. Abbott, J. Chromatogr. B 666 (1995) 269.
[13] N.V.S. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, M. Santosh, J. Chidambara, B.R. Kumar, Rapid Commun. Mass Spectrom. 19 (2005) 1970.
[14] V. Pucci, E. Monteagudo, F. Bonell, Rapid Commun. Mass Spectrom. 19 (2005) 3713.
[15] T. Mino, M. Nakajima, H. Wakabayashi, S. Yamato, K. Shimada, Anal. Sci. 17 (2001) 999.
[16] V. Pucci, E. Monteagudo, F. Bonelli, Rapid Commun. Mass Spectrom. 19 (2005) 3713.
[17] K. Matsuura, T. Ohmori, M. Nakamura, Y. Itoh, K. Hirano, Biomed. Chromatogr. 22 (2008) 387.
[18] H. Cheng, Z. Liu, W. Blum, J. Byrd, R. Klisovic, M.R. Grever, G. Marcucci, K.K. Chan, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 850 (2007) 206.
[19] M.D. Rukhadze, M.V. Gonashvili, A.N. Zirakishvili, N.V. Okudzhava, Pharm. Chem. J. 36 (2002) 45.
[20] H. Amini, M. Javan, A. Ahmadiani, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 830 (2006) 368.
[21] Y. Zhong, Z. Jiao, Y. Yu, Biomed. Chromatogr. 20 (2006) 319.
[22] S. Hara, M. Kamura, K. Inoue, M. Fukuzawa, N. Ono, T. Kuroda, Biol. Pharm. Bull. 22 (1999) 975.
[23] M.C. Lin, H.S. Kou, C.C. Chen, S.M. Wu, H.L. Wu, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 810 (2004) 169.
[24] J.P. Moody, S.M. Allan, Clin. Chim. Acta 127 (1983) 263.
[25] G. Kamalinia, M.R. Rouini, P. Ghaeli, Chromatographia 70 (2009) 569.
[26] C. Lucarelli, P. Villa, E. Lombaradi, P. Prandini, A. Brega, Chromatographia 33 (1992) 37.